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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, platform, poster / discussion, workshops, roundtables, and poster sessions of the 34th Annual Meeting of the Society of Toxicology, held at the Baltimore Convention Center, Baltimore, Maryland, March 5-9, 1995.

An alphabetical Author Index, cross-referencing the corresponding abstract number(s), begins on page 327.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 351.

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1 TOXICANT-INDUCED ALTERATION OF GENE EXPRESSION

C McQueen, R F Novak. *University of Arizona, College of Pharmacy, Tuscon, AZ; Institute of Chemical Toxicology, Wayne State University, Detroit, MI*

A variety of stimuli such as growth factors, cytokines, and hormones, as well as physical, chemical or metabolic events, including oxidative stress and altered Ca^{2+} homeostasis, alter signal transduction pathways either through receptor-mediated signaling pathways or through other signaling processes. These signaling events frequently culminate in changes in gene expression. Whereas enhanced gene expression occurs through transcriptional activation, mediated by the binding of transcription factor(s) to the 5′ regulatory region of the gene, there exist numerous examples in which down regulation of gene expression also occurs. This symposium will feature presentations on the enhanced expression and suppression of gene expression in different systems in response to chemical agents, the mechanism(s) underlying altered gene expression and the potential role of this cellular response in modulating toxic injury and cellular homeostasis.

2 METALLOTHIONEIN GENE TRANSCRIPTION IN RESPONSE TO TOXIC METALS AND OXIDATIVE STRESS

D J Thiele. *Department of Biological Chemistry, University of Michigan Medical School, Ann Arbor, MI*

Metallothioneins (MTs) are low molecular weight, cysteine-rich metal binding proteins which are found throughout nature and are biosynthetical elevated, at the level of gene transcription, in response to a wide array of environmental stresses. Yeast cells provide excellent experimental model eukaryotic systems in which to study the precise molecular mechanisms whereby toxic environmental stresses are sensed by signal transduction molecules and appropriate transcriptional responses are activated. The *CUP 1* gene of the baker's yeast *Saccharomyces cerevisiae* encodes an MT protein which plays a critical role in protecting yeast cells from toxic copper concentrations and serves as a line of defense against oxygen-derived damaging free radicals. Consistent with MT serving as a major cellular defense mechanism against metal and free radical toxicity, the *CUP 1* gene is transcriptionally activated in response to both elevated environmental copper concentrations and by conditions or chemicals which generate oxygen-derived free radicals. Our laboratory has cloned two genes encoding toxic metal-sensing transcription factors which are activated, through copper coordination, to bind to specific metal responsive sites in the yeast MT gene promoters. The mechanisms whereby these toxic metal sensing transcription factors activate the expression of metal detoxification genes, and the interplay of other metal homeostatic factors with these transcription factors are critical aspect of metal detoxification in yeast. Furthermore, yeast cells utilize distinct transcription factors to sense oxidative stress for the activation of yeast MT gene transcription.

3 DIFFERENTIAL EFFECTS OF HORMONES AND XENOBIOTICS ON RAT LIVER SULFOTRANSFERASE GENE EXPRESSION

M Runge-Morris. *Institute of Chemical Toxicology, Wayne State University, Detroit, MI*

Hydroxysteroid sulfotransferase-a (HST-a) and aryl sulfotransferase IV (ASTIV) detoxify xenobiotics, activate carcinogens and modulate intra-tissue active hormone levels. Since xenobiotics and endocrine changes may alter sulfotransferase gene expression, the effects of streptozotocin (STZ), 3-methylcholanthrene (3-MC), phenobarbital (PB) and dexamethasone (DEX) on rat hepatic HST-a and ASTIV mRNA and protein levels were characterized. Mature female Sprague Dawley (SD) rats with STZ-induced diabetes displayed suppressed hepatic HST-a mRNA levels (up to 65%), which was partially reversible with insulin treatment. Hepatic HST-a mRNA levels in mature female SD rats were dose-dependently suppressed following treatment with 3-MC (75% suppression at 80 mg/kg ip). In mature male rats, hepatic HST-a and ASTIV mRNA levels were suppressed (≈50–70%) by PB (80 mg/kg ip) and induced by DEX (50 or 100 mg/kg ip). These results suggest that both xenobiotic and endocrine-related signals affect sulfotransferase gene expression. Supported by NIEHS Grant ES05823.

4 TRANSCRIPTIONAL AND POST-TRANSCRIPTIONAL REGULATION OF GENE EXPRESSION BY DIOXIN

W F Greenlee, Y-Y P Wo, Y M Tang. *Dept. of Pharmacology and Toxicology, Purdue University, West Lafayette, IN*

Many of the toxic responses elicited by dioxin and related compounds result at least in part from modulation of gene expression mediated by activation of the intracellular *Ah* receptor. Multiple regulatory mechanisms have been documented. These include: direct transcriptional activation (CYP1A1), mRNA stabilization (TGF-α), and transcriptional repression (TGF-β_2). Recently, four human dioxin-responsive cDNA clones have been isolated from a keratinocyte line. One of the clones was identified as plasminogen activator inhibitor-2 (PAI-2). Another clone was shown to be the first member of the cytochrome P450 1B sub-family. This gene (designated CYP1B1) contains nine potential dioxin-responsive enhancer (DRE) binding motifs located within a 2.5 kb genomic fragment 5′-ward of the transcription initiation start site (TIS). In contrast to CYP1A1, CYP1B1 lacks a consensus TATA box in the promoter. The CYP1B1 promoter contains a TATA-like sequence (TTAAAA) 27 bp upstream from the TIS and is similar to other TATA-less genes that require an initiator and Sp1 element for efficient transcription. Comparison of the rates of gene transcription with the level of mRNA transcripts for CYP1B1 and PAI-2 indicate the involvement of multiple regulatory mechanisms that include direct transcriptional activation, interaction with other transcription factors, and altered mRNA turnover. (Supported by a grant from the American Forest and Paper Association.)

5 ROLE OF P450-CATALYZED CCl_4 METABOLISM AND HEPATIC INJURY IN THE EXPRESSION OF IMMEDIATE-EARLY GENES, TRANSCRIPTION FACTORS, AND THE Ca^{2+}-ACTIVATED NEUTRAL PROTEASE (CANP)

R F Novak, A Gruebele, K Zawaski, D Kaplan. *The Institute of Chemical Toxicology, Wayne State University, Detroit, MI*

Research was initiated to examine the role of cytochrome P450-catalyzed CCl_4 metabolism, and the resultant oxidative damage to hepatic tissue, in the enhanced expression of c-fos and c-jun and the nuclear levels of the AP-1 and NFκB transcription factors. The expression of CANP was also monitored under various conditions. Animals were treated either with CCl_4 alone (160 mg/kg, 0.5 to 24 hr) or pre-treated with P450 inducers [pyridine (PY) (100 mg/kg, 24 hr), phenobarbital (PB) (100 mg/kg, 72 hr)] or inhibitors / antioxidants [diallyl sulfide (DAS) (200 mg/kg, 3 hr), N-acetylcysteine (NAC) (600 mg/kg, 3 hr)] prior to treatment with CCl_4 and sacrificed. Hepatic poly(A)$^+$ RNA samples were analyzed by Northern blot and/or RT-PCR analysis. CCl_4 administration resulted in a pronounced time- and dose-dependent increase in c-fos and c-jun mRNA levels and an elevation in nuclear AP-1 and NFκB levels, as monitored by gel shift analysis. This response was followed by an elevation in the expression of CANP mRNA 18–24 hours post-treatment. A marked decline in hepatic c-fos and c-jun mRNA levels was monitored in animals pre-treated with either DAS or NAC prior to administration of CCl_4, relative to those monitored in hepatic tissues of animals treated with CCl_4 alone. In contrast, pre-treatment with PY or PB resulted in a significant elevation in c-fos and c-jun mRNA levels over those monitored in CCl_4-treated animals. The data suggest that hepatic injury results in the expression / generation of immediate-early genes and transcription factors which regulate CANP expression, a proteinase which also has been implicated in the degradation of receptors and transcription factors. (Supported by NIH Grant ES02521)

6 IS CARNITINE A KEY TOXICOLOGICAL TARGET? INTRODUCTION

J E Garst. *Alamogordo, NM*

Increasingly R-(L)-carnitine (Cn) and/or short-chain O-acyl Cn esters (SCCn) are reported to ameliorate various adverse effects of diverse chemicals and drugs, including halothane, 2,4,5-T, MPTP, valproic acid, adriamycin, aflatoxin B 1 and even hyperbaric oxygen. Besides affecting chemical/drug-induced toxicity, Cn and its SCCn esters can protect against metabolic diseases such as Reye's and Reye's-like syndromes, diabetic nephropathy, and even Alzheimer's disease. Moreover, the toxicity of various substances seems mediated in part by actions that alter carnitine and/or its enzymes. These include CO, various heavy metals, and the antibiotic cephaloridine. Cn and its esters may play a direct role in homeostasis, influencing protein translation and function and physiological signaling pathways involving calcium ion flux, calpains II, and protein kinase C. This symposium will explore new avenues suggest-

ing that the Cn system may be a key toxicological target. The first speaker will review the biochemistry and function of Cn. The second speaker will review Cn biosynthesis, deficiency (CnD), and its growing use in clinical medicine. The third speaker will review work from the juvenile visceral steatosis (jvs) CnD mouse. Besides showing signs similar to Reye's syndrome in humans, these mice show an increased expression of *c-jun* and *c-fos* proto-oncogenes. The fourth speaker will review evidence of protection by SCCn esters against radical-mediated loss of enzyme activity and against DNA-strand breakage. These data associate SCCn (carnitine acetyltransferase?) with prevention of programmed cell death or apoptosis. By revealing how Cn changes can explain the tumorigenicity of peroxisome proliferating agents (PPA), the last speaker will explore whether Cn might participate in a signaling system by which a cell responds to the organism's external environment.

7 BIOCHEMISTRY AND FUNCTIONS OF L-CARNITINE

L L Bieber. *Michigan State University, East Lansing, MI.* Sponsor: J Garst

More than a decade ago, five roles for L-carnitine were proposed in addition to its well-established function in mitochondrial β-oxidation of long-chain fatty acids (*Fed. Proc.* 41, 2858-2861 (1982)). It now is well-established that L-carnitine can: (a) modulate cellular acyl-CoA/CoASH ratios; (b) detoxify a large array of poorly or nonmetabolizable acyl residues via conjugation to the β-hydroxyl group; (c) shuttle acyl residues from one cellular compartment to another; d) affect the rate of specific α-keto acid oxidations; and (e) serve as a reservoir of activated acyls, primarily acetyl. The above-mentioned processes are mediated by a family of carnitine acyltransferases capable of reversibly catalyzing short-, medium-, and long-chain acyl interchange between CoASH and the β-OH group of carnitine. The multi-organelle association of the carnitine acyltransferases has been extensively investigated, including use of different kinds of organelle proliferators. Both multi-organelle and specific organelle effects occur; i.e. the specific activity of microsomal, mitochondrial and peroxisomal short-chain and medium-/long-chain carnitine acyltransferases of liver all increase due to dietary clofibrate, but none increase due to dietary phenobarbital. Some xenobiotics or metabolic abnormalities in humans can produce nonmetabolizable acyl-CoAs that are detoxified via conjugation of the acyl moiety to L-carnitine; in some cases, the urinary acylcarnitine profile can be used for diagnostic purposes. (Supported in part by grant DK-18427 from NIH).

8 CARNITINE BIOSYNTHESIS, DEFICIENCY, AND USE IN CLINICAL MEDICINE

C J Rebouche. *University of Iowa, Iowa City, IA.* Sponsor: J Garst

L-Carnitine is synthesized from lysine and methionine, via protein-lysine N-trimethylation, protein hydrolysis, hydroxylation at the three position of ε-N-trimethyllysine, aldol cleavage, oxidation, and finally hydroxylation at carbon 3 of γ-butyrobetaine. Carnitine biosynthesis in mammals is regulated by the rate of formation of ε-N-trimethyllysine and its ability to penetrate to cellular compartments where it is hydroxylated. Carnitine "deficiency" (low circulating and/or tissue concentrations of carnitine) is associated with numerous diseases and other medical conditions (e.g., acyl-CoA dehydrogenase deficiencies and other defects of organic acid metabolism, glycogen storage diseases, urea cycle defects). Moreover, use of some drugs (e.g., valproic acid, prodrugs containing pivalic acid) also leads to carnitine depletion. Mechanisms by which these effects occur are in many cases not well understood, but are often associated with hyperexcretion of specific acylcarnitine esters (e.g., propionylcarnitine in propionic acidemia), or decreased efficiency of reabsorption of both carnitine and acylcarnitine esters. Although often suggested, a decreased rate of carnitine biosynthesis as a cause of carnitine deficiency has not been demonstrated in association with any disease or other medical condition. L-Carnitine or its esters have been shown to be effective in treatment of some chronic diseases (e.g., Alzheimer's disease, diabetic nephropathy requiring hemodialysis).

9 PATHOPHYSIOLOGY OF CARNITINE-DEFICIENT JVS MICE

T Saheki. *Kagoshima University, Kagoshima, Japan.* Sponsor: J Garst

Juvenile visceral steatosis (jvs) mice were described to suffer from fatty liver, hypoglycemia and hyperammonemia (*Lab. Anim.* 22: 83–87 (1988)). The hyperammonemia results from decreases in all hepatic urea cycle enzymes found to be caused by suppression of the gene transcription (*FEBS Lett.* 260: 119–121 (1990)). Further work revealed that c-fos and c-jun mRNAs are increased in the liver of jvs mice (*FEBS Lett.* 311: 63–66 (1992)) and that gene activation through the glucocorticoid pathway might be inhibited by AP-1, causing suppression of glucocorticoid-dependent genes in the liver. Jvs mice show a generalized carnitine deficiency (*Biochem. Biophys. Res. Commun.* 174: 1090–1094 (1990)) and suppression of the gene expression is prevented by administration of carnitine (*J. Biol Chem.* 267: 5032–5035 (1990)). Jvs mice were also found to suffer from carnitine-responsive cardiac hypertrophy (*FEBS Lett.* 326: 267–271 (1993)) associated with disturbance in expression of some genes. Possible mechanisms of abnormal gene expression in liver and heart caused by carnitine deficiency will be discussed.

10 OXYGEN-RADICAL MEDIATED ENZYME AND DNA DAMAGE IS AMELIORATED BY CARNITINE AND SHORT-CHAIN ACYLCARNITINE ESTERS

M E Boerrigter. *Harvard Medical School & Beth Israel Hospital, Boston, MA.* Sponsor: J Garst

The degenerative changes which are seen during senescence are ultimately the consequence of an organism's failure to maintain the integrity of key macromolecules such as DNA, RNA and proteins. L-carnitine, which acts as an essential transmitochondrial carrier of acetyl and long-chain acyl groups to be used in fatty acid oxidation, is one substance which might be applied as an agent in the prevention and/or amelioration of age-related DNA and protein damage. The mechanisms by which L-carnitine and its derivatives may exert their beneficial influence on, e.g. cardiac performance, cell survival and DNA repair, are largely unknown. Our studies have shown that (i) *in vitro* treatment of human peripheral blood lymphocytes with L-carnitine or acetyl-L-carnitine confers an increased DNA repair capacity following exposure to oxygen radicals or the alkylating agent *N*-ethyl-*N'*-nitrosourea, and (ii) *in vivo* treatment of mice with propionyl-L-carnitine partially restores the observed age-related decrease in the activity of cardiac carnitine palmitoyl transferase-I activity.

11 DOES CARNITINE LINK OXIDATIVE STRESS, GENE EXPRESSION, AND APOPTOSIS INTO A TOXICOLOGICAL SIGNALING SYSTEM FOR PEROXISOME PROLIFERATING AGENTS?

J E Garst. *Alamogordo, NM*

Carnitine (Cn), its esters, and enzymes for which they are substrates may be major regulators/integrators of the cellular response to its organism's environment. Non-genotoxic tumor development by diverse peroxisome proliferating agents (PPA) illustrate this point. Five facts suggest that changes in balance between Cn and its esters, termed Cn-perturbations (CnP), may better explain PPA tumorigenicity than other proposed theories. *First*, data increasingly separate peroxisomal changes and oxidative processes from PPA-mediated tumorigenicity. Yet, carnitine acetyltransferase induction remains the most sensitive measure of PPA. *Second*, major increases in the antioxidant acetylcarnitine (AcCn) could explain the absence of a sustained oxidative stress after PPA exposure. *Third*, substantial formation of hepatic AcCn causes the rapid loss of Cn by all PPA so far investigated. Cn-deficiency (CnD) develops as peroxisomal β-oxidation produces increasingly more acylCn. It is hypothesized that CnD would worsen unabatedly, except that CnD triggers hepatic gene expression (GE) and proteolysis to restore Cn to normal. *Fourth*, the effects of PPA show remarkable similarities to CnD in *jvs* mice and to CnD, Reye's-, and Reye's-like syndromes in humans. *Fifth*, CnP can explain PPA-tumorigenicity. Tumor development is often mediated by increased proto-onco-GE and decreased apoptosis (APO). Low AcCn can activate xanthine oxidase, forming oxygen-radicals that break DNA strands, and cause calcium flux, activating endonuclease; both may lead to APO. Conversely, high AcCn reduces APO. Since both PPA and PPA-mediated CnP reduce APO and foster GE, are CnP mechanistically responsible for PPA-linked tumorigenicity? Since CnP can and do occur in humans, might they also underlie tumor development in humans?

12 DDTC RELEASES CS_2 *IN VIVO* RESULTING IN THE EXCRETION OF A UNIQUE URINARY METABOLITE, TTCA

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It has been previously postulated that 2-thiothiazolidine-4-carboxylic acid (TTCA) is formed by reaction with glutathione or cysteine upon exposure to CS_2. This study was conducted to determine if TTCA is formed as a result of dithiocarbamate exposure and if TTCA formation is the result of the CS_2 released *in vivo* from diethyldithiocarbamate (DDTC). [^{13}C]-DDTC was given

i.p. to male Sprague-Dawley rats, 1.4 mmol/kg/day for 3 days, and the urines collected. TTCA was extracted from acidified urine with ethyl acetate. Pooled extracts were concentrated by rotoevaporation and the samples were methylated with diazomethane and purified through a silica gel column. TTCA was detected by GC/MS as the dimethyl derivative of TTCA. Purification by silica gel treatment yielded a 5-fold increase in measurable TTCA. In these experiments, all the TTCA formed from DDTC-treated rats was labeled, whereas no TTCA was obtained from control urine. The formation of TTCA in the urine of DDTC-treated rats is consistent with the direct reaction of CS_2, produced from the *in vivo* metabolism of DDTC, with glutathione or cysteine to form trithiocarbonic acid or dithiocarbamate. These experiments demonstrate that TTCA is a unique metabolite of CS_2 since CS_2 is required for the production of TTCA and lend plausibility to the use of TTCA measurement as a potential biomarker for exposure to dithiocarbamates. Supported by NIEHS grants ES06387 and (DJJ) ES07031.

13 USE OF HUMAN LYMPHOBLASTOID CELLS EXPRESSING HUMAN UDP-GLUCURONOSYL- TRANSFERASES FOR MECHANISTIC STUDIES OF HUMAN DRUG METABOLISM

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For the human risk assessment of many drugs/xenobiotics, bioactivation must be weighed against competing detoxification pathway(s). As a major route for the elimination of many xenobiotics, glucuronidation can be a critical variable (de Morais et al. (1992). *Toxicol.Appl.Pharmacol.* 117: 81–87 and de Marais et al. (1992) *Gastroenterology,* 102: 577–586). We are developing human lymphoblastoid cell lines stably expressing individual isoenzymes of UDP-glucuronosyltransferases(UGTs) to complement commercially-available cell lines expressing human cytochrome P450's. The cDNAs for UGT01*6(phenol-form), UGT2B10, and UGT(bilirubin) have been cloned and sequenced, and cell lines stably expressing UGT01*6 and UGT2B10 have been partially characterized. We tested the potential of this *in vitro* system for mechanistic studies using parachloro-meta-xylenol (PCMX), a topical antimicrobial known to be both bioactivated and glucuronidated (ISSX Proceedings, ABST 72). As assessed by several morphological criteria, a dose-dependent decrease in toxicity was observed in the lymphoblastoid cell line containing UGT01*6, compared to both the control and UGT2B10-transfected cell lines. A dose-dependent increase in toxicity was observed in the CYP2E1 cell line. We conclude that CYP2E1 may mediate the formation of oxidative metabolites of PCMX, but that glucuronidation is an efficient detoxification mechanism *in situ*, as it is *in vitro* and *in vivo*. The lymphoblastoid cDNA expression system may be useful for mechanistic studies of human drug metabolism and risk assessment.

14 DMPS IN HUMANS IS BOUND TO PLASMA ALBUMIN AND CYCLIC POLYMERIC DISULFIDES OF DMPS ARE FOUND IN URINE

R M Maiorino, Z F Xu, H V Aposhian. *Department of Molecular and Cellular Biology, University of Arizona, Tucson, AZ*

The binding of 2,3-dimercapto-1-propanesulfonate (DMPS) to plasma proteins was determined in 3 normal adults after p.o. administration of 300 mg of DMPS. By 5 hr after DMPS administration, 62.5% of the total DMPS in the plasma was bound to plasma proteins via disulfide linkage mainly to albumin. The remainder in the plasma consisted of non-protein bound DMPS disulfides (36.6%) and unaltered DMPS (0.9%). The DMPS bound to plasma protein was a DMPS-albumin complex (84%) and a higher molecular weight protein complex (16%), most likely aggregates of albumin. The isolated DMPS-albumin complex was converted to unaltered DMPS by treatment with dithiothreitol, which indicated that DMPS was bound to albumin via disulfide linkage. DMPS was biotransformed rapidly and extensively to disulfide forms. By 9 hr after DMPS administration, 10% of the urinary total DMPS was unaltered DMPS and 90% was altered DMPS. Altered DMPS was converted to DMPS by treatment with dithiothreitol, which indicated that the altered DMPS was disulfides. The DMPS metabolites in 2-4 hr urine were isolated by TLC, extraction and HPLC. The DMPS disulfides consisted of cyclic polymeric DMPS disulfides (97%), a DMPS-cysteine (1:2) mixed disulfide (2.5%) and an acyclic DMPS disulfide (0.5%). There were two cyclic polymeric DMPS disulfides based on TLC separation, one was 91.5% and the other was 5.5% of the DMPS disulfides. (Supported in part by NIEHS Grant #ES-03356.)

15 XENOBIOTIC METABOLISM BY HUMAN LIVER MICROSOMES: POTENTIAL PITFALLS DUE TO LOW AFFINITY, HIGH CAPACITY METABOLISM BY CYP3A ENZYMES

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Two techniques are commonly used to identify which human P450 enzyme is responsible for metabolizing a drug. One involves the use of human liver microsomes, the other involves the use of cDNA-expressed P450 enzymes. The latter technique has tended to underestimate the catalytic capabilities of CYP3A4, which is known to play a major role in drug metabolism. Recent studies in our laboratory suggest that the former technique can potentially overestimate the role of CYP3A enzymes in drug metabolism. This can occur when human liver microsomes are incubated with drugs at concentrations that far exceed those encountered clinically. Under such conditions, drug metabolism can be dominated by the CYP3A enzymes, which can act as low affinity, high capacity enzymes, as the following examples illustrate. Based on clinical studies in poor metabolizers of S-mephenytoin, CYP2C19 has been implicated in the metabolism of omeprazole and diazepam. A major role for CYP2C19 in the *in vitro* metabolism of these drugs is apparent only when human liver microsomes are incubated with micromolar substrate concentrations. At these pharmacologically relevant concentrations, CYP3A contributes negligibly to certain pathways of omeprazole and diazepam metabolism. However, at substantially higher concentrations ($<20\ \mu M$), all pathways of omeprazole and diazepam metabolism are dominated by CYP3A enzymes, which overshadow and completely obscure the role played by CYP2C19. The results of these studies underscore the importance of selecting pharmacologically relevant concentrations for *in vitro* studies of drug metabolism. Supported by NIH grants ES03765 and ES07079.

16 EVALUATION OF PRIMARY HUMAN HEPATOCYTES AS AN EXPERIMENTAL MODEL FOR DRUG INTERACTIONS: EFFECTS OF CYP3A4 INDUCERS

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The hepatic cytochromes P450 (CYP) are a superfamily of microsomal hemoproteins that catalyze the oxidation of endogenous and exogenous compounds including many drugs. One of the major mechanisms of drug interactions is drug modification (induction or inhibition) of CYP activity. Because of the significant species difference in CYP isozyme profile, it is difficult to predict human drug interactions using laboratory animals. In our laboratory, we are evaluating the use of primary human hepatocytes for drug interaction studies. Our initial approach is to evaluate the response of human hepatocytes towards CYP inducers, especially CYP3A4 inducers. A reproducible procedure for the induction of CYP3A4 was developed. Primary human hepatocytes were isolated from fresh surgical specimen and cultured for 3–4 days on collagen-coated plastic and then followed by a 1–2 day treatment with known CYP3A4 inducers, including rifampicin, dexamethasone, and phenytoin. The 3–4 day culture period allows the CYP3A4 level to fall to a relatively stable basal level. Induction was monitored via measurement of lidocaine metabolism to monoethylglycinexylidide (MEGX) and of 6-β hydroxylation of testosterone *in situ*, and by Western blot analysis of microsomes. Our results showed that rifampicin and phenytoin were potent inducers, while dexamethasone appeared to be less potent. As of this writing, CYP3A4 induction by rifampicin was observed in 4/4 of the human hepatocyte isolates studied. Our results suggest that human hepatocyte in culture is an appropriate experimental model for the evaluation of CYP3A4-induction potential of drugs and drug candidates.

17 ENHANCEMENT OF CYTOCHROME P450 2E1 (CYP2E1) IN RAT KIDNEY BY CIPROFIBRATE

R C Zangar, K J Woodcroft, R F Novak. *Institute of Chemical Toxicology, Wayne State University, Detroit, MI*

Recent evidence has demonstrated that treatment of primary cultured rat hepatocytes with ciprofibrate (CIPRO), a peroxisomal proliferator, increases the expression of CYP2E1 mRNA. We examined the effects of CIPRO treatment on the expression of CYP2E1 and CYP4A mRNA and protein in rat kidney. Rats were injected intraperitoneally with 20 mg CIPRO/kg/dy and sacrificed at 18, 48 and 120 hr. Northern and Western blot procedures were used to analyze renal CYP2E1 mRNA and protein levels, respectively. CYP2E1 and CYP4A mRNA levels were increased ≈3- to 4-fold at all time points. Immunodetectable CYP2E1 was increased ≈4- to 6-fold at all time points, while

CYP4A was increased ≈1.5-fold at all time points. The results of this study showed that CIPRO treatment enhanced the expression of renal CYP2E1 mRNA and protein *in vivo* and that maximal expression occurred by 18 hr after treatment. This observation is in contrast to effects observed for most other chemical agents, which increase CYP2E1 protein but not mRNA levels. Supported by NIH center grant ES03656 from the National Institutes of Environmental Health Sciences.

18 DIFFERENTIAL INDUCTION OF CYP2B1, 2B2, 3A1, 1A1 AND 1A2 BY 4-N-ALKYL-METHYLENEDIOXY-BENZENES IN PRIMARY RAT HEPATOCYTE CULTURES

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A primary rat hepatocyte culture system (faithfully reproducing *in vivo* phenobarbital (PB)-inducible and liver-selective gene expression) was used to examine induction of CYP450 gene expression by a series of methylenedioxybenzenes (MDBs; alkyl side-chain lengths 0, 1, 3, 4, 6, 8). Hepatocytes were treated for 24, 48, or 72 hr. with the series of MDBs (0–500 μM). Total RNA and protein from each treatment was evaluated by Northern (slot) and Western blotting. Treatments with MDBs of increasing side-chain length (C_0–C_8) resulted in dose- and structure-dependent increases of CYP2B1, 2B2, 3A1, 1A1, and 1A2 expression. The most effective MDB congeners, C_6 and C_8, were more efficient than PB at an equivalent concentration (100 μM) for induction of CYP2B1, 2B2 and 3A1 gene expression. Levels of MDB-inducible CYP2B1 and 2B2 mRNA were highly correlated with CYP2B1/2 apoprotein levels. The present study reveals potential discrepancies to our previous *in vivo* study (*Arch. Biochem. Biophys.* 277:8, 1990) which suggested a different structure-activity relationship for P450 induction, and non-parallel increases in CYP2B mRNA and apoprotein. These discrepancies may be attributable to differences in pharmacokinetics between the *in vivo* and *in vitro* systems, the hepatocyte model being more representative of potential induction mechanisms. This study reveals a previously unreported induction of CYP3A by MDBs, and suggests similar mechanisms for MDB- and PB-mediated induction of CYP2B1 and 2B2 expression. (ES 05311[1] and GM 32281[2]).

19 XENOBIOTIC-ENHANCED EXPRESSION OF CYTOCHROME P450 2E1 AND 2B IN PRIMARY RAT HEPATOCYTE CULTURE

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CYP2E1 and 2B expression was examined in cultured primary rat hepatocytes, using Western and Northern blot analyses, in response to chemicals known to enhance hepatic 2E1 expression *in vivo*. Hepatocytes were cultured in Vitrogen-coated sealable flasks using modified Chee's medium for 72 hr prior to initiation of treatment. Treatment (24 hr) with 100 mM ethanol (E) or isopropanol (ISO) enhanced expression of 2E1 protein by 3- and 2.3-fold, respectively, while 50 mM acetone (A), pyrazine (PN), or thiazole (TH) resulted in increases of 1.7-, 2.4-, and 3.3-fold, respectively, compared to untreated cells. Corresponding 2E1 mRNA levels were either unchanged (ISO, A, PN) or slightly decreased (E, TH) relative to untreated levels. Xenobiotic treatment also increased 2B protein levels by ≈10-fold (A, ISO, PN) and 18-fold (TH), and was accompanied by increases in 2B mRNA of 9-(TH), ≈14-(A, PN), and 32-(ISO) fold. Pyridine (PYR) treatment of cultured hepatocytes enhanced expression of 2E1 and 2B protein in a concentration- and time-dependent manner. A concentration of 0.2 mM PYR increased 2E1 levels by 1.7-fold, while this concentration had no effect on 2B expression. 2E1 and 2B were increased by ≈2.5-fold by 1 mM PYR, and 2.7- and 3.7-fold, respectively, by 7.5 mM PYR. 2E1 levels were unchanged after 6 hr of treatment with 7.5 mM PYR and were maximal at 24 hr, whereas 2B levels were increased 1.6-, 3-, and 1.4-fold at 6, 12, and 24 hr, respectively, and declined to undetectable levels after 48 hr. In contrast, treatment with 0.1 mM phenobarbital increased 2B protein levels 1.2-, 17-, and 48-fold at 6, 24, and 48 hr. These data demonstrate that 2E1 and 2B are inducible by known 2E1 inducers in cultures of primary rat hepatocytes and parallel the results reported *in vivo*. Supported by NIH Grant ES03656.

20 CORRELATING STYRENE METABOLISM AND DISTRIBUTION WITH HEPATOTOXICITY

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Styrene has been evaluated as a possible human carcinogen. Styrene induces mortality in rats (>1500 ppm) and in mice (250 to 500 ppm), but on repeated exposure mice become tolerant. Biochemical mechanisms of styrene toxicity were examined by comparing its metabolism, distribution, and hepatotoxicity in male F344 rats, B6C3F1 mice, and CD1 mice following single and repeated exposures. Rats and mice were exposed (6 hr) to 125, 250, and 500 ppm [7,8-^{13}C]-styrene, and transferred to metabowls for the collection of urine. Rats and mice were also exposed to styrene at 250 ppm for 6 hr/day for 1 to 5 days. After each 6 hr exposure, liver and/or blood were collected from 3 rats and 3 mice. Severe congestion, centrilobular necrosis, and elevated plasma alanine aminotransferase activity occurred for both mouse strains. Following 0, 2, or 4 days of exposure to styrene, additional rats and mice were exposed (6 hr) to a 250 ppm mixture of [7,8-^{13}C]-styrene (90%) and [7-^{14}C]-styrene (50 μCi/mmol). Urine (0–48 hr), feces, CO_2, and tissues were collected. Rats and CD1 mice eliminate ^{14}C at a faster rate than B6C3F1 mice following a single 250 ppm exposure. After 3 or 5 days of exposure, rats and mice have a similar elimination rate, where total ^{14}C increased over that measured for one exposure. For these dosing regimens, a large percentage of the metabolites excreted by rats (70%) and mice (50%) are formed following conversion of styrene to styrene oxide (SO) and further metabolism via epoxide hydrolase. Metabolism also occurred via conjugation of SO with glutathione for rats (25%), B6C3F1 mice (33%), or CD1 mice (22%). Metabolites formed presumably from addition of water to styrene occurred for both strains of mice (15%–22%). Correlating individual metabolites with liver toxicity is currently under investigation. This work was supported in part by the Styrene Information and Research Center.

21 FORSKOLIN-MEDIATED INDUCTION OF CYP3A1 GENE EXPRESSION IN PRIMARY RAT HEPATOCYTES IS INDEPENDENT OF ADENYLATE CYCLASE ACTIVATION AND INCREASES IN INTRACELLULAR CAMP

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In a previous study we demonstrated that stimulation of intracellular cAMP by classic activators of adenylate cyclase, e.g. glucagon and forskolin, resulted in a dramatic concentration-dependent inhibition of phenobarbital-(PB) inducible CYP2B gene expression in primary rat hepatocyte cultures. In contrast however, forskolin treatment alone resulted in a concentration-dependent induction of CYP3A1 gene expression. The latter gene is prototypically inducible by steroids and PB. In the present study we examined whether the CYP3A1 inductive effect was mediated by forskolin activation of adenylate cyclase. Primary rat hepatocytes were treated with a range of concentrations (0–100 μM) of forskolin, a water-soluble forskolin analog (7β-desacetyl-7β-(N-methylpiperazine)butyl-dihydrochloride), and a 1,9-dideoxy-forskolin analog — the latter an inactive derivative devoid of adenylate cyclase stimulatory activity. Total cellular RNA and protein was isolated for each treatment and evaluated by Northern and Western blot techniques with specific oligonucleotide and antibody probes. Using a specific ELISA assay for assessment of intracellular cAMP levels, forskolin and its water-soluble analog were equipotent in stimulating adenylate cyclase activity. In contrast, forskolin and its inactive 1,9-dideoxy analog were active in inducing CYP3A1 expression in a concentration-dependent manner. Induction potential was compared to other known inducers of CYP3A1, e.g. dexamethasone (Dex) and PB. Forskolin was less potent than Dex but more potent than PB with respect to concentration-effect. As with the inactive 1,9-dideoxy derivative, both Dex and PB were ineffective in stimulating adenylate cyclase. These results demonstrate that CYP3A1-mediated induction by forskolin is independent of adenylate cyclase stimulation, and instead is likely to involve a direct chemical effect of this agent on the CYP3A1 induction pathway. Supported by NIH Grant GM32281.

22 USE OF STEREOLITHOGRAPHY TO CONSTRUCT A THREE-DIMENSIONAL PHYSICAL MODEL OF THE ANTERIOR F344 RAT NASAL PASSAGES

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An anatomically accurate mathematical dosimetry model was developed for the F344 rat nasal passages to predict site-specific response from inhaled xenobiotics (Kimbell et al., Toxicol. Appl. Pharmacol. 121:253–263, 1993). Solid metal casts and hollow acrylic molds of the rat nasal passages were used for comparison with mathematical model design and predictions, but are too small (1.6 cm) for detailed measurements. An enlarged (10x) physical model of the anterior F344 rat nasal passages was constructed using stereolithography, in which a series of layers is created by an ultraviolet laser moving across the surface of a liquid photopolymer bath. Wherever the laser beam scans the surface,

the resin hardens and an accurate reproduction of the layer is produced in solid plastic. The newly formed layer is lowered into the bath and liquid resin flows over the partially completed object. When the next layer is drawn, it adheres to the previous one and the process repeats until the entire object is constructed. Geometric data from a three-dimensional finite element grid (FIDAP, Fluid Dynamics Intl., Evanston, IL), reconstructed from serial step-sections through the anterior nasal passages of a 3-month-old male F344 rat, were scaled up and used as the basis of the stereolithography model. The stereolithography model will be tested for use in water-dye experiments for visualization and measurement of flow and uptake patterns and used to identify exact locations of toxicological interest in the nasal passages. This method can be applied to other anatomical structures for which there are geometric data (such as digitized CAT scans or images from MRI) and has the potential for producing accurate models useful for both experimentation and communication.

23 APPLICATION OF A LESION MAPPING STRATEGY TO STUDIES OF FORMALDEHYDE-INDUCED NASAL CARCINOGENESIS

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A lesion mapping strategy was developed for the analysis of a 2yr formaldehyde (HCHO) pathogenesis study to determine whether site-specific increases in nasal epithelial cell replication, number of epithelial cells in selected target sites, and/or regional HCHO-dosimetry estimates, correlate with sites of induction of HCHO-induced neoplastic responses. Male F-344 rats were exposed by inhalation (6 hr/d, 5 d/wk) to a range of HCHO concentrations (0, 0.7, 2, 6, 10, or 15 ppm) with numerous necropsy time points (1d to 2yr). The sites of origin of nasal neoplasms, and other selected responses to HCHO, were mapped using 30 nasal diagrams (Mery et al., Toxicol. Pathol., in press, 1994) modified for this study. Lesion locators on these diagrams were coded both by anterior/posterior level and selected sites around the airway perimeter within each section. Combination of data from selected groups of lesion locators allowed correlation of sites (anatomically by structure or epithelial type) with lesions, sites of cell proliferation indices, number of epithelial cells within each site, and published information on regional formaldehyde dosimetry (Kimbell et al., Toxicol. Appl. Pharmacol., 121, 1994). For example, a HCHO concentration-related trend in incidence of keratinizing dysplasia was seen in all nasal locations ($p < 0.001$). Site analysis of incidence of keratinizing dysplasia in the lateral meatus showed incidence for nasal levels 4, 5, 6, 7, and 8 of 2%, 4%, 0%, 0%, and 0% for 6 ppm; 3%, 12%, 3%, 1% and 1% for 10 ppm; and 10%, 16%, 16%, 8% and 3% for 15 ppm, indicating both concentration and site relationship. Detailed mapping of nasal responses clearly facilitates regional correlation of dosimetry, target cell population size, and cell replication rate with site-specific incidence of nasal lesions.

24 AN INSTRUMENT FOR DETERMINING NASAL AND LUNG ABSORPTION OF GASES DURING VARIABLE CYCLIC BREATHING

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The absorption of inhaled gases in the nose and lung airways is an important defense for the pulmonary tissue. Knowing how much inhaled gas is removed from the air stream by upper airway absorption is necessary for determining dose for both the pulmonary tissue and the airways themselves. Using a dog model, we have shown the dependence of nasal uptake on the blood/air partition coefficient (*TAP, 109,* 276, 1991). This required development of a complex instrument that included means for control of respiration, sets of solenoids for separate sampling of air during inhalation and exhalation in the trachea and breathing zone, and a respiration monitor to control the solenoids (*FAAT 16,* 81, 1991). Adapting this instrument for use with the rat was desirable because rats are less expensive, easier to obtain than are dogs, and most inhalation toxicology studies are carried out in rodents. However, the low air flows, rapid breathing rates, and constricted anatomy of the rat respiratory tract required major modifications of the original instrument, including reciprocating syringes activated by a small animal respirator for sampling tracheal air during inhalation and exhalation, low-flow, variable-speed pumps to push sampled air through a gas chromatograph, and inline solenoids. The resulting instrument allows accurate determination of gas uptake over a wide range of respiratory parameters.

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25 OBTAINING ACCURATE GEOMETRIC COORDINATES OF NASAL AIRWAYS FOR DOSIMETRY MODELING

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Understanding the effects of toxic agents depends upon knowledge of the dose delivered to the target tissue, but this is often difficult to quantify accurately. This is true for many toxic agents that are inhaled, since time-dependent airflow patterns, mucociliary translocation, and the uptake characteristics of different epithelial types have the potential to influence local xenobiotic mass flow. Computer simulations using three-dimensional airway geometry are being used to study how some of these factors affect the delivered dose. These computer simulations are dependent upon having an accurate reconstruction of the airway geometry. A method was developed to obtain nasal geometric coordinates in a form that facilitates the creation of accurate computer meshes. A tissue specimen (the upper respiratory tract of a rhesus monkey) was embedded in a frozen, contrast-enhancing medium. The frozen block was serially sectioned and photographed at 100 μm intervals. Video images of the photographic negatives were digitized and image analysis was used to obtain geometric coordinates for each nasal passage perimeter. Calibration images and embedded alignment marks were used to scale and align sequential sections. This method improved on previous reported approaches. Undistorted airway geometry was obtained by photographing the cut block face. Automatic thresholding to obtain coordinates during image analysis was made possible by the contrast-enhancing medium. Automatic scaling and alignment of sections was facilitated by the use of calibration images and alignment marks. Computer visualization of the sections shows that the shape of the airways over a 10 cm distance from the nostrils through the larynx was faithfully reproduced. This method is applicable to other regions of the respiratory tract and may have wider applicability to the reconstruction of other sectioned specimens of biological passageways.

26 COMPUTATIONAL FLUID DYNAMIC (CFD) MODELING OF INHALED GAS DOSIMETRY IN THE RAT: USE OF A SATURABLE BOUNDARY AT NASAL AIRWAY WALLS

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Previous simulations of rat nasal airway dosimetry have used a linear boundary condition for transport (flux) of chemical into the airway wall (Kimbell *et al.,* Toxicol. Appl. Pharmacol. 121:253–263, 1993). For some toxicants, such as formaldehyde, disposition in the tissues lining the nasal airway is known to be non-linear with inhaled concentration. In this work, a saturable boundary condition was applied at the walls of a three-dimensional model of the anterior nasal passage of the F344 rat (Kimbell et al., *ibid.*). CFD simulations were carried out using FIDAP (Fluid Dynamics International, Evanston, IL) on a 'result-independent' mesh, for a constant inspiratory flow rate of 600 ml/min. A FIDAP user subroutine was written to apply a boundary condition with the Michaelis-Menten form:

$$\text{flux} = V_{max} \times \text{Conc.}/K_m + \text{Conc.}$$

Simulated flux at the airway wall was calculated for a series of increasing concentrations of an arbitrary inhaled toxicant, corresponding to the cases of less than, equal to, and greater than K_m. As the inhaled concentration was increased from concentrations well below K_m to concentrations saturating the system, gas uptake into the airway wall was observed to increase in the expected non-linear manner, finally reaching a maximum at concentrations well above K_m. This result confirmed the capability of FIDAP to implement a non-linear boundary condition in the rat nasal mesh. This approach is being extended to produce physiologically-realistic descriptions of formaldehyde disposition in the airway wall.

27 A PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODEL FOR NASAL UPTAKE AND METABOLISM OF ACETALDEHYDE VAPOR

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The aim of the current study was to develop a PBPK model for uptake and metabolism of inspired acetaldehyde in the nose of the F344 rat. Model predictions were compared to uptake data previously obtained in this laboratory (TAP 114: 140–146, 1992). The model is based on the approach previously used for non-reactive vapors (TAP 123: 120–129, 1993) and, in the current study,

included terms for: 1) non-uniform nasal ventilation patterns, 2) diffusion of vapor through nasal tissues, 3) non-uniform distribution of nasal metabolic capacity (for both the high and low affinity forms of aldehyde dehydrogenase, AIDH), and 4) direct reactivity with and potential depletion of tissue substrates. Uptake data obtained a 1, 10, 100 or 1000 ppm could be reasonably described by this model. Model predictions suggest that the high affinity AIDH pathway is saturated at inspired concentrations of about 10 ppm, however the low affinity pathway is not saturated even at inspired concentrations of 1000 ppm. Predicted free (non reacted) acetaldehyde concentrations in respiratory and olfactory epithelial tissues were not linearly related to inspired concentration, rather, tissue concentrations at 1 ppm were predicted to be many-fold less than those estimated by linear extrapolation of the tissue concentrations predicted for 1000 ppm. The predictions of this model may aid in mechanistic studies on acetaldehyde-induced nasal carcinogenesis. *Supported by R0103676 from NIEHS.*

28 EFFECTS OF M-XYLENE INHALATION ON CYTOCHROME P450 ISOZYMES IN RAT NASAL MUCOSA

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Previous studies of *m*-xylene inhalation demonstrated selective inhibition of lung microsomal cytochrome P450 isozymes without alteration of liver isozymes. Benzyloxyresorufin dealkylase (CYP 2B1;BROD) activity was decreased in the lung while ethoxyresorufin dealkylase (CYP 1A1; EROD) activity was unaltered. The benzo(a)-pyrene metabolism ratio [CYP 2B1(detoxication)/CYP 1A1(toxication)] was also decreased in the lung after *m*-xylene. The present study examined the effects of *m*-xylene inhalation (300 ppm; 6h) on cytochrome P450 isozymes but this time in nasal mucosa microsomes. CYP 2B1 and CYP 1A1 activities were both decreased following solvent exposure. CYP 1A2 activity was also decreased significantly after *m*-xylene inhalation. Decreases in both 2B1 and 1A1 activity would result in an overall decrease in BaP metabolism. The BaP detoxication/ toxication ratio (2B1/1A1) was increased in mucosa indicating a shift towards detoxication. A decrease in CYP 1A2 activity could result in decreased metabolism of heterocyclic amines which would result in fewer carcinogenic metabolites being produced in the mucosa. A fourth isozyme CYP 2E1, was also inhibited in the mucosa. CYP 2E1 is responsible for the metabolism of several compounds including NDMA (nitrosodimethylamine) and benzene. Inhibition of 2E1 would also result in decreased formation of reactive metabolites of these compounds in the mucosa. All isozymes examined were inhibited suggesting a greater sensitivity of mucosa to low levels of solvent as compared to the lung, which in the case of the carcinogens above would result in decreased formation of reactive metabolites.

29 METABOLISM OF VINYL ACETATE BY HUMAN NASAL TISSUES USING A MINI VAPOR UPTAKE TECHNIQUE

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Vinyl acetate (VA) is used in the manufacture of polymers for the paint, adhesives and paper board industries. Chronic inhalation bioassays show that VA causes nasal tumors in rats and non-neoplastic nasal lesions in mice. Several lines of evidence suggest that nasal metabolism of VA is a critical step in the mechanism of action. Development of physiological models to describe VA metabolism require metabolic rate constants that are reflective of the *in vivo* situation. An *in vitro* mini vapor uptake system was developed for use with freshly isolated intact human nasal respiratory and olfactory tissue to measure critical metabolic constants describing VA metabolism. Loss of VA from and the appearance of acetaldehyde into the headspace were modeled, using Simu-Solv, as a one compartment description containing removal terms for VA (carboxylesterase) and acetaldehyde (aldehyde dehydrogenase). Preliminary results showed that carboxylesterase V_{max}/K_m was 4–5 fold lower in nasal tissues of humans relative to that of rats. Analysis of data from olfactory tissue of rats suggests VA vapor is extensively metabolized in the superficial sustentacular cell layer with very little penetration to the Bowman's glands. This observation explains discrepancies between results obtained here and those obtained using homogenates of olfactory tissue. In the latter, esterases in Bowman's glands of olfactory tissue become available to the substrate yielding V_{max} values that are remarkably greater than those obtained with the intact tissue procedure. This work was supported in part by the Vinyl Acetate Toxicology Group.

30 ATROPHIC RHINITIS INDUCED BY A 1-MONTH EXPOSURE OF F344/N RATS TO 0.25 & 0.5 PPM OZONE

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Repeated exposures to ozone induce nasal epithelial lesions in laboratory animals. The effects of ozone exposure on subepithelial tissues (i.e., lamina propria and bone) of nasal turbinates have not been thoroughly examined. The purpose of this study was to determine the morphological changes of the lamina propria and bone in rat maxilloturbinates following a 1-month exposure to ambient levels of ozone. Eighteen Male F334/N rats were exposed to either 0, 0.25, or 0.5 ppm ozone, 8 h/day, 7 d/wk, at night, for 1-month. Rats were sacrificed 12 h post-exposure, and the nasal airways were prepared for light microscopic analysis. Amounts of the three major tissue components (i.e., surface epithelium, lamina propria, and bone) were determined using image analysis and standard morphometric techniques. Rats exposed to 0.5 ppm, but not 0.25 ppm, ozone displayed significant changes when compared to controls. While the total turbinate area remained unchanged, there was a 68% increase in the amount of surface epithelium (hyperplasia) and a 30% loss in the amount of turbinate bone (atrophy) of rats exposed to 0.5 ppm ozone. Although there was no significant difference in the amount of lamina propria between 0.5 ppm ozone-exposed and control rats, exposed rats did exhibit a 58% reduction in the lumenal area of blood vessels. In addition, there was a thickening of blood vessel walls and a mixed inflammatory cell infiltrate in the lamina propria of these ozone-exposed rats. The ozone-induced changes in turbinate structure detected in this study suggest that the normal function of nasal airways may be chronically altered when subjected to repeated exposures to ambient concentrations of ozone. Research supported by NHLBI Grant No. R01 HL51712-01 and the U.S. DOE/OHER under Contract No. DE-AC04-76EV01013.

31 A 14-DAY REPEATED DOSE INHALATION TOXICITY STUDY OF 1,2-BIS (TRIMETHOXYSILYL) ETHANE IN ALBINO RATS

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1,2-bis (trimethoxysilyl) ethane has been shown to be very toxic via inhalation. The 4-hour LC_{50} value is 2.4 ppm (unpublished data). To assess the repeated dose inhalation toxicity of 1,2-bis (trimethoxysilyl) ethane, Fischer 344 rats were exposed to target concentrations of 0, 50, 100, and 200 ppb for 6 hours a day, 5 days/week for 14 days. Statistically significant decreases in body weights were observed in males exposed to 100 and 200 ppb and females exposed to 200 ppb. Microscopic examination revealed test article-related changes limited to respiratory tract and occurred in all rats exposed to 50, 100, or 200 ppb of 1,2-bis (trimethoxysilyl) ethane. The nares, nasal cavity, and pharynx were the most common sites of lesions with the trachea and larynx only infrequently affected. Squamous metaplasia and acute inflammation of the nasal cavity were common findings in animals exposed to 200 ppb and also occurred, with somewhat reduced incidence and severity, in male and female rats exposed to 50 and 100 ppb. An increased height and prominence of the mucus goblet cells with formation of microcysts was observed in all sections of the nasal cavity of rats exposed to 50 and 100 ppb. Other test article related changes included erosions/ulcers, suppurative exudate, and chronic inflammations.

32 COMPARISON OF 125-IODODEOXYURIDINE (^{125}I) AND ^{3}H-THYMIDINE FOR ASSESSING CELL PROLIFERATION IN THE MURINE LOCAL LYMPH NODE ASSAY

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The murine local lymph node assay (LLNA) evaluates the sensitization potential of chemicals by assessing the cell proliferation occurring in the auricular lymph nodes following topical application of a chemical to the ears. Traditionally, ^{3}H-thymidine (^{3}H) is used to measure proliferation. Due to it's long half-life and resulting expense of disposing of ^{3}H waste, the usefulness, reliability, and sensitivity of 125-Iododeoxyuridine (^{125}I) (half-life of 60 days) was evaluated in the LLNA. An optimal response was obtained using the following: exposure on the dorsum of both ears for 3 days; iv injection of 2 μCi/mouse ^{125}I 3 days later; removal of nodes after 5 hr. Female CBA/J mice (n = 5) were exposed to vehicle or methyl salicylate (MS), potassium dichromate (PD), or dinitrochlorobenzene (DNCB); injected iv with 20 μCi ^{3}H or 2 μCi ^{125}I; and proliferation in the draining pooled nodes from individual mice assessed. A positive response was considered to be either a stimulation index (SI) $\geq$ 3 or statistical significance. For the nonsensitizer MS, neither a SI $\geq$ 3 nor statistical significance was detected using ^{125}I or ^{3}H. Both isotopes detected a statisti-

cally significant effect at 0.5% and 0.25% PD, while ^{125}I also detected statistical significance at 0.1%. A SI $\geq$ 3 was observed by both isotopes at 0.5% PD, while ^{125}I detected a SI $\geq$ 3 at 0.25% PD. Significance was detected for ^{3}H at 0.025, 0.05, 0.1, and 0.25% DNCB and at 0.1 and 0.25% DNCB for ^{125}I. A SI $\geq$ 3 was observed at 0.05, 0.1, and 0.25% DNCB for ^{3}H and at 0.1 and 0.25% DNCB for ^{125}I. ^{125}I may prove to be a suitable cost-effective alternative to ^{3}H for assessing cell proliferation in the LLNA.

33 INFLUENCE OF VEHICLE ON MURINE LYMPH NODE ACTIVATION FOLLOWING SKIN SENSITIZATION

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Effective skin sensitization is dependent upon immune activation of lymph nodes draining the site of exposure; a phenomenon that forms the basis of the local lymph node assay, a method for the prospective identification of contact allergens. In the present study, the influence of vehicle formulation on the vigor of lymph node proliferative responses to 2,4-dinitrochlorobenzene (DNCB) has been examined. Mice (BALB/c strain) were exposed topically to 0.5% DNCB dissolved in either acetone or propylene glycol (PG). A substantially greater proliferative response by lymph node cells was induced by DNCB in acetone. The observed differences were not attributable to variations in the numbers of immunostimulatory dendritic cells accumulating in the draining nodes following sensitization. In parallel studies, the absorption and cutaneous disposition of DNCB dissolved in acetone or PG was measured *in vitro* using static diffusion cells and full thickness mouse skin. Although flux of DNCB through the skin was comparable with both vehicles at 24 hours, the absorption rate of the allergen during the first 4 hours of exposure was significantly faster when acetone was used as the vehicle. Localization of DNCB in the epidermis was greater with acetone at both 4 and 24 hours. These data indicate that the sensitization potential of contact allergens is influenced significantly by the nature of the vehicle used, possibly due to consequential effects on chemical absorption and disposition.

34 EFFECTS OF DOSE AND TIME ON THE PHENOTYPIC CHANGES OBSERVED IN DRAINING LYMPH NODES OF MICE FOLLOWING TOPICAL EXPOSURE TO ALLERGENS OR IRRITANTS

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The murine local lymph node assay (LLNA) uses in vivo cellular proliferation in draining lymph nodes (DLN) following topical exposure to chemicals for assessing contact sensitization potential. However, chemically induced cellular proliferation has been observed with some irritants. In an effort to distinguish allergen from irritant induced proliferation, we examined the phenotypic changes which occurred in the DLN of mice following topical exposure to test chemicals. Test materials examined included the allergens, 1-chloro-2,4-dinitrobenzene(DNCB) and 1-chloro-2,4,6-trinitrobenzene (TNCB); and the irritants, benzalkonium chloride (BC) and nonanoic acid (NONA). Mice were treated on the ears for three consecutive days with either allergen, irritant or vehicle. Cell number and phenotype were determined at various time points after the last application of test material. By 48 h post treatment, differences in cell numbers and %B220^{+} cells were observed between test and vehicle control groups as well as between allergen and irritant treated mice. In general, mice treated with allergen had a larger increase in the percentage of B220^{+} cells relative to vehicle control than irritants which demonstrated only a slight increase (NONA) or a decrease (BC) over control. In dose response studies which examined the changes at 48 hours only, mice treated with either allergen or irritant demonstrated a concentration related increase in cell number in the DLN. However, only allergen treated mice showed increasing percentages of B220^{+} cells with increasing concentration. These preliminary results suggest that 48 hours post-treatment is an appropriate time point for examining phenotypic differences in the cells of the DLN of allergen and irritant treated mice.

35 DETECTION OF CONTACT SENSITIZATION BY LARGE MOLECULAR WEIGHT PROTEINS WITH THE MURINE LOCAL LYMPH NODE ASSAY

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Female CBA/j mice (5/group) were dosed on each side of each ear once each day for four consecutive days. Dosing solutions included carriers (water, acetone, or DMSO), ovalbumin, two α-amylase and two lipase preparations. Ovalbumin was suspended in DMSO or water. The α-amylases (75 units/g from *Bacillus subtilis;* amylase 1, and 314,000 units/g from *B. subtilis;* amylase 2) were suspended in DMSO or water. The lipases (Type XI from *Rhizopus arrhizus;* Lipase 1, and Type XIII from *Pseudomonas* sp.; Lipase 2) were suspended in DMSO or acetone. On Day 5, the mice received 20 μCi of ^{3}H-thymidine via intravenous injection 5 hours before the auricular lymph nodes were removed and incorporated radioactivity determined. Ovalbumin suspended in DMSO (2.5% wt/vol) resulted in a significant increase in cellular proliferation as measured by uptake of ^{3}H-thymidine. No significant increase was found at concentrations of 0.25 or 0.025%. No significant cellular proliferation was detected with either lipase preparation suspended in DMSO or acetone at concentrations of 0.1 or 10%. Amylase 2 was positive in the local lymph node assay using both water and DMSO as carriers at the 10% concentration level. No significant changes were seen at a dose concentration of 0.1%. Amylase 1 with a specific activity of only 75 units/g was not positive under the same conditions. These data suggest that the use of appropriate carriers may allow the local lymph node assay to detect certain large MW protein sensitizers.

36 DIFFERENTIAL STIMULATION OF CUTANEOUS IL-6 PRODUCTION BY CHEMICAL ALLERGENS AND IRRITANTS

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Epidermal cells produce constitutively, or can be stimulated to produce, a variety of cytokines including interleukins, colony-stimulating factors and growth factors. It has been proposed that the cytokine products of epidermal cells play important roles in the induction of both contact sensitization and skin irritation. In the present study we have examined the influence of topical exposure to oxazolone (a contact allergen) and to sodium lauryl sulfate (SLS; a skin irritant) on the production by murine skin of interleukin 6 (IL-6), a cytokine expressed constitutively by Langerhans cells and, following stimulation, by keratinocytes. Mice (BALB/c strain) were exposed to chemical, or to an equal volume of vehicle alone, on the dorsum of both ears. At various times thereafter mice were sacrificed, ears removed and homogenates of the dorsal surfaces prepared. The concentration of IL-6 was measured by enzyme-linked immunosorbent assay (ELISA). Skin prepared from naive mice contained low levels of constitutive cytokine (75–125 pg/ear). Treatment of mice with vehicles (4:1 acetone:olive oil; AOO or water) did not at any time influence the IL-6 content of the skin. However, treatment with oxazolone (1% in AOO) induced a rapid and sustained up-regulation of cutaneous IL-6. Increased production of IL-6 was detectable within 2 hours of treatment, was maximal at 4 hours, reaching between 450 and 600 pg/ear, and remained elevated for up to 24 hours. In contrast, exposure of mice to the non-sensitizing skin irritant SLS (30% in water) failed over the same period to influence IL-6 expression. These data suggest that chemicals differ with respect to the nature and/or tempo of epidermal cytokine induction following topical administration. It is proposed that the rapid stimulation of epidermal IL-6 production may provide a selective marker for skin allergens.

37 INTERFERON-γ PRODUCTION: A MOLECULAR READOUT FOR THE LOCAL LYMPH NODE ASSAY

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The murine local lymph node assay (LLNA) identifies contact allergens as a function of proliferative responses induced in lymph nodes draining the site of topical application. The purpose of the present study was to investigate opportunities for the development of a molecular, rather than cellular endpoint for the LLNA; interferon γ (IFN-γ) production by allergen-activated lymph node cells (LNC) *in vitro.* Five days following topical exposure of mice to a range of skin sensitizing and non-sensitizing chemicals, single cell suspensions of draining (auricular) LNC were prepared and cultured. LNC proliferation was measured by 24 hour thymidine incorporation and supernatants derived from parallel cultures after 72 hours were analyzed for IFN-γ by ELISA. The production of IFN-γ correlated closely with LNC proliferative activity, with only those chemicals known to cause skin sensitization eliciting measurable (>130 pg/ml) IFN-γ expression. In contrast, control LNC isolated from vehicle-treated mice or from animals exposed to non-sensitizing skin irritants, including sodium lauryl sulfate, failed to elaborate detectable levels of IFN-γ. These data suggest that the production of IFN-γ by allergen-activated LNC is a selective marker of lymph node activation and may provide an alternative, molecular, endpoint for the LLNA.

38 DIVERGENT CYTOKINE PRODUCTION CHARACTERIZES IMMUNE RESPONSES TO CONTACT AND RESPIRATORY CHEMICAL ALLERGENS

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We have reported previously that, following topical exposure of mice, the contact allergen oxazolone and the respiratory allergen trimellitic anhydride (TMA) elicit divergent antibody responses consistent with the preferential stimulation of T_{H1}- and T_{H2}-type immune responses, respectively. We have now examined the potential of allergen-activated draining lymph node cells (LNC) to produce the T_{H1} cytokine interferon γ (IFN-γ) and the T_{H2} cytokines interleukins 4 and 10 (IL-4 and IL-10). Primary immunization resulted in spontaneous IFN-γ production by both oxazolone- and TMA-activated LNC and restimulation *in vitro* induced in each case substantial IL-4 and IL-10 secretion. Following more chronic exposure, however, IFN-γ production by TMA-stimulated LNC was reduced while the potential to express IL-4 and IL-10 was enhanced markedly. In contrast, oxazolone-activated LNC retained the capacity to secrete IFN-γ but IL-4 and IL-10 production was inhibited. Thus, selective cytokine production, consistent with preferential T_H subset activation, becomes apparent after temporal evolution of the immune response. These data suggest that it may be possible to distinguish between different classes of chemical allergen as a function of induced changes in the pattern of cytokine expression as the immune response matures.

39 ROLE OF INTERFERON-γ IN CUTANEOUS DELAYED TYPE HYPERSENSITIVITY IN MICE

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Sponsor: J S Bus

The role of interferon γ (IFNγ) in contact hypersensitivity induced by the haptens oxazolone and 2,4,6 trinitrochlorobenzene (TNCB) was investigated in mice with a targeted disruption of the IFNγ receptor (IFNγ-R−/−). The 24 hours ear-swelling response was only slightly reduced in IFNγ-R−/− mice. The dermal mononuclear infiltrate and epidermal microabscesses, however, were significantly reduced in the mutant mice. Immunofluorescence analysis revealed reduced expression of intercellular adhesion molecule-1 (ICAM1) and MHC class I in IFNγ-R−/− mice as compared to wild-type mice.It is concluded that oxazolone and TNCB induced hypersensitivity is partially dependent on a functional IFNγ system. While the cutaneous oedema is not significantly diminished, the mononuclear cell infiltration and epidermal microabscesses are clearly reduced. The reduction ICAM1 and class I antigen expression in IFNg-R−/− mice is likely responsible for reduced monocytes and T and B cells recruitment.

40 USE OF THE IMMUNOGLOBULIN RESPONSE TO DISTINGUISH RESPIRATORY FROM CONTACT SENSITIZING CHEMICALS

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Certain industrial chemicals, such as toluene diisocyanate (TDI) and trimellitic anhydride (TMA), are known to cause predominantly respiratory sensitivity. Others, such as oxazalone (oxaz) and dinitrochlorobenzene (DNCB), cause predominantly skin allergy. The reason for this apparent organ-directed response is unknown. We have reported development of murine models for both pulmonary and dermal sensitivity. We report here an investigation of the value of serologic determinations in distinguishing pulmonary from contact sensitizing chemicals. BALB/c mice were exposed epicutaneously to 1% solutions of either a respiratory sensitizer (TDI, TMA, or ampicillin), or a contact sensitizer (oxaz, DNCB, or dicyclohexylmethane 4,4′-diisocyanate [HMDI]) each dissolved in acetone-olive oil (4:1). Blood was obtained from mice on day 14. Total IgE was determined using a sandwich ELISA. The geometric mean total IgE for the naive animals was 3.1 μg/ml. Other values were: 2.7 (vehicle-treated controls), 1.4 (DNCB), 1.6 (TDI), 2.2 (TMA), 2.5 (ampicillin), 2.8 (HMDI), and 3.2 (oxaz). Values were not significantly different from those of the vehicle-treated control group. A series of hapten-modified mouse serum albumin (MSA) test antigens was prepared to evaluate the presence of specific antibodies in the sera. The average hapten adduction of the proteins was 25 mol hapten/mol MSA (range 16–33). ELISA indicated no hapten-specific IgE in any group. By contrast, hapten-specific IgG was detected. Titers to two of the respiratory sensitizing chemicals, TDI and TMA, averaged 355, and 80, respectively. No IgG was detected to ampicillin. Similarly, no hapten-specific IgG was detected to the contact sensitizers, DNCB or HMDI. However, exposure to the contact sensitizer, oxazalone, resulted in all animals producing hapten-specific IgG, with the mean titer being 320. These results indicate that, under the exposure conditions employed, immunologic responses to respiratory sensitizers did not differ from contact sensitizers. Supported by NIH ES05651.

41 IMPAIRED DELAYED-TYPE HYPERSENSITIVITY IN MICE WITH A TARGETED DISRUPTION OF THE Rel/NF-κB FAMILY MEMBER RelB

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RelB, a member of the Rel/NF-κB family of transcription factors, has been implicated in the constitutive expression of κB-regulated genes in lymphoid tissues. Mice homozygous for the disrupted *rel*B locus, *rel*B$^{-/-}$, have severe multiorgan inflammation and hematopoietic abnormalities. We examined the response of *rel*B$^{-/-}$ mice to a delayed-type hypersensitivity reaction in the skin. FITC (0.5% in 1:1 acetone/dibutyl phthalate) solution was applied epicutaneously (EC) to the shaved abdomen of 6–8 week old, wild-type and *rel*B$^{-/-}$ mice. After 6 days (afferent phase), the right ear thickness was measured. FITC was then applied to the ear, and ear thickness and histopathologic analysis was performed after 24 hours (elicitation phase). Naive wild-type (control) mice that were not sensitized had a very mild increase in ear thickness and minimal histopathologic changes, whereas sensitized wild-type animals responded strongly to the exogenous antigen with ear lesions characterized by moderate to marked dermal edema, a moderate to marked cellular infiltrate, and multifocal microabscesses consisting of discrete neutrophil aggregates within the epidermis. In contrast, the ear swelling, and the edema and cellular infiltrate were markedly reduced in sensitized *rel*B$^{-/-}$ animals, although there was pronounced interanimal variation. The results of this study indicate that *rel*B$^{-/-}$ mice have impaired cellular immunity.

42 RENAL TUMORS IN MALE B6C3F1 MICE EXPOSED TO LEAD ACETATE DURING GESTATION AND LACTATION OCCUR WITH MINIMAL CHRONIC NEPHROPATHY

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Lead is a high priority hazardous substance in humans and a renal carcinogen in adult rodents. This study assessed the carcinogenic potential of gestational/lactational lead exposure in B6C3F1 mice. Effects of a renal tumor promoter (Barbital, BB) on lead-induced lesions were also studied. Pregnant female C57BL/6N mice (10 per group) previously bred with C3H/HeN males were given lead acetate (0, 500, 750 and 1000 ppm Pb) *ad libitum* in the drinking water starting on gestation day 12 and continuing to 4 wks post-partum. Offspring were then weaned and divided into same sex groups of 25 and observed for 72 wks. Other groups received lead and then continuous BB (500 ppm) *ad libitum* in the drinking water from weaning onward. To date only male mice are sufficiently analyzed to allow reporting. In controls (0 Pb/0 BB) renal proliferative lesions (RPLs; defined as atypical tubular hyperplasia or tumor) occurred only rarely (1 lesion bearing mouse/23 mice examined, 4%) and did not include tumors. RPLs increased in a dose-related fashion with lead exposure (500 Pb/0 BB, 3/20, 15%; 750 Pb/0 BB, 5/22, 23%; 1000 Pb/0 BB, 10/24, 42%) and were often multiple. All lead treated groups had renal tumors, including carcinoma, but these were most common at the highest dose (1000 Pb/0 BB, 5/24). Lead-induced renal tumors arose in the absence of the extensive chronic nephropathy typically seen with lead carcinogenesis in rats exposed as adults. BB exposure had no effect on RPL incidence (e.g., 1000 Pb/500 BB, 8/25, 32%). Thus lead exposure during the gestational/lactational period has carcinogenic potential.

43 TISSUE Pb DISTRIBUTION IN RATS SUBCHRONICALLY EXPOSED TO LOW LEVELS OF Pb

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Long-Evans hooded (21-day-old, male) rats were exposed to 0, 25, 50 and 500 ppm Pb as lead acetate in deionized distilled water for 90 days. At the end of the exposure period, rats were sacrificed and kidney, liver, heart, and various brain regions; and blood samples were collected for the analysis of Pb.

Brain regions collected included frontal cortex, striatum, nucleus accumbens, hypothalamus, hippocampus and brainstem, while the kidneys were analyzed as medulla and cortex. The average total intake of Pb during 90 day exposure period for the 25, 50 and 500 ppm Pb treated groups were found to be 50, 107 and 1020 mg/rat, respectively; which resulted in the blood Pb concentrations of 13, 15 and 49 μg/dl. respectively. The control group drinking sodium acetate for the same exposure period showed blood Pb levels of 4 μg/dl. Analyses of the data on tissue Pb distribution revealed that the order of Pb accumulation in various tissues was kidney > liver > brain > heart. Further, a dose-dependent increase in lead accumulation with exposure was found in liver and kidney tissues. Nucleus accumbens exhibited significantly higher levels of Pb accumulation as compared to other brain regions. There were no significant differences in Pb accumulation among the other the brain regions studied. Although, there were no significant differences in kidney cortex and medulla regions at the two lower exposure levels (25 and 50 ppm Pb), Pb accumulation in medulla was found to be significantly higher as compared to cortex in 500 ppm Pb exposed rats. Supported by ATSDR/MHPF Grant No. U50/ATU 398948-02.

44 LEAD INHIBITS VITAMIN-D_3-INDUCED Ca^{2+} TRANSIENTS AND OSCILLATIONS IN RAT OTEOBLASTS ANALYZED BY DIGITAL IMAGING FLUORESCENCE MICROSCOPY

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The hormonal form of vitamin-D_3, 1α,25-dihydroxyvitamin-D_3 (i.e., VD_3), exhibits both genomic (transcriptional activation) and non-genomic (signal transduction) mechanisms of action. We have begun to characterize the rapid, non-genomic influence of VD_3 on $[Ca^{2+}]_i$ in rat osteoblast-like osteosarcoma cells (ROS 17/2.8) at the single cell level by using the fluorescent Ca^{2+} indicator dye Indo-1 and digital imaging fluorescence microscopy. Under optimal Indo-1/AM loading conditions, established empirically, ROS cells exhibited a mean basal $[Ca^{2+}]_i$ of 60 nM and responded maximally to ionomycin. Physiological concentrations of VD_3 (0.12–12 nM) induced transient Ca^{2+} spikes (range Δ40 nM to Δ120 nM) in about 50% of ROS cells within 20 to 40 seconds after hormone stimulation. Some cells also displayed periodic, transient Ca^{2+} spikes (oscillations). The VD_3-induced Ca^{2+} spiking activity was heterogeneous within a cell population. Preincubation of ROS cells with 10 or 25 μM Pb-glutamate for 24 hrs prior to Indo-1/AM loading and VD_3 stimulation markedly blunted VD_3-induced Ca^{2+} spikes. Pb-exposed cells did exhibit gradual increases in fluorescence upon VD_3 stimulation, but these increases clearly differed with respect to amplitude and duration from the Ca^{2+} spikes stimulated by VD_3 treatment, alone. Pb had no effect on the $[Ca^{2+}]_i$ elevations induced by 3 μM ionomycin or by 2 μM thapsigargin, indicating that Pb did not interfere with, or quench, intracellular Ca^{2+}-Indo-1 fluorescence. Although a functional role for the non-genomic effects of VD_3 in osteoblasts has not been clearly established, alteration of the Ca^{2+} messenger system by Pb has been linked to its mechanism of toxicity. We intend to further characterize the mechanisms of VD_3-induced Ca^{2+} oscillations and their inhibition by Pb, and to integrate the effects of Pb on the non-genomic and genomic mechanisms of VD_3 action. Supported in part by ES04040.

45 LEAD INDUCED RISE IN INTRACELLULAR FREE CALCIUM ($[Ca^{2+}]_i$) IS MEDIATED BY PROTEIN KINASE C (PKC)

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Lead characteristically perturbs processes linked to calcium and the calcium messenger system. The PKC regulates Ca^{2+} fluxes and responds to the local distribution of Ca^{2+}. This study was undertaken to determine the role of PKC in the Pb^{2+} induced rise of $[Ca^{2+}]_i$. The $[Ca^{2+}]_i$ in ROS 17/2.8 cells was measured using the divalent cation indicator, 1,2-bis(2-amino-5-fluorophenoxy) ethane N,N,N′,N′-tetraacetic acid (5F-BAPTA) and ^{19}F NMR. Cells were monitored for 40 min to determine baseline $[Ca^{2+}]_i$ and then treated as noted for 40 min in a flow through NMR tube apparatus during which $[Ca^{2+}]_i$ was measured. Treatment with 10 μM phorbol 12-myristate 13-acetate (PMA), a known, direct activator of PKC, produced a rise in $[Ca^{2+}]_i$ from a baseline of 125 nM to 210 nM. Pb^{2+} treatment at 1 and 5 μM produced a rise in $[Ca^{2+}]_i$ to 170 nM and 230 nM, respectively. Pretreatment with calphostin C, a potent and specific inhibitor of PKC activation, produced no change in $[Ca^{2+}]_i$ but prevented any significant rise in $[Ca^{2+}]_i$ in response to Pb^{2+} treatment. These data indicate that PKC activation elevates $[Ca^{2+}]_i$, and Pb^{2+} treatment elevates $[Ca^{2+}]_i$, while inhibition of PKC activation prevents the Pb induced rise in $[Ca^{2+}]_i$. It follows, therefore, that the Pb^{2+} induced elevation in $[Ca^{2+}]_i$ is mediated through the activation of PKC.

46 LEAD ACTIVATES PROTEIN KINASE C (PKC) IN RAT OSTEOSARCOMA (ROS 17/2.8) CELLS

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The Ca^{2+}/phospholipid-dependent kinase, PKC, has been identified as a high affinity intracellular target for lead in brain tissue. In this study, we sought to identify the sensitivity of PKC as a potential target in ROS 17/2.8 cells. We measured the Pb^{2+}-dependent activation of phosphatidylserine/dioleindependent incorporation of ^{32}P from ATP into histone and endogenous TCA precipitable proteins in the 100,000 × g supernatant from homogenized ROS 17/2.8 cells. The specific free concentrations of both Pb^{2+} and Ca^{2+} were set using the divalent cation chelator and indicator 1,2-bis(2-amino-5-fluorophenoxy)ethane N,N,N′,N′-tetraacetic acid (5F-BAPTA). This enables us to directly confirm the $[Ca^{2+}]_i$ and $[Pb^{2+}]$ in the PKC reaction mix by ^{19}F NMR. We found that free Pb^{2+} activates PKC in the range from 10^{-11} to 10^{-7} M, with an activation constant of 1.1×10^{-10} M, while Ca^{2+} activates PKC in the range from 10^{-8} to 10^{-3} M, with an activation constant of 3.6×10^{-7} M. We also examined the effect of Pb on the intracellular distribution of PKC in ROS 17/2.8 cells. We found that Pb treatment (5 μM) induced a translocation of PKC from the cytosol (100,000 × g supernatant) to the membrane (100,000 × g pellet) within 3 to 10 min that was sustained for at least 30 min. Together these data are consistent with the hypothesis that lead acts directly to induce translocation and activation of PKC in ROS 17/2.8 cells.

47 PROTEIN KINASE C (PKC) IN RAT BLOOD-CEREBRO-SPINAL FLUID (CSF) BARRIER

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PKC is a regulatory enzyme mediating cellular proliferation, differentiation and function by phosphorylating other cellular regulatory proteins. Lead (Pb) exposure stimulates PKC activity in brain extracts, the blood-brain barrier, and peripheral blood vessels. It has been suggested that Pb-induced cerebral dysfunction may result from PKC modulation by Pb. We have previously reported that the choroid plexus (CP), where the blood-CSF barrier resides, sequesters Pb. We are testing the hypothesis that the accumulation of Pb in the CP may alter PKC activity, resulting in functional damage to the blood-CSF barrier. We have evaluated the expression of PKC and its subtypes in rat choroid plexus, cerebral cortex and hippocampus using monoclonal antibodies (PKC α, β, γ and ε) and Western blot analysis. The CP of Sprague-Dawley rats expressed all four PKC subtypes. The expression of α and γ subtypes in the CP appeared to be equal to those found in the cortex and hippocampus, while the expression in the β and ε subtypes was much lower in the CP than in the cortex. This study is the first to demonstrate the presence of PKC in the CP. The effect of Pb exposure on the expression pattern and the activity of PKC is under investigation. (Supported in part by Calderone Award and Grant P20-ES-06831-01).

48 LEAD STIMULATES NITRIC OXIDE SYNTHASE OF CULTURED CEREBELLAR GRANULE CELLS: ANOTHER TOXIC MECHANISM DUE TO ACTIVATION OF PROTEIN KINASE C?

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The immature cerebellum is a sensitive target for the neurotoxic effects of low level lead (Pb) with neurotoxicity and developmental arrest attributed to excessive stimulation of the *N*-methyl-*D*-aspartate (NMDA) receptors. The hypothesis that Pb can have an effect similar to NMDA on the activation of nitric oxide synthase (NOS) was tested in cerebellar granule cells from 7 day-old rat cerebellum cultured at 25 mM KCl, conditions of depolarization that favor granule cell growth. The production of 3-H citrulline from 3-H arginine was measured as an established index of NOS activation. Pb $\leq$ 1 μM and NMDA 10–25 μM evoked comparable effects. The responses to ultra low concentrations of Pb suggest that this may be another manifestation of the activation of brain Protein Kinase C (PKC) by Pb at concentrations lower than that required for activation by Ca. This is tested by the effect of Pb on the responses to phorbol-12-myristate-13-acetate (TPA), inactive TPA and the TPA inhibitor 1-(5-isoquinoline sulfonyl)-2-methyl piperazine (H-7) at EC_{50} of Pb and NMDA. *Supported in part by University of Nebraska Medical Center Edna Ittner Pediatric Research Support Fund.*

49 LEAD MAY AFFECT HORMONAL INDUCTION OF TYROSINE AMINOTRANSFERASE IN CULTURED HEPATOMA CELLS THROUGH INHIBITION OF PROTEIN KINASE C

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Previous results have shown that exposure (24–48 hr) of hepatoma cells (H4-II-E-C3) to 1–10 μM lead (Pb) acetate inhibited dexamethasone induced tyrosine aminotransferase (TAT) specific activity by 52–61%. A possible mechanism of the Pb-induced inhibition may be perturbation of calcium/phospholipid dependent protein kinase (PKC), a family of isozymes which mediate signal transduction responses to a variety of growth factors, neurotransmitters and hormones. To determine whether PKC plays a role in glucocorticoid-induced TAT levels, hepatoma cells were incubated with dexamethasone (10 nM) in the absence or presence of the PKC inhibitor H-7 (10–300 nM). TAT specific activity was dose-dependently inhibited from 17.3 to 81.4%. Treatments with the PKC-activating agent phorbol myristate acetate (PMA) 30 nM–1 μM, alone did not induce TAT. Extended treatment with PMA alone or with dexamathasone decreased basal and induced TAT specific activities, respectively. When cells were incubated with dexamethasone for 18 hr, then 2 hr with 30, 100 or 300 nM PMA, TAT specific activity was potentiated by 19.6, 37.1 and 46.5%, respectively. In 48 hr Pb-exposed cells, potentiation by those same PMA treatments was reduced to 0.6, 21.7 and 23.4%, respectively. Pb exposure for 24 hr likewise resulted in a significant loss of PMA-induced potentiation of enzyme activity. These results suggest that PKC is involved in the hormonal signal transduction events leading to TAT induction, and exposure of cells to the environmental pollutant Pb may inhibit PKC activity. One or more PKC isozymes may be molecular targets of Pb. (Supported by ATSDR and NIH 03320).

50 DIFFERENTIAL MODULATORY EFFECTS OF HEAVY-METAL TOXICANTS ON CONSTITUTIVE NITRIC OXIDE SYNTHASE ACTIVITY

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We have recently demonstrated that constitutive nitric oxide synthase (cNOS) requires superoxide anion for conversion of L-arginine to nitric oxide (NO) and is inhibited by pretreatment with calcium ion [BBRC. 193, 126–132 (1993) and BBRC. 203, 8–15 (1994)]. Since cNOS is a Heme(Fe^{2+}) containing flavoprotein which also exhibits electron transport properties, the present studies were designed to evaluate the in vitro effects of transition heavy-metals on brain cNOS activity. NOS activity was determined in cytosolic fractions of rat cerebral hemispheres by conversion of ^{3}H-L-arginine to ^{3}H-L-citrulline. Different concentrations of mercury (Hg^{2+}), manganese (Mn^{2+}), nickel (Ni^{2+}), zinc (Zn^{2+}) and cadmiun (Cd^{2+}) were tested on NOS activity. All the metals caused significant inhibition of cNOS activity. However, significant differences were observed in the apparent Ki values for various ions [Hg^{2+} = 23 μM; Mn^{2+} = 3.5 mM; Ni^{2+} = 0.36 mM; Zn^{2+} = 0.25 mM; Cd^{2+} = 0.22 mM]. In contrast to calcium ion-mediated inhibition which requires preincubation in the absence of sustrates, the transition metals caused cNOS inhibition without preincubation treatment. Also, the preincubation of cytosolic preparations with calcium ion in combination with heavy-metals led to additive inhibitory response. These data indicate that while calcium ion modulate cNOS activity at regulatory sites, inhibitory influence of heavy-metals is exerted directly on the catalytic site(s). [Supported by RCMI 2G12RR03045-09 and MBRS 2S06 GM08061 from NIH].

51 EFFECTS OF LEAD ON DOPAMINE β HYDROXYLASE ACTIVITY IN THE PC12 CELL MODEL

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Lead poisoning following chronic exposure is injurious to the learning abilities of exposed children. The mechanisms surrounding this toxic effect are still unclear. We hypothesize that lead influences the optimal functioning of the enzyme system involved in neurotransmitter synthesis and thereby, alters the levels and or activities of both dopamine and norepinephrine. We report studies on the effects of lead on commercial grade and PC12 cells derived dopamine β hydroxylase, the enzyme involved in the formation of norepiniphrine from dopamine. Lead was shown to have marginal effects on cell viability at lead ions concentrations relevant to those found in chronically exposed children. There was no significant effect on dopamine β hydroxylase enzyme activity at 0.1–1000 μg/dL lead, but a 20–40% reduction in Km in the presence of lead. Lead's neurotoxic effects, therefore, may not be solely dependent on its effects on dopamine β hydroxylase per se. We are currently investigating its influence on the rate limiting enzyme tyrosine hydroxylase. This work was supported by an ATSDR Grant No. U50/ATU39894802.

52 THE EFFECT OF LEAD (PB) ON INTERLEUKIN-6 (IL-6) PRESENCE IN MOUSE SPLEEN AND BRAIN

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IL-6 is a cytokine that has diverse biological activities including induction of acute phase proteins, stimulation of T cells, differentiation of B cells to plasma cells, and activation of the hypothalamic-pituitary-adrenal (HPA) axis. The heavy metal Pb has immunomodulatory effects, and Pb-induced suppression of host defense mechanisms at low doses in laboratory animals is well documented. However, the effect of lead on the production of IL-6 has not been characterized. The *in vivo* effects of Pb on the production of IL-6 in the saline-perfused spleen and brain upon LPS challenge were assessed. B6C3F1 female mice (5 mon) that were exposed to 2 mM Pb in their drinking water for 3 mon were used for the experiments. ELISA quantification of IL-6 demonstrated that both Pb-exposed and age and sex matched control mice exhibited the same kinetic patterns upon i.v. challenge with 25 μg LPS. IL-6 peaked 2 hr and 3 hr after LPS challenge in spleen and brain, respectively. The highest amounts of IL-6 found were approximately 480 pg and 240 pg/mg protein in spleen and brain, respectively. When the amounts of IL-6 detected were compared at 2 and 6 hr post-LPS, Pb-exposed and non-exposed spleen samples did not show any difference. However, substantially higher amounts of IL-6 were detected at both time points in the brains of mice exposed to Pb. Since IL-6 activates the HPA axis and this activation results in the induction of glucocorticoids that have a profound suppressive effect on the immune system, the enhanced IL-6 level observed in the brain of Pb-exposed mice could be, in part, the mechanism responsible for Pb-induced immunosuppression upon infection. (Supported by NIH grant ES03179)

53 HEAVY METAL LEAD AFFECTS THE EXPRESSION OF CYTOKINES IN HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC)

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Lead has been implicated as a causative agent for several immune function abnormalities. In order to find some direct evidence for lead poisoning effects on the human immune system, PBMC isolated by Ficoll-hypaque from healthy donors were cultured with medium, 50 μM lead chloride (Pb), 1% (vol/vol) phytohemagglutinin (PHA), PHA + Pb, 1 ng/ml lipopolysaccharide (LPS), and LPS + Pb. After 24h, the supernatants were assayed for cytokines using enzyme linked immunosorbent assays (ELISAs), and the cells were used for mRNA isolation. RNA-polymerase chain reaction (PCR) was done to detect the mRNA levels of different cytokines. The secretion of interferon γ (IFN-γ), interleukin 2 (IL-2), and interleukin 4 (IL-4) were significantly increased by Pb in the PHA-activated PBMC. The secretion of tumor necrosis factor α (TNF-α) was significantly increased by Pb in the LPS-activated PBMC. There was no detectable IL-2, IL-4, or TNF-α in the supernatants of non-treated and Pb-treated PBMC. Without PHA or LPS treatment, the mRNA levels of IL-1β and IL-2 were upregulated by Pb. We did not see any change at the mRNA levels of IL-4, IL-10, IFN-γ, and TNF-α. In the PHA or LPS activated PBMC, Pb did not substantially alter the mRNA levels of these cytokines except IFN-γ which was downregulated by Pb. These results suggest that Pb can modulate cytokine secretion at both transcriptional and post-transcriptional levels. Supported by ES03179.

54 NERVOUS SYSTEM DAMAGE PRECEDES IMMUNE SYSTEM INVOLVEMENT IN MALE MICE EXPOSED TO PB

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Previously we presented evidence that Pb may provoke an immune response against nervous system (NS) structures. However, it is not known whether Pb-induced neural damage must precede the immune response. To resolve this question, male and female CBA/J mice were exposed to 820 ppm Pb in the drinking water. Endpoints included: serum autoantibody (AuAb) titers to nervous system proteins (NSP): neurofilaments (NF), glial fibrillary acidic protein (GFAP) and myelin basic protein as well as interleukin 2 and 6 (IL-2,IL-6) levels from supernatants of lectin-stimulated splenocytes. GFAP was used as a

marker of neurotoxicity. GFAP levels were elevated in the hippocampus and cerebellum of male mice at 5d but recovered to baseline afterward. In males, serum AuAbs to NSP were mostly directed against the NFs and peaked predominately at 10d. These early AuAb changes accompanied IL-6 elevations (10d), followed by IL-2 elevations (28d). Analogous effects were not seen in female mice. These results suggest that NS damage, as indicated by elevated GFAP levels, precedes AuAb production in male mice suggesting that Pb-induced neural damage precedes the immune response. This study also confirms that males are more sensitive to Pb neurotoxicity. However, these differences cannot be accounted for on the basis of Pb exposure since weight gain and water consumption did not differ between the genders. (Sponsored by ES-04895).

55 MORPHOMETRIC ANALYSIS OF CORTICAL ASTROCYTES OF JUVENILE RATS FROM LEAD TREATED DAMS

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The objective of this study was to determine the maternal effect of very low doses of lead on the area of astrocyte of juvenile rats as determined by morphometeric analysis. Female Sprague-Dawley rats were treated orally with 25 ppm and 50 ppm lead acetate (PbAc) in drinking water for 5 weeks. Treatment was completed prior to mating with untreated males. Litters were culled and 21 day pup brains were sectioned and Golgi-stained. The sections were then analyzed using the BioQuant Advanced Image Analysis System. Parameters measured were the area of the astrocyte and its perimeter. Preliminary data collected and analyzed showed a clear and significant difference in the statistical population distribution of the astrocyte area with a shift from larger to smaller areas with treatment. Detailed analysis using more sections showed a significant trend.

56 COMPARISON OF LEAD UPTAKE IN MATURE AND IMMATURE ASTROGLIA

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There is a growing concern about the effects of low level lead (Pb) exposure on the developing central nervous system in children. Cognitive, attentional and behavioral deficits have been reported, and a no-effect level for Pb has not been determined. As a result, current research is focused on identifying cellular markers of toxicity. Because astroglia have been shown to accumulate Pb and are necessary for maintaining the brain microenvironment, our studies have focused on these cells. The present study reveals a differential uptake of Pb by astroglia that is dependent upon age in culture at which Pb exposure is initiated. Primary rat astroglia were exposed to 1 μM Pb beginning at 7 days (D7), 14 days (D14) and 21 days (D21) in culture. By seven days of exposure the D7 and D14 cells showed a significantly greater accumulation of Pb when compared to the D21 cells. Also, an additional 10 μM glutathione in the medium had no effect on Pb accumulation except in the D14 astroglia which showed a significant reduction in Pb content. In association with this, only the D14 calls showed an elevated Pb to calcium ratio when compared to both D7 and D21 astroglia. Future studies will be directed at exploring these age-dependent differences in astroglia in order to better understand the toxicological implications of Pb exposure.

57 VISUALIZATION OF LEAD UPTAKE IN BRAIN CAPILLARY ENDOTHELIAL CELLS USING A FLUORESCENT DYE

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The mechanism by which lead crosses the blood-brain barrier is not known. The blood-brain barrier is comprised of brain capillary endothelial cells which form tight junctions with each other; lead must traverse these cells to reach the brain. We have developed a new method to monitor lead transport into cultured bovine brain capillary endothelial cells using Indo-1, a fluorescent dye commonly used as a Ca^{2+} indicator which is quenched upon binding to lead. Uptake was monitored using spectrofluorimetric and digital fluorescence imaging techniques. For spectrofluorometric measurements, Indo-1-loaded cells were monitored at ex 336 and em 450 nm. Addition of 1–10 μM lead caused quenching which was time- and concentration-dependent. Fluorescence was only partially recovered by the addition of the cell-impermeable heavy metal chelator diethylenetriaminepentaacetic anhydride, and was fully recovered upon the addition of the permeable chelator tetrakis(2-pyridylmethyl)ethylenediamine, indicating that quench was due to intracellular lead. Single-cell imaging confirmed this observation. Dye-loaded cells grown on glass coverslips were placed in a chamber on a microscope, and images were recorded before and after the addition of lead. Lead caused quench of fluorescence first in the nucleus, then in cytoplasm of endothelial cells. Fluorescence was recovered by the addition of heavy metal chelators. This novel approach to studying lead uptake may facilitate investigations of the transport mechanism in brain capillary cells, and may be extended to other cell types as well. Supported by ES05855, ES07026 and ES06484.

58 LEAD TOXICITY TARGETS TO PYRROLOQUINOLINE QUINONE (PQQ) IN MITOCHONDRIAL COMPLEX I AND IN RED CELLS

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We have demonstrated that lead toxicity involves inhibition of mitochondrial complex I; higher levels of lead causes hemolysis of red cells. Our focus is on the prevention of both of these effects by the addition of the natural product PQQ. PQQ is a redox cofactor originally discovered in methylotrophic bacteria and more recently shown by our laboratory to be present in mammalian systems in protein-free dialysates of biological fluids, tissue homogenates, red and white cells and mitochondrial sonicates. In mitochondria, PQQ is the metabolic target for a variety of complex I inhibitors. In a diaphorase assay, electron transport inhibited at complex I by rotenone, iodonium compounds or *N*-methylphenylpyridinium (MPP+) is restored with PQQ. Lead inhibits oxygen uptake in intact mitochondria in the presence of complex I NADH-generating substrates, but not with the complex II FADH-generating substrate. We demonstrated the reversal of rotenone inhibition by addition of a lipoidal agonist of PQQ. This approach is currently being applied to mitochondria inhibited with lead. We have also shown that high levels of lead cause hemolysis of red cells by sequestering endogenous PQQ. This action is prevented by adding exogenous PQQ. These findings may lead to novel approaches for treatment of lead toxicity using PQQ and PQQ agonists. Research Support: National Dairy Promotion & Research Board Grant, MRC and AHFMR (JM).

59 EFFECT OF LEAD ON INSULIN BINDING AND RESPONSIVENESS

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The effect of lead exposure on the binding and cellular responsiveness of the hormone insulin to its cell surface receptor has been investigated using 3T3-C2 mouse fibroblasts, an insulin-responsive cell line. Specific binding of insulin to cell surface receptors increased with 48 h treatment of 10^{-4} M lead acetate. Data from Scatchard analysis of equilibrium binding isotherms indicate that the increased binding is not due to a change in affinity, but to a change in the number of receptors. To assess changes in insulin sensitivity of lead treated cells, glucose uptake and the ability of treated cells to downregulate their insulin receptor was investigated. No significant change in glucose uptake was observed. However, data from downregulation studies indicate that treated cells decrease receptor number to the same steady state level but the time required to attain the steady state level is shorter in lead treated cells. Results from these studies will increase the understanding of the cellular effects of toxic metals and aid in delineating whether the effects of lead on insulin receptor binding and metabolic processing impacts the health status of an exposed population.

60 CHARACTERIZATION STUDIES OF HUMAN BRAIN LEAD-BINDING PROTEINS (PBBPS)

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The recognition that metal-binding proteins may exert homeostatic control over the intracellular bioavailability of metal ions is of central importance for both essential and toxic elements. Our group has previously reported the existence of PbBPs in several species and in various tissues. In this study we present results of Pb-binding studies which confirm that the PbBPs isolated from human brain cytosol (Quintanilla-Vega et al., 1994, The Toxicol. 14, 255) bind Pb with a K_d of $\cong 10^{-9}$ M. Similar chemical, chromatographic and Pb-binding

characteristics have been observed among PbBPs from different tissues and other species suggesting they are related molecules which share the same role in Pb toxicity. Results of the present studies demonstrate that the Pb-binding characteristics of human brain PbBPs are similar to those previously reported for the rat. The data also provide further quantitative evidence for the hypothesis that these proteins are major molecular determinants for mediating the intracellular bioavailability of low levels of Pb to sensitive biochemical processes in brain tissue of humans. (Supported in part by the Maryland Department of the Environment through a grant from ATSDR and Centro de Investigacion y Estudios Avanzados-IPN, Mexico, DF Mexico).

61 IDENTIFICATION OF A NOVEL CYTOSOLIC COPPER BINDING PROTEIN IN INDIAN CHILDHOOD CIRRHOSIS LIVER

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Indian Childhood Cirrhosis (ICC) is a lethal liver storage disease characterized by swelling and degeneration of liver cells along with the presence of orcein staining deposits for copper and copper binding proteins. The copper content in the cytosol of ICC liver was about 22 fold higher than control cytosol levels. Elution profile of ICC liver cytosol on sephadex G-75 column showed three peaks viz. peak I, peak II and peak III. About 70.4% of the total cytosolic copper was found in peak II with highest Cu/O.D. 280 ratio. Further purification on anion exchange chromatography showed a major copper binding protein (Cu BP). The molecular weight of major copper binding protein on SDS PAGE was found to be about 48 KD with 8 g atom/ml of Cu bound to the protein. Appearance of this specific CU BP in ICC liver cytosol in response to massive accumulation of hepatic copper indicates that this protein may be involved in pathophysiology of Indian childhood cirrhosis.

62 ESTIMATION, ISOLATION AND STRUCTURAL CHARACTERIZATION OF A NATIVE ZINC BINDING PROTEIN FROM HUMAN PLACENTA

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A cytosolic low molecular weight, native zinc binding protein (LMZP) has been isolated and purified from human normal term placenta using gel filtration, ion-exchange and fast protein liquid chromatography. The purified placental LMZP was homogenous on SDS-PAGE with a single band. UV-spectrum of LMZP showed an absorption maxima at 257 nm which disappeared at pH 2. Molecular weight of LMZP was found to be 6 kD and it was calculated that one gram atom of zinc was bound to one mole of the LMZP. Unlike classical metallothionein, the amino acid composition of placental revealed the presence of aromatic amino acids, lower content of cysteine and higher content of histidine, glutamic acid and aspartic acid (10,9 and 5 residues/mole respectively. The levels of placental LMZP were increased with gestational age and a significant ($p<0.001$) increase (60%) was observed between 2nd and 3rd trimester. It is speculated that LMZP might have some role to play in homeostatic control or transport of zinc from placenta to the fetus.

63 THE SKELETON AS A SOURCE OF LEAD TO THE CIRCULATION IN ENVIRONMENTALLY-EXPOSED HUMANS

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Clinical and experimental studies provide evidence that the skeleton is an important endogenous source of lead to the circulation and soft tissues, and this role may be increasing over recent years due to declines in industrial and environmental lead exposures. Stable lead isotope systematics may be used to distinguish circulatory lead derived from current exposures from "older" remobilized skeletal lead that was accumulated in bone years or decades earlier. Differences in the stable lead isotopic compositions of paired blood and trabecular bone samples recovered from environmentally-exposed individuals (PbB levels 1–6 μg/dl, bone Pb 1–15 μg/g dw) undergoing total hip or knee arthroplasty were used to evaluate the contribution of bone lead to the total lead in circulation. These preliminary data indicate that 30–65% of the lead in circulation is derived from the skeleton, based on a stable lead isotopic signature of current exposures derived from collected particulate aerosol samples. Variability between individuals in both bone and blood lead isotopic compositions also demonstrates different sources of past and current lead exposures. In summary, these data provide direct evidence of the skeleton's role as an endogenous source of lead to the circulation during normal homeostasis, and they suggest that the skeleton may serve as the predominant source of lead exposure during periods of accelerated bone loss, such as osteoporosis and pregnancy/lactation. (Supported by NIEHS Grant #ES04850).

64 BLOOD LEAD AND BLOOD PRESSURE IN CHILDREN AGED 5.5 YEARS

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Cross-sectional studies in adult populations suggest small increases in blood pressure (BP) with increasing blood lead concentrations (BPb). We examined this association in children (N = 282), residing in two towns in Kosovo, Yugoslavia; one town is heavily exposed from a smelter, refinery and battery plant. BPb was measured from birth onward at six month intervals; mean BPb at age 5.5 was 37.3 (s.d. 12.0) and 8.7 (s.d. 2.8) ug/dl, in the two towns, respectively. Six BP measurements were obtained at age 5.5 using an automated monitor (Dinamap); the average of the last two measures was used to estimate resting BP. Overall means were 99.4 (s.d. 10.4) and 58.7 (s.d. 7.1) mm Hg for systolic and diastolic BP, respectively. After controlling for potential confounders, neither concurrent BPb nor an integrated average of BPb from birth to age 5.5 were associated with either systolic or diastolic BP. Controlling for height, body mass index, gender, ethnic group and birth order, a 10 ug/dl increase in BPb was associated with a 0.5 (95% confidence interval –0.2, 1.3) mm Hg increase in systolic BP. Similarly, controlling for waist circumference, ethnic group and birth order, a 10 ug/dl increase in BPb was associated with a 0.4 (95% C.I. –0.1, 0.9) mm Hg increase in diastolic BP. The magnitude of the regression coefficients decreased with adjustment for hemoglobin concentration and/or maternal blood pressure. Results were similar when data from each town were analyzed separately and when the logarithmic transformation of BPb was used as the exposure. Statistical power to detect differences between towns on the order of 2 mm Hg was low; hence, these results do not preclude the existence of small associations between BPb and BP.

65 MEASUREMENT OF OXIDATIVE STRESS IN BLOOD SAMPLES OF MEXICO CITY NEWBORNS

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The environment is continuously polluted by industrial products, which include several toxic substances. High concentrations of lead and cadmium, for example, cause genotoxicity, alterations of the nervous system, and peroxidative damage. The aim of this study was to measure the lipid peroxidation products in relation with the level of some environmental pollutants, as lead and cadmium, in a sample of 250 newborns from Mexico city. We estimated the lipid peroxidation products by two diferents indexes: lipid flourescence products (LFP) and ultraviolet absorbance (UA). Our results showed that LFP formation in the blood samples from the newborns were significantly correlated with UA ($r = 0.72$). But, there were no significant correlation between lead concentrations and lipid peroxidation products in blood ($r = 0.21$ for LFP and 0.05 for UA). These results indicated the need of a correction for confusing variables that allow us to observe a relationship among metals and lipid peroxidation.

66 CHANGES IN BLOOD LEAD CONCENTRATION AND ISOTOPE RATIO ASSOCIATED WITH PREGNANCY AND LACTATION

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Measurements of blood lead concentration (PbB) and $^{206}Pb/^{207}Pb$ ratio (IR) and the IR of the diets of women during pregnancy and lactation may be used to test the hypothesis that a greater than normal release of Pb from the skeleton occurs during these changed physiological conditions. Although the IR of their skeletons are not known, a rise in PbB accompanied by a shift in IR away from the diet IR would indicate Pb coming from a second and presumably endogenous source. To this end 22 Omaha women (8 breast feeders) have been followed from mid-pregnancy. Initial PbB ranged from 0.5 to 3.0 μg/dL. The PbB of about half remained constant while those of the rest had risen by as much as 50% at parturition, but by 9 mos. post partum bottle feeders had fallen to initial values. The PbB of breast feeders on average doubled during lactation and slowly fell to initial values thereafter. Subtle changes in IR

accompany pregnancy and lactation. At the present time dietary analyses are complete for one breast feeder whose PbB rose from 1.8 at the end of her first trimester to 5.5 μg/dL after 6 mos. of breast feeding, while her blood IR decreased from 1.203 to 1.195. Her diet averaged 13 μg Pb/day with an IR of 1.177. The shift in her blood IR is towards her diet and is thus not definitive. Supported by NIEHS R01 ES04762.

67 QUANTITATIVE ATOMIC ABSORPTION SPECTROMETRY STUDY OF THE TOXIC EFFECTS OF A HEAVY METAL, LEAD (PB^{2+}), ON FUNDULUS GRANDIS IN CONTAMINATED AQUATIC ENVIRONMENTS

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Quantitative Atomic Absorption Spectrometry (AAS) is used to assess the toxic effects of acute and chronic exposure to the heavy metal, lead (Pb^{2+}), on the estuarine fish, *Fundulus grandis* in contaminated aquatic environments. In order to ascertain if the data on the cellular effects of Pb^{2+} are relevant to human tissues and the reported lower *in vivo* concentrations, we are investigating the effects of different concentrations of aquatic Pb^{2+} including 1 microMole, which is the accepted environmentally relevant concentration. We report here some of the data on cellular responses to acute Pb^{2+} exposure. The uptake and distribution of Pb^{2+} in plasma membrane, mitochondria, nuclei, and cytosol isolated from fish liver following exposure to 200 ppm of $PbCl_2$ contaminated sea water was determined by AAS. Fish were exposed to $PbCl_2$ as in a previously reported marine system (Brown, I V and Browne, J M. In: *Radiation Carcinogenesis and DNA Alterations*. Eds.: F J Burns, et al., N.Y.: Plenum Press, 1986). Intracellular stores of Pb^{2+} and fraction purity were monitored by electron microscopy. Subcellular fractions of liver, obtained by sucrose density gradient centrifugation, were subjected to nitric acid hydrolysis followed by AAS analysis. The highest concentration of Pb^{2+} was found in the cytosol fraction with decreasing amounts in nuclei, mitochondria, and plasma membrane, respectively. These Pb^{2+} accumulations corresponded to the ultrastructural observations of cellular toxicity seen as numerous membrane-bound apoptotic bodies and lysosomes which contained particulate material, putatively, the AAS identified Pb^{2+}. These data will be used in biomonitoring lead contamination of the aquatic environment. Supported by NIH/NCRR grant No. G12RR03062.

68 PHYSIOLOGICALLY-BASED MODELING OF LEAD EFFECTS ON RAT PHOTORECEPTORS

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Fox and colleagues have found that developmental lead (Pb) exposure results in a decrease in cGMP-phosphodiesterase (PDE) activity in rat rod photoreceptors. We seek to relate these biochemical findings to photoreceptor physiology evaluated with the corneal electroretinogram (ERG). We recorded ERGs from dark-adapted control (n = 6) and Pb-exposed (n = 8) adult male Long Evans rats. The treated group was exposed from birth through weaning by nursing from dams maintained on 0.2% lead acetate in drinking water. Flash intensities varied from 1.24 to 3.34 log scotopic trolands. A-wave amplitudes in Pb-exposed animals were significantly lower than those in controls. The difference in b-wave amplitudes did not reach statistical significance. Preliminary analysis indicates that responses from both groups can be modeled with a 2-component response model which postulates a linear primary stage and secondary stage characterized by a static, saturating non-linearity (Hood and Birch, 1990, 1993). In further modeling, it should be possible to incorporate terms representing discrete biochemical steps of phototransduction (Lamb and Pugh, 1992), including the rate of cGMP hydrolysis by activated PDE. This may help uncover changes in transductional efficiency due to lead exposure. Supported by EPA cooperative agreement CR817643 with CEMLB, UNC-CH.

69 CHARACTERIZING SOURCES OF VARIABILITY IN THE U.S. E.P.A. IEUBK LEAD MODEL BY MONTE CARLO SIMULATION

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The Integrated Exposure, Uptake, and Biokinetic (IEUBK) Model for Lead in children was released by U.S. EPA in 1994 to assist risk managers at Superfund sites in evaluating the potential exposure of young children to environmental lead. The IEUBK Model starts with a deterministic algorithm using algebraic and differential equations to describe environmental lead intake, absorption, and elimination. The deterministic calculation is converted into a blood lead distribution by assuming that the calculated blood lead is the geometric mean of a lognormal distribution whose variability is characterized by an assumed geometric standard deviation (GSD). The GSD is the same as that estimated from epidemiology studies at Superfund sites. We studied the importance of random variability in different components of the model by use of Monte Carlo simulations. The simplest simulations used independent random variables for the most sensitive parameters of exposure, absorption, and biokinetics, with the random parameter values drawn once at the beginning for each child. More realistic simulations change parameters at each simulation time point, but are far more computer-intensive and may require specifying the multivariate correlation structure. Some preliminary simulations suggest that the log-normal approximation may be adequate for the middle part of the blood lead distribution, but that the extreme percentiles are more sensitive to assumptions about the range of input parameter values. Measurement errors in input variables and parameters can substantially attenuate the relationship between input and predicted blood lead distribution.

70 A PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODEL FOR METHYL MERCURY (MEHG) IN MONKEY AND HUMAN

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A PBPK model for MeHg was developed which coherently describes MeHg pharmacokinetics in the adult rat, monkey and man, and predicts fetal levels of MeHg from in utero exposure. The model includes a description of enterohepatic recirculation of MeHg, conversion to inorganic mercury in tissues and intestinal flora, slowly reversible incorporation of mercury in tissues, and excretion of both organic and inorganic mercury into urine, feces, and hair. The adult submodel includes compartments representing the red blood cells (RBC), plasma, brain, liver, kidney, gut intestinal lumen, gut tissue, hair, richly and slowly perfused tissues, and placenta. The fetal submodel includes compartments representing RBC's, plasma, brain, and remaining body mass. Two features of the model structure which are critical to prediction of the kinetics of MeHg in different species is the use of separate RBC and plasma compartments, allowing the use of species specific RBC/plasma ratios, and biliary excretion with enterohepatic recirculation. Published tissue and blood MeHg concentrations were used to derive the partition coefficients and RBC/plasma ratios to adjust for species differences in MeHg distribution. Validation involved comparing the model simulations with data from repeated dosing studies in animals and humans, and from accidental human exposures. The model will be used to investigate maternal MeHg intake as it relates to measured blood and hair MeHg concentrations, and to fetal exposure.

71 INDUCTION OF SERUM AUTOANTIBODIES TO NERVOUS SYSTEM PROTEINS BY NEUROTOXIC ORGANOMETALS: COMPARISON BETWEEN LEAD AND MERCURY

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Exposure to inorganic lead has been shown to result in the production of autoantibodies to proteins of the neurofilament triplet (NF68;NF160;NF200), myelin basic protein (MBP) and glial fibrillary acid protein (GFAP), providing potential markers of subclinical neurotoxicity. In the present study, the effects of trimethyl lead (TMPb) and methyl mercury (MeHg) on the autoantibody response were compared in male Fisher 344 rats exposed to 16 ppm of metal in the drinking water. Both metals resulted in the induction of autoantibodies to the protein antigens at 7 and 14d of exposure. For both, IgM against the NFs and GFAP antigens dominated over IgG ($p < 0.001$), indicating recent damage and antigen release. In both exposures, anti-NF68 was the most prevalent anti-NF detected. In comparison, however, this was significantly higher ($p < 0.05$) at 7d and 14d for TMPb. In addition anti-NF200 and GFAP titers at 14d were significantly ($p < 0.001$) higher in the sera of TMPb-exposed rats compared to those exposed to MeHg. While no IgM against MBP was detected in sera of MeHg-exposed rats at 7 or 14d, very high levels were detected in the sera of TMPb-exposed rats at 14d. Brain levels of GFAP, a marker of neurotoxicity, were determined in the cortex, hippocampus and cerebellum. At 14d, significant GFAP increases were seen in the cortex of MeHg rats and in the hippocampus of TMPb rats. This study suggests that autoantibodies against nervous system proteins may provide a means of assessing the early neurotoxic effects of organometals and that titer profiles may differ with different neurotoxicants. (ES-00260, ES-04895 & API).

72 INCREASED EXPRESSION AND LOCALIZATION BY *IN SITU* HYBRIDIZATION OF γ-GLUTAMYLCYSTEINE SYNTHETASE (GCS) mRNA IN MOUSE BRAIN FOLLOWING METHYLMERCURY TREATMENT

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In previous studies (ABB 296:350–353, 1992), we reported that methyl mercury (MM) treatment of rats elicited a 2–3 fold increase in renal glutathione (GSH) content and a 4-fold increase in the mRNA encoding GCS, the rate-limiting enzyme in GSH synthesis. Since MM is a potent neurotoxicant, we investigated the effect of MM treatment on GSH synthesis and the distribution of GCS expression in the brain. Male C57Bl mice were treated for 3 days with MM (3 mg/kg/day, IP). GSH levels in whole brains were increased by 3-fold 24 hrs after the 1st injection and remained at that level after 2 subsequent MM treatments. Despite only a 1.2-fold increase in GCS enzyme activity, GCS mRNA levels were highly expressed after the 3 day MM treatment. In situ hybridization using an antisense GCS riboprobe showed that GCS expression was localized to the cerebellum and hippocampus, with no apparent expression in other brain regions. These findings are consistent with the known distribution of MM in the brain. Available evidence indicates that Purkinje neurons within the cerebellum resist cytotoxic MM damage through ability to sequester MM in inactive form. The present findings suggest that such resistance may reflect the ability of Purkinje cells to transform MM to Hg^{+2}, which, in turn, induces the synthesis of Hg^{+2}-scavenging GSH molecules. Supported by ES03628.

73 METHYLMERCURY CAUSES RELEASE OF ZINC FROM SOLUBLE SYNAPTOSOMAL PROTEINS

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Methylmercury (MeHg) elevates the free concentrations of Ca^{2+} and Zn^{2+} within synaptosomes from rat brain. The increases in Ca^{2+} concentration were strictly dependent upon extracellular Ca^{2+}, while the elevations in Zn^{2+} were due to release of endogenous synaptosomal Zn^{2+}. We are attempting to identify the source of the MeHg-induced elevations in intrasynaptosomal Zn^{2+} concentration ($[Zn^{2+}]_i$) using protein chromatography. Synaptosomes are homogenized in 50 mM HEPES, and centrifuged to separate the particulate and soluble fractions. Fura-2 pentapotassium salt is added to aliquots of the soluble fraction and particulate suspension, and excitation spectra of fura-2 free acid are acquired before and after MeHg treatment. These excitation spectra are then compared for MeHg-induced changes in fura-2 fluorescence consistent with increases in free Zn^{2+} concentration. Addition of MeHg to the soluble fraction causes greater shifts in fura-2 fluorescence than MeHg addition to the particulate suspension. Thus, the factor(s) responsible for the MeHg-induced elevations in $[Zn^{2+}]_i$ are predominantly soluble. The soluble fraction is concentrated, and subjected to either anion exchange or gel permeation chromatography. Fractions are collected continously, then assayed for MeHg-induced Zn^{2+} release as described above. Following High-Q anion exchange chromatography three peaks which release Zn^{2+} are observed. Gel permeation chromatography of the soluble fraction using BioGel-P100 yields one high molecular weight and one low molecular weight peak. Thus, it is apparent that more than one soluble protein is responsible for the MeHg-induced elevation in $[Zn^{2+}]_i$. Supported by NIH grants R01-ES03298 and T32-ES07255. MFD is a recipient of an SOT fellowship sponsored by the Hazelton, Co.

74 METHYLMERCURY (MEHG) MODULATION OF WHOLE CELL OUTWARD CURRENT IN CEREBELLAR GRANULE CELLS

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MeHg is an environmental toxicant which has been shown to interfere with numerous cellular homeostatic mechanisms. Voltage-gated ion channels contained in the plasma membrane are one of the potential targets for MeHg *in vitro*. Previous studies using electrophysiological and fluorescence measurement techniques have shown an ability of MeHg to block currents carried by Na^+, K^+ and Ca^{2+} through their respective ion channels, an action which may contribute to the ultimate pathogenesis of MeHg. Among cells of the central nervous system, cerebellar granule cells are particularly susceptible to MeHg and thus provide an excellent model for studying neurotoxic effects of this compound. We are interested in determining possible reasons for the heightened sensitivity of this cell type to MeHg. Initial experiments were designed to measure peak and steady state outward currents in primary granule cell cultures using the whole cell voltage clamp method. Positive voltage pulses (100 ms duration) from a holding potential of −80 mV generated currents which remained stable over approximately 10 minutes. Addition of 20 μM MeHg decreased beth peak and steady-state outward current when measured after 3 minutes of exposure. Thus it is possible that MeHg may modify normal potassium channel function in cerebellar granule cells. Supported by NIH grants R01-ES03299 and T32-ES07255.

75 ALTERATIONS IN RAT BRAIN NEUROTRANSMITTER LEVELS INDUCED BY MERCURIC CHLORIDE EXPOSURE

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Inorganic mercury at low doses has been shown to induce behavioral alterations in rats and to affect the receptor binding of certain neurotransmitter systems within the rat brain. These effects are seen at doses that do not show overt signs of central nervous system toxicity. Previous reports from our laboratory have indicated that low levels of mercuric chloride ($HgCl_2$) induce alterations in brain norepinephrine and dopamine levels 24 hours post injection. The present study was designed to determine whether low level $HgCl_2$ exposure produces alterations in neurotransmitter levels two weeks post injection. Male Sprague-Dawley rats were administered mercuric chloride (1.0 and 2.0 mg/kg) i.p. for 7 consecutive days. Control animals received saline. Two weeks later, animals were sacrificed by decapitation, their brains were immediately removed, dissected and frozen for later analysis of neurotransmitter levels by high performance liquid chromatography with electrochemical detection. $HgCl_2$ exposure of 1.0 mg/kg resulted in significant increase of homovanillic acid (HVA) in the frontal cortex. Significant decreases were observed in dopamine (DA) levels in the caudate nucleus of treated rats. Decreased concentrations of serotonin (5HT), 5-hydroxyindoleacetic acid (5HIAA) and HVA occurred in the hippocampus of exposed rats. $HgCl_2$ exposure at 2.0 mg/kg produced a significant increase in 3,4-dihydroxyphenylacetic acid (DOPAC) in the hypothalamus and significant increases of DA levels in the caudate nucleus, hippocampus, and cerebellum. 5HT, 5HIAA and HVA were decreased in the hippocampus as well as DOPAC and HVA levels in the cerebellum and brain stem of treated rats, respectively. These data suggest that low level exposure to mercuric chloride produces alterations in specific neurotransmitter systems and that these effects may be related to mercuric chloride-induced behavioral effects. (Supported by Florida Education Fund and NIH grants RR08111 and RR03020)

76 *IN VITRO* METAL INHIBITION OF NMDA-SPECIFIC GLUTAMATE RECEPTOR BINDING IN RAT BRAIN

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We have recently reported that metals such as mercury and lead alter the calcium homeostasis in neuronal cells by interacting with inositol polyphosphate receptors. Since the receptor-mediated channels control the influx of calcium into the cell, the *in vitro* effects of mercury and lead on NMDA-specific glutamate receptor binding was investigated in rat brain. The Synaptic plasma membranes (SPMs) were prepared from rat brain by sucrose gradient. The SPMs were incubated at 37° for 30 min., followed by a washing with the buffer and this procedure was repeated three times to reduce the endogenous glutamate. The assay of receptor binding was done with washed SPMs by incubating at 2°C with (^{3}H)-glutamic acid in the presence of micromolar concentrations of mercuric chloride, methyl mercury chloride, lead chloride and lead acetate. All metal salts inhibited NMDA-specific glutamate receptor binding in a concentration-dependent manner with IC_{50}values of 389.1, 97.2, 560 and 390 um respectively. The organic salts of mercury and lead are found to be more potent than their inorganic forms and this might be due to their lipophilic nature. This investigation demonstrates that mercury and lead inhibit receptor-mediated calcium channels in brain. (Supported by NIH/NIGMS/MBRS 08169).

77 NEUROPSYCHOLOGICAL ASSESSMENT OF A PRACTICING DENTIST WITH ELEVATED URINARY MERCURY

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Dentists are exposed to mercury from amalgam (composed of 52% mercury, 8% copper, 37% silver) used as implants for dental restoration. A recent study of 32% of Singapore's dentists found mercury dose-related neuropsychological deficits when compared with controls. The current subject, a dentist practicing for 14 years in the US, was referred by his physician for neurobehavioral examination. The subject presented with symptoms including fatigue, GI

problems, sweats, tremors, rashes, bowel dyscontrol, and heart arrhythmia. Tremors of the hand and eyelid affect the subject's hand stability while working. Results of the *Neurotoxicity Screening Survey* showed a profile of symptoms consistent with neurotoxicity. Urine sampling (24 h) over the past year found 2.1–99 ppb mercury, averaging 35 ppb (expected range < 2 ppb; atomic fluorescence spectrometry). High readings may be related with chelation therapy. Current IQ was at the 87% (% = percentile; 91–96% or greater estimated premorbidly), consistent with his presentation as an intelligent, competent dentist. In contrast to a relatively high IQ, appropriate neuropsychological testing revealed that the subject has deficits in immediate memory (16%); visuospatial perception (50%); visual memory; visual figure-ground perception (50%); verbal learning (2–5%); mental flexibility (12%); attentional processes (19%); logical memory ($p < 0.004$); and non-verbal recognition memory (50%). Neuropsychological testing helped confirm mercury toxicity affecting multiple organ systems. Such deficits impact the subject's ability to practice dentistry, and raises questions as to effects of mercury in other US dentists, as well as possible subclinical or clinical effects among the estimated 80–90% of Americans with mercury amalgam implants.

78 ACCIDENTAL METALLIC MERCURY INTOXICATION THROUGH ORAL MUCOSA AND ITS SEQUELAE. CASE REPORT

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The intoxicatiòn by metallic mercury is unusual, mostly occur by accident. We are describing an interesting case of accidental subcutaneous metallic mercury deposit with granuloma formation. There are very few cases in the literature. *Case Report:* A 9 year old girl was seen with a two month swelling (0.5/cm diameter) on the anterior midline of the neck, at tyroid region. From its clinical course and physical exam, it was considered to be a thyroglossal duct cyst, as usually seen in the age group. Lab pre-op test were ordered and surgery performed under general anesthesia. Surprisely, free metametallic mercury and small pieces of glass were found. Retrospectively it was established that the children suffered a minor trauma that broke an oral thermometer while temperature was taken, and the mercury bulb was never recovered. The explanation of the event was that the thermometer penetrated the mouth floor, which eventually healed. The bulb "buttoned" on the subcutaneous layer of the tyroid region. In the post-op, X ray were taken and minimal drops of metal were noticed; she was re-operated on twice to remove it. The urinary level of mercury was 40 mcg/l prior to these re-operation. The patient has remained asymptomatic and is being followed up.

79 LEVELS OF MERCURY IN AUTOPSY TISSUES OF INHABITANTS OF TARRAGONA PROVINCE, SPAIN

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Tarragona Province is an area of Catalonia (NE Spain) with a notorious industrial and agricultural activity. The study described herewith was conducted to determine mercury (Hg) concentrations in the kidney, liver, lung, bone, and brain of 42 autopsied subjects living in Tarragona Province. The results were analyzed in terms of the place of residence, age, sex, and drinking and smoking habits of the subjects. Mercury concentrations were determined by the cold vapor atomic absorption method in a cold vapor atomic absorption spectrophotometer. The highest Hg levels were found in liver (214 μg/kg) and kidney (208 μg/kg), whereas the lowest concentrations of Hg were detected in bone (15 μg/kg). No significant differences in Hg tissue levels were observed in relation to the place of residence (i.e., whether it was a rural or an industrial area), whereas Hg concentrations in males exceeded those of females. On the other hand, in a recent study on the dietary intake of Hg by the inhabitants of Tarragona Province, we showed that the total Hg intake would be 142.8 μg/week (54.6–256.9 μg/week). Taking into account those results as well as the tissue levels of Hg found in the present study, neither the Hg environmental exposure of the general population from that area, nor the daily intake of Hg would be considered a hazard to the health of that population.

80 THE LOW DOSE TOXIC EFFECT OF MERCURIC SULFIDE *IN VIVO*

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Cinnabar, a natural mercuric sulfide, has been used in Chinese herbal medicine for over 2000 years. Today, it is still used in proprietary herbal medicine for children. Due to its insolubility, cinnabar is not considered to be toxic as compared to other notorious mercuricals. Recently, mercuric sulfide has been claimed to be absorbed in vivo. This study is then designed to examine the toxic potential of mercuric sulfide in vivo. Wistar rats were gavaged with 0, 5, 50, 250 and 500 mg/kg/day mercuric sulfide in methyl-cellulose for 3 days. The liver and kidney were checked for the presence of lipid peroxidation, as evidenced by thiobarbituric acid reactive substance (TBARS) and GSH/GSSG content, as well as oxidative DNA damage, as measured by 8-hydroxydeoxyguanosine (8-OH-dG) level. We did not detect any evidence of lipid peroxidation and oxidative DNA damage in hepatic tissues. In kidney tissues, we did not observe any changes in GSH and TBARS levels; however, we noticed the oxidative DNA damage as evidenced by a dose dependent elevation of GSSG, 8-OH-dG and mercury content. Electron microscopy revealed an increase of lysosomal bodies in kidney proximal tubular cells. In conclusion, this study indicated that mercuric sulfide may induce kidney cell damages in vivo as a result of oxidative stress.

81 MERCURY SPECIATION: DIFFERENCES BETWEEN INORGANIC MERCURY AND MERCURY-THIOL COMPLEXES IN $LLCPK_1$ CELLS

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In vivo, Hg(II) is generally thought to exist complexed with endogenous ligands and not as a free ion. Hg(II) compounds whenever identified in biological media, have been found complexed with molecules containing at least one sulfhydryl group. We have modeled Hg(II) ligand complexes using two independently developed computer programs (Martell & Motekaitis, and May). Models generated at physiological pH using published Hg(II) ligand Kfs for a variety of ligands suggest that Hg(II) will primarily exist as a $Hg(thiol)_2$ complex [$Hg(SG)_2$ or $Hg(Cys)_2$]. $Hg(SG)_2$, $Hg(Cys)_2$ and $Hg(CysGly)_2$ were then synthesized and identified (by NMR). $HgCl_2$, $Hg(SG)_2$, $Hg(Cys)_2$, $HgCl_2$ + 2GSH, $HgCl_2$ + 2Cys, $HgCl_2$ + 2CysGly, $HgCl_2$ + GSH, $HgCl_2$ + Cys and $HgCl_2$ + albumin were incubated with $LLCPK_1$ cells (a cultured pig kidney cell line) for 20 hrs. The Hg(II) concentrations tested ranged from 20–120 μM. Toxicity was determined by the XTT assay. In $LLCPK_1$ cells, 40–60 μM $HgCl_2$ caused complete cell death. Both the $Hg(SG)_2$ and $Hg(Cys)_2$ complexes protected from Hg(II) toxicity throughout the dosing range tested. Hg(II) transport studies are being conducted (supported by grant ES 4940).

82 EVIDENCE FOR TWO MECHANISMS INVOLVED IN THE RENAL TUBULE UPTAKE OF MERCURY

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The role of renal tubular γ-glutamyltransferase and organic anion transport on the renal uptake and accumulation and intrarenal distribution of injected inorganic mercury was investigated. Dispositional data were collected from, control rats, rats pretreated with two 10 mg/kg doses of acivicin, rats pretreated with a 10 mmol/kg dose of p-aminohippurate (PAH), and rats pretreated with both acivicin and PAH. The renal disposition of inorganic mercury (Hg^{++}) was evaluated in all four groups of rats one hour after a non-toxic, intravenous, 0.5 μmol/kg dose of mercuric chloride was administered. Radiolabelled inorganic mercury ($^{203}Hg^{++}$) was added to the injection solution containing the mercuric chloride to make it possible to quantitate the accumulation and disposition of mercury using standard isotopic methods. The renal concentration of mercury was significantly lower ($p < 0.05$) in the rats pretreated with either acivicin (10.0 ± 0.44 %dose/g; mean ± S.E.) or PAH (8.08 ± 0.83 %dose/g) than in the control rats (19.9 ± 1.06 %dose/g). More importantly, the renal concentration of mercury in the rats pretreated with both acivicin and PAH was significantly lower (3.44 ± 0.06 %dose/g) than in any other group. In addition, the data from the evaluation of the intrarenal distribution of mercury indicated that the renal cortex was the main zone in which the uptake of mercury was significantly affected by both acivicin and PAH. The findings from this study indicate that the renal tubular uptake of administered inorganic mercury, mainly along segments of the proximal tubule, is linked to the transport of organic anions and to the action of γ-glutamyltransferase. Thus, at least two mechanisms appear to be involved in the renal tubular uptake of inorganic mercury.

83 PARA-AMINOHIPPURATE (PAH) INHIBITS THE RENAL UPTAKE OF INORGANIC MERCURY

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In the present study, we evaluated in rats pretreated (intravenously) with a 10 mumol/kg dose of PAH the renal and hepatic disposition of inorganic mercury (Hg^{++}) 1 hour after the intravenous administration of a 0.5 μmol/kg dose of mercuric chloride. The PAH was administered either 5, 30 or 60 minutes prior to the injection of mercuric chloride. Radiolabelled inorganic mercury ($^{203}Hg^{++}$) was added to the injection solution containing the mercuric chloride to make it possible to quantitate the accumulation and disposition of mercury using standard isotopic methods. The content of mercury in the kidneys was significantly greater in control rats than in rats pretreated with PAH at all times of evaluation. When the dose of PAH was administered 5 or 30 minutes prior to the injection of mercuric chloride, the renal burden of mercury was as much as 1/3 less than that in corresponding control rats. Evaluation of the intrarenal distribution of mercury revealed that decrease in the renal content of mercury in the rats pretreated with PAH was due mainly to a decrease in the accumulation of mercury in the cortex. Some decrease in the accumulation of mercury was also observed in the outer stripe of the outer medulla. No statistically significant differences were detected in the disposition of mercury in the renal inner stripe of the outer medulla and papilla, liver or blood between the corresponding groups of control rats and the rats pretreated with PAH. Our findings demonstrate that the renal accumulation of mercury, particularly in the cortex, is significantly decreased during the initial hour after exposure inorganic mercury. These findings tend to indicate that the transport of the organic anion PAH interferes with the uptake of inorganic mercury along the proximal tubule. Perhaps inorganic mercury is transported into proximal tubular epithelial cells in some organic anionic form.

84 TRANSEPITHELIAL TRANSPORT, CELLULAR ACCUMULATION AND TOXICITY OF INORGANIC MERCURY IN ISOLATED-PERFUSED RABBIT CORTICAL COLLECTING DUCTS

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Rabbit cortical collecting ducts (CCD) were isolated and perfused *in vitro* with a saline solution containing 9.65 μM inorganic mercuric chloride ($^{203}HgCl_2$). Mercury transepithelial transport was assessed by the disappearance of $^{203}Hg^{++}$ from the lumen, its appearance in the bathing solution and its accumulation in the epithelial cells. Toxicity was assessed by the lumen-to-bath leak of the volume marker ^{3}H-L-glucose and the cellular uptake of the vital dye FD & C green. There was no evidence of acute toxicity during 50 minutes of perfusion. This was evidenced by a lack of vital dye uptake and by minimal leak (4.0 ± 1.1 fmol min^{-1} mm^{-1}) of ^{3}H-L-glucose. There was minimal transepithelial lumen-to-bath transport of mercury (6.16 ± 1.38 fmol min^{-1} mm^{-1}). Mercury disappearance rate from the luminal fluid was 33.5 ± 2.8 fmol min^{-1} mm^{-1}. The total cellular accumulation of mercury was 1518 ± 344 fmol mm^{-1}. Of this amount, $14.3 \pm 4.6\%$ was associated with the trichloroacetic acid (TCA) soluble fraction of the tubule, while $85.8 \pm 4.6\%$ was associated with a TCA-precipitable fraction of the tubule. This level of cellular accumulation and distribution of the mercury in the perfused CCD is similar to that found in segments of the proximal tubule perfused with 18.4 μM inorganic mercury. One major difference, however, is that inorganic mercury induced very severe cellular injury resulting in large rates of ^{3}H-L-glucose leak ($\cong$600 fmol min^{-1} mm^{-1}) in the proximal tubule. We conclude that there are substantial differences in the toxicity and transport of inorganic mercury between isolated perfused CCDs and proximal tubule segments.

85 METHOD FOR SCREENING DRUG AND CHEMICAL EFFECTS IN LABORATORY RATS USING COMPUTERIZED QUANTITATIVE ELECTROENCEPHALOGRAPHY

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A noninvasive, non-anesthetic method of quantitative electroencephalography (qEEG) that parallels human techniques of naturalistic stimulation, was developed and validated for regulatory testing of drugs and chemicals in rats. Sprague-Dawley rats (n = 6) were utilized in a treatment-by-subjects design to determine qEEG target parameter changes due to the M_2 autoreceptor agonist, oxotremorine. Repeated doses (0.05, 0.1, 0.2 mg/kg SQ) of oxotremorine caused significant increases in beta contribution (RB), a right shift in spectral edge frequency (SF90, MT50, TB90) and a decrease in alpha contribution (RA). In a second study, Fischer 344 rats (n = 48) were utilized in a randomized block design, to measure qEEG target parameters due to cholinesterase inhibition. Physostigmine was administered at doses of 0.05, 0.2 and 1.0 mg/kg IP, resulting in mean cholinesterase inhibitions in plasma of 28%, 38% and 70%, in erythrocytes of 19%, 24%, 36% and in terminal brain cholinesterase of 2%, 10%, 31%, which correlated well with increased total power and amplitude changes. Significant dose-related effects noted were a decrease in relative alpha and beta, an increase in relative delta, and spectral edge frequency was shifted to the left. Electrophysiology data correlated well with receptor-specific and general muscarinic effects, making a reliable contribution to analysis.

86 EPIBATIDINE: A PROBE FOR IDENTIFYING DIVERSE NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS (NACHRS)

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Neurotoxins isolated from the skin of several species of frogs have been useful for the structural and functional characterization of membrane ion channels. Recently, Spande *et al.* (J. Am. Chem. Soc. 114: 3475, 1992) isolated a neurotoxin, which they named epibatidine, from the Ecuadorean frog *Epipedobates tricolor.* This toxin was found to be a potent analgesic, and was proposed to act via a nicotinic mechanism. We have explored the agonist activity of epibatidine enantiomers on hippocampal neurons, which express multiple subtypes of functional nAChRs, using the whole-cell patch clamp technique. Epibatidine activated both type IA and type II currents that are blocked by methyllycaconitine and dihydro-β-erythroidine, respectively (Alkondon and Albuquerque, J.P.E.T. 265: 1455, 1993). Epibatidine acted as a potent and full agonist in eliciting type IA currents, with EC_{50}s of 2.9 μM and 4.3 μM for the (−) and (+) enantiomers, respectively. These effects were comparable to that of (+)anatoxin-*a* (AnTX). Epibatidine also behaved as a potent and full agonist in evoking type II currents, with EC_{50}s of 16 nM and 11 nM for the (−) and (+) enantiomers, respectively. However, AnTX was only a partial agonist in eliciting type II currents, with an EC_{50} of 100 nM. Among several agonists tested (including acetylcholine, AnTX, epibatidine, (−)nicotine, DMPP, and cytisine), epibatidine appears to have the highest selectivity in discriminating between the two types of currents, as revealed by the potency ratios for the two current types. (Support: NINDS Grant NS 25296; NIEHS Grants ES05730 and T32-ES07263).

87 *IN SITU* LABELING OF CEREBELLAR GRANULE CELLS FOR APOPTOSIS FOLLOWING EXPOSURE TO STYRENE OXIDE AND ACRYLAMIDE

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Administration of styrene oxide plus acrylamide (SO/ACR) to adult rats results in a cell-specific lesion in the anterior cerebellum characterized by pyknotic granule cells without involvement of adjacent Purkinje and glial cells. No changes are observed with either SO or ACR alone. This neuronal injury exhibits morphological and biochemical features of apoptosis. Ultrastructural studies performed 24 hrs after SO/ACR treatment (212 mg/kg SO, ip; followed 2 hrs later by 85 mg/kg ACR, ip) revealed condensation of granule cell chromatin into uniformly dense masses, shrinkage of cytoplasm with preservation of mitochondrial morphology in early stages, followed by cytoplasmic vacuolization and formation of cytoplasmic blebs. DNA fragmentation typical of apoptosis was assessed immunohistochemically using *in situ* tagging of nicked 3'-OH DNA ends with digoxigenin-ll-dUTP/dATP by terminal deoxynucleotidyl transferase. Immunoperoxidase staining of nicked DNA was confined primarily to granule cells of the anterior cerebellum (especially in the the depths of the sulci), corresponding to the distribution of the lesion. Glial cells also stained for DNA nicks in SO/ACR-treated animals, but did not exhibit morphological changes. Purkinje cells were not involved. While morphology and DNA nicks are not sufficient to prove apoptosis, data from this study suggest that apoptotic mechanisms are involved in SO/ACR-induced granule cell death in the adult cerebellum. (Supported by NIH ES-06356, ES-05022, ES-05955 and ES-04976.)

88 DIFFERENTIAL EFFECTS OF ACRYLAMIDE AND BIS-ACRYLAMIDE ON EXPRESSION OF GST ISOZYMES IN RAT CEREBELLUM

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Acrylamide, a neurotoxicant which is metabolized partly via the GSH conjugation pathway, has been reported to inhibit glutathione-s-transferase (GST) activity in brain and liver when administered chronically. The effects of treatment with acrylamide and its non-neurotoxic analogue, bis-acrylamide, on GST activity and GST isozyme protein expression in cerebellum and liver were examined in male Sprague-Dawley rats (250–350 g) administered either acrylamide or bis-acrylamide (50 mg/kg; i.p.) or saline for 10 days. Cytosolic 100,000xg fractions were prepared for GST (CDNB) assays and for Western blot analyses. Acrylamide treatment caused a statistically significant increase in GST activity in cerebellum (135 ± 6% of control) whereas bis-acrylamide treatment did not (93 ± 9%; $p < 0.005$). Western blot analyses showed that acrylamide treatment increased expression of GST α (Ya) and GST π by 3-fold and that of GST α (Yc) by 2-fold in cerebellum. Bis-acrylamide treatment did not change expression of GST α (Ya) or GST π in cerebellum, but there was a 1.5-fold increase in expression of GST α (Yc). In liver, bis-acrylamide treatment resulted in a significant increase in GST activity (161 ± 8.3%), whereas acrylamide treatment did not (124 ± 9%; $p < 0.005$). Both acrylamide and bis-acrylamide increased expression of liver GST α (Ya) and GST α (Yc) by 2-fold, but decreased GST μ expression by 30 and 50% respectively. Levels of GST π in liver and GST μ in brain were below levels of detection. In conclusion, acrylamide appears to differentially enhance cerebellar GST isozyme expression, an effect that is not elicited by its non-neurotoxic analogue, bis-acrylamide. This suggests a role for GST's in the neurotoxicity of acrylamide. (Supported by NIH grants ES-06356, ES-05022, ES-05955).

89 A COMPARATIVE STUDY OF THE EFFECTS OF TRI-ORTHO-TOLYL PHOSPHATE AND TRIPHENYL PHOSPHITE ON THE CENTRAL NERVOUS SYSTEM OF THE JAPANESE QUAIL

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The neurotoxic effects of single oral doses of tri-*ortho*-tolyl phosphate (TOTP) (500 mg/kg BW) or single subcutaneous injections of triphenyl phosphite (TPP) (62.5–500 mg/kg BW) were investigated in the Japanese quail (*Coturnix coturnix japonica*). Oral doses of TOTP resulted in no detectable clinical signs while injections of TPP resulted in mild ataxia to severe paralysis depending on dosage level. At 24hr postdosing, whole-brain activity of neuropathy target esterase (NTE) was inhibited by 90% in birds exposed to TOTP and by 11–87% in birds injected with TPP. Oral doses of TOTP resulted in only sparse Fink-Heimer silver-impregnated degeneration in the white matter of the cerebellum with no degeneration noted in any other area of the brain. Injections of TPP resulted in widespread degeneration in large numbers of brainstem nuclei and tracts, all cerebellar foliae and deep nuclei, and in midbrain and forebrain nuclei and tracts related to vision and audition. These results indicate that the Japanese quail responds differentially to exposure to TOTP and TPP. Oral doses of TOTP do not result in clinical signs or significant amounts of CNS degeneration even though NTE activity is inhibited by 90%. In contrast, injections of TPP at higher dosage levels yield severe clinical signs, widespread axonal degeneration, and significant inhibition of NTE activity. The presence of degeneration in forebrain visual and auditory centers in addition to that seen in the brainstem and cerebellum indicates that exposure to TPP may also affect the transmission and processing of higher order visuomotor, visual sensory, and auditory sensory information. The sharp dichotomy in the relative sensitivity to TOTP and TPP in the Japanese quail suggests that each compound may have its own unique effect on CNS structure and function. In addition, the relationship between levels of NTE inhibition and onset of clinical signs or neuropathology remains unclear.

90 EFFECTS OF CARBON TETRACHLORIDE AND GALACTOSAMINE ON RAT BRAIN NITRIC OXIDE SYNTHASE

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Previous studies have established that the administration of carbon tetrachloride (CCl_4) or galactosamine (Gal-N) to experimental animals is associated with severe damage not only to the liver but also to several other organs. Nitric oxide(NO) is a novel messenger that plays an important physiological role and has been found to attenuate several types of tissue injury caused by ischemia, re-perfusion and endotoxemia. The aim of this study was to determine *in vivo* the effects of both CCl_4 and Gal-N on rat brain nitric oxide synthase (NOS). NOS activity was determined by monitoring the formation of ^{3}H-citrulline in the brains of rots treated with either CCl_4 (0.5 ml/kg) or Gal-N (0.4/kg) 24 hours prior to sacrificing the animals. The results showed that NOS activity was reduced significantly in CCl_4 treated rats as compared to controls. Whereas, the Gal-N treated rats showed increased NOS activity. It is apparent that CCl_4 caused a significant inhibition of NOS whereas Gal-N displayed a stimulatory effect. In conclusion: Both CCl_4 and Gal-N have profound effects on rat brain NOS activity that are diametrically opposite.

91 ISOLATION OF HIBERNATION RELATED PROTEINS

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Our previous work indicates that a Hibernation Induction Trigger (HIT) molecule derived from the plasma of deeply hibernating woodchucks may act as an endogenous opioid. When infused I.C.V. or I.V. into primates, it exerts profound effects mimicking a hibernation-like state. These effects are all reversed or retarded by the infusion of the opiate antagonists naloxone or naltrexone. Furthermore, the *delta* opiate receptor agonist D-Ala^2-D-Leu^5-Enkephalin (DADLE) is able to induce hibernation in summer-active ground squirrels in a manner similar to those injected with HIT, suggesting this *delta* opioid mimics the action of the HIT molecule.

Plasma albumin fractions, isolated via affinity chromatography using Affi-Gel Blue as the matrix, from both summer-active and winter hibernating woodchucks were compared for compositional and concentration differences using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Proteins from the hibernating woodchuck albumin fractions at 175 and 89 kD and from the summer-active woodchuck albumin at 77.5 kD were selected for isolation and purification. Proteins were purified by anion exchange chromatography using Fast Performance Liquid Chromatography, Preparative SDS-PAGE, and Electro-Elution.

Purified proteins were subjected to proteolytic cleavage. Major components were isolated by HPLC and molecular weight determined by mass spectrometry. N-terminal sequence analysis of internal sequences was performed using the Edman degradation technique. Full protein determination and testing involving cloning and antibody preparation is ongoing. (This work is supported in part by the U.S. Army Res. and Devel. Command under Contract No. DAMD 17-92-C-2026, the VA Med. Research Funds, and the Univ. of KY Med. Research Fund).

92 FUMONISIN B1 (FB1) INCREASES SPHINGANINE (A PRECURSOR OF CERAMIDE SYNTHESIS) IN THE BRAIN AND SPINAL CORD OF DEVELOPING RATS

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FB1, a metabolite from *Fusarium moniliforme*, induces neurologic disorders in horses and carcinogenesis in rats. FB1 has been reported to alter sphingolipid metabolism in vivo and in vitro. Brain tissue contains abundant sources of sphingomyelin and gangliosides, the key components in the myelination of axons in brain and spinal cord. The objectives of this experiment were to test the hypothesis that FB1 alters sphingoid base levels and myelin synthesis in the central nervous system of the developing rat. FB1 (98% purity, 0.1–1.0 mg/kg/day) was subcutaneously administered from postnatal day (PND) 3 to PND 12. Sphingosine (So) and sphinganine (Sa) levels in the forebrain (FRB), brainstem (BS), and spinal cord (SC) were measured and myelin deposition was quantified in corpus callosum (CC) of FRB. FB1 0.4 mg/kg/day (0.4FB1) and 0.8 mg/kg/day FB1 (0.8FB1) resulted in 86% and 70% survival, and in 63% and 52% body weight gain of saline controls, respectively. Sa levels were significantly increased in FRB (5-fold), BS (3-fold), and SC (2-fold) in the 0.8FB1 group. FB1, however, had no effects on So levels. Hence, 0.8FB1 increased the Sa/So ratio in FRB (4-fold) and 3-fold in both BS and SC. The Sa/So ratio in BS of 0.4FB1 rats also increased significantly. In order to confirm effects of limited nutrition (LN) on Sa levels, 0.8FB1 and LN groups were compared to controls. Sa levels and Sa/So ratios were increased significantly in the 0.8FB1 group, but were unchanged in the LN group, indicating that sphingolipid metabolism in the central nervous system of developing rats is

vulnerable to FB1. Myelin deposition decreased significantly in CC of FRB in both the LN and FB1 groups. These data indicate that FB1 and LN alter myelin deposition by different mechanisms.

93 ASSESSMENT OF IVERMECTIN'S ANTICONVULSANT MECHANISM BY ITS EFFECT ON CONVULSIONS INDUCED BY ANTIGABAERGIC OR NON-ANTIGABAERGIC CONVULSANTS

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To elucidate the mechanism whereby ivermectin mediates its anticonvulsant action against seizures induced by intermittent photic stimulation in photosensitive epileptic chickens, 10 epileptic and 10 non-epileptic chickens were pretreated with an anticonvulsant dose of ivermectin (5.0 mg/kg, subcutaneously) 6 hours after injection of the antiGABAergic convulsants bicuculline, methyl-β-carboline-carboxylate(β-CCM), picrotoxin, pentylene-tetrazol (PTZ), and the non-antiGABAergic convulsant strychnine. These induced convulsions in all tested chickens (ED_{100} dose). The maximal anticonvulsant dose of ivermectin exerted little or no effect on the incidence of seizures induced by the ED_{100} dose of the bicuculline and β-CCM. Similarly, ivermectin provided no protection against seizures produced by the ED_{100} dose of the non-antiGABAergic convulsant strychnine. However, ivermectin provided a significant decrease in the incidence of seizures induced by picrotoxin and pentylenetetrazol. These findings suggests that the anti-convulsant action of ivermectin may be achieved by its action at the chloride channel moiety or the PTZ component of the benzodiazepine-GABA-chloride ionophore complex of $GABA_A$ receptors.

94 NEUROPROTECTIVE EFFECT OF KYNURENINE + PROBENECID AGAINST QUINOLINATE-INDUCED NEUROTOXICITY

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Quinolinic acid (QUIN) is an endogenous excitotoxin acting on NMDA-type glutamate receptors. When administered intra-striatally to rats, QUIN destroys GABA-ergic neurons specifically. On the other hand, kynurenic acid (KYNA) is able to block QUIN neurotoxicity. KYNA levels in brain increase after the administration of kynurenine (KYN) its metabolic precursor and also after probenecid injection. We found that KYN injection protected rats against QUIN neurotoxicity at 300 but not at 150 doses. Probenecid was also neuroprotective at 200 and 300 mg/kg doses. KYN + Probenecid combination treatment significantly protected rats at 150 + 100 mg/100 mg/kg doses respectively. Results suggest that increased KYNA levels induced both by probenecid and KYN are able to prevent QUIN neurotoxicity. Supported by CONACYT (México) grant 3525M.

95 ABILITY OF VITAMIN D_3 SULFATE TO DECREASE LIGAND BINDING AT THE CENTRAL BENZODIAZEPINE RECEPTOR

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Vitamin D_3, through its metabolite the 1,25-dihydroxy derivative of vitamin D_3 [$1,25(OH)_2D_3$], functions as a steroid hormone in mammals. Ligand binding studies indicate that there are distinct receptors for $1,25(OH)_2D_3$ in the brain. In present study the ability of vitamin D_3 sulfate (cholecalciferol sulfate) to decrease ^{3}H-flumazenil specific binding in homogenate of rat cerebral cortex was determined. Incubations were conducted at 0–4°C. Specific binding was measured as the difference between the ^{3}H-flumazenil bound in the absence and presence of 1 uM clonazepam. Vitamin D_3 sulfate produced a dose dependent decrease in ^{3}H-flumazenil specific binding. However, 1 uM of vitamin D_3 sulfate only reduced the specific binding by one third. Provitamin D_3 (7-dehydrocholesterol) was slightly less active in decreasing ligand binding at the central benzodiazepine receptor site. In summary, cholecalciferol sulfate and 7-dehydrocholesterol can only weakly decrease ^{3}H-flumazenil binding at the central benzodiazepine receptor site.

96 AN AUTORADIOGRAPHIC STUDY ON [^{3}H]FLUNITRAZEPAM(FNZ) BINDING SITES IN THE BRAIN OF RAT MADE TOLERANCE TO AND DEPENDENCE ON PENTOBARBITAL(PB)

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The changes in benzodiazepine binding sites in 37 discrete brain regions of rat brains made tolerant to and dependent on PB were examined. Animals were made tolerant by infusion with pentobarbital (300 μg/hr, i.c.v., for 6 days) through pre-implanted cannulae. The PB-dependence was assessed 24 hr after abrupt withdrawal from PB. Tissue sections were incubated with 1 nM [^{3}H]FNZ at 4°C for 90 min. One-way ANOVA was used to determine statistical significance among four groups. In the PB tolerant rats, a significant increase in [^{3}H]FNZ binding sites was found in layer IV of frontal cortex, caudate-putamen and the molecular layer of olfactory bulb, as compared to the control group. In the PB dependent rats, [^{3}H]FNZ binding sites were significantly increased in globus pallidus, ventral pallidum, and olfactory tubercle, in addition to those regions found in the tolerant group. The changes in [^{3}H]FNZ binding sites were also significantly different from those in the tolerant rats. There was, however, no significant difference in the cerebellum and hindbrain in both of the PB-treated groups. These results suggest that benzodiazepine receptors in the forebrain may be involved in the development of PB intoxication, and that tolerance to and dependence on PB may be mediated through different mechanisms. (Supported by NIDA 4480).

97 INVOLVEMENT OF PROTEIN KINASE C IN THE DEVELOPMENT OF DEPENDENCE ON OPIOIDS

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The effects of a protein kinase inhibitor, H-7, on the behavioral signs of naloxone (opioid antagonist) precipitated withdrawal syndrome and the protein kinase C (PKC) activity in the pons/medulla regions in butorphanol ($\mu/\delta/\kappa$ mixed opioid agonist)- and morphine (μ-opioid agonist)-dependent rats were investigated. Appearance of physical dependence produced by i.c.v. infusion of butorphanol (26 nmol/μl/hr) or morphine (26 nmol/μl/hr) for 3 days, as evaluated by naloxone (5 mg/kg, i.p.)-precipitated withdrawal signs, was dose-dependently reduced by concomitant infusion of H-7 (1 and 10 nmol/μl/hr). In the same condition, butorphanol and morphine chronic treatment enhanced (26.3% and 28.1% enhancement over the saline treated group, respectively) cytosolic PKC activity in the pons/medulla, but not in the membrane fraction. Furthermore, concomitant infusion of H-7 inhibited the enhancement of PKC activity. These results suggest that the enhancement of cytosolic PKC activity in the pons/medulla might play an important role in the development of dependence on opioids. (Supported by NIDA DA-05828).

98 ANATOMICAL DISPOSITION, RECEPTOR BINDING AND CLEARANCE OF THE NEUROCONVULSANT TRIMETHYLOLPROPANE PHOSPHATE (TMPP)

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TMPP, a potent convulsant produced through the pyrolysis of certain synthetic lubricants, progressively induces EEG paroxysms, sub-clinical and clinical seizures, and lethality in rats and other species. Unlike similar chemical convulsants, such as pentylenetetrazol (PTZ), repeated dosing of TMPP kindles EEG epileptiform activity without development of behavioral seizures. Single doses of TMPP, below those required to observe EEG paroxysms, reliably potentiate full-blown audiogenic seizures in several non-susceptible rat strains. TMPP administration has recently been shown to significantly enhance hyperactivity response to amphetamine challenge several weeks post-injection, and may provide a model for CNS sensitization.

The present study investigates the anatomical disposition and clearance of radiolabeled TMPP in both major brain regions and body tissues. TMPP, shown to produce no detectable metabolites, was 99%+ cleared from all body tissues 24 hours post-administration, with no accumulation of TMPP in any major tissue, including fat, after 5 daily administrations. At the time of clinical seizure the highest ^{14}C-TMPP counts in the CNS were in cerebellum, striatum and hippocampus. Autoradiographic analysis verified these results. Silver stain analysis indicated no detectable cellular damage in rats dosed with TMPP at a level sufficient to induce clinical seizures. Ligand binding studies indicated limited binding of ^{14}C-TMPP at $GABA_A$ and $GABA_B$, but not muscarinic alpha-1 or alpha-2 receptors.

99 TOXICOKINETICS OF THE NEUROTOXIN DOMOIC ACID IN COMMERCIAL SEAFOOD SPECIES

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Domoic acid (DA) is a naturally occurring secondary amino acid that exhibits high affinity for glutamate receptors of the central nervous system. Human DA intoxication and subsequent memory loss following consumption of intertidal mussels and clams has now been termed amnesic shellfish poisoning. DA is produced by several species of *Pseudonitzschia,* a diatom ubiquitous in coastal marine waters. Filter feeding organisms such as mussels, which readily consume phytoplankton in their diet, can concentrate and serve as a vector of the potent neurotoxin to human consumers. Additionally, crabs can obtain DA through the direct ingestion of detritus or through food chain transfer by consuming toxic mussels. In order to determine the fate of DA, uptake and depuration rates were determined in the edible mussel *Mytilus edulis* and the Dungeness crab, *Cancer magister;* rates were compared between exposures to aqueous DA versus dietary DA. DA was quantified by high-pressure liquid chromatography following precolumn derivatization and fluorescence detection of exposure media, algal suspensions, and animal tissues. Aqueous exposures of both mussels and crabs consisted of DA in filtered seawater, followed by clean seawater exposure; for feeding trials, batch cultures of DA-producing diatoms, originally isolated from local waters, were maintained under controlled laboratory conditions. Diatom cultures producing known amounts of DA were fed to replicate groups of mussels for 48 to 96 h, followed by clean filtered seawater for an additional 48 to 120 h, in a flow-through exposure system; aqueous DA exposures followed the same time regimes as dietary exposures. Additionally, mussels exposed to toxic diatoms were fed to individual crabs, to mimic natural food chain transfer of DA. Uptake and depuration increased in feeding trials versus aqueous exposures, possibly due to the low water solubility of DA. Ultimately, the determination of DA toxicokinetics in edible species will assist in setting regulatory levels for consumption of impacted species and the establishment of adequate quarantine periods following seasonal blooms of toxin-producing diatoms.

100 REQUIREMENT FOR ADEQUATE GLYCINE FOR THE DETOXIFICATION OF BENZYL ACETATE AND MAINTENANCE OF ITS DETOXIFICATION PATHWAYS

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Because glycine plays a large role in the detoxification of benzyl acetate (BA), a deficiency of this amino acid may arise when excessive doses of BA are administered to the growing animal. We studied the influence of supplemental glycine on BA induced toxicity including BA-induced brain, skeletal muscle, liver, and kidney lesions as well as biochemical intermediates in glycine requiring pathways. Groups of male F344 rats were fed NIH-07 diet containing 0, 20,000, 35,000, or 50,000 ppm BA for up to 28 days. Two additional groups were fed NIH-07 diet with 50,000 ppm BA and 27,000 ppm glycine or 50,000 ppm BA and 32,000 ppm alanine. The supplemental alanine and glycine were equimolar. A third additional group was fed NIH-07 with 32,000 ppm alanine and served as an isonitrogenous control. The alanine groups were added in order to show that the effect seen with glycine was glycine specific and was not simply due to added amino nitrogen. Mortality rates were: 0%, 0%, 10%, and 100% for rats receiving 0, 20,000, 35,000, or 50,000 ppm BA as compared to 0%, 10%, and 100% for rats receiving 32,000 ppm alanine, 50,000 ppm BA and 27,000 ppm glycine or 50,000 ppm BA and 32,000 ppm alanine. BA caused a dose-related body weight loss that was alleviated by glycine but not alanine. Neurobehavioral signs such as ataxia and convulsions were observed in all groups receiving BA alone or in combination with alanine. None were seen in rats receiving BA and glycine. The BA induced histopathologic lesions in the brain, skeletal muscle, liver, and kidney lesions were correlated to the levels of benzoic acid, hippuric acid, and pyroglutamic acid in the urine, and glycine, serine, and pyroglutamic acid in plasma, and serine, glycine and benzoyl Coenzyme A in the brain.

These results show that adequate levels of glycine protect against the toxicity of BA and related compounds normally detoxified by glycine requiring pathways.

101 COMPARATIVE ACUTE TOXICITY AND IRRITANCY OF 2 CHEMICAL FORMULATIONS CONTAINING N,N,N′,N′-TETRA- METHYLETHYLENEDIAMINE (TMEDA)

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Crude TMEDA, an industrial intermediate and tertiary amine mixture with 49.8% TMEDA, was tested for acute toxicity and irritancy. Aqueous dilutions of Crude TMEDA produced peroral LD50s of 469 mg/kg (female rats) and >500 mg/kg (males). Clinical signs of neurotoxicity were observed; however, no treatment-related microscopic lesions were seen. The percutaneous LD50s for rabbits were 1.75 g/kg (females) and <2.0 g/kg (males). A single static inhalation exposure to saturated vapor resulted in an LT50 of 2.12 hr (both sexes). Crude TMEDA was corrosive to rabbit skin in 4- and 1-hr irritation tests, but not in a 3-min test. Because clinical signs indicative of neurotoxicity were apparent in the peroral LD50 test with Crude TMEDA, a more pure formulation (98.9%) was tested. Peroral LD50s for female and male rats dosed with aqueous dilutions of TMEDA (98.9%) were 406 and 891 mg/kg, respectively. Peroral doses of undiluted TMEDA (98.9%) to rats produced LD50s of 268 mg/kg (females) and >250 mg/kg (males). Neurotoxic signs, as well as brain, spinal cord and/or peripheral nerve lesions, were observed microscopically in both peroral tests with TMEDA (98.9%). Percutaneous LD50s in the rabbit were 1.23 g/kg (females) and >1.0 g/kg (males). In the static inhalation test, the LT50s for rats were 30.8 min (females) and 25.9 min (males). A dynamic inhalation exposure to saturated vapor (rats) resulted in LT50s of 1.44 hr (females) and 1.25 hr (males). Corrosion was evident in all 3 rabbit skin irritation tests with TMEDA (98.9%). Both TMEDA formulations (0.01 ml) produced severe rabbit eye injury. *In conclusion*, the peroral LD50s (using dilutions), percutaneous LD50s and eye irritation results were essentially similar for both formulations. TMEDA (98.9%) was markedly more toxic than Crude TMEDA by acute inhalation and more irritating to rabbit skin. In addition, the peroral neurotoxic potential was more apparent with TMEDA (98.9%) than with Crude TMEDA. (Supported by OSi Specialties, Inc., Sistersville, WV.)

102 EVALUATION OF GENETIC SUSCEPTIBILITY TO LEAD INTOXICATION USING AUTO-ANTIBODY TITERS TO NERVOUS SYSTEM PROTEINS

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Lead intoxication is one of the most widespread environmentally-induced afflictions in the human population, yet many questions remain regarding the mechanisms of lead-induced neurotoxicity and its effects on immune responses. We hypothesize that lead may cause neurotoxicity by disruption of the blood-brain barrier (BBB). BBB dysfunction may expose antigenic proteins of the nervous system to circulating immune cells and produce changes in antibody titers in the serum.

To explore this hypothesis, we have used two mouse strains which differ in the gene coding for δ-aminolevulinate dehydratase (ALAD), an enzyme implicated in susceptibility to lead intoxication in humans. Although the genetic difference we find in these mouse strains appears to be quantitative rather than qualitative, we show that this model parallels observations made in humans. Our findings show that mice with a duplication of the ALAD gene (strain D) accumulate more lead in their blood than mice with a single copy of the gene (strain C) when exposed to the same acute oral doses of lead during adulthood. Also, female mice showed higher titers than males.

Assessment of BBB function with intravascular tracers and immunocytochemistry for extravasated serum proteins showed increased BBB permeability in strain D animals. These animals also showed higher antibody titers to many CNS proteins as compared to strain C.

The data reported here suggest dysfunction of the BBB can occur in susceptible animals exposed during adulthood and that these animals are also more likely to produce higher auto-antibody titers to CNS components.

103 SELECTIVE IMMUNOLOGICAL TARGETING OF PURKINJE CELLS BY OX7-SAPORIN

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Saporin, a plant toxin derived from *Saponaria officianalis,* disrupts protein synthesis by inactivating the 60S portion of the ribosome. OX7 is a mouse monoclonal antibody directed against the Thy-1 receptor selectively expressed on

subpopulations of central nervous system neurons including Purkinje cells. Covalent attachment of OX7 to saporin optimizes delivery of saporin to target neurons. OX7-saporin was used to study the behavioral and morphological consequences of selective Purkinje cell destruction. Male S-D rats (250–300 g) were bilaterally injected i.c.v. with 1- or 2 μg OX7-saporin or 8 μl artificial CSF. Rats were tested for behavioral changes 1, 2, and 8 weeks post-treatment. Behavioral assessment demonstrated dose- and time-dependent changes in motor performance and activity, but no significant effect on foot splay or geotaxis. Following behavioral testing cerebella were prepared for microscopic examination and Purkinje cells in anatomically matched sections counted. OX7-saporin induced dose-dependent death of Purkinje cells, particularly in the anterior and superior portions of cerebellar folia 1–6 and folium 9. Other brain regions were unaffected. Data suggest that OX7-saporin is an effective model with which to examine the consequences of selective Purkinje cell destruction. (Supported by ES 04976 and NSF IBN 9222097).

105 ANATOMICAL AND PHYSIOLOGICAL ASPECTS OF MICROPIG EYE AS A POSSIBLE TARGET ORGAN IN TOXICOLOGY

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Gross examination, ocular reflexes, esthesiometry, indirect ophtalmoscopical and biomicroscopical examinations and tonometry were performed in eighteen 6 to 8-week-old microbiologically defined and in forty-nine 2 to 10-month-old conventional Göttingen micropigs. Pachymetry was performed in 3 Göttingen and 10 Yucatan micropigs.

Ophtalmological findings often consisted of embryonic remnants (hyaloid artery, pupillary membrane), the incidence of which seemed to decrease with age although not statistically. The most noteworthy findings were as follows in decreasing incidence: hyaloid artery remnants (microbiologically defined: 83.3%, conventional micropigs: 76.5%), tigroid fundus (microbiologically defined: 72.2%, conventional: 75.5%), slight lens opacitis (microbiologically defined: 38.9%, conventional: 41.8%) and pupillary membrane remnants (microbiologically defined: 33.3%, conventional: 21.4%). The most important findings were either considered to be congenital in origin or of undetermined etiology.

Interestingly, the visual capabilities of the micropigs were not affected. Moreover, the aspects of micropig eye showed a number of anatomical and physiological similarities with the human eye. The micropig is therefore considered a good animal model for the assessment of adverse ocular effects in eye toxicity studies as well as in general toxicity studies with the eye as a possible target organ.

106 NEUROTOXICOLOGIC EXAMINATION OF RATS EXPOSED TO 1,1,2,2-TETRACHLOROETHYLENE (PERCHLOROETHYLENE) VAPOR FOR 13 WEEKS

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Large evoked potential and EEG changes occurred in a pilot study in Fischer 344 rats *during* exposure to 2000 ppm of 1,1,2,2-tetrachloroethylene (perchloroethylene; 'perc'), a cleaning solvent with anesthetic properties. In the main study, rats were evaluated for persistent nervous system effects the week following exposure to 0, 50, 200 or 800 ppm perc for 6 hours/day, 5 days/week, for 13 weeks.

The only effect possibly related to treatment was in the flash evoked potential (FEP), recorded from the visual cortex. The longer latency potentials (N_3) of the FEP had a somewhat greater amplitude in the 800-ppm perc group. The FEPs were of normal shape and latency. Although mild neurotoxicity could not completely be ruled out, amplitude changes in N_3 can occur for a variety of psychophysiological reasons other than neurotoxicity. Consequently, as a stand-alone finding, there was no toxicologic significance attributed to the larger FEP in the 800-ppm exposure group.

Other data did not support a diagnosis of neurotoxicity. No treatment-related alterations were noted in the functional observational battery, in the FEP recorded from the cerebellum (as opposed to visual cortex FEP), or in auditory, somatosensory or caudal nerve evoked potentials. No treatment-related lesions were noted during histopathologic examination of eyes, optic nerves, optic tract, or multiple sections of brain, spinal cord, peripheral nerves or limb muscles. Sponsored by the Halogenated Solvents Industry Alliance.

107 RETINAL ATROPHY AND VISUAL DYSFUNCTION WITH AGING IN FISCHER 344 RATS

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To investigate age-related changes in the retina and visual function, male and female Fischer 344 (F344) rats of 4, 9, 12, 15, 18, 24, and 30 months of age were examined histopathologically and electrophysiologically. Histopathologic examination of the retina revealed atrophy of photoreceptor cells on the outer nuclear and photoreceptor layers with aging. Immunohistochemically, increased reactivity to anti-glial fibrillary acid protein antibody was observed in the retina of the animals which were over 15 months old, but no changes were seen in reactivity to anti- S-100 protein or neuron specific enolase antibodies. Electroretinograms showed reduced amplitudes in rats over 18 months old and were not evoked in 30-month old rats. The selection rate for the dark area in a T-shaped test box was 80% in rats of 30 months of age, and ability of light-dark discrimination was markedly reduced. These results indicate that the number of photoreceptor cells is decreased with increasing age in F344 rats, resulting in the reduced reactivity to light and the depressed light-dark discrimination.

108 EFFECTS OF ETHANOL ON THE RESPONSE OF THE BOVINE ISOLATED IRIS TO PHARMACOLOGICAL AGENTS

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Alcohol-induced changes in pupil diameter has been used as an index of autonomic nervous system dysfunction in humans (Rubin et al. J. Stud. Alcohol. 41: 611, 1980). Since the pharmacological/toxicological basis of this response is unknown, the present study investigated the effects of ethanol on the response of the bovine iris to pharmacological agents. Iris smooth muscle was isolated from bovine eyes, set up in 25 ml organ baths containing Krebs solution at 37°C and gassed with 95% O_2; 5% CO_2. Isometric tension was recorded via Grass FTO3 transducers and displayed on a polygraph. Ethanol (10–100 mM) caused a concentration-dependent attenuation of maximal contractile responses to carbachol, pilocarpine and prostaglandin $F_{2\alpha}$ and a 2.5-fold decrease in pilocarpine potency at the highest ethanol concentration tested. Furthermore, ethanol (100 mM) caused a 30% decrease in the contractions produced by KCl (50–100 mM). Under conditions of induced tone, ethanol (100 mM) produced significant ($p < 0.001$) right-ward shifts of concentration response curves to the relaxants isoproterenol and nicotine, but had no significant effect on relaxations caused by papaverine. We conclude that ethanol impairs both contractile and relaxant responses of the bovine iris smooth muscle to pharmacological agents.

109 FOLATE AND 10-FORMYLTETRAHYDROFOLATE DEHYDROGENASE (FDH) IN RAT CENTRAL NERVOUS SYSTEM (CNS)

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Methanol poisoning is characterized by CNS toxicity from formate accumulation. The final step in formate metabolism requires tetrahydrofolate and FDH. Total tissue folate was quantified by microbiological assay. FDH was localized by immunohistochemistry (IHC) in matured, primary cultured neonatal cells and in sectioned adult CNS. Folate levels in retina, optic nerve, and brain regions were 0.8, 1.1, and 2.0–3.5 nmol/g, respectively. Folate levels correlated with levels of the folate-binding FDH. The presence of both folate and FDH in CNS tissues suggests that formate metabolism occurs in the CNS. IHC of CNS cells showed FDH expression in purified cultures of cortical astrocytes or neurons and in mixed spinal cord cultures. CNS sections showed that glia were intensely immunostained, whereas neurons were only weakly stained. These differences suggest that glia serve a protective role against formate toxicity, and may also explain why neuronotoxicity results from methanol poisoning. Supported by a grant from the NutraSweet Co. (TRT), and by NIH grants AA06916 (MWM), AA07568 (MWM), and NS29844 (STM).

110 10-FORMYLTETRAHYDROFOLATE DEHYDROGENASE (FDH) AND FOLATE IN HUMAN RETINA AND BRAIN REGIONS

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Ocular toxicity resulting from methanol poisoning occurs as a consequence

of formate accumulation in sensitive species, such as humans and primates. Studies were conducted to determine whether components of formate oxidation, FDH and folate, were present in human retina and brain. Levels of FDH were quantified by Western blot analysis; total tissue folate was determined by microbiological assay. Results from postmitochondrial and mitochondrial preparations of six human retinae showed mean FDH levels of 0.8 ± 0.4 and 0.9 ± 0.3 ng/μg protein ± S.D., respectively. Results from three human retinae showed mean folate levels of 0.14 ± 0.04 nmol/g tissue ± S.D. Levels of FDH and folate from various regions of one human brain ranged from 2.2–5.9 ng/μg protein and 1.4–2.8 nmol/g tissue, respectively. The presence of FDH and folate demonstrate the capacity for formate oxidation in tissues sensitive to methanol (formate) poisoning. Supported by a grant from The NutraSweet Co.

111 INHIBITION OF RETINAL MITOCHONDRIAL FUNCTION IN METHANOL INTOXICATION

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Formic acid is the toxic metabolite responsible for the metabolic acidosis and visual toxicity characteristic of methanol poisoning. Formic acid has been hypothesized to disrupt visual function by inhibition of the mitochondrial enzyme, cytochrome oxidase (C.O.). To test this hypothesis, we measured C.O. activity and ATP concentrations in retina, liver, kidney and heart in a rodent model of methanol-intoxication developed in our laboratory (Eells, 1991). Methanol-intoxicated rats developed formic acidemia, metabolic acidosis and retinal dysfunction. Morphologic studies coupled with C.O. histochemistry revealed mitochondrial disruption and a profound reduction in retinal C.O. activity in methanol-intoxicated rats compared to control animals. Biochemical measurements also showed a significant reduction in retinal C.O. activity (43.2 ± 6% of control) and retinal ATP concentrations (59.6 ± 1.5% of control) in methanol-intoxicated rats relative to control animals. Surprisingly, no differences from control values were observed in hepatic, renal or cardiac C.O. activity or ATP concentrations in methanol-intoxicated rats. The reduction in retinal function, inhibition of retinal C.O. activity, depletion of retinal ATP and mitochondrial disruption produced in methanol-intoxicated rats are consistent with the hypothesis that formate acts as a retinal mitochondrial toxin in methanol poisoning. (Supported by The American Petroleum Institute and NIH grants RO1-ES06648 and P30-EYO1931).

112 ROLE OF THE MÜLLER CELL IN METHANOL-INDUCED RETINAL TOXICITY

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Previously we reported that the folate reduced (FR) rat is an excellent animal model which mimics the characteristic human methanol (M) toxicities. The present study examines the role of the Müller cell in the initiation of M-induced retinal toxicity and compares blood and ocular levels of formate at a sub-maximal M dose. A dose-related reduction in electroretinogram (ERG) b-wave amplitude is observed 48 hrs after methanol administration in FR rats. This effect is observed after treatment with either M, the Müller cell toxin α-aminoadipic acid (α-AAA) or the glutamate analogue 4-aminophosphonobutyrate (APB). However, the Müller cell can be depolarized *in situ* by increasing the regional extracellular [K^+] after APB treatment, but not after M or α-AAA treatment suggesting that direct Müller cell toxicity is responsible for the ERG changes observed after M treatment. Decreases in retinal ATP levels are also similar in M and α-AAA treated rats. Pharmacokinetic studies demonstrate that blood (B), vitreous humor (VH) and retinal (R) tissue concentrations of formate are maximal 48 hrs after 2.0 g/kg M. However, R formate levels remain elevated while those of B and VH decrease by 72 hrs. The ERG b-wave amplitude is decreased at 48 hrs and remains depressed at 72 hrs. Both the ERG response and formate concentrations return to normal by 168 hrs.

113 THERMALDENATURATION OF BOVINE EYE LENS PROTEINS AS INFLUENCED BY NUTRITIONAL TRACE METALS (Co, Cu, Mn, AND Zn) AND NON NUTRITIONAL TOXIC METALS (Cd, Pb, NiAND Ag)

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Cataract, a cloudiness in the eye lens interfering with vision, is caused by oxidation and subsequent polymerization of the proteins. These changes in protein have been attributed to several factors, mostly unrelated. In laboratory animals, thalium compounds have been used to produced drug-induced cataracts. In the present study the effect of various nutritional and toxic metals on thermaldenaturation of bovine eye lens proteins have been investigated using *in vitro* models.

The 100,000xg supernatant of homogenate in water was adjusted to 9 mg/ml concentration of proteins and incubated with different concentrations of metal salts at 60° from 0 to 8 hrs. The denaturation caused was monitored turbidometrically at 555 nm and by protein and sulfhydryl (SH) content determination in soluble and insoluble fractions at 0, 2, 4, and 8 hours of incubation. The nature of the soluble and precipitated proteins was evaluated by SDS-PAGE.

The results indicate that the rate of denaturation by metals as measured by following the turbidity changes differed with the metals studied. High rates of denaturation were observed with Zn, Cd, and Cu. Ni, Co and Ag caused denaturation at lower rates while the rates with Pb and Mn were intermediate. The soluble protein concentration decreased and the amount of precipitated protein increased with duration of incubation. The SH content decreased with time in the following order: Cu < Ag < Cd, Zn, Co, Pb < Mn, and Ni. SDS-PAGE revealed that the protein profiles for soluble and insoluble proteins were different and were influenced by the metal used. Interestingly no high molecular weight polymers were formed by any of the metals studied.

These results indicate that the metals tested in this study, like thalium, if ingested, absorb and accumulate in the lens, potentially leading to lens protein oxidation. Thus, metal may possibly play a role in cataract formation.

114 LEAD DIFFERENTIALLY DECREASES RETINAL AND RENAL Ca^{2+}-ACTIVATED Mg^{2+}-DEPENDENT (Ca^{2+},Mg^{2+})-ATPaseACTIVITY

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Developmental lead exposure (0–21 days of age: doa) results in the inhibition of rat retinal, but not renal, Na^+,K^+-ATPase activity whereas in vitro Pb^{2+} inhibits both sites: albeit with differing sensitivities. The current studies on Ca^{2+},Mg^{2+}-ATPase, another important ion-motive P-type ATPase, were designed to: 1) determine the short-term (21 doa) and long-term (90 doa) dose-response effects of low and moderate level developmental lead exposure, 2) determine the concentration-dependent effects of in vitro Pb^{2+} exposure and 3) compare results with those on Na^+,K^+-ATPase. Oligomycin-insensitive, vanadate- and calmidazolium-sensitive Ca^{2+},Mg^{2+}-ATPase activity was examined under V_{max} conditions in homogenates of rat neural retina and renal cortices. Like Na^+,K^+-ATPase, developmental lead exposure produced short- and long-term dose-dependent decreases in retinal Ca^{2+},Mg^{2+}-ATPase activity whereas activity in the kidney was unchanged. In vitro, the retinal and renal enzymes were maximally inhibited at 10^{-3} M Pb^{2+} (≈50% and ≈20%, respectively) with IC_{50} values of 3–4 × 10^{-5} M. The in vitro data revealed that (Ca^{2+},Mg^{2+}-ATPase was 2–3 log units less sensitive to inhibition by Pb^{2+} than Na^+,K^+-ATPase. Kinetic analysis indicated mixed competition which suggests that the inhibition was direct and/or associated with calmodulin. In conclusion, the lead-induced inhibition of retinal Ca^{2+},Mg^{2+}-ATPase may also contribute to the elevation of retinal calcium and thus to the observed retinal deficits and apoptotic cell death. Supported by NIH Grant ES03183.

115 EXPRESSION OF PHOTORECEPTOR-SPECIFIC mRNAS IN DEVELOPMENTALLY LEAD-EXPOSED RATS

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Adult rats exposed to lead only during neonatal development exhibit selective rod electroretinographic deficits and apoptotic cell death. Elevated levels of retinal cGMP, resulting from decreased rod-specific cGMP phosphodiesterase (cGMP-PDE) activity, are thought to be responsible for the photoreceptor alterations and cell death. To determine if there were any long-term changes in photoreceptor-specific mRNAs associated with phototransduction, we examined mRNAs coding for the three subunits of cGMP-PDE (α, β and γ) and

for the proteins involved in the process of activation (opsin, and the α and β subunits of transducin) and deactivation (48 kD protein and recoverin) of this enzyme in the retinas of 90 day old control and neonatally lead-exposed rats (n = 18 retinas per group). Total neural retinal RNA was isolated and Northern blots were performed according to published laboratory procedures. The cDNA probes were labeled with [^{32}P]dCTP by the random primer extension method. Quantification of radioactivity was carried out using the Ambis Radioanalytic Imaging System. All the results were normalized to data obtained with the cDNA probe for the α-subunit of tubulin. The transcript sizes of all the mRNAs appeared normal. The mRNA levels of the α and β subunits of cGMP-PDE were decreased significantly whereas the levels of the remaining mRNAs were normal. These results suggest that persistent abnormalities in cGMP-PDE protein expression may underlie the lead-induced rod photoreceptor functional deficits and apoptotic cell death. Supported by NIH Grants ES03183, EY02651 and EY08285.

116 TOLUENE INDUCED LENGTH CHANGES IN ISOLATED OUTER HAIR CELLS

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Toluene causes hearing loss in rats and is thought to cause hearing loss in humans. The mechanism for this loss is not known, but outer hair cell damage is thought to be a cause of the hearing loss. Outer hair cells are believed to change length in response to sound enhancing the sensitivity of the inner ear. In this study outer hair cells were isolated from pigmented guinea pigs and examined under a phase-contrast microscope in order to determine whether toluene exposure *in vitro* could alter cell length. An additional purpose of this project was to determine whether a toxicant with very low solubility in aqueous media could be studied using a functional *in vitro* assay. The length of the outer hair cell was measured at 5 min intervals for 60 min while perfused with toluene in artificial perilymph. The concentrations of toluene tested in this study were 7.27 mM (saturated), 3.64 mM (half-saturated), 0.73 mM (one-tenth saturated), and 0.24 mM (one-thirtieth saturated). The degree of contraction of the outer hair cells was dependent on the concentration. The lowest dose of toluene found to cause contraction in the outer hair cells was 0.73 mM toluene. The lowest dose tested, 0.24 mM toluene, showed an increase in outer hair cell length when compared to the control. Supported by NIH Grant ES02852.

117 EFFECT OF NIFEDIPINE ON THE ELEVATION OF $[Ca^{2+}]_I$ IN SPIRAL GANGLION CELLS AND OUTER HAIR CELLS BY TRIMETHYLTIN

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Trimethyltin (TMT), an ototoxicant, disrupts hair cells both *in vivo* and *in vitro*. It elevates the compound action potential (CAP) sensitivity without changing cochlear microphonic (CM) within 30 min of exposure, suggesting the inner hair cell (IHC) and/or spiral ganglion cell (SGC) as the initial target. However, at longer intervals (hours) following TMT injection, a significant depression in the amplitude of the cochlear microphonic (CM) is observed with only modest impairment of CAP sensitivity, suggesting that the ototoxic target for TMT may shift from IHC and/or SGC to the outer hair cell (OHC). Evidence suggests that the effect of TMT on OHC and IHC/SGC may involve different mechanisms. Previous *in vitro* laboratory studies have indicated that TMT increases the intracellular calcium level ($[Ca^{2+}]_i$) of OHC, IHC and SGC although with somewhat different patterns. In this experiment we have investigated the effect of nifedipine (100 μM), an "L-type" calcium channel blocker, on the $[Ca^{2+}]_i$ increase of OHC and SGC. Guinea pigs were sacrificed and the otic capsules were rapidly removed. To obtain OHCs, the organs of Corti were incubated in 4 μM fura-2 AM artificial perilymph then washed in artificial perilymph and triturated. The tissues containing SGC were incubated in artificial perilymph containing 4 μM fura-2 AM and 0.1 mg/ml collagenase and 0.2% bovine serum albumin for 25 min, then washed in artificial perilymph and triturated. The OHCs and SGCs were exposed to 100 μM TMT in artificial perilymph or to artificial perilymph for controls. In the nifedipine treatment group the OHCs and SGCs were pretreated with 100 μM Nifedipine in artificial perilymph (300 mmol/kg, pH 7.3 ± 0.1) for 3–5 min and then exposed to TMT. Using a digital imaging fluorescent microscope we have monitored dynamic changes of $[Ca^{2+}]_i$ in post-TMT exposed single SGC and OHC. We have replicated the increase in $[Ca^{2+}]_i$ in SGC and OHC after exposure to 100 μM TMT. Results show that nifedipine inhibits $[Ca^{2+}]_i$ increases in SGC but not in OHC.

This study is supported by NIH grant ES02852.

118 THE EFFECTS OF DURATION ON THE OTOTOXICITY INDUCED BY INHALATION EXPOSURE TO TRICHLOROETHYLENE IN THE RAT

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Inhalation exposure to high concentrations of 1,1,2-trichloroethylene (TCE) has been shown to damage hearing in the mid-frequency range in the rat. The present study directly evaluated the adequacy of short-term exposures to TCE for predicting neurotoxicity produced by longer duration exposures. Adult male LE rats (n = 10–12 per group) were exposed to TCE via inhalation (whole body) in 1 m^3 stainless steel flow-through chambers for 6 hr/day. The following exposures were used: Acute (2000–10000 ppm); one week (800–4000 ppm); four weeks (400–3200 ppm); and 13-weeks (800–3200 ppm). Air-only exposed animals served as controls. Auditory function was assessed 3–5 wks after exposure using reflex modification audiometry. Auditory thresholds were determined for 1, 4, 16, 24, 32 and 40 kHz tones. Results replicate previous findings of mid-frequency hearing loss for all exposure durations. The lowest effective concentrations for 16-kHz thresholds were 8000, 3200, 3200, and 2400 ppm for the acute, 1-, 4-, and 13-week exposures, respectively. These data suggest that solvent-induced ototoxicity may depend on the concentration rate of exposure, and that the ototoxicity of TCE was less than that predicted by a strict concentration X time relationship.

119 STRUCTURE-ACTIVITY STUDIES ON THE AUDIO-VESTIBULAR AND OLFACTORY TOXICITY OF NITRILES

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We report here evidence that some organonitriles are toxic to the auditory, vestibular, and olfactory systems. Adult male Long-Evans rats were administered (ip) the following nitriles (dose range in mg/kg/day) for three consecutive days: 3,3′-iminodipropionitrile (IDPN; 200–400), crotononitrile (CNT; 75–125), *N,N*-bis(2-cyanoethyl)formamide (BCEF; 500–2000), butyronitrile (BYNT; 70–110), 3-butenenitrile (3-BNT; 22–47), 2-pentenenitrile (2-PNT; 100–175), 3-pentenenitrile (3-PNT; 20–60), glutaronitrile (GNT; 70–100), 3,3′-iminodiacetonitrile (IDAN; 66–133), and N-hydroxy-3,3′-iminodipropionitrile (HO-IDPN; 80). The following were tested for olfactory effects only: 2-methyl-3,3′-iminodipropionitrile (MIDPN; 218), 2,2-methyl-3,3′-iminodipropionitrile (2-MIDPN; 241), and *cis* and *trans* 2-pentene. Neurotoxicity was assessed using reflex modification audiometry, nasal cavity histopathology for assessment of olfactory epithelial degeneration, and behavioral tests of vestibular function. IDPN, HO-IDPN, and 2-PNT affected all three systems. CNT and BNT affected auditory and vestibular systems, but did not cause olfactory epithelial degeneration. The methylated IDPN derivatives caused olfactory mucosal toxicity, but the other chemicals tested were negative for the three end points. The mechanism(s) of neurotoxicity of these chemicals is currently unknown, but structure-activity and metabolism studies suggest that olfactory and audiovestibular effects may result from distinct mechanisms.

120 DEVELOPMENT OF A BEHAVIORAL OLFACTOMETER FOR THE STUDY OF CHEMICAL-INDUCED OLFACTORY NEUROTOXICITY

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The olfactory system is adversely affected by a number of compounds of toxicological interest. Despite this potential for sensory neurotoxicity, few studies have evaluated chemical-induced olfactory neurotoxicity. This is due in part to the difficulty in generating, controlling, and measuring olfactory stimuli. The purpose of this study is to develop behavioral tests of olfactory function. An apparatus to generate olfactory stimuli and measure behavioral responses has been developed. This olfactometer consists of a modified shuttle box capable of delivering diluted odorant vapors to either of two chambers separated by a physical barrier consisting of a downward directed airwall sandwiched between two exhaust panels. An odorant, such as acetaldehyde, is delivered (>55 L/min) through a series of solenoids so that the front edge of the plug flow travels the length of either 4.5 L chamber within 5 seconds. Solenoids are programmed to alternate during a trial to prevent animal training to solenoid-based auditory cues. Rats will be trained (>90% positive response) via a coupled odor-foot shock paradigm. This system will be used to compare auditory versus olfactory-cued learning and memory. Olfactory thresholds for acetaldehyde will then be established, and animals will be exposed to 3-methylindole

to induce olfactory epithelial damage. This system will provide a functional assessment of chemical-induced olfactory dysfunction.

121 PARAQUAT ABSORPTION AND TOXICITY: EFFECT OF AROMATIC SULPHONATES AS A POTENTIAL TREATMENT TO POISONING

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Treatment of acute paraquat poisoning has not been successful largely since the bipyridyl herbicide is rapidly absorbed from the gastrointestinal (GI) tract and is actively accumulated in pulmonary epithelial cells. Current approaches to treatment include gastric lavage and oral administration of adsorbents and purgatives. Our paraquat research has examined a range of aromatic sulphonate compounds, which have the ability to form charge-transfer complexes with paraquat, with a view to the generation of *in situ* non-absorbable complexes in the lumen of the GI tract. Our objective was to optimize structure activity and to understand more about the mechanism of action of these compounds. From six naphthalene derivatives, sodium 2,6-naphthalene disulphonate was the most effective treatment in mice and rats when given up to 4 hours after a potentially lethal paraquat (PQ) dose. In rats, plasma PQ levels were significantly reduced and the amount of PQ recovered from the stomach, small intestine and large intestine was altered. There was a progressive reduction in gastric PQ and elevation of colonic PQ from 2–48 hours which is consistent with the reduction in oral bioavailability. This was also associated with a marked reduction in both lung and kidney PQ levels 24 and 48 hours after dosing. Parenteral administration of the sulphonate did not alter PQ plasma and tissue kinetics either alone or when combined with its effective oral dose. These studies have demonstrated that PQ bioavailability can be effectively reduced by complexing the bipyridyl *in situ* thereby reducing GI absorption and may offer an additional and more specific treatment following human paraquat poisoning.

122 DITHIOCARBAMATES PESTICIDES INHIBIT THYROID PEROXIDASE IN FRTL-5 CELLS TRANSFECTED WITH THYROID PEROXIDASE (TPO) ENZYME

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The acute toxicity of dithiocarbamates (DTC) fungicides is low and the persistance in the environment is modest. However, in the rat, thyroid is the target organ of ethylenebisdithiocarbamates (EBDCs) toxicity. The inhibited synthesis of the thyroid hormones, induced most probably by EBDCs metabolite ethylenethiourea (ETU), leads through a feedback mechanism to an increased thyroid weight and hyperplasia of the organ. The molecular target of ETU *in vivo* is believed to be the TPO enzyme that catalyzes the transfer and the organification of iodine. To verify the validity of this hypothesis, we transfected Chinese Hamster Ovary (CHO) and Fisher Rat Thyroyid (FRTL-5) cells with the TPO gene (kindly supplied by Dr. B. Rapoport, San Francisco). These genetically manipulated cell lines were then exposed both to ETU and to different DTC: ziram a dialkyldithiocarbamate and two EBDCs (zineb, mancozeb). The results showed that TPO enzyme was inhibited in both the cell lines by methimazole 25 μM (MMI), a known TPO inhibitior, and ETU 50 μM. The ETU effect was always reversible, while MMI irreversibly inhibited the enzyme in CHO cells in opposition to FRTL-5 cells. Surprisingly either ziram, zineb and mancozeb *per se* inhibited TPO at 5 μM concentration, already 5 min after the exposure, suggesting a direct effect rather than an effect mediated by ETU. Even if ziram is electively toxic toward thyroid cells, the observed inhibition of TPO is probably due to an interference with the enzyme activity. In fact at these doses and time of exposure no general toxicity or cell functional impairment was evident, as assessed by LDH leakage and protein synthesis measurement. The effect of EBDCs was counteracted by the presence of an high concentration of iodide (NaI 100 μM), confirming iodine importance in the reconstitution of the functional TPO form. In conclusion, the *in vitro* evaluation of such compounds on a specific target, like FRTL-5 transfected with a functional TPO enzyme, has provided a useful tool to study selectively the molecular mechanisms involved in DTC toxicity. Our results, in fact, demonstrated for the first time in a thyrodal cell line that TPO might be the primary target of DTC toxicity.

123 THE EFFECTS OF 2,4-DICHLORPHENOXYACETIC ACID (2,4-D),2-METHYL-4-CHLOROPHENOXYACETIC ACID (MCPA) AND 2,4-D + MCPA IN DOGS AFTER SINGLE DOSE ORAL ADMINISTRATIONS

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There is little information about the clinical effects, pharmacokinetics, and renal elimination resulting from interactions between the chlorinated phenoxyacetic acid herbicides although these chemicals are often applied as mixtures. Six female dogs were administered single oral doses of 2,4-D (25 mg/kg b.w.), MCPA (25 mg/kg b.w.), and 2,4-D plus MCPA (25 mg/kg b.w. each), each dose separated by a 2–3 week washout period. Dogs were observed for abnormal clinical signs (*i.e.*, myotonia, weakness, vomiting, diarrhea) for 48 hours after dosing. Blood samples were collected at 1, 2, 4, 6, 8, 12, 18, 24, 36 and 48 hours after dose administration for chemical analyses of 2,4-D and MCPA. Free catch urine was collected from 0–24 hours and 24–48 hours after dose administration. Blood samples were also collected before dose administration and at 24 and 48 hours after dose administration for determination of clinical chemistry parameters. 2,4-D and MCPA concentrations in plasma and urine were determined using a modification of the HPLC method of Suzuki and Watanabe. (*J. Chromatogr.* 541: 359–364, 1991) Dogs were observed as normal after each administration and there were no changes in any of the measured clinical chemistry parameters.

124 2,4-D HERBICIDE (2,4-DICHLOROPHENOXYACETIC ACID): ONE-YEAR ORAL NEUROTOXICITY STUDY IN FISCHER 344 RATS

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This study was conducted according to USEPA-FIFRA Guideline 83-1 (1991) and was conducted concurrently with a one-year chronic toxicity study. Ten male and female Fischer 344 rats per dose level were fed diets to provide 0, 5, 75 or 150 mg/kg/day of 2,4-D acid. Rats were evaluated preexposure, and at months 3, 6, 9 and 12 by a functional observational battery (FOB), forelimb and hindlimb grip performance, landing foot splay, and an automated test of motor activity. After completion of 12 months of exposure, neuropathologic examination of perfusion fixed central and peripheral nervous tissues was conducted on 5 rats/sex from the control and high-dose groups.The only finding of neurotoxicologic significance was retinal degeneration in females of the 150 mg/kg/day dose group. Also at 150 mg/kg/day: Body weights were about 10% lower; there was more urination during open field examination in females; forelimb grip performance was significantly increased in both sexes when analyzed as a ratio of grams of grip/grams body weight, but not when analyzed as grams of grip only (and not a ratio with body weight). Body weights were about 4% lower than controls in the 75 mg/kg/day group. Based on body weight effects, the No-Observed-Effect-Level (NOEL) for the neurotoxicity subgroups of rats was 5 mg/kg/day.

125 PHYSICAL AND BIOCHEMICAL EVENTS IN THE CYTOSKELETAL DAMAGE INDUCED BY ORGANOTIN COMPOUNDS

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The mechanisms involved in the toxic action of chemicals on the cytoskeletal microfilaments were investigated. Human leukemia cells chemically differentiated to neutrophils were exposed for different times to a concentration 10 μM of two pesticides, triphenyltin chloride (TPT) and tributyltin chloride (TBT). Both compounds decreased the amount of polymerized (F)-actin, the basic component of the microfilaments, after very short time of exposure, 1–5 min. The effect of TPT was reversible, while TBT caused eventually cell death. In spite of the damage in the exposed cells actin polymerization was still detectable after stimulation with the chemiotactic peptide fMLP (100 nM, 1 min). The possible oxidation of thiol groups was investigated. TPT and TBT caused a depletion of SH-protein in the cytosol of the cells, but an increase of -SH groups in the cytoskeletal fraction was detectable. Organotin compouns were also tested directly on purified F-actin 0.5 mg/ml in a cell-free system. TBT and TPT did not cause the depolymerization of the F-actin up to 18 h of incubation and did not inhibit the polymerization triggered by KC1 20 or 40 mM. On the basis of these results we hypothesized that TPT and TBT effect on F-actin in the cells could be due to intracellullar edema. Incubation of the cells in ipotonic buffer causing cell swelling, caused a dramatic depolymerization of

F-actin in 1 min; in the mean time actin still polymerized after stimulation with fMLP, similarly to the results obtained with organotin compunds.

126 EFFECT OF THALLIUM (TH) EXPOSURE ON THE CHOLINERGIC ENZYME IN THE GASTROINTESTINAL (GI) TRACT AND BRAIN REGIONS OF THE RAT

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Exposure to *TH* has been shown to produced neurological disorders which may lead to death. In addition, *TH* is known to cause *GI* disturbances in human and animals. The present study examines the effect of acute and repeated exposure to *TH* on the cholinergic enzyme (Acetyl Cholinesterase, *AChE*) activity in various segments of the *GI* and brain regions. Adult male Sprague-Dawley rats (N = 8/group) were injected (i.p.) with either vehicle, 0.1, 1.0 or 10.0 mg/kg of *TH* acetate, twice daily for 5 days or a single dose of 25 mg/kg. *AChE* was measured 24 hours after the last dose. The results of this study shows that the highest dose of *TH* was fatal since 6 rats died before the 5th day. In most brain regions and segments of the *GI*, *TH* caused a decrease in *AChE* activity. However, the single dose of 25 mg/kg was more potent since it resulted in a significant decrease in *AChE* activity in the hypothalamus and the duodenal segment of the *GI*. This data may indicate the involvement of the cholinergic system in *GI* toxicity induced by *TH*. (Supported by CDC/ATSDR/MHPF award #U50/CCU 304522-04).

127 EVALUATION OF N,N-DIETHYL-M-TOLUAMIDE (DEET) FOR CHRONIC TOXICITY, ONCOGENICITY AND REPRODUCTIVE EFFECTS

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Four studies have been completed recently in which the insect repellent DEET was evaluated for chronic toxicity, oncogenicity and reproductive effects. Chronic toxicity evaluations were conducted in a two-year study in rats and a one-year study in dogs. Oncogenicity was evaluated in a two-year study in rats and an 18-month study in mice. The potential for DEET to produce reproductive effects was evaluated in a two-generation reproduction study in rats. All studies were conducted by the oral route of administration at the "maximum tolerated dose". No specific target organ toxicity, oncogenicity or reproductive effects were observed.

128 EVALUATION OF THE PYRETHROID INSECTICIDE DELTAMETHRIN IN THE MPTP/C57 BLACK MOUSE MODEL OF PARKINSONISM

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Behavioral and neurochemical changes in C57 retired breeder male mice were investigated following 3 nonlethal doses of deltamethrin (6 mg/kg) alone or in combination with a single dose (20 mg/kg) of the parkinsonian neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Deltamethrin, but not MPTP, caused abnormalities in motor coordination as measured in a pole traction test, and neither compound affected open field ambulation at the doses tested. MPTP depleted about 50% of the dopamine from the striatum of treated mice, but deltamethrin showed only a slight reduction that was not statistically significant. In contrast, deltamethrin increased striatal DOPAC 77% (dihydroxyphenylacetic acid, a dopamine metabolite), suggesting an increase in dopamine turnover *in vivo*. MPTP alone had no effect on DOPAC levels, and when given in combination, the effect of deltamethrin was not expressed. For all treatment groups, there was increased synaptosomal dopamine uptake compared to controls. Radiolabelled neurotransmitter release studies with striatal synaptosome preparations *in vitro* found that deltamethrin potentiated release of dopamine and glutamate in a tetrodotoxin-sensitive manner. Thus, exposure to subconvulsive doses of deltamethrin has significant effects on behavior and striatal neurochemistry. These findings suggest a possible role for exposure to environmental neurotoxins in neurodegenerative disorders such as idiopathic Parkinson's Disease.

129 ROLE OF P-GLYCOPROTEIN IN INSECTICIDE RESISTANCE

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Multidrug resistance (MDR) is the resistance to structurally unrelated therapeutic agents and is mediated by the overexpression of an ATP-dependent glycoprotein, P-glycoprotein (P-gp). We have previously demonstrated that insecticides interact with P-gp; therefore, we wanted to determine its role in insecticide resistance. Immunoblot analysis of microsomes prepared from tobacco budworms (*Heliothis virescens*) resistant to specific carbamates, synthetic pyrethroids, and/or organophosphorus insecticides revealed at least a six fold increase in the levels of P-gp compared to the susceptible strain. P-gp was localized primarily in the cuticle > fat body ≫ mid gut. A topical application bioassay was used to pretreat 3rd instar cypermethrin resistant tobacco budworm larvae with 4 μl of P-gp inhibitor, quinidine (0.1 μg/g). We challenged these larvae with various concentrations of chlorpyrifos and thiodicarb. Mortality was not observed in the control groups, tobacco budworms treated topically with acetone or quinidine (0.1 μg/g). After 24 hours, a topical application of 0.3 μg/g or 3 μg/g chlorpyrifos-acetone solution with quinidine pretreatment resulted in 13 and 29% mortality, respectively, while no mortality was seen without pretreatment. A topical application of 3 μg/g or 33 μg/g thiodicarb-acetone solution with quinidine pretreatment resulted in 25 and 43% mortality respectively, while 0 and 20% mortality was seen without pretreatment. Finally, tobacco budworms treated with 0.1 μg/g quinidine were allowed to feed on cotton leaves dipped in concentrations of thiodicarb ranging from 25 to 200 ppm. Mortality ranged from 10 to 60% with the inhibitor while only 0 to 8% mortality was seen without the inhibitor. These results indicate that quinidine synergizes the action of pesticides and suggest that P-gp is involved in insecticide resistance.
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130 *IN VITRO* AND *IN VIVO* HEAT SHOCK PROTEIN (HSP) GENE EXPRESSION BY SELECTED PESTICIDES

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The polyhalogenated cyclic hydrocarbons endrin and chlordane, the organophosphate pesticides chlorpyrifos and fenthion, and the chloroacetamide insecticide alachlor induce lipid peroxidation and DNA damage. No information is available regarding whether these pesticides can induce the expression of Hsp genes. The pesticides were administered p.o. in two 0.25 LD_{50} doses at 0 hr and 21 hr, the rats were killed at 24 hr, and liver, brain, heart and lung tissues were removed to examine the induction of Hsp by Northern and Western blot analysis. In a separate series of experiments, cultured PC-12 cells treated 24 hr with 50, 100 or 200 nM concentrations of these pesticides. Endrin, chlorpyrifos, fenthion and alachlor induced Hsp89α and Hsp89β in the hepatic and brain tissues, and as well in cultured PC-12 cells. Expression of Hsp89α was observed only in the hepatic tissues of the chlordane treated rats. Some expression of Hsp89β was observed in the lung tissues of alachlor and endrin treated rats. None of the pesticides induced the expression of Hsps 27 and 60. The results indicate striking tissue differences in the patterns of the Hsp by these pesticides which were used.

131 INHIBITION OF ESTROGEN METABOLISM BY THE ORGANOPHOSPHORUS INSECTICIDE FENITROTHION

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Phosphorothioate insectides such as fenitrothion (FS) are suicide substrates of cytochromes P450 (P450). These compounds undergo oxidative desulfuration by P450 resulting in the release and subsequent binding of atomic sulfur to the enzyme. Consequently, the P450-dependent metabolism of endogenous steroids could be inhibited by exposure to these insecticides. Formation of 2-hydroxyestrone (2-OH), 4-hydroxyestrone (4-OH), and 16α-hydroxyestrone (16α-OH) in mammals occurs by P450-dependent hydroxylation of estrone at the various positions on the steroid nucleus. In the present study, incubation of 50 μM estrone with mouse hepatic microsomes for 20 min @ 37° resulted in NADPH-dependent production of 120.28 pmols/mg protein/min ± 14.37 (mean ± SD) 2-OH and 27.73 pmols/mg protein/min ± 4.32 16α-OH, as determined by high performance liquid chromatography. Inclusion of FS (50 μM) in the incubation significantly reduced ($p < 0.05$) production of 2-OH to 83.87 pmols/mg protein/min ± 0.933, and 16α-OH to 9.29 pmols/mg

protein/min ± 5.42. Pretreatment with 500 mg/kg FS decreased 2-, 4-, and 16α-hydroxylation in a similar fashion. These data suggest that exposure to insecticides that undergo oxidative desulfuration by P450 could interfere with metabolism of naturally-occurring steroids like estrone and its metabolites. (Supported by NIEHS Grant ES04355 and NIGMS Grant GM15150.)

132 TOXICITY AND PHARMACOKINETICS OF ISOFENPHOS IN MALE HARTLEY GUINEA PIG

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The toxicity and the pharmacokinetics of isofenphos (IFP) in male Hartley guinea pigs (average weight 465 g) were investigated. In the toxicity study, 28 guinea pigs were injected ip with IFP at 0, 5, 10, 20, 30, 40 and 50 mg/kg. At 24 hrs, the ED_{50} values for cholinesterase (AChE) inhibition in red blood cells, plasma, and whole blood were determined to be 24.2, 4.2, and 8.9 mg/kg, respectively. In the pharmacokinetics study, 36 animals were injected ip with ^{14}C-IFP (7.74×10^6 DPM/animal) at a dosage of 22.0 mg/kg and animals were sacrificed at 0.5, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, and 168 hrs. Blood and major organs were analyzed for ^{14}C content by liquid scintillation spectrometry. In addition, four guinea pigs were injected with the ^{3}H-ethyl-labelled-IFP, 22.0 mg/kg (126.7×10^6 DPM/animal) and urine was analyzed for isofenphos metabolites with a High Performance Liquid Chromatograph (HPLC) equipped with an ion exchange column and radioactive detector. The pharmacokinetics elimination constants from tissues for ^{14}C-IFP equivalents were determined with a two-compartment retention model. ^{14}C-IFP equivalents were eliminated rapidly from kidney, liver, plasma, liver, and heart and at moderately slower rates from fat, carcass, and brain. The half-lives for these tissues were 5.4, 5.9, 7.7, 7.5, 10.9, 11.5, and 15.3 hrs, respectively. IFP was eliminated mainly in the urine as water soluble metabolites (84% of dosage in 168 hrs). Urine analysis by HPLC indicated that four major metabolites were present in the urine and these metabolites are likely to be the glycine conjugates of the IFP, IFP-oxon, d-N-IFP, and d-N-IFP-oxon. These glycine conjugates need to be identified.

133 EFFECT OF LAUNDERING ON ABILITY OF GLOVE FABRICS TO INHIBIT THE PENETRATION OF ORGANO-PHOSPHATE (OP) INSECTICIDES THROUGH AN ARTIFICIAL SKIN MODEL

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Two glove fabrics (100% cotton and 100% polypropylene) were examined new and after laundering for their capability to decrease the penetration of the OP insecticides, azinphos-methyl and paraoxon, at field concentrations (3000 and 15 ppm, respectively), though an artificial skin model (Skin2®). Capability of the media under the skin to inhibit acetylcholinesterase (AChE) in hen brain homogenates was used to indicate penetration. Protection from azinphos-methyl penetration was provided both by skin and by new and laundered fabric and the effects of both were greater than either alone. (AChE activity 58% ± 1 of control under skin; 59% ± 7 under cotton; 85% ± 4 under skin + cotton; 49% ± 3 under polypropylene; 80% ± 4 under skin + polypropylene.) For paraoxon solution, however, new cotton glove fabric prevented absorption, and this protective effect was lost when the fabric was laundered. These results indicate that the pesticide formulation needs consideration when protective fabrics are chosen, and that, for cotton, the best protection may be provided before it is laundered.

134 ASSAY CONDITIONS FOR MONITORING HUMAN BLOOD CHOLINESTERASES

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Monitoring of blood cholinesterase (ChE) levels is required in California after use of selected anticholinesterase pesticides. A recent survey by Cal-EPA found several procedures in use without interlaboratory standardization. Most assays were based on the pH test of Michel (1948) or the thiocholine assay of Ellman, et al. (1961). This report compares conditions from two commercial kits and the optimum conditions for the Ellman assay using blood from volunteer human subjects. The studies showed that lower ChE values were obtained when the kits' conditions were used. The kits used a pH 7.2 buffer, resulting in a 25% decrease in measured activity compared to use of the optimal pH 8.0 buffer. One kit used propionylthiocholine as the substrate, but erythrocyte acetylcholinesterase (RBC AChE) has a poor affinity for it. The other kit used acetylthiocholine (as recommended by Ellman), but at a concentration (5.5 mM) high enough to result in substrate inhibition of RBC AChE. Plasma cholinesterase (BChE) activity increased with increasing substrate concentration, up to 10 mM. In addition, one of the kits often was used with an automated commercial method which measures absorbance at 480 nm, instead of 410 nm, resulting in diminished sensitivity. This study supports the recommendation that a standardized assay be developed for clinical laboratories, so that workers can be accurately monitored as they move from region to region.

135 ANTICHOLINESTERASIC DRUG EFFECTS

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The actions of anticholinesterasic drugs, particularly organophosphorus compounds (OPs), have troubled many scientists for years. We present here a synopsis of multiple actions based upon extensive literature summarizing the observed effects of OPs, carbamates, oximes, and pyridiniums on the CNS and on the neuromuscular junction, including the electrophysiological studies from this laboratory. Since the actions of OPs are not uniform, each compound merits individual consideration. Low-level and long-term effects on cognitive performance deserve further attention. Atropine clearly provides partial protection against acute lethality; its protection of the CNS respiratory mechanisms may be accomplished by protection of muscarinic receptors against the direct muscarinic action of toxins or against an excess of acetylcholine secondary to inhibition of acetylcholinesterase. Although atropine may prevent the proconvulsive actions of physostigmine and acetylcholine, the seizures induced by OPs are more difficult to control. While it has been suggested that cholinergic hyperstimulation is the primary event in OP toxicity and is followed by recruitment of other neurotransmitter systems, other evidence shows direct actions of OPs on glutamatergic and GABAergic transmission, and also direct stimulation of neurotransmitter release that is triggered by an unidentified Ca^{2+}-dependent process. Neuromuscular blockade accounts for the lethality of OPs after atropine treatment. Direct actions of anticholinesterasic drugs on nicotinic receptors (stimulation and ion-channel blockade) and presynaptic nerve terminals are highly, significant. A specific binding site, conserved on all nicotinic receptor α-subunits and distinct from the agonist binding site, may account for the prophylactic action of physostigmine, whose protection against OP-lethality is not dependent on the inhibition of cholinesterase. A perspective encompassing multiple actions of anticholinesterasic and antidotal drugs may facilitate the discovery of novel antidotes. (Supported by DAMD-17-88-C-8119, NIH grants NS 25296 and T32-ES07263.)

136 MUSCARINIC ACETYLCHOLINE RECEPTOR (MACHR) DENSITY IN NESTLING STARLINGS DURING CHRONIC DOSING WITH DIAZINON

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Chronic exposure to anti-cholinesterases (anti-ChEs) has previously been demonstrated to cause a decrease in the density, i.e. down-regulation, of mAChRs in the brains of rats, mice, and guinea pigs. Determination of the developmental pattern of mAChRs in birds, and the possible response of chronic anti-ChE exposure on ChE activity and receptor density in young birds has not yet been investigated. Two chronic (day 2 through 16) dosing regimes with diazinon were used to contrast enzyme response and mAChR density levels in European starlings. Doses were administered in a 3-tiered approach, and increased as nestlings matured to maintain a constant level of ChE inhibition. Developmental patterns of mAChR levels in controls increased over time, reaching half of adult levels by day 16. A similar pattern was observed for plasma ChE activity. Brain ChE was inhibited an average of 15% and 72% at the low and high doses respectively. A trend in decreased density levels of mAChRs was observed in the high dose birds beginning on day 13. The use of this method to determine chronic anti-ChE exposure in birds appears to hold greater promise in older nestlings. Supported by NIEHS ES04696.

137 OCULAR TOXICITY FOLLOWING 13-WEEKS OF EXPOSURE TO THE ORGANOPHOSPHATE INSECTICIDE FENTHION

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Reports of ocular toxicity including retinal degeneration following long term (1–2 yrs) exposure to organophosphates led us to investigate the effects of short term repeated treatment with fenthion. Male, 60-day old, Long-Evans rats were given s.c. injections of 0 (corn oil vehicle), 10, 25, or 50 mg/kg fenthion twice/week for 13 weeks. Dependent measures taken during and/or after the course of treatment included: biochemical (brain, retina and blood cholinesterase (ChE) activity; retinal and brain muscarinic receptor density (mChR); carbachol-stimulated release of inositolphosphates (IP) as an index of muscarinic receptor function; and glial fibrillary acidic protein (GFAP) as an index of reactive gliosis); electrophysiological (electroretinograms (ERG), indicative of outer and middle retina; and flash and pattern visual evoked potentials (FEP and PEP), indicative of entire retina and visual pathway); histological (retina and brain routine stains and special stains for GFAP and phosphorylated neurofilaments); and ophthalmological (clinical exam). Results included: ChE inhibition in all tissues and at all dose levels; decreased mChR in retina and brain; decreased IP release in retina and brain which recovered in brain, but not retina by 104 days after the last dose; increased GFAP levels in retina but not in brain, which were more elevated 6 weeks than 4 days after the last dose; significant alterations in FEPs, nonsignificant trends in PEPs, and no indications of changes in ERGs; no gross ophthalmological or histopathological changes, but altered staining of GFAP and phosphorylated neurofilaments in the optic nerve. The results indicate that 13-weeks exposure to fenthion produced early indications of both structural (GFAP and neurofilaments) and functional (IP, mChR, FEP) toxicity to the retina. The results (IP, mChR, FEP but not ERG) also suggest a site of early dysfunction in the inner retina.

138 A STUDY ON THE BIOLOGICAL EXPOSURE INDEX OF FENITROTHION IN RATS

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Fenitrothion (FNT) is an organophosphate pesticide that readily penetrates skin and inhibits serum cholineasterase (ChE) in mammals. A study was carried out to evaluate the relationship between urinary excretion of 3-methyl p-nitrophenol (3MpNP), the in vivo metabolite of FNT, and its inhibition of serum ChE. Male Wistar rats, 6 per dose group, were housed individually in metabolic cages and gavaged with FNT in Mazola corn oil at 0, 3, 6, and 12 mg/kg b.wt. Serum ChE was determined both at 24-h before and 24-h after dosing. Concentrations of urinary 3MpNP were determined for samples collected for 24-h after dosing. A linear correlation was observed for the excretion of urinary 3MpNP and the inhibition of ChE. Based on the linearity obtained and the criteria set by the American Conference of Governmental Industrial Hygenists, that 3MpNP of ≈ 2 μg/mg creatinine, which corresponded to a 30% inhibition of serum ChE, would be the biological exposure index of FNT in rats. (Supported by CMRP 357, Chang Gung Med Coll and NSC 82-0412-B182-083, Taiwan.)

139 URBAN RESIDENTIAL PESTICIDE USAGE AND STREAM SURVEY

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In 1992, a multi-year project was initiated to evaluate nonpoint source pesticide pollution of urban streams in Baltimore. During the first year, two activities were conducted: 1) a pesticide usage survey of residents within selected Baltimore watersheds and 2) a survey of streams located within those watersheds which monitored for the presence of 27 pesticides. The usage survey identified 47 pesticides as receiving prominent usage, of which appropriate analytical methods were available or 25. Pesticide usage in residential areas was common, with 80% of all surveyed residents stating that they used pesticides. Two out of three residents used pesticides outdoors, while 40% used pesticides indoors. The frequency of residential usage varied considerably, but a seasonal pattern of usage predominated, with spring and summer pesticide usage being most popular. In addition, fourteen of the 27 pesticides monitored during the urban stream survey were detected in at least one surface water sample; the remaining thirteen pesticides were not detected in any samples. By consolidating the list of 47 prominently used residential pesticides with the list of pesticides detected during the urban stream survey, a target list of 35 pesticides was developed for monitoring.

140 OXIDATIVE DNA DAMAGE CAUSED BY ETHINYL ESTRADIOL IN CULTURED FEMALE RAT HEPATOCYTES

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Ethinyl estradiol (EE) is a strong promoter and weak hepatocarcinogen, but the mechanism(s) is not fully understood. Our working hypothesis is that catechol metabolites of EE cause oxidative damage which contributes to its carcinogenicity. Female rat hepatocytes cultured in serum-free Chee's essential medium supplemented with insulin, selenium, transferrin, dexamethasone and dimethylsulfoxide for 24 hrs were exposed to EE. At 60 μM, EE treatment caused a time-dependent increase in 8-OH-dG levels. The maximum increase was approximately 2 to 3-fold between 6 and 24 hrs. 8-OH-dG levels had returned to control levels (14.3 ± 2.0 [mean $\pm$ SEM] 8-OH-dG/10^6dG) by 42 hrs. The 2-hydroxylated metabolite of estradiol at 100 μM caused a 2-fold increase in 8-OH-dG within 15–60 min. Studies are under way to characterize the kinetics and dose-response for 8-OH-dG formation and repair. Other indicators of oxidative damage are also being investigated. The observed increases in 8-OH-dG adducts in DNA, which can cause GC to AT transitions, could contribute to the promoting activity and/or the carcinogenicity of EE. (Supported by PHS Grants CA36701, ESO7141, ES06052 and ESO3819).

141 CATECHOL ESTROGEN OXIDATION & GLUTATHIONE CONJUGATION IN SYRIAN HAMSTERS *IN VIVO*

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Hormones can play an important role in the initiation and promotion of cancer, and hormonal carcinogenesis is often characterized by both tissue and species specificity. For example, male Golden Syrian hamsters develop renal carcinoma following prolonged exposure to 17-β-estradiol (E_2). The basis for this specificity is unclear. Although catechol estrogens have been proposed as putative redox active metabolites contributing to the carcinogenic effects of E_2, detailed information on the disposition of the catechol estrogens is lacking. Following administration of E_2 (5.4 and 100 μmol/kg, ip) to hamsters, 9.3% and 2.3%, respectively, of the dose was recovered in bile as a combination of 2-hydroxy-1-glutathion-*S*-yl-E_2 > 2-hydroxy-4-glutathion-*S*-yl-E_2 > 2-hydroxy-1,4-*bis*-glutathion-*S*-yl-E_2 > 4-hydroxy-1-glutathion-*S*-yl-E_2, within 5 hours. At 500 μmol/kg E_2, $7.7 \pm 2.1\%$ (n = 4) of the dose was recovered as GSH conjugates in the urine after 24 hours. Treatment of urine with β-glucuronidase increased the recovery of GSH conjugates by 2–3 fold, indicative of mixed GSH/glucuronide conjugate formation *in vivo*. The addition of GSH to the catechol estrogens had no effect on their oxidation potentials, as assessed by cyclic voltammetry. Thus, GSH conjugates of 2- and 4-hydroxy-E_2 remain as redox active as the parent catechols. In summary, we have demonstrated that oxidation of catechol estrogens followed by GSH conjugation occurs *in vivo* and these conjugates may possess the same capacity as the parent catechol to cause oxidative damage. Supported by CA 58036.

142 TOBACCO SMOKE INITIATES OXIDATIVE STRESS IN LUNG CELLS

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We hypothesize that the diverse biological effects of tobacco smoke (TS) are caused by oxidative stress. Adult rat lung cells (ARL) plated on coverglasses with plastic wells (NUNC) grew for 48hrs in F12k medium with 10% fetal bovine serum to an adherent layer. Oxidative stress was measured by preincubating the ARL cells with 10 μM 2,7-dichlorofluorescin diacetate (DCFDA) for 10 min and rinsing free of excess dye. DCFDA oxidizes to a fluorescent product on reaction with active oxygen species. Cells were treated with Hank's balanced salt solution pH 7.4 containing no TS, main stream TS or side-stream (environmental) TS. We simulated the concentration of TS reaching the lung lining fluid by bubbling one of the two types of TS through 75 ml BSS (near the fluid volume of an adult lung). TS from a single filtered cigarette caused a dose-related increase in oxidative stress rapidly over 10 min. Side stream TS was more potent than main stream. Cells treated about 10 min and washed free of TS solution recovered about 30% of the maximum. We suggest that these measurements show that TS initiates oxidative stress directly in lung cells and that the concentrations needed are well within the range of environmental tobacco smoke exposures.

143 THE EXPERIMENTAL CONDITIONS AND PROPOSED MECHANISM OF ALUMINUM-FACILITATED IRON-MEDIATED LIPID PEROXIDATION

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Brain lipid peroxidation (LP) contributes to the etiology and pathogenesis of neurodegeneration. Brain LP might be initiated by the hydroxyl radical or by an iron-oxygen complex. Iron (Fe) plays an important role in both hypotheses. Aluminum (Al) is not a transition metal, but it may facilitate Fe-mediated LP. LP of bovine brain extracts and pure phospholipids was produced by addition of varying concentrations of Fe(II) and Al at pH 5.5 and 7.4. LP was evidenced by generation of malondialdehyde in the form of thiobarbituric acid reactive substances. The results confirmed the presence of Al-facilitated Fe-mediated LP. The magnitude of LP was dependent on Al and Fe concentrations. The Al facilitation of LP was greater at pH 5.5 than 7.4. Al facilitated Fe-mediated LP of bovine brain Folch fractions I and III, bovine brain phosphatidylserine, and egg yolk phosphatidylcholine. There was no Fe-mediated LP, nor any Al effect, with Folch fraction V, bovine brain phosphatidylethanolamine, phosphatidylcholine, or sphingomyelin. Among the above phospholipids, phosphatidylserine was the most susceptible substrate for Al-facilitated Fe-mediated LP. We propose: (1) Fe(II) initiates LP, which depends on the number of double bonds in the fatty acid. (2) Al facilitates LP, via the head group of the phospholipid, *e.g.* phosphatidylserine. (3) Acidic pH increased LP by increasing free Al concentration and decreasing Fe(II) autoxidation to Fe(III). (4) The lysosome and the mitochondria, which have acidic pH, might be the targets for this oxidative injury. Supported by NIH ES 4640.

144 1,2-DICHLOROBENZENE INDUCES CONJUGATED DIENE FORMATION IN MALE FISCHER-344 RATS

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1,2-Dichlorobenzene (1,2-DCB) is an environmental contaminant and a potent hepatotoxicant in male Fischer-344 (F-344) rats. Previous data from our laboratory have demonstrated that the Kupffer cell is involved in modulation of 1,2-DCB hepatotoxicity. This modulation is due, at least in part, to increased reactive oxygen species formation, which can ultimately lead to lipid peroxidation. This study was conducted to determine if conjugated dienes, a marker for lipid peroxidation, are formed after ip administration of 1,2-DCB. Rats were administered either corn oil or 3.6 mmols/kg 1,2-DCB; livers and serum were then harvested at various time points following treatment (0, 3, 12, 16, 24 hours). In liver, conjugated diene levels, (measured spectrophotometrically and expressed as change in absorbance/nmole phosphate), were significantly elevated beginning at the 16 hr time point, and was maximal 24 hrs after 1,2-DCB administration. These increases were over two-fold after 24 hours. Similarly, in serum, conjugated diene levels were also significantly increased after 16 and 24 hours (1.9 and 4.2-fold, respectively). This time-dependent increase in conjugated diene levels are consistent with increases in serum ALT activities. These data, along with previous findings, suggest that Kupffer cells, though production of reactive oxygen species, participate in the hepatocellular damage from 1,2-DCB administration in F-344 rats. Supported in part by NIEHS ES 06095 and Center Grant #P30 ES06694.

145 NITRIC OXIDE EXPOSURE AND SULFHYDRYL MODULATION ALTER PLASMA MEMBRANE-DEPENDENT L-ARGININE TRANSPORT IN PULMONARY ARTERY ENDOTHELIAL CELLS

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L-arginine (L-arg) transport in pulmonary artery endothelial cells (PAEC) is regulated by Na^+-dependent (+ Na) and Na^+-independent (−Na) carrier-mediated processes. Because exposure to nitric oxide (NO) results in S-nitrosylation of proteins, we sought to determine the effects of NO exposure and sulfhydryl reactive chemicals, N-ethylmaleimide (NEM) and acrolein (ACR), on L-arg transport in PAEC. Confluent monolayers of porcine PAEC were exposed to 7.5 ppm NO in 95% N_2-5% CO_2 or to 95% N_2-5% CO_2 (control) at 37°C for 4–24 hr or to NEM (10–50 μM) or ACR (0.5–1.5 μM) at 37°C for 30 min. After exposure, +Na and −Na L-arg transport was measured. Exposure of PAEC to NO for 4 hr resulted in a significant ($p < 0.05$) reduction of +Na but not −Na L-arg transport. More prolonged exposure for 12–24 hr reduced both +Na and −Na transport of L-arg with maximal loss of transport after 18 hr of exposure ($p < 0.02$ for both). When cells were exposed to NEM or ACR, a dose-dependent reduction of L-arg transport by both +Na and −Na processes was observed. +Na L-arg transport was more susceptible to inhibition by NO, NEM, and ACR. These results demonstrate that exposure to exogenous NO is responsible for reduction of plasma membrane-dependent L-arg transport mediated by +Na and −Nat transport processes. Modulation of the sulfhydryl status of plasma membrane proteins involved in L-arg transport, such as L-arg transporters and/or Na^+/K^+-ATPase, may be responsible for reduction of L-arg transport in PAEC.

146 ANTIOXIDANT-INSENSITIVE FREE RADICAL PRODUCTION BY WHITE BLOOD CELLS (WBCS) ISOLATED FROM ALCOHOL TREATED LIVERS AFTER TRANSPLANTATION

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Alcohol treatment or high fat diet results in poor graft survival after transplantation. Recently, we detected superoxide dismutase (SOD)/catalase-insensitive free radicals exhibiting a 6-line spectrum by electron paramagnetic resonance (EPR) analysis of blood 5 minutes after transplantation of livers receiving high fat or alcohol containing diets. Further, electron microscopy revealed an increase in WBCs sticking to the sinusiod 15 minutes after transplantation. This study examined the source of radicals associated with graft failure. Sticking WBCs were isolated 30 minutes after liver transplantation under conditions of normal and poor graft survival. Briefly, a balanced salt soution was perfused to rinse out free blood elements. The liver was then perfused with calcium free buffer containing 1 mM EGTA to free the adherent WBCs and the effluent was collected. The number of WBCs isolated was higher under poor survival conditions ($\approx 5 \times 10^7$ cells/liver) compared to survival conditions ($\approx 2.5 \times 10^7$ cells/liver). Light microscopy revealed that monocytes from livers transplanted under poor survival conditions were enriched 4- to 5-fold compared to whole blood. WBCs were incubated with PBN, a free radical spin trap, and radical adducts were measured by EPR. SOD/catalase-insensitve free radicals exhibiting a 6-line spectrum similiar to radicals isolated from blood following transplantation, were produced by WBCs. Radical species were increased slightly by PMA and decreased by pretreatment with GSH. We hypothesize activated, adherent monocytes are a major source of early free radicals in fatty liver which may be responsible for graft failure. (AA-09156).

147 CELL LINES GENETICALLY OVEREXPRESSING GLUTATHIONE PEROXIDASE-1 ARE HIGHLY RESISTANT TO PARAQUAT

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SV- and EBV-derived eukaryotic expression vectors for human selenium-dependent glutathione peroxidase-1 (GPX1) were constructed and transfected into various murine and human cell lines. Murine cells overexpressed GPX1 indicating that the murine cellular component responsible for selenocysteine insertion into the opal codon in GPX1 recognizes the human "selenocysteine insertion sequences" (SECIS) located in the far 3′ untranslated region of the human GPX1 cDNA. The majority of clones initially displayed increased glutathione peroxidase activity, but eventually the activity was lost in the majority of clones despite continuous G418 selection. Several clones were isolated that retained elevated GPX1 activity. The increase in GPX1 did not alter activity of endogenous SOD1, SOD2, CAT, GPX2, GPX4, NADPH-reductase, GSH, or GSSG. GPX1 expressing clones were resistant to hydrogen peroxide and organic peroxides. The clones were markedly resistant to paraquat. The ratio of paraquat resistance to increase in GPX1 activity was higher than ratios previously reported for SOD1 and SOD2 expressing lines. Supported by NIH ES04989.

148 ROLE OF GSH LEVELS IN THE OXIDATIVE STRESS RESPONSE

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Intracellular sensing of oxidative stress generates a cellular response that reduces the toxicological impact of continued stress. We used L-buthionine S,R-sulfoximine (BSO), a non-electrophilic inhibitor of γ-glutamylcysteine

synthetase (GCS), to examine the permissive effects of glutathione (GSH) depletion on 1) Phase II enzymes of the [*Ah*] gene battery; and 2) GSH level rebound following depletion. Exposure of mouse Hepa-1 cells to BSO produced an 80% depletion of GSH levels, proportional to the inhibition of GCS activity. Concomitant increases in aldehyde dehydrogenase (ALDH3c, 170%), NAD(P)H:menadione oxidoreductase (NMO1, 110%), uridine diphosphoglucuronosyl transferase (UGT1*06, 60%) and glutathione transferase (GSTA1, 30%) occurred as a result of transcriptional activation. N-Acetylcysteine produced increased levels of intracellular cysteine but not GSH, in the absence or presence of BSO. Since N-acetylcysteine or other thiols did not reverse the induction of NMO1 by BSO, it appears that lower GSH *per se* mediated increased NMO1 activity. To examine the rebound of GSH to greater than control levels following BSO depletion, a GCS gene cDNA fragment was prepared by RT-PCR using mouse mRNA as template. The labeled fragment was used as a probe for Northern blot analysis to show that the GSH rebound resulted from transcriptional activation of the GCS gene. These studies provide further support that the GCS gene is regulated in a coordinate fashion with the Phase II genes of the [Ah] battery in response to oxidative stress. (Supported by Grants ES06096, ES04908, ES06321, AG09235).

149 REGULATION OF γ-GLUTAMYL TRANSPEPTIDASE AND γ-GLUTAMYLCYSTEINE SYNTHETASE EXPRESSION BY TERTIARY-BUTYLHYDROQUINONE (TBHQ) IN RAT LUNG EPITHELIAL L2 CELLS

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TBHQ is a major metabolite of the antioxidant, tertiary-butylhydroxyanisole. TBHQ can be oxidized to the semiquinone, which then autooxidizes to produce O_2^-. This leads to subsequent generation of H_2O_2 and hydroxyl radical. The mRNA level of two enzymes of the γ-glutamyl cycle, γ-glutamylcysteine synthetase (catalytic subunit) (γGCS) and γ-glutamyl transpeptidase (γGT), were increased in L2 cells by exposure to 25–100 μM TBHQ. This was accompanied by an increase in intracellular GSH. Both acivicin, an inhibitor of γGT, and buthionine sulfoximine, an inhibitor of γGCS, prevented the increase in GSH by TBHQ. Although Northern blots showed that the steady state level of γGT-mRNA increased by a greater extent than did γGCS-mRNA, nuclear run-on analysis indicated that an increase in the transcriptional rate occurred for γGCS-mRNA but not for γGT-mRNA. Thus, although these two enzymes act together to elevate GSH in response to oxidative stress, their regulation appears to differ. Supported by ES05511 from NIEHS.

150 POTENTIAL MECHANISMS OF POLYCHLORINATED BIPHENYL DISRUPTION OF AGONIST-STIMULATED INOSITOL PHOSPHATE ACCUMULATION

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We reported previously that exposure of cultured cerebellar granule cells to the *ortho*-substituted, neuroactive polychlorinated biphenyl 2,2'-dichlorobiphenyl (DCBP), potentiated (30–50 μM) then inhibited (100 μM) carbachol (CB)-stimulated inositol phosphate (IP) accumulation, perturbed intracellular Ca^{2+} homeostasis, and caused protein kinase C (PKC) translocation. We now report that a structurally related compound, 2,4,4'-trichlorodiphenyl ether (3–100 μM), also potentiated then inhibited CB-stimulated IP accumulation. We characterized further effects of DCBP by examining norepinephrine (NE)- and quisqualate (QUIS)-stimulated IP accumulation in granule cells. Differential effects of DCBP on IP accumulation (1 mM agonist) were observed: compared to control, 30 μM DCBP potentiated CB-, but not NE- or QUIS-stimulated IP accumulation, whereas 100 μM DCBP inhibited IP accumulation by all three agonists. We examined whether direct or indirect activation of PKC may underlie the inhibition of agonist-stimulated IP accumulation. DCBP (100 μM) did not alter PKC activity in cytosolic or membrane fractions of cell homogenates. In intact cells, 50 nM phorbol myristate acetate inhibited CB-stimulated IP accumulation by 75%, an effect which was blocked completely by the PKC inhibitor bisindolylmaleimide (2 μM; BIM). However, DCBP inhibition of CB-stimulated IP accumulation (75%) was not affected by BIM. DCBP (100 μM) had no effect on, potentiated, and inhibited CB-stimulated IP accumulation at 0.1, 1.0, and 10 mM extracellular [Ca^{2+}], respectively. These results suggest that: (1) DCBP differentially affects agonist-stimulated IP accumulation, (2) activation of PKC is not important in DCBP effects on IP accumulation, (3) perturbation of Ca^{2+} homeostasis may be involved in DCBP effects on IP accumulation, and (4) other non-coplanar biphenyl compounds also disrupt agonist-stimulated IP accumulation in granule cells.

151 ACTIVATION OF PROTEIN KINASE C ALPHA (PKC-α) AND INHIBITION OF INTERCELLULAR COMMUNICATION BY POLYCHLORINATED BIPHENYLS (PCB) IN RAT LIVER EPITHELIAL CELLS

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Aberrant regulation of phosphatidyl inositol messenger system has been implicated in the carcinogenesis process. Tumor promoting phorbol esters are potent activators of a central component of this system, namely the calcium and phospholipid dependent protein kinase C (PKC). These and other tumor promoting xenobiotics also down regulate gap junctional intercellular communication (GJIC) that is presumed to be involved in tumor promotion. In the present studies we investigated the modulation of PKC and inhibition of GJIC by a polychlorinated biphenyl (PCB) mixture, Aroclor-1254, and two tetrachlorobiphenyl (TCB) congeners in a rat liver epithelial cell line, WB F-344. At noncytotoxic concentrations (5 or 10 μg/ml) A-1254 and 2,4,2',4'-TCB inhibited GJIC and induced partial (40–50%) translocation of PKC to the plasma membrane while 3,4,3',4'-TCB was ineffective. The onset of these changes was rapid and sustained for at least 24 hours. Western blot analysis of cellular lysates from treated cells showed the expression of PKC-α isozyme in these cells. The results suggest that PKC-α may be involved in the inhibition of GJIC by these agents possibly through phosphorylation of connexin43, the major gap junction protein expressed by these cells. (Supported by NIEHS Superfund grant no. ES-04911).

153 ENHANCEMENT BY CYSTEINE AND SOME OTHER NATURAL AMINO ACID LIGANDS OF NICKEL(II)-MEDIATED OXIDATION OF FREE 2'-DEOXYGUANOSINE WITH H_2O_2

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Oxidation of 2'-deoxyguanosine (dG) by H_2O_2 to 8-oxo-dG, facilitated by $NiCl_2$, is increased by complexing Ni(II) with L-histidine (His), a major, but not the sole amino acid tissue carrier for transition metals. The aim of the present study was to determine if the enhancement could likewise be produced by other L-amino acids: arginine, aspartic acid, cysteine (Cys), glutamic acid, tyrosine, asparagine, serine, threonine, methionine, tryptophan (Trp), and phenylalanine. The amino acids were tested at 2 mM concentrations for effects on oxidation of 0.75 mM dG with 10 mM H_2O_2 plus 1 mM $NiCl_2$ at pH 7.4 (100 mM phosphate buffer), during 24-h incubation at 37°C. Only Cys produced a strong effect by increasing the yield of 8-oxo-dG more than 15-fold versus that by $NiCl_2$ alone. A mixture of Cys and His was even more active, producing a 40-fold increase. The remaining amino acids had no significant influence on Ni(II), except for a slight enhancement by Trp. Thus, Cys and His, two principal low molecular weight tissue carriers of Ni(II), increase this metal's capacity to mediate oxidation of dG both individually and mutually. The mechanisms of the enhancing effects may involve formation of reactive oxygen intermediates, including free radicals and/or ligand-coordinated H_2O_2.

154 GC-MS ANALYSIS OF VITAMIN E AND ITS OXIDATION PRODUCTS IN BIOLOGICAL SAMPLES

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We have developed a capillary gas chromatography-mass spectrometry (GC-MS) assay for vitamin E (α-tocopherol, TH) and its three principal oxidation products, α-tocopherol-quinone (TQ), 5,6-epoxy-α-tocopherolquinone (TQE1), and 2, 3-epoxy-α-tocopherolquinone (TQE2). Deuterium-labeled internal standards (5,8-[$^2H_3$-methyl]-TH (d_6-TH), 2,5-[$^2H_3$methyl]-TQ (d_6TQ), 2,5-[$^2H_3$-methyl]-TQE1 (d_6TQE1), and 2,5-[$^2H_3$-methyl]-TQE2 (d_6-TQE2)) are added to cell or tissue homogenates. The products then are extracted with hexane and converted to O-trimethylsilyl derivatives with N,O-(bis-trimethylsilyl)-trifluoroacetamide/trimethylchlorosilane (10:1, v/v) in dimethylformamide at 25°C for 60 min. Products separated on a 0.25 μm × 30 m DB-5ms column are detected by selected ion monitoring in electron ionization mode of characteristic fragment ions for TH/d_6-TH (m/z 237/243),

TQ/d_6-TQ (m/z 165/171), and both TQE1/d_6-TQE1 and TQE2/d_6-TQE2 (m/z 309/315). Standard curves for TH and the products were linear from 10 fmol to 4 pmol. TH oxidized in microsomes treated with Fe^{2+}/cumene hydroperoxide was quantitatively accounted for as TQ (81% of TH consumed) and TQE1/2 (19%). This method permits quantitative studies of the antioxidant actions and turnover of TH in tissues and cells. (Supported by USPHS Grant CA59585).

155 DIFFERENTIAL MECHANISMS OF CELL KILLING BY REDOX CYCLING AND ARYLATING QUINONES

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The role of mitochondrial dysfunction in quinone-induced cytotoxicity was evaluated. Hepatocyte suspensions were treated with 2,3-dimethoxy-1,4-naphthoquinone (DiOMeNQ), menadione (MQ), 1,4-naphthoquinone (NQ) or 1,4-benzoquinone (BQ) in the presence of cyclosporine A (CyA), ruthenium red (RR), fructose or fructose plus oligomycin. All of the quinones caused concentration-dependent cell killing that was temporally correlated with a loss of mitochondrial membrane potential. For all four quinones, neither CyA nor RR protected against cell killing or loss of mitochondrial membrane potential. In contrast, fructose protected against all four quinones. For DiOMeNQ and MQ, both of which are "redox cycling" quinones, oligomycin reversed the protection afforded by fructose. However, the cytoprotective effect of fructose against the arylating quinones, NQ and BQ, was not reversed by oligomycin. In summary, despite the temporal correlation between loss of membrane potential and cell killing inhibitors of the mitochondrial membrane permeability transition did not protect hepatocytes from any of the quinones. For the redox cycling quinones, a critical factor leading to cell death appears to be mitochondrial membrane potential and not ATP content per se. In contrast, mitochondrial membrane potential does not appear to be critical to the cell killing of hepatocytes by arylating quinones. These findings demonstrate that differential mechanisms of cell killing exist for quinones of differing chemical reactivities.

156 MEASUREMENT OF MITOCHONDRIAL MEMBRANE POTENTIAL IN ISOLATED HEPATOCYTES: A NEW APPROACH

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Experimental evidence indicates that the mitochondrion represents a preferred and important target for numerous cytotoxic agents. We report a simple fluorometric method for the continuous monitoring of mitochondrial membrane potential in isolated cells exposed in vitro to cytotoxic agents. Suspensions of freshly isolated hepatocytes (10^6 cells/ml) pre-loaded with the rhodamine 123 (Rh123, 100 μM) are transferred to a thermostatically-controlled mixed cuvette to which is added the desired cytotoxic agent and fluorescence (excitation 505 nm; emission, 525 nm) recorded continuously. Rh123 is actively accumulated by cell suspensions in direct proportion to mitochondrial membrane potential. Treatment with 50 μg/ml digitonin caused the release of 55% ± 2% of the cytosolic LDH but not Rh123. Subsequent addition of FCCP (5 μM) resulted in the immediate and complete release of Rh 123 without any further leakage of LDH indicating that the release of Rh123 is not a consequence of immediate cell death. This procedure provides a simple and specific technique for monitoring mitochondrial membrane potential in freshly isolated cells based on the stoichiometric redistribution of Rh123 from the mitochondrion upon membrane depolarization. The advantage of this new method is that there is no need to separate cells from the incubation medium making it possible to record real-time changes in mitochondrial membrane potential throughout the in vitro exposure period.

157 QUINOLONE ANTIBACTERIAL PHOTOTOXICITY AS MEASURED BY OXIDATIVE DNA DAMAGE

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Quinolones, a class of synthetic broad spectrum antibacterial agents are widely used as oral preparations and have been under development since the discovery of nalidixic acid in the 1960's. Starting with nalidixic acid, quinolones have caused phototoxicity in humans. Of currently prescribed quinolones, Lomefloxacin (LMX) is associated with the highest number of cases. The purpose of this study was to determine a mechanism for the UVA-induced phototoxicity caused by LMX. Several studies have suggested that UVA-induced quinolone phototoxicity is mediated by reactive oxygen species such as singlet oxygen, OH radical, superoxide anion and H_2O_2. Since singlet oxygen as well as OH radical results in the formation of 8-Oxo-dG in DNA, we quantified 8-Oxo-dG levels from DNA digests of cultured rat epithelial cells (ARL-18) exposed to UVA and LMX by HPLC/UV/EC. Our data demonstrate a dose dependent increase in 8-Oxo-dG up to 2.5 fold with UVA exposure at fluences from 3 J/cm^2 to 24 J/cm^2. Exposure to LMX (50–400 μM) alone in the dark also induced between a 1.3–2 fold increase in 8-Oxo-dG over dark control. A dose dependent increase from 1.1–4 fold in 8-Oxo-dG levels was found with increasing concentration of LMX (50–400 μM) together with 20 J/cm^2 UVA compared to UVA alone at 20 J/cm^2 which induced increased levels to 2 × dark control. We suggest that a potential mechanism for UVA-induced phototoxicity by LMX is through an oxidative pathway.

158 OXIDANT INDUCED CHANGES IN RED AUTOFLUORESCENCE IN LYMPHOCYTES AND LYMPHOBLASTOID CELL LINES CORRELATES WITH DECREASED GLUTATHIONE AND NAD(P)H

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Many toxicants act by inducing reactive oxygen species which may damage DNA, alter protein thiols, and disrupt lipid membranes. We have observed an increase in autofluorescence above 560 nm (red autofluorescence, RAF) with oxidative stress in lymphocytes. Peripheral blood lymphocytes (PBLs) and lymphoblastoid cell lines (Jurkat and Ramos) demonstrate unimodal distributions of RAF, while PHA blasts develop a bimodal distribution as they age and undergo IL-2 deprivation-induced apoptosis. The population of bright RAF cells has increased basal calcium and much lower intracellular GSH compared to the dim RAF cells. We tested the hypothesis that intracellular oxidation increases the RAF of lymphocytes, using monochlorobimane to measure GSH content and blue auto-fluorescence to measure reduced NAD(P)H. Resting 7-day PHA blasts with a bimodal RAF distribution were treated with 7.5 mM H_2O_2 for 30–90 minutes. The proportion of cells in the bright population increased, and both populations became brighter with increasing incubation time. The increase in RAF was accompanied by a decrease in GSH. PBLs treated similarly acted as a single population, showing increased RAF and decreased GSH following incubation. Jurkat and Ramos cells were treated with 0.5 to 10 mM H_2O_2; RAF, NAD(P)H, and GSH were measured following 2 hour incubation. Dose-response curves indicate that intracellular reducing equivalents are decreased before an increase in RAF occurs. Thus, red autofluorescence may be a novel biomarker of oxidative stress, and may be predictive of xenobiotic-induced immunotoxicity. This work was supported by ES04696 and Bristol-Meyers Squibb Pharmaceutical Research Institute.

159 GENERATION OF HYDROXYL FREE RADICALS AND CALCIUM OVERLOADING IN HUMAN LEUKEMIC T-LYMPHOCYTE MOLT4 CELLS TREATED WITH CHROMIUM(VI) AND VANADIUM(V)

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Chromium(VI) [Cr(VI)] compounds have been shown to be potent cytotoxicants and genotoxicants. Although the mechanisms of chromate-induced genotoxicity is not well understood, ·OH radicals have been considered as the ultimate agents in chromate genotoxicity. Chromate cytotoxicity and genotoxicity have been suggested to be occurring via two different mechanisms. Recently ·OH radicals have been shown to exert their cytotoxic effects through alteration of Ca^{2+}-homeostasis. We have studied the mechanism of chromate cytotoxicity and potential relationship between chromate cytoxicity and intracellular Ca^{2+} level. Because vanadium(V) has similar cellular uptake mechanism and intracellular reductive metabolism as Cr(VI), we have compared the vanadium(V) effects with Cr(VI) in regulating intracellular Ca^{2+}. Hydroxyl radicals were detected using a ESR spectrometer and 5,5-dimethyl-1 pyrroline N-oxide (DMPO) as spin trap. Intracellular Ca^{2+} levels were determined using Indo-1 in a Flow cytometer. Cellular ATP levels were determined using the fire fly luciferase reaction and chemiluminescence was monitored using a Scintillation counter. Potassium chromate (a Cr(VI) compound), Cr(V) (generated by reaction of Cr(VI) with NADPH), and Cr(III) chloride hexahydrate (a Cr(III) compound) were found to generate ·OH when reacted with H_2O_2 at pH 7.4. Treatment of cells with potassium chromate (0–100 μM) or sodium orthovanadate (0–100 μM) increased the intracellular Ca_{2+} in a dose-

dependent manner and after 1 hr treatment, cellular Ca_{2+} level reached to 140% and 160% of the control, respectively. Treatment of cells with the metals also decreased the intracellular ATP in a dose-dependent manner within 1 hr of treatment. These results suggest that the above metals may be inhibiting the energy-dependent Ca_{2+} regulating process by interacting with Ca_{2+}-ATPase pumps and this calcium overloading could, at least in part, be mediating the metal-induced cytotoxicity.

160 SUPERIOR PROTECTION AGAINST OXIDATIVE INJURY WITH HYDROPHILIC TOCOPHEROL ESTERS IN RAT HEPATOCYTES

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It has previously been demonstrated that hepatocytes incubated with tocopheryl succinate (TS) are completely protected from the toxic effects of a wide variety of toxic insults including oxidative injury. In contrast, protection was not observed with the administration of unesterified tocopherol (T) or tocopheryl acetate (TA). To test the possibility that TS cytoprotection results from the intact anionic TS molecule we examined the effect of the non-hydrolyzable derivatives of TS, tocopheroxyl butanoate and tocopheryl 3-methyl succinate, on ethyl methanesulfonate-induced cell death and lipid peroxidation. Cytoprotection was not observed with these anionic derivatives indicating that the cellular accumulation and hydrolysis of TS (releasing tocopherol) are responsible for TS protection. Next, we investigated the hypothesis that the superior protective abilities of TS result from the increased hydrophilicity of this molecule. We examined the protective capacity of other hydrophilic tocopherol derivatives, such as TS-PEG ester, tocopheryl phosphate, tocopheryl glutarate, and trolox. Each of these compounds completely protected hepatocytes from oxidative damage, suggesting that hydrophilic tocopherol derivatives are superior cytoprotective agents in vitro, during an acute oxidative insult (as compared to T and TA). We suggest that ability of hydrophilic tocopherol derivatives to rapidly replenish subcellular sites with a functional antioxidant is responsible for protection against oxidative injury. (Supported by NIEHS grant #05452).

161 TRICHLOROETHYLENE: FREE RADICAL STUDIES IN B6C3F1 MOUSE LIVER SLICES

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Electron paramagnetic resonance EPR/spin trapping techniques were used to test the hypothesis exposure of liver to trichloroethylene (TCE) causes increased formation of free radicals. Precision cut liver slices from B6C3F1 mice were incubated for 20 min. with TCE at headspace concentrations ranging from 0–10,000 ppm with and without the spin trap α-phenyl-tert-butyl nitrone (PBN). PBN (10 mM) did not interfere with K^+ levels, lactic dehydrogenase, aspartamine transaminase or alanine transaminase. Free radicals were measured using a Bruker ESP300E spectrometer. A solution of 3-carbamoyl — 2,2,5,5 tetramethyl-1-yloxy free radical was used as a standard to quantitate radicals. All data was normalized to liver wet weight. Total radicals (TR) increased linearly with increasing TCE head space concentration (TR = 4.9E – 04[TCE] + 6.84X10E14, r = 0.99 P < 0.001). EPR/spin trapping results were corroborated by conjugated diene measurements and analysis by gas chromatography.

162 TIME-COURSE OF LIPID PEROXIDATION FOLLOWING TRICHLOROETHYLENE GAVAGE IN B6C3F1 MICE

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Trichloroethylene (TCE), a common groundwater pollutant, is is suggested to produce free radical species during its metabolic degradation (Steel-Goodwin et al., 1994). This may account for the evidence of lipid peroxidation, seen as lipofuscin accumulation, observed in acute TCE exposures (Elcombe et al., 1985) and in chronic studies using the major metabolite trichloroacetic acid (TCA) (Bull et al., 1990). Present time course data for TCE-induced lipid peroxidation is limited to a few hours. To extend that dataset, TCE was administered by corn oil gavage to male B6C3F1 mice 5 days a week for 8 weeks at doses of 0, 400, 800 and 1200 mg/kg. Liver homogenates were prepared from harvests at multiple time points throughout the study and analysed for thiobarbituric acid reactive substances (TBARS). TBARS rise rapidly to reach a peak of approximately 140 nmoles per gram of liver tissue at three days. Between three and five weeks the levels drop to 50 nmole/g liver and then gradually rise to 100 nmole/g by the eighth week. In this study, the time course was independent of TCE concentration.

163 SUPEROXIDE DISMUTASE MIMETICS PROTECT AGAINST PARAQUAT-INDUCED INJURY BOTH *IN VITRO* AND *IN VIVO*

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We have employed low molecular weight superoxide dismutase mimetics based on substituted manganic meso-porphyrins to protect against a known pulmonary toxicant, paraquat, which is thought to produce injury by redox cycling with diaphorases and molecular oxygen producing the reactive superoxide radical. SOD mimetics were first tested in an *in vitro* cell culture model using calf pulmonary endothelial cells (CPA-47) and rat lung epithelial cells (L2). Cell injury was assessed by measuring release of the cytosolic enzyme lactate dehydrogenase (LDH) into the cell medium. Paraquat exposure produced a dose dependent release of LDH in both types of lung cells. The epithelial cells were more resistant than the endothelial cells to paraquat-induced injury. The SOD mimetics (MnTBAP and MnTMPyP) protected both cells types in a dose dependent manner. Neither compound appeared toxic to either cell type at the highest doses used in these studies. The protective effect of the SOD mimetics appears to be due to their dismutase activities since the zinc forms which do not possess SOD activity did not protect the cells against paraquat-induced injury. We next assessed whether MnTBAP would protect *in vivo* from a bolus dose of paraquat. Six mice where given either saline (10 ml/kg, ip) or paraquat (45 mg/kg, ip) and exposed to nebulized saline or MnTBAP (5 mg/ml) for 30 minutes twice daily. Mice were killed 48 hours after paraquat treatment. Lung injury was assessed by measuring LDH, protein and % PMN's in bronchoalveolar lavage. Paraquat increased levels of lavage LDH, protein and number of PMNs. MnTBAP did not affect any of these parameters in control animals, but significantly decreased both LDH and protein levels in the group that received paraquat. These studies suggest that these SOD mimetics may be useful agents in preventing injury associated with the generation of reactive oxygen species.

164 ALTERED EFFECTS OF THE SUPEROXIDE ANION ON ISOLATED COCHLEAR OUTER HAIR CELL SHAPE BY SUBSTITUTION OF EXTRACELLULAR SODIUM WITH N-METHYL-D-GLUCAMINE

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Exposure of the guinea pig cochlear outer hair cell (OHC) *in vitro* to reactive oxygen species generating systems induces xieotic or potocytotic bleb formation at the infranuclear region similar to that seen following certain ototoxicant exposures. In neurons, replacing extracellular sodium (Na^+) in the medium, with N-methyl-D-glucamine ($NMDG^+$), a relatively impermeant cation, reverses oxidative stress induced cell surface bleb formation. This study exposed OHCs to a superoxide anion (O_2^-) generating system, xanthine-xanthine oxidase (X-XO), and bathing media containing either $NMDG^+$ or Na^+, in order to determine the potential of preventing or reversing O_2^- induced bleb formation. OHCs were bathed with artificial perilymph containing 1.5 mM $CaCl_2$, 1.5 mM $MgCl_2$ and 10.0 mM D-glucose, in 1.0% modified Dulbecco's PBS (Na^+AP; 300–310 mOsm, pH = 7.2 ± 0.1), or iso-osmotic artificial perilymph in which Na^+ was substituted by equimolar $NMDG^+$ ($NMDG^+$AP). Based upon pre-exposure (30 min)/exposure (90 min)/post-exposure (60 min) conditions, groups included: a) Na^+AP/ X-XO in Na^+AP/ Na^+AP, b) $NMDG^+$AP/ X-XO in $NMDG^+$AP/ $NMDG^+$AP, c) Na^+AP/ X-XO in Na^+AP/ $NMDG^+$AP and d) $NMDG^+$AP/ X-XO in $NMDG^+$AP/ Na^+AP. Group (a) cells developed blebs during the X-XO in Na^+AP exposure which lasted to the experiment end. OHCs in group (b) did not develop blebs during X-XO in $NMDG^+$AP infusion or subsequent $NMDG^+$AP reinstatement. Group (c) cells developed infranuclear blebs during the X-XO in Na^+AP exposure, all of which disappeared upon superfusion with $NMDG^+$AP. OHCs in group (d) did not develop blebs during X-XO in $NMDG^+$AP infusion, but did upon Na^+AP superfusion. This suggests that cell surface bleb formation in OHCs due to O_2^- generation is reversible and depends upon the presence of extracellular Na^+ but that the absence of Na^+ does not protect the plasma membrane against O_2^- induced damage.

165 HUMAN MANGANESE SUPEROXIDE DISMUTASE TRANSGENIC MICE ARE PROTECTED AGAINST ADRIAMYCIN-INDUCED CARDIAC TOXICITY

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Previous studies have suggested that free radical mechanisms are involved in the toxicity of many xenobiotics. Mitochondria are major sites of oxidative metabolism, and thus, represent the first target for free radical insult. Manganese superoxide dismutase (MnSOD) is the primary mitochondria antioxidant enzyme that scavenges superoxide radicals. We have produced human MnSOD transgenic mice by introducing a transgene which contains the human β-actin promotor and the human MnSOD cDNA. Mice bearing the transgene were identified by Southern analysis of DNA isolated from tail tissues. Three founders with different increased levels of human MnSOD were obtained. Northern blot, activity gel, and enzymatic activity assays showed that human MnSOD was actively expressed in heart, lung, muscle, brain, and skin. The MnSOD activities in the heart in the three transgenic mice lines are increased approximately 1.5, 2.5, and 5 folds respectively compared to non-transgenic littermates. Immuno-electron microscopy has confirmed that the human MnSOD is properly transported into the mitochondria. The development of transgenic mice with a range of MnSOD activities provides a valuable model to study the role of MnSOD in conferring protection against free radical-mediated toxicity. We chose adriamycin (ADR)-induced cardiotoxicity as a test model. ADR is a widely used anticancer drug, but significant cardiotoxicity limits its use. Free radical generation has been suggested as a mechanism for the cardiotoxicity, but is not proved. The present study demonstrated that transgenic mice expressing high level of MnSOD are significantly protected from ADR at the dose of 25 mg/kg, while the non-transgenic littermates showed severe cardiac toxicity as demonstrated ultrastructurally by mitochondria damage and injury to myofilaments. (Supported by an NIH grant CA 49797, a KTRB grant 5-4113, and a VA merit award.)

166 EFFECT OF ETHANOL AND TNF ON MANGANOUS SUPEROXIDE DISMUTASE IN HUMAN HEPATOMA CELLS

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There is a growing body of evidence that indicates the involvement of free radical processes in ethanol-induced liver injury. A major physiological source of oxygen radicals are the mitochondria, the site of cellular electron transport and oxidative phosphorylation. One of the antioxidant defense systems in mammalian cells is a mitochondrial enzyme, manganous superoxide dismutase (MnSOD). This enzyme scavenges superoxide radicals and can be induced by agents that cause oxidative stress. To investigate the involvement of free radical processes in ethanol-induced liver injury, the effect of ethanol on MnSOD mRNA, protein and activity was studied in HepG2 cells, a human hepatoma cell line. Acute (72 hours) and chronic (5 and 10 weeks) doses of ethanol, at a concentration of 25 mM, were administered to HepG2 cells. An acute dose of TNF, a known inducer of MnSOD, was also administered, as a positive control. Northern blot analysis was used to investigate steady-state mRNA levels, Western blot analysis was performed to identify MnSOD protein and MnSOD activity was determined using the nitroblue tetrazolium method. It was found that acute ethanol dosage significantly increased the enzyme activity and protein levels of MnSOD. However, there was no corresponding increase in the mRNA level. Chronic ethanol treatment also resulted in a slight increase in MnSOD activity but this effect was not judged to be significant. We conclude that acute ethanol and TNF are capable of enhancing MnSOD activity, presumably by causing an increase in oxidative stress. However, TNF causes a true induction of MnSOD, while acute ethanol dosage increases MnSOD activity by a translational process. (Supported by NIH grants CA-49797 and 3MO1 2602 07S1, CRC #MO1RRO26O2-O7, THRB 5-41113 and the Veterans Administration.)

167 RNA-PROTEIN CROSSLINKAGE, 8-HYDROXY-GUANINE AND PROTEIN CARBONYL FORMATION AS MARKERS OF EXPOSURE TO METHYLENE BLUE PLUS LIGHT

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Methylene Blue (MB) has long been used medically in the treatment of methemaglobinemia and has recently been introduced as a photoactive antiviral agent in the treatment of blood products. Upon exposure to white light (L) MB produces singlet oxygen, a strong oxidizer, which is most likely to be the active species in viral inactivation. We have measured several parameters of oxidative damage following inactivation of the RNA bacteriophage Qβ by MB + L. Previously, we have shown that 8-hydroxy-guanine formation in the phage RNA is insufficient to account for the number of lethal events (which reside in the RNA) following MB + L exposure. In this report we show that formation of protein carbonyls and RNA-protein crosslinking also correlate with phage inactivation, as does 8-OHGua formation. We also show that the double-stranded nature of Qβ RNA is important in the efficiency of 8-OHGua formation in the RNA by MB + L. This activity is in contrast to a dye of similar structure to MB, thiopyronin, which forms high levels of 8-OHGua in DNA and RNA, but with no preference for double-stranded RNA. Supported by NIH CA 42854 and HL 53585.

168 NADH, NADPH OXIDOREDUCTASE (DT-DIAPHORASE) MAY CATALYZE MENADIONE (MD) HEPATIC, MICROSOMAL LIPID PEROXIDATION (LP)

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MD toxicity has been attributed to its 1 e^- reduction to the semiquinone. This damages cells either by reducing O_2 to reactive species or nonenzymatically alkylating cellular thiols. We have compared *MD* and 2,3-dimethoxy-1,4-naphthoquinone (*DMNQ*) effects on hepatic microsomal *LP* determined by HPLC-chemiluminescence. *DMNQ* is much less hepatotoxic than *MD*. Their NADPH dependent O_2 uptakes were the same. In the remaining studies NADPH was not included, except as noted. At toxic concentrations ($\geq$100 μM), *MD*, but not *DMNQ*, caused *LP*. *LP* was not observed in *MD* (150 μM) incubations without microsomes, with heated microsomes or only red cell ghosts or soybean, phosphatidylcholine liposomes. *LP* was linear to 30 min with a Vm = 35 nmol/min/mg prot and Km = 200 μM. *LP* was unaffected by low [NADPH] (0–10 μM), but was markedly increased at higher [NADPH]s. GSH and mercaptoethanol (5 mM) added after incubation reduced all of the lipid peroxides. *LP* was inhibited by superoxide dismutase (55%), catalase (37%), mannitol (40%), EDTA (39%), desferoxamine (36%) and N-ethyl-maleimide (25%) suggesting a partial role for reactive O_2. Dicourmarol (30 μM) inhibited *LP* 74%, suggesting a role for microsomal, DT-diaphorase. The correlation between the [*MD*] dependence of *LP* formation and its hepatotoxicity suggest that this toxicity is partially mediated by NADPH-independent, DT-diaphorase catalyzed *LP*. In contrast, *DMNQ*, which is not hepatotoxic, caused no *LP*.

169 INFLUENCE OF GLUTATHIONE AND pHON BIOMARKERS OF CELLULAR OXIDATIVE STRESS

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Cellular oxidative stress is associated with such pathological conditions as atherosclerosis, inflammatory diseases and cancer. The oxidation of 2′,7′-dichlorofluorescin (DCFH) to dichlorofluorescein (DCF), as well as 2-deoxyribose (DOR) oxidation, are used to assess the status of oxidative stress in cells and tissues. Since high levels of reduced glutathione (GSH) and lower pH values have been associated with protection of cells from chemical lethality, we evaluated the influence of these parameters on the oxidation of DCFH and DOR, as well as parameters of cellular response to oxidative stress. We used a cultured hepatocyte line (*chlch* cells) that is susceptible to damage by chemicals that produce oxidative stress. A hydroxyl radical generating system consisting of H_2O_2, ascorbate and ferrous ion produced a pH-dependent lethality, with complete cell killing at pH 7.4 and none at pH 6.8. Lethality correlated with the depletion of intracellular GSH, and with an increase in DNA fragmentation. The influence of GSH and pH was assessed for DCFH and DOR oxidation. The oxidation of both probes was inhibited by GSH, with about 4-fold greater inhibition at pH 6.8, relative to pH 7.4 [IC_{50} values for pH 6.8 and 7.4, respectively: DCFH = 7 μM and 30 μM; DOR = 125 μM and 490 μM]. These data suggest that the protonated form GSH is the inhibitory species, rather than GS^-. Since the intracellular GSH concentration is approximately 1 mM, either hydroxyl radical may not be the primary intracellular oxidant of these probes, or a burst of hydroxyl radical generation may overcome the inhibition by GSH. (Supported by Grant ES06096).

170 THE USE OF CLINICAL AND CONSUMER EYE IRRITATION DATA TO EVALUATE THE RABBIT LOW VOLUME EYE TEST

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Eye irritation evaluation of products is necessary to ensure consumer safety if inadvertent accidental exposure occurs. Thus, it is important to assess periodically the reliability of eye irritation tests to accurately predict consumer response. We reviewed consumer eye irritation comments from 1985 through 1992 on over 75 marketed beauty/personal care, laundry and household cleaning products. Twenty-four products evaluated during this time period were also tested in the rabbit Low Volume Eye Irritation Test (LVET). A comparison was made of the time needed for consumer and rabbit eye irritation to clear after exposure to one of the 24 products. Additionally, the results of clinical eye studies on two prototype shampoo formulations were evaluated against parallel LVET data. The clinical data and consumer experience showed less eye irritation in humans from exposure to these products than in rabbits. The consistency of the data over a 13 year period presents strong evidence that human eye irritation is less severe than predicted by the rabbit LVET and that the LVET correlates well with human eye exposure from beauty/personal care, laundry and household cleaning products. We concluded that the LVET is suitable for predicting human eye irritation potential of these types of products.

171 STATUS OF SAFETY PHARMACOLOGY (SP) IN THE PHARMACEUTICAL INDUSTRY — 1995

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SP identifies adverse drug effects on organ functions and is required for drug registrations. We surveyed 28 pharmaceutical companies (18 US; 10 UK) for how and why they conduct SP. All conduct/contract SP studies; 10 place SP in Development, 18 in Research. 20 have SP Units with 2 to 18 staff/unit (ave. ≈7). Most (n) conduct cardiovascular (28) and CNS (27) studies, less evaluate respiratory (22), GI (21) and renal (17) studies; 8 conduct a ligand binding/activity panel; most use the 1991 Japanese SP guidelines only as a guide. 10 use an MTD for SP studies, 18 use multiples of pharmacologic or therapeutic doses. 14 conduct SP studies to Good Laboratory Practices (GLPs). Resources to complete a company's standard SP program are ≈1.3 person year/compound. Clinicians are most often cited as the primary client for SP studies, followed by research and development scientists, then regulators. The results suggest that most companies conduct SP for its contribution to risk assessment and clinical critical care management. Companies are increasingly conducting SP studies to GLPs, reflecting the EEC position on pharmacodynamic testing for adverse effects.

172 LACK OF A DIFFERENCE IN TOXICITY BETWEEN AD LIBITUM (AL) OVERFEEDING AND MODERATE DIETARY (CALORIC) RESTRICTION (DR) IN SPRAGUE-DAWLEY (SD) RATS TREATED WITH 5 PHARMACEUTICAL DRUGS

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The objective of this study was to compare the effects of feeding Purina 5002 diet under moderate DR [16 g/day (49 Kcal/day) for females, 21.5 g/day (66 Kcal/day) for males] vs. AL [24 g/day (74 Kcal/day) for females, 32 g/day (98 Kcal/day) for males] on the responses to 5 drugs given to 10 rats/sex/group at a toxic dose. Phenobarbital (100 mg/kg/day), clofibrate (500 mg/kg/day), L-647,318, an HMGRI compound (150 mg/kg/day), cyclosporin A (25 mg/kg/day), and MK-0458, a dopamine agonist, (4 mg/kg/day) were administered to SD rats for 14 wks. Clinical signs, BW, clin path parameters, liver microsomal cytochrome P450 content, and drug metabolizing enzyme activities were evaluated. With the large doses given, anticipated manifestations of toxicity of these compounds were observed. While quantitative differences were seen relative to the dietary regimen, in general, the responses were qualitatively very similar for each compound under AL and moderate DR (which is within the range of AL food consumption for other labs). There were no significant differences in cytochrome P450 contents or enzyme activities in samples from rats on AL or DR in control or treated groups. These results show that moderate DR did not significantly affect the toxicologic response induced by diverse drugs.

173 REDUCED BODY WEIGHT GAIN AND MAMMARY TUMOR INCIDENCE: A CASE FOR A NON-RELATIONSHIP

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The existence of a significant relationship between body weight and mammary tumor incidence in females rats was proposed by Haseman and his colleagues (Rao et al., 1987). The existence of this relationship has been subsequently cited as 'fact' by several authors. A closer look at this work revealed that the relationship is minimal, and that additional factors must be considered when examining this hypothetical relationship. The analysis presented by Rao *et al.* was re-created. Examination of the studies analyzed revealed that the selection criteria appeared to be arbitrary, some studies with possibly invalid data were included, and many had confounding factors, e.g., survival effects, competing tumor response. A new analysis was performed by examining data from control groups in 142 NTP studies. The correlation between percent tumor incidence and percent body weight gain was 0.037, indicating a minimal relationship. Examination of subsets of studies revealed that other factors (survival, toxicity, competing tumor, positive mammary tumor response) had differing effects on the hypothesized relationship, and that there were marked differences between studies with dietary or gavage routes of administration. Thus, the hypothesis of a relationship between body weight and mammary tumor incidence in female rats is not supported, and alternate hypotheses, including the effects of isocaloric restriction on mammary tumor incidence, must be explored.

174 FDA CENTER FOR VETERINARY MEDICINE ANIMAL DRUG ADVERSE REACTION MONITORING PROGRAM

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The Center for Veterinary Medicine monitors adverse reactions for all animal drug products. A computer database currently containing >8000 reports of adverse reactions was established in 1987 to support this activity. The database information on report source, species, breed, age, sex, number of animals involved, dosage regimen and route, reason for drug use, concomitant medical problems, concomitant drugs administered, general state of animals health, detailed description of adverse reaction, previous exposure, drugs used to treat adverse reaction, attending veterinarian's comments as well as follow-up reports is used to provide the basis for regulatory decisions. Causality between products and adverse reactions is evaluated using an algorithm process adapted from Kramer et al. (1979). The algorithm decision strategy provides an objective mechanism for evaluating a potential cause-effect relationship for reported adverse drug reactions.

175 *AD LIB* CONSUMPTION: A MEANS OF ESTIMATING SNACK CHIP CONSUMPTION IN THE GENERAL POPULATION FOR THE EVALUATION OF FOOD ADDITIVE EXPOSURE

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Critical to the potential safety evaluation of food additives or food contaminants is an accurate estimate of exposure. This study was developed as a means to estimate the possible range of food additive exposure to humans eating snack chips. Human subjects made selections from 2 oz. units of 4 vegetable oil snack chip types to minimize bias from taste related under-consumption. Subjects were instructed to eat from 1 to 21 bags *ad libitum* over each study week. Peel-off bag labels were mounted in a daily diary to track consumption patterns. To provide a reflection of the general U.S. population, subjects were recruited using a two-way stratification scheme. Adult test subjects were stratified by age and gender. Subjects were further stratified by self-reported snack chip eating frequency (light to heavy snackers). 71 subjects completed the two week study. During Week 2 an average of 14.6 oz. (per 7 days) snack chips were consumed. All available 42 oz./week of test article was consumed by a few 99th percentile individuals. In this study, 38 oz./week or 5.4 oz./day was observed to be a conservative 95th percentile estimate of chronic snack chip consumption by adults. The daily range of consumption included 0 to 18 oz. of chips. *Ad lib* consumption patterns were analyzed using chaos theory as a means to reduce exposure estimation uncertainty due to data variation.

176 STRUCTURE-TOXICITY RELATIONSHIPS FOR RODENTS: A MECHANISTICALLY BASED MODEL FOR PREDICTING LC50S FOR INDUSTRIAL CHEMICALS WHICH INDUCE LETHAL NARCOSIS

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The 4-hour LC50 of volatile industrial chemicals which induce lethality by narcosis can be predicted by a model developed at Haskell Laboratory. Structure-toxicity relationships indicate the model is binary, based on air/water and octanol/water partition coefficients. The model is highly predictive for many chemical classes including: linear and cyclic ethers, alkylsulfides, halogenated alkenes and alkanes, simple and substituted aromatics, and substituted aliphatics. This model supports the hypothesis that lethal narcosis in mammals is non-specific and results from partitioning of a chemical between an organism's environment (air) into critical central nervous system tissue. The model demonstrates that lethal narcosis in mammals is directly comparable to that of fish when expressed as chemical activity. This mammalian toxicity model is in theoretical agreement with lethal narcosis models previously developed for aquatic animals.

177 COMPARATIVE RISK ASSESSMENT: VIRTUALLY SAFE DOSE VS. THERAPEUTIC DOSE FOR SOME COMMON DRUGS

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Risk assessment uncertainty analysis should provide qualitative and quantitative comparisons which allow the reader to understand uncertainty arising from the assumptions utilized in a risk assessment. A particularly useful uncertainty analysis tool is comparative risk assessment where risks projected from the risk assessment are quantitatively compared to actual risks (lifestyle factors, dietary habits, etc.). We have determined oral cancer slope factors for a number of drugs, derived virtually safe doses (VSD; assumed 1×10^{-6} risk) based on their slope factors and compared these VSD values to doses normally received during a therapeutic regimen. Tumor incidence data for clofibrate, dapsone, disulfiram, isoniazid, methimazole, metronidazole, oxazepam, phenobarbital, rifampin, and sulfisoxazole were abstracted from the Carcinogenic Potency Database (L S Gold et al., 1984, *seq.*) or NTP technical reports and subjected to multistage analysis using Tox_Risk version 3.5 using current EPA methodologies including scaling between animals and humans based upon surface area. Geometric mean slope factor values were prepared in cases where more than one incidence data set per compound was analyzed. VSD values for the drugs considered ranged from 22 to 0.001 μg/kg/day; VSD values were generally 5 orders of magnitude below therapeutic doses. Though the comparison between therapeutic dose and VSD is based upon the assumption of lifelong daily therapy, even if one were to assume only one day's treatment over a lifetime, the resulting lifetime average therapeutic dose would still be 2 to 80 times greater than the VSD. Thus, in a comparative sense, therapeutic use of some drugs carries projected risks greater than guidelines for acceptable environmental risks.

178 AN APPROACH FOR DETERMINING TOXICITY VALUES FOR DERMAL EXPOSURE

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Oral toxicity data are available for many chemicals allowing for the calculation of Oral Reference Doses (RfD) for noncarcinogenic effects and slope factors (q_1*) for carcinogenic effects. In contrast, dermal toxicity data for long-term exposure are not available for most chemicals which precludes calculation of dermal RfDs and slope factors. Health risks from dermal exposure to chemicals may be estimated by modification of the oral RfD or slope factor by a chemical-specific gastrointestinal absorption factor following SUPERFUND guidelines. This transforms the administered doses (oral RfDs and slope factors) into absorbed doses for comparison to intake amounts determined from dermal exposures. A computer program was developed to calculate the dermal RfD and slope factors from absorption data obtained from the published literature. When no quantitative data were available, estimates were made using structural analogs or a scheme developed to assign quantitative values to qualitative data. When quantitative data were available, the most conservative absorption factor from the species phylogenetically closest to the human was selected. The computer program uses two data bases for calculating dermal toxicity values. The first, updated quarterly, contains oral and inhalation risk values for over 600 chemicals. The second contains the absorption factor and the reference citation for over 100 chemicals. The program compares the CAS numbers of all chemicals in both data bases. When a match is found and if oral data and absorption factors are present, the program computes the dermal RfD and slope factors, prints the calculated results, the reference citations, and the source of the oral data (IRIS or HEAST). Using this methodology, RfDs ranging from 3.00E−08 to 4.00E+00 mg/kg/day and slope factors ranging from 7.89E−03 to 4.30E+02 $(mg/kg/day)^{-1}$ have been derived for over 60 chemicals.

* Managed by Martin Marietta Energy Systems, Inc., for the U.S. Department of Energy under Contract No. DE-AC05-84OR21400.

179 COMPARISON OF ANIMAL AND HUMAN DATA TO ESTIMATE THE NO ADVERSE EFFECT LEVEL (NOAEL) IN HUMAN

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Permissible life time exposure levels for humans are usually calculated by extrapolating NOAELs after subchronic and chronic studies and dividing the values downwards by 10 X. In the present investigation the relationship between results from animal and human exposures were investigated. The data bases for two environmental pollutants: (dinitrophenol, carbon disulfide) and four drugs (acetaminophen, ranitidine, isoniazid and zidovudine) were investigated. The drugs were chosen for the present investigation due to the presence of a large number of data on the effect of these classes of chemicals on both animal and human populations. The ratio between NOAEL (animal)/NOAEL (human) for dinitrophenol and carbon disulfide was ≤ 4. The results of the studied drugs on experimental animal and human populations suggest that a factor of ≤ 1 may be required for extrapolation of the data of exposure levels from animals to human. The present investigation suggests that evaluation of the use of uncertainty factors of 10X in every chemical is not warranted. (Supported by NSF-HSMRC-NJIT-NJ).

180 EVALUATION OF THE CURRENT METHOD FOR EXTRAPOLATING SUBCHRONIC AND CHRONIC TEST RESULTS FOR ESTIMATION OF LIFE TIME EXPOSURES LEVELS

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The current guidelines for non-carcinogenic exposure is based on identification of the No Observed Adverse Effect Level (NOAEL). This data is usually adjusted by using default factors of 10 X (uncertainty factors) to estimate the values for life time exposure. In the present investigation data sets on the following pesticides were investigated: Chlordecone, Mirex, hexachlorobutadiene, bromomethane, Disulfotone and methyl parathion. Examination of the experimental results revealed that the ratio of Low Observed Adverse Effect Level (LOAEL)/NOAEL for Chlordecone, Mirex and bromomethane was 1–5 for studies performed on different species. All LOAEL/NOAEL ratios for hexachlorobutadiene was ≤ 3.5. Using the same parameters, other data also showed that the ratios of NOAEL (subchronic)/NOAEL (chronic) were less than 10. Data sets of Disulfoton and methyl Parathion is in agreement with the above results (≤ 4). The present investigation suggests that the use of uncertainty factors of 10 X has no experimental merits and could be excessive. (Supported by NSF-HSMRC, NJIT, NJ).

181 AVAILABILITY AND USE OF HUMAN DATA IN THE DEVELOPMENT OF USEPA REFERENCE DOSES AND REFERENCE CONCENTRATIONS

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Reference doses (RfDs) and reference concentrations (RfCs) developed by the USEPA are typically used to assess the risk of adverse human health effects from exposure to environmental chemicals. RfDs and RfCs are derived in the majority of cases by adjusting a no-effect or low-effect level (NOAEL or LOAEL) in animals by a number of uncertainty and/or modifying factors to arrive at an estimate of a safe human dosage. Despite the existence of human exposure information for many commonly-used industrial chemicals, information which many toxicologists would consider substantial, a consistent USEPA practice is to rely almost exclusively on animal data in the derivation of an

RfD or RfC. This practice is best illustrated by the chemicals dimethylaniline and acrolein. For these chemicals, the endpoints selected as the basis for the reference values are blood (dimethylaniline) and irritation (acrolein) effects in mice and rats, respectively. Large (1,000 or greater) uncertainty factors are applied to LOAELs from short-term animal studies (13 weeks for dimethylaniline and 62 days for acrolein) to develop a safe human dose estimate, even though chronic and/or occupational human studies are available in the published literature for these chemicals. In fact, workplace threshold limit values (TLVs) recommended by the American Conference of Governmental Industrial Hygienists for these chemicals were based on studies in humans which considered the same endpoints upon which USEPA derived the corresponding reference values (i.e., blood for dimethylaniline and irritation for acrolein). Thus, we demonstrate through these and other examples that the USEPA has chosen an unnecessarily conservative approach by placing little emphasis on available human data in deriving RfDs and RfCs or for modifying the magnitude of the uncertainty factors applied to animal data.

182 THE RELATIONSHIP BETWEEN CONCENTRATION AND EFFECT LEVELS IN SUBCHRONIC AND CHRONIC EXPOSURES USING EC_{10} RATIOS

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This analysis used parallel subchronic (SC) and chronic (C) inhalation studies in rats exposed to CS_2 (NTP, 1990). The EC_{10}, the concentration producing a response rate 10% different from controls, was used in lieu of NOAELs. The SC/C EC_{10} ratios for body weight loss (1.9 for ♂ & 3.8 for ♀) were comparable to the SC/C NOAEL ratio of 3 for both sexes. Analysis of the C lesions in the respiratory tract showed that EC_{10}s increased in direct relation to the depth of the lesion, from 0.17–0.67 mg/m^3 in the nasal region, to 1.1 mg/m^3 in the laryngeal region, to 1.1–3.2 mg/m^3 in the pulmonary region, suggesting the nasal region to be the most sensitive. In comparing results from lesions in this most sensitive region, EC_{10}s for the SC study were between 0.10–0.24 mg/m^3, comparable to the C results. These results suggest that use of the default value of 10 for extrapolation of SC to C duration may be conservative for some endpoints but not for others. Additional chemicals will require similar evaluation to better understand what generalizations have the most support.

(NTP, 1990. Technical Report 377, Toxicology and Carcinogenicity of Carbon Disulfide [CS_2].)

183 NEUROTOXICITY AS THE BASIS FOR CHRONIC DOSE-RESPONSE ESTIMATES

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Derivation of an inhalation reference concentration (RfC) or an oral reference dose (RfD) requires selection of a critical effect from available toxicity data. The Integrated Risk Information System (IRIS) was searched to create a database containing chemicals for which the critical effect(s) were based on neurotoxicity. Critical effects were assigned the following categories: behavioral, physiological, biochemical, and pathological. As expected, chronic studies were cited more often (47%) than subchronic (36%) or acute exposures (14%). Analyses revealed that 21% of all RfDs and 43% of all RfCs are based on evidence of neurotoxicity. As percent of total citations, behavioral (39, 37%) and pathological (33, 54%) data were cited more often than biochemical data (28, 4%) (% of RfDs and RfCs, respectively). A neurophysiological effect was cited only once. All neurochemical effects were based on cholinesterase inhibition. For RfCs, 63% were based on olfactory epithelium changes. Dosimetry and physicochemical characteristics may explain such lesions; 50% of these chemicals also showed effects in respiratory epithelium. Specific mechanisms of olfactory toxicity (e.g., local metabolism by carboxylesterase) likely account for another 16%, but 33% showed other neurotoxicity in addition, suggesting a sentinel role for olfactory effects. These data demonstrate the importance of neurotoxicity to risk assessment and support routine testing for this endpoint.

184 SINGLE EXPOSURE CARCINOGENS

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A single exposure to over 300 chemical carcinogens representing more than 30 chemical classes has been found to cause cancer in mammalian models. This phenomenon has been reported in more than 30 mouse strains, 24 rat strains as well as in other less frequently used models such as hamsters, gerbils, guinea pigs, rabbits, and fish. Similarly, this phenomenon is not restricted to a given tumor type, a specific age, or sex nor is it limited to extremely high doses as judged by percentage of the LD_{50}. Epidemiologic evidence indicating that brief exposures (e.g. < 1 year) to some chemical carcinogens are associated with enhanced cancer risk add weight-of-evidence support to the animal model findings. The implications of these findings for carcinogen testing and risk assessment procedures especially those concerning less than lifetime exposures are addressed in light of potential mechanistic interpretations by which single dose carcinogens act.

185 PROPOSED METHOD OF CATEGORIZING MATERIALS FOR REPRODUCTIVE AND DEVELOPMENTAL HAZARDS IN THE WORKPLACE

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A proposed method is being evaluated to identify and control occupational reproductive and developmental hazards in the workplace since uniform procedures have not been developed for categorizing such materials. According to this method materials of greatest concern are those which: a) human epidemiological studies have established associations between exposure and adverse effects, b) animal studies produced effects under exposure conditions similar to those encountered in the workplace or c) a weight of evidence evaluation suggesting toxicity based on similarity to other chemicals. These materials would be categorized in Group 1 as either *known or presumed to impair fertility, cause developmental toxicity or affect the quantity or quality of breast milk.* Group 1 materials would be labeled and mandatory workplace controls implemented following standard industrial hygiene practices with employee training. Materials which produced effects in animal studies under exposure conditions highly unlikely to be encountered in the workplace would be categorized in Group 2 as *possible but unlikely to either impair fertility, cause developmental toxicity or affect the quantity or quality of breast milk* and also labeled. Materials which produced effects in animal studies not relevant to humans would be categorized in Group 3 as *having effects in animals but not expected in humans.* Materials in Groups 2 and 3 would be handled with standard industrial hygiene practices with monitoring conducted only if needed as aids for control.

186 SCREENING AND RANKING OF HEALTH HAZARDS IN ENVIRONMENTAL STEWARDSHIP PROGRAMS

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Monsanto Company published its pledge to reduce hazardous releases and emissions, working toward an ultimate goal of zero effect on the environment. In order to pursue this goal, a practical tool is needed to measure the potential effects of chemicals. While standard risk assessment methods can measure potential environmental impact, the complexity of evaluating large numbers of chemicals with varying data makes such methods impractical. However, an appropriate screening approach can provide appropriate prioritization for detailed evaluations.

Monsanto developed, in cooperation with the U. of Tennessee, an overall screen for potential environmental effects which included human health concerns, aquatic and wildlife concerns and environmental persistence. Key in this screen is evaluation of toxicological properties. In the health hazard evaluation, emphasis was placed on use of external referenced data, with internal scientific evaluations or appropriate defaults for missing data. The scoring system for potential human health effects is based on evaluations of 3 components: chronic toxicity (50% of scoring) based on the RfD evaluation; carcinogenicity (25% of scoring) based on EPA or IARC cancer evaluations; other effects (up to 25% of scoring) including evaluations of acute toxicity, based on the LD50, and sensitization/corrosivity.

The screen described provides a reasonable evaluation of potential human health hazards. It can be incorporated into an overall environmental effects model for screening/ranking evaluations of chemical uses, chemical releases and products. The model can serve as a tool to focus on the materials of highest concern in pollution prevention and product stewardship programs.

187 UTILIZATION OF A COMPUTER-BASED TOXICOLOGY RESOURCE CENTER TO ASSIST IN HUMAN HEALTH IMPACT ANALYSES FROM EXPOSURE TO AROMATIC HYDROCARBON CONTAMINANTS — INTEGRATION OF EXPOSURE DATA FROM ANIMAL STUDIES WITH ACUTE HUMAN EXPOSURE

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The human health impact of acute human exposure to aromatic hydrocarbons (benzene, xylene, toluene) following a technological disaster involving exposure to elevated aerosol concentration of the compounds was analyzed using a microcomputer/telecommunications-based-technology resource center. The center provided a focal-point for immediate acquisition, rapid assessment and efficient dissemination of toxicological and biomedical information. The program utilized a microcomputer with toxicological/biomedical software as well as a telecommunication linkage to electronic retrieval databases to provide the toxicologist with capabilities to evaluate laboratory, environmental and human/animal health data. The outcome of the analyses not only provided an assessment of the human health impact but also determined the extent and nature of any required medical surveillance. For the exposure to aromatic hydrocarbons the medical surveillance included a urine phenol scan. The results and recommendations of the analysis have become a standard component of evaluation of the risk of acute exposure to aromatic hydrocarbons.

188 SKIN IRRITATION IS NOT INCREASED IN INFANTS WEARING CLOTH DIAPERS LAUNDERED WITH SODIUM HYPOCHLORITE BLEACH

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Sodium hypochlorite bleach (SHB) is an effective laundry additive to provide disinfectancy to cloth diapers by killing fecal bacteria that may otherwise survive typical home laundering conditions. Data from historical studies indicate that skin irritation was not significantly different in infants wearing control versus SHB-treated diapers. As a result of considerable changes in home laundering technology and consumer laundering habits over the past 30 years, an additional study was conducted to confirm that the use of SHB in home laundry does not increase the skin irritancy potential of cloth diapers. A six week double-blind parallel group home use test was chosen as the study design. Parents/guardians were provided with cloth diapers, a standard marketed detergent, and either SHB or a SHB placebo to use exclusively when washing diapers for the six week study duration. Parents whose infant was 18 months or younger, typically wore cloth diapers, and was without active dermatitis or skin infection were eligible for the study. A baseline skin irritation reading was taken for all participating infants, with interim irritation readings taken at two week intervals. A diary was also used by the parent/guardian to document the diaper change frequency and skin irritation scores from a daily evaluation of the child's diaper area. Data were evaluated by analysis of variance. Results indicate no significant differences comparing skin irritation scores of infants wearing control or SHB-treated diapers. However, as was observed in the historical data, a directional decrease in skin irritation was observed in infants who wore diapers laundered with SHB thereby indicating a possible benefit to reduce skin irritation by using SHB.

189 STUDY OF THE INGESTION FREQUENCY AND OUTCOME OF SCENTED AND NON-SCENTED SODIUM HYPOCHLORITE BLEACH PRODUCTS

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The ingestion frequency and severity of sodium hypochlorite bleach (scented and non-scented) was monitored using data from regional poison control centers (PCC) and Clorox Consumer Services. Exposure data were collected at 4 PCCs from May 1, 1989 to October 31, 1989. Clorox Consumer Services call data were collected from 1/92–8/94. Some of the data evaluated included product type, patient age, description of incident and symptoms. The PCC calls received 342 calls: 73% involved Regular Clorox Bleach (RCB), 12.3% involved Fresh Scent (FS) and 10.5% involved Lemon Fresh (LF). Of the PCC calls received, 240 involved children under the age of six. Analysis of Clorox Consumer Services reports adjusted for market share, indicate that 9 times as many ingestion calls are received for RCB as for either FS and LF. The PCC data revealed the 69.6% of ingestions were asymptomatic, 10.2% vomited, 2.6% experienced throat irritation and 17.6% were classified as either other or unknown. These data are consistent with 1992 American Association of Poison Control Center data which show that 50–55% of Clorox bleach (RCB, FS, LF) ingestions were classified as no or minor effect, <1% as moderate or major and 45–50% as unknown or unrelated. Scented hypochlorite products are not ingested at a higher frequency than unscented products based on calls received by Clorox Consumer Services. Accidental ingestion of household bleaches predominantly results in either no or minor effects.

190 RISK ASSESSMENT OF BORIC ACID (BA): A NOVEL PIVOTAL STUDY

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Previous risk assessments of BA have been based on reproductive toxicity observed in dog studies (Weir and Fisher, 1972). A recent series of NTP-sponsored developmental toxicity studies in mice, rats and rabbits suggested the most sensitive endpoint of toxicity is decreased fetal body weight (FBW) in the offspring of rats fed BA (Heindel et al., 1992); however, the original rat study failed to establish a NOAEL. In order to define a NOAEL for developmental toxicity in rats, a massive follow-up study was conducted in the same laboratory. Groups of 60 Sprague-Dawley derived rats were fed diets of 0, 0.025, 0.050, 0.075, 0.100 or 0.200% (approx. 0, 19, 36, 55, 76 or 143 mg BA/kg/day) from gestation days 0 to 20. Half the dams were sacrificed on day 20 for fetal exams; the remaining dams were allowed to deliver and their offspring were studied through postnatal day 21. No evidence of developmental toxicity was observed at 55 mg BA/kg/day. At 76 and 143 mg BA/kg/day, reduced FBW and increased incidences of short rib XIII and wavy ribs were seen in a dose related manner. Average FBW was decreased from the low through high dose groups for this (and the previous) study: 99%, 98%, 97%, 94% (94%), 88% (87%), (63%) and (47%). Benchmark doses (BMD) were calculated for the two studies alone or in combination (Allen and Strong, 1994). By combining the FBW data from the two studies, it was concluded that the appropriate BMD for risk assessment is 59 mg BA/kg/day, a value similar to the NOAEL of 55 mg BA/kg/day. These values should form the basis of future risk assessments of BA.

191 DIMETHYL METHYL PHOSPHONATE (DMMP): REPRODUCTIVE TOXICITY AS THE BASIS FOR A REFERENCE DOSE (RFD) AND DRINKING WATER HEALTH ADVISORY (HA)

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DMMP is used by the military to simulate nerve agents for testing of chemical agent detection equipment and to evaluate techniques for handling organophosphate nerve agents. Military uses of DMMP and production facility effluents could contaminate the environment. In 1985, the Department of the Army and the Environmental Protection Agency (EPA) established a Memorandum of Understanding for the EPA to develop drinking water HAs to provide guidance in an effort to minimize health effects in persons exposed to munitions-contaminated water. To study DMMP, rats were treated at doses ranging from 179 to 714 mg/kg/day (mkd). Adverse effects, such as sperm abnormalities and resorptions in females impregnated by DMMP-treated males, were noted in all groups. Based upon fetal resorptions, an RfD of 0.2 mkd was derived. The lifetime HA of 0.1 mg/l uses this RfD and an extra 10-fold UF as DMMP is a Group C Chemical. We will examine the reproductive toxicity of DMMP and use of this data to develop a drinking water HA.

192 RISK ASSESSMENT OF BERYLLIUM SAFETY PROCEDURES USING THE BERYLLIUM ANTIBODY ASSAY

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The Rocky Flats Environmental Technology Site (RFETS) is devoted to developing and applying new technology to the mission of cleaning-up this former nuclear weapons plant. Development and use of novel technology to protect worker health and safety is also being promoted. In addition to the challenges created by radioactive and chemical waste, there is also the problem of cleaning-up the element beryllium. Beryllium was used extensively during the manufacture of nuclear weapons' components. More specifically, beryllium was processed in numerous rooms within 39 separate Rocky Flats' buildings. This lightweight metal can cause chronic beryllium disease in approximately 1% of employees who inhale it in its microscopic particle form (a result of the manufacturing process). Since one cannot see or smell such particles, it

is difficult to fully comprehend the potential exposure danger. The Beryllium Antibody Assay was developed at RFETS as a screening assay for exposure to beryllium. This assay is now being used to assess risk to employees who are assigned beryllium clean-up jobs. This paper provides an assessment of risk involving two employees who are cleaning a beryllium plenum which has not been cleaned in more than ten years. Individual immune systems reveal whether or not one is able to enter, clean, and depart without being internally exposed to beryllium.

193 THE MAXIMUM ALLOWABLE LEVEL FOR WHITE MINERAL OIL IN POTABLE WATER

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Direct additive use of white mineral oils is currently being assessed by NSF International under ANSI/NSF Standard 60: Drinking Water Treatment Chemicals — Health Effects. NSF has worked with the American Petroleum Institute to obtain the health effects data that are necessary for establishing exposure limits under this consensus standard. The NSF evaluation of the toxicological data on mineral oils led to the establishment of maximum allowable level (MAL) values for several grades (naphthenic and paraffinic) and viscosities of white mineral oils that apply to exposures through potable water.

In accordance with Standard 60, genetic toxicology data (Level I requirements) were evaluated first and the overall lack of mutagenicity for polyaromatic hydrocarbon (PAH) free oils supported their presence in potable water at concentrations of up to 10 ppb. Evaluation of acceptable subchronic studies (Level II requirement) of oils with different viscosities then raised the allowable level to 21 ppb for low viscosity oils and 50 ppb for high viscosity oils. The critical effect was the occurrence of granulomatous macrophage syncytia in the mesenteric lymph nodes of female Fischer-344 rats.

194 NITRATE IN DRINKING WATER: METHEMOGLOBINEMIA AND REPRODUCTIVE/DEVELOPMENTAL TOXICITY

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This assessment represents an update of earlier assessments conducted in 1987 and 1992. For methemoglobinemia, worldwide incidences are compiled with information on dates, locations, age of subjects involved, clinical findings, microbial contamination, and nitrate concentrations in water. Methemoglobinemia had occurred in the U.S. only in infants and at nitrate levels exceeding the maximum contaminant level (MCL) of 45 ppm. Animal studies showed reproductive toxicity nitrate at dose levels (calculated based on nitrite exposure) of more than 100 times higher than the combined nitrate intake from diet and drinking water containing nitrate at the MCL. Teratology studies were negative. Epidemiologic studies in India, South Australia, New Brunswick/Canada, and New Jersey/United States showed a suggestive but not definitive association between ingestion of nitrate from drinking water and neural robe defects (a developmental effect of the central nervous system), but a causal relationship is lacking. Factors for consideration are offered for evaluating the appropriate limit set for human exposure.

195 RISK ASSESSMENT FOR MULTIPLE SOURCE EXPOSURES

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We propose quantitative methods for evaluating cumulative risk from multiple source exposures such as those from military bases. The principal method, expressing risk isopleths on a coordinate system, allows one to sum and visualize risks from multiple environmental media, operable units, or sites. The usual process of risk assessment and remediation for individual operable units is demonstrated with a military base in California (Site A). Subsequently the cumulative basewide risk from all operable units must be evaluated. Site A is also located near a plant which has released toxic chemicals. This creates potential risks to common receptors from independent sources, a frequent occurrence for residences located in industrial areas. Site B illustrates regional ground water contamination with solvents from at least 11 clustered industries. At another military base (Site C) people get multiple exposures from living, working and fishing on the base. Full assessment of risks from hazardous chemicals requires consideration of both mobility of receptors and mobility of contaminants in air and water.

196 USE OF A PHARMACOKINETIC MODEL FOR ASSESSING LEAD RISK IN EAST HELENA, MT

K M Serl, J S Tsuji. *Kleinfelder, Inc., Seattle, WA.* Sponsor: K C Cater

The U.S. Environmental Protection Agency (EPA) Lead Model was applied to assess lead risks to children in East Helena, MT, after planned remediation of soil. This Superfund site includes an active lead smelter and residential area. Environmental investigations between 1983–1993 measured lead concentrations in soil for 772 yards, as well as concentrations in house dust, vegetables, grain, beef cattle, air, creek water, and sediments. The EPA Lead Model was used to assess if remaining risk met EPA's target goal for lead at Superfund sites. The EPA Lead Model combines lead exposure from multiple sources with pharmacokinetic information to predict blood lead levels in children under 7 years old. The EPA target goal for Superfund sites is for no more than 5% of children to exceed 10 μg/dl. In addition, U.S. EPA Region VIII's goal is for no more than 5% of yards in East Helena to have a greater than 5% risk of exceeding 10 μg/dl. All predicted mean blood lead levels for the community yards were less than 10 μg/dl using the batch mode of the model. The community risk of exceeding 10 μg/dl was 0.5–2%. 3–13% of children had a greater than 5% chance of exceeding 10 μg/dl depending on the amount of individual variation in blood lead assumed. For the available data on yards with soil levels $\leq$1,000 ppm, no effect of soil lead on blood lead was apparent.

197 PARTNERING WITH AGENCIES TO ESTABLISH RISK-BASED SOIL CLEANUP LEVELS

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Various state, county, city and other local agencies participated in a Technical Working Group (TWG) which evaluated the Work Plan and resulting risk-based calculations for soil cleanup levels (SCLs). The SCLs were developed for petroleum products which leaked more than 18 years ago from pipelines under the main street in a small CA beach town. Formation of the TWG had 4 main objectives: 1) Include exposure concerns of interest to the community 2) Foster communication and trust between diverse interest groups 3) Enhance understanding by the community of the scientific and regulatory process 4) Reduce public hostility and anxiety regarding SCLs. Risk Assessment expertise of TWG members ranged from highly skilled to no previous knowledge. Explanation and presentation of information had to satisfy the complex technical needs of the regulators and still be understood by a non-technical audience. Populations evaluated included humans using residential, commercial, and public beach properties as well as ecological receptors. The primary community concern was the impact of soil cleanup levels on property values rather than health risks. This perception impacted the scientific and regulatory process. Leachability tests rather than modeling were used to assess potential future soil impacts to ground water. A very intrusive child was one unique exposure scenario included in the evaluation. Potential future beach erosion patterns and/or natural creek bed movement were considered in addressing fate and transport. This paper discusses the benefits and risks of using the TWG approach in an emotionally charged and technically complex case. In general, early involvement and participation enhanced communication and confidence of diverse interest groups in the scientific and ethical validity of the process.

198 ASSESSMENT OF THE U.S. EPA IEUBK MODEL PREDICTION OF ELEVATED BLOOD LEAD LEVELS

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The Integrated Exposure, Uptake, and Biokinetic (IEUBK) Model for Lead in Children, which was designed to predict the proportion of a population of children with elevated blood leads (μg/dL) on a site-specific basis was examined for its use as a risk assessment tool for regulatory purposes. This was carried out with existing data sets relating environmental and blood lead levels on a per individual basis by using the IEUBK Model to generate blood lead predictions from the measured environmental lead levels. These predicted blood lead levels were then compared with the measured blood lead levels, by comparing geometric mean blood leads and proportions observed or expected to have elevated blood lead levels. All studies used for this examination had data of sufficient quality and quantity to characterize the environmental lead levels in each residential home and yard (i.e., for each participant: blood lead; soil, dust, water, interior and exterior paint lead; and demographic/behavioral survey data covering other aspects of lead exposures). The model results and observed blood lead levels were reasonably concordant, with similar geometric

mean predicted and observed blood leads, and similar population proportions with elevated blood lead levels.

199 THREE POTENTIAL PROBLEMS WITH THE UPTAKE COMPONENT OF THE EPA'S INTEGRATED EXPOSURE/UPTAKE/BIOKINETIC (LEAD IEUBK) MODEL

J W Appling. *Woodward-Clyde Consultants, Denver CO.* Sponsor: B J Kelman

Reduced bioavailability of some forms of lead in soil may explain why the IEUBK model overestimates blood lead levels at mining and other sites. However, several structural components of the model may systematically overestimate exposure, uptake, and tissue accumulation of lead. This presentation explores three issues regarding the uptake component of the model: (1) why the rat lead absorption study, the basis for the equation used in the IEUBK to describe active/passive lad absorption in the model, may not be appropriate for evaluating high intakes of lead; (2) why the infant duplicate diet study, used to identify parameter values including the half-saturation intake for the absorption from lead in soil children aged 1–6; and (3) why there is a potential error in the uptake equation itself. EPA used the infant study data to derive coefficients to use in the model absorption equation, which is derived from the rat study. When we derive an alternate, better fitting set of coefficients to the infant data, IEUBK output changes: if a certain soil is 15% bioavailable, a target level derived with default values would be 1,000 ppm Pb: but with a passive component of 5% and a half-saturation intake of 45 μg/day, the target soil level becomes 1,225 ppm. These and other concerns indicate a large degree of uncertainty in using the model output as a basis for remedial decisions.

200 A RISK ASSESSMENT OF DEVELOPMENTAL METHYLMERCURY EXPOSURE

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Methylmercury (MeHg) is a global environmental problem and a well recognized neurotoxicant, particularly for the developing organism. Human exposure to MeHg primarily occurs through the consumption of contaminated foods such as fish and whale meat, although catastrophic exposures due to industrial pollution have occurred. High-level prenatal MeHg exposure in humans results in offspring effects such as cerebral palsy and mental retardation while chronic low-level exposure is related to decreased birthweight and early sensorimotor dysfunction. Neurobehavioral studies with nonhuman primates and rodents and have produced similar neurobehavioral and neuropathological effects. The US EPA has established a reference dose (RfD) for methylmercury at 0.3 μg/kg/day. The FDA level for fish consumption is 1 μg/g (1 ppm). Given the vulnerability of the fetus to MeHg and the persistent problem of human exposure, a risk assessment of the developmental effects of MeHg exposure was undertaken. Based on results from human and animal studies of developmental neurotoxicity, the reference dose (RfD) was calculated to be 0.025–0.06 MeHg μg/kg/day. This reference value emphasizes the developmental neurotoxic potential of MeHg. Based on these calculations, it is recommended that pregnant women and women of childbearing age be advised to limit their exposure to potential sources of MeHg. Supported in part by grant #ES03745 from NIEHS.

201 DETERMINATION OF A NOAEL FOR IN UTERO EFFECTS OF METHYLMERCURY

K S Crump, A Silvers. *ICF Kaiser Inc, Ruston, LA; EPRI, Palo Alto, CA.* Sponsor: M E Andersen

Applying a hockey stick parametric dose-response model to data on late or retarded development in Iraqi children exposed *in utero* to methylmercury, with mercury (Hg) exposure characterized by the peak mercury concentration in mothers' hair during pregnancy, Cox *et al.* (1989) calculated the "best statistical estimate" of the threshold for health effects as 10 ppm Hg in hair with a 95% range of uncertainty of between 0 and 13.6 ppm. Our reanalysis of these data using the Cox *et al.* methodology shows, to the contrary, that the best estimate of the threshold is 114 ppm Hg in hair with an upper 97.5% confidence bound of 294 ppm. This corrected result is consist with results of trend tests and benchmark analyses applied to the Iraqi data. However, the acute nature of the exposures, as well as other limitations in the Iraqi data suggest that data from a New Zealand study by Kjellstrom *et al.* (1989) on scores from psychological tests administered to six-year-old children of mothers exposed to methyl mercury in fish may be more appropriate for determining acceptable human exposures to methylmercury. A preliminary benchmark analysis of the New Zealand data suggests NOAELs in the range of 10–90 ppm Hg in mother's hair. More definitive analyses of the New Zealand data are currently being conducted.

202 A RISK ASSESSMENT FOR MANGANESE IN WATER

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An evaluation was made of the dose-response relationship between exposure to manganese concentrations and the incidence of Parkinson-like neurological symptoms in an elderly population. Individual subject values from the study described by Kondakis et al (1989; *Arch Environ Hlth 44*: 175–178) were used as a basis for the assessment. This study yielded neurological scores ranging from 0 to 64 which were intended to be indicative of Parkinson's disease. The subjects were all aged 50 and over and were exposed to manganese in well water ranging from approximately 0.1 to 2 ppm for at least 10 years. The data set was modeled with 242 alternative equations which differed in the dose-response model employed, the population (variability) model employed, and in the presence of background (dose-independent) terms. The model providing the best fit of the data set, based on a weighted least-squares criterion was a three parameter model with an exponential dose-response component, a logistic population component, and no dose-independent terms. The results of the modeling efforts were used as the basis of a two-dimensional (variability and uncertainty) Monte-Carlo simulation in which the incidence of Parkinson-like symptoms was estimated at 5 different manganese concentrations in drinking water ranging from 0.05 to 1 ppm. Model uncertainty was represented by using model selection as a component of the uncertainty dimension, with the frequency of selection based on goodness-of-fit criteria. At 0.05 ppm manganese, the average estimate over the range of uncertainty yielded a population distribution (variability) with an average individual response score of 3.9, a median of 1.9, and a 95th percentile of 12.9. The corresponding values for a population consuming drinking water at 1.0 ppm were 5.4, 2.8, 18.2, respectively. The corresponding values for the net difference between 0.05 and 1.0 ppm are 1.5, 0.8, and 5.2. These results, or other similar comparisons that could be made, may be useful for gauging effort expended in avoiding exposure to manganese from dietary sources.

203 COST-BENEFIT ANALYSIS OF CALIFORNIA POLICY ON CANCER RISK: A CASE STUDY OF CHROMIUM (VI)

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In a striking departure from the worldwide consensus regarding Cr(VI) carcinogenic potency, in 1987 the California EPA classified Cr(VI) as an oral carcinogen (slope factor = 0.42 mg/kg-day^{-1}) and has regulated it as such since then. In addition, CalEPA has determined that the USEPA inhalation slope factor for Cr(VI) (41 mg/kg-day^{-1}) is not adequately health protective and they have developed and enforced a more conservative value (510 mg/kg-day^{-1}). Because of their position, and due to California's stringent "right-to-know" legislation, it is mandated that many industries in California that currently use Cr(VI) compounds will be required to publish official community warnings for exposures that would be considered negligible health risks in other states. In addition, industries that are attempting to address soil and groundwater contamination are faced with Cr(VI) cleanup standards that are far below other health-based standards and/or existing limits of detection. We conducted a preliminary evaluation of the financial resources required for selected industries in California to: 1) control airborne Cr(VI) emissions to CalEPA's "risk-based" levels, and 2) remediate all Cr(VI)-containing soil and groundwater to CalEPA's "risk-based" cleanup standards. Our analysis suggests that, compared to other states, millions of additional dollars will be paid by California industries and tax payers due to these more stringent clean-up values which are based on weak or inappropriate data.

204 ENVIRONMENTAL MERCURY CONTAMINATION OF LOW LEVEL EXPOSURE FROM FISH INGESTION AND AIR INHALATION IN ALTA FLORESTA — AMAZONIA

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Mercury has been used in the Amazonian ecosystem as a result of gold mining activities over the past 15 years. During this period, 1500 tons of Hg have been released into the Amazon Basin. Metallic Hg is used to amalgamate gold. During the amalgamation process, about 65 to 85% of the total Hg emission enters directly into the atmosphere and the rest will find its way into the aquatic and terrestrial systems. This constituted an important source of environmental contamination and human exposure to mercury in the Amazon basin, whose impact on the environment and on human health is difficult to predict. Alta Floresta is the most important gold trading center in Amazonia. The urban population is exposed to Hg concentration in air, ranging from 23 to 5788 ng/m^3 with average value of 882 ng/m^3 and contaminated fish varying from 0.5

to 3.57 mg/kg. This study was undertaken to provide quantitative estimates of exposure via fish and water ingestion, as well as Hg laden air inhaled by the urban population including the risk group. This investigation was designed to select a random population sample of about 150 people all of whom were required to have lived in their present residence for at least the previous six months and to have consumed contaminated fish at least once/month during the previous three months. It was possible to identify how the demographic and social variables and dietary habits may affect the exposure levels of some groups of the population. Assuming an average fish consumption rate of 31 grams per day, a weighted average of Hg fish concentration of 1.2 mg/kg, an average body weight for adults of 60 kg and an average exposure duration of 8 years, the estimated incorporation of 0.63 μg of Hg per kg of body weight would imply that attention should be given to critical groups such as woman in the childbearing age, since the fetus is more sensitive to Hg contamination than are adults.

205 BIOAVAILABILITY AND BIOTRANSFORMATION OF [RING-G-^{3}H]-3-HYDROXYBENZO(A)PYRENE (3-OHBAP) IN AN *IN SITU* INTESTINAL PREPARATION IN UNINDUCED AND β-NAPHTHOFLAVONE-INDUCED CHANNEL CATFISH

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The diets of fish from polycylic hydrocarbon-polluted environments may include hydroxylated aromatics. In this study, an isolated *in situ* preparation of catfish intestine was used to examine the transfer of 3-OHBaP and its intestinal biotransformation products from intestinal contents to the systemic blood. When each fish preparation was stable, a solution of 3-OHBaP, 98% radio-chemically and chemically pure and nominally 2 or 20 μM, was introduced into the lumen. Efferent blood was collected over the course of 1 hour, when the preparation was terminated and the isolated segment excised. Samples of the intestinal contents (post-infusate), scraped mucosa and blood were analysed for [^{3}H] content by scintillation counting and for chemical composition by extraction under N_2 and isocratic HPLC analysis with radio-chemical and fluorescence detection. There was a dose-dependent transfer of 3-OHBaP and metabolites into blood. At 2 μM or 20 μM 3-OHBaP, blood contained 28–39 or 180–236 pmole 3-OHBaP equiv./ml blood respectively. At each concentration, there were no significant differences between induced and control fish, regarding uptake into mucosa, transfer into blood or metabolite composition of the compartments. The post-infusate contained 84–88% 3-OHBaP, 6–9% BaP-3-sulfate, 2–3% BaP-3-glucuronide and 2–3% BaP-3,6-dione. Mucosa contained 38–47% 3-OHBaP, 24–41% BaP-3-sulfate and 18–24% BaP-3-glucuronide. Blood contained traces of 3-OHBaP, 44–56% BaP-3-sulfate, 1–2% glucuronide and 38–49% radioactivity that could not be extracted by ethyl acetate. Thus, low concentrations of 3-OHBaP were extensively biotransformed, predominantly to the sulfate conjugate, in intestine prior to transfer into the blood. Funded in part by USPHS grant ES-05781.

206 IMMUNOTOXICITY OF POLYCYCLIC AROMATIC HYDROCARBONS IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES: STRUCTURE-ACTIVITY RELATIONSHIPS

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The immunotoxicity of many polycyclic aromatic hydrocarbons (PAHs) is well defined in rodents, but poorly understood in humans. Differential sensitivity to PAH immunotoxicity is indicated by the fact that human lymphocytes are 100 to 1000-fold more sensitive than murine cells to suppression of mitogen responses by benzo(a)pyrene (BaP) (Mudzinski, *Toxicol. Appl. Pharmacol., 119*:166, 1993). To further define the immunotoxicity of PAHs in humans, we measured phytohemagglutinin-induced mitogenesis of human peripheral blood mononuclear cells that were incubated with or without BaP, 3-methylcholanthrene (3-MC), 7,12-dimethylbenz(a)anthracene (DMBA), dibenz(a,c)anthracene (DAC), dibenz(a,h)anthracene (DAH), 9,10-dimethylanthracene (DMA), benzo(e)pyrene (BeP), benz(a)anthracene (BA), anthracene (ANT), or vehicle control at concentrations of 10^{-5} to 10^{-8} M. BaP, 3-MC, DMBA, DAC, and DAH suppressed mitogenesis at concentrations as low as 10^{-8} M. We have shown that DMBA and BaP directly inhibit sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPases, and this effect is partially reversed by ANT. Therefore, we co-incubated DMBA, BaP, or 3-MC with ANT in cultures, and found that ANT partially reversed inhibition by DMBA and BaP, but not by 3-MC, suggesting competition for binding sites. We also found partial reversal by α-naphthoflavone of immunotoxicity of DMBA, BaP, and 3-MC, suggesting that P450 metabolism or Ah receptor binding may play a role in some of the immunotoxicity of these PAHs. Our data demonstrate toxic effects of PAHs at low levels in human lymphocytes, and illustrate the need to further define the immunotoxicity of PAHs in humans. (NIH RO1 ES-05495)

207 EFFECT OF NEONATAL EXPOSURE TO DIETHYLSTILBESTEROL (DES) ON BENZO(a)PYRENE (BAP) METABOLISM IN RAT REPRODUCTIVE TISSUES

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This study seeks to elucidate the role of DES on metabolism in the male reproductive tissues. Offspring of Sprague-Dawley rats were neonatally treated on days 2, 4, and 6 postpartum with 1.45 umoles of DES. Ten weeks after birth, the adult rats were challenged with ^{3}H BaP (10 umol/kg), and sacrificed 2 hours after Bap exposure. Prostate, testes and tissues were removed and extracted using a mixture of H_2O, MeOH and $CHCl_3$. The extracts were analyzed by reverse phase HPLC. In the organic phase, 3-OH Bap, 9-OH Bap, 4,5-dihydrodiol, 7,8-dihydrodiol and 9,10-dihydrodiol were the major metabolites. In the aqueous phase, OH-glucuronides, OH-sulfates, 7,8-diol GSH, 9,10-diol GSH, 7,8-epoxide GSH, and 9,10-epoxide GSH were the major metabolites. The distribution of metabolites appeared to be organ specific and were significantly decreased in DES treated animals. Both the 3-OH and 9-OH metabolites were found in the control and DES prostates, but not in DES treated testes or liver. It appears that DES induces a chemopreventive effect.

208 INHIBITION OF PROTEIN KINASE C-RELATED SIGNAL TRANSDUCTION IN VASCULAR SMOOTH MUSCLE CELLS BY 3-HYDROXY-BENZO(A)PYRENE

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Recent studies in this laboratory have shown that acute *in vitro* exposure of vascular (aortic) smooth muscle cells (SMCs) to benzo(a)pyrene (BaP) interferes with protein kinase C (PKC)-related signal transduction. Because 3-hydroxy BaP is the major oxidative metabolite of BaP in these cells, experiments were conducted to examine the impact of this metabolite in the PKC response. Exposure of cycling SMCs to 30 μM BaP or 3-hydroxy BaP was associated with inhibition of PKC activity in both the cytosolic and particulate compartments. While the inhibition of PKC activity by BaP in both compartments was comparable, 3-hydroxy BaP preferentially inhibited particulate activity. Northern analysis revealed that challenge of growth-arrested SMCs with either hydrocarbon for 1 hr decreased serum-induced c-fos expression. Depletion of PKC activity by long-term (24 h) PMA (100 ng/ml) treatment abolished serum-induced c-fos expression confirming that this gene is regulated through a PKC-dependent pathway. Interference with subsequent PKC signaling was suggested by the ability of BaP and 3-hydroxy BaP to inhibit AP-1 binding to a phorbol ester responsive element. Collectively, our data suggest that the ability of BaP to modulate PKC-related signal transduction pathways in vascular SMCs may involve the formation of oxidative metabolites. (Supported by NIH grants ES 04849 and ES 00213).

209 INDUCTION OF CYP1A-1 AND CYP1A-2 GENE EXPRESSION BY A RECONSTITUTED MIXTURE OF POLYNUCLEAR AROMATIC HYDROCARBONS IN B6C3F1 MICE

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The potential non-additive interactions of polynuclear aromatic hydrocarbon (PAH) mixtures as inducers of *Cyp1a-1* and *Cyp1a-2* gene expression were investigated in B6C3F1 mice using a reconstituted PAH mixture containing 17 individual congeners. The ≥4-ring PAH fraction and reconstituted mixture induced hepatic microsomal ethoxyresorufin *O*-deethylase (EROD) activity and Cyp1a-1 mRNA levels whereas the 2- and 3-ring PAHs were only weakly active. Direct comparison of the potencies of the reconstituted mixture and ≥4-ring PAHs showed that the Cyp1a-1 induction activity of the reconstituted mixture was due to the ≥4-ring PAHs. The reconstituted PAH mixture and ≥4-ring PAHs also induced Cyp1a-2 hepatic mRNA levels and microsomal methoxyresorufin *O*-deethylase (MROD) activity; however, their dose-response curves indicated that the reconstituted PAH mixture was more potent as a Cyp1a-2

inducer than the ≥4-ring PAHs. The differences in potency were due to 3-ring PAHs which were found to be strong inducers of hepatic Cyp1a-2 mRNA levels and microsomal MROD activity at the lowest dose administered (37 mg/kg). The 3-ring PAHs did not competitively bind to the mouse hepatic cytosolic aryl hydrocarbon (Ah) receptor suggesting that 3-ring PAHs are a new class of Cyp1a-2 inducers which do not act through the Ah receptor.

210 INDUCTION OF MICRONUCLEI IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES TREATED WITH BENZO(a)PYRENE

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Our research utilized the micronucleus assay to study the effect of Benzo(a)pyrene (B(a)P) on human chromosomes *in vitro*. We used peripheral blood lymphocytes (PBL's) isolated from two healthy donors (Donor 01 was female and Donor 02 male, both were age 26). PBL's were mitogen stimulated and either remained untreated (control) or after 24 hours received B(a)P (4–40 μM) or Dimethyl Sulfoxide (DMSO; solvent control). After a total of 88 hours in culture, cells were harvested and stained with Acridine Orange (AO) and fluorescence microscopy was utilized to investigate the frequency of MN. The number of MN/100 mononucleated cells was determined for a total of 1000 cells scored per treatment; n = 10. The Kruskall-Wallis test (Dunn's Method) was used to determine if treatment means differed from controls. No statistical difference was observed between replicate experiments therefore the values reported here represent the mean MN/100 cells ± s.e.m; n = 20. Donor 01 PBL's were found to have a background of 0.4 ± 13. No significant increase over controls was detectable for any of the treatments($p > 0.05$). In contrast to 01, donor 02 displayed a highly significant ($p < 0.001$) dose dependent increase in MN. Background micronucleus frequency was 0.3 ± 0.27. We saw the following increases over control: 4 μM = 5.8 fold; 10 μM = 8.3 fold; 20 μM = 16 fold and 40 μM = 9.8 fold. We believe that saturation of the P-450 enzyme system may explain the diminished response observed at 40 μM. Because these results were based on the mononucleated method which lacks kinetic sensitivity i.e., uncertainty as to number of cell divisions; we repeated these experiments on PBL's from Donor 02 utilizing the binucleated (BN) method. Cells were stimulated with mitogen and 24 hours later treatment administered, at 48 hours Cytochalsin B was added to block cytoplasmic division, at 72 hours cells were harvested and stained with AO. The background micronucleus frequency was 1.0 ± 0.26. We were only able to detect significant increases over the control at 20 and 40 μM (3.2 fold $p = 0.01$ and 2.8 fold $p = 0.02$ respectively). Because the dose response was much less than expected we believe the Cytochalasin B may interact with the chemical to inhibit formation of MN. The results indicate that the micronucleus assay is a sensitive tool which can be used to investigate B(a)P effects on chromosomes in a human cell system. (Supported jointly by the Smokeless Tobacco Research Council and the Eastman Kodak Co.)

211 INHIBITION OF BENZO[a]PYRENE METABOLISM IN HEPA-1 CELLS BY CYP1A1 ANTISENSE OLIGONUCLEOTIDES

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Specific cytochrome P450 isozymes are important for the metabolic activation of polycyclic aromatic hydrocarbons. To determine how the induction of CYP1A1 is involved in the metabolic activation of benzo[*a*]pyrene (BaP) in cells, antisense oligonucleotides are being developed to inhibit this specific P450 isozyme in Hepa-1 cells. Preliminary studies demonstrated that treatment of cells with phosphorothioate antisense oligonucleotides provided limited inhibition of BaP metabolism. To increase the efficacy of the oligonucleotides, liposomes which complex with nucleic acids (Lipofectamine™) are being used to facilitate the uptake of the oligonucleotides into the cell. Lipofectamine alone was found to be cytotoxic at a concentration of 10 μg/mL after 4 hours, however when used in combination with 1 μM of phosphorothioate oligonucleotides cell survival was 100%. Treatment of Hepa-1 cells with 1 μM phosphorothioate oligonucleotide which recognizes a splice site in the CYP1A1 mRNA in the presence of 10 μg/mL Lipofectamine inhibited BaP metabolism by 28% at 4 hours. The same oligonucleotide, in the presence of 20 μg/mL Lipofectamine, inhibited BaP metabolism by 32–36% at 2, 4 and 6 hours. Uptake studies with [^{33}P]-5′-end labeled phosphorothioate oligonucleotide indicated that only 5% of the labeled oligonucleotide was associated with the cell after 24 hours in the absence of Lipofectamine, however, with 10 μg/mL Lipofectamine 58% of the labeled oligonucleotide was recovered with the cell. 72% of the labeled oligonucleotide in Lipofectamine treated cells was present in the nucleus and 28% remained in the cytoplasm after 24 hours. Gel electrophoresis indicated greater degradation of the oligonucleotide in the cytoplasm than in the nucleus. Lipofectamine increases oligonucleotide uptake to both the cytoplasm and nucleus and facilitates the inhibition of BaP metabolism in cells by CYP1A1 antisense oligonucleotides. Supported by grant CA40228 NCI DHHS.

212 BACTERIAL MUTAGENICITY OF POLYNUCLEAR AROMATIC HYDROCARBONS IN MIXTURES REPRESENTATIVE OF COAL TAR

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The mutagenic interactions of PAH mixtures representative of a model coal tar were investigated. The PAH mixture were prepared in 2, 3, and 4 ring groups in the percentages as that of a model coal tar. A representaive reconstituted PAH mixture was also made. Each of the two, three, and four ring mixtures, and the reconstituted mixture mutagenic potentials were evaluated with and without metabolic activation. Each sample was tested at 5 consecutive dose levels in the *Salmonella*/Microsome assay using tester strains TA98 and TA100. The metabolically activated 4 ring, and reconstituted showed positive dose related mutagenic responses in both strains. The overall mutagenic response was enhanced by TA100. At the highest dose of 18 mg/ml, the 4 ring mixture had an eight fold larger mutagenic response (809 net revertants) than the reconstituted mixture which showed a toxic response (69 net revertants) at the same dose. This indicated a toxic and possibly inhibitory effect. At the lowest dose level of 0.09 mg/ml the 4 ring showed a negative response with 38 net revertants, while the reconstituted showed a mutagenic response with 517 net revertants. At 1.8 mg/ml the 4 ring induced an expected mutagenic response with 474 net revertants, but the reconstituted exhibited a mutagenic response of 935 net revertants. The mutagenic potential of the representative PAH mixtures was evaluated in combinations as 2 and 3, 3 and 4, and 2 and 4 ring mixtures using tester strain TA100. The 2 and 4 ring, and 3 and 4 ring combinations induced unexpected inhibited mutagenic responses. This data suggests that PAH mutagenic interactions may not be additive. Interactions vary depending on amount of chemical, individual PAHs, and metabolic activation.

213 BENZO[a]PYRENE-INDUCED HEPATIC DNA ADDUCT FORMATION IN B6C3F1 MICE: MODULATION BY CHRYSENE AND ACENAPHTHYLENE

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Manufactured gas plant-derived polynuclear aromatic hydrocarbons (PAHs) are a complex mixture in which benzo[a]pyrene (BaP) is only a minor component (0.17%). The mixture contains 2-, 3- and ≥4-ring PAHs and, in studies with a reconstituted PAH mixture, the 3-ring PAHs induced hepatic Cyp1a-2 gene expression whereas the ≥4-ring PAH mixture induced both Cyp1a-1 and Cyp1a-2. The interactive effects of 3- and ≥4-ring PAHs on BaP-induced DNA adduct formation was investigated in male B6C3F1 mouse liver after treatment with BaP (1 mg/kg) alone, BaP (1 mg/kg) plus acenaphthylene (200 mg/kg) or BaP (1 mg/kg) plus chrysene (40 mg/kg). Acenaphthylene and chrysene were selected as prototypes for the 3- and ≥4-ring PAHs, respectively. In mice treated with BaP (1 mg/kg) alone, there was a < 2-fold induction of hepatic microsomal methoxyresorufin *O*-demethylase (MROD) and ethoxyresorufin *O*-deethylase (EROD) activities; in animals cotreated with acenaphthylene plus BaP, there was a 3.5-fold induction of MROD activity and both EROD and MROD activities were induced by BaP plus chrysene (coadministered). In parallel studies, hepatic DNA adducts were examined using the [^{32}P]postlabeling procedure. The major hepatic DNA adduct observed in animals treated with BaP alone was identical to the guanine adduct derived from the anti-diolepoxide BaP metabolite. Quantitation of this DNA adduct in mice cotreated with the binary PAH mixtures showed that acenaphthylene, a model tricyclic PAH, did not alter levels of the major BaP-DNA adduct. In contrast, the BaP-DNA adduct was decreased by >50% in mice cotreated with chrysene plus BaP. Current studies are focused on the interactions of BaP with more complex PAH mixtures and the effects of these interactions on DNA adduct levels and development of liver tumors in B6C3F1 mice.

214 EXPOSURE OF KILLIFISH TO BENZO[a]PYRENE: COMPARATIVE METABOLISM, DNA ADDUCT FORMATION AND ARYL HYDROCARBON (Ah) RECEPTOR AGONIST ACTIVITIES

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Benzo[a]pyrene (BaP) elicited a dose-response induction of hepatic CYP1A1 gene expression in killifish characterized by increased CYP1A1 mRNA levels, ethoxyresorufin *O*-deethylase (EROD) and aryl hydrocarbon hydroxylase (AHH) activities. There were marked differences in the maximally-induced EROD (1529 pmol/mg/min) and AHH (377 pmol/mg/min) activities and these differences were observed at all time points and at concentrations of BaP from 1 to 50 mg/kg. Treatment of killifish with BaP did not significantly affect binding of [^{125}I]epidermal growth factor (EGF) to hepatic plasma membranes containing the EGF receptor. There was a dose-dependent increase in formation of DNA adducts in killifish treated with 1 to 50 mg/kg of BaP. Two-dimensional thin-layer chromatographic analysis of the [^{32}P]labeled adducted nucleotides revealed one major spot. In a time course study over a period of 2 to 14 days, the relative DNA adduct levels in fish treated with 5 mg/kg BaP were constant. The most sensitive indicator of exposure to BaP was the dose-dependent formation of biliary metabolites in which there was a >40-fold increase (compared to untreated animals) in fluorescent metabolites formed at the lowest dose of BaP (1 mg/kg) used in this study. Killifish expressed relatively high levels (203 fmol/mg) of the nuclear aryl hydrocarbon (Ah) receptor which was isolated from nuclear extracts of fish treated with [^{3}H]2,3,7,8-tetrachlorodibenzo-*p*-dioxin. The nuclear Ah receptor complex sedimented at 6.0S which was similar to that observed in other animal species. Based on their CYP1A1 induction response to BaP and Ah receptor levels, the results of this study indicate that killifish are highly Ah-responsive.

215 EFFECT OF SOIL ON THE BIOAVAILABILITY OF CHEMICAL COMPONENTS OF MANUFACTURED GAS PLANT RESIDUE

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The urinary excretion of PAH metabolites and chemical:DNA adduct formation in various tissue sites were used to assess the bioavailability of chemical components present in soil contaminated with manufatured gas plant residue (MGP). Groups of female CD1 mice were maintained on various powder diets adulterated with either MGP contaminated soil or non-contaminated control soil (0.2% or 20% soil wt/dry food wt). Diets were fed for a total of 15 days. Urine was collected on day 1, 7, and 14 of diet administration. 1-Hydroxypyrene levels in urine were determined using standard extraction methods, while levels of phenanthrene were determined following hydroiodic reduction of urinary metabolites. HPLC with fluorescence detection was used for each analysis. 1-Hydroxypyrene levels were in the range of 0.03–0.16 μg and 8.2–12.9 μg for animals maintained on a 0.2% and 20% diet adulterated with MGP contaminated soil, respectively. In contrast, phenanthrene (29–41 μg) was only detected in urine of animals that ingested a 20% diet. Both 1-hydroxypyrene and phenanthrene levels in urine reached a maximum within 7 days of diet administration. DNA isolated from lung and forestomach tissue were analyzed for chemical:DNA adducts using ^{32}P-postlabeling and multidimensional TLC. Chemical:DNA adducts were not detected in lung or forestomach tissue under the conditions employed in this study. The ability to easily detect PAH metabolites in urine along with the inability to detect chemical:DNA adducts in various tissue sites, suggests that urinary PAH metabolites may not provide an accurate assessment of the bioavailability of genotoxic compounds. The data obtained in the present study will be compared to the results of studies conducted with neat MGP and mixtures of pure polycyclic aromatic hydrocarbons.

216 DEVELOPMENT OF A PRIORITY LIST OF CHEMICAL MIXTURES OCCURRING AT 1,188 HAZARDOUS WASTE SITES, USING THE HAZDAT DATABASE

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Under the CERCLA 104 mandate, as amended by SARA (42 USC 9604(i)(2)), ATSDR is to identify individual substances and combinations of substances that pose the greatest hazard to public health at hazardous waste sites. This led to certain mandated activities by the Agency, including development of toxicological profiles, identification of data gaps, and, ultimately, establishment of a research agenda. ATSDR has developed HazDat, a database that captures pertinent information from public health assessments that are performed for hazardous waste sites. As a preliminary step, data from 1,188 sites were analyzed to identify the potential combinations of chemicals found in various environmental media. The following were the most frequently found combinations: TCE and perc in water (23.5% of sites); Cr and Pb in soil (20.5%); benzene and toluene in air (3.5%); perc, 1,1,1-TCA, and TCE in water (11.6%); Cr, Cd, and Pb in soil (12%); and benzene, perc, and TCE in air (2.2%). To improve on inherent shortcomings of this analysis, factors such as toxicity, source contribution, and likelihood of human exposure should be incorporated into an algorithm that could be similar to the algorithm utilized to identify the Agency's priority list of 275 single substances (59 FR 9489). Assessment of the impact on human health of mixtures of chemicals occurring at waste sites is a formidable task. An equally demanding challenge is the estimation of the role of chemical interactions in the overall expression of toxicity of chemical mixtures. Since limited published data exists to evaluate chemical interactions, alternative methods including predictive approaches and in vitro techniques need to be developed and employed.

217 SIGNIFICANCE OF TRICHLOROETHYLENE AND ETHANOL INTERACTION FOR PUBLIC HEALTH ASSESSMENT

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Trichloroethylene (TCE) is found at most hazardous waste sites and is the most common groundwater contaminant near such sites. Drinking ethanol (EtOH) can affect TCE metabolism. Because EtOH both induces and competes with TCE metabolism, the effect of these two chemicals' interaction depends on the exposure protocol. Preexposure to EtOH induces microsomal oxidation of TCE to its short-lived metabolite, chloral hydrate. This induction is maximal when EtOH exposure precedes TCE exposure by 18 hours. Simultaneous exposure causes competition for enzymes and cofactors in TCE metabolism, resulting in suppressed serum trichloroethanol and trichloracetic acid (TCA), and consequently, decreased potentiation of TCE-induced CNS depression (due to trichloroethanol). If the interval between preexposure to EtOH and exposure to TCE is 3 hours, the EtOH is metabolized and not available to compete for enzymes and cofactors, and induction predominates. However, if the interval is 1 hour, competition by unmetabolized EtOH predominates. Simultaneous exposure potentiates cardiac arrhythmias, while increasing the exposure interval decreases this potentiation and increases liver toxicity. TCA, a TCE metabolite, binds to plasma proteins that also bind the EtOH metabolite, acetaldehyde. This sequestration of plasma protein binding sites causes increased free plasma acetaldehyde when habitual drinkers are acutely exposed, first to TCE, and then to EtOH. The resulting severe and sometimes fatal intoxication is called "degreaser's flush". Chronic EtOH exposure increases microsomal metabolism of an acute dose of TCE. EtOH generally damages the liver, with greater susceptibility to injury by activation of TCE and binding of TCE metabolites. Thus, susceptible populations such as chronic alcoholics with declining organ function are more vulnerable to TCE than healthy people.

218 A STATISTICAL MODEL TO ANALYZE KINETIC CHANGES IN F344 RATS FOLLOWING EXPOSURE TO A CHEMICAL MIXTURE AND ITS SUBMIXTURES

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A one-month study was performed to investigate kinetic changes in F344 rats after repeated exposures to low-levels of a chemical mixture of seven groundwater contaminants. The seven chemicals used are among the most frequently detected contaminants associated with hazardous waste sites; arsenic, benzene, chloroform, chromium, lead, phenol, and trichloroethylene. A branched study design was used to separate the complete mixture into submixtures of the organic or inorganic chemicals, trichloroethylene and chloroform together, benzene and phenol together, and the various individual components. Following exposure, gas-uptake pharmacokinetic experiments were performed to assess the impact of each mixture on enzyme kinetics and to determine what type of interactions were present (additive, synergistic, and/or antagonistic). Based on classical pharmacokinetic techniques, only phenol, lead, arsenic, and the organic submixture resulted in a statistically significant difference ($p < 0.05$) in the metabolism as compared to controls. Of these components, lead and phenol increased the metabolic activity while arsenic reduced the activity. A statistical model was built which utilized the slow slopes as unique data points and the various mixtures as qualitative variables. Preliminary analysis using the

statistical model indicated that the interactions among the various components of the complete mixture and various submixtures are best described using an additive model ($p < 0.05$). The present model demonstrates the first step in "predictive toxicology" by coupling statistical modeling with pharmacokinetic information to allow prediction of metabolic changes for any combination of chemicals in the mixture.

219 TIME-COURSE OF ELEVATED HEPATIC CYP2E1 ACTIVITY AND ENHANCED CARBON TETRACHLORIDE (CCl_4) HEPATOTOXICITY FOLLOWING EXPOSURE TO INHALED METHANOL (MeOH)

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Acute (single, 6 hr) exposure of rats to inhaled MeOH results in potentiation of the hepatotoxicity of CCl_4 that is dependent on the time of CCl_4 challenge relative to MeOH exposure. Marked potentiation results when CCl_4 challenge occurs 18 hr after termination of MeOH exposure, but not when CCl_4 exposure occurs 42 or more hr after MeOH. This is consistent with previous data (Allis et al., 1992) on the effect of inhaled MeOH on CYP2E1 activity at these two time points. The objective of the present study was to examine in detail the time course of increased CYP2E1 activity resulting from exposure to inhaled MeOH, with comparison to the time-course of enhancement of CCl_4 toxicity. Adult, male F-344 rats were exposed by inhalation to 0, 5000 or 10,000 ppm MeOH for 6 hr. Hepatic microsomes were isolated immediately upon termination of MeOH exposure (0 hr) as well as 6, 12, 18, 30 and 42 hr later. CYP2E1 activity was determined by measurement of p-nitrophenol hydroxylase activity. Present results indicate little or no increase in CYP2E1 activity at 0 hr, statistically significant increases at 6, 12 and 18 hr for both 5000 and 10,000 ppm MeOH, and little/no increase at 30 and 42 hr. Consistent with the CYP2E1 data, CCl_4 toxicity was potentiated when CCl_4 administration occurred 6 or 18 hr after termination of MeOH exposure, but not when CCl_4 challenge occurred at 42 hr. It is noteworthy that 5000 ppm MeOH potentiated CCl_4 hepatotoxicity at 0 hr, a time when increased CYP2E1 activity was not readily apparent. These results suggest that more than one mechanism may be involved in MeOH potentiation of CCl_4 toxicity. (This abstract does not necessarily reflect EPA policy.)

220 POTENTIATION OF CARBON TETRACHLORIDE HEPATOTOXICITY BY INHALED METHANOL IN MICE

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In rats, exposure to methanol (MeOH) by inhalation results in concentration-dependent potentiation of the hepatotoxicity of orally administered carbon tetrachloride (CCl_4). Relative to the amount of research that has been conducted in the rat, there is a paucity of data in the mouse. Thus, the objective of the present study was to examine the interaction of inhaled MeOH with CCl_4 in the mouse. Adult, male CD-1 mice were exposed to 0, 625, 1250 or 2500 ppm MEOH by inhalation for 6 hr, and gavaged the next day with 0, 7.5, 15 or 20 ul CCl_4/kg. Hepatotoxicity was assessed ≈24 hr after CCl_4 challenge. Based on serum alanine aminotransferase and hepatic histopathology, MeOH alone was not overtly hepatotoxic. Prior exposure to MeOH resulted in an apparent increase in CCl_4 hepatotoxicity, dependent on the concentration of MeOH and CCl_4. In summary, these data suggest that exposure to inhaled MeOH potentiates CCl_4 hepatotoxicity in the mouse as well as the rat. (This abstract does not necessarily reflect EPA policy.)

221 QUANTITATION OF 1,3-BUTADIENE AND STYRENE METABOLIC INTERACTIONS IN B6C3F1 MICE

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Co-exposure of workers to 1,3-butadiene (BD) and styrene occurs in many polymer industries. BD and styrene require metabolic activation to epoxides to exert their toxic effect; therefore, metabolic interactions may influence their genotoxicity. Styrene can inhibit BD metabolism, since both are substrates for P4502E1, and styrene has a higher solubility in blood and tissues. To assess metabolic interactions, male B6C3F1 mice (25–30 g) were exposed for 8 hrs to 1000 ppm BD and 0–250 ppm styrene in a dynamic inhalation system. The rate of metabolism of BD was quantitated by measuring the difference between the inlet and outlet BD concentrations. Following exposure, butadiene monoepoxide (BMO), butadiene diepoxide (BDE), and styrene oxide were measured in the blood by GC-MS. As the styrene co-exposure concentration increased, the metabolism of BD decreased 10 to 30%. At all co-exposures the blood concentration of BMO remained constant; however, the blood concentration of BDE decreased 35%. The results indicate that styrene inhibited the oxidation of BD to BMO, as well as BMO to BDE. Elimination of BMO by hydrolysis or glutathione conjugation may also have been inhibited by styrene oxide. If BDE is the metabolite responsible for the carcinogenicity of BD, co-exposure with styrene could reduce the genotoxic potency of BD. These data were incorporated into a PBPK model describing disposition of BD and styrene, which ultimately will be linked to genotoxic response following co-exposure. The validated model can be extrapolated to humans to assess carcinogenic risk in workers exposed to mixtures of BD and styrene.

222 TOXICITY OF AROCLOR 1254 AND CADMIUM MIXTURE IN DEER MICE: PRELIMINARY STUDY

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Male and female *Peromyscus maniculatus* were exposed to Aroclor 1254 (20 ppm) and cadmium (400 ppm), singly and combined, for 20 and 6 days respectively. In this preliminary study, weights of the kidneys, hearts, testes, and uteri were collected and evaluated for indication of general toxicity. The results indicated that Aroclor 1254 caused a significant increase in the weight of kidneys above all treatment groups except the females treated with cadmium-alone. Exposure of females to Aroclor 1254 and cadmium in combination resulted in a significantly lower kidney weight than those of females exposed to Aroclor 1254 or cadmium alone. In the males, kidney weights at 0.0189 g following exposure to Aroclor 1254 and cadmium combination was significantly lower than the male control group. They were also lower than the control and treated female groups, except the females exposed to the combination of Aroclor 1254 and cadmium. Interestingly, kidney weights for females exposed to Aroclor 1254 alone and cadmium alone were found to be significantly higher than those of males in similar treatment groups. There were no statistical difference in the weights of the hearts, testes, and uteri. The average weight range of the heart was 0.1220 to 0.1523 g, while the uteri and testes were 0.0323 to 0.0547 g and 0.084 to 0.190 g, respectively. Analysis for the induction of porphyrin profiles will be carried out to further characterize the toxicity of environmental contaminants. This work was supported by NIH, NIGMS grants GM08117, GM08387 and NIEHS ES04696.

223 THE EFFECTS OF AROCLOR 1254 AND CADMIUM ON SELECTED TISSUE AND BLOOD CELLULARITY

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Forty adult male and female *Peromyscus maniculatus* were used to explore the potential interaction between two environmental toxicants on tissue and blood cellularity. Four experimental treatments were used: 20 ppm Aroclor 1254 (exposure day 1–20) with and without 400 ppm cadmium (exposure day 15–20), 400 ppm cadmium only (exposure day 15–20), and negative control. Red blood cell count (RBC), packed cell volume (PCV), spleen mass and cellularity, and leukocyte (WBC) and leukocyte subpopulations were measured on day 21. Spleen mass and cellularity (total splenocytes and splenocytes per mg spleen) did not vary among treatments or between sexes. WBC appeared to be influenced by treatment ($P = 0.1024$) and sex ($P = 0.0662$). Total leukocytes were higher for mice exposed to PCB only ($3.8 \pm 0.4 \times 10^3$/ul) compared to mice exposed to PCB and cadmium ($2.2 \pm 0.2 \times 10^3$/ul). RBC and PCV were significantly influenced by cadmium exposure ($6.8 \pm 0.3 \times 10^6$/ul, $39 \pm 2\%$, respectively) but not PCB ($8.4 \pm 0.5 \times 10^6$/ul, $42 \pm 1\%$, respectively). Except for WBC, no apparent chemical interactions were observed, given the endpoints measured. Additional information on leukocyte subpopulations is forthcoming. This work was supported by NIH, NIGMS grants GM08117, GM08387 and NIEHS ES04696.

224 PRELIMINARY STUDIES OF LIVER TOXICITY, CYTOCHROME P540 AND PORPHYRIN PROFILES IN AROCLOR 1254 AND CADMIUM (Cd) IN MALE AND FEMALE DEER MICE

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Male and female mice were exposed to Aroclor 1254 (20 ppm) in feed and Cd

(400 ppm) for days 1–20 and 15–20 respectively, both single and in combination. The female liver weight ranged from 0.592 to 1.047 g, while the males ranged from 0.716 to 1.043 g. Aroclor 1254 significantly increased the liver weight of males and females over the controls. Interestingly, the liver weight of the males exposed to Cd-alone (0.716 g) was significantly lower than males and females exposed to PCB-alone 1.043 g and 1.047 g respectively, as well as, males exposed to PCB-Cd in combination. However, PCB-Cd group seem to reflect the toxicity of the PCB exposure. While the liver weights of the male and female controls were not significantly different, the females exposed to the combination of PCB-Cd was significantly lower than males in the same treatment group, as well as females exposed to PCB-alone. Unlike the males, the PCB-Cd combination in the females seem to reflect the toxicity of Cd. Preliminary indication of cytochrome P450 and porphyrin profiles are in progress and suggest that further research is needed to characterize the toxicity of environmental contaminants both single and in combination in males and females. This work was supported by NIH, NIGMS grants GM08117, GM08387 and NIEHS ES04696.

225 DYNAMIC ORGAN CULTURE TO ASSESS THE TOXICITY OF CHROMIUM/HALOGENATED HYDROCARBON MIXTURES

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Perchloroethylene (PCE), trichloroethylene (TCE) dichloroethylene (1,1-DCE), and chromium are common groundwater contaminants. Precision cut rat liver slices were used to evaluate the relative toxicities of the halogenated hydrocarbons in the presence of chromium containing compounds (Cr^{+3}, Cr^{+6}). The relative uptake of each form of chromium was evaluated using ^{51}Cr. Uptake of chromate (CrO_4^{-2}) via the sulfate anion transporter was assessed. Liver slice viability was measured by quantitating potassium content and leakage of lactate dehydrogenase (LDH) into the medium. Slices were exposed to gas phase concentrations of 2.5–100 ul halogenated hydrocarbon/L and 1×10^{-7} M–5×10^{-3} M sodium chromate or chromium chloride in the medium. Sodium chromate (Cr^{+6}) showed additive toxicity when present in the medium of slices exposed to TCE, DCE or PCE. Conversely, chromium chloride exhibited some protection against toxicity when administered in the medium of slices exposed to TCE, DCE or PCE. Uptake of ^{51}Cr as sodium chromate was not increased by addition of TCE, DCE, or PCE. This is contrary to our previous *in vivo* results. Minimal uptake of $^{51}CrCl_3$ was demonstrated in the presence *or* absence of TCE, DCE or PCE. Increasing the concentration of sulfate anion in the medium inhibited chromate uptake. Addition of 0.5 mM 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid (DIDS), an inhibitor of anion transporters, reduced chromium uptake by nearly 50%. These results imply that chromate can enter cells via anion transport. TCE and DCE reduced the viability of rat liver slices in a dose responsive manner with DCE exerting its toxic effect at lower concentrations than TCE. However, PCE showed an interesting dose response in liver slices. It was less toxic than DCE or TCE until a 'threshold' concentration (≈50–70 ul PCE/L in 95% O_2/5% CO_2) where it became more toxic than either of the other halogenated hydrocarbons. This response may reflect depletion of a critical cofactor such as glutathione at the threshold concentration. (AHA AZ Affiliate #AZG-12-93; NIEHS #NIH ES-04940, The University of Arizona Undergraduate Research Grant Program.)

226 EVALUATION OF RESPIRATORY IRRITATION OF A METALWORKING FLUID (MWF) VS. ITS INDIVIDUAL COMPONENTS USING MICE

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Currently, there is a renewed interest in defining human health effects from exposure to MWFs. Some health-related concerns are due to the change from traditional "cutting oils" to newer types of MWFs (synthetics, semi-synthetics, solubles) which are complex chemical mixtures. Little toxicological information is available on newer MWFs or on components used in these fluids. The present study focused on the respiratory irritancy of individual components used in MWFs. Specifically, this study was based on previous results with aerosolized MWF "A" which was a synthetic fluid with 11 components. It evoked sensory and pulmonary irritation in mice and had an RD_{50} (concentration evoking a 50% decrease in mean respiratory frequency, *f*) of 119 mg/m^3 (Schaper and Detwiler, Fundam.Appl.Toxicol. 16, 309–319, 1991). In the present study, mice were exposed for 3 hr to aerosols of each component in MWF "A". Sensory and pulmonary irritation were evoked by each component, except a defoamer. For the 10 "active" components, concentration-response relationships (using *f*) and RD_{50} values were obtained. The 3 most potent components were tolutriazole, a preservative, and fatty acid alkanolamide condensates; all had RD_{50} values similar to that for MWF "A". It was concluded that these 3 contributed to the respiratory irritancy of MWF "A". This approach should be useful to identify irritants in other MWFs. Supported by UAW-GM.

227 HOST RESISTANCE TO BACTERIAL, VIRAL, OR TUMOR CELL CHALLENGE FOLLOWING EXPOSURE TO WOOD STOVE (WS) AND OIL FURNACE (OF) EMISSIONS

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Increasing use of WSs to supplement conventional heating systems has had an impact on air quality. WSs emit a complex mix of CO, SO_2, NO_x's, and organic compounds, whereas OF generally emit fewer constituents at lower concentrations. Effects of emissions from these sources on several host resistance models were compared. Mice received a single 6 hr exposure to emissions and were challenged immediately thereafter by aerosol with *Streptococcus zooepidemicus*. Mortality was enhanced in mice exposed to WS but not in those exposed to OF emissions. Mice also were exposed 6 h/day for 4 days and challenged after the 2nd exposure with influenza virus or B16 melanoma tumor cells. Neither OF nor WS emissions affected mortality, virus titers, or lung wet weight following influenza infection. However, significantly more B16 tumors were detected in lungs of mice exposed to WS emissions. More tumors were also seen in mice exposed to OF emissions although the difference was not statistically significant. Results indicate that WS emissions enhance susceptibility of mice to both bacterial and tumor cell challenge, whereas OF emissions had little or no effect. (This abstract does not reflect EPA policy.)

228 COMPARATIVE AND PREDICTIVE ACUTE TOXICITY AND PRIMARY SKIN IRRITANCY OF CRUDE ACETALDEHYDE SAMPLES (CONTAINING >2% ACROLEIN AND <2% ACROLEIN)

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The acute toxicity and primary skin irritancy of crude acetaldehyde (CA), an industrial by-product, were evaluated. Acrolein content in CA varies; therefore, 2 representative samples (containing 3.1% or 1.7% acrolein) were tested and the results compared. Perorally, the CA-3.1% had an LD50 of 297 mg/kg for both male and female rats, while the CA-1.7% had LD50s of 595 and 683 mg/kg for males and females, respectively. By the cutaneous route (female rabbits), the LD50 for the CA-3.1% was 933 mg/kg; that for the CA-1.7% was 771 mg/kg. For both materials, moderate to severe skin irritation (including superficial to full-thickness necrosis) resulted after a 4-hour application of 0.5 ml to occluded rabbit skin. As expected, doubling the acrolein content in CA essentially doubled the peroral toxicity. However, the predicted peroral toxicity of CA, calculated from reported LD50s for acetaldehyde (1930 mg/kg) and acrolein (46 mg/kg), was considerably less than observed in these studies (based on both additive and individual toxicity from the 2 components). Conversely, prediction of individual component toxicity from the observed CA results greatly overestimated their toxicity. It was concluded that the peroral toxicity of CA was largely related to acrolein toxicity, but that the toxic effects were more than additive (synergistic). Although clinical signs of peroral toxicity were similar for both CA samples, the CA-3.1% produced more rapid death. Cutaneous toxicity and irritancy were similar for both samples, perhaps indicating that volatility of the samples limited the amount of absorption.

229 THE EFFECTS OF JP-8 JET FUEL ON SPRAGUE-DAWLEY MALE RATS AFTER A 90-DAY EXPOSURE BY ORAL GAVAGE

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The U.S. Air Force is converting from JP-4 jet fuel to a less volatile JP-8 jet fuel similar to commercial Jet A. A 90-day inhalation study with JP-8 vapor conducted by this laboratory using F-344 rats and C57BL/6 mice resulted in no treatment-related adverse effects except for the alpha 2-microglobin nephropathy in male rats. In the present study male rats were dosed with neat JP-8 (0, 750, 1500, 3000 mg/kg) daily by gavage for 90 days in an effort to further characterize the kidney lesion and assess any additional adverse effects which may be associated with the prolonged exposure to this fuel. Results of this study revealed a significant dose dependent decrease in body weights of

rats exposed to JP-8. The male rat specific alpha 2-microglobin nephropathy was observed by histopathologic examination. A number of significant changes were also seen in blood and urine that were not dose dependent and may be related to the male rat specific alpha 2-microglobulin protein droplet nephropathy. Although there was no report of histopathological or liver weight changes in the liver of exposed rats, there was an increase (179, 165, 146; 246, 242, 210% control) in the liver enzymes AST and ALT, respectively.

230 EFFECTS OF SUBCHRONIC DERMAL EXPOSURE OF F344 RATS TO AIR FORCE JET FUEL (JP-4)

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Military and other personnel may be exposed to Air Force Jet Fuel (JP-4) primarily by the dermal route. There is little information on the toxicity of this material which is a complex crude oil distillate mixture of alkanes, benzenes, naphthalenes, and other agents. F344 rats were exposed to undiluted JP-4 (100 μl) applied on shaved portions of the dorsal lumbar skin for 2 weeks, 1 month, 3 months, or 6 months. Control rats received deionized water in the same region. The pharmacokinetics of trichloroethylene (TCE) was studied in exposed rats using an *in vivo* gas uptake system. Also evaluated were *in vitro* metabolism of TCE by liver microsomes, liver cell proliferation, growth, and hematologic, clinical chemical and histopathological findings. Body weight was significantly reduced in rats treated with JP-4 for 3 months. Thymus weight was significantly reduced after 2 weeks and 1 month of JP-4 treatment, and was lower, although not significantly, in the involuting organ at 3 months. Total blood erythrocyte and leukocyte counts were decreased after 3-month JP-4 treatment. Skin irritation was prominent at the site of JP-4 application. Supported by U.S. Air Force contract F33615-91-C-0538.

231 COORDINATION AND IMPLEMENTATION OF BIOTECHNOLOGY PRODUCT SAFETY ASSESSMENT BY THE FEDERAL GOVERNMENT: INTRODUCTION

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This workshop will: 1. inform SOT members how various agencies conduct safety assessments of biotechnology products, 2. provide key contacts at the important federal agencies, and 3. encourage an open discussion among federal and industrial toxicologists. Regulation of biotechnology products is a dynamic process; thus, EPA, USDA, and FDA regulatory scientists are developing principles for flexible regulation. The lack of prospectively defined standard testing requirements such as those in existing guidelines, however, creates uncertainty. Each regulatory agency must establish tests and procedures consistent with its statutory authorities and appropriate to the products for which it is responsible. An agency's ability to develop predictable, generic testing requirements with minimal regulatory impact may be limited by the rapidly increasing number and diversity of new products, a desire to maintain a flexible oversight system, and the need to address public concerns regarding creditability of the regulatory framework and safety of approved products. This workshop will promote the concept of codevelopment of scientific principles for toxicological evaluations for the regulation of novel products by industry and government.

232 PROTECTING THE FOOD SUPPLY FROM ENVIRONMENTAL CONTAMINANTS: SCIENTIFIC AND ECONOMIC CONSIDERATIONS

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The use of chemicals to increase productivity in many areas such as agriculture and industry, and their potential adverse effects on public health, constitutes a major conflict in today's society. This conflict gains greater proportion when economic growth and progress are weighed in. For example, the assumption used to assess risk is that compounds such as pesticide residues and veterinary drugs can potentially reach the food chain "at their highest permitted level over the life time of the consumer." Do these compounds remain bioavailable to the consumer long enough at high concentration? Another public concern is over air pollution and acid rain originating from mobile and stationary sources which most often lead to enactment of new regulations. Such regulations can be viewed, by some, as economic obstacles stifling growth and development, and by others as necessary to limit an otherwise uncontrollable environmental pollution. This conflict between protecting the environment and economic development is even stronger in developing countries where producing enough food for the society is thought to be more important than safety of the environment or the individual consumer. This symposium will bring together scientists, economists, regulators and safety advocates to discuss this timely issue.

233 BIOLOGICALLY-BASED, QUANTITATIVE RISK ASSESSMENT OF NEUROTOXICANTS: INTRODUCTION

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The need for biologically-based, quantitative risk assessment procedures for non-cancer endpoints such as neurotoxicity has been discussed in reports by the United States Congress (Office of Technology Assessment, OTA), Nation Research Council and a federal coordinating council. According to OTA, current attention and resources allocated to health risk assessment research are not commensurate with its impact on public health and the economy. Methods to include continuous rather than dichotomous data for neurotoxicity endpoints, biomarkers of exposure and effects, pharmacokinetic and mechanistic data have been proposed for neurotoxicity risk assessment but require further review and validation before acceptance. The purpose of this Symposium is to examine procedures to enhance the risk assessment process for neurotoxicants and to discuss techniques to make the process more quantitative. Accordingly, a review of the currently used safety-factor risk assessment approach for neurotoxicants will be provided along with specific examples of how this process may be enhanced with the use of the benchmark dose approach. The importance of including physiologically-based pharmacokinetic data in the risk assessment process and specific examples of this approach will be presented for neurotoxicants. The role of biomarkers of exposure and effect and mechanistic information in the risk assessment process will also be addressed. Finally, quantitative approaches with the use of continuous neurotoxicity data will be demonstrated and the outcomes compared to those generated by currently used risk assessment procedures.

234 ASSESSMENT PROCEDURES FOR NEUROTOXICANTS: COMPARISON OF THE CURRENTLY USED AND BENCHMARK DOSE APPROACHES

K S Crump. *ICF Kaiser Engineers, Ruston, LA.* Sponsor: W Slikker, Jr.

Acceptable levels of exposure to non-carcinogens are often determined by dividing a no-observed-adverse-effect-level (NOAEL) by uncertainty factors that reflect data limitations. A statistical lower bound on the benchmark dose (BMD) has been proposed as a replacement for the NOAEL in this process. A BMD is the dose of a substance that corresponds to a prescribed increase (the benchmark risk or BMR) in an adverse effect. For example, if 5% of unexposed subjects have an abnormal response and the BMR is 10%, the BMD is the dose that results in an abnormal response in 15% of exposed subjects. Whereas the determination of a NOAEL generally involves comparing responses at discreet doses to responses in control subjects, the benchmark approach involves fitting a dose-response model to all of the dose-response data. Several potential advantages of the benchmark over the NOAEL have been identified: (1) The benchmark makes better use of dose-response information. (2) The benchmark reflects sample size more appropriately. (3) The BMD is not constrained to be one of the experimental doses. (4) A benchmark can be defined from a data set in which a NOAEL does not exist. (5) A benchmark calculation does not require the data to be categorized into a few distinct dose groups. Issues that need to be addressed in adopting the benchmark procedure for regulatory use include selection of dose-response model, selection of the BMR, and determination of approaches that treat quantal and discreet data evenly.

235 THE USE OF PHYSIOLOGICALLY-BASED PHARMACOKINETICS (PB-PK) IN THE RISK ASSESSMENT OF NEUROTOXICANTS

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Biologically-based risk assessments for neurotoxicant are based on presumptions about (1) the chemical-tissue interactions that result in toxicity and (2) the biological processes involved in delivery of toxic chemicals to target tissues. The former come from the study of the mechanisms of toxicity; the latter from the study of pharmacokinetics. The nature of the toxic chemical, i.e., parent compound, metabolite, tissue adduct, etc., is usually inferred from the mechanisms of toxicity. PB-PK models are attractive for estimating target tissue dose

of toxic chemicals in biologically-based risk assessments since they are based on physiological, biochemical, and anatomical characteristics of the animal species. In principle, PB-PK models for neurotoxicants permit extrapolation of tissue dosimetry from high dose animal studies to ambient exposure concentrations, from test animals to people, and from the exposure scenario and route used in animal studies to the scenario and route of concern in the human population. This paper examines the development of PB-PK models for three classes of neurotoxic compounds: organophosphates with their pervasive effects on cholinergic transmission, hexane-induced peripheral neuropathics, and central nervous system accumulation and toxicity of organic acids. The discussion with each class of neurotoxicant emphasizes PB-PK model development, the strategies envisioned for interspecies scaling for risk assessment, and the potential role of PB-PK models in improving the basis of the present uncertainty factor approaches for neurotoxicity risk assessment. (This abstract does not necessarily reflect EPA policy.)

236 THE ROLE OF BIOMARKERS IN THE RISK ASSESSMENT OF NEUROTOXICANTS

David Bellinger. *Children's Hospital, Boston, MA.* Sponsor: W Slikker, Jr.

Epidemiologic studies of children using continuously distributed measures of behavior (e.g., IQ) as indices of adverse effect have been given considerable weight in risk assessments of environmental neurotoxicants such as lead. Several features of these data complicate the application of quantitative methods of risk assessment. Commonly used biomarkers of exposure (e.g. blood lead, tooth lead, bone lead) do not measure dose delivered to the target tissue site, nor do they adequately reflect critical features of exposure history. Defining useful biomarkers of effect for identifying a NOAEL or LOAEL for a continuously distributed function has also proved difficult. Changing the focus of endpoints used in human neurotoxicity studies from global indices such as IQ to endpoints more similar to those used to assess animal learning will (1) increase our understanding of the mechanisms of human behavioral neurotoxicity and (2) facilitate cross-species comparisons. New methods are needed for using dose-effect relationships observed in human neurotoxicity studies to estimate the prevalence of responses of varying severity at different doses. Characterizing the variability of individual responses as a function of dose will also provide the opportunity to identify markers of susceptibility to neurotoxicity.

237 QUANTITATIVE RISK ASSESSMENT OF NEUROTOXICANTS WITH THE USE OF CONTINUOUS DATA

W Slikker, Jr., D W Gaylor. *National Center for Toxicological Research/FDA, Jefferson, AR*

Quantitative approaches that include the use of continuous, pharmacokinetic and mechanistic data have been recently proposed for neurotoxicity risk assessment but require further validation. One approach requires four steps: a) determination of a dose-response model, b) determination of the distribution of measurements (variability) about the model, c) determination of an adverse, or abnormal level, and d) estimation of the probability that a measure is beyond the abnormal level as a function of dose. In order to enhance our understanding of the relationship between a biomarker of effect and dose of administered chemical, several biological factors need to be considered as exemplified by examining the effects of the neurotoxicant methylenedioxymethamphetamine (MDMA). The use of continuous rather than dichotomous data for neurotoxicity risk assessment may be problematic because a distinct value that separates normal levels from those associated with toxicity is seldom obvious. With data generated from 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) exposed animals, a method will be presented for defining the abnormal range and for estimating the proportion (risk) of animals in the abnormal range as a function of dose. Comparison of risk assessment outcomes between the currently used and the presented quantitative approaches provides the framework for validation of these new approaches.

238 SECOND MESSENGERS: THEIR ROLE IN IMMUNTOXICITY: INTRODUCTION

N E Kaminski. *Michigan State University, East Lansing, MI*

The objective of this symposium will be to describe the role of second messengers in immune regulation and how changes in intracellular signaling by immunotoxic agents results in immune dysfunction. Several well characterized immunotoxicants will be discussed in the context of the signal transduction pathways they disrupt and the immunological functions which are consequently altered. Changes in immunocompetence following chemical exposure has been established for a wide variety of unrelated agents. For the vast majority of immunotoxic compounds thus far identified, it is clear that disruption of normal immune function is mediated through direct interactions between the agent or its metabolite and immunocompetent cells. Regardless whether this interaction occurs at the level of the cell membrane or at intracellular sites, basic regulatory processes mediated by second messengers are often altered ultimately resulting in immunologic dysfunction. For a number of immunotoxic compounds, the specific disruptions in intracellular signaling they produce have now been identified leading to a basic understanding of their mechanism of action. Equally important, through the application of these agents as biological probes, tremendous insight has been gained pertaining to which intracellular signaling processes control which cellular functions. Although a number of signaling pathways common to all cell-types have been identified, the actual functional processes they control may differ remarkably from cell-type to cell-type. Each of the speakers will focus on the role of specific signal transduction pathways and how their perturbation by chemicals results in altered immunocompetence.

239 INTRODUCTION TO SIGNAL TRANSDUCTION

J W Putney, Jr.. *NIEHS, Research Triangle Park, NC.* Sponsor: N Kaminski

Signal transduction mechanisms provide the means for cells to respond to signals from the internal as well external environment. A wide variety of carcinogens and other toxic substances produce their effects by disrupting or otherwise modifying cellular signalling systems. This includes signalling systems controlled by heterotrimeric G-proteins, as well as those controlled by receptor-tyrosine kinases. The tyrosine kinase-linked receptors are well known as regulators of cell growth and differentiation, and disruption of these pathways can lead to aberrant controls on cell division or cell death, leading to cancer. Although less well appreciated, this is also likely true for G-protein regulated pathways. Calcium signalling and calcium regulation are especially important targets for toxic interventions. Under normal conditions, the Ca^{2+} concentration in the cytoplasm is buffered within narrow limits (*ca.* 100 nM) by the actions of active transport mechanisms in the plasma membrane and in the limiting membranes of intracellular organelles. Perturbation of the mechanisms for Ca^{2+} homeostasis or signalling can lead to inappropriate cell responses, cell growth, cellular necrosis, or inappropriate initiation of programmed cell death or apoptosis.

240 REDOX REGULATION OF LYMPHOCYTE ACTIVATION

J A Ledbetter, G L Schieven. *Bristol-Myers Squibb, Seattle, WA.* Sponsor: N Kaminski

We have previously shown that glutathione depletion inhibits calcium mobilization signals induced by antigen-receptor stimulation in T and B lymphocytes, while simultaneously increasing basal calcium concentration. CD3 activation-dependent tyrosine phosphorylation of PLCγ1 was prevented after glutathione depletion while basal levels of tyrosine phosphorylation in whole cell lysates increased. Therefore oxidation can have both inhibitory and stimulatory effects on lymphocyte signal transduction pathways involving protein tyrosine kinase (PTK) activation.

Ionizing radiation and UV radiation induce early responses in lymphocytes, but also result in prolonged immune suppression. Ionizing radiation rapidly induces tyrosine phosphorylation of cellular proteins, and PTK inhibitors block radiation included NF-κB and c-jun induction indicating that PTKs mediate cellular radiation responses. To investigate the redox-sensitive PTKs, we nave used H_2O_2, a potent generator of reactive oxygen intermediates that readily crosses the plasma membrane. H_2O_2 rapidly induced tyrosine phosphorylation and calcium mobilization in lymphocytes, and the response was augmented by the protein tyrosine phosphatase (PTP) inhibitor vanadate. Tyrosine kinases associated with lymphocyte antigen receptors include src-family kinases fyn, lyn and blk and syk-family kinases ZAP-70 and $p72^{syk}$. In H_2O_2 treated cells, ZAP-70 or $p72^{syk}$ kinases were rapidly activated to higher levels than after antigen-receptor stimulation. H_2O_2 did not activate $p72^{syk}$ if the kinase was immunoprecipitated before treatment, implicating the cellular proteins that interact with $p72^{syk}$ as free radical targets. Based on these results we propose that reactive oxygen species mediate stimulation and suppression of PTK activation in lymphocytes by uncoupling the normal interaction between src-family and syk-family kinases.

241 ROLE OF CALCIUM IN 2,3,7,8-TETRACHLORO-DIBENZO-p-DIOXIN (TCDD)-INDUCED SUPPRESSION OF B-CELL FUNCTION

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Because the B-lymphocyte is a primary cell target, we characterized possible effects of TCDD on the B-cell antigen receptor, surface IgM. TCDD suppresses proliferation in response to anti-IgM, and IgM secretion induced by anti-IgM plus lymphokines. A mechanism of action downstream of the receptor was suggested by two observations. First, TCDD had no effect on either the increased expression of surface Ia or the increase in intracellular Ca^{2+} following treatment with anti-IgM. Second, the proliferation of B-cells in response to PMA + ionomycin was suppressed by TCDD. Because the suppression was dependent on the concentration of ionomycin, but not PMA, an important role by Ca^{2+} was suggested. Additional support came from studies which showed that calcium-independent models of B-cell activation (CD40 and PWM) were refractory to TCDD-induced suppression, and from studies with fura-2 which showed that direct exposure to TCDD caused an increase in the levels of basal Ca^{2+} in isolated B-cells. Because we saw no effect of TCDD on calcium influx using radiolabelled ^{45}Ca, the mechanism may involve an effect on an intracellular Ca^{2+} pool. Comparative studies indicated that while thapsigargin induced an immediate 4–5-fold rise in intracellular Ca^{2+}, consistent with its ability to inhibit an ER ATPase, TCDD treatment did not produce rapid calcium mobilization; but did result in an elevation of nearly the same magnitude 18 hours post-exposure. The results indicate that the effect of inappropriate elevation of intracellular Ca^{2+} depends upon the nature of the activational stimuli, and suggest that TCDD can be used to further characterize Ca^{2+} handling in lymphocytes. (Supported by NIH Grant ES 02520.)

242 ROLE OF ALTERED CALCIUM HOMEOSTASIS IN THE IMMUNOTOXICITY OF POLYCYCLIC AROMATIC HYDROCARBONS

S W Burchiel. *The University of New Mexico College of Pharmacology, Toxicology Program, Albuquerque, NM*

Previous studies indicate that immunosuppressive and carcinogenic polycyclic aromatic hydrocarbons (PAHs) impair Ca^{+2}-dependent transmembrane signaling in human and murine lymphocytes. Many PAHs produce a rapid (secs to mins) and sustained (hrs) increase in intracellular levels of Ca^{+2}. Rapid Ca^{+2} mobilization appears to be associated with activation of protein tyrosine kinases, tyrosine phosphorylation of $PLC_{\gamma 1}$, and a transient increase in IP_3 formation leading to release of Ca^{+2} from store in the endoplasmic reticulum (ER). Studies with genistein, a PTK-inhibitor, demonstrate that this agent inhibits PAH-induced $PLC_{\gamma 1}$ tyrosine phosphorylation, but does not totally prevent the sustained (4 hr) Ca^{+2} mobilization observed in HPB-ALL cells. Therefore, other biochemical mechanisms are likely responsible for the sustained increase in intracellular Ca^{+2} induced by PAHs (such as DMBA, benzo(a)pyrene and dibenzanthracenes). Results of recent studies demonstrate that immunosuppressive PAHs produce a dose and time-dependent inhibition of sarcoplasmic/endoplasmic reticulum Ca^{+2}-ATPases (SERCA). The ability of PAHs to inhibit SERCA pumps appears to positively correlate with their known immunosuppressive and carcinogenic properties. The inhibition of SERCA pumps by PAHs appears to be a novel activity that may be associated with PAH-induced immunotoxicity as well as alterations in Ca^{+2} signaling by various antigen, mitogen, and growth factor receptors on lymphocytes and certain non-immune system cells.

243 ROLE OF ADENYLATE CYCLASE IN CANNABINOID-MEDIATED IMMUNE INHIBITION

N E Kaminski. *Michigan State University, East Lansing, MI*

Cannabinoid compounds, including the major psychoactive component of marihuana, Δ^9-THC, have been widely established as being immunosuppressive. Recently, the existence of G-protein coupled cannabinoid (CB) receptors similar to that originally identified in brain tissue have been confirmed on lymphoid cells through radioligand binding analyses and by the identification of CB receptor mRNA. Agonist stimulation of CB receptors on mouse spleen cells produces a rapid inhibition of adenylate cyclase resulting in the depletion of cellular cAMP and a concomitant inhibition of T-cell function. Although the role of the adenylate cyclase/cAMP second messenger system in lymphocyte function has been controversial, results from the present studies suggest that this pathway is essential for T-cell activation. Consistent with a critical role for cAMP, spleen cell activation by phorbal ester plus calcium ionophore produced a rapid but transient increase in cytosolic cAMP which was completely inhibited by immunoinhibitory concentrations of cannabinoids. Reversal of cannabinoid-mediated inhibition was achieved by concomitant treatment of splenoctes with membrane permiable cAMP analogs, dibutyryl cAMP and 8-Br-cAMP. Pertussis toxin pretreatment of spleen cells also abrogated cannabinoid inhibition of adenylate cyclase and immune suppression. Taken together, these results suggest that immune suppression by cannabinoids is mediated, at least in part, through the inhibition of adenylate cyclase upregulation which normally occurs during the early stages of T-cell activation. This inhibition of adenylate cyclase activity appears to be under the control of a pertussis toxin sensitive $G_{inhibitory}$-protein coupled cannabinoid receptor.

244 QUANTITATION OF PULMONARY AND PLEURAL FIBER BURDENS IN FISCHER 344 RATS FOLLOWING SHORT-TERM INHALATION OF REFRACTORY CERAMIC FIBERS (RCF-1)

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Although the pleura is a prime target of fiber-induced disease, it is not known whether fibers must be in direct contact with pleural tissues to exert pathologic effects. The object of these experiments is to determine the kinetics of fiber movement into pleural tissues following inhalation of RCF-1, a fiber known to be carcinogenic in laboratory rodents. Male F344 rats were exposed by nose-only inhalation to RCF-1 at 82 mg/m^3, 6 hrs/day for 5 consecutive days. Immediately following the final exposure and at 4 weeks post-exposure, animals were assayed for determination of pulmonary and pleural fiber burdens. Pleural fibers were isolated by selective digestion with agarose-SDS (Bermudez, *Inhalat. Toxicol.* 6, 115, 1994). Fiber populations were characterized based on the bivariate lognormal size distribution of fiber lengths and diameters. Mean fiber counts were 22×10^6/lung (23×10^3/pleura) at day 5 and 18×10^6/lung (16×10^3/pleura) at 4 wks post-exposure. Similar geometric mean lengths (GML) and diameters (GMD) of pulmonary fiber burdens were observed at both time points. Values were 4 μm for GML (geometric standard deviation GSD(L) $\cong$ 2.3) and 0.3 μm for GMD (GSD(D) $\cong$ 1.9), with a correlation between length and diameter (τ) of 0.2. Analysis of pleural samples demonstrated that small, thin fibers could be detected immediately following 5 days of exposure. The size distributions of pleural fiber burdens at both timepoints were approximately 1 μm GML and 0.08 μm GMD (GSD(L) $\cong$ 2.3, GSD(D) $\cong$ 1.9, $\tau \cong$ 0.2). Few fibers longer than 5 μm were observed at either time point. These findings demonstrate that fibers can rapidly translocate to pleural tissues; however, only short, thin (<5 μm length) fibers could be detected over the 5-week time course of this experiment.

245 THE EVALUATION OF SOLUBLE FIBERS USING THE INHALATION BIOPERSISTENCE MODEL, A NINE FIBER COMPARISON

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The biopersistence of nine fibers was evaluated using an inhalation model. The fibers studied were glasses with different in-vitro acellular solubility. Their compositions were based either essentially on sodium (commercial glass fibers) or calcium (commercial stone wools). Fischer 344 male rats were exposed to a well defined rat respirable aerosol (mean diameter of $\approx$1 μm) at a concentration of 30 mg/m^3, 6 h/day for 5 days with post-exposure sacrifices at 1h, 1d, 5d, 4w, 13w and 26w. At sacrifice, the whole lung was removed, weighed and frozen at −20 °C for subsequent digestion by low temperature plasma ashing. The number and bivariate size distribution of the fibers in the aerosol and lung were determined. At 1 h following the last exposure, the 9 fibers were found to have lung burdens ranging from 4.5–32 $\times 10^6$ fibers/lung with GMDs of 0.41–0.56 μm. The range of initial lung burdens was found to reflect the different bivariate distributions in the exposure aerosol. The fibers were found to be removed from the lung following the cessation of exposure with half-lives of approximately 5 to 65 days. An important difference in removal was seen between the long fiber (>15 μm) and short fiber (<15 μm) fractions depending upon composition. For most soluble compositions, the longer fibers were removed faster than the shorter fibers. It was found that the composition influenced the clearance of the different length fractions. Examination of the fiber length distribution and particles in the lung from 1 h through 5 d of exposure indicated that, especially for the more soluble fibers, fiber breakage was important in the removal of long fibers. For most fibers, the diameters did not change significantly over time. This agrees with the in-vitro findings that

dissolution of fibers can result in a leached layer which is thought to weaken the structure of the fiber thus contributing to breaking. These results demonstrate that the inhalation biopersistence model can be used in the process of evaluating the optimal composition for the reduction of fiber biodurability in the lung.

246 *IN VITRO* CYTOTOXICITY OF FIBERGLASS SAMPLES: IMPORTANCE OF PHYSICAL PROPERTIES

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The relative importance of chemistry vs morphology in determining the pathogenicity of fibers is a matter of great debate. The objective of the present study was to compare the *in vitro* cytotoxicity of two types of fibrous glass which exhibit strikingly different physical dimensions. Owens Corning building insulation fibers were 28× longer and 36× thicker than Manville Code 100 fibers. These factors resulted in a 6× greater surface area/mass and a 50× higher particle number/mass for the Manville fiberglass. The relatively thick Owens Corning fibrous glass exhibited little toxicity toward alveolar macrophages in culture over a 72 hr exposure period even at a dose of 1000 μg/ml. In contrast, the thin Manville fiberglass significantly decreased membrane integrity, increased leakage of cytosolic and lysosomal enzymes, and decreased particle-stimulated respiratory burst activity in macrophages after a 24 hr exposure to a dose of 100 μg/ml. Therefore, on both an equivalent mass and surface area basis, the Manville Code 100 fibrous glass was more cytotoxic *in vitro* than Owens Corning building insulation. These data emphasize the importance of size and morphology in determining the potential toxicity of man-made glass fibers.

247 INTRATRACHEAL INHALATION VS. INTRATRACHEAL INSTILLATION: DIFFERENCES IN PARTICLE EFFECTS

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Some questions have been raised about the relevancy of intratracheal instillation studies to inhaled exposures. While instillation studies allow the delivery of an exact dose to the lung, they do so with an uneven distribution and necessitate the use of a vehicle. We have developed and refined a method of intratracheal inhalation (ITIH) which allows for the administration of up to 1000 μg in a short time period (2 hours), and sought to determine if the effects of particles delivered by this system were akin to instilled or longer-term inhalation exposures. We exposed rats to fine (TiO_2-F, 250 nm) and ultrafine (TiO_2-D, 21 nm) sizes of titanium dioxide particles for 2 hours. An equivalent dose of each type of TiO_2 was intratracheally instilled into another test group. Animals were sacrificed at days 0, 1, 3, and 7 and lavaged. Lavage cell number, viability, cell differential, and lavage protein levels were determined. ITIH animals exposed to TiO_2-F showed few differences from unexposed or sham exposed groups. When a similar dose was instilled, an increase in PMN's and total cells was seen. Animals receiving TiO_2-D showed increases regardless of method of administration. Our results suggest that a dose delivered by inhalation over a time period as short as two hours may elicit a different response than one instilled directly.

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248 PULMONARY EFFECTS OF INHALED CARBON BLACK IN RATS

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Chronic rat inhalation studies with a number of highly insoluble low toxicity particles induced fibrogenic and tumorigenic responses. The objective of this study was to investigate mechanisms of response by establishing exposure-dose-response relationships for different endpoints following a 3-month subchronic inhalation exposure of Fischer-344 rats to carbon black. Rats were exposed for 6 h/day, 5 days/week to concentrations of 1; 7; and 50 mg/m^3 carbon black. The amount of carbon black retained in the lung at the end of the 3-month exposure was 353; 1923; and 7112 μg, respectively. A significant translocation of the particles to regional lymphnodes occurred in the middle and high exposure groups. Cellular and biochemical inflammatory parameters in lung lavage showed no response at the lowest carbon black exposure level but significant increases at the highest exposure concentration. Inflammatory influx of neutrophils appeared to be the most sensitive indicator with 0.24% (not different from control); 13%; and 39% of the lavaged cells, respectively. Epithelial cell proliferation also showed a dose-dependent response which appeared to parallel the neutrophil inflammatory response. We suggest that inflammatory cytokines and oxidants released by the inflammatory cells are causally involved in the different long-term adverse effects and that a threshold of particulate lung burden exists below which no adverse long-term effects occur.

249 INFLAMMATION, CYTOKINE EXPRESSION AND MUTAGENESIS IN RAT LUNG AFTER CARBON BLACK INHALATION

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Chronic inhalation of carbon black (CB) by rats results in lung tumors. The mechanisms underlying this response are poorly understood, however, we have proposed that inflammatory cells and their associated mediators (i.e., oxidants, cytokines) are contributing factors. Here we report the exposure-response relationships for the effect of CB inhalation on pulmonary inflammation, expression of the neutrophil chemotactic cytokine, MIP-2, and mutagenesis in rat lung epithelial (RLE) cells. Groups of rats were exposed to air, 1, 7 and 50 mg/m^3 CB, 6 hr/d, 5 d/wk for 13 wks. Inflammation was characterized by analysis of BALF and MIP-2 mRNA levels were determined using RT-PCR techniques. Mutation in RLE cells was assessed by their *ex vivo* culture in 6 thioguanine containing media. After exposure to air or 1 mg/m^3 CB, MIP-2 mRNA expression was not detected nor were any increases in inflammatory cells observed. In contrast, both MIP-2 mRNA and BALF neutrophils were increased after exposure to 7 and 50 mg/m^3 CB. No change in hprt mutation frequency was seen at the lowest level of CB exposure. Exposure to 7 and 50 mg/m^3 Cb resulted in mutation frequencies which were 3.2 and 4.2 fold greater, respectively, than those in air control rats. The concurrence in the exposure–response relationships for CB-induced MIP-2 expression, neutrophilic inflammation and RLE mutagenesis is consistent with a role for inflammation in the lung tumor response to CB. In addition, the absence of any observed changes at the lowest CB exposure level supports the existence of a threshold for the above described effects.

250 PROLIFERATIVE ACTIVITY OF KERATOACANTHOMA AND DUST-INDUCED KERATINIZING SQUAMOUS LESIONS OF THE LUNGS IN RATS AS ASSESSED BY THE PROLIFERATING CELL NUCLEAR ANTIGEN

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Cystic and keratinizing squamous lesions have been produced in the lungs of rats following chronic inhalation exposure to high concentrations of a number of dusts. There is disagreement among pathologists as to whether, in the absence of clear morphological criteria of malignancy, these lesions should be designated as cysts or as benign neoplasms. Some investigators have suggested that morphological similarities between these cystic keratinizing lung lesions and keratoacanthoma, a benign skin tumor, are supportive of a diagnosis of benign neoplasia for the lung lesions. To evaluate the comparative proliferative activities of keratoacanthomas (KA) and dust-induced cystic keratinizing squamous lesions (SL) in rats, 5 KAs and 6 SLs were stained immunocytochemically for the proliferating cell nuclear antigen (PCNA), an endogenous cell replication marker. PCNA staining of SLs of the lung was segmental along the cyst wall and was primarily limited to the basilar most layer of epithelium. Staining of KAs was also segmental but in most areas was more extensive and staining intensity was greater than that seen in the SLs. In contrast to the lung lesion, the KAs had multifocal areas with staining of multiple layers of squamous epithelium in the basilar region. These results suggest that the proliferative activity of KAs of the skin in rats is greater than that of dust-induced cystic keratinizing squamous lesions of the lung.

251 PERSISTENT PULMONARY INFLAMMATION AND CELLULAR PROLIFERATION IS LIKELY TO RESULT FROM "OVERLOAD" EXPOSURES IN RATS INHALING LOW TOXICITY, LOW SOLUBILITY DUSTS

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Chronic inhalation exposures to high dust burdens can produce tumors or proliferative keratin cysts in the lungs of exposed rats. We postulated that concs. which overwhelm lung clearance mechanisms correlate with persistent pulmonary inflammation and cellular proliferation. Male rats were exposed to titanium dioxide (TiO_2) or carbonyl iron (CI) particles for 4 weeks

at concentrations of 5, 50 and 250 mg/m^3. After exposure, the lungs of sham and dust-exposed animals were lavaged or assessed for cell proliferation or particle clearance immediately after, as well as 1 week, 1, 3, and 6 months postexposure. Exposures to TiO_2 or CI at 250 mg/m^3 produced lung burdens of 10 mg titanium and 15 mg/lung of iron and this correlated with sustained pulmonary inflammatory responses and increased BrdU labeling of terminal airway and pulmonary parenchymal cells. Clearance/retention studies demonstrated a substantial impairment of particle clearance in rats exposed to 250 mg/m^3, and was accounted for by phagocytic and chemotactic deficits in exposed macrophages. Histopathology studies revealed many aggregates of particle-laden macrophages contained within alveoli and alveolar ducts. Cellular hypertrophy and hyperplasia was evident. The results of this study clearly demonstrate that exposure to excessive dust concentrations of two innocuous particle-types produced sustained pulmonary inflammation, enhanced pulmonary cell labeling, impairment of particle clearance, and the development of pulmonary lesions. (Supported by the CMA TiO_2 Panel).

252 COMPARATIVE PULMONARY CARCINOGENICITY OF BERYLLIUM IN A/J AND C3H/HeJ MICE

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Beryllium (Be), a metal generally negative in short-term genotoxicity assays, is a potent pulmonary carcinogen in F344 rats. Although the epidemiological evidence is weak, Be is classified as a suspect human carcinogen. This study compared the pulmonary carcinogenicity of Be metal in A/J and C3H/HeJ mice, which are sensitive and resistant, respectively, to lung tumor formation. Female, 6–8 wk old, mice (206 each strain) were exposed once, nose-only, to Be metal or to filtered air (controls; 50 each strain). Despite simultaneous exposures of both strains, Be initial lung burdens (ILBs/g body wt) were 63 μg (3.4 μg/g) for C3H/HeJ mice and 47 μg (3.0 μg/g) for A/J mice. Clearance half-times were 108 and 97 days for C3H/HeJ and A/J mice, respectively. Differences between strains in ILBs and clearance half-times were statistically significant. Be exposure reduced survival significantly for C3H/HeJ mice and marginally for A/J mice. There was no difference in tumor incidence, multiplicity, or latency in Be-exposed C3H/HeJ mice compared to controls. When mice sacrificed before 11 mo on study were excluded (sacrifices to determine ILBs and study preneoplastic lesions), the incidence of lung neoplasia from 11 to 22 mo was slightly increased in Be-exposed A/J mice. Six percent of control and 19% of Be-exposed A/J mice had more than one neoplasm. Analyses of K-*ras* mutation patterns in neoplasms from exposed and control A/J mice suggest that the increased multiplicity was due to promotion of spontaneously initiated cells. Further analyses will define whether these increases are statistically significant and if tumor latency was decreased. (Research sponsored by the Assistant Secretary for Defense Programs and the U.S. DOE/OHER under Contract No. DE-AC04-76EV01013.)

253 LUNG TUMOR SYNERGISM BETWEEN $^{239}PuO_2$ AND CIGARETTE SMOKE INHALED BY F344 RATS

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Male and female CDF®(F344)/CrlBR rats were exposed to inhaled $^{239}PuO_2$ and cigarette smoke alone and in combination to better define potential lung tumorigenic interactions between these agents. Animals exposed to filtered air (FA) served as controls. Exposures were begun at 6 wk of age and continued for 6 hr/day, 5 days/wk, for 30 months in whole-body exposure chambers to mainstream smoke from 1R3 research cigarettes diluted to concentrations of either 100 or 250 mg total particulate matter/m^3 (LCS or HCS, respectively). A single, pernasal, acute exposure to $^{239}PuO_2$ was given to 50% of the rats at 12 wk of age. Exposure to smoke caused significantly reduced body weight gains. Compared to controls, survival was slightly increased in the LCS groups and decreased in the HCS groups. Neither weight nor survival was significantly affected by $^{239}PuO_2$ exposure. Smoke exposure decreased the pulmonary clearance of radiolabeled tracer particles in an exposure concentration-dependent manner; data for ^{239}Pu clearance are not yet available. Preliminarily, the prevalence of benign or malignant lung tumors in rats exposed for at least 12 mo to LCS or HCS without $^{239}PuO_2$ are similar to values in controls (prevalence range from 0 to 2%), except for an increase in female rats exposed to HCS (prevalence of 7%). However, prevalence in groups of rats exposed to only $^{239}PuO_2$, or to $^{239}PuO_2$ combined with LCS or HCS are 22, 41, and 64%, respectively. Interestingly, the combined exposures have produced bronchiolar tumors and relatively more squamous cell carcinomas (compared with adenocarcinomas). These results suggest a synergistic carcinogenic interaction between cigarette smoke and ^{239}Pu in the rat. (Research supported by the Assistant Secretary for Defense Programs and U.S. DOE/OHER under Contract No. DE-AC04-76EV01013.)

254 EXTRAPOLATION FROM PREGNANT MICE TO RATS OF A PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODEL DESCRIBING 2-METHOXYACETIC ACID DISPOSITION AFTER 2-METHOXYETHANOL ADMINISTRATION

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Accurate prediction of embryo dosimetry in laboratory animals is an important step toward improved risk assessment for exposure to chemicals during human pregnancy. We have used the potent developmental toxicant 2-methoxyethanol (2-ME) as a prototypical compound to develop a hybrid PBPK model that describes the disposition of its primary metabolite and proximate toxicant, 2-methoxyacetic acid (2-MAA), in the pregnant CD-1 mouse. The revised model primarily uses actual tissue compartments to describe 2-ME distribution and lumped compartments to describe 2-MAA distribution. The revised model describes the disposition of 2-MAA following gavage or i.v. bolus administration of 2-ME in both mice and rats. Pharmacokinetics and tissue partition coefficients of 2-ME and 2-MAA in maternal plasma, embryo and extraembryonic fluids (EAF) were determined in mid-organogesis in rats on gestation days (gd) 13 and 15 (gd 0 = sperm positive day). Other species-specific parameters (2-ME gastrointestinal absorption rate, 2-MAA intra-conceptus mass transfer coefficients, 2-MAA elimination rate constant) were obtained by optimization based upon rat data. The revised rodent model for 2-MAA divides the maternal body into richly and poorly perfused compartments. An active transport model of the conceptus, in which mass transfer rates into and out of the conceptus are unequal, provided the best simulations of the pharmacokinetic data collected on gd 13 and 15. Since the mechanism by which 2-MAA accumulates in the embryo and EAF has not yet been elucidated, this mathematical description is empirical. The successful interspecies extrapolation of the rodent gestation PBPK model encourages further development of the model for 2-MAA and its extrapolation to human pregnancy.

255 PHYSIOLOGICALLY-BASED PHARMACOKINETICS OF THE DERMAL ABSORPTION OF 2-BUTOXYETHANOL VAPORS IN HUMANS

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A recent study suggested that dermal uptake accounts for as much as 75% of the total uptake of 2-butoxyethanol (BE) by humans during whole body exposures to BE vapors (Johanson and Boman, *Br. J. Ind. Med.* 1991, 48, 788). This conclusion is unusual in light of the known physiological differences between the lungs and skin as they relate to the absorption of xenobiotics. The possibility that portal of entry effects confounded the finger prick blood sampling technique used in the Johanson and Boman study was tested using a PB-PK model. To validate the model, six human volunteers exposed one arm each to 50 ppm ^{13}C-BE vapors for two hr. Blood samples were taken from both the exposed arm (finger prick; end exposure only) and unexposed arm (catheter; during and after exposure) and analyzed for both BE and its major metabolite, butoxyacetic acid (BAA). As predicted, the finger prick technique for blood sampling was not representative of systemic blood for the parent compound, BE, but was representative of systemic blood concentrations of the metabolite, BAA, under these experimental conditions. In addition, urine was collected for 24 hr and analyzed for free and total BE, BAA and ethylene glycol (EG). Unlike rats, very little EG or conjugated BE was found in the urine. Also unlike rats, BAA was extensively (≈60%) conjugated, presumably with glutamine, and excreted in the urine. This study demonstrated that no more than 21%, not 75%, of the total uptake of BE by humans in a whole body exposure can be attributed to percutaneous absorption.

256 VALIDATION OF A CARBON TETRACHLORIDE PBPK MODEL USING REAL-TIME KINETIC DATA

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A physiologically based pharmacokinetic (PBPK) model to predict the behavior of inhaled carbon tetrachloride (CCl_4) has been modified to simulate an

intravenous (IV) route of CCl_4 exposure in the male Fischer 344 rat and used to predict the clearance of CCl_4 via exhaled breath. This PBPK model for the rat adequately described the concentrations of CCl_4 in real-time exhaled breath data collected from rats exposed to CCl_4 via a single IV injection. The data is collected using an innovative system that links the exhaled breath from the rat directly with a mass spectrometer. The system generates a new data point every 1.6 seconds. Thus, this methodology can aid in describing the rapid exponential emptying of the blood compartment that occurs immediately following peak exposure. Coupling the real-time collection of biokinetic data with a PBPK model should prove useful in elucidating complex, rapidly occurring biological reactions. This is clearly demonstrated by additional data on the real-time exhalation of CCl_4, chloroform, ethane, pentane, and acetone in the rat following a gavage dose of CCl_4.

257 DEVELOPMENT OF A SIMPLE INDEX FOR REPRESENTING THE DEGREE OF DISCREPANCY BETWEEN PBPK MODEL SIMULATIONS AND EXPERIMENTAL DATA

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The application of appropriate statistical tests provides a means of evaluating whether or not the model simulations are significantly different from the experimental data. Regardless of the outcome of such statistical analyses, it is often necessary and useful to be able to represent, in a quantitative manner, the extent to which the model simulations differ from the experimental data. The objective of the present study was to develop an index that provides a measure of the degree of discrepancy between PBPK model simulations and experimental data. The approach involved the calculation of the root mean square of the error (representing the difference between the individual simulated and experimental values for each sampling point in a time course curve), and of the absolute difference between the simulated and experimental values of area under the blood/tissue concentration vs time curve (AUC), and the time to attain maximal blood concentration of the metabolite or parent chemical (t_{max}). These absolute values were then normalized by dividing them by the corresponding experimental values to yield a measure of deviation of the PBPK model simulations of (i) each data point in the time-course curve, (ii) AUC and (iii) t_{max} from the corresponding experimental data. The resulting numerical values were then adjusted with a weighting factor (which depends on the state of the knowledge of mechanism of action of the chemical) specific to each term and consolidated to yield a composite index. The application of this kind of a "quantitative" method should help remove the ambiguity in evaluating/communicating the discrepancy between the PBPK model simulations and experimental data.

258 STATISTICAL APPROACHES FOR THE VALIDATION OF PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODELS

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The validation step in the PBPK model development process involves comparing the model predictions with experimental data to accept, refute, or refine the model. Most commonly, an 'inspection' approach (i.e., comparing model output with experimental data without the use of a formal statistical test) is used for these purposes. The objective of the present study was to investigate the statistical methods potentially applicable for the validation of PBPK models. The approaches considered include: correlation/regression, tests of hypotheses, residual analysis, and multivariate analysis. The various statistical tests were applied to verify the adequacy of several PBPK models developed previously. The correlation/regression approaches represent an inappropriate way of determining the adequacy of a model, since few data points at the extremities of the line often influence the outcome. In using hypothesis tests, the objective should not be to test the null hypothesis that the system (i.e., animal) and the model are the same; rather, it should be to verify if the differences between the simulated and experimental values are significant. In this context, a lack of fit F test is useful in specific cases. Since most PBPK modeling efforts involve comparing simulations with experimental data obtained in several species following administration of various doses of chemicals by more than one route, the use of selected forms of multivariate analysis of variance/regression appears to be appropriate. The systematic verification of the adequacy of PBPK models during the validation step should enhance the consistency and confidence in their use in health risk assessments.

259 A POPULATION PHARMACOKINETIC MODEL FOR NICOTINE AND ITS APPLICATION TO HUMAN EXPOSURE

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A population pharmacokinetic model for nicotine was developed to study the variability in plasma nicotine concentrations between individuals. Population pharmacokinetic parameters such as total clearance and volume of distribution were determined after nicotine administration through IV infusion, transdermal patch delivery and cigarette smoking. The nicotine concentration-time profile was best described by a two compartment model. Nicotine absorption for trandermal delivery was described by two first-order constants in the patch and a first-order absorption from the skin depot compartment. Nicotine uptake from smoking cigarettes was approximated through a series of IV infusions. Population mean pharmacokinetic parameters were 75 L/hr for total clearance and 251 L for volume of distribution. Inter-individual variations in nicotine distribution and metabolism explained large variations of plasma nicotine concentrations among the studied population. In addition, smoking behaviors influence the bioavailability of nicotine during smoking and fewer sampling points are required by Bayesian regression to estimate nicotine uptake from smoked cigarettes. Population pharmacokinetic models are useful tools for understanding the pharmacology of nicotine.

260 PHYSIOLOGICALLY-BASED MODEL FOR THE RELEASE OF LEAD FROM BONE AFTER MENOPAUSE

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The possible health impact of lead released from bone as a result of the net bone loss that takes place in older persons has been a cause of expressed concern. A physiologically-based model of lead kinetics (O'Flaherty, E.J., *Toxicol. Appl. Pharmacol. 118,* 16–29 (1993)) has been adapted to predict blood and bone lead in women after menopause. Changes in bone turnover during aging were incorporated into the model. Bone formation rate (BFR) after menopause was assumed to be constant (Jowsey. J., *et al, J. Bone Jt. Surg. 47-A* 785–806 (1965)). The increase in bone resorption rate (BRR) and the age-dependence of bone turnover rate (BTR) after menopause were defined based on the International Commission on Radiological Protection (ICRP) model of Reference Man (*ICRP Publication 23*(1975)). The differences between BRR and BTR in normal and osteoporotic bone are illustrated. The model was applied to the estimation of blood lead concentration after menopause, when lead is being released from bone at an increased rate. Comparison of mean lead concentrations demonstrates good agreement between model predictions and observations made in women (Silbergeld, E., *et al, Environ. Res. 47,* 79–94 (1988)) before and after menopause. This work was supported by a grant from the Atlantic Richfield Corporation.

261 EVALUATION OF PHARMACOKINETIC MODELS FOR USE IN CANCER RISK ASSESSMENT: EXAMPLE WITH TRICHLOROETHYLENE (TCE)

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TCE produces lung and liver tumors in the mouse and kidney tumors in the rat, but well conducted epidemiological studies have found no association with cancer in the human. In this study, mechanistic information, pharmacokinetic data, and bioassay data on TCE and its metabolites were analyzed to determine the most appropriate dose metrics for use in a pharmacokinetic cancer risk assessment for TCE. Based on this analysis, the dose metrics recommended for the three rodent target tissues are area under the tissue concentration curve for chloral in the lung, dichlorovinylcysteine in the kidney, and trichloroacetic acid in the liver. A physiologically based pharmacokinetic model of TCE and its principle metabolites was developed and applied to explore the key uncertainties associated with extrapolating animal data to estimate human risk from exposure to TCE. Incorporation of pharmacokinetics alone was not sufficient to predict observed species differences in the dose response for the various tumors. An adequate approach for tumor dose-response modeling may require incorporation of information on the impact of cell proliferation secondary to recurrent toxicity and mitogenicity, or other mechanistic considerations. At present, safe human exposure could be estimated from the animal data based on the target tissue exposure which is below the threshold for the appearance of toxicity or focal hyperplasia in the most sensitive animal species. Additional pharmacokinetic data on TCE and its metabolites, particularly in the human, would be required to perform this extrapolation accurately. (Funded by OSHA/HSP and EPA/OHEA).

262 DEVELOPMENT OF A PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODEL FOR METHYL t-BUTYL ETHER (MTBE) AND t-BUTANOL (TBA) IN MALE RATS

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MTBE, and its metabolite TBA, both cause renal tumors in chronically exposed male rats. Knowledge of the kinetic behavior of MTBE and TBA will aid in assessing human risk. The objective of this study was to first develop and validate a PBPK model for MTBE and TBA in rats as a basis for developing a human model. Physiological parameters such as blood flows, tissue volumes, and alveolar ventilation were obtained from the literature. Chemical-specific parameters, such as the solubility of MTBE and TBA in blood and selected tissues, and metabolic rate constants to describe whole-body metabolism of MTBE in rats, were measured using vial equilibration and gas uptake techniques, respectively. Metabolism of MTBE was saturable with a $V^{maxC} \approx 4.8$ mg/hr/kg, and $K_M \approx 3.3$ mg/l. Using these parameter values, the model predicted published data on MTBE levels in blood of rats exposed to MTBE by inhalation (400 and 8000 ppm, 6 hours), iv (40 mg/kg) and oral (40 or 400 mg/kg, $K_a = 2.7\ hr^{-1}$) administration. Using a model for TBA, which was linked to the MTBE model by metabolism and consisted of two compartments, liver and total body water, TBA blood levels were only predicted at low MTBE exposure levels. This suggests that the kinetics of TBA are more complex than MTBE. A quantitative description of the important determinants of MTBE and TBA dosimetry will enable a better assessment of the potential toxic and cancer risk for humans exposed to MTBE.

263 INCORPORATION OF HETEROGENEOUS ENZYME DISTRIBUTION INTO A PHYSIOLOGICAL MODEL FOR BENZENE, PHENOL AND HYDROQUINONE

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Phenol (PH) and hydroquinone (HQ), sequentially oxidized metabolites of benzene (BZ), undergo detoxification by sulfation and glucuronidation. Available data suggest that, in combination, PH and HQ are responsible for the myelotoxicity associated with benzene exposure. When mice are given a comparable (≈1 mg/kg) dose of BZ or PH by gavage, a greater percentage of the dose is excreted as HQ glucuronide (HQG) after administration of BZ (43%) compared to PH (7%). We hypothesize that HQ formation is a favored pathway after BZ exposure due to the pericentral and periportal localization of enzymes in liver acini responsible for oxidation (e.g., P4502E1) and sulfation, respectively. Heterogeneous enzyme distribution was incorporated in a physiological (PBPK) model for BZ, PH and HQ by describing the liver as three compartments linked in series by hepatic blood flow. PH sulfation was constrained to the periportal (zone 1) compartment and oxidation was constrained to the pericentral (zone 3) compartment. The resultant model was able to simulate observed experimental data for HQ levels in blood and liver and excretion of HQ conjugates in urine of mice after BZ (inhalation, gavage) or PH (intravenous) administration. Preferential removal of PH by sulfation in zone 1 explains why more HQ and conjugates are produced after BZ exposure compared to PH; and may also explain why PH does not produce the myelotoxicity observed after BZ administration. Incorporation of mechanism-based descriptions of metabolism into physiological models can significantly improve predictions of target tissue dosimetry and provide better estimates of risks associated with chemical exposure in the environment or workplace.

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264 A MULTI-COMPARTMENT GEOMETRIC MODEL OF THE RAT LIVER: APPLICATIONS WITH ENZYME INDUCERS

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In recent years physiologically-based pharmacokinetic (PB-PK) models have been developed for chemicals metabolized to reactive metabolites by hepatic cytochrome P450 isozymes and for compounds that induce these enzymes. These models treat the liver as a single compartment with evenly distributed constituents. Toxic effects and induction, however, are often highly localized. Induction generally begins in the centrilobular region and moves periportally with increasing doses of inducers. In addition, the boundary between fully induced cells and uninduced cells is abrupt. We have developed a sequential, 5-compartment PB-PK model of the liver, progressing from periportal to centrilobular regions. Blood flows sequentially through these 5 compartments, each of which has a specific volume (8, 32, 33, 20, and 7% total liver, respectively) and an associated surface area in the two-dimensional hexagonal geometry of the acinus. Regional enzyme concentrations are used to 'paint' the two-dimensional acinus. These geometric pictures can be qualitatively compared with immunohistochemical localization of enzymes. The present study examines model behavior for compounds that induce in a centrilobular-to-periportal manner via receptor-mediated mechanisms. The combination of binding parameters and cooperative interactions required to produce a sharp moving boundary are estimated and model output compared to the single-compartment liver model. These multi-compartmental models are important for compounds like dioxin, peroxisomal proliferators, and other inducers to fully appreciate their hepatic interactions and toxicity on a quantitative level. (This abstract does not necessarily reflect US EPA policy).

265 PHYSIOLOGICALLY BASED PHARMACOKINETIC/ PHARMACODYNAMIC (PBPK/PD) MODELING OF THE TOXICOLOGIC INTERACTION BETWEEN KEPONE AND CARBON TETRACHLORIDE (CCl_4)

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PBPK/PD modeling was utilized to quantitatively describe the interaction between Kepone and CCl_4. The PBPK/PD model was calibrated with previously published hepatotoxicity data and tested with experimental lethality studies performed at our laboratory. Sprague-Dawley rats were given either control diet or diet containing 10 ppm Kepone. On the sixteenth day, rats in the Kepone treated group were given ip doses of 0, 10, 50, and 100 μl/kg of CCl_4 (n = 9) while control rats were exposed to 0, 100, 1000, 3000, and 6000 μl/kg of CCl_4 (n = 9). Lethality was observed at the 1000 (1/9), 3000 (4/9), and 6000 (8/9) μl/kg doses for the control group and at the 50 (4/9) and 100 (8/9) μl/kg of the treated group. In the PBPD model, a first order rate equation describes the rate of cellular injury in relation to the amount of CCl_4 metabolites. The model continues to estimate the fraction of dead cells by considering an induced rate of death. When the percentage of dead cells reaches 50% of the original number of normal hepatocytes, the model considers the animal to be dead. LD_{50} estimates for CCl_4 toxicity with and without Kepone pretreatment were 47.1 and 2890 μl/kg, respectively. Monte Carlo analysis coupled with the PBPK/PD model, produced lethality proportions which were not significantly different from the observed mortality ($p > 0.1$) with the exception of CCl_4 at high doses ($p = 0.014$). This deviation may be attributed to extra-hepatic systemic toxicities of CCl_4 at high doses which were not included in the model. Furthermore, to address the question of the dose effect of Kepone on the interaction with CCl_4, a separate PBPK model was constructed to establish a target dose-interaction response between Kepone and CCl_4.

266 HSP70 INDUCTION IN HEPG2 CELLS IN RESPONSE TO HEPATOTOXICANTS

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Hyperthermia, as well as a variety of other stressful stimuli, has been observed to result in increased expression of a family of proteins termed the heat shock proteins (hsps). Across species and cell types, one of the most commonly increased hsps is an inducible form of approximately 70 kDa (hsp 70I). The objective of this study was to examine hsp 70I expression in response to a series of hepatotoxicants using the HepG2 human liver cell line. Incubation of cells for 24 hrs with cytotoxic concentrations of cadmium acetate (Cd), diethylnitrosamine (DEN), and bromobenzene (BB) resulted in significant increases in hsp 70I. No increases were observed following incubation with carbon tetrachloride (CCl_4) or KCN; and cyclophosphamide (CP) and cocaine produced significant decreases in hsp 70I levels. For comparison purposes, the largest increases in hsp 70I levels produced by toxicant exposure (6- to 8-fold, by Cd and DEN) were about half of the increase produced by sub-lethal heat shock (SLHS; incubation at 43° C for one hour). A 24-hour SLHS pretreatment reduced cytotoxicity of BB, Cd, DEN, CP, and lethal heat shock (46.5°C for 3 hrs; included as a positive control). Toxicity of CCl_4, cocaine, and KCN were unaffected by SLHS pretreatment. In general, cytoprotection against hepatotoxicants afforded HepG2 cells by SLHS pretreatment was correlated with the ability of the hepatotoxicant to induce hsp 70I. CP, the only apparent exception, increased HSP 70I mRNA, but also blocked protein synthesis, thereby preventing expression. (Supported by NIH Grant ES-07213.)

267 CARBON TETRACHLORIDE INDUCES STRESS PROTEIN SYNTHESIS IN PRECISION-CUT RAT LIVER SLICES

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Previously the expression of stress proteins in precision-cut rat liver slices by subtoxic doses of sodium arsenite was reported. Stress protein induction was observed before other viability markers were affected, indicating that it is a more sensitive biomarker of toxicity. Therefore, these studies were extended to investigate whether the model hepatotoxin carbon tetrachloride (CCl_4) causes stress protein induction in precision-cut rat liver slices at doses which do not alter intracellular K^+ levels or incorporation of ^{3}H-leucine into newly synthesized proteins. Liver slices from male Sprague Dawley rats were incubated for 2–8 hr with 0.1–1.0 μl CCl_4 (evaporated into a total volume of 25 ml) in methionine free medium to which ^{35}S-methionine has been added. Labeled proteins were separated by SDS-PAGE and detected by fluorography. Immunoblot analysis was performed using monoclonal antibodies for HSP 72 and HSP 90 and immunoperoxidase staining. Viability was not affected by 1.0 μl CCl_4 up to 24 hr as indicated by nmole K^+/μg DNA (23.4 ± 5.1 vs 25.97 ± 5.8 control), while ^{3}H-leucine incorporation was decreased by 39% by the above dose at 24 hr. However, both HSP 70 and HSP 90 were increased with lower doses of CCl_4 at earlier times. Densitometric analysis of fluorographs indicated an increase in HSP 70 (4-fold) and HSP 90 (8-fold) at 4 hr by 0.25 μl CCl_4. Immunoblot analysis demonstrated that 0.25 μl CCl_4 increased HSP 72 by 31% and 46% at 4 & 8 hr, respectively. HSP 90 was increased by 45% as early as 2 hr by 0.5 μl CCl_4. At 8 hr, both HSP 72 and HSP 90 were increased 2-fold by 0.5 μl CCl_4. It is interesting that this pattern of stress protein induction is different from that of the classic stress protein inducer arsenite which increased HSP 72 by 5-fold and decreased HSP 90 to 15% at 8 hr. (NIH ES05790)

268 HEPATIC AND RENAL STRESS PROTEINS AS BIOMARKERS OF CHEMICAL INTOXICATION

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Early screening of target tissues for chemical intoxication at the molecular level can be achieved if a sensitive yet accurate system can be developed to reliably assess the appearance and magnitude of biochemical alterations specifically associated with the chemical exposure. Recent studies in this laboratory and by others suggest that two-dimensional polyacrylamide gel electrophoresis of proteins (2DE) possesses significant utility in the detection of chemical toxicity and in providing information regarding toxic mechanism. Accordingly, identification of a set of specific biomarkers of *exposure* or *effect* with a proclivity for a particular target tissue in a variety of species is essential. In this regard, stress proteins, such as the heat shock and glucose-regulated proteins (Hsp and Grp), are appropriate candidates. These gene products are highly conserved, respond to both thermal and metabolic stress, are known to be intimately involved as chaperones in the biogenesis and disposition of cellular protein products, and have increasing potential relevance in environmental toxicology, clinical medicine, and human health. The present investigation was undertaken to 1) identify these stress proteins on conventional format 2D electrophoretic gel patterns representing different rodent target tissues and 2) compare the effect of structurally diverse peroxisome proliferators on stress protein expression. The following stress proteins were identified, their *x, y* coordinate positions mapped, and abundance statistically analyzed and compared: Hsp25, Hsp60, Hsc70, Hsp70, Hsp90, Grp75, Grp78, Grp94, protein disulfide isomerase (PDI), and ER-60. We report here that the stress response to peroxisomal proliferators is tissue-, toxicant-, and stress protein class-specific and, in the case of perfluorocarboxylic acids, it is dose-related. Furthermore, because nearly all of the proteins studied were constitutively expressed at detectable levels in both liver and kidney, the 2D stress protein pattern is well-suited to future toxicologic screening applications. (Supported by the Air Force Office of Scientific Research, Grant F49620-93-1-0297).

269 STRESS RESPONSE AND HISTONE DOWN-REGULATION IN RESPONSE TO QUINONE-THIOETHERS IN RENAL PROXIMAL TUBULAR EPITHELIAL CELLS (LLC-PK1)

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Administration of 2-bromo-(di-glutathion-*S*-yl)hydroquinone (2-Br-(diGSyl) HQ) to rats causes rapid and extensive karyorrhexis and karyolysis, and rapidly induces DNA single strand breaks in LLC-PK1 cells. In response to this damage the expression of several stress genes is induced, such as *gadd-153* and *c-myc*. Additional stress genes, such as nuclear-associated heat shock protein 70 (hsp70) and endoplasmic reticulum-associated glucose-regulated protein (grp78) respond to a variety of stimuli that cause abnormalities in protein conformation, and may serve as sensitive biomarkers of toxicity. We therefore investigated the cellular response to quinone-thioether-mediated protein alkylation. Both 2-Br-(diGSyl)HQ and 2-Br-6-(GSyl)-HQ caused a time- and concentration-dependent induction of *hsp-70;* maximum expression occurred at 200 μM. In contrast, no significant effect on *grp-78* mRNA was observed. The different effects on the expression of *hsp-70* and *grp-78* are consistent with *in vivo* data showing that 2-Br-(diGSyl)HQ has adverse effects on the nucleus. In particular, reactive metabolites of 2-Br-(diGSyl)HQ bind covalently to nuclear proteins, and downregulate *histone H2B* expression. We extended these studies and found that 2-Br-(diGSyl)HQ and 2-Br-6-(GSyl)HQ downregulate both linker (H1) and core (H2A, H2B, H3, and H4) histone gene expression. The common 3′ end on all "replication-dependent" histone mRNAs permits their coordinate regulation, in tune with DNA synthesis. However, the link between quinone-thioether mediated growth arrest (⇑ gadd-153) and histone down-regulation requires investigation. (ES 04662).

270 INCREASED MITOCHONDRIAL HSP60 RECOGNITION OF ADDUCTED AND FUNCTIONALLY INACTIVATED DEHYDROGENASES DURING CHEMICALLY-INDUCED RENAL PROXIMAL TUBULE CELL DEATH

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We have previously shown that the potent mammalian nephrotoxicant tetrafluoroethyl-L-cysteine (TFEC) covalently modifies a select group of mitochondrial proteins prior to cell death [1,2]. More recently we have identified these adducted proteins as subunits of mitochondrial dehydrogenase (DH) multienzyme complexes which are involved in key regulatory steps of cellular respiration [3]. Most importantly, the E2 and E3 subunits of α-ketoglutarate DH and E3 subunit of branched chain keto-acid DH complexes are adducted. We report here that the consequence of adduction is an inhibition of DH complex activity *in vivo* (*ca.* 80% for α-ketoglutarate DH) whereas the activity of an unadducted DH complex, pyruvate DH, was not altered. These studies indicate that covalent modification specifically alters cellular metabolism prior to cell death. Furthermore, immunoprecipitation studies have indicated an increased association of adducted DH subunits with mitochondrial HSP60 *in vivo*. Thus, adduction perturbs protein structural integrity sufficiently to allow for HSP60 recognition. These data also suggest that, in our mammalian system, HSP60 appears to act in the identification and maintenance of protein integrity as has been previously established for simpler eukaryotic systems [4].

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271 ACTIVATION OF HEAT SHOCK FACTOR (HSF) BY AN ALKYLATING AGENT IODOACETAMIDE (IDAM) INVOLVES GLUTATHIONE (GSH) DEPLETION AND PROTEIN THIOL OXIDATION

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The transcriptional activation of heat shock genes in response to stressful insults is mediated by the transcription factor HSF. Using IDAM as a model compound, we investigated the mechanisms for HSF activation by alkylating agents. Treatment of renal epithelial LLC-PK1 cells with IDAM resulted in cell death and induction of the 70-kD heat shock protein (hsp70) at the mRNA and protein levels. Electrophoretic mobility shift assays showed that IDAM induced the binding of HSF to the heat shock element present in the promoter of the hsp70 gene. Nuclear run-on analysis demonstrated that the hsp70 gene was transcriptionally activated. Moreover, inhibition of protein synthesis blocked HSF binding as well as hsp70 mRNA accumulation. A thiol reducing agent, dithiothreitol, prevented IDAM toxicity and abolished the binding of HSF, whereas antioxidants (N, N-diphenyl-p-phenylene diamine) or iron chelators (desferroxamine) inhibited cell death but not the binding activity of HSF. Furthermore, covalent binding and thiol assays indicated that IDAM induced alkylation of protein thiols and oxidation of protein thiols secondary to GSH depletion. In preliminary experiments we observed that IDAM caused the formation of protein aggregates. We propose that HSF activation by alkylating agents may depend on continued protein synthesis, protein thiol oxi-

dation and protein aggregation. Ongoing studies will determine the signals mediating IDAM-induced protein aggregation.

272 ANALYSIS OF THE STRESS RESPONSE IN UNSTIMULATED AND CON A-ACTIVATED HUMAN T LYMPHOCYTES DEPLETED OF GLUTATHIONE

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Glutathione depletion achieved by exposure of mitogen-activated human T lymphocytes to L-buthionine-(*S,R*)-sulfoximine, a specific inhibitor of γ-glutamylcysteine synthetase, leads to a marked inhibition of the proliferative response. Con A-activated T cells treated with buthionine sulfoximine (BSO) failed to exhibit the increase in glutathione content normally observed in activated T cells and were depleted of cellular glutathione over four days. Total RNA synthesis (as measured by [^{3}H]-uridine incorporation) was dramatically reduced in the glutathione-depleted cells being inhibited by 26, 61, and 82% on days 2, 3, and 4, respectively. We looked for evidence of a cellular stress response in the BSO-treated T cells. In glutathione-depleted unstimulated cells, Northern hybridization analysis of steady-state mRNA levels revealed an increase in mRNA levels of the two stress response genes, HSP70 and gadd 153. However there was no significant difference in the levels of transcripts seen in RNA from the Con A- versus the BSO/Con A-treated cells. Perhaps because Con A stimulation, itself, induces upregulation of a cellular antioxidant defense system. Interestingly, we observed an increase in the steady-state levels of mRNA encoding the catalytic subunit of γ-glutamylcysteine synthetase (GCS), the enzyme inhibited by BSO. We detected a 2.1-fold and 3.5-fold increase in the levels of GCS transcripts in BSO cells versus untreated cells at 16–24 and 48 h, respectively. We also detected a consistent 22% increase in the level of GCS transcripts in BSO/Con A versus Con A cells at 16–48 h. Thus, induction of GCS expression appears to be part of the cellular response to GSH depletion. The data suggest that a feedback mechanism may be involved in regulation of the mRNA expression of GCS. (Supported by the Colgate-Palmolive Postdoctoral Fellowship in *In Vitro* Toxicology and NIH grants ES05542 and ES03778).

273 TISSUE SPECIFIC INDUCTION OF HSP72 IN HEAT TREATED RAT EMBRYOS

B R Fisher [1], K M Brown [2]. [1] *Ctr Devices & Radiol Hlth, FDA, Rockville, MD;* [2] *Dept of Biological Sciences, The George Washington University, Washington, DC.* Sponsor: P Goering

We are studying alterations in protein synthesis as biomarkers of developmental toxicity using heat as a model teratogen. Previous studies have demonstrated that heat exposure on gestation day 10 resulted in disrupted somite development in rat embryos 24 hr after exposure and thoracic skeletal malformations in neonates examined 3 days postpartum. The production of abnormal somites was correlated with the location of affected skeletal elements. Heat also induces marked and rapid changes in genetic expression of heat shock proteins (hsps). The enhanced synthesis of hsps enables cells to mount a protective response against adverse stimuli, but little research has been conducted to address whether hsp synthesis represents an early event in the toxic process. To address this issue, immunohistochemical studies were performed on serial sections of heat-treated embryos to determine if hsps are induced in affected tissues. Embryos were cultured in vitro and exposed to 42–42.5°C for 15 min; conditions which disrupt somite segmentation. Results from this study demonstrated that hsp72 accumulates in neuroectodermal tissues of the embryo and not the somite mesoderm. These data argue against the direct involvement of hsp72 with abnormal somite segmentation. Conversely, tissues which do not express hsps may be susceptible to heat and other developmental toxicants.

274 TISSUE SPECIFIC STRESS RESPONSES OF GENETICALLY ENGINEERED MOUSE AND HUMAN CELL LINES TO CHEMICAL INSULTS

K Wun-Kim, G Christoph. *Haskell Laboratory, DuPont Company, Newark, DE.* Sponsor: S Loveless

All organisms, from bacteria to humans, respond to adverse environmental conditions by producing a family of stress proteins. These stress-induced proteins protect cells from damage that can be caused by the insults. We have transfected and stably transformed mouse and human cell lines derived from various organs and tissues with a reporter gene (fire-fly luciferase gene) controlled by the human hsp70 promoter. The stress response of cells was measured by assaying for luciferase activity in the presence of the substrate, luciferin. The long-term goal of this approach is to develop a general toxicity screen that would not only identify the relative toxicity of different agents, but would also identify the target organ most at risk. Although the work is at an early stage, we have demonstrated that tissue specific responses are associated with different test chemicals. The threshold of stress response as well as the concentration of chemical that can cause the maximum response differs among tissues. We have also developed a method that can measure the stress response in intact cells; this method allows study of the cellular response to stress at various time points within the same cells. For example, heat shock exposure (42.5 °C for 2 hours) causes luciferase activity of liver cells to increase as early as 1 hour post heat shock and reach maximum level at 3 hours post heat shock. Four hours after heat shock, the activity level decreases quite rapidly. The ability to easily track the time course and study the kinetics of the stress response may prove highly valuable in differentiating the toxic potential of novel compounds in a screening system.

275 DETECTING DNA DAMAGE IN MAMMALIAN CELLS USING THE CAT-TOX (L) ASSAY

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The purpose of the current study was to determine stress gene responses in the CAT-Tox (L) assay when human liver cells were exposed to DNA damaging agents. Different genotoxic agents were tested in the CAT-Tox (L) assay system. These included agents which were known to generate DNA adducts covalently (*e.g.* methyl methanesulfonate), to cross link intra-and interstrands of DNA (e.g. mitomycin C), to block replication by disrupting the stacking of bases in the DNA helix (*e.g.* ethidium bromide) or by inhibiting DNA synthesis (*e.g.* hydroxyurea). Genotoxins known to require biotransformation (*e.g.* 2-acetyl aminofluorene) were tested in the presence and absence of rat liver S9 extract. Fourteen stable human liver cells lines were generated using stress gene- or response element-chloramphenicol acetyl transferase (CAT) constructs. A broad range of promoters and response elements was chosen for the assay including promoters responsive to DNA damage, growth arrest, tumor promotion, mitogens, and changes in intracellular cyclic AMP. The activity of a given promoter is measured simply by the accumulation of CAT protein, quantified using a CAT ELISA detection system. Exposures to test chemicals ranged between 4 hours and 48 hours. Different classes of DNA damaging agents resulted in different stress gene profiles. Most genotoxins tested transcriptionally up-regulated several of the DNA damage responsive stress gene-CAT fusion constructs such as the GADD153 and GADD45, FOS, GRP78 promoters, and the p53 response element. In addition to these indicators of DNA damage, other cellular responses were measured in the CAT-Tox (L) assay. DNA damage-responsive constructs also responded when compounds such as benzo[a]pyrene and 2-acetyl aminofluorene were biotransformed to their toxic metabolites using rat liver S9 extract.

276 COMPUTER-ASSISTED INSTRUCTIONAL MATERIALS FOR PHARMACOLOGY AND TOXICOLOGY IN THE CLASSROOM AND LABORATORY

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Traditional approaches to teaching higher-order biological processes in living organisms are frequently inadequate, many biological events are dynamic, complex processes requiring coordinated integration of many independent steps in individual cells and tissues over time these processes. Utilizing recent advances in microcomputer technology and software, we have developed customized computer-assisted instructional materials for teaching principals of xenobiotic absorption, distribution, metabolism and excretion to undergraduate students in the life and health sciences. These instructional modules are utilized both as lecture materials and self-paced review materials. We have also developed a number of interactive laboratory exercises. One of these laboratory exercises simulates xenobiotic metabolism and excretion in the laboratory rat, replacing a previous exercise that utilized live animals. A *key* feature of these learning exercises is the capacity for the students to interact in real time with the simulations, alter model parameters and immediately observe and quantitate the result. Examples of other modules developed to date include models of acetylcholinesterase inhibition by insecticides, protein structure and function, molecular orbital models, and simulations of chemical reactions.

277 POISONOUS PLANT BIBLIOGRAPHIC FILE IN INTERNET

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The Poisonous Plant Bibliographic File, which contains references to over 32,000 publications, is one of a set of working files of scientific information

about the animal and human toxicology of vascular plants and herbal products of the world (PLANTOX). Citations start in the 1800s and cover both primary and secondary literature. Emphasis is on naturally occurring and experimental intoxications, but some chemical and botanical aspects are also covered. This initial file will be supplemented in the future with other files of plant names and synonyms, plant uses, geographic distributions, animal species affected, signs, and lesions. The information is in the public domain and has no official status. The file will be updated periodically, but no claim is made that the information for this large area of toxicology is totally comprehensive or error-free. Access is either by Anonymous FTP at VAX8.CFSAN.FDA.GOV and get ANON.FILES.PUBLIC.PLANT-TOX PLANTOX.REF or through World Wide Web. For further information provide your Internet address to: D. Jesse Wagstaff; Ph: 202-205-8705; DJW@FDACFSSW.DHHS.GOV.

278 RISKY BUSINESS: LIVING IN A CHEMICAL WORLD

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Public understanding of environmental health issues has tremendous social and economic implications, and general education in this area must begin with our children. The purpose of this project is to develop curricular materials that incorporate basic principles of environmental health sciences, especially toxicology, into existing high school science curricula (*e.g.*, Biology, Chemistry, Health, Environmental Sciences). Specifically, we are developing five interactive, computer-based, multimedia teaching units that can be easily inserted into high school science lessons. These units teach basic biology and chemistry, but also incorporate principles of environmental health and toxicology, utilizing examples from toxicology to help make science relevant to the student. The materials integrate animation and graphics with interactive text, and allow the student to explore how the environment interacts with basic biology in ways that affect our health. The first unit under development, "Cell Biology and the Environment", explores basic cell biology (structure & function of organelles; cell division, including meiosis & mitosis; structure & function of DNA, chromosomes, genes, proteins, enzymes), and interfaces these biology concepts with toxicological concepts. Later units will connect this basic unit with "The Environment and Cancer", "The Environment and Birth Defects", and "The Environment and Organ System Function/Dysfunction". The fifth unit, "Risky Business: Risk/Alternatives Assessment", will integrate the concepts of biology, chemistry, and environmental health developed in previous units to show how daily decisions influence our own personal risks. (Supported by NIH grant R25 ES06938).

279 COMPUTER-BASED TRAINING IN USE OF NLM'S TOXICOLOGY BIBLIOGRAPHIC DATABASES

J C Goshorn, M Perkins. *National Library of Medicine, Bethesda, MD.* Sponsor: I. Yager

The Toxicology and Environmental Health Information Program (TEHIP), established in 1967 as the Toxicology Information Program at the National Library of Medicine in response to recommendations by the President's Science Advisory Committee, has as its objectives the creation of automated toxicology data banks and the dissemination of toxicology information and data services. TEHIP provides online database services, responds to queries, sponsors publications, and supports the information requirements of other Federal agencies. This demonstration will focus on computer-based training developed to teach how to search some of the toxicology databases that TEHIP provides on MEDLARS, NLM's online search system for biomedical literature. These programs run on IBM or compatible machines, and are provided in DOS format as well as a Windows format, complete with audio. Searchers can obtain the programs from NTIS or download them from NLM's gopher server on the Internet. Three programs will be demonstrated: a TEHIP Files Demo Disk, intended to introduce potential database users to all the NLM toxicology-related files; ELHILL Learn, designed to teach the use of the MEDLARS search and retrieval system; and TOXLEARN, developed to cover the basic aspects of searching the TOXLINE family of bibliographic databases covering a wide range of toxicology topics. While these packages are designed primarily for new users, their menu-driven structure permits their use as quick reference tools to reinforce previously learned search techniques.

280 RETRIEVAL DATA BASE ON SINGLE EXPOSURE CARCINOGENS

E J Calabrese. *Environmental Health Sciences Program, School of Public Health, University of Massachusetts, Amherst, MA.* Sponsor: J Yager

Approximately 5,000 single exposure carcinogen studies have been identified and are being incorporated into a computer assisted retrieval data base. The studies indicate toxicological evidence for approximately 350 chemicals within 40 chemical classes that cause cancer in animal models with a single dose. The presentation will provide a demonstration of how the single exposure carcinogen data base is organized and may be searched. Applications of the data base for use in cancer risk assessment for issues dealing with less than lifetime exposures to carcinogens, and cumulative dose versus dose rate will be discussed.

280a USE OF COMPUTER-BASED RESOURCES AS CLASSROOM SUPPLEMENTS FOR TEACHING TOXICOLOGY

D A Eastmond. *Environmental Toxicology Graduate Program, University of California, Riverside, CA*

In recent years, a number of software and computer-based training programs have been developed which can be used to complement classroom instruction in toxicology and risk assessment. This presentation will overview my experiences over the past four years using several of these software programs in teaching introductory toxicology classes and a risk assessment class. The primary focus will be on the use of the computer-based training program entitled, "Fundamental Toxicology and Risk Assessment" that was developed by Dr. Ann Anderson and Michael Dailey through a grant awarded to the National Environmental Health Association by the US Environmental Protection Agency. This software was designed to provide workers in the field of environmental health with a basic background of the principles underlying toxicology and risk assessment. Originally developed as a stand-alone course, the program can be easily utilized as a complement to university-level classroom instruction. The program consists of 9 separate lessons which briefly introduce the field of toxicology, describe the biological fate of toxicants in the body, discuss pathological responses to toxic substances and then present the basic steps of risk assessment. The program is simple to use, relies upon self-paced learning, employs effective graphics and is moderately interactive, as the student is periodically faced with multiple-choice quizzes and tests. Based on my experiences, computer-based training can be a valuable supplement to classroom instruction in teaching toxicology and risk assessment.

280b USE OF RISK*ASSISTANT PERSONAL COMPUTER SOFTWARE TO TEACH TOXICOLOGICAL RISK ASSESSMENT AND EXPOSURE ASSESSMENT

W R Muir, J S Young, C M Benes. *The Hampshire Research Institute, Alexandria, VA*

The RISK*ASSISTANT software system was developed to help environmental professionals conduct and/or review site-specific human health risk assessments for exposures to toxic chemicals. Key features of the system are the provision of standardized exposure scenarios with default parameters, which the user can modify to fit specific exposure conditions for a given context. The resultant estimates of exposure and dose can then be automatically combined with estimates of toxic hazards promulgated by the US Environmental Protection Agency (Slope Factors, Unit Risks, Reference Doses and Reference Concentrations) to generate estimates of risk.Because it was intended to address a user community with diverse educational backgrounds, the software not only documents all equations and default data, but also embodies detailed explanations of the risk assessment process. Accordingly, in addition to hundreds of users in the professional community, the software system is explicitly used for instructional purposes at several universities that have educational licenses, and is used in risk assessment training conducted by EPA in "Eastern Bloc" countries. A new version of the software for the Windows™ operating environment, which significantly enhances the instructional potential of the software, will be completed by March.This presentation will describe the application of the software for educational and training purposes.

280c EDUCATIONAL SOFTWARE FOR ENVIRONMENTAL DECISION MAKING

A Lane-Cline, A R Gotsch, B V Parks. *EOHSI, Piscataway, NJ.* Sponsor: I. Yager

Trash Encounter, an innovative computer based instructional software program, designed to provide students with the challenge of operating their own county solid waste plan in an interactive and market driven environment that

simulates contemporary solid waste municipal issues will be demonstrated.The new software is one component of a high school curriculum designed to provide students with the skills for problem solving and decision making in the context of solid waste. *Trash Encounter* demonstrates how interdisciplinary factors must be integrated for the development of an effective solid waste management strategy, including factors such as the rules we are required to follow, the budget and other resources available to us, and the cast of characters with which we must deal.The curriculum was developed by the Public Education Risk Communication Division at the Environmental and Occupational Health Sciences Institute in New Jersey through their National Environmental Health Education Campaign. The campaign's goal is to increase the public's awareness and literacy in environmental issues.

280d INTERACTIVE MULTIMEDIA COMPUTER PROGRAMS AS ALTERNATIVES TO TRADITIONAL EXPERIMENTS IN EDUCATION

C E Branch. *Department of Physiology and Pharmacology, Auburn University, AL.* Sponsor: J. Yager

The education of health profession students in physiology, pharmacology, surgery, and toxicology traditionally has used live animal experiments. Due to constraints of time, finances, and other pressures, these experiments have been curtailed or eliminated at many schools. Many educators feel that this has deprived students of valuable hands-on experiments. The purpose of this project has been to provide supplemental experience, as well as alternative experiences for those schools which have elected to eliminate such experiments. The work in this laboratory has focused on the development of programs using videodisc and other media to present realistic demonstrations of traditional experiments in cardiovascular physiology, such as cardiac catheterization, autonomic control, fibrillation, heart sounds, and the cardiac cycle. In a separate project, CONVINCE, a consortium of all veterinary schools in North America, has supported multimedia developments projects at 13 different schools. These projects provide similar alternative experiences in other areas of surgery, anatomy, physiology, and pharmacology. The cardiovascular physiology programs developed in this lab, as well as samples from some of the CONVINCE projects, will be available for hands-on use.

280e EXTOXNET — A UNIQUE APPROACH TO ENVIRONMENTAL TOXICOLOGY OUTREACH

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EXTOXNET is an *ad hoc* consortium of university toxicologists, environmental chemists and scientists from other disciplines who are interested in promoting public education and university outreach programs related to toxicants in the environment. This consortium was formed in 1983 and now has been institutionalized with funding from the the USDA National Pesticide Impact Assessment Program. EXTOXNET now consists of four partners: the National Coordination Center at University of California-Davis, the Western Regional Access Center at Oregon State University, the Eastern Regional Access Center at Cornell University and the Midwest Regional Access Center at Michigan State University. EXTOXNET has developed a variety of written materials including almost 150 pesticide profiles, 25 toxicology information briefs, a number of issue documents and other written resources and databases. These were originally available only in hard copy form but can now also be accessed by computer via ALMANAC, GOPHER and MOSAIC systems administered at the Western Regional Center. In addition to providing written information, EXTOXNET members also respond to phone, mail and computer inquiries. EXTOXNET is set up to provide a coherent, rapid, national response to media reports, such as recent television programs about pesticides in foods. Expansion of EXTOXNET to include regional centers in other sections of the country is planned.

281 HAPLOTYPE DETERMINATION OF HUMAN MICROSOMAL EPOXIDE HYDROLASE PROTEIN POLYMORPHISMS

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Microsomal epoxide hydrolase (mEH) is a pivotal enzyme in the metabolism of a broad range of xenobiotic substrates. Interindividual variation in mEH enzymatic activity has been observed in human populations, and this may be a risk factor for chemically-induced toxicities. Using *in vitro* expression approaches, we have demonstrated that mEH enzymatic activity profiles are influenced by polymorphisms at amino acid positions 113 (Tyr or His in exon 3) or 139 (His or Arg in exon 4). In order to determine the inherited combinations of amino acid variants we have developed a polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP)-based haplotype assay. A bank of approximately 50 human livers was characterized for mEH enzyme levels, and total RNA was purified from each sample and reverse-transcribed. PCR reactions were performed on these cDNA templates using mEH-specific amplification primers that encompass the variable amino acids in exons 3 and 4. The PCR products were digested with *RsaI* (which is diagnostic for the exon 4 polymorphism) and size-separated in duplicate on agarose gels. Following DNA transfer to nylon membranes, blots were probed with exon 3 allele-specific oligomers. Resulting restriction and hybridization patterns enabled determination of amino acid linkage. Each of the 10 expected diploid haplotypes was detected in the liver bank samples. Of the 4 possible haplotypes, Tyr/His (exon 3/4) was most frequently observed (55%), followed by Tyr/Arg (19%), His/His (16%), and His/Arg (10%).

Supported by NIH grant ES-04978.

282 CLONING AND EXPRESSION OF HYDROLASE S, A SERUM CARBOXYLESTERASE SECRETED BY RAT LIVER

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Carboxylesterases play an important role in the metabolism of drugs and other xenobiotics, and are the major determinant of species differences in susceptibility to the toxic effects of organophosphorus compounds. Four carboxylesterases, designated hydrolases A, B, C and S, have been cloned from rat liver, three of which are microsomal enzymes. The 4th enzyme, hydrolase S, is hypothesized to be secreted from liver into serum. To test this hypothesis, cDNA for hydrolase S was cloned into a pUC18 plasmid and subcloned into a pMAL expression vector at a site downstream from the *mal* E gene, which encodes maltose binding protein (MBP). A fusion protein (rat hydrolase S linked to MBP) was produced in *E. coli*, and purified by affinity chromatography on an amylose column (which binds MBP). The purified fusion protein was cleaved with Factor Xa. Free MBP was removed by affinity chromatography, and an antibody against purified hydrolase S was raised in rabbits. This antibody cross-reacted strongly with purified hydrolases A and B (Mr 57–59 kDa) due to the structural relatedness (≈70%) among these carboxylesterases. The antibody recognized a 71-kDa protein (hydrolase S) in rat serum, as well as precursor form (67-kDa) in rat liver microsomes. In addition to rat serum, hydrolase S was also detected in the extracellular medium of cultured rat hepatocytes. Immunoprecipitation of hydrolase S from rat serum decreased esterase activity toward 1-naphthylacetate and *para*-nitrophenylacetate. The results of this study support the hypothesis that hydrolase S is a rat carboxylesterase secreted from liver into serum. Supported by NIH grant ES04996.

283 MOLECULAR CLONING AND CHARACTERIZATION OF THE RABBIT FMO1 GENE

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The flavin containing monooxygenases (FMO) are a family of microsomal xenobiotic-metabolizing enzymes present in several tissues. They participate in the oxidation of sulfur, nitrogen, and phosphorous atoms within numerous compounds. Studies by several groups including our own have demonstrated differential expression of FMO isoforms in various tissues. The FMO also are under hormonal and dietary control. We have cloned and partially characterized the rabbit FMO1 gene as a first approach to understanding the mechanisms controlling its expression. An FMO1 cDNA probe was used to screen a rabbit λEMBL3 library. Five overlapping clones were obtained spanning a 44 kbp region. These clones were restriction endonculease mapped and fragments subcloned. DNA sequence and Southern blot analyses revealed the isolated clones contain 14 kbp of 5′ flanking information and approximately 30 kbp of the structural gene but do not include the 3′ end. Primer extension analysis demonstrated a major transcription start site defining the beginning of a non-coding 73 bp exon 1. The ATG start codon was located 6 bp downstream of the beginning of exon 2. The latter was separated from exon 1 by a 4.5 kbp intron. A minor transcription start site was also demonstrated by primer extension. 5′ RACE revealed this site defined the beginning of a 68 bp exon 0 followed by a 132 bp intron. Processing of this transcript results in a 25 bp 5′ extension on exon 1. Neither a TATA-box or GC islands were found in either promotor region. Searching 2 kbp of upstream sequence did reveal regions exhibiting identity (65%–85%) with known promotor sequences. The major transcription start site also exhibits properties of known INR elements.

284 MOLECULAR BIOLOGY OF RAT LIVER CARBOXYLESTERASES

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We recently purified two carboxylesterases, hydrolases A and B, from rat liver microsomes. High levels of hydrolase A are present in rat liver and testis, whereas high levels of hydrolase B are present in rat liver and kidney, as determined by Western immunoblotting and enzyme kinetics. To establish the molecular basis for the multiplicity of carboxylesterases, cDNA libraries from rat liver, kidney and testis were constructed or purchased, and screened with antibodies against hydrolase A or B. To obtain full length cDNAs, the same libraries were screened by two step-polymerase chain reaction (PCR) with primers based on the sequence of the cDNAs isolated by immunoscreening. Four full-length cDNAs encoding distinct rat carboxylesterases were isolated; they are designated hydrolases A, B, C and S. The sequences of hydrolases A, B, C and S are ≈70% identical at both the amino acid and nucleotide level, with the exception of hydrolases B and C, which are ≈95% identical. Based on alignment of amino acid sequences, and based on the mechanism of catalysis of serine proteases, the catalytic triad in hydrolases A, B, C and S is apparently composed of the nucleophile, Ser_{203}, the basic amino acid, His_{448}, and the acidic amino acid, Asp_{97} or Glu_{228}. Northern blotting of mRNA confirmed that hydrolase A is expressed in liver and testis, where hydrolase B is expressed in liver and kidney. In contrast, hydrolase C and S are apparently expressed only in liver. In contrast to hydrolases A-C, which are present in liver microsomes, hydrolase S lacks the HXEL consensus sequence for retaining proteins in the endoplasmic reticulum, and this esterase contains 5 glycosylation sites (3 more than in hydrolases A-C). These differences can be rationalized by our recent finding that hydrolase S is a serum carboxylesterase synthesized in the liver. Hydrolases A, B, C and S have different substrate specificities, but the toxicological, pharmacological and physiological functions of these four carboxylesterases remain to be determined. Supported by NIH grant ES04496.

285 THE USE OF SITE-DIRECTED MUTAGENESIS TO INVESTIGATE ORGANOPHOSPHORUS HYDROLASE ACTIVITY

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Organophosphorus Hydrolase (OPH) is encoded by the *opd* gene of *Pseudomonas dimunita* MG and by an identical gene isolated from *Flavobacterium sp.* OPH is a metalloenzyme with one to two divalent metal cations per enzyme molecule in its native form. It has been shown to be capable of the cleavage of P-O, P-S, P-F, and P-CN bonds in a wide variety of OP neurotoxins with varying substrate specificities. Histidinyl and cysteinyl residues potentially involved in maintaining active site geometry, binding metal ions, and participating in the catalytic mechanism have been investigated via regional random and site-directed mutagenesis and chemical modification studies. Site-directed mutagenesis experiments have led to the identification of some enzymes with altered metal binding stoichiometry, altered catalytic turnover rates (k_{cat}), and/or altered substrate specificity constants (k_{cat}/K_M). Several of these substitutions have resulted in enhanced capability of the enzyme to directly detoxify the chemical warfare agents soman and VX.

286 BIOLOGICAL CONSEQUENCES OF SITE SPECIFIC BENZ[a]ANTHRACENE-DNA ADDUCTS

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The biological effects of site specific DNA adducts which would result from the interaction of benz[a]anthracene metabolites at the N6 position of adenine have been examined. Using a prokaryotic *in vivo* replication system, we have shown that both stereoisomers of the non-bay region anti-*trans* benz[a]anthracene adducts are essentially non-mutagenic.

These same adducts were examined in an *in vitro* primer extension assay using various polymerases. A distinct pattern of extension was observed which was dependent on the stereochemistry about the adduct forming bond.

A differential pattern of exonucleolytic digestion was also observed depending on the absolute stereochemistry of the adduct. Studies from other groups have found that the spatial orientation of the pyrenyl moiety determines the pattern of exonucleolytic digestion. In light of these findings, predictions are made about the structure of the adducts studied.

287 RETROVIRAL-MEDIATED TRANSFER AND EXPRESSION OF HUMAN MGMT GENE IN MURINE HEMATOPOIETIC STEM CELLS

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O^6-Methylguanine-DNA methyltransferase (MGMT) is an important DNA repair protein that plays a key role in cancer chemotherapy by alkylating agents such as carmustine (BCNU) and dacarbazine (DTIC). BCNU and DTIC are the most effective chemotherapeutic agents against malignant tumors. However, therapy by BCNU and DTIC is compromised by dose-limiting hematological toxicity that is due mainly to the very low MGMT activity in bone marrow cells. A clinical strategy in which MGMT is introduced into hematopoietic precursors by gene therapy methods may increase the dose of BCNU and DTIC and therefore achieve a better therapeutic response in malignant tumors. In this study, we have constructed a retroviral vector containing a human MGMT gene. Packaging cell lines have been generated to produce high-titer retrovirus. Transduction of this MGMT retroviral vector into murine multipotent hematopoietic stem cells, FDCP-1, resulted in the expression of high level of MGMT activity. In comparison with control cells that were transduced with the parent vector, the MGMT-expressing cells were considerably more resistant to the cytotoxic effects of the methylating agents *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) and *N*-methyl-*N*-nitrosourea (MNU), as well as the chloroethylating agent chloroethylnitrosourea (CNU). The protection provided by MGMT could be eliminated by the MGMT inactivator, O^6-benzylguanine (O^6-BG). Thus, the principal lethal lesion produced by alkylating agents in murine hematopoietic stem cells and the MGMT deficiency in these cells can be complemented by retrovirus-mediated gene transfection.

288 *IN VITRO* ANALYSIS OF MECHANISMS OF MUTAGENESIS BY BENZO[a]PYRENE DIOLEPOXIDE

L Cheng, K Vasunia, M Carty, K Dixon. *Department of Environmental Health, University of Cincinnati, Cincinnati, OH.* Sponsor: E O'Flaherty

Mutations in proto-oncogenes or tumor suppressor genes can eventually lead to tumor formation. To investigate the molecular mechanisms of mutation fixation, *in vitro* DNA replication systems have been used in our laboratory to study UV mutagenesis. Here we describe the use of this same *in vitro* system to study mutagenesis resulting from a chemical that forms bulky adducts with DNA, anti-benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide (BPDE). BPDE is generally considered to be the most important mutagenic and carcinogenic metabolite of benzo[a]pyrene (B[a]P), which is a member of the polycyclic aromatic hydrocarbon family. A simian virus 40-based shuttle vector plasmid, pZ189, was treated with BPDE and then replicated *in vitro* using hypotonic extracts of human HeLa cells; the replication efficiency was monitored by the incorporation of radioactive nucleotides. The plasmids were then treated with DpnI to inactivate unreplicated plasmids and transfected into *E. coli.* MBM7070 to screen for mutants. The mutants were isolated and the mutated SupF genes were sequenced to determine the mutational spectrum of BPDE. Our results show that BPDE-damaged plasmids undergo replication in the *in vitro* system and that the mutation frequency increases in a BPDE dose-dependent pattern. This study further validates the *in vitro* DNA replication system by demonstrating the mutagenicity of bulky adducts of BPDE in addition to UV-photoproducts. This work was supported in part by NIEHS grants: ES05400 and ES04908.

289 MUTANT FREQUENCY AND MUTATION SPECTRUM OF LACI FROM THE LIVER OF TRANSGENIC MICE EXPOSED TO BENZO(a)PYRENE

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The Big Blue® transgenic mouse mutation system permits the study of the role of pharmacodynamics on mutant frequency following *in vivo* xenobiotic exposure. We have studied the effects of cellular proliferation on the mutant frequency (MF) of *lacI* in the liver of male Big Blue® mice following 40% partial hepatectomy. Mice (3) were injected with 40 mg/kg benzo(a)pyrene (B(a)P) in corn oil or corn oil alone on three consecutive days followed by hepatectomy on the fourth day. Three days later the animals were sacrificed and the MF in the liver was compared to the MF observed in the liver of the same mouse recovered at the time of hepatectomy. The spontaneous MF in vehicle-treated mice was not significantly different at the time of hepatectomy (4.4×10^{-5})

compared to the MF at the time of sacrifice (3.0×10^{-5}). Although the MF (1.2×10^{-4}) was significantly higher in B(a)P-treated animals than in the control animals, there was no significant difference between the MF in the B(a)P-treated animals at the time of hepatectomy (1.2×10^{-4}) and sacrifice (1.1×10^{-4}). Interestingly, both control and treated samples gave rise to sectored mutant plaques. Sectored plaques result from mutations arising in *lacI* during phage growth in *E. coli.* Since only some of the phage progeny are mutant, only part of the plaque is blue. The sectored spontaneous MF at sacrifice was 1.4×10^{-5} and the sectored MF from B(a)P-treated mice was 6.7×10^{-5}. These data suggest that bulky adducts may remain in mouse genomic DNA and be packaged and ultimately fixed as mutations in *E. coli.* The mutation spectra of B(a)P in the *lacI* mutants recovered at hepatectomy and sacrifice resembles that which has been reported for B(a)P in other systems. Thus, transgenic mouse mutagenesis systems can be used to enhance our understanding of the mechanisms of mutation *in vivo.*

290 *IN VIVO* MUTANT FREQUENCY OF LACI IN LIVER DNA OF TRANSGENIC MICE FOLLOWING DIETARY EXPOSURE TO THE CARCINOGEN-NONCARCINOGEN PAIR 2,4- AND 2,6-DIAMINOTOLUENE

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The present investigation represents a continuation of an effort to explain sources of nonconcordance in *in vitro* mutagenicity and chronic studies of carcinogenesis. The carcinogen-noncarcinogen pair 2,4- and 2,6-diaminotoluene (DAT) are equally mutagenic in the Ames/ *Salmonella* assay in the presence of S9. They are equally absorbed, metabolized and excreted in rodents. In NTP rodent bioassays, 2,4-DAT is a potent hepatocarcinogen, whereas 2,6-DAT is not tumorigenic either in rats or mice even when administered at a higher dose. Our previous work demonstrated that 2,4-DAT induces cell proliferation in liver whereas 2,6-DAT does not. The Big Blue® mouse allows for recovery of mutations induced *in vivo* at a stable chromosomally integrated *lacI* gene. Mice were fed 2,4- or 2,6-DAT in the diet at 0 or 1000 ppm. Groups of 5 mice were sacrificed at 30 and 90 days of continuous dietary exposure. A positive control group of mice was treated with five daily ip injections of dimethylnitrosamine (DMN) at 6 mg/kg/day and sacrificed 15 days later. Results showed that mutant frequency was essentially identical for the control and DAT treated groups at 30 days. However, the mutant frequency for animals treated with the carcinogen 2,4-DAT ($12.10 \pm 1.43 \times 10^{-5}$) was significantly higher ($p < 0.01$) than age-matched control animals ($5.65 \pm 2.93 \times 10^{-5}$) or 2,6-DAT treated animals ($5.56 \pm 2.35 \times 10^{-5}$) at 90 days. DMN increased the mutant frequency to 38.4 $\pm 4.8 \times 10^{-5}$ ($p < 0.001$). Results indicate that *in vivo* mutagenesis is induced only by the carcinogenic isomer of DAT and that assay duration is a significant variable for detecting mutant frequency. It is speculated that mutations induced *in vivo* in proliferating cells may account for the carcinogenicity of 2,4-DAT.

291 A NOVEL MODEL FOR DRUG RESISTANCE DURING VIRALLY INDUCED HEPATOCARCINOGENESIS

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Hepatocellular carcinomas (HCC) are intrinsically resistant to cancer chemotherapy due to multifactorial cellular events. Woodchucks chronically infected with woodchuck hepatitis virus (WHV) are being developed as a drug resistance model for hepatitis B virus (HBV) since both are DNA viruses linked to the development of HCC. Coinduction of multidrug resistance gene(s) (MDR) and glutathione-S-transferase pi (GST-P) have been observed in chemically and retrovirally induced HCC. In addition, HBV transgenic mice and human HCC overexpress MDR. The purpose of this study was to determine if WHV induced HCC also overexpress these resistance markers. An increase in mRNA for MDR was observed in HCC based on RT-PCR, though there was no indication of gene amplification by slot blot analysis of genomic DNA which suggested transcriptional activation or message stabilization. The drug binding properties of the woodchuck MDR gene product were confirmed by competitive photoaffinity labeling experiments using 3H azidopine and various anticancer drugs. Additionally, GST-P was overexpressed in HCC which may further aid in intracellular binding and translocation. In contrast to GST-P, GST alpha and mu were decreased in HCC. The results demonstrated that the drug resistance markers MDR and GST-P were coinduced in HCC during chronic WHV infection. The woodchuck is singularly useful as a model for HBV because aside from viral and pathological similarities between WHV and HBV, the woodchuck is the natural host for WHV which eleviates host/agent extrapolations.

292 CELL LINES FROM A MOUSE SQUAMOUS CELL CARCINOMA WITH DIFFERENT MUTANT c-Ha-*ras* GENE DOSAGES CHARACTERISTIC OF DIFFERENT STAGES OF CARCINOGENESIS

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Increasing gene dosage of mutated c-Ha-*ras* occurs with progression of multistage chemical carcinogenesis in mouse skin. Papillomas promoted from 7,12-dimethylbenz[a]anthracene (DMBA)-initiated skin have an activating A^{182}->T mutation of one allele of c-Ha-*ras*, while squamous and spindle cell carcinomas often have excess mutated and undetectable normal c-Ha-*ras* alleles. However, the difficulty in attributing sequential oncogenic changes to multistage progression of a tumor stem cell using intact tumor with heterogeneous cell types has led us to use multiple cell lines isolated from foci of the same cultured squamous cell carcinoma (SCC). One DMBA-initiated SCC with a normal and mutated (A^{182}->T) c-Ha-*ras* from a mouse co-promoted with mirex plus 12-O-tetradecanoylphorbol-13-acetate (TPA) yielded two types of cell lines, both reactive with an anti-pan cytokeratin, with different morphologies in low Ca^{2+} (0.05 mM) medium. MT2.5s were polygonal cells and MT2.6s were fusiform cells. Early passage cells had different allelotypes of c-Ha-*ras*; MT2.5s exhibited the heterozygous normal plus mutant (papilloma-like) allelotype, while MT2.6s exhibited the mutant only (carcinoma-like) allelotype. Also, MT2.5s growth arrested, but reversibly, in 1.2 mM Ca^{2+}, while MT2.6s grew better in 1.2 mM Ca^{2+}. These differential c-Ha-*ras* allelotypes and Ca^{2+} responses indicate that MT2.5s are a less advanced stage of progression than MT2.6s. Hence, this pair of cell lines will be a useful cellular model for study of c-Ha-*ras* gene dosage during squamous cell carcinogenesis. (PHS grant ES00044).

293 DEVELOPMENT AND CHARACTERIZATION OF VARIANT T47D HUMAN BREAST CANCER CELLS WITH ALTERED ESTROGEN AND ANTIESTROGEN RESPONSIVENESS

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New breast cancer cell models were derived from the genetically unstable T47D human breast cancer cell line by culturing both low and high passage cells in estrogen-deficient growth conditions for several years. The emerging cell models, designated L(hE-) and H(hE-) cells, exhibited altered growth patterns, accelerated growth rates, increased DNA content and variant estrogen responsiveness. Both models expressed elevated estrogen receptor (ER) binding (8–14x wild type) although ER mRNA levels and binding to a synthetic estrogen responsive element (ERE) oligonucleotide was equivalent to or lower than that of wild type cells (L). The H(hE-) cells remained responsive to 17β-estradiol (E_2) stimulation while the L(hE-) cells were inhibited by E_2. The latter inhibition was accompanied by a significant reduction of S phase cells and distinct morphological changes. The differential responsiveness of the variant cell models to a battery of steroidal and non-steroidal antiestrogens was evaluated with a cell proliferation assay. The reversibility of the observed changes was studied by reverting growth conditions to that of wild type cells. Possible mechanisms underlying altered hormone responsiveness will be discussed. These new breast cancer cells are useful models for the study of ER-positive breast cancer tumors that progress from a state of hormone responsiveness to a state of hormonal autonomy.

294 THE EFFECT OF HORMONES ON SULFOTRANSFERASE GENE EXPRESSION IN PRIMARY RAT HEPATOCYTE CULTURES

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Because of their demonstrated importance in sulfotransferase regulation, the effects of several hormones on hydroxysteroid sulfotransferase-a (HST-a) and aryl sutfotransferase IV (ASTIV) mRNA expression were investigated in primary monolayer cultures of rat hepatocytes. Hepatocytes were isolated from adult male Sprague Dawley rats and cultured on Matrigel-coated dishes in Williams' Medium E containing 0.25 U/ml insulin, 100 U/ml penicilin and 100 μg/ml streptomycin. Hepatocyte cultures were treated for 72 hours with dexamethasone (DEX), dihydrotestosterone (DHT), 17β-estradiol (E2), or human growth hormone (hGH), and then harvested for isolation of total RNA. HST-a and ASTIV mRNA expression each increased in a dose dependent manner in response to DEX treatment. However, whereas ASTIV mRNA levels were maximal at 10^{-8} M DEX, HST-a mRNA levels continued to increase through 10^{-5} M DEX. In cultures incubated with medium containing 10^{-7}M DEX, ASTIV and HST-a mRNA levels were further increased by ≈2-

and ≈6-fold, respectively, following treatment with 10^{-5} M E2. Neither DHT nor hGH treatment altered HST-a or ASTIV mRNA expression substantially. These results provide evidence that the rat hepatic HST-a and ASTIV genes are regulated by gonadal and extragondal hormones through a direct action on the hepatocyte. Supported by NIEHS Grant ES05823 (MRM) and NIH Grant HL50710 (TAK).

295 SULFOTRANSFERASE GENE EXPRESSION IN RAT KIDNEY

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Rat hepatic hydroxysteroid sulfotransferase-a (HST-a) and aryl sulfotransferase IV (ASTIV) gene expression has been shown to be modulated by aging, hormones and streptozotocin (STZ) induced diabetes. However, HST-a and ASTIV gene expression in critical extrahepatic toxicant target tissues is not yet well characterized. Hence, the effects of aging, gender, dexamethasone (DEX) treatment, and STZ induced diabetes on rat renal HST-a and ASTIV mRNA expression were investigated. Poly(A)$^+$RNA was isolated from the livers of prepubertal and mature male and female Sprague Dawley rats, and HST-a and ASTIV mRNA levels relative to β-actin mRNA levels were analyzed on Northern blot. Unlike in rat liver, rat renal HST-a and ASTIV mRNA expression did not alter substantially in response to aging and gender. In rat liver, and in primary cultured rat hepatocytes, HST-a and ASTIV mRNA expression has been shown to increase following DEX treatment. In rat kidney, mature male rats treated with DEX (100 mg/kg ip) demonstrated an ≈4-fold increase in renal ASTIV mRNA levels relative to vehicle treated controls. In contrast, rat renal HST-a mRNA levels did not increase substantially in response to DEX treatment. Mature female rats with STZ induced diabetes demonstrated a suppression of rat renal HST-a mRNA expression, and increased levels of ASTIV mRNA. These results suggest that DEX hormone treatment and insulin dependent diabetes can alter rat renal HST-a and ASTIV gene expression, and that HST-a and ASTIV may be differentially regulated in extrahepatic relative to hepatic tissues. Supported by NIEHS Grant ES05823.

296 CHROMIUM(VI)-INDUCED DNA DAMAGE AFFECTS HORMONE- AND METAL-INDUCIBLE GENE EXPRESSION IN CHROMIUM(VI)-TREATED CULTURED RAT FAO CELLS

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The effect of DNA damage induced by the carcinogen chromium(VI) [Cr(VI)] on the expression of constitutive and inducible genes was examined in rat hepatoma Fao cells. Cr(VI) had no effect on steady-state mRNA levels of the constitutively expressed genes for metallothionein (*mt I* and *mt II*), β-actin or α-tubulin. Also, Cr(VI) treatment for 2 h had no effect on basal and dexamethasone (Dex)-inducible expression of tyrosine aminotransferase (*tat*) and phosphoenolpyruvate carboxykinase (*pepck*) which correlates with the absence of DNA-protein crosslinks (DPC) and single-strand breaks (SSB), although significant levels of chromium were bound to DNA at this time. In contrast, Cr(VI) treatment for 4 h had a significant inhibitory effect on the basal and drug-inducible expression of *tat* and *pepck*. Cr(VI) completely eliminated the basal expression of *tat* and *pepck*, and suppressed the induction of *tat* and *pepck* expression by Dex by 88% and 100%, respectively, and the time of this effect correlated with Cr-DNA adducts and DPC, and no detectable SSB. The comparative induction of *mt I* and *mt II* by cadmium(II) and Dex was determined in Cr(VI)-treated Fao cells. Cr(VI) suppressed the cadmium(II)-induced expression of *mt I* and *mt II* over control or Cr(VI)-treated constitutive levels by 70% and 47%, respectively, and Dex-induced expression by 97% and 79%, respectively, which correlated with the presence of Cr-DNA adducts and DPC. These results suggest that Cr(VI)-induced DNA damage affects both homone- and metal-inducible gene expression through a general pathway.

297 DIFFERENCES IN DNA METHYLATION INDUCED BY A CHOLINE-DEVOID, METHIONINE-DEFICIENT DIET OR BY PHENOBARBITAL CORRELATE WITH LIVER TUMOR SUSCEPTIBILITY IN MICE

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We are testing the hypothesis that hypomethylation of DNA (a decreased 5-methylcytosine (5MeC) content) is an epigenetic, nongenotoxic mechanism that can facilitate the abnormal oncogene expression involved in tumorigenesis. 5MeC is not incorporated directly into DNA; instead the methyl group is donated from S-adenosylmethionine (SAM). A choline-devoid, methionine-deficient (CMD) diet causes rodent liver tumors. Our hypothesis is being tested using strains of mice that differ in sensitivity to liver tumor formation and information may be gained regarding the ability to maintain status of specific genes and of DNA globally. We compared the effects of either CMD diet administration or phenobarbital, a known rodent liver tumor promoter, on the liver tumor sensitive B6C3F1♂ and the liver tumor resistant C57BL/6♂, after 1, 2 or 4 weeks of treatment, a time when cell proliferation is increased. The effect of the CMD diet on the methylation status of the protooncogenes Ha-*ras* and *raf* was assessed using methylation sensitive restriction enzymes and Southern analysis. Hypomethylation of *raf* was evident in the B6C3F1♂ mouse, but not the resistant C57BL/6♂, after 4 weeks on the CMD diet. Bacterial methylases were used to assess the global DNA methylation status by measuring the incorporation of ^{3}H-CH_3 from SAM into unmethylated CpG sites. Hypomethylation of liver DNA was evident at an earlier time and to a larger extent in the sensitive B6C3F1♂ mouse under both treatments. We conclude that the B6C3F1♂ mouse may have a decreased capacity to maintain, and/or decreased fidelity of, maintenance methylation compared to the liver tumor resistant C57BL/6♂. Supported by NIH ES05299.

298 THE METHYLATION STATUS OF HA-RAS: A COMPARATIVE ANALYSIS

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Differential methylation of DNA (i.e., 5-methylcytosine (5MeC) v. cytosine) is a determinant of chromatin structure and the methyl group provides a signal which is recognized by trans-acting factors that regulate transcription. Hypomethylation of DNA (i.e., decreased 5MeC) is necessary but not sufficient for transcription. We are testing the hypothesis that hypomethylation is an epigenetic mechanism involved in tumor promotion. The B6C3F1 mouse is used as a model because it is sensitive to development of liver tumors (MLT) and relevant comparisons can be made with the sensitive paternal (C3H/He) and resistant maternal (C57BL/6) strains. Ha-ras appears to be involved frequently in MLT. It has characteristics of a housekeeping gene and, therefore, its 5′ flanking region is expected to be unmethylated. However, our previous studies indicated that 5MeC is present in a 500 bp segment of this region. We use a PCR-based approach involving restriction of DNA with a methylation-sensitive enzyme (e.g., HpaII, MspI and HhaI) prior to amplification (i.e., amplification will only occur if the site(s) of interest is methylated) to assess the methylation status of liver DNA. We extended our initial studies by examining a 495 bp portion of the 5′ flanking region; a segment closer to the transcription start site. This region does not appear to be methylated and unlike our earlier studies, no strain variation in methylation status was observed. This region may play a role in maintaining basal transcriptional activity. Taken together, these data indicate that methylation may affect Ha-ras expression in the mouse and failure to maintain nascent methylation of the gene could facilitate its aberrant expression. Additionally, using published data, we compared the sequence of the 5′ flanking region in the vicinity of the transcription start site of mouse and human Ha-ras. The similarity is less than 60%. This indicates that regulation of mouse Ha-ras may not be the same as that of the human gene. Supported by NIH ES05299.

299 IMPACT OF c-Ha-*ras*^{EJ} TRANSFECTION ON MUSCLE-SPECIFIC GENE EXPRESSION IN CULTURED AORTIC CELLS

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We investigated the potential reciprocal interaction between smooth muscle-specific genes and unregulated proliferative characteristics by examining the influence of c-Ha-ras oncogene transfection on α-smooth muscle (α-SM) actin and α_{1B}-adrenoceptor (α_{1B}-ADR) expression. Cultured rat aortic SMCs during early passage (P4) were transfected by lipofection with either c-Ha-ras^{EJ} in a pSV2neo vector (ras LF-1) or pSV2neo alone (neo LF-1) and selected in G418. Consistent with previous observations, c-Ha-ras^{EJ} transfection enhanced ^{3}H-thymidine incorporation relative to vector controls and was associated with a significant reduction in α-SM and β-actin mRNA in randomly cycling cultures. Serum-deprivation of ras and neo LF-1 SMCs for 72 hr increased α-SM actin mRNA in neo LF-1 relative to cycling cells, whereas slight decreases were observed in ras LF-1 cells. Randomly cycling cells grown on elastin were exposed to stretch at a rate of 120 cycles/min for 72 hr. We observed a modest increase in α-SM actin mRNA levels in stretched neo LF-1 SMCs whereas ras LF-1 expression remained constant. Overall α_{1B}-ADR

mRNA was higher in ras LF-1 relative to neo-LF-1 SMCs, however, stretch increased α_{1B}-ADR expression in neo LF-1 cells with no change observed in ras LF-1. No differences between neo-LF-1 and naive SMCs in the expression of these genes were found suggesting that these responses may be due to ras transfection. Stretch inhibited DNA synthesis in both neo and ras LF-1 cells. Serum-deprivation reduced ^{3}H-thymidine incorporation in both neo and ras LF-1 cells but differentially modified α-SM actin mRNA levels in neo LF-1 cells suggesting that inhibition of proliferation in ras-transfected cells did not restore the expression of this gene. Collectively, these results suggest that c-Ha-ras^{EJ}-transfection is associated with down-regulation of α-SM actin and enhanced expression of α_{1B}-adrenoceptor gene expression.

300 SPONTANEOUS AND TRANSFECTION-RELATED TUMORIGENICITY OF VASCULAR SMOOTH MUSCLE CELLS (SMCs) *IN VIVO*

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Atherogenesis and carcinogenesis share common molecular events in the induction of altered cellular phenotypes. We have recently characterized the impact of oncogenic c-Ha-ras transfection in rat aortic SMCs and shown that oncogenic ras induces a phenotype reminiscent of transformed cells. To determine if ras transfection was indeed associated with transforming potential, experiments were conducted to examine the tumorigenicity of parental, vector control, and ras-transfected cell strains in nude mice and to characterize the histogenesis of the induced tumors. Naive cultured rat aortic SMCs during early passage (P4) were transfected by lipofection with c-Ha-ras^{EJ} in a pSV2 neo vector or with pSV2 neo vector alone and selected in G418 over a six week period. All cell strains, including the parental line, induced tumors in nude mice. However, the latency of the response was shorter and the degree of anaplasia was greater in ras-transfected cells relative to either vector or parental strains. The SM origin of all tumors was verified by positive α-SM actin, vimentin and desmin and negative myoglobin immunoreactivity. These findings suggest that a SMC with inherent transforming potential resides within the aortic wall and that ras transfection modulates their tumorigenic potential. (Supported by NIH grant ES 04849).

301 *Ras* AND P53 GENE EXPRESSION IN UV RADIATION INDUCED SKIN SQUAMOUS CELL CARCINOMAS AND SPINDLE CELL TUMORS IN SENCAR MICE

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The present study was carried out to investigate the specific role of the alterations of ras and p53 gene expressions in skin tumor development and progression. Seventy three female Sencar mice were exposed to ultraviolet radiation (2.9×10^4 J/m^2, three times a week) from Dermallight 2001 (80% UVA, 17% UVB and 3% UVC). Superficial squamous cell carcinomas (SCC_{sf}), well differentiated squamous cell carcinomas (SCC), and malignant spindle cell tumors (SCT) appeared sequentially after 16–20 weeks of exposure. Ras oncogene and p53 expression were examined in the tumor tissue by using the antibodies specific for ras p21 protein and p53 protein. Overexpression of ras-p21 was observed in 5/13 (38%) of SCC_{sf} and 4/11 (36%) of SCC tumors. Only 1/14 (7%) of SCT were ras-p21 positive. Contrary to the observed expression of ras in SCC, p53 over expression was observed in 6/14 (43%) of SCT while only 1/12 (8%) of SCC_{sf} and 0/9 (0%) of SCC positively expressed p53 protein. These results indicate that ras oncogene overexpression may be an early event in skin carcinogenesis induced by UV radiation while mutations in p53 may play an important role in the later stages of tumor progression.

302 DETECTION OF P53 GENE DELETIONS

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Defining associations between environmental exposures and genetic alterations found in a variety of human tumors is a challenge facing molecular toxicologist. In this context, it should be noted that commonly used PCR protocols do not reveal gene deletions. The absence of genetic material at one allele can be masked by the second wild-type allele. We have developed a method that uses *p53* processed pseudogenes in rats and mice as internal controls for detecting allelic deletion of the functional *p53* gene. This application is possible since the pseudogenes retain sequence homology to the functional *p53* genes and their PCR products are distinguishable based on size. We used DNA from two *p53* knock-out mouse models as control PCR templates. By comparing relative amounts of gene and Pseudogene amplicons, we show the procedure detects *p53* deletions and distinguished between heterozygous and homozygous deletions within the gene. The method is generally applicable to detecting deletions of genes for which there are corresponding processed pseudogenes present in the genome.

303 EFFECT OF CARCINOGEN TREATMENT ON LIVER TUMOR PROGRESSION IN HETEROZYGOUS F1 ALB.SV40 TRANSGENIC RATS

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Transgenic (TG$^+$) rats containing the alb.SV40 construct present with a 100% incidence of hepatocarcinogenesis by 6–8 months of age. The TG$^+$ rats express large T antigen which acts to sequester p53 resulting in an organ-specific (liver), null phenotype for this putative tumor suppressor gene. To address the effect of carcinogens on a p53 deficient background in the liver, female Sprague-Dawley rats were mated with heterozygous alb.SV40 male rats. At 5 days of age, the progeny were treated with solvent, diethylnitrosamine (3–30 mg DEN/kg), aflatoxin B1 (0.05, 0.2 mg AFB1/kg), or γ-irradiation (300 rad). The non-transgenic (TG$^-$) littermates were used as a control. At weaning, the rats were fed a basal diet or a diet containing 0.05% phenobarbital (PB). All of the rats were sacrificed at 3 months of age and liver sections were obtained for histopathology. The TG$^-$ littermates did not exhibit carcinomas with any treatment regimen under the conditions of this study. Treatment with irradiation, AFB1 or PB did not increase the incidence of hepatocellular carcinomas above the 10% level observed in solvent-treated TG$^+$ rats. Treatment with DEN (±PB) resulted in an increased incidence and decreased latency of liver tumor formation in TG$^+$ pups compared with TG$^-$ littermates and solvent treated TG$^+$ rats. Thus, the alb.SV40 TG$^+$ rats provide a model for examination of carcinogen action in the absence of functional p53 protein. Supported by CA-22484-17.

304 NON Ha-*ras* 7,12-DIMETHYLBENZ[a]ANTHRACENE (DMBA)-INDUCED GENETIC ALTERATIONS COOPERATE WITH v-Ha-*ras* TO ACCELERATE MULTISTAGE CARCINOGENESIS

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TG.AC transgenic mice harbor a v-Ha-*ras* transgene and also retain two normal c-Ha-*ras* alleles and are susceptible to skin tumor formation by TPA treatment. In order to determine if normal c-Ha-*ras* antagonizes the oncogenic potential of the mutant v-Ha-*ras* transgene and/or if additional DMBA target genes exist in mouse epidermis which could cooperate with mutant v-Ha-*ras* to influence promotion and/or progression, we treated TG.AC mouse skin with a subthreshold dose of DMBA. This was followed by limited TPA promotion to select for cells with additional genetic alterations. DMBA-treated/TPA-promoted TG.AC mice demonstrated a greater papilloma incidence (100 vs. 30%), a 10 fold increase in the number of papillomas per mouse, and an increased papilloma growth rate when compared to TPA-promoted TG.AC mice not treated with DMBA. DMBA-treated TG.AC mice not promoted with TPA did not develop any tumors. DMBA-treated/TPA-promoted TG.AC mice developed their first squamous cell carcinoma (SCC) at 16 weeks, and by 23 weeks 30% of these mice developed SCCs compared to no incidence of SCC in TPA-promoted TG.AC mice not treated with DMBA. Surprisingly, none of the DMBA-treated/TPA-promoted TG.AC papillomas or carcinomas contained the characteristic DMBA-induced A^{182}->T mutation in c-Ha-*ras*. No mutations in the *p53* gene were found by immunohistochemical staining. These results indicate that there exist unidentified DMBA target genes that cooperate with mutant Ha-*ras* to accelerate multistage carcinogenesis and that promoter treatment does influence premalignant progression by selecting for cells containing multiple genetic alterations.

305 ACUTE AND CHRONIC *IN VIVO* PHENOBARBITAL TREATMENT EFFECTS ON GRE AND AP-1 DNA BINDING PROTEINS

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The barbiturate, phenobarbital (PB), has been shown to induce a variety of

biotransformation enzymes. Although the mechanism(s) of induction by PB is not fully understood, current research is focusing on the role PB may play in altering the binding of nuclear proteins to critical DNA response elements. The objective of this study was to determine the effect of acute and chronic *in vivo* PB treatment on protein binding to various DNA regulatory elements including a TPA Response Element (TRE) and a Glucocorticoid Response Element (GRE). Rats received either a 70 mg/kg i.p. injection of PB for four days and sacrificed on day five, or 100 mg/kg PB i.p. 18 hours before sacrifice. Electrophoretic mobility shift assays (EMSAs) demonstrate no qualitative or quantitative differences between acute and chronic *in vivo* PB treatment regimens. Consistent with previous *in vitro* findings in other laboratories PB treatment *in vivo* enhanced AP-1 binding activity to a TRE. In contrast, PB had no effect on protein binding to the GRE. Our results show that AP-1 binding occurs following acute PB exposure *in vivo* and persists during chronic treatment, thus providing further evidence to support a role of AP-1 in the induction mechanism of PB. *Acknowledgements:* A.L.R. supported by NIEHS Training Grant 5 T32ES07266. Research supported in part by Sigma Xi Grant-in-Aid of Research Award (ALR).

306 CHARACTERIZATION OF CYTOCHROME P450 AND GLUTATHIONE TRANSFERASE ACTIVITIES IN SV40-IMMORTALIZED UROEPITHELIAL CELL LINES

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An *in vitro/in vivo* transformation system has been developed as a model for bladder tumorigenesis (Bookland et al, Cancer Res 52: 1606–1614). SV40-immortalized human uroepithelial cells are exposed to putative carcinogens and then implanted into athymic nude mice to test for tumorigenesis. Studies with 4-aminobiphenyl (4-ABP) demonstrated that one cell line was sensitive to chemical-induced transformation, SV-HUC-PC, and another line, SV-HUC-BC, was refractory. We are currently testing this system as a model to identify occupational carcinogens and develop biomarkers of effects. This study examined the P450-dependent metabolism, glutathione transferase, and the effects of chemicals on DNA synthesis and repair in SV-HUC-PC and SV-HUC-BC. Activities for CYP1A1/1A2, CYP3A and CYP2B1/2B2 were estimated by determining o-deakylation of ethoxy-, benzoxy- and pentoxy-resorufin, respectively. Coumarin hydroxylase and p-nitrophenol hydroxylase were used to estimate CYP2A and CYP2E1, respectively. SV-HUC-PC microsomes had 5 fold greater CYP1A1/1A2 activity and 2 fold higher CYP3A activity than SV-HUC-BC. CYP2B1/2B2- and CYP2A-activities and glutathione transferases were not different between the two cell lines. DNA synthesis and repair, by BrdU incorporation, was not different between the two lines when MMNG or other reactive metabolites were tested; however, SV-HUC-PC was more sensitive to n-nitrosodimethylamine, 4-ABP and MOCA. The data demonstrate that while these cells have retained form-specific P450 activities, SV-HUC-PC has greater CYP1A1/1A2 and CYP3A activities.

307 EVALUATION OF THE ROLE OF CYP2C19 GENOTYPE IN BLADDER CANCER

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CYP2C19 has been shown to be polymorphic in humans. The enzyme coded by this gene is responsible for the metabolism of the anticonvulsant drug *S*-mephenytoin and a variety of other drugs. However, its role in the metabolism of carcinogens and in cancer risk has not been investigated. We have reported two major genetic defects in *CYP2C19* (*m1* and *m2*) that are responsible for the polymorphism and have developed PCR-based genotypic assays. The *m1* defect is a G→A mutation that creates an aberrant splice site in exon 5, and the *m2* mutation is a G→A mutation that creates at stop codon in exon 4. To investigate the role of *CYP2C19* genotype in bladder cancer, we used the PCR-RFLP assays to analyse 221 bladder cancer cases and 200 sex, race, and age frequency-matched controls for the *m1* and *m2* defects. In Caucasian controls, the frequency of the *m1* allele was 0.15. The *m1/m1* homozygotes comprised 1% of Caucasian controls, but 4% of Caucasian cases. In previous studies, the *m2* allele comprised 15–30% of the defective alleles among Orientals. However, *m2* was not observed in this study of a total of 392 Caucasian cases and controls nor in 29 African-American cases and controls. Among Caucasians, the risk of bladder cancer was 3.9-fold (95% CI = 0.8–18.5, p = 0.07) higher among persons with the *m1/m1* (poor metabolizer) genotype relative to persons with the *wt/m1* or *wt/wt* (extensive metabolizer) genotypes. Stratifying by smoking status reveals no increased risk of the *m1/m1* genotype among non-smokers (OR = 0.99, 95% CI = 0.3–30, p = 0.99) and an increased risk among smokers (OR = 6.0, 95% CI = 0.7–48, p = 0.06) although there was no evidence of a dose-response among different levels of smokers. These preliminary results suggest a possible association between *CYP2C19* genotype, environmental exposures, and bladder cancer risk.

308 Ah-RECEPTOR DEPENDENT MODULATION OF GENE EXPRESSION BY AGED AND DILUTED SIDESTREAM CIGARETTE SMOKE (ADSS)

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Sidestream smoke is known to induce CYP 1A1 gene expression in rat and mouse lung as confirmed by increased enzyme activity and increased protein and message levels. The exact mechanism of how such induction occurs is based in logical speculation rather than experimentally supported proof. In this study we examined the mechanism of gene expression induction by ADSS in mouse hepatoma hepa1c1c7 (hepa1) cells. Recombinant hepa1 cells stably transfected with a luciferase gene under control of several dioxin responsive elements (DREs) were exposed to ADSS and the luciferase gene expression was examined. Induction of gene expression was observed with whole ADSS. No induction was observed with filtered ADSS suggesting that the particulates in the smoke are the inducing agent(s). α-naphtoflavone, an Ah-receptor antagonist, suppressed ADSS mediated induction of the luciferase gene. Nuclear protein extract from cells exposed to ADSS was found to bind to DRE as detected by gel shift assay. These findings strongly support the hypothesis that the Ah-receptor mediates induction of gene expression by ADSS. Supported UCTRDRP grant #4RT-0213.

309 PURIFICATION OF RAT LIVER NUCLEAR FACTOR THAT BINDS TO 5′-UP STREAM REGION OF CYP 1A1 GENE

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A negative regulatory element (NRE) has been localized in the 5′-up stream region of the CYP1A1 at −843 to −746 bp from the site of transcription. This element has >80% sequence identity to the 26 bp fos/jun-octamer binding motif of the c-myc NRE. Here we describe the purification of Oct-1 factor from rat liver nuclei and its effects on CYP1A1 expression. Specific binding to an octamer containing motif in the 5′-up stream region of CYP1A1 was used in the purification of the factor to near homogeneity. The purification procedure included conventional chromatographic methods and DNA site recognition affinity chromatography. We have also demonstrated the suppression of reporter gene activity in hepatoma cells in to which a fusion construct containing the octamer binding sequence had been transfected. From the data we conclude that we have purified the nuclear factor Oct-1 that has a role in the down regulation of the rat CYP1A1 gene.

310 THE POTENTIAL INFLUENCE OF INTERINDIVIDUAL VARIATION IN PHASE I AND PHASE II METABOLISM OF BENZENE ON BENZENE TOXICITY

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In humans, myelotoxicity of benzene (BZ) likely results from oxidative metabolism to reactive products. However, susceptibility to toxic effects of BZ may be related to a balance between activation (Phase I) and detoxication (Phase II) reactions. In the present study, we have estimated kinetic parameters of BZ oxidation and two detoxication reactions, phenol (PH) sulfation and hydroquinone (HQ) glucuronidation, in liver samples from ten humans. In these samples, initial rates of oxidative metabolism of benzene catalyzed by cytochrome P450 (CYP) 2E1 varied from 0.402 to 5.252 nmoles/mg/min. Initial rates of PH sulfation varied 3-fold (range: 0.309 to 0.919 nmole/mg/min), as did initial rates of HQ glucuronidation (range: 0.101 to 0.281 nmole/mg/min). *In vitro* bone marrow studies have demonstrated that HQ can be oxidized to 1,4-benzoquinone, a highly lipophilic compound that undergoes extensive protein and DNA binding. That reaction is enhanced in the presence of PH. Therefore, a physiologically-based pharmacokinetic model that incorporates measured rates of oxidation and conjugation reactions was developed in order to predict steady-state concentrations of PH and HQ in blood of the ten human donors during a low BZ exposure. Predicted steady-state concentrations of PH and HQ varied 8-fold and 3.4-fold, respectively. In all simulations, predicted concentrations of HQ exceeded those for PH, and in all but one human the difference was greater than 10-fold. Predicted concentrations of HQ at

steady-state did not correlate with measured CYP 2E1 activity, underscoring the importance of a balance between activation and detoxication reactions in determining relative risk of benzene toxicity.

311 CHARACTERIZATION OF THE HUMAN CYP1B1 PROMOTER

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Human *CYP1B1* previously has been demonstrated to be a dioxin inducible gene (Sutter et al., *J. Biol. Chem.* 269: 13092, 1994). Here, we report on a characterization of the essential transcription elements in the promoter of *CYP1B1*. A series of constructs containing various 5-deletions in the *CYP1B1* regulatory region fused to the structural gene for chloramphenicol acetyltransferase (CAT), were transfected into human keratinocytes and the transfectants assayed for CAT activity. A region from −101 to +25 (relative to the transcription initiation site, TIS) contained full promoter activity. This region lacked a consensus TATA box or a CCAAT sequence. A TATA-like sequence (TTAAAA) was found 27 bp upstream from the TIS. Mutation or deletion of the TATA-like sequence, an initiator-like sequence (TTGACTCT) surrounding the TIS (−3 to +5), or two Sp1 binding motifs (GGGCGG) located at −84 and −68, antisense strand, significantly decreased the promoter activity. *In vitro* transcription studies showed that the mutation of the initiator-like sequence resulted in multiple transcription initiation sites. These results indicate that the *CYP1B1* promoter is similar to other TATA-less genes that require an initiator and Sp1 element for efficient transcription. (Supported by a grant from the American Forest and Paper Association).

312 CYTOCHROME P450-MEDIATED REGIOSPECIFIC HYDROXYLATION OF LAURIC ACID BY RAINBOW TROUT

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Our previous research established that fatty acids such as lauric acid were metabolized primarily at the *w*- and (*w*-1)-positions by rainbow trout hepatic microsomes. Purified trout P450s LMC1 and LMC2 (CYP2K1) were shown to catalyze the *w*- and (*w*-1)-hydroxylation of lauric acid, respectively. A recent report (*Lipids* 27, 187, 1992), however, indicates that sea bass liver microsomes hydroxylate lauric acid at several subterminal positions. Therefore, we have reexamined the hydroxylation of [^{14}C]-lauric acid by trout liver microsomes and reconstituted trout P450s, using a new HPLC system that gave improved separation of hydroxylated lauric acids. Under these conditions, hepatic microsomes from yearling juvenile trout were shown to form *w*, *w*-1, *w*-2, *w*-3, *w*-4, *w*-5 and *w*-6 hydroxylaurates as verified by GC/MS analysis. As in the sea bass, the *w*-1 hydroxylaurate was the major metabolite (23–29% of total radioactivity) in trout and no difference was observed between males and females. By contrast, liver microsomes from 3-year-old sexually mature trout produced small quantities of only the *w*-1, *w*-2 and *w*-6 hydroxylation products. Lauric acid also was incubated with reconstituted trout LMC1 and LMC2 (CYP2K1) and with hepatic microsomes from juvenile trout in the presence of rabbit polyclonal antibodies raised against the two purified trout P450s. Based on these latter studies, we have concluded that LMC1 catalyzes the *w*-6 hydroxylation of lauric acid while the trout LMC2 (CYP2K1) form produces mainly *w*-1 hydroxylaurate together with a smaller quantity of the *w*-2 hydroxylation product. Studies are underway to identify other trout P450 forms that are responsible for the regiospecific hydroxylation of fatty acids. (Supported by NIH Grants ES00210, ES03850, ES04766 and ES05533).

313 COMPARISON OF HUMAN, RHESUS MONKEY AND DOG HEPATIC METABOLIC ACTIVITIES

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Sponsor: J Coleman

A primary component of metabolism research in drug development is the use of animal models, such as dog and Rhesus monkey, to predict the human biological fate of xenobiotics. Microsomes were prepared from liver tissue of eight Beagle dogs, three Rhesus monkeys and thirteen humans. Several well-characterized P450 substrate assays were used as indices of the relative P450 isozyme activities in the microsomes (acetamidophenol (1A), coumarin (2A), tolbutamide (2C), bufuralol (2D), testosterone (3A)). Studies with specific P450 inhibitors were also conducted to support the substrate specificity in the three species studied.

For example, in studies for P450 3A, the activity varied significantly in human (0.1 to 3.3 nmol/mg protein/min), while the activities in the dog and Rhesus were not as variable (0.3 to 0.5 nmol/mg protein/min and 1.7 to 2 nmol/mg protein/min, respectively). In the P450 2D experiments, the activity observed in the human (0.2 to 0.7 nmol/mg protein/min) and dog (0.3 to 0.6 nmol/mg protein/min) were similar but the activity in Rhesus monkey was significantly higher (2.5 to 3.4 nmol/mg protein/min). Such data contribute to the data base of information which will provide for reliable extrapolation of biotransformations from animal models to human.

314 ROLE OF MURINE CYP1A2 GENE IN TOXICITY: STUDIES IN KNOCKOUT MICE

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We have produced an embryonic stem (ES) cell-derived transgenic mouse line having a disruption in both copies of the functional *Cyp1a2* gene. We have knocked out one copy of the *Cyp1a2* gene in ES cells with a *Cyp1a2* homologous recombination construct and injected the targeted ES cells into the blastocoele cavity of embryos. Surviving blastocysts were transferred to a foster mother by uterine implantation. PCR and Southern blot analyses have confirmed that the targeted gene has been correctly brought into the germline. Heterozygous mice carrying a disrupted *Cyp1a2* allele are viable and fertile and appear phenotypically normal. Homozygous mutants derived from interbred heterozygotes are indistinguishable from the heterozygote and wild-type mice by appearance and mortality rates up to 5 weeks of age. Longevity and fertility of the homozygotes will be discussed, as well as the susceptibility of *Cyp1a2* (−/−) mice toward aflatoxin B_1 and acetaminophen.

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315 ISOLATION, MUTATION, AND EXPRESSION OF GOAT PULMONARY CYP2F3 CDNA

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Exposure to environmental toxins and their bioactivation *in situ* have been implicated as important mechanisms that contribute to pulmonary toxicities. 3-Methylindole (3MI), a pulmonary specific toxin contained in cigarette smoke and foods, is bioactivated by P450s to form covalently binding metabolites that produce selective lung toxicity. Previous studies have shown that human pulmonary *CYP2F1* participates in 3MI bioactivation. As goats are the most susceptible species to 3MI-induced toxicity, in order to explain the species- and tissue-specific toxicity of 3MI to goat lung, *CYP2F3* cDNA that contains 1473 bp in the transcribing region and encodes a polypeptide of 491 amino acids was isolated from goat lung λZAPII cDNA library by screening with the human *CYP2F1* cDNA probe. Sequencing of the full-length transcribing region of *2F3* and comparing its amino acid with that of human *2F1* and mouse *2f-2* showed 82% similarity with both enzymes. Northern blot analysis of goat lung and liver mRNA with the insert of *2F3* revealed that a 2.0 kb transcript was detected in a highly tissue-specific manner only in lung but not in liver. This cDNA clone was mutated at the 5′-terminus to favor the bacterial codon bias and was subcloned into the pTrc 99A expression vector then used to transform *E. coli* XL1-blue cells. The expressed P450 content(91 nmol/L) was measured by its CO-difference spectrum and characterized by western immunoblotting using a rabbit anti-mouse 2f-2 polyclonal antibody. Further studies with the expressed enzyme will allow us to undertake more detailed metabolism studies to establish the relationship between this enzyme and its bioactivation of pulmonary specific toxins. These studies will provide important information about the members of the *CYP2F* subfamily and their participation in pulmonary specific toxicity. (Supported by USPHS grant HL13645 from the National Heart, Lung, and Blood Institute).

316 DIFFERENTIAL EXPRESSION OF RAT MICROSOMAL EPOXIDE HYDROLASE GENE BY AZOLE ANTIFUNGAL AGENTS: THE EFFECTS OF KETOCONAZOLE, CLOTRIMAZOLE, FLUCONAZOLE AND ITRACONAZOLE

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Previous studies showed that imidazole antifungal agents induce microsomal epoxide hydrolase (mEH) with transcriptional activation. In order to establish whether imidazole antifungals, ketoconazole and clotrimazole elevate mEH mRNA levels in a dose-related manner and whether triazole antifungal agents, fluconazole and itraconazole affect mEH gene expression to similar extents,

hepatic mEH mRNA levels were quantified in rats by RNA blot analyses. Treatment of male rats with ketoconazole caused ≈4-, 10- and 11-fold increases in mEH mRNA levels at 24h post-treatment at the doses of 50, 100 and 200 mg/kg, respectively, whereas mEH mRNA levels were increased ≈18-fold at 24h after a single administration of clotrimazole at 50 mg/kg b.w. or at greater doses with EC_{50} value being noted as 20 mg/kg. Fluconazole elevated mEH mRNA levels by 2-, 3- and 6-fold at the doses of 50, 100 and 200 mg/kg b.w., respectively. However, hepatic microsomes isolated from rats treated with fluconazole for 3 days failed to exhibit significant elevation in mEH protein levels. No significant increase in mEH mRNA nor protein levels was observed at 1 or 3 day(s) after treatment of rats with itraconazole (200 mg/kg/day). To characterize gender-related differences in hepatic mEH gene expression in response to azole antifungal agents, the effects of ketoconazole and clotrimazole were examined in females. Ketoconazole marginally affected hepatic mEH gene expression in females and clotrimazole caused 70%–90% less increase in mEH mRNA levels in females than in males, representing notable sex specificity. These results demonstrated that clotrimazole was the most potent and efficacious agent in mEH gene expression among the azole compounds examined and that triazole antifungal agents, fluconazole and itraconazole, were not as effective as imidazole-based compounds in stimulating mEH gene expression. (Supported by research grant 931-0700-002-02 from KOSEF).

317 COMPARISON OF OMEPRAZOLE HYDROXYLATION USING RECOMBINANT HUMAN CYP2C ENZYMES EXPRESSED IN YEAST

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Omeprazole (OMP), a potent inhibitor of gastric acid secretion, is used for the treatment of severe oesophagitis, the zollinger-syndrome, and refractory peptic ulcers. OMP oxidation is a major metabolic pathway of this drug and is catalyzed by cytochrome P450s (CYP). Although the identity of the CYP enzyme responsible remains controversial, there is evidence that OMP hydroxylation cosegregates with the polymorphism of S-mephenytoin 4'-hydroxylation in humans. Present studies were undertaken to assess OMP metabolism using human CYP2C recombinant enzymes expressed in yeast. Yeast microsomes were incubated with ^{14}C-OMP, the reaction was terminated with methanol, and the supernatant was analyzed by HPLC. A single metabolite with the same HPLC retention time as that of 5-hydroxyomeprazole (5OH-OMP) was the only metabolite formed by CYP2C19, CYP2C8, and CYP2C18. The rate of 5OH-OMP formation by these enzymes were 19.2 ± 0.9, 2.2 ± 0.1, and 1.5 ± 0.1 nmol/min/nmol P450, respectively. In contrast, CYP2C9 did not produce 5OH-OMP. Incubation of OMP with microsomes from AHH-1 (TK±) cells containing recombinant human CYP3A4 resulted in, depending on the incubation conditions, some OMP metabolism. In conclusion, these data indicated that CYP2C19, the enzyme responsible for mephenytoin polymorphism, is also a major OMP hydroxylating enzyme.

318 EFFECTS OF α-NAPHTHOFLAVONE ON AFLATOXIN B_1 OXIDATION BY HUMAN P450S

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Aflatoxin (AF) B_1 oxidation was studied with purified recombinant human P450 1A2, 3A4, and 3A5 in reconstituted systems. Analysis of the AFB_1 oxidation products indicated that P450 1A2 formed AFM_1, a small amount of AFQ_1, and equal amounts of *exo*- and *endo*-8,9-oxides; P450 3A4 formed AFQ_1 and *exo*-8,9-oxide. P450 3A5, with 85% amino acid sequence identity to P450 3A4, formed mainly the *exo*-oxide and only a small amount of the detoxication product AFQ_1. The *exo*-oxide formation rates of 1A2 and 3A5 were ≈14% and 43% of P450 3A4. The *endo*-oxide is essentially non-genotoxic in the *umu* test, as found previously with mutations (Iyer *et al.*, 1994). These results indicated that P450 3A enzymes are more effective than P450 1A2 in the bioactivation of AFB_1. α-Naphthoflavone (α-NF) caused potent inhibition of all oxidation reactions catalyzed by P450 1A2. In contrast to P450 1A2, addition of α-NF to P450 3A4 resulted in a 2-fold increase of oxide formation but decreased AFQ_1 formation. α-NF also stimulated epoxide formation by P450 3A5. Plots of AFQ_1 and epoxide formation rates vs AFB_1 concentration were sigmoidal for P450 3A4 and were shifted to a more hyperbolic nature in the presence of α-NF. α-NF can be oxidized by P450 3A4 to its 5,6-oxide but there was no significant effect of AFB_1 on the oxidation of α-NF, even under concentrations where α-NF altered AFB_1 oxidation. α-NF may be an allosteric effector of AFB_1 oxidation of P450 3A enzymes. (Supported in part by USPHS grants CA44353 and ES00267).

319 DETECTION OF CYTOCHROME P450 ISOFORM EXPRESSION IN RAT LIVER, SPLEEN AND PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC) USING RT-PCR AND IMMUNOBLOTTING TECHNIQUES

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Reverse transcriptase-polymerase chain reaction (RT-PCR) and immunoblotting techniques were used to detect expression of the cytochrome P450 isoenzymes IIB, IIE, 1A, IIC, 3A and 4A in male Sprague Dawley rats. Comparisons were then made between the expression of P450 isoforms in liver with that found in PBMC, and spleen, to determine if lymphoid tissue can be used to monitor rat P450 induction during in-life toxicity studies. Phenobarbital (PB; 80 mg/kg), β-naphthoflavone (BNF; 80 mg/kg) and dexamethasone (DEX; 100 mg/kg) were used as P450 enzyme inducers. For RT-PCR, specific primers were selected for each P450 isoenzyme and tested for mRNA detection under optimized linear amplification conditions. The induction index (P450 isoform expression:constitutive gene expression) of each P450 isoform was determined by densitometric quantitation of the amplified DNA products. For immunoblotting, monoclonal antibodies selective for the P450 isoenzymes IIB, IIE, IA, IIC and 3A were obtained from commercial sources and used for detection of protein levels in liver microsomal preparations. Analysis of P450 expression in the liver revealed that the major isoforms induced by PB, BNF and DEX were IIB, IA, and 3A, respectively, and were in agreement by the two detection techniques. The remaining isoforms (IIE, IIC and 4A) were found to also change in an inducer-specific manner. The only P450 isoform found to be expressed and induced in the spleen and PBMC was 1A, which was only detectable by RT-PCR. These results further demonstrate the utility of RT-PCR for the analysis of P450 isoenzyme expression following exposure to xenobiotics, particularly in non-hepatic tissues having low copy number expression.

320 CYTOCHROME P-450 2E1 AND SPECIES DIFFERENCES IN 1,2-DICHLORO-1,1,2-TRIFLUOROETHANE BIOTRANSFORMATION

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1,2-Dichloro-1,1,2-trifluoroethane (HCFC-123a) is a potential alternative to replace ozone depleting chlorofluorocarbons. The metabolism of HCFC-123a was studied in microsomes and in rats and mice *in vivo*. Rat, mouse and human liver microsomes metabolized HCFC-123a to fluoride and chlorodifluoroacetic acid. Fluoride formation was dependent on time, NADPH, HCFC-123a and protein concentration. Microsomes from unpretreated rats oxidized HCFC-123a at low rates (0.49 nmol fluoride/20 min × mg protein). Pretreatment of rats with pyridine and ethanol, inducers of cytochrome P-450 2E1, increased the rates of fluoride release. In mouse liver microsomes, the rates of HCFC-123a oxidation to release inorganic fluoride were significantly higher (1.68 nmol fluoride/20 min × mg) than in rat liver microsomes. Coincubation of HCFC-123a with microsomes and diethyldithiocarbamate (100 μM), an inhibitor of cytochrome P-450 2E1, reduced fluoride production by more than 70%. In different samples of human liver microsomes, rates of fluoride formation were between 2 and 4 fold higher than observed in liver microsomes from unpretreated rats. In rats and mice exposed to concentrations of HCFC-123a up to 5 000 ppm in a closed recirculating exposure system, chlorofluoroacetic acid and inorganic fluoride were identified as urinary metabolites. The biotransformation of HCFC-123a in rats was saturated after exposure to more than 2 000 ppm HCFC-123a for 6 hours, whereas no saturation was evident in mice exposed to concentrations of up to 5 000 ppm. The obtained results suggest a major role of P-450 2E1 in the oxidation of HCFC-123a and in the different capacities for oxidative biotransformation of HCFC-123a in rodents. Mice may thus be more sensitive to toxic effects of HCFC-123a depending on biotransformation.

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321 HYDROPEROXIDE ENHANCED XENOBIOTIC CYTOTOXICITY AS A NOVEL *IN VITRO* TOXICOLOGY TECHNIQUE

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Previously we showed that hydroperoxides act as an oxygen donor for xenobiotic metabolism (Pharmacol. Ther. 2: 517-536, 1978). Hepatotoxins e.g. 4-hydroxyanisole which were not toxic to hepatocytes at 10 mM became toxic (LC_{50} 2hrs, 2 mM for trypan blue exclusion) on addition of a non-toxic concentration, of t-butylhydroperoxide (0.15 mM). The hydroperoxide

enhanced 4-hydroxyanisole cytotoxicity and GSH depletion was prevented by CYP2E1 inhibitors with uninduced hepatocytes and by CYP1A inhibitors with 3-methylcholanthrene induced hepatocytes. GSH conjugate formation now readily occurred and protein covalent binding was attributed to benzoquinone. Hydroperoxide also enhanced 4-nitroanisole cytotoxicity ten fold (LC_{50} = 0.5 mM) and cytotoxicity was prevented by CYP2E1 inhibitors. 4-Nitroanisole O-demethylation by hepatocytes was also much faster with hydroperoxide and was prevented by CYP2E1 inhibitors. ATP depletion (due to nitrophenol formation) was also much more extensive. CYP3A2 inhibitors also prevented hydroperoxide enhanced estradiol cytotoxicity. This cellular peroxidase/peroxigenase technique therefore acts as a probe for P450 isozyme specificity in intact cells. The increased level of covalently bound intermediates also enables us to identify reactive intermediates. (Supported by Nat. Sc. Eng. Res. Council of Canada).

322 OXIDATION OF BUTADIENE MONOXIDE TO MESO- AND (±)-DIEPOXYBUTANE BY MOUSE AND RAT LIVER MICROSOMES AND HUMAN cRNA-EXPRESSED CYTOCHROME P450S

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Previously we have shown that 1,3-butadiene is metabolized by rat, mouse and human liver microsomes and human cDNA-expressed cytochrome P450s to butadiene monoxide (BM). In this study, the ability of BM to be further oxidized to diepoxybutane (DEB) was examined. Reference *meso-* and (±)-DEB were synthesized by the chemical oxidation of BM by *m*-chloroperoxybenzoic acid and characterized by GC-mass spectrometry. Incubations of rat or mouse liver microsomes with racemic BM (0.25–5 mM) revealed two new peaks upon analysis by capillary GC. Formation of these peaks was dependent on the presence of NADPH as well as protein and BM concentrations. GC retention times of the enzymatically produced peaks matched those of the chemically synthesized compounds and GC-mass spectra gave fragmentation patterns similar to those of reference DEB. The ratio of *meso-* to (±)-DEB was dependent on both the species and concentration of BM. Because previous studies showed that cDNA expressed cytochrome P450s 2A6 and 2E1 were efficient catalysts of 1,3-butadiene oxidation, the ability of these enzymes to metabolize BM was examined. The results from these experiments show that both *meso-* and (±)-DEB can be formed, however 2E1 appears to be the more efficient catalyst of BM oxidation. (Supported by NIH grant ES06841).

323 SPONTANEOUS REDUCTION OF CYTOCHROME P450IA1 ACTIVITY IN CULTURED RAT EPIDERMAL CELLS

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3-Methylcholanthrene is toxic to rat epidermal cells in primary culture but is not by passage 15 as seen in colony growth assays. The ability of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) to induce P450IA1 message and polycyclic aromatic hydrocarbon (PAH) metabolism is greatly reduced in high compared to low passage cultures. To examine the mechanism of the loss of sensitivity, alterations in Ah receptor levels and function were investigated. [^{3}H]TCDD ligand binding assays revealed comparable Ah receptor levels (37 fmol TCDD bound/mg protein) in low and high passage cells. Transient transfection experiments were carried out with a TCDD-inducible luciferase reporter construct containing 4 dioxin responsive elements linked to a mouse mammary tumor virus promoter. The results revealed comparable TCDD-inducible luciferase activity (4 and 5-fold) in low and high passage cells, respectively, indicating the presence of a functional Ah receptor. On the other hand, using a reporter construct which contains the full 5′ flanking region of P450IA1, TCDD-enhanced luciferase expression was observed only in low passage cells, comparable to the effect observed with the endogenous P450IA1 gene. These results suggest that alterations in the activity of transactivating factors important in the expression of P450IA1, other than the Ah receptor, are responsible for the loss of PAH sensitivity in high passage rat epidermal cells.

324 METABOLISM OF NAPHTHALENE (NA) TO NA-GLUTATHIONE CONJUGATES AND 1,2-DIHYDRODIOL IN MICRODISSECTED AIRWAYS OF TOLERANT MICE

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We previously demonstrated decreased metabolism of NA to glutathione conjugates and 1,2-dihydrodiol in pulmonary microsomal preparations from mice given seven daily doses of naphthalene (200 mg/kg). Although microsomal metabolism was depressed in tolerant mice, metabolite production in isolated microdissected airways at saturating substrate concentrations was no different in corn oil (CO) vs NA treated controls (0.5 mM NA final concentration). This study was designed to determine whether the kinetics of NA metabolism were different in microdissected airways of tolerant mice at low substrate concentration. Proximal (trachea), and distal airways (minor daughter with distal bronchioles) of tolerant and control mice were incubated with varying concentrations of NA (0.005, 0.01, 0.025, 0.05, and 0.10 mM). The highest rates of metabolism were observed in distal airways where diol and conjugates were generated at nearly 10 fold higher rates than proximal airways. No striking differences in the rates of NA metabolism were noted between control and NA tolerant animals at 0.1 mM or greater NA concentrations. However, at lower substrate concentrations approximately 20 fold lower rates were observed in both proximal and distal airways of tolerant mice compared to controls. We conclude that tolerance due to NA may be, in part, a function of alterations in NA activation at low substrate concentrations. Supported by NIEHS ESO5578, and ES04311.

325 5′ FLANKING REGION OF CYP2E GENES FOR P450 CYTOCHROMES THAT PROMOTE LIPID PEROXIDATION AND METABOLIZE NUMEROUS FOREIGN COMPOUNDS, INCLUDING CHEMICAL CARCINOGENS

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Ethanol-inducible P450 2E cytochromes are the low-K_m enzymes for N-nitrosodimethylamine metabolism and promote lipid peroxidation, especially during CCl_4 metabolism. The upstream sequence of the rabbit 2E1 and 2E2 genes was analyzed to understand the basis for their differential transcriptional regulation in neonatal liver, where rabbit 2E2 is expressed immediately after birth, two weeks before 2E1, and in the mature animal, where both genes are expressed in the liver, but where only 2E1 mRNA occurs in the kidney. The two genes differ by 2–3% except for large deletions in 2E2 that correspond to −150 to −310 in 2E1; in that region, 2E1 contains a 32-base direct repeat, which in 2E2 is present as a single, inexact element. The 32-base pair element (32-BPE) has an estrogen regulatory element half-site, which comprises a 13-nt sequence that is also present in the human and rat 2E1 genes. The upstream region of 2E genes has an HNF1 sequence, which is known to control hepatic gene expression. Of relevance to 2E induction in diabetic animals was the finding of a putative insulin-responsive sequence (IRS) in the rabbit 2E genes between the TATA box and transcription start site; the 2E2 IRS has only one mismatch relative to that of the PEPCK gene. Band shift assays reveal interaction of the HNF1, IRS, and 32-BPE sequences with nuclear protein from HeLa and HepG2 cells, or from adult liver and kidney. [Supported by AA-06221 (M.J.C.) from the NIAAA; S.J.P. was a Postdoctoral Trainee of the NIEHS, Grant ES-07062.]

326 KINETICS AND INHIBITION OF DICHLOROACETIC ACID METABOLISM *IN VITRO*

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Dichloroacetic acid (DCA) is a contaminant of groundwater, may be identified in finished drinking water at appreciable levels and may arise from the in vivo metabolism of trichloroethylene, another ground water contaminant. DCA administered to rodents at levels which do not produce peroxisome proliferation resulted in rodent hepato-carcinogenesis. The metabolism of DCA may be a determinant of toxicity and has been thought to proceed via reductive dehalogenation by cytochrome P450. To produce a more efficient extrapolation of rodent toxicity data to the human, an evaluation of the mechanism and extent of DCA degradation in the mouse, rat and human was undertaken. In this report we detail the kinetic parameters associated with DCA degradation in hepatic cytosol of the rat, mouse and human and detail the results of inhibition studies involving substrates of known cytosolic enzymes. Kinetic analyses indicate that rat and mouse cytosol catalyze DCA degradation at similar rates (Km approximately 300 μM and Vmax approximately 12 nmol/min/mg). The results indicate that a high degree of interindividual variability exists in the human. These data indicate that the human may be poorly equipped to metabolize DCA, a factor which should be included in assessing human risk to DCA. Funded by the Strategic Environmental Restoration Defense Program.

327 DIET-RELATED REDUCTION IN HEPATIC MICROSOMAL P450 AND RELATED CATALYTIC ACTIVITY IN HATCHERY-RAISED RED DRUM

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Specific hepatic microsomal parameters were investigated in redfish, a popular U.S. gulf coast aquaculture and gamefish species. Constitutive and induced levels of microsomal cytochrome P450 and P420, 7-ethoxyresorufin-O-deethylase and NADPH cytochrome C reductase catalytic activities, and the extent of membrane lipid peroxidation were compared in sexually-immature farm-raised and wild-caught animals. Our data indicate a diet-related reduction in active cytochrome P450 and an increase in cytochrome P420, the denatured form of cytochrome P450, in farm-raised redfish. Lipid peroxide formation was significantly higher and EROD catalytic activity was decreased in farm-raised redfish whereas wild-caught fish exhibited significantly higher levels of cytochrome P450 and EROD activity. No significant alterations in NADPH cytochrome C reductase activity were noted in farm-raised fish. The hepatopancreii from farm-raised redfish have increased levels of cellular lipid which correlate with increased levels of lipid peroxides and are apparently associated with the loss of active cytochrome P450. These data indicate that farm-raised fish fed a lipogenic diet have a reduced metabolizing capacity for endobiotics, drugs and hydrocarbon pollutants, and suggest that the existence of significantly increased hepatic lipid deposition may have potentially dangerous therapeutic and/or toxicological implications for these animals and their ultimate consumers.

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328 MECHANISM-BASED INHIBITION OF MOUSE P4502B AND 1A BY SELECTED ARYLALKYNES

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Suicide inhibitors of P450 families are excellent tools to predict which isoforms mediate the metabolism/activation of a variety of chemical agents. We compared the inhibitory effects of several arylalkynes on mouse P450 with published data in the rat model. The inhibition of P4502b specific dealkylation of benzyloxy-resorufin and 1a specific deethylation of ethoxyresorufin were measured in hepatic and pulmonary microsomes from male Swiss mice by 2-ethynylnapthalene (2-EN), 5-phenyl-1-pentyne (PPY), 4-phenyl-1-butyne (PBY) and 9-ethynylphenathrene (9-EPH). Mice were treated with 1,4-bis[2-(3,5-dichloropyridyloxl)]-benzene (TCPOBOP) to induce cytochrome P450 2b or 2,3,7,8-TCDD to induce P450 1a1 and 1a2. The IC50 was calculated for both liver and lung microsomes and compared to published values of K_i reported in the rat. PPY, PBY and 9-EPH were equally effective inhibitors of mouse 2b and the rat isoform. 2-EN was a 10-fold less potent inhibitor of mouse 2b, as compared to rat. By comparison, 2-EN was as effective an inhibitor of P450 1a2 in the mouse as reported for rat 1A2 in TCDD-induced liver microsomes. These data suggest that the active site of the mouse 2b enzyme is similar to the rat isoform 2B, with the exception of the disparity in IC50 for 2-EN. The potency of 2-EN for P450 1a2, however, was equivalent between mouse and rat hepatic microsomes.

329 STAPHYLOCOCCAL ENTEROTOXIN B (SEB) INHIBITS CYTO-CHROME P450-MEDIATED 2E1 ACTIVITY IN MICE

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It is well known that inflammatory stimuli can depress concentrations and activities of hepatic cytochromes P450. This phenomenon has been studied with gram-negative bacterial cell wall endotoxin, but has not yet been reported with gram-positive bacterial products. Because critically ill septic patients are more likely to have infections with gram positive organisms (often staph sp.) we decided to see if purified SEB (Toxin Tech, Inc. — 3 mg/kg) would affect P450s in male C3H/HeN mice (25 g) and compared it to endotoxin [lipopolysaccharide (LPS), *E. coli* 0111:B4–0.5 mg/kg]. Both agents were injected ip and microsomes prepared from mice killed 24h later. Total P450 concentrations were 0.95 ± 0.09 nmol/mg protein in saline controls, 0.62 ± 0.09 after LPS, and 0.66 ± 0.06 after SEB. Paranitro-phenol hydroxylase, a specific activity of P450 2E1, was 74 ± 19 pmol/mg/min in saline controls, 28 ± 12 after LPS, and 40 ± 9 after SEB. (Values are mean ± SD for 5 mice). We conclude that a gram-positive stimulus like SEB can also depress cytochrome P450 concentrations and at least the activity of the 2E1 isozyme in mice.

330 EFFECTS OF INDUCERS AND INHIBITORS OF STYRENE METABOLISM ON ITS TOXICITY IN MICE

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Styrene is metabolized by cytochromes P450 to the active metabolite styrene-7,8-oxide (SO) which is then detoxified primarily to the dihydrodiol by epoxide hydrolase. Non-Swiss albino mice were administered pyridine (PYR, 200 mg/kg, ip) to induce CYP2E1, phenobarbital (PB, 80 mg/kg/d × 4d, ip) to induce CYP2B, or β-naphthoflavone (β NF, 40 mg/kg/d × 3d, ip) to induce CYP1A. The hepatotoxicity of styrene (600 or 800 mg/kg, ip) was assessed by measurement of serum sorbitol dehydrogenase (SDH) and pulmonary toxicity by determination of lactate dehydrogenase and γ-glutamyl transpeptidase in bronchoalveolar lavage fluid (BALF). All 3 inducers increased the pulmonary toxicity of styrene. PYR and βNF increased the hepatotoxicity of styrene. PB caused a nonsignificant ($P > 0.05$) increase in SDH. The biochemical lesions were confirmed by histological analysis. The results suggest that induction of P450 isozymes enhances styrene toxicity. The epoxide hydrolase inhibitor trichloropropane oxide (161 mg/kg, ip) increased the hepatotoxicity of racemic SO and the R- and S-enantiomers as well as the pneumotoxicity of the enantiomers demonstrating potentiation of SO toxicity by inhibition of its detoxification. (Supported in part by NIH grant ES04362 and the Styrene Information and Research Center).

331 TIME COURSE STUDIES OF HEPATIC MICROSOMAL METABOLISM OF ORGANOPHOSPHORUS INSECTICIDES IN RATS TREATED WITH β-NAPHTHOFLAVONE

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The time dependent changes in parathion (P = S) and chlorpyrifos (C = S) metabolism were investigated after the administration of β-naphthoflavone (BNF) to female rats for 3 days. Ethoxyresorufin *O*-deethylase (EROD), pentoxyresorufin *O*-dealkylase (PROD) and aliesterases were also studied. Hepatic aliesterases from BNF-treated rats displayed lower activity than controls. EROD and PROD activities were induced by about 82 fold and 20 fold, respectively, after three days of BNF treatment. The BNF treatment significantly increased the rate of activation and detoxication of P = S by about 3.5 fold and 2 fold, respectively, and C = S by about 10 fold and 1.6 fold, respectively. All activities monitored had returned to control levels 7 days after the last BNF injection except EROD. With the activation reactions, 10 μM and 50 μM concentrations of C = S and P = S displayed similar profiles, with a peak of activity on day 3. However, there was a more rapid decrease in the activity of P = S activation than C = S. EROD activity, reflecting CYP1A1 levels, were substantially induced by BNF treatment. The induction of P = S and C = S metabolism, which followed the same time course as EROD, indicates that CYP1A1 can catalyze the activation and the detoxication of both compounds, although the patterns with the two compounds were different. The induction of phosphorothionate insecticide metabolism and the decrease in the protective aliesterases suggest that BNF treatment could alter the level of insecticide toxicity. (Supported by NIH RO1 ES04394).

332 EXPOSURE MODELING AND VALIDATION STUDIES FOR AEROSOLS: A CASE STUDY OF DIOXIN EXPOSURE DURING ROADSIDE WEED ABATEMENT WITH 2,4,5-T

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Aerosol exposure modeling creates unique challenges for exposure assessment due to the wide-ranging results that can be obtained by applying many assumptions to the modeling process. Our objective was to characterize the probable dioxin exposure that a roadside weed abatement worker would experience from blowback of aerosol while spraying 2,4,5-T solution from inside the cab of a truck. Our first step was to record worker observations regarding blowback exposure. Second, we determined upperbound and 'most-likely' estimates of exposure with an evaporative loss model based on exercise physiology principles. Third, we conducted a series of experiments measuring aerosol accumulation under varied wind speeds and spraying postures. The results of our analyses indicate that dioxin exposure from aerosol blowback is probably negligible for the roadside spraying routine simulated here. This is consistent with

our literature review which indicates that such aerosol exposures are likely nominal in comparison to direct contact events such as mixing/loading of the herbicide.

333 FURTHER STUDIES ON THE SIMILARITIES OF EFFECTS OF TCDD AND ITS STRUCTURAL ANALOG, CHLORPROMAZINE (CPZ)

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Male Sprague-Dawley rats (260–280 g) were implanted with slow release pellets (200 mg CPZ/21 days) whereas pair-fed controls were sham-operated. Feed intake and body weight were monitored daily. Groups of 5 animals were sacrificed 1, 2, 4, 8, and 21 days after surgery. CPZ concentration in serum was determined by HPLC. Ethoxyresorufin O-deethylase (EROD) activity in liver microsomes was determined fluorometrically. Phosphoenolpyruvate carboxykinase (PEPCK) activity in liver cytosol was determined luminometrically. CPZ treatment resulted in reduced feed intake and body weight loss for up to 8 days; thereafter animals resumed eating and body weights began to increase. The concentration of CPZ in serum was highest one day after implantation of the pellets and then decreased in an exponential fashion; there was no measurable level by day 21. EROD activity in liver was increased, reaching a maximum of 3-fold induction by day 4, then declining to control levels by day 21. PEPCK activity in livers of CPZ-treated animals showed a 50% reduction on day 8 of dosing but had returned to control levels by day 21. PEPCK and EROD activities in pair-fed animals remained unchanged throughout the study period. The time courses of CPZ concentration and EROD activity suggest that CPZ induces drug-metabolizing enzymes including those that metabolize CPZ itself. Nevertheless, after 8 days of dosing the AUC of CPZ concentration was sufficiently large to reduce PEPCK activity in the liver. These results provide further evidence that CPZ, at least in part, acts in similar or identical ways as its structural analog TCDD.

334 VALIDATION OF PRECISION-CUT LIVER SLICES IN DYNAMIC ORGAN CULTURE AS AN *IN VITRO* MODEL FOR STUDYING CYP1A1 AND CYP1A2 INDUCTION

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The utilization of precision-cut liver slices as an *in vitro* model was validated by comparing the induction of the biomarker responses following *in vitro* (rat liver slice) and *in vivo* exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The biomarker responses investigated were cytochrome P-450s 1A1 and 1A2 (CYP1A1 and CYP1A2) mRNA, protein, and activities. Precision-cut rat liver slices were incubated in dynamic organ culture for 24 hr with medium containing between 0.001 nM and 10 nM TCDD. The resultant mean TCDD concentration in the slices ranged from 19 to 80,925 ppt (wet wt). A dose dependent induction of CYP1A1 mRNA, protein, and activities and CYP1A2 mRNA was observed in liver slices at all doses of TCDD. The O-deethylation of 7-methoxyresorufin, a marker for CYP1A2 activity, was induced at TCDD doses of 0.01 nM and greater, while CYP1A2 protein was a less sensitive response being induced only at the higher doses. In comparison, similar liver concentrations of TCDD (8 to 64,698 ppt wet wt) were achieved at 24 hr following a single *in vivo* exposure to TCDD at doses ranging from 0.002 to 5 μg/kg, po. Additionally, similar tissue dose-response relationships for induction of CYP1A1 and CYP1A2 were also observed following *in vitro* and *in vivo* exposure to TCDD, although the magnitude of induction was significantly greater for *in vivo* exposure. The data lends support to the use of liver slices in dynamic organ culture for assessing the relative *in vivo* potency of a compound to induce CYP1A1 and CYP1A2. Human tissue can also be readily utilized in this *in vitro* model to predict the biological and toxicological effects of a given *in vivo* exposure to TCDD. (Supported by NIEHS grant R01 ES06556-01, Research Triangle Park, NC).

335 INDUCTION OF CYTOCHROME P4501A1 AND 1A2 (CYP1A1 AND CYP1A2) IN RAT AND HUMAN LIVER SLICES BY 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN (TCDD)

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Precision-cut liver slices in dynamic organ culture were utilized as an *in vitro* model to compare biomarker responses in rat and human tissue exposed *in vitro* to TCDD for 24 hr. The biomarker responses investigated include quantification of CYP1A1 and CYP1A2 mRNA, protein, and activities in rat and human liver slices. Incubation of liver slices for 24 hr with medium containing TCDD (0.001 nM to 10 nM) resulted in linear uptake of TCDD into the rat slices with a resultant mean TCDD concentration in the slices ranging from 19 to 80,925 ppt (wet wt). The uptake of TCDD in human slices was more variable over the same medium concentration of TCDD and resulted in mean TCDD levels in the slices ranging from 236 to 72,283 ppt (wet wt). A dose dependent induction of CYP1A1 mRNA, protein, and activity and CYP1A2 mRNA in rat slices was observed at all doses of TCDD. The O-deethylation of 7-methoxyresorufin, a marker for CYP1A2, was induced at TCDD doses of 0.01 nM and greater, while CYP1A2 protein was a less sensitive response being induced only at the higher doses. Preliminary human liver slice data suggest dose dependent induction of CYP1A1 mRNA at all doses of TCDD, whereas CYP1A2 mRNA and CYP1A1 and CYP1A2 activities were induced only at the higher doses of TCDD. Interestingly, the levels of CYP1A2 protein were not induced at any of the TCDD doses. These results indicate that precision-cut liver slices in dynamic organ culture is a useful and sensitive *in vitro* model to directly compare enzyme induction in rat and human liver. While these results suggest some species difference, additional studies are required, particularly in the assessment of biomarker responses following longer periods of exposure to TCDD. (Supported by NIEHS grant R01-ES06556-01).

336 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN: DOSE RESPONSE, TIME COURSE AND DIETARY ALTERATIONS IN LIVER CELL METABOLISM

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The environmental contaminant, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), is a very potent carcinogen and immunosuppressant agent. However, the possible actions of TCDD on hepatic parenchymal cell biometabolism, pertaining to dose response, time course and dietary alterations of TCDD-induced toxicity, have not been determined. The pharmacokinetics of TCDD, administered in a single oral dose of either 10 μg/kg or 25 μg/kg, using male Sprague Dawley rats as the experimental models were studied. Control animals received an single, equimolar, oral dose of corn oil/acetone (95%/5%, v/v). Three days, post-oral dose administration, selected animals were fasted for 24-hours. On day 4 hepatic parenchymal cells were isolated from either fed or 24-hour fasted animals and the isolated cells incubated (0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110 or 120 minutes) for determination of gluconeogenic and ureogenic alterations, as well as total liver cellular protein contents. TCDD, administered at the higher oral dose (25 μg/kg), as compared to the lower oral dose (10 μg/kg), produced significant alterations in the hepatic parenchymal cellular gluconeogenic (>35% decrease) and ureogenic contents (>20% decrease), in the fed as compared to the fasted animals, with a corresponding decrease in protein metabolism. Additionally, the alterations were dose, time course and dietary dependent. The control values were not significantly different. These studies indicate that various factors, including hepatic cellular gluconeogenesis, ureogenesis and total protein, in addition to other hepatic biochemical processes, might be involved in TCDD-induced biochemical alterations. (Supported by CDC/ATSDR/MHPF #U50/CCU 304522-04).

337 ADDITIVE INTERACTIONS OF MIXTURES CONTAINING POLYCHLORINATED DIBENZO-p-DIOXINS (PCDD), DIBENZO FURANS (PCDF) AND BIPHENYLS (PCB)

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PCDDs, PCDFs and PCBs are found in the environment as components of complex mixtures. Toxic Equivalency Factors (TEFs) have been developed to estimate the toxic potential of a mixture of these chemicals. The ability of TEFs, to predict the potency of mixtures containing PCDDs, PCDFs and PCBs was tested by comparing the induction potency of these mixtures to that of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Mixtures of these chemicals were prepared based on the relative concentrations of these chemicals found in food samples. The first mixture (MIX1) contained 2,3,7,8-TCDD (1 ng/ml), 1,2,3,7,8-PeCDD (1 ng/ml), 2,3,7,8-TCDF (1.5 ng/ml), 1,2,3,7,8-PeCDF (0.5 ng/ml), 2,3,4,7,8-PeCDF (2 ng/ml), 1,2,3,4,6,7,8,9-OCDF (5 ng/ml). The second mixture was prepared by adding the following PCBs to MIX1; 77 (150 ng/ml), 105 (6,000 ng/ml), 118 (30,000 ng/ml), 126 (45 ng/ml), 156 (1000 ng/ml), and 169 (30 ng/ml). Female B6C3F1 mice received either 0, 1.5, 15 or 150 ng

TCDD/kg/d or 0, 1.5, 15 or 150 ng TCDD equivalents/kg/d of the mixtures. Chemicals were administered by gavage in corn oil, 5d/wk for 13 wks. EROD activity was determined in liver, lung and skin3d after the last dose. Liver EROD activity was significantly induced at the lowest dose tested for TCDD and both mixtures. EROD activity in mice treated with 1.5 and 15 ng MIX1/kg were within 82% of animals treated with equivalent doses of TCDD. The high dose of MIX1 was 68 ± 5% of mice treated with TCDD. The low dose of MIX2 was 63 ± 8% of animals treated with a comparable dose of TCDD, while the two higher doses of MIX2 were at least 90% of animals treated with TCDD. Similar results were seen in skin and lung. The TEFs accurately predicted the potency of the mixtures suggesting that the interaction of these chemicals in environmentally relevant mixtures is additive. (This abstract does not necessarily represent EPA policy).

338 DIOXIN ACTIVATES HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 GENE EXPRESSION BY AN OXIDATIVE STRESS PATHWAY THAT REQUIRES A FUNCTIONAL CYTOCHROME P450 CYP1A1 GENE

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We have studied the effect of several xenobiotic compounds on the transient expression of a *cat* reporter gene directed by the U3 promoter sequences in the long terminal repeat (LTR) of the human immunodeficiency virus type 1. Aflatoxin B_1, benzo[a]pyrene, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD; dioxin) caused a significant increase of CAT expression in mouse Hepa-1 hepatoma cells. CAT induction after TCDD treatment was abolished by administration of N-acetyl-L-cysteine or 2-mercaptoethanol and required a functional cytochrome P450 Cyp1a1 enzyme, since it did not take place in a mutant cell line that lacks Cyp1a1 enzymatic activity. Linker scanning mutational analysis of transcription factor binding sites revealed that both the NFκB and an adjacent aromatic hydrocarbon response element (AhRE) were required for TCDD-dependent CAT expression in Hepa-1 cells. In addition, mutation of the NFAT/AP-1 binding sites in the negative regulatory region of the promoter, increased the magnitude of the TCDD effect. We conclude that induction of a functional Cyp1a1 monooxygenase by TCDD stimulates a pathway that generates thiol-sensitive reactive oxygen intermediates, which in turn are responsible for the TCDD-dependent activation of genes linked to the LTR.

339 TCDD-INDUCIBLE PROTEIN-DNA INTERACTIONS IN HUMAN B LYMPHOCYTE CELL LINES

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2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) has been shown to inhibit mitogen-stimulated proliferation and immunoglobulin production in normal human B lymphocytes. In order to identify possible molecular targets for TCDD in human B lymphocytes, we are using the gel mobility shift assay (MSA) to characterize the DNA-binding properties of the aryl hydrocarbon receptor (AhR) in the Epstein-Barr virus-positive human B lymphocyte cell lines, IM-9 and PJS-91. Nuclear extracts were prepared from IM-9 and PJS-91 cells treated for 3 hours with 25 nM TCDD and then analyzed by MSA with a 30 bp synthetic oligonucleotide probe containing a xenobiotic response element (XRE1) from the upstream region of the human *CYP1A1* gene. A TCDD-inducible protein-XRE1 complex was detected in both human B cell lines that comigrated with the TCDD-dependent band present in nuclear extracts from the AhR-positive human hepatoma cell line, HepG2. The TCDD-inducible band was competed for by excess unlabeled XRE1, but not by excess unlabeled XRE1 with a single base pair mutation in the AhR "core" binding motif. Pre-incubation of nuclear extracts from TCDD-treated HepG2 and PJS-91 cells with a human AhR polyclonal antisera (kindly provided by Dr. Chris Bradfield) prevented the formation of the TCDD-inducible complex. Saturation binding experiments with the XRE1 probe followed by Scatchard analysis showed the K_d of the AhR:XRE1 complex as well as the maximal amount of XRE1-binding activity (B_{max}) was similar in HepG2 and PJS-91 cells. In competition experiments, high concentrations (approximately 100-fold greater than the IC_{50} for the unlabeled XRE1) of two synthetic oligonucleotides with binding sites for a B cell transcription factor that resemble the AhR binding site partially inhibited AhR binding to the XRE1 probe in PJS-91 nuclear extracts. In conclusion, a TCDD-inducible XRE-binding activity has been identified in two human B lymphocyte cell lines using MSA, that by several criteria appears to be the AhR. Analysis of AhR:DNA interactions using MSA should prove useful in elucidating the molecular mechanism by which TCDD inhibits immunoglobulin production in human B cells.

340 EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN (TCDD) ON CELL-MEDIATED IMMUNITY IN SPRAGUE-DAWLEY RATS

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Recently the possibility of occurrence of immunological effects in humans at very low doses of TCDD has gained increasing attention. Extensive studies on the immunosuppressive effect of TCDD have been conducted in mice, but those results cannot explain some of the epidemiological data obtained in exposed humans. Therefore studies in other laboratory animal species were needed. The aim of this experiment was to examine effects of TCDD on cell-mediated immunity in rats using a delayed-type hypersensitivity (DTH) assay. Rats were immunized with keyhole limpet hemocyanin (KLH)-Freund's complete adjuvant (FCA) on day 0, challenged with heat-aggregated KLH on day 7, and sacrificed on day 8. The degree of reaction was measured as the difference in swelling of the footpad between the antigen-challenged site and the control site. A time-course study demonstrated that TCDD treatment on day −5 relative to immunization (day 0) produced the greatest effect on cell-mediated immunity. In a dose-response experiment, rats were treated with 1, 3, 10, 20, 30, 40 and 90 μg TCDD/kg. The effect of TCDD on cell-mediated immunity displayed an inverted U-shaped dose-response curve, in that low doses enhanced and high doses suppressed this immune function. This is the first study to demonstrate an U-shaped dose-response curve of TCDD on the immune system. This appears to be of importance for the risk assessment of dioxins because U-shaped dose-response curves cannot be modeled by currently used methods.

341 IMMUNOMODULATORY EFFECT OF 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN (TCDD) DEMONSTRATED BY THE POPLITEAL LYMPH NODE (PLN) ASSAY

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Drugs and other chemicals that have the potential to induce or exacerbate systemic autoimmune diseases in humans are of great concern. The aim of this study was to examine the immune disregulating potential of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) by using the popliteal lymph node (PLN) assay. Chlorpromazine (CPZ) was used as a reference compound for two reasons: (1) CPZ is known to elicit a positive response in this assay and (2) CPZ is a structural analogue of TCDD. Male Sprague-Dawley (S-D) rats were injected subcutaneously with either TCDD or CPZ into the right hind footpad whereas vehicle alone was injected into the contralateral footpad. Control rats were injected with vehicle in both hind footpads. Animals were sacrificed on day 7 and their PLNs were removed, weighed and fixed in 10% formalin. The PLN weight index (the weight ratio of right PLN over left PLN) was significantly higher in both CPZ- and TCDD-treated rats than in controls. Histological examinations of PLNs in the CPZ- and TCDD-treated rats revealed similar pathological changes in both groups, e.g. mild follicular hyperplasia with no evidence of an acute inflammatory response. These results indicate that TCDD has the potential to induce or exacerbate autoimmune-like reactions. Results also suggest that drugs may be useful surrogates to study the mechanism of toxicity of environmental chemicals which cannot be administered to humans.

342 2,3,7,8-TCDD-INDUCED ALTERATIONS IN ADULT AND HATCHLING CHICKEN IMMUNE FUNCTION

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Times Beach MO is undergoing remediation at present. Areas that contain TCDD levels of 1 ppb or higher have the upper layers of soil removed and replaced with clean backfill. Areas with less than 1 ppb TCDD are left as is. There are significant populations of wild turkey and geese on the site and there is concern about the effects of the remaining TCDD on these species. White Leghorn hens and roosters, as surrogate species, were dosed for six weeks with levels of 2,3,7,8-TCDD reflecting exposure to areas of Times Beach, MO. The eggs produced were collected and incubated. Approximately 1/3 of the weekly egg production was reserved for embryonic analysis. The remaining 2/3 of

the egg production was allowed to incubate to hatching. The chicks were injected with sheep red blood cells on day 1 and 8 following hatching. Adult birds were euthanized after 6 weeks of dosing and chicks were euthanized at 14 days of age. Blood samples and spleens were collected and processed as required for each assay. Hemagglutination titers were measured on the plasma and CD4/CD8 subset populations measured in lymphocytes isolated from the blood samples. Mitogen-induced T and B cell proliferation was measured in single cell preparations made from the spleens. A significant decrease in hemagglutination titer was observed at the higher dose in the hatchlings. Alterations in CD4/CD8 subset populations as well as T and B cell proliferation were also observed in the adults as well as the hatchlings. Supported by NIEHS ES04696.

343 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN (TCDD) DOWNREGULATES THE EXPRESSION OF THE CYCLIN DEPENDENT KINASES CDK1 AND CDK2 IN MCF-7 HUMAN BREAST CANCER CELLS

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Previous research has demonstrated that TCDD exhibits potent antiestrogenic activity and inhibits development of mammary tumors in rodents. This study examined the expression of cyclin dependent kinases (CDKs), a family of cell division control enzymes, in human breast cancer cells (MCF-7) exposed to TCDD or estradiol (E2). MCF-7 cells were cultured in the presence of 0, 0.01, 0.1, 1, 10, 100 or 1000 nM TCDD or E2 for 24 to 120 hours and whole cell lysates were prepared. The resulting cell lysates were separated on an SDS PAGE gel and CDK1 and CDK2 were identified from immunoblots using α-CDK1 and α-CDK2 polyclonal antibodies. An ELISA technique was used to quantify total cellular CDK1 and CDK2. TCDD decreased the expression of CDK1 and CDK2 in a dose-dependent manner in the MCF-7 cell lysates with maximal decreases at approximately 1000 nM (51%) and 100 nM (54%) nM, respectively. Half-maximal decreases of CDK1 and CDK2 were estimated to occur at 100 and 12 nM TCDD. Release of CDK1 and CDK2 into the culture medium by MCF-7 cells exposed to TCDD was decreased 50 to 80%, respectively, over the doses tested. In contrast to TCDD, E2 increased the expression of CDK1 and CDK2 in the MCF-7 cell lysates 1.6- and 1.9-fold. Release of CDK1 and CDK2 into the culture medium by MCF-7 cells exposed to E2 was increased 1.5- and 1.7-fold, respectively. The results demonstrate that TCDD downregulates and E2 upregulates proliferative signals in MCF-7 cells.

344 2,3,7,8-TCDD-INDUCED ALTERATIONS IN CHICK EMBRYO VIABILITY, MORPHOLOGY AND VITELLINE VASCULATURE

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Reproductively active White Leghorn hens and roosters were dosed for six weeks with levels of 2,3,7,8-TCDD reflecting exposure to the areas of Times Beach MO that will be remediated (≈200 ppb TCDD) and those areas that will not be remediated (<1 ppb TCDD). The eggs produced were collected and incubated. Approximately 1/3 of the weekly egg production was reserved for embryonic analysis. These eggs were removed from the incubator on embryonic days 2 and 4 and the embryos removed and analyzed. In addition, those embryos appearing non-viable at embryonic days 5, 9, 14 and 18 were removed and examined. The yolk, albumin and chorionic allantoic membranes from these eggs were retained for future residue analysis. The remaining 2/3 of the egg production was allowed to incubate to hatching. In a related study, fertile eggs were injected with doses of TCDD from 2 pg/g egg to 20 ng/g egg and treated as above to provide quantitative dose response relationships by which TCDD transferal from hens to eggs could be estimated. Time and dose-dependent alterations in embryo viability, morphology and vitelline vasculature were observed. Changes were apparent at the lowest dose of TCDD used to directly inject the eggs. Supported by NIEHS ES04696.

345 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN-INDUCED ALTERATIONS IN ADULT AND HATCHLING CHICKEN ENDOCRINE FUNCTION AND CYP1A1/2 ACTIVITY

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Areas of Times Beach MO currently contain ≈200 ppb TCDD and these will be remediated to <1 ppb TCDD. This site contains populations of wild turkey and concern has been voiced as to the impact of these levels of TCDD on this and other wildlife species. Reproductively active White Leghorn hens and roosters were selected as surrogate species and dosed for six weeks with levels of 2,3,7,8-TCDD reflecting exposure to the site. Approximately 1/3 of the weekly egg production was reserved for embryonic analysis and remaining 2/3 of the egg production was allowed to incubate to hatching. Adult birds were euthanized after 6 weeks of dosing and chicks were euthanized at 14 days of age. Blood samples, livers, egg shell glands and testes were collected in both cases and processed as required for each assay. 17β-estradiol and testosterone levels were measured by radio immune assay. Estrogen and progesterone receptor binding activity was measured in preparations from the egg shell gland. Cyp1A1/2 activities were measured in the liver samples. A dose and time dependent increase was observed in EROD and MROD activities in both hatchlings and adult birds. A dose-dependent decrease was observed in adult chicken plasma 17β-estradiol levels and egg shell gland ER and PR binding. No significant changes were observed in adult rooster testosterone levels or hatchling estradiol -to- testosterone ratios. Supported by NIEHS ES04696.

346 RESPONSIVENESS OF THE HUMAN OVARIAN CARCINOMA CELL LINE, BG-1 TO 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN (TCDD)

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Evidence indicates that ovarian hormones play a role in the hepatocarcinogenic activity of TCDD in the rat. This suggests that the ovary may be a target organ for TCDD-mediated effects. We investigated the responsiveness of the human ovarian carcinoma cell line, BG-1 to *in vitro* exposure to various concentrations of TCDD. TCDD caused a dose-dependent increase in cell proliferation as indicated by increased cell number following an 18 hour exposure. A similar dose-dependent induction of CYP1A1 mRNA and protein occurred with TCDD treatment. The EC50 for cell-proliferation and induction of CYP1A1 activity was approximately 200 picomolar TCDD. The results demonstrate that the BG-1 cell line is extremely sensitive to the effects of TCDD, and therefore possesses a functional Ah receptor system. In addition, BG-1 has been shown to express functional receptor systems for estrogen and epidermal growth factor. Therefore, this cell line may be useful as a model system to investigate the interactions of various receptor systems that control cell growth and differentiation.

347 EFFECTS OF GESTATIONAL AND LACTATIONAL EXPOSURE TO PCB 77, PCB 126 OR TCDD ON THYROID HORMONES IN WEANLING SPRAGUE-DAWLEY RATS

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Adult exposure to coplanar PCBs or TCDD has been shown to depress thyroid hormone concentrations, but doses in the toxic range are required to achieve sizable effects. This study measured the effects of gestational and lactational exposure to low levels of these chemicals on thyroid hormone levels at weaning. Time-mated Sprague-Dawley rats were exposed to 2 or 8 mg/kg/day PCB 77 (33′44′-tetrachlorobiphenyl), 0.25 or 1.0 μg/kg/day PCB 126 (33′44′5-pentachlorobiphenyl), 0.025 or 0.10 μg/kg/day TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) or corn oil vehicle via gavage on gestation days 10–16. No effects on maternal weight gain, gestation length, litter size, % live births, birth weights or pup survival were observed. At weaning, plasma thyroxine (T^4) levels in female pups from the PCB 77 and TCDD high dose dams were significantly depressed, but the changes were modest (15–20% lower than controls). T^4 levels in PCB 126-exposed female pups and male pups from all three high dose groups were also depressed slightly, but the changes were not statistically significant. Triiodothyronine (T^3) and thyrotropin (TSH) levels were unchanged in all groups. In contrast, ethoxyresorufin-O-deethylase (EROD) activity was markedly induced in all exposed groups and thymus weights were decreased in the high dose groups. UDP-glucuronosyl transferase (UDP-GT) activity towards 4-nitrophenol was also increased in the high dose groups. Thus, the modest decreases in plasma T^4 levels in these groups could be due in part to increased T^4 glucuronidation by UDP-GT. The doses caused a sizable Ah response and striking behavioral changes (Seo et al., 1994 *Neurosci Abs,* 20:1661), but only minor changes in T^4. We conclude that changes in thyroid hormones are not one of the more sensitive effects of perinatal exposure to these compounds. Supported by Health Canada #4028 to SLS and NIH #ES01332 to REP.

348 EFFECT OF 2,3,7,8-TETRACHLORODIBENZODIOXIN (TCDD) ON EGF RECEPTOR BINDING AND CYP1A1 EXPRESSION IN AN HUMAN ENDOMETRIAL CELL LINE

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At present the biological action of 2,3,7,8-tetrachlorodibenzodioxin (TCDD) in humans is controversial. Chronic exposure to low levels of TCDD has been recently reported to increase the incidence of endometriosis in female Rhesus monkeys (Rier et al, 1994). The present study was undertaken to investigate the potential role of TCDD in uterine growth. An human endometrial adrenocarcinoma cell line (RL95-2) was utilized in these studies. Western immunoblot analysis using an antiserum against CYP1A1/CYP1A2 showed that maximal induction of CYP1A1 was observed at 1 nM TCDD. ^{125}I-EGF radioligand binding studies performed on control RL95-2 cells showed an increase in total specific binding from 10% to 20% during the first 48h followed by a return to 10% by 96h. Exposure of confluent cultures to 10 nM TCDD in serum-free media for 48h resulted in a 14% increase in total specific binding over controls. The EC_{50} for competitive displacement with unlabelled EGF for control and 10 nM TCDD treated cells was 1.6 nM and 1.8 nM, respectively. Treatment of sub-confluent cultures over a 96h period with 1–100 nM TCDD showed a time-dependent alteration in specific ^{125}I-EGF binding. Cell membrane preparations of the control and TCDD treated RL95-2 cultures were analysed in western immunoblots using an EGF receptor antisera. Densitometric analysis showed a 20%–30% increase in TCDD treated (100 nM) over controls at 72h. TCDD did not have any effect on the proliferation of these cells as determined by the non-radioisotopic MMT assay. In summary, the data showed a dose dependent induction of CYP1A1 by TCDD, but no apparent down-regulation of EGF binding or membrane protein in this cell line. (Supported by Grant ES 04435).

349 EXPRESSION OF Ah RECEPTOR AND ARNT IN MOUSE EMBRYONIC URINARY TRACT

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2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is an environmental contaminant that induces hydronephrosis after either pre- or postnatal exposure in C57BL/6N mice. TCDD induces hyperplasia of ureteric epithelium and blocks flow of urine. TCDD binds to the AhR and forms a heteromeric complex with the AhR nuclear translocator protein (ARNT). This complex binds to the xenobiotic response element in the nucleus, regulating transcription of targeted genes. The present study localizes expression of AhR and ARNT in the developing urinary tract of control and TCDD-exposed embryos. Pregnant C57BL/6N mice were dosed on gestation day (GD) 10 with either corn oil or TCDD at 12 μg/kg. Fetuses were removed on GD 12, 13, and 14 and the lower dorsal torso was dissected and fixed in 3% paraformaldahyde (PA), embedded in paraffin and sectioned. Ten specimens of each age and treatment were assayed immunohistochemically with polyclonal antibodies against mouse AhR (G. Clark, 8 μg/ml) and human ARNT (M.R. Probst, 1.1 μg/ml). In controls, the expression of both AhR and ARNT in epithelial cells of ureter and metanephric collecting ducts increased from GD 12, when there was little or no expression, to stronger expression on GD 14. At a cellular level, AhR expression shifted over GD 12–14 from blotchy cytoplasmic, to evenly cytoplasmic with low nuclear expression, to more uniform localization in both nucleus and cytoplasm. ARNT was initially cytoplasmic and nuclear on GD 12–13 and by GD 14 was more pronounced in the nucleus. In TCDD-treated urinary tracts, AhR and ARNT had a similar developmental pattern and there were no strong treatment effects on expression of these proteins. Thus, urinary tract epithelial cells expressed AhR and ARNT and these proteins may play a role in the normal maturation and function of the kidney and ureter. (This abstract does not necessarily reflect EPA policy.)

350 IMMUNOLOCALIZATION OF ARYL HYDROCARBON RECEPTOR (AhR) IN FEMALE RAT REPRODUCTIVE ORGANS

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Exposure of female rats to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) causes reduced fertility and litter size and possible suppression of the estrous cycle. TCDD acts through the AhR, but the distribution of AhR in female reproductive organs was unknown. Therefore, our objective was to immunohistochemically localize AhR in adult female rat reproductive organs using an antibody against mouse AhR. Ovary, oviduct, uterus, and vagina from 9-week-old Holtzman rats were fixed in Bouin's, embedded in paraffin and sectioned at 5 μm. Deparaffinized sections were treated with normal goat serum, then incubated with polyclonal rabbit anti-mouse AhR (1 μg/ml) or preabsorbed anti-mouse AhR. Antibody binding was detected using streptavidin-peroxidase. Sections treated with proabsorbed anti-AhR were negative while those treated with anti-AhR were positive. In all organs, smooth muscle in blood vessels was strongly positive. In the ovary, oocytes were very strongly stained for AhR while granulosa cells were negative. Corpora lutea and connective tissue were mildly positive. In oviduct and vagina, epithelium and smooth muscle were positive, but the fibroblastic stroma was negative. Uterine myometrium was positive, glandular and luminal epithelium were lightly positive and fibroblastic stroma was negative. These results suggest that ovary, oviduct, uterus, and vagina may be target organs of TCDD and that TCDD may have direct effects on these organs. The intense immunostaining for AhR in oocytes also indicates these cells may be a major target of TCDD, and this may partially explain TCDD effects on fertility. (Supported by NIH grant ES01332)

351 IN UTERO AND LACTATIONAL EXPOSURE TO 2,3,7,8-TETRA CHLORODIBENZO-p-DIOXIN (TCDD) AFFECTS THE GROWTH AXIS IN 21 DAY OLD FEMALE RATS

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The secretion of growth hormone (GH) is controlled primarily by growth homone-releasing hormone (GHRH), and inhibited by somatostatin (SS). Recently, a novel neuropeptide, galanin (GAL) has also been shown to be a modulator of GH activity both at the level of the hypothalamus and the pituitary. Acute administration of TCDD has been shown to inhibit GH secretion in adult male rats. This study was done to examine the effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on the mRNA expression of GHRH, SS, GAL, and GH in an effort to characterize the disruption of the GH-axis caused by TCDD during development. Pregnant rats were orally dosed with 1.0 μg/kg TCDD or vehicle on day 15 of gestation. Female offspring were subsequently studied on day 21 of age. Messenger RNA was measured using an RNAse protection assay. At the hypothalamic level, *in utero* and lactational exposure to TCDD decreased GHRH mRNA while SS mRNA was not altered as compared to controls treated rats. At the level of the pituitary, TCDD increased GAL mRNA as compared to control rats while there was no change in GH mRNA. At the systemic level, TCDD increased serum GH concentrations versus control rats as measured by GH RIA. This study suggests that the changes in GAL at the level of the pituitary may play an important role in the mechanism of growth disruption seen with TCDD. (The GAL clone was a gift from M. Vrontakis. This work was supported in part by grants ES04184, ES01332 and DK01874 from the NIH).

352 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN (TCDD) INCREASES RELEASE OF GONADOTROPINS (LH AND FSH) FROM THE PITUITARY OF IMMATURE FEMALE RATS *IN VIVO* AND *IN VITRO*

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Recent findings that serum levels of LH and FSH were dramatically increased in weanling female Sprague-Dawley rats treated with TCDD prompted a more detailed study to determine the time course and dose-responses of these effects. Single doses (0.03–30 μg/kg) of TCDD, dissolved in corn oil, were administered orally by gastric intubation to 22 day old female rats; control animals received vehicle only. Trunk blood was collected after decapitation at various time intervals during the following 72hr. Concentrations of LH and FSH were determined in serum by radioimmunoassay (RIA) using kits supplied by NIH. Two distinct peaks of both hormones were detected. The first, at 1hr, appeared to be a nonspecific response to dosing as it was present in both control and TCDD-treated animals. The second peak, at 24hr, occurred only in animals dosed with TCDD. Gonadotropin levels in these animals were dose-dependently elevated. The ED50 was about 5 μg/kg with a maximum elevations of 15 and 20 fold, for LH and FSH, respectively. The effect of TCDD

was also examined in pituitaries excised from immature female rats. Pituitaries were cut in half and incubated for 4hr in Krebs-Ringer bicarbonate buffer with or without gonadotropin releasing hormone (GnRH) and/or TCDD, dissolved in DMSO. The amount of LH and FSH released into the media was measured by RIA. TCDD caused a dose-dependent release of both LH and FSH with a ED50 of about 0.1 nM. The effect of combined GnRH/TCDD treatment was additive, suggesting similar mechanism(s) of action.

353 INDUCTION OF CYP1A1/2 ACTIVITY AND ANTIESTROGENIC EFFECTS BY 3-MC AND 2,3,7,8-TCDD IN THE DEER MOUSE (*PEROMYSCUS MANICULATUS*)

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Previous research in this laboratory has shown that exposure of deer mice to both halogenated aromatic hydrocarbons and polynuclear aromatic hydrocarbons resulted in significant immuno-suppression at dose levels at or less than those which produced significant CYP1A1 induction as measured by ethoxyresorufin-O-dealkylase (EROD) activity. Similar results were obtained whether exposure was by IP injection or by exposure to contaminated soil. The doses required for these effects were 10- to 100-fold less than reported for the C57B1/6 mouse. PAHs and HAHs have been shown to inhibit 17β-estradiol-induced responses in laboratory rodents, although at higher doses than required for significant induction of CYP1A1 activity or immune suppression. Deer mice were injected with either 3-methylcholanthrene (3-MC) or 2,3,7,8-TCDD in combination with 17β-estradiol to determine the relative sensitivity of antiestrogenic effects as measured by changes in uterine wet weight, estrogen receptor (ER) and progesterone receptor (PR) binding activity to the induction of CYP1A1/2 activities as quantitated by EROD and MROD measurements. Although significant changes in uterine wet weight, ER and PR binding were observed following 3-MC and 2,3,7,8-TCDD treatment, there was no substantial increase in the relative sensitivity of these endpoints as compared to CYP1 activity or immuno-suppression as biomarkers for field assessments. Supported by NIEHS ES04696.

354 REGULATION OF GENE EXPRESSION AND CELL PROLIFERATION IN HUMAN PLACENTAL CHORIO-CARCINOMA CELLS BY 2,3,7,8-TETRACHLORO-DIBENZO-p-DIOXIN

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2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) has been shown to alter the expression of a number of specific growth regulatory factor genes in human keratinocytes (Gaido et al, 1992). The present study characterized BeWo and JEG-3 human placental trophoblast choriocarcinoma cells as a potential model for study of TCDD-related changes in gene expression. Northern blot analysis detected mRNA transcripts for epidermal growth factor receptor (EGFR), plasminogen activator inhibitor-2, interlukin-1β, transforming growth factor-α and -β1. Exposure to TCDD for 48 h induced cytochrome P450 IA1 (CYP IA1) mRNA levels in a concentration dependent manner in both cell lines. The mRNA level of CYP IA 1 was maximally induced 50-fold in BeWo cells and 20-fold in JEG-3 cells at 10 nM TCDD, respectively, with levels progressively increasing from 6 h to 72 h following TCDD exposure. In contrast, the steady state mRNA level of EGFR was not changed by TCDD treatment in either cell line. Cell proliferation in both cell lines was unchanged following exposure to 0.1–100 nM TCDD in the presence of serum. However, proliferation under serum-free conditions was decreased by 24–35 % in BeWo cells, and by 23–40 % in JEG-3 cells following exposure to 50 and 100 nM TCDD, respectively. In summary, the data indicate that it is feasible to investigate TCDD-regulated genes and to explore the potential placental biomarkers in the BeWo and JEG-3 trophoblastic choriocarcinoma cell lines. (Supported by ES04435).

355 MODULATION OF RAT HEPATOCYTES GAP JUNCTIONAL INTERCELLULAR COMMUNICATION BY 2,3,7,8-TETRACHLORODIBENZO-[p]-DIOXIN (TCDD)

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TCDD has been widely studied over the past years in an effort to understand its toxic and carcinogenic potential. Chronic feeding studies of TCDD to F344 rats resulted in an increased hepatic tumor incidence. TCDD appears to function as a nongenotoxic carcinogen and when tested in an initiation/promotion protocol was shown to be a liver tumor promoter. Most tumor promoters examined to date have the ability to modulate gap junctional intercellular communication (GJIC) in the target tissue. TCDD, however has been previously reported to have no effect on GJIC in 10T1/2 and V79 cell lines. In the present study the effect of TCDD on primary cultured rat hepatocyte GJIC was examined. TCDD at subcytolethal concentrations (0.01, 0.1, 1.0 and 10.0 nM), produced a dose responsive decrease in rat hepatocyte GJIC after 4, 24 and 48 hrs of treatment. Removal of TCDD following a 4 hr treatment, failed to return GJIC to control values. Coincubation with either alpha-naphthoflavone or 8-bromo cAMP prevented blockage of GJIC by TCDD. Northern blot analysis of hepatocytes treated with TCDD showed a 2X decrease in GJ Cx 32 mRNA when compared to control. GJ Cx 26 mRNA showed no change. These results indicate that TCDD has the ability to inhibit GJIC in primary cultured rat hepatocytes in a dose responsive manner and that the inhibition of the GJIC may be related to modulation of gap junction gene expression by TCDD.

356 INTERACTIONS BETWEEN TCDD AND WEIGHT INCREASING FACTORS

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2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) causes a wasting syndrome with hypophagia and weight loss in rats. TCDD was studied with factors causing obesity. Ventromedial hypothalamic (VMH) lesions were made causing metabolic changes and hyperphagia leading to obesity. The bilateral lesions were performed by passing 1.0–1.3 mA anodal current in VMH under ketamine-medetomidine anesthesia and stereotaxic control. The wasting syndrome caused by TCDD appeared to be interactively aggravated in VMH-lesioned rats irrespective if the rats were already obese due to the lesions or not. This was the case in a TCDD-susceptible and a TCDD-resistant rat strain. Like VMH-lesioned rats, genetically obese Zucker rats showed aggravated wasting syndrome. In contrast, rats obese due to intragastric feeding or high-energy food lost weight equally or less than their lean counterparts after TCDD. Death was delayed with high-energy food after a lethal TCDD dose.

It is concluded that VMH is not the site of action of TCDD. In addition, weight increasing factors had either interactive or additive effects with TCDD. Thus, approach with TCDD and another weight changing factor could reveal new information about both the regulation of body weight and the mechanisms of wasting syndrome. (Supported by the Academy of Finland.)

357 TCDD-INDUCED CHANGES IN TRYPTOPHAN (TRP) IN RATS: ASSOCIATION WITH TCDD LETHALITY

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We have previously shown that TCDD increases brain TRP and subsequently serotonin metabolism in TCDD-sensitive L-E but not in TCDD-resistant H/W rats. Since TRP is normally bound to albumin in the circulation, the ratio of bound/free TRP regulates its tissue levels. Indeed, free TRP increased in parallel with brain TRP in TCDD-treated rats and the changes emerged in the dose range known to elicit lethality in L-E rats. A good correlation was found between changes in plasma bilirubins and free fatty acids with free TRP in L-E rats treated with TCDD. However, only free fatty acids were able to increase free tryptophan *in vitro* and they did so in a physiologically relevant concentration range. In TCDD-treated L-E rats, the maximum binding capacity of albumin for tryptophan was decreased. Again, a similar decrease was seen, if free fatty acids were added into rat plasma *in vitro*. Furthermore, when the plasma of TCDD-treated rats was extracted with aether to remove free fatty acids, the increase in free TRP could be reversed. These data show that increase in free TRP and TCDD lethality are tightly associated. Furthermore, the increase in free TRP may be mediated via disturbed lipid metabolism in TCDD-treated rats. Supported by the Academy of Finland.

358 SUBCHRONIC TOXICITY OF A MIXTURE OF FOUR CHLORINATED DIBENZO-p-DIOXINS (CDDs) IN SPRAGUE-DAWLEY RATS. PART II: BIOCHEMICAL OBSERVATIONS

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Groups of 20 male and 20 female rats were given five different oral doses

of a mixture of 2,3,7,8-tetraCDD, 1,2,3,7,8-pentaCDD, (PCDD) 1,2,3,4,7,8-hexaCDD (HxCDD) and 1,2,3,4,6,7,8-heptaCDD divided into four daily loading doses and six biweekly maintenance doses (see Viluksela et al. ibid). The dosing period was 13 weeks after which half of the rats was necropsied and the rest allowed to recover for a period of another 13 weeks. At the end of the dosing period liver ethoxyresorufin O-deethylase activity was dose-dependently increased whereas the activity of phosphoenolpyruvate carboxykinase was dose-dependently decreased in rats dosed with the mixture as well as in the positive control groups. Serum thyroxine (T4) concentrations also showed a dose-dependent decrease in rats given the mixture and were also decreased in the PCDD and HxCDD dosage groups, with virtually no sign of recovery in any treatment group after 13 weeks off-dose. Serum triiodothyronine (T3) levels were not affected. The results demonstrate that comparable biochemical changes occur after single as after multiple dosing with CDDs containing chlorine substituents in the 2,3,7 and 8-positions and that TEFs derived from acute studies can be used to predict the toxicity of mixtures of CDDs regardless whether they are administered as single compounds or as a mixture. This study strongly supports the validity of the concept of additive toxicity for CDDs currently used in the risk assessment of these compounds. (This abstract does not necessarily reflect EPA policy.)

359 SUBCHRONIC TOXICITY OF A MIXTURE OF FOUR CHLORINATED DIBENZO-p-DIOXINS (CDDs) IN SPRAGUE-DAWLEY RATS. PART I: STUDY DESIGN AND GENERAL OBSERVATIONS

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Groups of 20 male and 20 female rats were given five different doses of a mixture of 2,3,7,8-tetraCDD (TCDD), 1,2,3,7,8-pentaCDD, (PCDD) 1,2,3,4,7,8-hexaCDD (HxCDD) and 1,2,3,4,6,7,8-heptaCDD (HpCDD). The doses were selected based on the relative potency factors derived from acute toxicity data and a previous subchronic study with HpCDD. The mixture was constituted such that each of the homologues contributed one fourth of the total toxicity. Total doses were divided into four daily loading doses and six biweekly maintenance doses. The highest total dose for males was 17.5 μ/kg of TCDD, 87.5 μg/kg of PCDD, 350 μg/kg of HxCDD and 2500 μg/kg of HpCDD. Positive controls were administered PCDD (350 μg/kg) or HxCDD (1400 μg/kg). Females were given 1.5 times lower doses. The dosing period was 13 weeks, after which half of the rats was necropsied and the rest allowed to recover for another 13 weeks. Body weight gain was dose-dependently reduced throughout the study. Mortality rates in the recovery groups were 80 and 60% for the two highest dose groups (mixture) in males and 70 and 0% for females. Clinical signs and necropsy findings suggested that the cause of death was related to wasting, hemorrhage or anemia. Liver weights were increased, whereas thymus weights were decreased. Prothrombin times were prolonged and platelet counts decreased in some rats receiving high doses. The outcome of this study provides *in vivo* support for the validity of the assumption of additive toxicity of CDDs currently used in the toxicity equivalency factor (TEF) approach to estimate the toxicity of mixtures of CDDs. (This abstract does not necessarily reflect EPA policy.)

360 ELIMINATION PROFILES IN A SUBCHRONIC STUDY OF 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN (TCDD) IN MICE

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Studies in our laboratory have examined the effects of subchronic exposure on the dose-response relationships of various polyhalogenated aromatic hydrocarbons in the female B6C3F1 mouse. Repeated low-level exposures mimic the most probable modes for human exposure, including dietary consumption. In addition, the steady-state kinetics resulting from repeated dosing permits more accurate assessment of the relative potency of these compounds. The present study describes the elimination of TCDD in female B6C3F1 mice treated by oral gavage with either 1.5 or 150 ng [^{3}H]-TCDD/kg/day (Mon–Fri) for 13 wks. Animals were housed in individual metabolism cages, and daily urinary and fecal excretion of TCDD-derived radioactivity were measured throughout the treatment period. Over the 91 days of treatment, cumulative excretion of TCDD-derived radioactivity (as % administered dose) for low and high dose groups were 12 and 14% (urinary) and 35 and 46% (fecal), respectively. The feces from various time points were extracted (soxhlet), and the extract was analyzed by reverse-phase HPLC. The relative amounts of parent and metabolite in the feces reflected the dosing regimen and the approach, over time, of steady state conditions. During the final week of treatment, the high dose group had between 20 and 40% of the radioactivity in the feces present as parent compound. (This abstract does not necessarily reflect EPA policy.)

361 SUBCHRONIC DISPOSITION OF 2,3,7,8-TETRACHLORO-DIBENZO-p-DIOXIN (TCDD) IN MICE

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In order to evaluate human health risks to dioxins, it is necessary to know tissue levels consequent to human exposure. Food consumption is a major daily source of body burden in humans. Therefore, information is needed to estimate steady-state concentrations at low, repeated exposures. The present study was designed to determine tissue distribution and elimination of TCDD following oral gavage of 1.5 or 150 ng [^{3}H]-TCDD/kg/day (Mon–Fri) for 13 wks. During treatment, female B6C3F1 mice were housed in individual metabolism cages for 91 days with daily collection of excreta. At termination, more than 20 tissues were collected. Total radioactivity was measured in tissues & excreta. TCDD demonstrated dose-dependent kinetics. Dose-related differences in liver-to-fat concentration ratios were noted (0.36 vs 4.10, low and high doses respectively). Cumulative excreta of % dose for low and high doses were 12 and 14 (urinary) and 35 and 46 (fecal), respectively. The excreta profiles suggested TCDD was at or near steady state conditions. Preferential distribution of dioxin in fat at the low dose under steady-state conditions may be similar to what occurs in man at low environmental concentrations. Thus, studies employing steady-state exposure of dioxin and its congeners should be used for high-to-low dose extrapolations in risk assessment.

(This abstract does not necessarily reflect EPA policy.)

362 SUBCELLULAR DISTRIBUTION OF 2,3,7,8-TETRACHLORO-DIBENZO-p-DIOXIN (TCDD) IN MICE

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In order to fully understand the potential health risks associated with dioxin exposure, it is necessary to determine a dose response relationship. The most potent compound among the polyhalogenated aromatic hydrocarbons is TCDD. The present study was designed to understand better the subcellular distribution of TCDD in liver and kidney in mice. Animals received 1.5 or 150 ng/kg/day of [^{3}H]-TCDD for 120 days by oral gavage and were killed 123 days after the first treatment. During necropsy, the kidney and liver were removed and homogenized. The homogenate was centrifuged at 9000 × G for 20 minutes to separate the pellet fraction (P9) and supernatant (S9). The S9 fraction was further centrifuged at 100,000 × G for 60 minutes to separate the cytosol fraction (S100) and the microsome fraction (P100). Aliquots of the P9, S9, P100, and S100 fractions were oxidized and radioactivity determined to quantitate the amount of TCDD present in each. The results show dose-dependent distribution in liver, but not in kidney. The relative amount of TCDD is greater in liver P9 than in liver S9 at the low dose, but is smaller in P9 than S9 at the high dose. The relative amount of TCDD is greater in liver P100 than S100 at both doses, but is significantly increased at the high dose. The relative amount of TCDD is greater in kidney P9 than in kidney S9, and greater in kidney S100 than kidney P100. There was no apparent increase in the deposition of TCDD from low to high dose in the kidney. Therefore, the deposition of TCDD is more concentrated in the microsomes of the liver than in the microsomes of the kidney, which may be caused by the sequestration of dioxin by binding proteins present in the liver. This data is consistent with the hypothesis of an inducible, microsomal binding site in the liver believed to be CYP1A2.

(This abstract does not necessarily reflect EPA policy.)

363 DISTRIBUTION OF TCDD BETWEEN BLOOD COMPONENTS

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The distribution of 2,3,7,8-tetrachlorodibenzodioxin (TCDD) in human and

rat blood was investigated. Blood was collected from healthy male human volunteers or male Sprague-Dawley rats. 1 ml of whole blood was incubated with 0.2 ng ^{3}H-TCDD (0.68 fmol; 0.02 μCi, spec. act. 28 Ci/mmol) for 1 h and plasma separated from red blood cells (RBC) by centrifugation (1500 g). 10 ml of plasma were incubated with 2.2 ng ^{3}H-TCDD (6.8 fmol) for 1 h and separated by density gradient ultracentrifugation in 2 steps (step 1: 2h, 288000 g, yielding 3 fractions: VLDL, LDL, HDL + plasma proteins; step 2: twice 2h, 412000 g, yielding 2 fractions: HDL, plasma proteins). Fractions were examined for purity by cellulose acetate electrophoresis. Radioactivity was quantitated in a scintillation counter. Cholesterol, triglycerides, phospholipids and free glycerol were determined enzymatically, protein concentrations either by the biuret method or after reaction with pyrogallol red. The distribution of ^{3}H-TCDD was in human blood: 24 ± 4% in RBC, 12 ± 2% in plasma proteins and 61 ± 5% in lipids; in rat blood: 37 ± 8% in RBC, 24 ± 6% in plasma proteins and 36 ± 11% in lipids. The amount of TCDD recovered in the respective lipid fractions was directly proportional ($r^2 > 0.97$) to the sum over their neutral lipid content. 50 μl of lipoprotein free plasma was incubated with 1.1 ng ^{3}H-TCDD (0.34 fmol; 0.1 μCi) for 1 h, fractionated by agarose gel electrophoresis and the distribution of ^{3}H-TCDD assessed in remelted gel slices. 50–70% of the radioactivity recovered from the gel was associated with albumin. The remainder was evenly distributed between the point of application and the albumin peak.

364 THE SUBCHRONIC TOXICITY OF LIPOSOME ENCAPSULATED NYSTATIN IN THE CANINE

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Nystatin is a macrocyclic lactone antifungal antibiotic. As this material is absorbed only very poorly from the gastrointestinal tract, producing minimal blood levels, its utility has been limited to clinical cases treatable only via local application. However, there is therapeutic value in a systemically deliverable form of Nystatin. The purpose of this study was to investigate the toxicology of liposome encapsulated Nystatin (Nystatin^{LF}) administered intravenously to dogs for a ninety day time period. In this study, isolated animals showed statistically significantly increased red blood cell counts, hematocrits and hemoglobin values after an exposure to 1.0 mg Nystatin^{LF}/kg/day for one week. Dogs were also observed to demonstrate statistically significant elevations in serum creatinine and blood urea nitrogen after four weeks exposure to a dose of 2 mg Nystatin^{LF}/kg/day. Histopathological changes were noted in the kidneys of all dogs, in a dose related manner after ninety days exposure of dogs to both 1.0 and 2.0 mg Nystatin^{LF}/kg/day. The histomorphological changes included tubular regeneration, tubular dilatation and nephrosclerosis. The renal changes were observed to persist after a 28 day recovery period and were not completely reversible. However, no signs of delayed toxicity were observed to develop in the test animal population during the post exposure recovery period. The data from this study suggest a no observed adverse effect level (NOAEL) of 0.2 mg/kg/day.

365 *IN VITRO* NEUROTOXICITY ASSESSMENT OF OXIDATIVE AND REDUCTIVE METABOLITES OF HALOPERIDOL

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Previous studies suggested that the development of movement disorders (tardive dyskinesia) in schizophrenic patients given haloperidol (HP) results from the neurotoxic action of the pyridinium metabolite, HPP$^+$. We have explored the effects of HPP$^+$, reduced HPP$^+$ (RHPP$^+$), and the tetrahydropyridine derivative (HPTP) on biogenic amine neurochemistry in mouse brain synaptosomes and compared them to the actions of the dopaminergic toxin 1-methyl-4-phenylpyridinium (MPP$^+$). All the compounds blocked serotonin and dopamine uptake, and with the exception of MPP$^+$, had a higher inhibitory potency in the serotonergic system. In virtually all cases, the inhibition of transport was reversed by recentrifugation of the synaptosomes to remove free drug, suggesting that inhibition was via interaction with the transporter, and not through loss of synaptosome viability. MPP$^+$ stimulated the release of preloaded dopamine and serotonion, and this effect was blocked by the transport inhibitors mazindol and fluoxetine, respectively. HPP$^+$ and HPTP were more effective in releasing dopamine and serotonin than RHPP$^+$, but their effects were insensitive to transport antagonists. This finding indicates that although they interact with the transporters to block uptake, the haloperidol metabolites are apparently poor substrates and must use a different route of entry into cells. The parent compound HP was less active than HPP$^+$ or HPTP, which confirms the role of bioactivation for expression of toxicity.

366 CHRONIC TOXICITY ASSESSMENT OF RECOMBINANT HUMAN BASIC FIBROBLAST GROWTH FACTOR IN CYNOMOLGUS MONKEYS

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Recombinant growth factors are currently being developed to provide healing of a variety of wound types (e.g. pressure sores). Recombinant human basic fibroblast growth factor, rhbFGF, is a single chain polypeptide derived via DNA recombinant techniques from Escherichia coli. To evaluate the chronic toxicity of rhbFGF, 48 kennel-raised cynomolgus monkeys (*Macca fasicularis*) received subcutaneous administrations of 0, 5, 15, and 45 mg/kg daily for 26 consecutive weeks. Daily injections were given via 4 sites in the dorsal aspect at dose volumes of 0.1 or 0.3 mL/kg. Physiological and laboratory investigations were undertaken at regular intervals. Blood samples for antigenicity and proof of absorption investigations were obtained at regular intervals during the course of the study. At the end of the 26 week treatment period, 2 animals/sex were retained for a 13 week recovery period. Apart from subcutaneous thickening at the injections sites, there were no clinical reactions to treatment. There were no physiological or clinical pathology changes at the end of the dosing period. Two animals were sacrificed before termination of dosing, the cause for the demise of these animals remains unclear.

367 RETINOID-INDUCED EPIPHYSEAL CLOSURE IN MALE HARTLEY GUINEA PIGS

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Retinoid treatment has been reported to cause premature epiphyseal closure in humans and guinea pigs (Keene et al., 1991). The purpose of the present study was to extend the finding of retinoid-induced epiphyseal closure in guinea pigs and to study the mechanism of this effect. Groups of weanling male Hartley guinea pigs (n = 5) were treated i.p. with 5 or 25 mg/kg/d of all-*trans*-retinoic acid (ATRA) or vehicle for 10 days and euthanized on day 11. An additional group of controls was pair-fed with the high dose ATRA group. Mean body weight change over 10 days was +10%, −2%, +5%, and −18% for the *ad libitum* controls, pair-fed controls, low dose ATRA, and high dose ATRA groups, respectively. Examination of hematoxylin & eosin-stained sections of the distal femur and proximal tibia revealed disruption of the epiphyseal plate only in the high dose ATRA group. Histopathological changes included narrowing of the remaining epiphyseal plate, loss of basophilia in the extracellular matrix of epiphyseal chondrocytes, and invasion of the epiphyses by osteoclasts. In a subsequent study in which guinea pigs were treated i.p. with 0 or 25 mg/kg/d of ATRA for 10 days and euthanized on day 11 or 40, elongation of the femur and tibia between days 11 and 40 was observed only in controls. These data confirm that guinea pigs provide an animal model of retinoid-induced epiphyseal closure and show that this closure is associated with osteoclast activation and phenotypic changes in epiphyseal chondrocytes.

368 TOXICOLOGY FINDINGS IN THE USE OF AN OIL-IN-WATER ADJUVANT, MF59

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BIOCINE® MF59 Adjuvant is a novel oil in water emulsion composed of squalene (a metabolizable oil) two surfactants (polysorbate 80 and sorbitan trioleate) which are microfluidized with water to form a stable emulsion with a particle size below 230 nm. MF59 is given by intramuscular injection and has been found to increase the immunogenicity of proteins with which it is combined. This adjuvant is being used with recombinant vaccines produced by The Biocine Company. Immunogenicity studies have been performed in a wide variety of animals, including goats, baboons, guinea pigs, chimpanzees, dogs, and rabbits. Preclinical toxicology studies using dogs and rabbits and teratology studies with rats and rabbits have been performed with MF59 alone and in combination with several vaccine antigens including HIV and HSV. In toxicology studies, MF59 has produced no systemic toxicities in test animals at the doses and schedules examined. Ophthalmic examinations showed no changes related to the test-article. No teratologic effects were found in studies performed in rabbits and rats. The primary toxicity seen with MF59 has been mild, transient, local irritation and inflammation observed in-life (Draize scoring) and histopathologically.

369 TOXICOLOGICAL ASSESSMENT OF A POTENTIAL AGENT FOR THE PREVENTION/TREATMENT OF OSTEOPOROSIS

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RWJ-26127 is a benzylmonophosphonate currently under investigation for the prevention/treatment of osteoporosis-induced bone loss. Aged female rats (8–9 months of age) administered RWJ-26127 for 12 weeks at dosages ranging from 0.01 to 10 mg/kg/day developed an increase of up to 4.5% (whole body) or 9.5% (AP spine) over pretreatment baseline bone mineral content. An increase in skeletal alkaline phosphatase and serum osteocalcin levels was evident with maximum effects at 0.1 and 1.0 mg/kg, respectively. Bone mineral content of the neck of the femur was also significantly increased in female dogs administered RWJ-26127 for 6 months at dosages ranging from 0.3 to 30 mg/kg/day. Drug-related adverse effects in rats administered the drug for 6 months were limited to a slight decrease in body weight gain; mild to moderate elevations in hepatic enzymes; and, mild hyperplasia of the stratified squamous epithelium of the limiting ridge of the stomach at 50 and 150 mg/kg/day. Mild increases in APTT, AST and ALT values also occurred in dogs administered RWJ-26127 at 100 mg/kg/day for 6 months. Drug-related effects at 30 mg/kg/day in this species were confined to slightly prolonged APTT values and emesis which occurred shortly after dosing. The no-observed-adverse-effect level (NOAEL) of 15 mg/kg in both species was 150 and 50 times greater than the dosage shown to increase bone mineral content in rats and dogs, respectively.

370 PRECLINICAL TOXICITY EVALUATION OF A DUAL CYCLOOXYGENASE 5-LIPOXYGENASE INHIBITOR, TEPOXALIN, IN SPRAGUE DAWLEY RATS AND BEAGLE DOGS

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Tepoxalin [5-(4-chlorophenyl)-N-hydroxy-1-(4-methoxyphenyl)-N-methyl-1H-pyrazole-3-propanamide] is an orally active dual inhibitor of cyclooxygenase/5-lipoxygenase, anti-inflammatory agent. The oral toxicity of tepoxalin was evaluated in one and six month rat (up to 50 mg/kg/day) and dog (up to 300 mg/kg/day) studies. Increased liver weight, centrilobular hypertrophy, and hepatic necrosis were observed in rats treated with ≥20 mg/kg/day. Renal changes indicative of analgesic nephropathy syndrome (i.e. papillary edema or necrosis, cortical tubular dilatation) were seen at ≥15 mg/kg. These hepatic and renal effects were largely reversible in rats treated for one month after a one month recovery period. Gastrointestinal erosions and ulcers were seen in female rats given 40 mg/kg/day for 6 months. Changes in clinical pathology parameters included decreases for red blood cell count, hemoglobin and hematocrit mean values, elevation in platelet counts and an increase in prothrombin and activated partial thromboplastin times. Mild increases in alanine aminotransferase, aspartate aminotransferase and cholesterol were also noted. In dogs, decreased erythrocyte parameters, increased leukocyte counts and decreased total protein, albumin and/or calcium were noted in some dogs in the 300 mg/kg/day group following 6 months of dosing. Small pyloric ulcerations were seen at 100 and 300 mg/kg/day dosages for up to 6 months. In both rats and dogs, no accumulation of tepoxalin or its acid metabolite was detected in plasma following multiple dosing over the dose ranges (5 to 50 mg/kg/day for rats and 20 to 300 mg/kg/day for dogs). Plasma concentrations of the acid metabolite were several fold higher than those of the parent compound. The no-effect dosages in rats (5 mg/kg/day) and dogs (20 mg/kg/day) were approximately one and six times the ED_{50} (3.5 mg/kg) for inhibition of anti-inflammatory effects (without gastric mucosal damage) in the adjuvant arthritic rat, respectively. It would appear that the relative lack of gastrointestinal side effects (within the estimated therapeutic dose range) in terms of severity and onset distinguishes tepoxalin from most marketed anti-inflammatory drugs.

371 COMPARATIVE CARDIOTOXICITY AFTER REPEATED DOX-SL ADMINISTRATION TO RABBITS

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Repeated dosing of doxorubicin (DX), induces cardiotoxicity in humans and animals at high cumulative doses. DOX-SL (Stealth® liposomal doxorubicin HCl) is DX encapsulated in long-circulating sterically stabilized liposomes that avoid the high plasma and tissue concentrations associated with target organ toxicity. Repeated dose toxicity studies of DOX-SL in rats and dogs have shown that DOX-SL is significantly less cardiotoxic than unencapsulated DX. In this study, the cardiotoxicity of DOX-SL and DX were compared in a rabbit model in which DX-related cardiomyopathy is histopathologically similar to that induced in humans. Groups of 25 male NZW rabbits were injected with 1 mg/kg DOX-SL or DX once every five days for a total of 14 or 21 treatments. Standard toxicological evaluations were performed throughout the study. Animals (n = 3–5/group) were necropsied 1 or 5 weeks after the 14th dose. Later necropsy time points included 13 weeks after the 14th and 21st dose. Hearts were fixed and embedded in glycol methylacrylate resin prior to histopathological analysis. Preliminary histology results indicate that cardiomyopathy was observed more frequently and was more extensive after DX treatment. Eight of 10 DX-treated rabbits had multifocal degenerative changes (vacuolization and atrophy of myocardial fibers) in the left and right ventricles, papillary muscle and septum and ranged from moderate to very slight in severity. In contrast, evidence of cardiomyopathy was found in only 1 of 17 DOX-SL-treated rabbits, and findings were limited to the left ventricle. Data from groups that received 50% more DX or had longer recovery period after 14 doses will also be presented. Based on histopathological data, DOX-SL was less cardiotoxic than DX at the same cumulative dose.

372 RELATIONSHIP BETWEEN CHANGES IN ECG INTERVALS AND PLASMA CONCENTRATION OF BIDISOMIDE, A NEW ANTIARRHYTHMIC AGENT

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Bidisomide is a unique K^+ and Na^+ channel-blocking antiarrhythmic drug, currently in Phase-III clinical trials for the treatment of supraventricular tachyarrhythmias. Preclinical safety studies in dogs, the model of choice due to the similarity of ECG effects of bidisomide between man and dog, indicate that toxicity is due to an exaggeration of the pharmacologic activity, which is increased atrial refractoriness and slowed cardiac conduction. Understanding the relationship between ECG intervals and bidisomide plasma concentrations in the dog may provide a strategy for monitoring plasma concentration in the clinic. Changes in QRS and P-wave duration, PR, QT and JT (QT - QRS) intervals were evaluated relative to bidisomide plasma concentrations following once/day oral dosing or continuous intravenous infusion in dogs. Increases in QRS and P-wave duration and PR interval correlated with increasing mean plasma concentrations of bidisomide. Increased QRS duration with simultaneous decrease in the JT interval resulted in the absence of change in the QT interval. At plasma concentrations in excess (e.g. >3x) of therapeutic levels, QRS duration increased ≥50%, an undesirable effect, given the therapeutic goal of treating supraventricular arrhythmias. This specific change is diagnostic of excessive bidisomide plasma concentrations. Careful monitoring of QRS-duration in particular, may provide a mechanism for monitoring plasma concentrations of bidisomide in the clinical setting.

373 SAFETY ASSESSMENT OF SC-54684A (SC), THE ESTER PRODRUG OF A POTENT ORALLY ACTIVE FIBRINOGEN RECEPTOR ANTAGONIST, IN BEAGLE DOGS AND SPRAGUE DAWLEY (SD) RATS

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SC-54684A (SC) is the ester pro-drug of a potent, orally-active fibrinogen receptor antagonist (SC-54701 [SCA]) designed to inhibit the platelet glycoprotein IIb/IIIa receptor and thus inhibit platelet aggregation independent of the proaggregatory stimulus. On the basis of in situ and in vitro assays, relative potency of SCA is very similar between dog and man. Rat platelets are much less sensitive to aggregation inhibition by this agent. Doses of SC up to 60 mg/kg/day and 750 mg/kg/day were evaluated in the dog and rat, respectively, for at least four weeks. In the dog, an excellent pharmacological model for human platelet aggregation inhibition, all potentially adverse effects associated with administration of SC were related to exaggerated pharmacology. These included reversible, dose-related decrements in platelet count with increases in mean platelet volume and template bleeding time and a tendency for increased bleeding events at higher doses. These events generally occurred in organs predisposed to physiological microtrauma or were associated with animal handling and/or venous blood collection. At clinically relevant doses, these bleeding events were not clinically significant. Platelet aggregation inhibition

in the dog was complete at all doses tested. At the higher doses, inhibition remained at 100% or greater for the entire duration of the studies. In the rat, no increased bleeding tendency was noted and there were no effects on platelet parameters. The toxicity of SC in rats was associated with the apparent precipitation of test article-related material in the renal tubules (material identified by microscopic IR spectroscopy) resulting in tubular obstruction and epithelial cell necrosis. This lesion occurred at large dose exaggerations (≥250 mg/kg/day) in the rat and was not observed in dogs at anticipated human dose or plasma level exaggerations of at least 35–40X. In conclusion, SC was well tolerated in both pharmacologically sensitive and non-responsive species at doses plasma levels (SCA) representing large exaggerations of the anticipated human doses/plasma levels.

374 THE SYSTEMIC TOXICOLOGY AND PHARMACOKINETIC PROFILE OF LISOFYLLINE IN MICE

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Lysofylline (CT-1501R) is a methylxanthine and immuno modulator used for the relief of severe inflammatory reactions caused by a variety of noxious stimuli, such as trauma, shock or the side effects of antineoplastic chemotherapy. The objective of this work was to evaluate the toxicity of orally dosed Lisofylline in mice. Animals received the test article by gavage, three times each day for 28 consecutive days. Test groups included untreated controls, vehicle controls, 100 and 750 mg Lisofylline per kg of body weight. Half of the test animal population was evaluated on the last day of dosing (study day 28). The remaining half of the test population was evaluated after a fourteen day recovery period (study day 42), during which time the animals remained untreated. Lisofylline was found to be rapidly absorbed at both doses, and measurable blood levels were detected within one minute after dosing with maximum blood levels achieved by 10 min post dosing. Concentration-time profiles were similar for each of the two dose levels, but Lisofylline elimination was found to be much more rapid for animals receiving 100 mg/kg than for those animals receiving 750 mg/kg. Although initial Lisofylline concentrations were approximately proportional to the administered dose, there was an absence of direct dose proportionality for total exposure. Clinical signs of toxicity were observed in both exposure groups, but were of greater severity in the 750 mg/kg group. No gross observations of toxicity were noted at either necropsy that were considered to be related to the administration of the test article. Although severely statistically significant differences were observed in the clinical chemistry and hematological data, these changes were within the range of normal and not considered to be biologically significant nor related to the administration of Lisofylline. Finally, no histomorphological changes were found in tissues, which could be attributable to the administration of Lisofylline.

375 EVALUATION OF THE CUTANEOUS AND SYSTEMIC TOXICITY OF A FLUORURACIL/EPINEPHRINE INJECTABLE GEL IN MICE

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MPI 5003 (M) is a combination of fluoruracil (FU, 30 mg) and epinephrine (0.1 mg) in an injectable collagen gel (1 ml). The material is currently being used for the injection into and treatment of basal cell carcinoma and *condylomata acuminata*. This specific preparation localizes the chemotherapeutic agent which allows the neoplasm to be destroyed via localized necrosis. The objective of this study was to evaluate the local cutaneous toxicity of M in mice after a thirteen week period of intradermal exposure. Test groups included doses of 2, 20 and 100 mg fluoruracil per kg and three control groups (saline, saline + collagen, and saline + collagen + epinephrine). Animals were injected once a week, because human dosing is anticipated to be once a week for 6 weeks. Necropsies were performed on mice at 1, 14 and 28 days after the last injection. Statistically significant increases in food consumption were observed in the mid and high dose groups. Varying degrees of dermal irritation were observed in all groups at various time points, except in the negative saline control group. However, by 28 days after the last i.d. dose, all skin lesions were resolving normally. Injections into the deeper subcutaneous tissues were also tolerated well. Histomorphological changes indicative of increased hematopoietic activity were observed in the livers, spleens and bone marrows of 20 and 100 mg/kg exposed animals. Furthermore, mice exposed to the high dose of test article showed a statistically significant neutrophilia, when compared to saline controls. The increased food consumption and hematologic effects were judged to be secondary to cutaneous reparative processes. Mice received 100 mg FU/kg/week and no evidence of direct systemic toxicity due to the administration of the test article was observed in study animals at any dose level.

376 A CYCLIC INTRACELLULAR PRODRUG OF THE ANTI-CMV RETINITIS NUCLEOTIDE ANALOG (S)-1-(3-HYDROXY-2-PHOSPHONYLMETHOXYPROPYL) CYTOSINE (HPMPC) WITH REDUCED NEPHROTOXICITY

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The dose-limiting nephrotoxic potential of 1-[((*S*)-2-hydroxy-2-oxo-1,4,2-dioxaphosphorinan-5-yl)methyl] cytosine (cHPMPC), a cyclic analog of antiviral HPMPC, has been evaluated in subchronic intravenous toxicity studies in Sprague-Dawley rats and cynomolgus monkeys. Rats (5/sex/group) received cHPMPC at 0, 2.5, 10 or 40 mg/kg daily for 30 days or 70 mg/kg once weekly for 4 weeks. Monkeys (4/sex/group) received cHPMPC at 0, 0.5, 2.5 or 10 mg/kg daily for 30 days or 17.5 mg/kg once weekly for 4 weeks. In rats and monkeys, the nephropathy LOELs were 40 and 2.5 mg/kg, respectively. The once weekly dosing regimens resulted in no nephrotoxicity. In previously conducted 30-day studies of HPMPC, nephropathy LOELs in rats and monkeys were 1.0 and 0.25 mg/kg, respectively. These data indicate a 10–40 fold reduced nephrotoxic potential for cHPMPC compared to HPMPC. In addition, a once weekly cHPMPC bolus dosing schedule appears to produce less nephrotoxicity than a fractionated daily dosing regimen.

377 TWO AND 13-WEEK TOXICITY STUDIES OF RWJ-26127, A BENZYLMONOPHOSPHONATE WITH POTENTIAL TO PREVENT/TREAT OSTEOPOROSIS

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RWJ-26127 stimulates osteoblastic proliferation/differentiation *in vitro* and increases bone formation *in vivo* in dogs and rats at dosages ranging from 0.02 to 0.3 mg/kg/day. Two and 13-week oral toxicity studies in rats and dogs were conducted to support phase I safety/pharmacokinetic studies and early phase II efficacy studies in postmenopausal women. In the 2-week rat study, 65% mortality occurred at the high-dosage of 1800 mg/kg/day; regenerative anemia and various alterations in clinical chemistry values were noted at 600 mg/kg; and gastric irritation was evident at the low-dosage of 30 mg/kg. Thirty mg/kg/day was the no-observed-adverse effect level (NOAEL) in dogs; 150 mg/kg produced elevations in hepatic enzymes; and, 600 mg/kg (high-dose) resulted in mortality, prolongation of the Q-T interval, and clinical and morphologic evidence of mild to moderate hepatotoxicity. In the 13-week rat study, no changes occurred at 30 mg/kg and the response to RWJ-26127 administration was limited to a slight decrease in body weight gain (150 mg/kg) and gastric irritation (600 mg/kg). Electrocardiographic changes did not occur in dogs administered RWJ-26127 for 3 months. Hepatotoxicity was more pronounced at the high-dosage of 150 mg/kg and extended to dogs in the 50 mg/kg dosage group. The NOAEL was 20 mg/kg. Absorption of RWJ-26127 was dose-dependent. Toxicity associated with the high-dosages in the 2-week studies was not evident following 13 weeks of RWJ-26127 administration. These studies define the low order of toxicity of RWJ-26127 relative to its pharmacologic effects.

378 DOCETAXEL (TAXOTERE®): 12-CYCLE INTRAVENOUS TOXICITY STUDY IN CYNOMOLGUS MONKEYS

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Docetaxel is a microtubule depolymerization inhibitor being developed for cancer chemotherapy. The toxicity of docetaxel was investigated using two groups of six Cynomolgus monkeys (3/sex) receiving a treatment regimen corresponding to the clinical use of docetaxel: single intravenous doses of 25 or 50 mg/m^2 (corresponding dose range on a body weight basis: 1.6–2.1 or 3.4–3.9 mg/kg) were administered in 21-day intervals for a total duration of 12 cycles (21-day interval equivalent to 1 cycle). A vehicle control group of 3 monkeys/sex received the vehicle (polysorbate 80/saline). 21 days following the 12th administration, the animals were euthanatized and necropsied. The dose levels were selected according to the results of a preliminary study which indicated that 50 mg/m^2 was in the range of the maximum tolerated dose. However, following the first dose of 50 mg/m^2 a range of adverse effects (body weight loss, hyporeactivity, severe hypothermia and severe leukopenia) occurred which were of a severity warranting termination of treatment at this dose level. The dose level of 25 mg/m^2 was relatively well tolerated, principal adverse effects including transient vomiting, diarrhea (within 3 to 5 days

following each administration), minimal changes in clinical chemistry parameters and moderate hematologic changes such as reduction in WBC counts (max. −69 and −78% of pretest mean values in females and males, respectively), and red blood cell parameters (max. −27% of pretest mean values). Hematologic effects tended to return to normal within the 21 days following each administration and did not increase in severity with progression of the study. No compound-related gross findings were noted at necropsy of the 25 mg/m^2 group and organ weights appeared unaffected. In conclusion, a dose level of 50 mg/m^2 docetaxel produced severe toxicity in Cynomolgus monkeys whereas findings at 25 mg/m^2 were limited to effects consistent with the pharmacological activity of this therapeutic class of compounds.

379 ASSESSMENT OF THYROID TOXICITY FOLLOWING ACUTE ADMINISTRATION OF IODINATED OILS TO HAMSTERS

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Iodinated aromatic compounds are commonly used as contrast enhancement agents for X-ray based diagnostic imaging. We have examined the acute oral tolerance in Golden Syrian hamsters, with particular attention to the thyroid gland, of sixteen tri-iodinated compounds, from the alkyl- and acyloxybenzene, alkoxy- and acyloxy-isophthalate, alkoxy benzoate, and alkyl ether classes. All hamsters received a single oral dose via gavage, based on a constant iodine content (4.6 g I/kg) with all compounds suspended in a light mineral oil based emulsion. Hamsters were examined for clinical signs of toxicity and mortality for 48 hours, followed by a necropsy, and subsequent histomorphologic and clinical chemistry assessment of the thyroid gland. One compound in the acyloxybenzene class was acutely toxic, producing severe clinical signs and morbidity in 4 of 5 hamsters and thus was not examined further; all other compounds tested were well tolerated. All compounds tested in the alkoxy benzoate, alkoxy isophthalate, alkyl ether, and acyloxy benzene classes exhibited decreased serum T4 values (all at least 40% lower than control) and histomorphologic changes consisting of pale staining colloid, increased follicular mitotic figures, necrosis and dilation. One exception to these findings was one compound in the alkyl ether class (which was the most thyroid-toxic),which exhibited increased T4 levels (~ 64% above control), but similar histomorphology to other compounds in this class. Only the alkyl benzenes produced no changes in T4 levels and thyroid histomorphology when compared to control hamsters. Plasma analysis for the parent alkyl benzene compounds failed to demonstrate measurable levels (detection limit 0.19 μg/ml), suggesting these compounds were poorly bioavailable. Intraperitoneal injection of the alkyl benzenes (0.6 gI/kg) into hamsters produced no changes in T4 or thyroid histomorphology, yet plasma levels of the drugs were detected (up to ~ 3 μg/ml). In summary, the tri-iodinated alkyl benzenes appear relatively inert towards producing thyroid toxicity following oral adminstration.

380 PULMONARY TOXICITY INDUCED BY POLYETHYLENE GLYCOL LINKED GADOLINIUM CHELATE

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Polymeric chelates of gadolinium are expected to possess superior properties, such as prolonged blood half-life, over monomeric chelates currently used in magnetic resonance imaging. The toxicity of a polymeric chelate of Gd diethylenetriamine penta-acetate linked to polyethylene glycol (PEG) was examined in Sprague Dawley rats (4/sex/group) following single i.v. doses of 0.2, 1, 2, or 4 g/kg of the polymeric Gd-chelate (PGdC). Forty eight hours after dosing, the lung exhibited the most obvious macroscopic and microscopic changes. At doses of PGdC $\geq$ 2 g/kg, either red foci or dark red discoloration were noted in the lungs, which correlated with the microscopic observations of alveolar hemorrhage and the presence of hemoglobin crystals. Additionally, at the 4 g/kg dose, mediastinal mineralization was detected in 50% of the rats. To define the contribution of the PEG moiety to pulmonary toxicity, rats were dosed with either 4 g/kg of PGdC or the equivalent amount of PEG (3.2 g/kg), both with average molecular weight (M) of 20 kiloDaltons (kD). Treatment with these two compounds led to alveolar hemoglobin crystals and the presence of alveolar macrophages. Only the PGdC treated rats showed mediastinal mineralization. One other notable difference between PGdC and PEG was that PEG induced more severe pulmonary hemorrhage, resulting in 40% mortality in this group, whereas no mortality occurred in the PGdC group. Lastly, low ($\approx$9 kD) and high ($\approx$80 kD) M constructs of PGdC were examined to assess the contributions of M on lung toxicity. The high M PGdC was more toxic to the lung, producing the lesions noted above, although the low M fraction caused a greater influx of alveolar macrophages. In summary, the PEG component of the PGdC appeared to contribute to the hemorrhage related lesions, likely because of relatively high viscosity conferred on the drug by PEG. The other lesions associated with PGdC are related to other as yet uncharacterized properties of the polymer.

381 BIOCHEMICAL AND MORPHOLOGICAL EFFECTS OF THE LTD_4 ANTAGONIST RG 12525 ON RODENT BROWN ADIPOSE TISSUE

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RG 12525 is a potent leukotriene D_4 antagonist developed for the treatment of asthma in man. Chronic treatment of rodents with RG 12525 produced a large dose-dependent increase in interscapular brown adipose tissue (IBAT) weight. Histologically and ultrastructurally, the effect of RG 12525 on brown adipocyte tissue was characterized by cellular hypertrophy and hyperplasia, intracellular lipid changes from micro to macro-vesiculation, nuclear eccentricity of brown adipocytes and an increase in connective tissue. Oral administration of RG 12525 to female rats at doses of 20, 150 and 400 mg/kg/day for 15 weeks produced a large increase in IBAT weight (5-fold increase at the high dose) as well as a dose-dependent increase in the mitochondrial uncoupling protein/citrate synthase ratio. In addition, a significant increase (+0.5°C) in core body temperature was observed in the high-dose group. In a four-week BrdU rat study (doses 125, 300 and 600 mg/kg/day), a significant hypertrophy and dose-dependent increase in BrdU incorporation in brown adipocytes for both sexes was observed at the interim (1-week) and final necropsies. Oral treatment of mice with RG 12525 at 800 mg/kg/day for 2 weeks produced a slight increase in brown adipocyte mitochondrial uncoupling protein without significant change in mitochondrial citrate synthase activity. Treatment of mice with two other LTD4 antagonists did not affect the biochemical or histopathologic characteristics of IBAT. The mechanism by which RG 12525 produced these biochemical and ultrastructural changes in rodent IBAT is currently under investigation.

382 BIOTRANSFORMATION OF A SOMATOSTATIN ANALOGUE IN PRECISION-CUT LIVER AND KIDNEY SLICES FROM RAT, DOG AND HUMAN

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SDZ 212 611 (SDZ CO 611) is a somatostatin analogue intended to retain the pharmacological properties of octreotide (SMS 201 995) but have improved oral bioavailability. In *in vivo* studies carried out in the rat, intrajejunal administration of SDZ CO 611 resulted in oral bioavailabilities of 1.09% (Albert *et al.*, 1993). Precision-cut organ slices maintained in culture are a useful model with which to compare biotransformation among species (Connors *et al.*, 1990) and among organs (Vickers *et al.*, 1992). *The objectives* of the present study were to compare: *in vitro* metabolism of SDZ CO 611 among rats, dogs and humans and among organs using liver, kidney, gut wall and gut contents. *Conclusions:* (1) Cleavage of the glucopyranosyl moiety of SDZ CO 611 results in the formation of the major metabolite, SDZ CO 610, in liver and kidney slices of rat, dog and human, as well as in liver S9 and cytosol of rat and human. (2) The rates of SDZ CO 610 formation (nmol/h/mg slice protein) for all three species in liver slices and the relative order were: rat (0.12) > dog (0.096) = human (0.095). The rates of SDZ CO 610 formation (nmol/h/mg slice protein) for all three species in kidney slices and the relative order were: rat (0.29) > dog (0.16) > human (0.10). (3) SDZ CO 610 was rapidly formed by rat gut contents in the absence of NADPH, possibly by disaccharide splitting enzymes. (4) Rat intestinal S9 homogenates also formed SDZ CO 610 but metabolism was dependent upon NADPH, suggesting a cytochrome P-450 dependent reaction.

383 ACUTE TOXICITY STUDIES OF BUTYLUREA — A POTENTIAL ANTI-VIRAL AGENT

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Alkylureas as a chemical class may have usefulness as anti-viral agents. Specifically there is an interest in developing butylurea (BU) as an anti-viral that may be useful against HIV. Since there is little published toxicity data on BU, a series of acute studies were conducted to elucidate a toxicity profile of BU. The

oral LD50 of BU in rats was determined to be 1.255 mg/kg. An oral dose of 250 mg/kg was not toxic. The LD50 of a single intravenous injection of BU in rats was 382 mg/kg. BU was essentially non-irritating after a 24 hour dermal application on rabbits of concentrations ranging from 2.5% to 100% (neat powder). Additionally, at these same concentrations, BU was not sensitizing as determined by a closed patch repeated insult sensitization study in guinea pigs. In a rabbit eye irritation study, neat BU powder caused a severe conjunctivitis which could be prevented by washing the eyes with saline within 15 seconds of initial exposure. A concentration of 5.81% BU was non-irritating to rabbit eyes. Finally, a 10 day vaginal exposure of rabbits to a concentration of 4.5% BU produced only minimal irritation to vaginal tissues. In conclusion, BU has a low order of dermal toxicity, has moderate toxicity when administered orally or intravenously and is an ocular irritant when applied undiluted but not if it is washed out or applied diluted.

384 IRRITANCY OF ACETATE AND SUCCINATE BUFFERS DURING SUBCUTANEOUS INFUSION IN RATS

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The purpose of this study was to assess the irritancy of potential vehicles for use in chronic toxicity studies, using continuous subcutaneous infusion. Groups of 5 male Wistar rats were infused with 0.01 M or 0.02 M sodium succinate or sodium acetate buffer (pH 4.75, osmolarity adjusted to 300 mOsm/kg), via a subcutaneous catheter surgically implanted in the dorsal region, at rates of 0.25 or 1.0 mL/kg/hr. Two additional groups received either 0.9% saline (negative control) or 0.05 M sodium phosphate buffer, pH 7.0 (positive control) at a rate of 1.0 mL/kg/hr. All animals were continuously infused 24 hours per day for 7 consecutive days. At the completion of dosing, the animals were euthanized and the area around the catheter tip was examined microscopically. Moderate granulomatous inflammation was observed in all positive control animals but not in any other group. Minimal to moderate inflammation and vasculopathy were noted with a dose rate related incidence in both acetate and succinate treated animals. Since similar incidences and severity were observed in saline treated animals, this observation may be related to an imbalance between the rate of infusion and the absorption capacity of the subcutis. Minimal to mild thrombosis was also observed in animals treated with either buffer at 1.0 mL/kg/hr. There were no apparent concentration related differences. It is concluded that both 0.01 M and 0.02 M sodium acetate and sodium succinate buffer would be suitable for use as vehicles in chronic toxicity testing, but consideration should be given to using the lowest dose rate possible.

385 SAFETY ASSESSMENT OF AN ANTI-HIV PROTEASE INHIBITOR (SC-52151) IN NEONATAL/JUVENILE RATS TO SUPPORT EARLY ENTRY INTO PEDIATRIC CLINICAL TRIALS

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SC-52151, a urea-based peptidomimetic which is highly potent and selective for in vitro inhibition of HIV protease, is currently in a Phase II clinical efficacy trial in adults for treatment of HIV infection. Because pediatric HIV infection has become a leading cause of morbidity and mortality in children we developed an aggressive strategy to evaluate safety and efficacy in this population before completion of adult Phase II/III studies. While no SC-52151 toxicity has been elicited via oral administration of test article in adult animal studies (rat and dog), the potential for toxicity in an immature animal is not known. Therefore, a 4-week study was conducted in immature rats. Starting at 3 days of age, SC-52151, in a self emulsifying drug delivery system (SEDDS) (SC-52151:Tagat To:ethanol:Neobee M5 oil, 5:35:35:25, w:w:w:w), was administered via intragastric gavage at 30, 100 and 250 mg/kg t.i.d. (clinical dosing frequency) (10 mL/kg/dose) for 30 consecutive days. Clinical observations, body and organ weights, urinalysis, clinical chemistries, gross and microscopic pathology and pharmacokinetics were assessed. The 250 mg/kg group was discontinued after the 2nd dose interval due to probable ethanol associated mortality, possibly augmented by SC-52151. Animals in the 30 and 100 mg/kg groups surviving beyond the first 2 days demonstrated no SC-52151 or ethanol associated toxicity. The mean plasma concentrations 0.5 hours post dosing for male and female rats were 0.303 ± 0.046 (N = 8) and 0.799 ± 0.114 μg/mL (N = 5), for the 30 and 100 mg/kg dose groups, respectively, which is 3 to 8 fold the EC95 for this compound. The results of this study demonstrate the low toxic potential of SC-52151 as well as the feasibility of conducting toxicity studies utilizing multiple times per day dosing in immature animals.

386 RETINAL ALTERATIONS IN DOGS AFTER CHRONIC ORAL ADMINISTRATION OF AN IONOPHORE ANTIBIOTIC

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Semduramicin, a carboxylic ionophore antibiotic intended for veterinary use, was administered as a 0.1% preblend in feed to Beagle dogs (5/sex/dose group) at doses of 0.1, 0.3 and 1.0 mg/kg/day for one year. Besides observations and examinations performed as part of standard protocols, ophthalmic examinations were performed on all dogs predosing, at 3 and 6 months, and at monthly intervals thereafter until the end of the study. At the end of dosing, animals were euthanized and necropsied, organs were weighed and tissues were collected for microscopic examinations. Affected eyes at the 1.0 mg/kg dose and one eye/sex/group at the other dose levels were also collected for ultrastructural examination. Following six months of dosing, 3/10 high dose (1.0 mg/kg/day) dogs (2 M, 1 F) had multiple, discrete, hyperreflective lesions visible in the tapetal fundus upon ophthalmic examination. At seven months, 1 additional high dose female had visible lesions; lesions in the 3 previously affected dogs were more numerous. Subsequent examinations indicated a dynamic process with lesion position and severity varying with duration of dosing. No clinical vision deficits were detected in any dog. At necropsy, diffuse, miliary granularity and/or pinpoint foci were observed grossly in the tapetal and nontapetal fundus of all 4 dogs. Retinal detachment, occurring between the rod and cone layer and the retinal pigmented epithelial layer, was observed histologically in the tapetal and nontapetal fundus of these animals. In addition, marked hyaline degeneration and fragmentation of the inner and outer segments of the rods and cones were observed microscopically. Ultrastructurally, swollen, abnormally shaped mitochondria along with mitochondrial-associated tubulovesicular cristae and myelin figures were observed in degenerated rod and cone segments. These mitochondrial changes have been previously reported as characteristic of ionophore toxicity in tissues other than the retina. The limited degree of these mitochondrial abnormalities and the observed ophthalmoscopic reversibility of the retinal detachment suggests the lesions may be reversible if compound exposure is terminated. There were no treatment related ophthalmoscopic, macroscopic or microscopic changes observed in male or female dogs from the other dose groups, resulting in a NOEL of 0.3 mg/kg/day, 250X the maximum potential human dietary exposure (0.0012 mg/kg/day).

387 TOXIC EFFECTS OF 1,2-DIMETHYL-3-HYDROXY-PYRID-4-ONE (L1) IN UREMIC RATS

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Deferoxamine is the chelator of choice for the treatment and prevention of iron (Fe) overload and for the treatment of patients suffering from aluminum (Al) accumulation disorders. However, this drug is expensive, ineffective by mouth and has the potential to produce serious adverse effects. In recent years, L1, a new chelating agent that can be administered orally, was synthesized and evaluated with success for its possible clinical use in removing Fe from the body. Recent studies have shown that L1 might also be effective in Al mobilization. However, toxicity testing of L1 produced apparently discrepant findings. In order to extend the data on the toxic effects of L1, this study was conducted to evaluate the short-term toxicity of this drug in rats. Because of the potential of Al to produce toxicity in renally impaired patients, L1 was tested in uremic rats. L1 was given by gavage to five groups of male SD uremic (5/6 N_x) rats at 0, 20, 40, 80, and 160 mg/kg/d for 4 weeks. There were no effects of L1 on food and fluid consumption, or in body weight gain (except a decrease at 80 and 160 mg/kg/d) during the dosing period. No differences between control and L1-exposed groups were noted in hematological and biochemical parameters. Spotty lesions with some pycnotic nuclei were observed in all the L1-treated groups. However, similar lesions were also seen in the control group, and therefore they were not attributed to treatment. According to these results, the use of L1 in uremic patients would seem rather inadvisable.

388 MATERNAL AND DEVELOPMENTAL TOXICITY OF DEFEROXAMINE IN MICE

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Deferoxamine (desferrioxamine, DFOA) is a chelating agent widely used for the treatment of iron and aluminum overloads. In this study we investigated the potential adverse developmental effects of this drug in mice. Pregnant

Swiss mice received i.p. doses of DFOA at 0, 44, 88, 176, and 352 mg/kg/day on days 6–15 of gestation. Monitors for maternal toxicity were body weight, food consumption and clinical signs. At sacrifice (day 18), maternal weight, liver and kidney weights, and gravid uterine weights were measured. Gestational parameters monitored were number of implantations, resorptions, dead fetuses and postimplantation losses. Live fetuses were weighed, sexed, and examined for external, internal, and skeletal malformations and variations. Maternal toxicity was noted at 88, 176, and 352 mg/kg/day as evidenced by decreased weight gain and food consumption during treatment. There was no evidence of embryotoxicity at 44, 88, and 176 mg/kg/day. However, the number of live fetuses per liter was significantly diminished at 352 mg/kg/day. The lowest-observed-adverse-effect level (LOAEL) for maternal toxicity was 44 mg/kg/day, whereas the no-observed-adverse-effect level (NOAEL) for developmental toxicity was 176 mg/kg/day.

389 SUBCHRONIC TOXICITY AND REVERSIBILITY OF THE ANTIMALARIAL DRUG WR238605 SUCCINATE IN RATS AND DOGS

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These studies examined the three month oral toxicity and reversibility of the antimalarial drug WR238605 succinate in CD® rats and Beagle dogs. Dose levels were 0, 0.5, 6 and 18 mg base/kg/day for rats and 0, 0.1, 2 and 6 mg base/kg/day for dogs. Thirteen week recovery groups were included. The toxic effects of WR238605 in both species included hemolytic anemia, methemoglobinemia, and pulmonary toxicity (alveolar proteinosis and subacute inflammation in dogs). Secondary responses to hemolytic anemia included reticulocytosis, induction of Heinz bodies (rats), splenomegaly, and microscopic changes in bone marrow, spleen, kidneys and liver, e.g. hemosiderosis, etc. Generalized toxic effects included decreased body weight gains and food consumption (rats), clinical signs of cyanosis (blue gums, tongue and sclera in dogs), leukocytosis and thymic lymphocyte depletion (rats). These effects were limited to mid and high dose animals, however, minimal microscopic changes secondary to the hemolytic anemia without corresponding alterations in hematology parameters were seen in low dose dogs. In addition, marginal to mild hepatotoxic changes manifested by serum chemistry changes, hepatomegaly (dogs) and hepatocyte necrosis (dogs) were observed in high dose animals. During the recovery period, chronic inflammation and hemosiderosis in alveolar macrophages (rats) developed in the lungs as part of the process of resolution of alveolar proteinosis. WR238605 toxicity was essentially reversible, except that pulmonary lesions and microscopic changes secondary to the hemolytic anemia were still in the process of resolution at the end of the 13 week recovery period. Supported by USAMRMC Contract No. DAMD17-92-C-2001.

390 CHARACTERIZATION OF HYDRATED SODIUM CALCIUM ALUMINOSILICATE AND AFLATOXIN B_1 BINDING

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Previously, a phyllosilicate clay (HSCAS, or NovaSil™) was shown to tightly bind aflatoxins and prevent aflatoxicosis in animals. Additional *in vitro* studies have shown, through the use of ligands with functional groups in common with aflatoxin B_1 (AfB_1), that the β-dicarbonyl (ketolactone) system may be involved in the mechanism of tight binding and selective chemisorption. In this study an index of chemisorption ($C\alpha$) was used to compare the strength of AfB_1 binding in a series of diverse aluminosilicates, a series of chemically modified HSCAS clays, and 3 major constituents of HSCAS to gain an insight into the molecular mechanism(s) and site(s) of AfB_1 adsorption. The interlaminar spacing of HSCAS was collapsed by heating in a furnace at 800°C for 1 hr. HSCAS was separated into three components (A, B, and C). A (beige color) and B (dark brown color) were obtained by macroscopic separation and C (black color) by silting, since it had a higher relative density. Also, the total organic carbon (TOC) of HSCAS was determined. The structures of AfB_1, various clays, and a proposed model for HSCAS were entered into a molecular modeling program. Collapsing of HSCAS lowered the chemisorption index from 0.78 to 0.37, suggesting that AfB_1 binding may occur (in part) in the interlaminar space. Component B showed a stronger affinity for AfB_1 ($C\alpha$ of 0.97) relative to the other components. Characterization of these components is ongoing. No significant amount of organic material was found to be associated with HSCAS (TOC < 0.05%) which minimized the importance of organic partitioning in the mechanism of binding of AfB_1 to HSCAS. Data obtained with molecular modeling supported the hypothesis that AfB_1 may bind at the edges, within interlayers of HSCAS, and on the basal surface of HSCAS particles. (Supported by USDA Animal Health and ATP 999902-004).

391 SELECTIVE CHEMISORPTION OF AFLATOXIN B_1 BY HSCAS: PREVENTION OF DEVELOPMENTAL TOXICITY IN THE RAT

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Previous research has demonstrated that hydrated sodium calcium aluminosilicate (HSCAS, or NovaSil™) tightly binds and immobilizes aflatoxins in the gastrointestinal tract of animals. This action markedly reduces the bioavailability and toxicity of these food and feedborne poisons. Since AfB_1 is a potent developmental toxin in rats, our objectives in this study were to evaluate the ability of HSCAS and a common zeolite (clinoptilolite) to protect the developing fetus. Aluminosilicates, HSCAS 1, HSCAS 2, and clinoptilolite, were added to the diet at a level of 0.5% (w/w) and administered to Sprague-Dawley rats throughout pregnancy. Test animals were dosed orally with AfB_1 at 2 mg/kg b.w. dissolved in corn oil during gestation days 6–13, and dams and feti were evaluated for various maternal, developmental and histological parameters on day 20. Clays, by themselves, were not toxic; AfB_1 alone resulted in 75% maternal mortality, 98% embryonic resorptions and typical liver and kidney lesions. Animals treated with HSCAS 1 and HSCAS 2 (plus AfB_1) were comparable to controls. Interestingly, clinoptilolite (plus AfB_1) resulted in very severe maternal liver lesions, more than AfB_1 alone, suggesting that this zeolite may interact with factors in the diet that modulate aflatoxicosis. The findings from this study clearly demonstrate the efficacy of HSCAS clay in protecting the developing fetus from exposure to AfB_1. (Supported by the Egyptian Scientific Channel System, USDA Animal Health, ATP 999902-004, and TAES H6215).

392 A CLAY-BASED METHOD FOR THE ADSORPTION AND INACTIVATION OF CYCLOPIAZONIC ACID

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In earlier work, an acidic phyllosilicate clay (P_1) was shown to strongly adsorb the mycotoxin, cylopiazonic acid (CPA) *in vitro*. The objectives of this study were to further investigate the mechanism(s) of this adsorption and to evaluate the effect of P_1 on the bioavailability of CPA. CPA was reacted with 1% P_1 or P_2 (a neutral phyllosilicate clay) in water at pHs ranging from 2–10 and temperatures of 25 and 37 °C. Binding studies were performed using ^{3}H-aflatoxin B_1 (AfB_1) and clay at a level capable of binding 15–20% of tracer. Equimolar concentrations of test ligands were added and reacted at room temperature for 1 hr. Percent inhibition of binding was determined from radioactivity in the supernatant. Male Sprague-Dawley rats were orally dosed with 10 mg of CPA/kg b.w alone or in combination with 0.5% P_1. Urine samples were collected after 48 hr and analyzed by HPLC. Indices of chemisorption ($C\alpha$s) were determined for CPA and were equal to 0.77, 0.9 and 0.87 (with P_1) and 0.75, 0.35 and 0.34 (with P_2) at pH 2, 7 and 10, respectively. No differences in adsorption of CPA by P_1 or P_2 were observed at 25 vs. 37 °C. CPA did not significantly inhibit the binding of ^{3}H-AfB_1 (< 1%) as opposed to authentic AfB_1 and congeners, which displaced 30 to 50% of the tracer. In rats, P_1 (in the diet) was shown to reduce the output of CPA in urine by 36%. Results from this study indicate that adsorption of CPA to phyllosilicate clay is strongly influenced by pH (i.e., acidic pH produces more effective binding of CPA). Also, the site of adsorption of CPA on clay is apparently different from AfB_1. Studies are ongoing to further characterize the mechanism of binding. (Supported by TAES H-6215 and ATP #999902-004).

393 INVESTIGATION OF CHOLERA TOXIN ADSORPTION BY PHYLLOSILICATE CLAYS

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An assay was developed to measure the ability of various sorbent materials to sequester cholera toxin (CT) *in vitro*. An aliquot (0.5 ml) of CT (40 μg/ml) was reacted with 25 μg of test material at 600 rpm for 1 hr at room temperature. The mixture was centrifuged for 10 min at 2000 rpm and the equilibrium concentration of toxin in the supernatant was determined by the Bradford protein assay. Pellets were washed to determine the concentration of toxin

that could be dissociated. Adsorption indices were calculated from the ratio of the amount of CT tightly bound (after desorption) over the initial amount added. Of the phyllosilicate clays, HSCAS and sodium bentonite, showed the strongest interaction with CT, with adsorption indices equal to 0.6 and 1.0, respectively. Based on this assay, sodium bentonite was the best adsorbent for CT, and these findings correlated well with results from a cytotonic assay using Y-1 adrenal cells. Attapulgite, kaolinite, zeolites (clinoptilolite and mordenite), alumina (boehmite), tectosilicates (silica and SIP-50S), activated charcoal and polyvinylpyrrolidone adsorbed less CT. These results suggest that aluminas, silicas and various clay and zeolitic minerals have different propensities for CT. Prioritized phyllosilicate clays that tightly bind CT *in vitro* (e.g., HSCAS and sodium bentonite), may be useful to sequester and immobilize CT in the gastrointestinal tract of animals. (Supported by ATP 999902-004 and TAES H6215).

394 GENOTOXICITY AND SUBCHRONIC TOXICITY STUDIES WITH HEAT STRESSED OLESTRA

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Olestra, a noncaloric and non-digestible fat replacer made from sucrose and conventional fatty acids, is heat-stable so it can replace fat in a wide variety of foods, including baked and fried foods. The rationale by which the safety appraisal of heated olestra was investigated will be presented. The rationale was based in part on establishing the genotoxicity and subchronic toxicity potential of olestra, which had been heat stressed by heating for 25–31 hours at 350–365°F. Following analytical confirmation that the heat stressed olestra contained no components not found in unheated olestra or in heat stressed triglyceride, the heat stressed olestra was evaluated in an *in vitro* genotoxicity battery consisting of 1) Salmonella/mammalian microsome mutagenesis assay, 2) L5178Y TK +/– mouse lymphoma assay, 3) unscheduled DNA synthesis in rat hepatocytes, and 4) cytogenicity study in Chinese hamster ovary cells. These tests were conducted in the presence and absence of liver enzyme (S-9) activation at concentrations up to 5 mg/ml. There was no evidence of genotoxicity. Furthermore, no evidence of chromosomal aberrations were shown with heat stressed olestra following examination of bone marrow in an *in vivo* cytogenicity assay conducted with Sprague-Dawley rats administered olestra by gavage at 0, 0.5, 1.7, and 5 g/kg and dosed for either 1 or 5 days. In the subchronic toxicity study, heat stressed olestra was dosed to Fischer 344 rats at 0, 1, 5, and 10% of the diet for 90 days. Survival, food consumption, feed efficiency, physical condition, body weight, organ weight, hematologic and clinical chemistry parameters, and histomorphology were evaluated. No adverse treatment-related effects were observed. The above studies, in combination with previously reported chronic studies in which dogs, rats, and mice were fed up to 10% olestra in the diet, indicate that olestra used in fried and baked foods is safe for human consumption.

395 APPLICATION OF POLYNOMIAL REGRESSION ANALYSES AND DIETARY RESTRICTION TO EVALUATE PALATABILITY-INDUCED DECREASES IN RAT BODY WEIGHT GAIN

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Interpreting the results of paired-feeding (PF) studies may be problematic when there are small decreases in food consumption (i.e., < 10% of control). PF studies are often performed to assess if decreased body weight gain (BWG), coupled with decreased food consumption (FC), is due to either an adverse effect or diet palatability. This study reports on the use of polynomial regression (PR) analyses to determine whether the complex relationship between BW and FC was affected by the test material, and whether the observed reduction in BWG was entirely attributable to FC. The test material was sucralose, a non-caloric sweetener. Diets containing high levels of sucralose are poorly palatable to rats. Sprague-Dawley rats were treated for 26 weeks. Group 1 was fed *ad lib*; Groups 2, 3 and 4 were offered 5, 10 or 15% less food than the mean FC of Group 1, respectively. Groups 5 and 6 were offered diet containing 1% or 3% sucralose *ad lib*, respectively. Groups 7 and 8 also received diet containing 1% or 3% sucralose, but were offered basal diet equal to 10% less than the mean FC of Group 1. A PR model set forth by FDA was fitted to the Week 26 BW data from the combined diet-restricted controls, Groups 1–4 (Group 0), and then to each of Groups 5–8, separately. BW was modelled as a function of initial BW and average weekly FC (linear, quadratic or cubic). Two statistical tests were performed. Test 1 assessed if the regression curves were similar for Group 0 and each treatment group. Test 2 compared expected BW for Group 0 and each sucralose group derived from the regression model in test 1. This method was capable of detecting an approximately 5% decrease in expected BW.

396 EVALUATING POTENTIAL RISK FROM EXPOSURE TO MULTIPLE PESTICIDE RESIDUES IN FOOD: POSSIBLE APPROACHES FOR ESTIMATING TOTAL DIETARY RISK

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Pesticide safety has traditionally been evaluated by considering the exposure data for each chemical separately. An NAS report and other sources have recommended that dietary risk due to chemicals, especially those that act by similar mechanisms, be considered *in toto*. We have calculated estimates for the total dietary risk from multiple pesticides using the pesticide residue data from the USDA, 1992. The data on the magnitude and frequency of occurrence of five carbamates/organophosphates on over 1500 samples of representative fruits and vegetables were used to estimate potential exposure from the entire distribution of residue levels. The toxic contribution from each component was adjusted for each pesticide residue using the "toxic equivalency" approach (EPA, 1989) to weight the relative toxicity of each pesticide on the food sample. Toxicity endpoints were based upon levels that caused inhibition of plasma, blood and/or brain cholinesterases in animal tests. Data examined to date indicate that several samples had from 0 to 8 different pesticide residues within an overall range of 3 to 2700 ppb. Dietary/exposure estimates were found to be significantly affected by the proportion and handling of samples with no quantifiable residues.

397 SUBCHRONIC ORAL TOXICITY OF THE CCK-B/GASTRIN RECEPTOR ANTAGONIST CI-988 IN CYNOMOLGUS MONKEY

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CI-988 is a selective, nonpeptide CCK receptor antagonist with nanomolar affinity for the CCK-B/gastrin receptor. Subchronic toxicity was evaluated in cynomolgus monkeys at doses of 5, 25, and 74 for up to 13 weeks. After four weeks of treatment, gastric gland degeneration was observed in two of four and four of four monkeys at 25 and 75 mg/kg, respectively. However, gastric changes were absent in six monkeys at each dose level when evaluated after 13 weeks of continued dosing. No gastric changes were observed at 5 mg/kg at either 4 or 13 weeks. Similarly, average serum gastrin levels in 75 mg/kg males were evaluated approximately 10-fold relative to controls at four weeks, but were comparable to controls after 13 weeks of treatment. Mean plasma concentrations of CI-988 at 5, 25, and 75 mg/kg, respectively, were 1.3, 3.7, and 7.5 ng/mL during Week 4 and <1.0, 9.2 and 7.8 ng/mL during Week 13. The reversal of stomach mucosal changes and normalization of serum gastrin levels in the absence of changes in CI-988 pharmacokinetics, suggest adaption to continued treatment.

398 TOXICOKINETIC COMPARISON OF A CCK ANTAGONIST GIVEN ORALLY AND INTRAVENOUSLY

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CI-988, is a novel peptoid cholecystokinin antagonist selective for the CCK-B/gastrin receptor. In the cynomolgus monkey, CI-988 induces mild gastric gland degeneration, possibly through inhibition of gastrin's trophic effect on the gastric mucosa. This effect of CI-988 appears to be transient in that the mucosal changes resolve with continued treatment which suggests some form of adaptive gastric response. Twenty-five mg/kg given daily for four weeks was the lowest oral dose resulting in gastric mucosal changes and produced Cmax and AUC values of 11.1 ± 7.8 ng/mL and 55.6 ± 43.4 nghrmL^{-1}, respectively. In contrast, intravenous dosing at up to 5 mg/kg for four weeks failed to induce gastric changes, despite achieving plasma concentrations as great as 1260 ± 210 ng/mL (5 min. postdosing) and yielding AUC values as high as 1220 ± 110 nghrmL^{-1}. These data indicate that plasma CI-988 concentrations are not predictive for, nor directly associated with gastric gland degeneration. Alternatively, the gastric gland degeneration may be related to local rather than systemic exposure to the CI-988.

399 DICHOTOMOUS GASTRIC EFFECTS OF A CHOLECYSTOKININ ANTAGONIST IN THE CYNOMOLGUS MONKEY

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The gastrointestinal hormone gastrin functions not only as a secretogogue for gastric acid, but also as a trophic factor for gastric mucosa. Oral administration of CI-988, a novel peptoid CCK-B/gastrin receptor antagonist, to monkeys at 25 mg/kg and above for four weeks resulted in transient, mild gastric gland degeneration. These histopathologic findings are consistent with competitive antagonism at the gastrin receptor with consequent inhibition of gastrin's trophic effects on gastric mucosa. In contrast, CI-988 given orally to monkeys at 10 mg/kg caused a minor stimulatory effect on gastric acid secretion, resulting in a transitory increase in acid output from 110 ± 54 μEq/hr to 585 ± 200 μEq/hr. This secretory effect also appears to be mediated through activity at the level of the gastrin receptor. Since the regulatory mechanism(s) governing gastrin's secretory and trophic effects are poorly understood, CI-988 may serve as a useful tool to dissociate these two distinct physiologic attributes.

400 INDUCTION OF FREE RADICALS AND HSP90 IN GASTRIC CELLS BY *HELICOBACTER PYLORI*, AND ANTIOXIDANT PROTECTION

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The induction of reactive oxygen species (ROS) in gastric cells (GC) in association with *H. pylori* (HP) was assessed by chemiluminescence (CL), cytochrome *c* reduction (CCR), nitrobluetetrazolium reduction (NBTR) and lactate dehydrogenase leakage (LDL). Following incubation of GC with HP (1:1), approximately 7.0- and 4.3-fold increases were observed in CCR and NBTR, respectively, as compared to GC in the absence of HP, demonstrating the production of superoxide anion. Dose-dependent enhanced CL was observed following incubation of GC with 1:1, 1:5 and 1:10 ratios of HP. Dose- and time-dependent LDL from GC was observed following incubation of GC with various proportions of HP. After incubation for 24 hrs, approximately 2.2- and 3.5-fold enhanced LDL were observed, respectively, at a GC:HP ratios of 1:1 and 1:10, respectively. Substantial inhibition of LDL from GC in the presence of HP as well as decreased CCR and NBTR were observed following co-incubation with selected ROS scavengers, antioxidants and histamine H_2-receptor antagonists (HHA). The histamine H_2-receptor antagonist cimetidine served as the best chemoprotectant. Incubation of GC with HP enhanced Hsp90 synthesis as detected by Western blot analysis. This study demonstrates that HP induces the formation of ROS and Hsp90 in GC, and enhances membrane damage.

401 MORPHOLOGIC AND MORPHOMETRIC ANALYSIS OF CELLULAR AND SUBCELLULAR STRUCTURES OF RAT GASTRIC MUCOSA AFTER E3810, A NOVEL PROTON PUMP INHIBITOR

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Gastric mucosal alterations were evaluated by light and electron microscopy after E3810, a novel proton pump inhibitor, was given subcutaneously to male Wistar rats at daily doses of 5, 10 and 20 mg/kg for 2 and 5 weeks. Plasma gastrin levels increased in rats given 20 mg/kg for 5 weeks. By light microscopy, a dose-related increase of the fundic mucosal thickness was noted at 5 weeks in treated groups. The enterochromaffin-like (ECL) cell density also increased at 20 mg/kg in the 2 week-treatment groups and at all doses in the 5 week-treatment groups. No vacuolization occurred in parietal cells even in rats given 20 mg/kg for 5 weeks. Electron microscopy of parietal cells showed that the volume and surface densities of tubulovesicles did not change in both the 2 and 5 week-treatment groups, whereas those of microvilli on secretory canaliculi significantly decreased in the 5 week-treatment groups. These results suggest that increases in the mucosal thickness and ECL cell density are attributed to the hypergastrinemia induced by E3810 administration, whereas the morphometric changes in microvilli of parietal cells result from the inhibitory effect of E3810 on the gastric proton pump. However, morphologic and morphometric changes in parietal cells after E3810 were distinctly mild, compared with those reported previously for omeprazole.

402 TOXICITY STUDIES OF PENTAERYTHRITOL

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Pentaerythritol (PE) is used in formulation of alkyd resins and varnishes and as an intermediate in the manufacture of plasticizers, explosives and pharmaceuticals; however, little data have been published on its toxicity. PE, 99.4% purity, was tested in a mouse micronucleus assay and a 90 day subchronic toxicity assay. In the micronucleus assay, groups of mice (5 M/5 F) were dosed i.p. for 2 consecutive days at 8, 14.2, 26, 45 or 80 mg PE/mouse. Slides were evaluated for micronuclei (MN) as compared to concurrent controls. There was no evidence of an adverse reaction in the mice, no depression in polychromatic erythrocytes (PCE), and no increase in micronuclei. The MN/PCE values were 5.83, 3.90, 5.77 and 5.66% for males and 3.88, 4.79, 1.99 and 1.93% for females in the 0, 26, 45 and 80 mg/mouse/day dose groups, respectively. In the subchronic toxicity assay, groups of adult rats (10 M/10 F) were dosed via oral gavage at 0, 500, 1250 or 2500 mg PE/kg/day for 90 consecutive days. No treatment related effect on body weights, food consumption or clinical signs were noted during the dosing period. At termination body weights were within 5% of controls and there were no treatment related effects on clinical chemistry or hematology values, or organ weights. A few statistically significant findings in clinical chemistry and food consumption data were considered normal variations and not treatment related. The only treatment- and dose-related effect was an enlargement of the cecum which correlated with an increased incidence of "soft, fluid contents". Microscopic examination revealed no histologic changes. Quantitative and qualitative analysis of the microbial content indicated no difference from control or from untreated rats similarly processed. In summary, PE was not clastogenic in the mouse micronucleus assay and, except for cecal enlargement, exhibited no toxicity when administered for 90 days to rats at the maximum doses attainable.

403 EFFECT OF DX-9065A, A NEW ANTICOAGULANT DRUG, ON PANCREAS

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DX-9065a (DX), a new anticoagulant drug, has a specific inhibitory action on activated factor X in blood, but it also has a relatively slight inhibitory action on trypsin. It is wellknown that oral administration of trypsin inhibitor induces pancreatic hypertrophy and stimulates pancreatic exocrine secretion. The present study was performed to investigate the effect of DX on pancreas in comparison with camostat (CA), a synthetic trypsin inhibitor. In vitro inhibition constant of DX with trypsin was 1 μM from Dixon-plot. Seven-consecutive-day oral administration of DX (100 mg (0.22 mmol) /kg) or CA (111 mg (0.22 mmol) /kg) increased pancreatic weights and protein and enzyme (trypsin, chymotrypsin, lipase) contents significantly in rats. Histopathologically, pancreatic acinar cell hypertrophy was noted. In cynomolgus monkeys, beagles and guinea pigs treated with DX (100 mg/kg) and CA (111 mg/kg) for 7 days, however, no increase in pancreatic weights and contents of protein and enzymes were observed. After the recovery period (14 days), pancreatic weights returned to control level in the rats previously treated with DX or CA. In conscious rats, oral administration of DX or CA stimulated the secretion of pancreatic juice, protein, trypsin and amylase. After a single oral dosing of DX or CA, CCK levels in plasma were increased 10-fold above controls in rats but only a slight increase was seen in monkeys and no increase was noted in dogs. MK-329, a competitive inhibitor of CCK receptor, reduced the trophic effects of DX and CA on rat pancreas. These results suggest that the trophic effect of DX on pancreas in rats is mediated by CCK in a similar way to CA.

404 CHARACTERIZATION OF ADENOSINE AND GUANOSINE ADDUCTS OF BUTADIENE MONOXIDE

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We have previously shown that butadiene monoxide (BM) reacts *in vitro* with guanosine to form two diastereomeric pairs of two regioisomeric N^7-guanosine adducts. Reactions with other nucleosides have not yet been characterized. In this study, four adenosine adducts were isolated from the reaction of BM with adenosine. These adducts were determined to be diastereomers formed by the attack of the N^6 of adenosine at the two oxirane carbons of the two enantiomers of BM, based upon their stability under alkaline conditions and characterization by UV- and proton NMR spectroscopy and fast-atom

bombardment mass spectrometry. Upon the reaction of racemic BM (200 mM) with adenosine (3 mM) in phosphate buffer (pH 7.4) at 37°C, the regioisomer N^6-(1-hydroxy-3-buten-2-yl)adenosine was formed preferentially (5.3-fold) over N^6-(2-hydroxy-3-buten-1-yl)adenosine, while the diastereomers appear to be formed in nearly equal amounts. When the reaction of BM with guanosine was reexamined, adducts other than the previously characterized N^7-adducts were detected. The rates of formation of these adducts were enhanced when the reaction was carried out at pH 10.0 instead of pH 7.4. These adducts are tentatively identified as N^1- and N^2-guanosine adducts based on their higher rates of formation under alkaline conditions, their UV- and proton NMR spectra, and stability studies carried out under basic conditions. (Supported by NIH grant ES06841 and an NSF Graduate Research Fellowship).

405 IDENTIFICATION AND QUANTITATION OF DNA ADDUCTS FROM CALF THYMUS DNA EXPOSED TO 1,2-EPOXYBUTENE

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This study was conducted to characterize the DNA adducts formed when adenine or guanosine is reacted with 1,2-epoxybutene (EB). HPLC separation coupled with diode array detection was employed to purify the adducts that were formed. Two EB-adenine products were identified as N^6-(2-hydroxy-3-buten-1-yl) adenine (I) and N^6-(1-hydroxy-3-buten-2-yl) adenine (II) by ^{1}H-NMR analysis. Two guanine adducts similar to those previously characterized by Citti et al. (Carcinogenesis, 5: 47–52, 1984) as 7-(2-hydroxy-3-buten-1-yl) guanine (I) and 7-(1-hydroxy-3-buten-2-yl) guanine (II) were also collected. Absorptivity coefficients for EB-adenine adducts were determined. All four adducts were used to construct standard curves to quantitate the corresponding adducts formed in DNA incubated *in vitro* with EB. EB-adenine and EB-guanine adducts were detected in EB-treated calf thymus DNA. The formation of EB-adenine adducts per adenine (1.0×10^{-3} EB-adenine I, 2.7×10^{-3} EB-adenine II) in calf thymus DNA was approximately one tenth that of EB-guanine adducts per guanine (4.2×10^{-2} EB-guanine I, 3.0×10^{-2} EB-guanine II). Since 1,3-butadiene and EB induce nearly equal numbers of point mutations at AT and GC base pairs (Cochrane and Skopek, Carcinogenesis, 15: 719–723, 1994), the N^6-substituted adenine adducts may represent important promutagenic DNA adducts involved in the BD-induced mutagenesis and carcinogenesis. (Supported in part by a grant from CMA).

406 DETECTION OF 1,N^6-ETHENOADENINE EXCRETED IN RAT URINE AFTER CHLOROETHYLENE OXIDE EXPOSURE

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The four etheno adducts of vinyl chloride binding to DNA, 1, N^6-ethenoadenine (εA), 3,N^4-ethencytosine (εC), 1,N^2-ethenoguanine and N^2,3-ethenoguanine have recently been reported to be released by a partially purified human ethenoA-binding protein and cell-free extracts containing the cloned human N-methyl purine-DNA glycosylase from DNA treated with CAA (Dosanjh et. al., 1994). Adducts excised from DNA by glycosylase are usually excreted in urine and can be assayed as a measure of DNA damage in exposed individuals. We have detected εA in the urine of rats exposed to CEO intraportally by a previously developed immunoaffinity chromatography method (Misra et. al., 1994), in which the enrichment of etheno adducts was achieved using immunoaffinity columns made of specific monoclonal antibodies coupled to Protein A-Sepharose. Following enrichment, individual ethenobase adducts were quantified by HPLC with UV and fluorescence detection. εA was present in urine of control and CEO-treated rats, with the latter having ≈50-fold greater amounts. The rate of excretion was time-dependent, being greatest 4–6 hours after exposure. While it is clear that CEO treatment results in increased excretion of εA, quantitation of total excretion will require further method development, since recovery is low. Nevertheless, this is the first demonstration of urinary excretion of etheno DNA adducts, providing evidence for *in vivo* repair by glycosylase activity. (Supported by NIEHS grant P42-ES0594803)

407 DNA ADDUCT FORMATION FROM METABOLITES OF 2,4-TOLUENEDIAMINE (2,4-TDA) AND 2,4-DINITROTOLUENE (2,4-DNT)

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Sponsor: D Roberts

Experiments were conducted with the goal of defining the metabolic activation pathways of 2,4-TDA to reactive metabolites that result in the DNA adducts observed *in vivo*. Metabolites of 2,4-TDA and 2,4-DNT were incubated with rat liver S9, cytosol, or, in the case of *N*-hydroxy derivatives, pH 5 citrate buffer and calf thymus DNA. DNA was isolated from the reaction mix and analyzed by ^{32}P-postlabeling. The resulting adduct pattern was compared to the adduct pattern in liver DNA from rats treated with 2,4-TDA. Initial experiments indicated multiple adduct patterns from the various metabolite and incubation conditions. An adduct comigrating with the major (≈95% of total) *in vivo* 2,4-TDA DNA adduct was found only in incubations of 2,4-TDA and 4-amino-2-nitrobenzyl alcohol with liver S9 and acetyl coenzyme A or 2-*N*-hydroxylamino-4-acetylaminotoluene and 2-nitro-4-acetylaminotoluene with liver S9 in the presence of acetyl coenzyme A or 3′-phospho-adenosine 5′-phospho-sulfate. The major *in vivo* adduct was not found in similar incubations with 2-acetylamino-4-*N*-hydroxylaminotoluene or 2-acetylamino-4-nitro-toluene. The data demonstrate that multiple pathways exist for the generation of DNA adducts from 2,4-TDA and 2,4-DNT. In addition, the results are consistent with the involvement of N-hydroxylation at the 2 position in the *in vivo* activation of 2,4-TDA. This latter observation contrasts with previous data that implicated 4-*N*-hydroxylation followed by *O*-acetylation in the metabolic activation of 2,4-TDA to a mutagenic species in *Salmonella typhimurium*. Work to characterize the adducts generated *in vitro* is continuing in order to better define the potential roles of *N*-acetylation and methyl oxidation in the activation of 2,4-TDA.

408 FIALURIDINE (FIAU) ACCUMULATES IN DNA OF DOGS, MONKEYS, AND RATS FOLLOWING LONG-TERM ORAL ADMINISTRATION

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Accumulation of the antiviral nucleoside analogue fialuridine (FIAU) in genomic DNA was examined using a modified version of a recently developed RIA for FIAU. DNA was obtained from tissues of dogs administered at 0, 1, 2 or 3 mg/kg/day FIAU for 90 days, monkeys administered 0 or 25 mg/kg/day FIAU for 30 days, and rats administered 0, 255, or 510 mg/kg/day for 70 days. FIAU incorporation was observed in all species. In the rat, FIAU was incorporated into DNA of all tissues examined with highest concentrations in the liver followed by jejunum, spleen, and heart. FIAU was also incorporated into sperm DNA. Incorporation rates were as high as 11,000 pmol FIAU/μmol thymidine or 1 FIAU molecule per 90 thymidine molecules. In dogs and rats, the extent of incorporation was dose-dependent. Across species, FIAU-DNA concentrations were not singly dependent on the total dose administered, but may also have been dependent on duration of exposure. These studies show that FIAU accumulates to high concentrations in genomic DNA of liver as well as other tissues during chronic oral administration and suggest that net accumulation of FIAU in DNA may be a critical step in FIAU-induced toxicity.

409 EVALUATION OF PEPTIDE SYNTHESIS AND PURIFICATION REAGENTS IN THE *SALMONELLA TYPHIMURIUM* (AMES) TEST

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Mutagenic activity of a substance can be caused by the presence of trace amounts of impurities or degradents. For example, some peptides are weakly mutagenic in the Ames tests, although structure-activity analysis suggests the peptides themselves should have no mutagenic activity.

A number of chemicals often used in the synthesis and purification of peptides were evaluated in the Ames test. These included o-(N-succinimidyl)-N,N,N′,N′-tetramethyluronium tetrafluoroborate (I), pentafluorophenyl trifluoroacetate (II), (S)-(−)-2-azetidinecarboxylic acid (III), benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (IV), N-(9-fluorenylmethoxycarbonyl)-L-Proline Pentafluorophenyl (V), N-(tert-Butoxycarbonyl)-L-Tyrosine (VI) and BOCON (VII).

Each chemical was tested at a minimum of 7 dose levels (0, 100, 500, 1000, 1250 2500 and 5000 ug/plate). At least 2 plates were used per treatment group.

Four *Salmonella typhimurium* strains (TA98, TA100, TA1535 and TA1537) were used in both the presence and absence of aroclor-induced rat liver S9 metabolic activation.

None of the chemicals showed mutagenic activity. Toxicity to the bacterial was found at dose levels of ≥100 ug/plate for III, of ≥1000 ug/plate for II, IV, and VII, and of ≥2500 for V.

These results show that any mutagenic activity of a peptide in an Ames test using these strains cannot be attributed to the presence of "contaminating" amounts of any of these chemicals.

410 MUTAGENICITY OF BATRACYLIN IN *SALMONELLA TYPHIMURIUM* EXPRESSING DIFFERENT LEVELS OF ACETYLTRANSFERASE ACTIVITY

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Batracylin (BAT), a heterocyclic amine developed for use as an antitumor agent, has been shown to be mutagenic in several *S. typhimurium* strains particularly TA 98. The mutagenicity of BAT required a rat liver S9 activating system. *In vivo* and *in vitro* studies suggested that acetylation was involved in the adverse effects of BAT. Studies were initiated to investigate the hypothesis that acetylation enhances the mutagenicity of BAT in bacteria. Mutagenicity tests were performed in *S. typhimurium* TA 98 and its derivatives; TA 98/1,8DNP$_6$, and YG 1024. At 0.1 μg BAT, 2062 ± 344 revertants/plate were seen in YG 1024, a strain expressing elevated bacterial acetyltransferase (AT) activity. In TA 98, which expresses basal levels of AT, 0.1 μg BAT induced 288 ± 83 revertants/plate, while 56 ± 16 revertants/plate were observed in TA 98/1,8DNP$_6$ which lacks AT. A similar response was seen in TA 1538 and its derivatives. The strain expressing elevated AT activity, YG 1012, was again the most sensitive to the mutagenicity of BAT. At 0.1 μg/plate, BAT induced 1958 ± 329 revertants/plate in YG 1012, and 576 ± 146 revertants/plate in TA 1538. Little mutagenicity was seen in TA 1538/1,8DNP$_6$, a strain deficient in AT. These data demonstrate that elevated levels of acetylation in bacteria enhance the mutagenicity of BAT. Assays were also performed in TA 1538/1,8DNP$_6$ strains which lack the bacterial enzyme but express the human N-acetyltransferase genes *NAT1* (DJ 400) or *NAT2* (DJ 460). BAT induced a significant number of revertants at 1.0 μg/plate in DJ 400 (1025 ± 112) and DJ 460 (1536 ± 135), compared to the strain that lacks AT activity, TA 1538/1,8DNP$_6$ (580 ± 233). This suggests that the human ATs are capable of bioactivating BAT. These studies demonstrate that acetylation enhances the mutagenicity of BAT and provides further support that the adverse effects of BAT are linked to acetylation. (Supported by NIEHS ES-05174 and Procter & Gamble predoctoral fellowship).

411 MUTATIONAL SPECTRA GENERATED IN *SALMONELLA TYPHIMURIUM* TESTER STRAINS

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A set of *Salmonella typhimurium* strains (TA7001-TA7006) designed to identify specific base substitutions upon reversion were used to determine mutational spectra of several polycyclic aromatic hydrocarbons. The quinone intermediates of benzo[a]pyrene and 2-aminoanthracene metabolism have been shown to be carcinogenic in rodents and mutagenic in several non-specific *Salmonella* tester strains (TA102, TA98 *etc.*) at 5–10 μg/ml. The TA700x strains were mixed to simulate a comparable test system to the non-specific strains. The mixture remained His due to the minimal genetic complementation among the six strains. While the reversion of the mixture was 4 fold of the spontaneous reversion, there was a further increase in reversion of over 100 times (497 fold) when rat liver S9 fraction (4.5%) was used at 10 μg/ml. When the specific strains were used separately, benzo[a]pyrene and its activated quinone intermediates induced C·G to G·C transversions 157 fold, C·G to A·T transversions 63 fold, G·C to A·T transitions 54 fold, and T·A to A·T transversions 11 fold. The frameshift mutants TA98 and TA1537 reverted at 10 fold and 6 fold of spontaneous levels respectively. We did not detect any A·T to G·C transitions or T·A to G·C transversions for benzo[a]pyrene. In comparison, 2-aminoanthracene and its activated derivatives induced C·G to G·C transversions 105 fold. G·C to A·T transversions 73 fold, C·G to A·T transitions 25 fold, T·A to A·T transversions 18 fold and reverted the frameshift mutants TA98 76 fold. There was no reversion in TA1537 at 10 μg/ml but there was a 25 fold induction at 1.0 μg/ml. There were no significant inductions (3 fold) for T·A to G·C transversions or A·T to G·C transitions. These mutational spectra are generally consistent with known adducts formed by these polycyclic aromatic hydrocarbons.

412 GENOTOXIC AND ALKYLATING ACTIVITY OF AMINO ACIDS AND FLAVONOIDS UPON NITROSATION

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Diet is regarded as an important factor in the aetiology of human gastric cancer, with emphasis on the formation of direct alkylating nitrosamines from dietary precursors. The normal human diet contains many constituents that can be nitrosated in the gastro-intestinal tract (particularly in the stomach) to potentially carcinogenic N and C nitroso compounds. We have studied the mutagenic activity of different amino acids and related compounds upon nitrosation at pH 3.0 in the Ames test (TA 100). The same set of nitrosated compounds were assessed in a colorimetric assay using 4-(p-nitrobenzyl) pyridine (NBP) as a nucleophilic scavenger of alkylating agents. The results were compared and correlated with the mutagenic assays. Polyphenols are also widely present in the human diet. Thus, in parallel we have studied the mutagenic activity of different flavonoids upon nitrosation in the Ames assay (TA 100) along with the NBP assay. The mutagenic activity of flavonoids upon nitrosation is related to the number of hydroxyl groups on the B ring. Quercetin (two OH groups on ring B) presented the higher mutagenic activity upon nitrosation (6.4 rev/nmole). Compounds without any OH group in the B ring were not genotoxic upon nitrosation. The glycosides of flavonoids studied shown a lower mutagenic activity compared with their respective aglicones. The structural requirements for direct mutagenicity of flavonoids is different from mutagenic activity upon nitrosation.

413 MUTAGENICITY AND INDUCIBILITY OF A TUMOR MARKER (GLUTATHIONE S-TRANSFERASE P-FORM) OF LOW-CHLORINATED DIBENZOFURANS

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Toxic effects of low-chlorinated dibenzofurans are unclear, although polychlorinated dibenzofurans and related compounds are known to be potent tumor promotors and teratogens. The mutagenicity of 1-, 2-, 3- and 4-chlorodibenzofuran (CDF) was tested using *Salmonella typhimurium* TA98 and TA100, and their *in vitro* inducibility of glutathione S-transferase P-form (GST-P), a marker of hepatocarcinoma, was examined. i) 3-CDF was markedly mutagenic, and the intensity of its mutagenicity in TA98 was about 1/5 and in TA100 about 1/20 of that of benzo[a]pyrene, while 2-CDF showed weak mutagenicity. ii) 3-CDF expressed GST-P mRNA dose-dependently (0.1–100 μ M) in primary cultured rat liver parenchymal cells, while 2-CDF was a weak inducer. Previously we reported that GST-P was induced by coplanar polychlorinated biphenyls (PCBs) in the liver cells. The induction of GST-P by both coplanar PCBs and 3-CDF was inhibited by dexamethasone. 3-CDF seems to possess the same effect on the liver cells as coplanar PCBs.

414 CELLULAR RESPONSES TO DIFFERENT SULFONIC ACIDS

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A series of stress gene and genotoxicity assays were developed to elucidate the molecular mechanisms of cellular response(s) toward toxic and potentially mutagenic and/or carcinogenic compounds. The Pro-Tox and CAT-Tox (L) Assays use promoter::reporter gene constructs in bacterial and mammalian cells, respectively, to detect cellular responses to compounds that damage DNA, proteins, and/or membranes. The Yeast DEL, Ames II, and *E. coli* Trp Assays detect damage to DNA by intrachromosomal recombination in yeast and by reversion of point mutations in bacteria. The endpoints of these assays were optimized and verified with known toxicants. Two known mutagens, methyl methanesulfonate (MMS) and ethyl methanesulfonate (EMS) were positive in the genotoxicity assays as expected. Both EMS and MMS elicited strong gene induction with promoters that respond primarily to DNA damage in the Pro-Tox and CAT-Tox (L) assays. In contrast, tricaine methanesulfonate (TMS) and sodium xylenesulfonate (SXS) were negative in all three genotoxicity assays. Consistent with these results, TMS and SXS did not induce genes which respond to DNA damage; however, there were strong inductions of promoters that implicate protein denaturation and cellular membrane damage and/or transport. Since changes in the structure of these sulfonic acids elicit distinct cellular responses, these studies verify the specificity and sensitivity of these assays to correlate structure to toxicity.

415 FORMATION OF BENZO[a]PYRENE-7,8-DIONE (BPQ) IN ISOLATED RAT HEPATOCYTES: A REACTIVE POLYCYCLIC AROMATIC HYDROCARBON METABOLITE

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Homogeneous dihydrodiol dehydrogenase catalyzes the oxidation of (±)-*trans*-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene (BP-diol) to yield BPQ. This reaction has also been documented in rat liver S_{100} fractions [Carcinogenesis, 13: 1575 (1992)]. In these studies [^{3}H]BP-diol (20 μM) was incubated with isolated rat hepatocytes (3×10^6 cells/ml) and BPQ formation was detected in the organic phase of the extracellular media by diode-array RP-HPLC. The identity of BPQ was further established by co-chromatography with an authentic standard and by EIMS. The biological properties of BPQ were examined. [^{3}H]BPQ (20 μM) was found to be mildly cytotoxic in hepatocytes (1.5×10^6 cells/ml) [30% cell death after 2 h]. In addition, radioactivity was rapidly sequestered into the cell pellet (30% of the total). Although only a small number of BPQ-DNA adducts were detected, examination of the hepatocellular DNA by agarose gel electrophoresis indicated that extensive fragmentation had occurred. The ability of BPQ to cause DNA strand scission was studied *in vitro* using ϕX174 SC DNA. It was found that BPQ caused strand scission under conditions of redox-cycling and Fenton chemistry (NADPH and $CuCl_2$) which was prevented by catalase, indicating that reactive oxygen species were responsible for the DNA fragmentation. BPQ was also found to be a direct-acting mutagen in the Ames test using *S. typhimurium* tester strains TA97a, TA98, TA100, TA102, and TA104 but was less efficient than (±)-*trans*-7,8-dihydroxy-*anti*-9, 10-epoxy-7,8,9,10-tetrahydrobenzo-[a]pyrene. These data suggest that BPQ is an authentic metabolite of BP-diol in whole cells and that it is cyto- and genotoxic. Supported by NCI Grant 39506.

416 CO-MUTAGENIC EFFECTS OF SULFITE AND BENZO(a)PYRENE IN A MAMMALIAN CELL LINE

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Sulfur dioxide, a ubiquitous air pollutant, increases the incidence of benzo(a)pyrene (BP)-induced respiratory tract carcinogenesis in rodents when exposed concurrently. The interactions between sulfite, the physiological form of sulfur dioxide, and (±)-7r,8t-dihydroxy-9t,10t-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene (*anti*-BPDE), the ultimate carcinogenic form of BP, results in an enhanced mutation rate in an *in vitro* model system. When V79 hamster lung fibroblasts are exposed to 1 or 10 mM sulfite immediately prior to the addition of *anti*-BPDE (0.05 uM), an increase in mutation rate of 50% and 150%, respectively over that of *anti*-BPDE alone was seen. Sulfite does not cause an increase in the mutation rate over that of the spontaneous rate. Distinct differences are seen in the kinetics of covalent binding of BPDE-derived material to nuclear DNA. The *hprt* coding region from isolated mutants resulting from the treatment with *anti*-BPDE and sulfite or *anti*-BPDE alone was examined by PCR sequencing techniques. The mutation profile for the *anti*-BPDE induced mutants was identical to that seen in recent published reports, however, 3 of 8 mutants formed after exposure to sulfite and *anti*-BPDE exhibited sequence changes not seen before in *anti*-BPDE mutation profiles. These results demonstrate that sulfite clearly increases the mutagenic potency of *anti*-BPDE and that this produces unique mutations at the *hprt* gene, not seen after *anti*-BPDE treatment alone. The mechanism(s) responsible for these findings is under investigation. (ES-04092 and ES-07079).

417 ASSESSMENT OF THE MUTAGENICITY OF METHYL-TERTIARY BUTYL ETHER AT THE HPRT GENE IN CD-1 MICE

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Methyl-tertiary butyl ether (MTBE) is being used in rapidly increasing amounts as an anti-knock compound and oxygenate in automobile fuels. Recent studies have found increased frequencies of renal tumors in male rats and liver tumors in male and female mice at high exposure levels. *In vitro* tests for genetic toxicity have been largely negative. Effects of long duration exposure on mutagenesis *in vivo* have not been reported. We assessed the mutagenic activity of MTBE at the *hprt* locus in mouse spleen lymphocytes. Male and female CD-1 mice were treated five days/week for three weeks by oral intubation with MTBE in corn oil at 0, 1, 10, 100, and 1,000 mg/kg (5 mice per sex per treatment group) and held for three additional weeks to allow mutation expression. Positive controls were treated once with ethylnitrosurea (ENU) 25 mg/kg, i. p. six weeks prior to assay. Lymphocytes were recovered from the spleen and assayed to determine the frequency of *hprt* mutants of each mouse by a clonal assay adapted from Skopek et al. PNAS, 89:7866 (1992). MTBE had no effect on the frequencies of mutant cells with values (mean ± SD per 10^6 evaluatable cells) of 2.01 ± 0.77; 2.17 ± 0.76; 2.02 ± 0.78; 2.93 ± 1.56; 2.69 ± 0.67 in the males at 0, 1, 10, 100, and 1,000 m/kg/day respectively. The females also showed no change in response to MTBE. In both sexes the response to ENU was an increase of about 10-fold over the negative control. Daily oral administration of MTBE for three weeks over a wide dose range does not appear to be mutagenic at the *hprt* gene locus in mouse lymphocytes. Supported by grants from ATSDR (DHHS U50/ATU6800079-01) and NIEHS (T32ES07254).

418 THE GENOTOXIC POTENTIAL OF NICOTINE AND ITS MAJOR METABOLITES

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Nicotine is a naturally occurring alkaloid found in members of the solanaceous plant family, which includes tobacco. Nicotine is metabolized by mammalian enzymes, primarily cytochrome P450s. Studies on the genotoxic potential of nicotine and its metabolites are limited. Nicotine and four of its major metabolites: cotinine, nicotine N′-oxide, cotinine N-oxide, and 3′-hydroxycotinine were evaluated for genotoxic potential in the *Salmonella* mutagenicity assay (strains TA 98, TA 100, TA 1535, TA 1537, and TA 1538) at concentrations ranging from 0 to 1000 μg/plate and in the Chinese hamster ovary sister chromatid exchange (SCE) assay at concentrations ranging from 0 to 1000 μg/ml. All assays were conducted with and without S9 metabolic activation. None of the five compounds increased the frequency of mutations or the frequency of SCEs. These results indicate that nicotine and its major metabolites are not genotoxic.

419 MUTAGENICITY OF VINYL CHLORIDE AND ITS METABOLITES, AND VINYL FLUORIDE IN METABOLICALLY-COMPETENT HUMAN LYMPHOBLASTOID LINE

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Vinyl chloride (VC), a known human and rodent carcinogen, is metabolically activated by cytochrome P450 to chloroethylene oxide (CEO), which rapidly rearranges to chloroacetaldehyde (CAA). To further understand the role of CEO and CAA in VC mutagenesis, their frequencies and types of mutations induced at the hypoxanthine (guanine) phosphoribosyltransferase (*hprt*) locus were examined in a human lymphoblastoid line containing cloned human cytochrome P450 2E1. Delivered doses of 2.5 to 15.0% of VC in air resulted in induced mutant frequencies ranging from 26 to 38×10^6 with 60 to 70% survival after 48 hr exposure. The mutagenic response showed a plateau between 2.5 and 7.5%, suggesting saturation of metabolic activation. Vinyl fluoride, another vinyl halide, did not induce statistically significant increases in mutations under the same assay conditions. Both CEO and CAA exhibited a dose-dependent increase in cell killing and mutant frequency. Treatments of 16 μM CEO and CAA for 24 hr resulted in survival of 75% and 5%, and induced mutant frequencies of 19×10^{-6} and 16×10^{-6}, respectively. The extent of structural alterations induced at the *hprt* locus in independent thioguanine-resistant clones was examined by Southern blot analysis; 80–90% of VC- and CEO-induced mutants showed no detectable alterations, compared to 60% of CAA-induced mutants. CEO and VC exposures produced similar toxicity and mutation induction, as well as similar types of mutations, while CAA was much more toxic and induced a significantly greater number of deletions. This evidence supports the hypothesis that the majority of mutations induced by VC occur through its metabolite, CEO. (Supported by NIEHS grant P42-ES05948-03).

420 GENOTOXICITY OF 2,4-DIAMINOTOLUENE (2,4-DAT) AND 2-AMINOFLUORENE (2-AF)

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2,4-DAT, a mammary carcinogen in rodents, can be released in situ from

the degradation of polyurethane foam covered breast implants. We have investigated 2,4-DAT genotoxicity, primarily utilizing peroxidase activating systems. Previously, we found that 2,4-DAT is a weak mutagen in the Ames' test when activated by cytochrome P450 but is non-mutagenic when activated by prostaglandin H synthase (PHS). However, 2,4-DAT enhances the mutagenic response to 2-aminofluorene (2-AF) in both systems. We believe that PHS-derived 2,4-DAT metabolites can damage bacterial DNA. Although these lesions are not mutagenic, they enhance 2-AF mutagenicity. This hypothesis was tested using the *umu* test, a less selective bacterial genotoxicity system, and by a DNA strand break assay (SBA). In the *umu* assay, 2,4-DAT is genotoxic, eliciting up to a 250% increase above controls in the PHS catalyzed system. 2-AF does produce a mutagenic response in both catalyzed systems. It increases β-galactosidase levels by up to 100% in the PHS catalyzed systems and by up to 280% in P450 catalyzed systems as compared to control levels. In the DNA SBA, ϕX174 supercoiled closed-circular DNA (type 1) was incubated with 2,4-DAT and horseradish peroxidase to determine if this system can cause single strand breaks thus forming nicked circular (type II) DNA. 2,4-DAT in this system increased the amount of type II DNA up to 170% above control levels at concentrations similar to those used in the bacterial studies. SBA assays run with several 2,4-DAT analogs did not produce significant strand breakage. In conclusion, 2,4-DAT produced a genotoxic effect in the *umu* assay and in the SBA assay in a peroxidase catalyzed system. This supports our hypothesis that peroxidative 2,4-DAT metabolites can damage DNA. Supported by NIH ES-07079.

421 *SALMONELLA* TEST-NEGATIVE CARCINOGENS INDUCE OXIDATIVE STRESS IN YEAST

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A large number of carcinogens, previously classified as 'non-mutagenic' on the basis of negative results in short-term tests such as the Ames *Salmonella* mutagenicity assay, induce intrachromosomal recombination between repeated sequences in *Saccharomyces cerevisiae*, resulting in deletion (DEL) of intervening sequences. We wished to investigate whether the generation of free radical species by *Salmonella* test-negative carcinogens may be responsible for their recombinagenic activity in *S. cerevisiae*. The free radical scavenger N-acetyl cysteine afforded protection against the lethal and recombinagenic effects of cadmium, aniline, o-anisidine, o-toluidine, auramine O, urethane, thiourea, chloroform and the oxidative mutagen formaldehyde. NAC did not protect against MMS, EMS or 4-nitroquinoline oxide. Sensitivity to the lethal effects of cadmium, aniline and formaldehyde, but not MMS, was greatly increased in strains deficient in superoxide dismutase. Intracellular oxidation of the free radical-sensitive reporter compound dichlorofluorescein to the fluorescent dichlorofluorescein in growing cultures of *S. cerevisiae* was induced by treatment with cadmium, aniline, o-toluidine, auramine O, benzene and carbon tetrachloride. MMS, EMS, 4-NQO and sodium azide did not increase fluorescence of the dye. The results indicate a role for oxidative stress in the toxicity and recombinagenicity of Ames negative carcinogens.

422 EFFECT OF *SALMONELLA* TEST POSITIVE AND NEGATIVE CARCINOGENS ON INTRACHROMOSOMAL RECOMBINATION IN CELL CYCLE ARRESTED YEAST CELLS

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A genetic system detecting deletions due to intrachromosomal recombination was constructed in the yeast *Saccharomyces cerevisiae* and named DEL recombination. It has been shown that DEL recombination is inducible by several *Salmonella* test positive and negative carcinogens. Important differences between *Salmonella* test negative and positive carcinogens have been observed. The *Salmonella* test positive carcinogens showed an almost linear dose response curve; significant increases of DEL recombination were seen at nontoxic doses. The *Salmonella* test negative carcinogens showed a threshold in their dose response curve below which no induction in DEL recombination was observed. Generally the *Salmonella* test negative carcinogens induced DEL recombination only at toxic doses. To investigate in which phase of the cell cycle DEL recombination is inducible in order to understand the mechanism of action of the *Salmonella* test negative carcinogens, we constructed two yeast strains that arrest in the G1 and G2 phase respectively, and cannot progress through the cell cycle unless they undergo DEL recombination. The *Salmonella* test negative carcinogens safrole and urethane induced DEL recombination in G2. However the *Salmonella* test positive carcinogens UV, EMS, MMS and 4NQO did not induce DEL recombination in G2. On the other hand, γ-radiation and bleomycin treatment induced DEL recombination in G2. This suggests that DNA double strand breaks are involved.

423 METABOLIC ACTIVATION OF 1-NITRONAPHTHALENE (1-NN) AND 2-AMINOANTHRACENE (2-AA) INDUCES THE BACTERIAL ALKYLATION ADAPTATION GENE (*ada*) *IN VITRO*

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Xenobiotics that generate reactive moieties capable of electrophilic attack, induce toxicity associated with adduct formation or covalent binding at nucleophilic centers in macromolecules. This current study was designed to detect *ada* gene activation and potential mutagenicity using bacterial gene expression (Pro-Tox, Xenometrics) and yeast recombination (Yeast-Del, Xenometrics) assays for future use in screening combinatorial libraries of novel compounds *in vitro*. With both 1-NN and 2-AA, metabolic activation using rat S9 (aroclor-induced) produced a 3–4 fold increase in *ada* gene expression relative to baseline levels (without metabolic activation). 1-NN which also produces toxicity through lipid peroxidative mechanisms, consistently induced oxidative stress promoters in the same assay. Fold-induction levels of both compounds after biotransformation were similar using the yeast recombination assay. These *in vitro* results are consistent with *in vivo* requirements for toxicity and mutagenicity.

424 USE OF AN EXCISON REPAIR DEFICIENT YEAST STRAIN IN THE YEAST DEL ASSAY

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The Yeast DEL Assay detects a broad spectrum of mutagens and carcinogens. This assay consists of a direct duplication of the *his3* gene *(his3Δ3′his3Δ5′)* where both copies are nonfunctional. Treatment of yeast with genotoxins induces homologous intra-chromosomal recombination, which results in formation of a *HIS3*$^+$ recombinant with subsequent deletion (DEL) of DNA between the two *his3* alleles. While the assay is capable of detecting many carcinogens it does not detect, or weakly detects, many compounds which form DNA adducts normally removed by excision repair. In this work we compare a yeast strain in which the DNA repair gene *RAD2* is either present or deleted (*rad2Δ*). The *rad2Δ* strain is more sensitive to the mutagens such as 2-aminoanthracene, 2-acetamidoflourine, 2-nitroflourene, and formaldehyde, which are not detectable with the *RAD2*$^+$ strain. The *rad2Δ* increased recombination 10 fold for 2-aminoanthracene, 10 fold for 2-acetamidoflourine, 5 fold for 2-nitroflourene, and 4 fold for formaldehyde. Dimethylnitrosamine, which was undetectable without S9 fraction in the *RAD2*$^+$ strain, induced recombination 10 fold in the *rad2Δ* strain. The compounds benzene, phenol, and cadmium sulfate, which are negative in bacterial reversion mutation assays, but are positive in the Yeast DEL Assay, induce recombination to the same degree in both the *RAD2*$^+$ and *rad2Δ* strains. This indicates these carcinogens induce recombination by means other than formation of DNA adducts recognized by the cells excision repair apparatus. Compounds such as dimethylsulfoxide, ethanol, and acetone, propanol, and butanol, which did not induce recombination in the *RAD2*$^+$ strain, did not induce recombination in the *rad2Δ* strain.

425 PRACTICAL METHOD OF LIVER MICRONUCLEUS TEST IN MICE

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We have developed a practical and simple method of mouse liver micronucleus assay and proposed a method combining liver and peripheral blood reticulocyte micronucleus assay as an advantageous screening to evaluate the clastogenic potential of new chemicals. Although this method does not require perfusion of liver, it showed a high sensitivity to detect short-lived mutagenic metabolites that were negative as tested in bone marrow (BM). The procedure primarily consists of chemical treatment, blood collection, partial hepatectomy, digestion of the liver, smear slide preparation and fluorecent staining. Test compounds used in the evaluation included: mitomycin C (MMC), cyclophosphamide (CP), N-ethyl-N-nitrosourea (ENU), diethylnitrosoamine (DEN), 4-dimethylamino-3′-methylazobenzene (3′-Me-DAB), 2-acetylaminofluorene (AAF), benzene (BEN), amsacrine (m-AMSA), 5-fluorouracil (5-FU), H_2SeO_3 and K_2CrO_4. As a result, the liver carcinogens, DEN and 3′-Me-DAB, increased micronuclei in the liver only. On the other

hand, BEN, known of its specific toxicity in BM, showed positive response in reticulocytes alone. The remainders showed positive response with similar incidence in both tissues. Moreover, with some of the compounds, there was persistent occurrence of micronuclei from 3 weeks (m-AMSA) to over 5 weeks (MMC and DEN) in the liver. DEN showed a maximum response more than 7 days after treatment, while the remainders showed peaks on day 1. These differences would be due to types of DNA damage caused. These results suggest that the liver is more prone to suffer from mutagens and the damage caused remains long. Further study to explore the use of liver in mutagenicity testing seems justified.

426 SMOKING-INITIATED MICRONUCLEUS FORMATION IN HUMAN BUCCAL CELLS: ENHANCEMENT VERSUS INTRA- AND INTER-SUBJECT VARIABILITY

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Preneoplastic assessment may be relevant to carcinogenesis, but published results of cellular micronucleus (MN) formation in smokers are conflicting and highly variable. To determine whether intra-subject variability and dose-response could account for these discrepancies, buccal cells were obtained repetitively from smokers and non-smokers, with at least seven days between sampling, and the cells were quantified for MN formation. Smokers had enhanced MN formation compared to non-smoking controls, although not all smokers were affected. This difference was evident only if the highest MN value of multiple samples was used for both smokers and non-smokers all of whom had upto a 5-fold intra-subject variability in MN over a 3-month sampling period. The affected smokers had MN counts greater than 3 standard deviations from the normal mean value. These results in total indicate that single samples do not accurately reflect mutagenic events in smokers, most but not all of whom do appear to have enhanced MN formation. (Support: Medical Research Council of Canada).

427 RELATIONSHIP BETWEEN FORMATION OF CARCINOGEN-DNA ADDUCTS [CA] AND MICRONUCLEI [MN] IN MOUSE KERATINOCYTES EXPOSED TO CARCINOGENS

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This project is concerned with determining the relationship between two biological markers of exposure [CA and MN] in viable keratinocytes following exposure to an environmental and occupational carcinogen; 7H-dibenzo[c,g]carbazole [DBC], in comparison to the carcinogen-7, 12-dimethylbenz[a]anthracene [DMBA]. Mouse keratinocytes were grown on collagen coated dishes [CA analysis] and slides [MN analysis] and dosed with the carcinogen. The dishes were trypsinized and the cells kept frozen at −80°C for subsequent DNA isolation and CA analysis [^{32}P-postlabelling]. The medium for the cells grown on slides was replaced with medium containing cytochalasin-B (drug which blocks cytokinesis — 3 μg/ml) and the cells were cultured for 72 hours, then fixed in 100% methanol and stained with acridine orange. DBC induced a dose-dependent [10–1000 ng/ml] increase in MN [36.2–105.8 MN/1000 binucleated [BN] cells] over solvent controls [32 +/− 3.7; N = 3] and in CA [10.9–37 (Relative Adduct Labelling [RAL] × 10^7 nucleotides) over solvent controls [5.7 +/− 2.9 (RAL × 10^7 nucleotides); N = 4]. DMBA induced a dose-dependent [32–256 ng/ml] increase in MN [46.6–110.1 MN/1000 BN cells] over solvent controls [21.3 +/− 10.4; N = 3]. DMBA-induced CA [3.5–10.8 (RAL × 10^7 nucleotides)] were very low when compared to solvent controls [2.4 +/− 0.7; N = 3]. The results indicate that the relationship between MN frequency and CA formation is unique for each compound tested. (Supported by NIOSH Grant No: RO3 OHO2880-02)

428 LACK OF GENOTOXIC ACTIVITY OF PHTHALATE ESTERS *IN VIVO*

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Phthalate esters are used extensively in the plastics industry and are also used in paints and other coatings. Due to their widespread uses, substantial amounts of toxicity data have been developed. Previous studies have indicated that Di-hexyl phthalate (DHP) was negative in the Ames *Salmonella* mutagenicity test, while Di-isodecyl phthalate (DIDP) was negative in both the Ames test and the mouse lymphoma assay. DHP and DIDP were tested in the mouse micronucleus assay in order to develop *in vivo* data for these materials. Male and female CD-1 mice were dosed by oral gavage with either 1250, 2500, or 5000 mg/kg of each test material. Cyclophosphamide was used as the positive control. Test animals were sacrificed at 24, 48, or 72 hours following dosing, and the bone marrow was harvested. Neither DHP nor DIDP induced a significant increase in micronuclei formation at any of the doses administered. Also, neither material produced evidence of bone marrow depression. These results correspond with published data indicating that phthalate esters, as a class, are non-genotoxic.

429 INVESTIGATION OF MICRONUCLEAR CONTENT AND ORIGIN USING MICRODISSECTION AND PCR-BASED TECHNIQUES

B E Peace, G K Livingston, J C Loper. *Department of Environmental Health, University of Cincinnati, Cincinnati, Ohio.* Sponsor: E O'Flaherty

Micronuclei have been tested and shown to be sensitive quantitative biomarkers of chromosomal damage in humans. The goal of this research is to extend the utility of this test by characterizing the DNA in micronuclei in order to determine the specific chromosomes, chromosome regions, or bands from which the micronuclei originate. The hypothesis of this work is that the distribution of DNA to the micronuclei is nonrandom and that our approach can identify patterns in the distribution. The procedures that have been developed use microdissection to remove a single micronucleus from a binucleated cell, followed by a PCR amplification of the DNA. The PCR uses single, 10-mer primers of arbitrary sequence, and is an adaptation of random amplification of polymorphic DNA (RAPD). This method has been applied to cultured binucleated lymphocytes from a single individual. This healthy, normal subject has consistently exhibited over 100 micronuclei per 1000 binucleated lymphocytes, indicating significant chromosomal instability of unknown etiology. This individual has served as an abundant source of micronuclei with which to develop this technique, as well as an outlier case for study. The amplification of DNA from several micronuclei from this subject has yielded short (150 bp) sequences which have been used as probes after labeling with digoxigenin-dUTP (DIG-dUTP). These probes hybridize to unique sequences in Southern blots of human genomic DNA.

430 URETHANE- AND RADON PROGENY-INDUCED MICRONUCLEI IN LUNG FIBROBLAST OF A/J AND C57BL/6J MICE

M A Khan, J D Saffer, K M Groch, A L Brooks. *Pacific Northwest Laboratory, Richland, WA.* Sponsor: D Mahlum

The genotoxicity of urethane and radon progeny was tested in A/J and C57BL/6J mice using the well-established lung fibroblast micronucleus assay. Three mice from each strain were either given a single intraperitoneal dose of 1 g/kg body weight urethane or exposed to 300 WLM radon progeny; two animals from each strain were kept as controls. Lung fibroblasts were isolated from individual experimental animals and cultured in the presence of cytochalasin-B, and slides were processed for micronuclei (biomarkers of genotoxicity). One thousand binucleated cells/animal were scored on coded slides and the number of micronuclei recorded. Urethane induced significant increases in micronuclei in both strains of mice (145 and 127 micronuclei/1000 for the A/J and C57BL/6J mice, respectively). The number of micronuclei was not significantly different between the two strains. For mice exposed to radon the increase was again significant (535 or 562 micronuclei/1000 binucleated cells for A/J and C57BL mice). This value (1.8 or 1.9 micronuclei/1000 cells/WLM for A/J and C57BL/6J respectively) was higher than observed for rats (0.53) and Syrian hamsters (0.80), and equal to that observed in Chinese hamsters (1.83). Our results in this study and those obtained in previous inhalation studies indicate that the lung fibroblast micronucleus assay has the potential to detect both genotoxic agents injected intraperitoneally or inhaled.

431 AN *IN VIVO/IN VITRO* METHOD OF ASSESSING CHROMOSOME DAMAGE IN RATS

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An in vivo/in vitro system using rat bone marrow cells (BMC) and spleen cells (SC) to assess micronucleus (MN) and structural chromosome aberrations (SCA) simultaneously was developed. In two separate experiments, two rats/dose/experiment were treated i.p. with 0, 5, 10 and 15 mg/kg chlorambucil (CA) or with mitomycin C (MMC) at 0, 1, 2, 4 mg/kg (experiment 1) or 0, 4, 6, and 8 mg/kg (experiment 2) and euthanized 6 hours later. Cultures were then established in the presence of growth stimulants (interleukin-3 and granulocyte-macrophage colony stimulating factor for bone marrow;

lipopolysaccharide and concanavalin A for spleen) and Cytochalasin B, a cytokinesis inhibitor. BMC were harvested 24 hours after establishment of cultures, while SC were harvested at 48 hours. In addition, SC were concurrently assayed for chromosome aberrations. With the MN endpoint, SC appeared more sensitive than BMC to the effects of CA due both to a lower background and an increased response. For MMC, BMC exhibited both a higher background of MN and a greater numerical response than did SC. However, on the basis of a fold-increase over control values, SC were more sensitive than BMC. In general, the MN endpoint appeared more sensitive than the SCA in SC after treatment with CA or MMC. Thus, the approach described here shows great potential in detecting genotoxicity.

432 COMPARISON OF CLASTOGENIC EFFECTS INDUCED IN RATS BY TAMOXIFEN AND SOME OF ITS ANALOGUES

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The clastogenic activities of tamoxifen, toremifene and droloxifene were compared in female Wistar rats. Each chemical was administered as a single dose by gavage (35 mg/kg) and hepatocytes isolated by collagenase perfusion 24h later. The cells were cultured in the presence of epidermal growth factor (40 ng/ml) for 48h, colchicine (10 μg/ml) being added for the final 3h of incubation. At least 100 chromosomal spreads were examined from each animal for the presence of numerical and structural abnormalities. The incidence of aneuploidy following treatment was: tamoxifen 81%, toremifene 46%, droloxifene 9.6%, control 4.7%. The incidence of structural abnormalities was : tamoxifen 11%, toremifene 7%, droloxifene 4%, control 5%. Aneuploidy results from chromosomal non-disjunction at mitosis caused by malfunction of the spindle. The induction of aneuploidy in the liver depends on the persistence of each compound, its affinity for spindle protein and its ability to cause cell division; whereas chromosomal structural damage is related to the interaction of the chemicals with DNA. Since tamoxifen is the only chemical in this group that is hepatocarcinogenic in rats the results suggest that while the initiation of liver tumours depends on the accumulation of DNA damage, the subsequent promotion and progression of the tumours depend, in part, on clonal expansion resulting from the selective advantage conferred on some initiated cells by chromosomal numerical changes.

433 AN EVALUATION OF MUSK KETONE IN A BATTERY OF SHORT-TERM GENOTOXICITY TESTS

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Musk ketone (CAS Number 81-14-1), a synthetic musk fragrance ingredient, was evaluated in a battery of short-term genotoxicity tests that included the mouse lymphoma assay, *in vitro* cytogenetics assay in Chinese hamster ovary (CHO) cells and *in vitro* unscheduled DNA synthesis (UDS) assay in primary rat hepatocytes. Musk ketone was tested in the mouse lymphoma assay at ten concentrations ranging from 700 to 4000 μg/ml and 2.0 to 35 μg/ml in the absence and presence of rat liver S9, respectively. No increased mutant frequencies were noted under any of the test conditions. Musk ketone was tested for *in vitro* chromosome aberrations in CHO cells at five concentrations ranging from 2.2 to 34 μg/ml and 0.16 to 10 μg/ml in the absence and presence of rat liver S9, respectively. Metaphase cells were harvested both at 24 and 48 hours. A statistically significant increase in structural chromosome aberrations was observed at the 24 hour harvest time in the S9 activated study at the high dose only. No statistically significant increase in structural chromosome aberrations was observed in the confirmatory assay. Based on the nonreproducibility of the statistically significant increase at a single dose level and no increases in numerical aberrations in other test systems, musk ketone was negative in the *in vitro* mammalian cytogenetic assay. Musk ketone was tested for the induction of UDS in primary rat hepatocytes at five concentrations between 0.5 and 50 μg/ml. No increases in net nuclear grain counts were noted at any of the test concentrations. Musk ketone gave uniformly negative results in these genotoxicity tests.

434 DIETHYLENE, TRIETHYLENE, AND TETRAETHYLENE GLYCOLS: ASSESSMENT OF GENOTOXIC POTENTIAL *IN VITRO* AND *IN VIVO*

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Previous studies showed ethylene glycol (CAS No. 107-21-1) to be devoid of genotoxic potential *in vivo*. We have now assessed the genotoxic potentials of diethylene glycol (DEG, CAS No. 111-46-6) and triethylene glycol (TEG, CAS No. 112-27-6) *in vitro* and of tetraethylene glycol (TETRA, CAS No. 112-60-6) using a battery of *in vitro* and *in vivo* tests. No mutagenic activity was detected in Ames tests or in CHO/HGPRT forward gene mutation tests using any of these 3 glycols. Neither DEG nor TEG produced increased frequencies of either sister chromatid exchanges (SCE) or chromosome aberrations *in vitro*. Weak positive responses were observed *in vitro* with TETRA in an SCE test and in a chromosome aberrations test, both in the absence and presence of an Aroclor 1254-induced rat liver S9 homogenate. However, no dose response relationship was observed in either of these test systems. TETRA produced significant increases in the frequencies of micronucleated polychromatic erythrocytes in the peripheral blood of male Swiss-Webster mice sampled 30 hr after a single intraperitoneal injection at doses of 2500 and 5000 mg/kg. However, no genotoxicity was observed in TETRA-treated female mice, or in male mice treated with 4000 mg/kg TETRA. Sprague Dawley® rats treated with 1250, 2500, and 5000 mg/kg TETRA as a single dose by gavage did not have significant, treatment-related increases in the incidence of chromosome aberrations in rat bone marrow. A previous drinking water study in TETRA-treated Fischer 344 rats showed no dominant-lethal effects. These results suggest that DEG, TEG and TETRA have no significant genotoxic potential.

435 *IN SITU* MEASUREMENT OF BLEOMYCIN-INDUCED DNA STRAND BREAKS IN CULTURED MURINE LUNG FIBROBLASTS

D G Hoyt. *Dept. of Pharmacology, Univ. of Pittsburgh, Pittsburgh, PA.* Sponsor: M Schaper

Murine strains that differ in response to toxic agents are useful experimental tools. C57Bl/6N (*C*) mice are *sensitive* and BALB/cN (*B*) mice are *resistant* to bleomycin (*BLM*)-induced pulmonary fibrosis. BLM causes DNA strand breaks. Here, DNA breaks were measured in individual mouse lung cells by *in situ* nick translation. Primary cultures of C and B lung fibroblasts were established and grown on chambered slides. Cells were treated 30 min with 0 to 1 mg BLM/ml. Cells were fixed with formaldehyde and permeabilized with 70% ethanol. Some untreated, permeabilized cells were also digested with 0 to 48 U Deoxyribonuclease I (*DNAse*)/ml for 30 min. DNA breaks were labelled with fluorescein-12-dUTP (*FdUTP*) by *E. coli* DNA polymerase I. Relative incorporation of FdUTP was determined by nuclear fluorescence intensity in 40–200 cells, measured with a Meridian ACAS 570C laser confocal microscope. In DNAse-treated cells, labelling depended on concentration of DNAse and the reaction time. An optimal relation between [DNAse] and labelling was observed for 60–90 min reaction at 37°. BLM-treated cells were labelled for 90 min. 0.5 and 1 mg BLM/ml increased labelling to similar levels in C and B cells ($p < 0.05$ vs untreated cells). Strand breaks in B lung cells treated with 0.05 mg BLM/ml were only 61.6% of the level in corresponding C cells (mean $\pm$ S.E. fluorescence units = 1250 $\pm$ 76.7 in B vs 2030 $\pm$ 137 in C, $p < 0.05$). Thus, cultured B lung cells appear more resistant than C cells to BLM-induced DNA strand breakage. Microscopic methods will be useful for study of genotoxicity in small samples from mice (Support by PhRMA Research Starter Grant).

436 CELLULAR RESPONSES TO IONIZING AND ULTRAVIOLET RADIATION IN ATAXIA TELANGIECTASIA

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Ataxia telengiectasia (AT) is a genetic disease characterized by a wide variety of symptoms. Of particular interest is a marked increase of cancer incidence and hypersensitivity to ionizing radiation (IR). Hypersensitivity is expressed as decreased cell survival, increased induction of chromosomal damage, radioresistant DNA synthesis and absence of G1 arrest following exposure of cells to IR. Our hypothesis is that the defect in AT lies in the regulation of DNA replication and control of the cell cycle. Fluorescence-activated cell sorting (FACS) analysis confirms the alterations of cell cycle control in AT cells following exposure to radiation. To investigate cellular responses of AT to ionizing and UV radiation on a biochemical level, an *in vitro* replication assay has been used. In this assay, cytosolic extracts of irradiated and unirradiated cells are incubated with template DNA; replication activity is measured as incorporation of radiolabelled nucleotide imo newly synthesized DNA. Replication activity in the *in vitro* system mimics *in vivo* DNA synthesis in that: (a) extracts from normal cells exposed to either 1 Gy ionizing or 10 J UV radiation show a dramatic decrease in replication activity, and (b) extracts from AT cells exposed to either type of radiation do not show such a decrease in replication activity. Phosphorylation changes of the essential replication protein, single strand binding protein (hSSB), have also been investigated. Previous work in the lab (Catty,

et al. *EMBO J.* 13: 2114; 1994) has shown, via immunoblotting techniques, that hSSB is hyperphosphorylated in HeLa cells following exposure to 10 J UV radiation. In AT cells, hyperphosphorylation of hSSB also occurs following 10 J UV radiation, but not 1 Gy IR. Further research is being conducted to examine the apparent uncoupling of DNA synthesis control and hyperphosphorylation of hSSB in UV-exposed AT cells. (Supported by Center for Environmental Genetics and AT Children's Project).

437 PROTECTIVE EFFECT OF METALLOTHIONEIN IN RADIATION- AND Cu(II)-INDUCED DNA DAMAGE *IN VITRO*

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DNA damage is a critical event in carcinogenesis. Exposure to γ radiation (30–150 Gy) *in vitro* caused damage to calf thymus DNA which could be protected by addition of glutathione (GSH, 2 mM) and vitamin C (0.08–8 mM). DNA damage was measured by loss of fluorescence with ethidium bromide, and by increased mobility of DNA in agarose gels. Addition of vitamin C (2 mM) in the presence of copper (50 μM) significantly enhanced radiation-induced DNA damage, but GSH did not. The copper chelating agents, Trien and bathocuproinedisulfonic acid could abolish the enhanced radiation-induced DNA damage by Cu(II) in the presence of vitamin C. These results suggest that vitamin C can act in two ways: as an antioxidant to protect DNA from damage due to ionizing radiation, similar to GSH, or as a reducing agent in the presence of copper to promote the reduction of Cu(II) to Cu(I) and thereby enhance the DNA damage. In another experiment, calf thymus DNA damage caused by Cu(II)-mediated free radicals in the presence of vitamin C and hydrogen peroxide could be inhibited by addition of metallothionein (MT, 100–1000 μg/ml) prior to addition of Cu(II). Cu(I) formation from Cu(II) is also inhibited by MT. As compared to MT, other proteins (histone or albumin) were not effective in protecting DNA from Cu(II)-induced damage in the presence of vitamin C and hydrogen peroxide. These results show that MT can protect DNA from damage by radiation and free radicals.

438 ^{32}P-POSTLABELING ASSAY FOR DETECTION OF ADDUCT FORMATION BY PHENOBARBITAL, CHLORDANE, AND POLYCHLORINATED BIPHENYLS IN MOUSE LIVER DNA

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Tumor promotion has been shown to be the predominant mechanism by which phenobarbital, polychlorinated biphenyls, and chlordane produce hepatocellular carcinomas in rodents. However, findings of a small number of positive genotoxicity tests have continued to raise concerns about DNA adduct formation and tumor initiation by these chemicals. To address this, we have studied DNA adduct formation in mouse liver using the ^{32}P-postlabeling technique. Test substances were administered to B6C3F1 male and female mice in a single dose or for 14 days in the diet as follows: phenobarbital, 200 mg/kg, 1000 ppm; chlordane, 50 mg/kg, 200 ppm; Aroclor 1260, 50 mg/kg, 200 ppm. Animals were killed 24 hours following the end of test substance administration, and DNA from the liver was extracted. DNA adducts were not found for any of the test substances using butanol extraction or nuclease P1 enhancement for ^{32}P-postlabeling. The positive controls 2-acetylaminofluorene and benzidine demonstrated that bulky adducts could be identified in the assay systems.

439 WINGSTAY™100: ASSESSMENTS OF GENOTOXICITY, DNA ADDUCT FORMATION, CELL PROLIFERATION IN LIVER AND BLADDER, AND SUBCHRONIC TOXICITY IN RATS

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WINGSTAY™100 [N,N′ diaryl-p-phenylene diamine (CAS#68953-84-4)] is used as an antioxidant/antiozonant in high durability rubber products including tires and hoses. This product (W-100) was subjected to mutagenicity tests in which a positive response in Ames Salmonella (+S9 in TA98 & TA1538) was observed. Results were negative in *in vitro* CHO chromosomal aberration and *in vivo* mouse micronucleus tests. F-344 rats (M/F) were subjected to oral gavage dosing of W-100 at 0.1, 0.3, 1.0 and 3.0 g/kg daily for 3 weeks. Test animals were observed for effects on survival, body weights, and clinical appearance. 1.0 and 3.0 g/kg W-100 induced lethality in test rats by Day 7, and ↓BW gain at ≥0.3 g/kg. Postmortem, 0.1 & 0.3 g/kg test animals were shown to have ↑liver weights and ↑hepatocellular proliferation as measured by immunohistochemistry (PCNA-PC10 clone). ^{32}P postlabeling assay indicated no DNA adducts in either liver or urinary bladder following 0.3 g W-100/kg exposures for 7 days, although the carcinogenic aromatic amine 2-AAF (10 mg/kg bw) was very active producing DNA adducts. It is concluded that the genotoxicity of W-100 is restricted to a weak mutagenic effect in bacteria, without evidence of adduct formation in rat liver or urinary bladder and a mitogenic effect in livers of exposed rats.

440 A SURVEY ON DIETHYLENE GLYCOL INTOXICATION AS A CONTAMINANT IN A PEDIATRIC ANALGESIC SYRUP

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Diethylene glycol (DG) derives from ethylene glycol, is highly toxic and is present in plastic, solvents, and antifreezing compounds. Recently, twelve deaths and cases of toxicity were reported in Venezuela due to the presence of DG in a pediatric (syrup) formulation of acetaminophen (Laboratorio Interamericano). After careful screening, it was found that the acetaminophen syrup was the common factor in cases (both in hospital and in ambulatory patients). Through analytic techniques it was possible to detect "DG" in the formulation. Patients deceased presented with acute renal failure and cerebral edema; typical effects of "DG" toxicity.

In the last years, WHO has received information of similar cases in Nigeria (September 1990) due to Paracetamol syrup with more than 100 fatalities; Argentina (August 1992), due to "Propoleo" (tonic) with 33 deaths and 10 cases of serious toxicity; Bangladesh (November 1992); due to Paracetamol syrup, several deaths. In all these reports, the common factor was the substitution of propylenglycol by "DG".

We conclude that is important to perform a rigorous analysis in pharmaceutical preparations — with propylenglycol as a solvent or vehicle.

This would avoid this type of accident.

441 REPEATED DOSE TOXICITY STUDY IN MICE WITH N-METHYLPYRROLIDONE (NMP)

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A 28-day feeding study was conducted to evaluate the toxicity of NMP after repeated administration to B6C3F1/CrlBr mice. Groups of 5 male and 5 female mice were fed diets containing 0, 500, 2500, 7500 and 10,000 ppm NMP. At 10,000 ppm one male animal died intercurrently on day 22 of the study. At this concentration serum alkaline phosphatase was decreased in both sexes. Histopathologically, cloudy swelling of the epithelia of the distal parts of the renal tubuli was observed in 4 males and 3 females at 10,000 ppm and in 2 male animals at 7,500 ppm. The urine was stained intensively dark yellow in animals receiving 2500, 7500, and 10000 ppm. This staining was assessed as being a sign of systemic availability of the test substance, but not as an adverse effect. Thus, based on histopathological findings, the NOAEL was 2,500 ppm for male and female mice.

442 REPEATED DOSE TOXICITY STUDY IN RATS WITH N-METHYLPYRROLIDONE (NMP)

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A 28-day feeding study was conducted to evaluate the repeated dose toxicity of NMP, a widely used industrial solvent, in Crl:CD®BR rats. Male and female rats were fed diets that contained 0, 2,000, 6,000, 18,000, or 30,000 ppm NMP. Decrements in mean body weight and body weight gain were exhibited by male rats at 18,000 and 30,000 ppm, and in female rats at 30,000 ppm. Decrements in mean daily food consumption and food efficiency in these rats were coincident with body weight effects. Clinical chemical changes, indicated possible compound-related alterations in lipid, protein, and carbohydrate metabolism at 18,000 and/or 30,000 ppm. No histopathological changes were judged to be directly related to NMP exposure. Hematologic (mild to moderate leukopenia) and histopathological alterations (hypocellular bone marrow, testicular degeneration and atrophy, and thymic atrophy) were judged to be secondary

to nutritional and body weight effects in male and/or female rats at 30,000 ppm. The NOAEL was 6,000 for male rats and 18,000 ppm for female rats.

443 COMPARATIVE TOXICITY OF ETHYLENE GLYCOL MONO-N-BUTYL ETHER: SELECTION OF AN APPROPRIATE ANIMAL MODEL

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Ethylene glycol mono-*n*-butyl ether (EGBE), an important industrial solvent, is moderately toxic to certain species by a variety of routes. In sensitive species [e.g. rat, mouse, rabbit], this toxicity is secondary to intravascular hemolysis caused by 2-butoxyacetic acid [BAA], the primary metabolite of EGBE. Hemolysis can be demonstrated *in vivo* and *in vitro*. Hemolysis was not seen when human volunteers were exposed to EGBE vapors, nor with BAA *in vitro* using blood from normal or potentially sensitive individuals under conditions that caused extensive hemolysis with rat blood. Studies in sensitive species indicate the following range of acute toxicity: rat oral LD_{50}: 0.5–3 g/kg; rabbit occluded dermal LD_{50}: 0.3–3 g/kg. In recent studies, the rabbit dermal LD_{50} under occlusion was 0.8 g/kg, but under semi-occlusion [more relevant to human exposure] was in excess of 2 g/kg. Studies in the guinea pig, an insensitive species, showed the dermal LD_{50} [occluded] to be in excess of 2 g/kg, and an oral LD_{50} of 1.4 g/kg. There was no evidence of hemolysis; gross necropsy indicated only gastrointestinal irritation by this solvent. Acute inhalation exposure of guinea pigs to a saturated vapor [633 to 691 ppm] of EGBE produced no mortality or signs of toxicity. A consideration of species differences suggests that any toxicity secondary to hemolysis should not be used in assessing human risks associated with exposure to EGBE.

444 SUBCHRONIC (90-DAY) INHALATION TOXICITY AND NEUROBEHAVIORAL STUDY OF PCBTF IN THE RAT

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Sprague-Dawley rats (25/sex/group) were exposed in inhalation chambers, 6 hr/day, 5 days/wk for 90 days to 0, 10, 50, and 250 ppm p-chlorobenzotrifluoride (PCBTF). Neuro-behavior studies, functional observation battery (FOB), and muscular activity (MA) on 5/sex/group were performed on days 0, 30, 60, and 90 of the study. Following 90 days of exposure 10/sex/group were sacrificed, selected organs were weighed and organ/body and organ/brain weight ratios calculated. Complete macroscopic postmortem and microscopic examinations of selected tissues were conducted. Following 90 days of recovery (10/sex/group), 5/sex/group were sacrificed and examined as indicated above. Whole-body perfusion and microscopic examination of selected nervous tissue was conducted after exposure and after recovery (5/sex/group). Body weight gain was unaffected. There were no PCBTF related macroscopic observations. Microscopically, PCBTF-related centrilobular hypertrophy was present only in the livers of males and females at the high dose (250 ppm) after 90 days of exposure. No centrilobular hypertrophy was observed at any level among recovery animals. There were no PCBTF effects on the nervous system, as measured by FOB, MA, and neuropathology.

445 THIRTEEN-WEEK ORAL TOXICITY STUDY OF METHANOL IN FOLATE-DEFICIENT LONG-EVANS RATS

B M Ryan, N S Hatoum, W D Johnson, J K Yermakoff. *IIT Research Institute and Amoco Corporation, Chicago, IL*

Methanol (MeOH) is being touted as an alternative fuel for the future. Its acute toxicity is well known, however, its potential subchronic toxicity has not been thoroughly evaluated in an appropriate animal model. Therefore, MeOH was administered in drinking water for 13 weeks at concentrations of 0.5%, 1.0% and 2.0% to three groups of 16 male and 16 female Long-Evans rats which had been rendered folate-deficient prior to MeOH exposure. Folate-deficiency was induced by supplying a folate-deficient diet with 1% succinylsulfathiazole (SST) for a period of 16 weeks. The study also contained two control groups of equal size; one of the groups was rendered folate-deficient and served as a sham control, while the other was provided a folate-sufficient diet (*i.e.,* basal diet) and served as an untreated control group. Ten rats/sex/group were subjected to a limited neurotoxicity assessment using a functional observational battery (F.O.B.). The F.O.B. was performed 1 week prior to treatment initiation and after 1, 28, 56, and 86 days of treatment. Select organ weights, histopathology, clinical pathology, blood gas profiles, blood MeOH, serum formaldehyde and formic acid levels were evaluated in rats designated for F.O.B. In addition, blood gas profiles, blood MeOH, serum HCHO and HCOOH levels were evaluated in 2 rats/sex/group following 3, 6 and 9 weeks of treatment.

Folic-acid deficiency resulted in mild hepatocellular degeneration, a reduction in body weight or body weight gain, increases in endogenous formic acid levels, and select changes in clinical chemistry and absolute and relative organ weights. Despite the effects of folic acid deficiency, the effects of MeOH could still be delineated. Repeated exposure to MeOH caused the accumulation of formic acid in all three treatment groups, and morbidity and mortality in the 1% and 2% groups. Food and water consumption were reduced in the 1% and 2% MeOH groups, while reduced body weight or body weight gain was observed in all three MeOH-exposed groups. Acid-base disturbances, hypochloremia and elevation of alkaline phosphatase levels were observed in the 1% and 2% MeOH groups, as well as increases in blood urea nitrogen levels, tubular nephrosis and epithelial hyperplasia of the stomach in the 2% MeOH-exposed rats. No microscopic pathologic lesions were observed in the retina or optic nerve. Neurotoxicity assessments reflected alterations in the 2% MeOH group; however, these changes were considered to be associated with the poor health status of these rats rather than a direct indicator of neurotoxicity.

446 INACTIVATION OF GLUTATHIONE PEROXIDASE BY BENZALDEHYDE AS THE POSSIBLE MECHANISM OF TOLUENE NEUROTOXICITY

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Toluene is one of the most extensively used industrial organic solvents. Chronic exposure to toluene is known to produce a spectrum of neurotoxic effects. Biotransformation of toluene has been shown to result in generation of reactive oxygen species (ROS). Benzaldehyde has been found to be the metabolite mainly responsible for ROS formation. We have investigated the effect(s) of benzaldehyde (and toluene) on the activity of several antioxidant enzymes as a possible pathway for neurotoxicity. Our results show that while benzaldehyde and toluene are virtually devoid of any inactivating effect on catalase, superoxide dismutase, glutathione reductase, and glutamine synthetase, benzaldehyde (and not toluene) inactivates glutathione peroxidase (GPX) rapidly and very effectively. Several other aldehydes were found to possess little or no inactivation ability toward GPX. Conformational changes resulting from nucleophilic reaction of amino groups at or near the active site with benzaldehyde may be responsible for inactivation. Since GPX is the main brain defense mechanism against toxicity of H_2O_2 and the ensuing ROS-related insults, inactivation of this enzyme will render the brain susceptible to oxidative damage with neurode-generation being the final outcome. Supported in part by NIH NS 23307 and AG 09690.

447 NEUROTOXICITY OF TOLUENE AND TRICHLOROETHYLENE IN RATS

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As a part of ongoing studies, the neurotoxicity of toluene (TOL) and trichloroethylene (TCE), two of the most frequently found chemicals at the superfund sites, are being investigated (The Toxicologist Vol 14(1), 1994). Groups of 10 male and 10 females Sprague Dawley (42 days old) were exposed by gavage to either TOL or TCE for 90 days. The dosing regimen was as follows: LOW (TOL 0.25 ml/kg or TCE 0.125 ml/kg), INTERMEDIATE (TOL 0.5 ml/kg or TCE 0.25 ml/kg), and HIGH (TOL 1.0 ml/kg or TCE 0.5 ml/kg). Spontaneous motor activity (SMA) using Digiscan apparatus, the forelimb and hindlimb grip strength, and the Functional Observational Battery (FOB), were measured on weeks 0, 4, 8, and 13. The observations were made before the administration of the test solvents at predefined interval. Thus, the toxicity attributable to the last dose was avoided and only the cumulative toxic effects were observed. When compared to saline controls, TOL-exposed male rats exhibited dose dependent decrease in SMA while the female rats exhibited a consistent decrease in SMA only at 1.0 ml TOL/kg dose level. TCE-exposed male rats showed a decrease in the horizontal (HA), stereotype (SA), and vertical (VA) activities at week 4 and 13 for HIGH exposure, while the females exhibited an increase in HA and SA. TCE-exposed female rats showed significantly ($p < 0.05$) greater response than the TCE-exposed male rats. At week 13, the forelimb grip strength was greater than control group in TOL-exposed females, while a decrease was observed in males. TCE-exposed females showed an increase in both the forelimb and hindlimb grip strength at weeks 4, 8, and 13 and such a response was not seen in the male rats. Significant

differences in toxicity were observed between the male and female rats. These gender-related toxicity differences may be due to variability in their respective pharmacokinetic parameters. Since TOL and TCE are found together at Superfund sites, further studies are planned to assess their combined toxicity. [Funded by ATSDR grant #U50/ATU398948-02].

448 13-WEEK ORAL TOXICITY EVALUATION OF POLYDIMETHYLSILOXANE FLUIDS IN SPRAGUE-DAWLEY RATS

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A 13-week dietary feeding study was conducted with three polydimethylsiloxane (PDMS) fluids. Twenty male and twenty female Sprague-Dawley rats were utilized in each of two control and nine test groups. Three dose levels (1, 5 and 10%) of PDMS fluid at three viscosities (35, 350 and 1,000 cs) were mixed in NIH-07 ground rodent chow. No overt signs of toxicity or changes in behavior were noted in any of the groups. Slight to marked anal leakage of fluid was observed in animals treated with the 10% PDMS for all viscosities. The amount of anal leakage decreased with increasing viscosity. No changes in stool consistency or coating of fluid were noted in any of the test animals. A significantly greater food consumption was observed in rats treated with 5 and 10% PDMS fluids. The increase of food consumption was considered to represent a compensation by the animals to the non-nutritive components of the diet. No significant changes were observed in the blood, clinical chemistry, bone marrow, or urinary parameters which could be considered to be of biological or toxicological significance. Treatment-related changes were seen in the eyes of rats. The most prominent lesions were corneal opacities and neovascularization. Histologically, the corneal lesions observed showed minimal to mild chronic inflammation of the cornea. Mineralization of the cornea was also present in some animals. These eye lesions were present in treated animals in a non-dose related pattern and may have been due to direct ocular irritation from PDMS fluid in the feed. Presently, additional dietary studies are in progress with PDMS fluids in Fischer 344 rats to further characterize the eye lesion.

449 FROM TEACHERS TO TOXICOLOGISTS: ANSWERING THE TOUGH QUESTIONS ABOUT ANIMAL RESEARCH

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It is becoming increasingly important for biomedical researchers to communicate the importance and value of our work to the general public and in particular to school students. Current deficiencies in science education and the lack of understanding of important scientific principles (e.g., dose-response, risk assessment) have resulted in large portions of the populace being susceptible to scientific misinformation and the efforts by animal activists to limit research activities. Several SOT groups have developed creative ways to communicate with the general public on important issues, including the value of animal research for human and animal health. Speakers will describe their experiences of giving presentations to various school age groups, speaking to community groups, or engaging in debates. The goal of this Workshop is to illustrate three different approaches that SOT members have developed to convey the value of animal research.

450 NATURALLY OCCURRING SUBSTANCES IN FOODS: A BALANCE OF TOXIC AND PROTECTIVE PROPERTIES

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Most natural food chemicals have not been studied for their protective or deleterious potential. Detoxification mechanisms in the body are numerous and effective, failing only when overwhelmed by excessive concentrations of a toxicant or a carcinogen. Thousands of toxicants are consumed each day without causing harm to the majority of the human population. To protect against environmental insults, specific enzymes have evolved, which modify structure to enhance clearance, to reduce activated oxygen species, and to repair DNA. Nutritional factors influence these activities and alter their expression. Dietary toxicants may alter nutrient bioavailability, or produce tissue damage when dietary intakes are high or when atypical consumers are affected. Two specific types of plant toxicants, phytoallergens and mycotoxin contaminants, will be discussed. A natural chemical may act as a toxicant, a protectant or both by affecting different physiologic reactions, e.g. phytoestrogens. The effects of harvest time, storage time and processing on natural chemicals will provide a better perspective on the risk and benefits of man made chemicals in health and disease.

451 NATURAL TOXICANTS: A PERSPECTIVE ON APPROPRIATE TOXICOLOGICAL EVALUATION

A G Ebert. *Robert H. Kellen Company, Atlanta, GA*

Although most people express little concern over the safety of the food supply, popular opinion continues to rank man-made substances, as more hazardous than naturally occurring toxicants. Due in part to the comprehensive research of Ames, and his colleagues, food toxicology has attained increased focus. Ames has reminded us that only a few of the myriad of naturally occurring compounds found in food have nutritive value and many are toxic. The chemical structure and stability of these food toxicants are quite diverse. Many are easily destroyed by the physical and chemical changes common to food processing, e.g. heat, pH, enzyme modification and other treatments. Detection of a toxicant in a food product is not an absolute predictor that it poses a hazard to humans. Foods may also contain certain other natural substances which block the effects of the toxicants thereby protecting the organism consuming the total food. Case reports indicate ingestion of foods, typically innocuous to the general population at normal consumption levels, may become toxic when increased intake leads to elevated toxicants exposure. A practical concomitant of the changing science is in the are of food regulation. FDA and other authorities charged with protection of the food supply have begun to realize classic animal studies examining single compounds administered at high doses, MDT or greater, are insufficient. The marked expansion of programs outlined in FDS's "Redbook" incorporate numerous specific studies, completion of which creates a profound escalation in the cost of food safety evaluation.

452 EFFECT OF NUTRITIONAL STATUS ON PHYSIOLOGICAL DETOXIFICATION MECHANISMS

W R Bidlack, W Wang. *Department of Food Science and Human Nutrition, Iowa State University, Ames, IA*

Following digestion, xenobiotic agents found in food are absorbed through the digestive track. The intestinal mucosal cells bind heavy metals, while chemical agents are absorbed and transported to the liver. Metabolic activities involved in detoxification include the mixed function oxidase (cytochrome P-450) and the conjugation enzymes (sulfotransferase, glucuronosyl transferase and glutathione transferase), which alter the chemical structure to enhance clearance. Many agents generate free radical intermediates which also produce activated oxygen species. Several enzyme systems (SOD, catalase, GSH peroxidase) and natural antioxidant compounds (vitamins C and E, B-carotene and sulfur amino acids) reduce these oxidizing agents protecting membrane lipids and protein sulphydryls from oxidative damage. The continuous repair of DNA damage provides a critical cellular defense mechanism. Specific nutrients are integrally involved in these reactions, and nutritional status affects the efficiency of these detoxification processes. (USDA/CSRS CDFIN grant 94-34115-0269)

453 SOY PHYTOESTROGENS; A DOUBLE-EDGED SWORD IN HUMAN HEALTH

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Soy contains high levels of genistein, a phytoestrogen which binds to the estrogen receptor with low affinity and can elicit estrogenic responses in animals and humans. As an estrogen agonist, genistein may be detrimental to women at high risk for breast cancer. Conversely, phytoestrogens may be beneficial to post-menopausal women at risk to bone density loss and cardiovascular disease due to low estrogen levels. We have conducted a series of *in vivo* and *in vitro* experiments to evaluate the estrogen agonistic effects of genistein. We have demonstrated that feeding genistein at varying levels in the AIN-76 diet results in a dose-dependent increase in uterine weight over that of control ($p \leq 0.01$). Expression of uterine *c-fos* an estrogen responsive gene was induced in both E_2 and genistein treated rats. An increase in mammary gland development was also observed in these animals, suggesting that genistein is an estrogen agonist in both uterine and mammary tissue. Since genistein is able to enhance mammary development, it may act to promote growth of estrogen-responsive tumors. We have conducted experiments using estrogen-responsive human breast cancer (MCF-7) cells. In general, these cells proliferate in response to estrogens. However, we have demonstrated that genistein is antiproliferative in MCV-7 cells. Possible reasons for this apparent paradox will be discussed.

454 PHYTOALLERGENS

S L Taylor, J A Nordlee. *University of Nebraska, Lincoln, NE.* Sponsor: W R Bidlack

A small, but significant, number (perhaps 1–2%) of consumers are allergic to specific foods. The symptoms in affected individuals range from mild cases of hives and gastrointestinal discomfort to severe, life threatening anaphylactic shock. While numerous mechanisms may be involved in individualistic adverse reactions, true food allergies involve abnormal immunological responses to foodborne substances, usually naturally occurring proteins. Plant foods, especially oilseeds, tree nuts, and grains are frequently involved in IgE-mediated allergies. Wheat and related grains are responsible for celiac disease, a cell-mediated immune reaction. Sensitive consumers can react to ingestion of very small amounts of the offending food, although the severity of reactions is related to the dose of exposure.

455 FUMONISINS AND OTHER MYCOTOXINS CONTAMINANTS

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Fumonisins (FBs) are a family of mycotoxins produced by *Fusarium moniliforme* and *F. proliferatum*, predominant corn pathogens. Although the FBs were only discovered in 1988, there is evidence suggesting their presence in corn for more than a century. The FBs are heat stable, resistant to ammoniation and, unlike most mycotoxins, are water-soluble. However, the FBs are usually not found alone in corn and other *Fusaria* mycotoxins such as deoxynivalenol and zearalenone may co-contaminant foods. There is evidence that the effects of combinations of these mycotoxins are greater than the sum of their individual activities. The levels ranging from > 2 ppm to < 20 ppb in corn and corn-containing foods will be presented. The effects of nixtamalization ($Ca(OH)_2$ treatment), bleach, water washing, and enzymatic and chemical modification will be described. The effects of these treatments have been evaluated both chemically and biologically.

456 SELECTIVE PROTEIN COVALENT BINDING AND TARGET ORGAN TOXICITY: INTRODUCTION

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Protein covalent binding by xenobiotic metabolites has long been associated with target organ toxicity but mechanistic involvement of such binding has not been widely demonstrated. Modern biochemical, molecular and immunochemical approaches have facilitated identification of specific protein targets of xenobiotic covalent binding. Such studies have revealed that protein covalent binding is not random but selective with respect to the proteins targeted. Selective binding to specific cellular target proteins may better correlate with toxicity than total protein covalent binding. Current research is directed at characterizing and identifying the targeted proteins and clarifying the effect of such binding on their structure, function and potential roles in target organ toxicity. This symposium will first describe the approaches employed to detect and identify the targeted proteins. Next, the association of selective protein covalent binding by acetaminophen and 2,5-hexanedione will be discussed in the context of specific cellular perturbations which may contribute to the target organ toxicity. Formation of xenobiotic-protein adducts with immunogenic properties in drug hypersensitivity and autoimmunity will also be described as an additional means by which selective protein covalent binding may mediate toxicity.

457 DETECTION AND IDENTIFICATION OF XENOBIOTIC-PROTEIN ADDUCTS

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Covalent modification of proteins is postulated as a mechanism for the toxicity of a variety of chemicals. It can be envisioned that the binding to proteins can disrupt critical functions within the cell. Covalent binding to proteins may also activate the immune system, resulting in either hypersensitivity reactions or production of autoimmunity. Recently, new immunochemical methods have been developed to detect and identify xenobiotics covalently bound to proteins. These methods have been utilized to investigate the mechanisms of toxicity of a variety of chemicals including acetaminophen, diclofenac, and halothane. An enzyme-linked immunosorbent assay can correlate the levels of adducts with the chemical's toxicity, while the cellular localization of the protein-conjugate can be determined utilizing immunohistochemistry. The relative molecular masses and isoelectric points can be determined using a Western blot. The major protein-conjugates can be isolated using biochemical techniques such as column chromatography, and the progress can be monitored using antiserum specific for the xenobiotic-protein adduct. Amino acid sequence information obtained from the isolated proteins can be used to compare homology with known proteins to aid in their identification. Utilizing specific antiserum that recognize chemicals bound to protein has greatly increased our knowledge of the interactions between chemicals and proteins.

458 CELLULAR CONSEQUENCES OF PROTEIN ADDUCT FORMATION

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Understanding the significance of drug covalent binding necessitates identification and characterization of the macromolecular targets. Acetaminophen (APAP) hepatotoxicity has been extensively studied to evaluate the consequences of drug binding to cellular proteins. Although a number of cytosolic, microsomal, mitochondrial, and plasma membrane proteins have been postulated to bind APAP, none have yet been shown to be critical targets for the hepatotoxicity. We have recently detected two APAP-binding nuclear proteins within 2 hrs after APAP administration (600 mg/kg, i.p.). The first has been identified immunochemically as nuclear lamin A. The arylation of lamin A was coincident with phosphorylation of this protein, disruption of the nuclear cytoskeleton, and disaggregation of the peripheral chromatin associated with the nuclear envelope. The second nuclear protein target has been shown to cross react with an antibody raised against the cytosolic 56–58 kDa acetaminophen binding protein (58ABP) which is a major target of APAP binding. Western blots of sub-nuclear fractions indicated that this protein translocates into the nucleus only upon arylation. Immunogold electron microscopy has confirmed these observations. These data suggest that the covalent binding associated with APAP hepatotoxicity is temporally related to a disruption of nuclear organization resulting from lamin A arylation and that the translocation of the 58ABP into the nucleus may serve as a signalling mechanism either for cellular repair or demise. (NIH GM-31460 and the UCONN Center for Biochemical Toxicology).

459 2,5-HEXANEDIONE TARGETS MICROTUBULES AND DISRUPTS SEMINIFEROUS TUBULE FLUID SECRETION

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2,5-Hexanedione (2,5-HD), the toxic γ-diketone metabolite of *n*-hexane, can react with tissue nucleophiles through sequential steps of pyrrole formation, oxidation, and crosslinking. 2,5-HD exposure produces a testicular injury with primary involvement of microtubule-rich Sertoli cells, the polarized epithelial cells which extend from the base to the lumen of the seminiferous tubule creating the appropriate milieu for male germ cell maturation. Following 2,5-HD exposure, the kinetics of microtubule assembly are altered and the ability of the microtubule motor kinesin to move along microtubules is inhibited. Since microtubule-dependent protein transport is likely critical to the formation and secretion of the Sertoli cell-derived seminiferous tubule fluid and since adluminal germ cells depend upon an appropriate seminiferous tubule fluid for their viability, disruption of the secretory process is one mechanism by which Sertoli cell dysfunction could result in testicular atrophy. In this working hypothesis, dysfunctional microtubules result in progressive failure of the Sertoli cell secretory pathway causing the appearance of Sertoli cell vacuoles, cessation of seminiferous tubule fluid secretion and germ cell necrosis.

460 METABOLIC AND IMMUNOLOGIC BASIS OF AUTOIMMUNE DRUG-INDUCED HEPATITIS

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It has been estimated that more than 600 drugs cause hepatic injury. The majority of cases appear to be idiosyncratic and are often thought to be caused by allergic reactions, directed against liver proteins that have been covalently altered by a reactive metabolite. Two reasons why very little progress has been made in the understanding of how drugs produce allergic hepatitis is that until very recently, little was known of the identity of hapten-carrier conjugates

associated with these diseases and how these antigens are processed in the liver. In the case of halothane hepatitis many of the hapten-carrier conjugates associated with this disease have been purified and characterized. They include trifluoroacetylated endoplasmic reticulum proteins of 100 kDa, 82 kDa, 80 kDa, 63 kDa, 59 kDa, 58 kDa, and 57 kDa. Serum antibodies of patients with a clinical diagnosis of halothane hepatitis have been shown to react with one or more of these hapten-carrier conjugates. Antibodies have been raised against each of these antigens and have been used to study their cellular biology in parenchymal cells of the liver, in order to help explain how they might come into contact with the immune system to induce immune reactions and immunopathology.

461 EVALUATION OF RESIDENTIAL EXPOSURE TO ARSENIC IN SOIL NEAR A SUPERFUND SITE

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Elevated surface soil arsenic levels over 100 mg/kg were identified in residential areas near a Superfund site in Fort Valley, GA, at which arsenical pesticides had been manufactured. Arsenic levels in drinking water and air were not significantly elevated. A baseline risk assessment suggested an increased potential for adverse health effects due to incidental ingestion of soil. To address community concerns and guide remediation goals, arsenic exposure in residents living and working near the facility was assessed through measurement of total urinary arsenic in random and 24-hour samples during the summer of 1993. Of 225 urine samples obtained from 115 individuals, over 90% had undetectable levels of arsenic (less than 10 μg/L). None exceeded 34 μg/L (laboratory normal value is less than 100 μg/L). The mean urinary arsenic concentration for participants was approximately 6 μg/L assuming a concentration of one-half the detection limit for samples in which arsenic was not detected. Urinary arsenic concentration was not associated with gender, age, race, home location, or property arsenic soil concentration. These results suggest there was not an increased level of arsenic exposure in the community.

462 URINALYSIS STUDY FOR EVALUATING ARSENIC EXPOSURE IN A POPULATION RESIDING ON MILL TAILINGS

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A urinalysis screening study for arsenic exposure was performed at a mining/milling site where homes had been constructed on tailings containing elevated levels of arsenic. Levels of arsenic in the top two inches of soil averaged about 200 ppm. Total and speciated arsenic in urine were measured in first morning void samples collected in September 1993, February 1994, and June 1994 (3 samples each time) from adults and children volunteers residing in the tailings area (study group) or residing elsewhere within the same community or in another city (control group). Mean speciated arsenic levels were 7 μg/L in the study group and 4.7 μgL in the control group ($p < 0.05$) in the February survey. Similar differences were observed as part of the June and September surveys. Mean speciated urine arsenic levels were similar between adults (7.1 μg/L) and children (7 μg/L) in the study group and in the control group (adults 5.2 μg/L and children 4.4 μg/L). While the speciated arsenic levels of the groups were quite stable in consecutive sampling events, the intra-individual variability was large. Fish consumption was not associated with elevated speciated urine arsenic. Because of the lack of seasonal variability and the lack of differences between adults and children in urine arsenic levels, we conclude that soils containing tailings arsenic had a limited, if any effect, on arsenic body burden. In addition, any impact was below levels of potential concern (50 μg/L) for adverse health effects.

463 INTERPHASE CYTOGENETICS OF WORKERS EXPOSED TO BENZENE

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Trisomy (hyperdiploidy) of chromosomes 8 and 21 and monosomy (hypodiploidy) of chromosomes 5 and 7 are common in human myeloid leukemia. Benzene is an established human leukemogen and causes chromosome aberrations in the peripheral blood of exposed workers. These aberrations have been reported to occur in specific chromosomes, especially of the C-group of 6–12 and X. Since standard karyotyping only allows for a few metaphase spreads to be examined, we have used fluorescence *in situ* hybridization (FISH) to perform interphase cytogenetic analyses of chromosomes 7, 8 and 9 in lymphocytes of workers exposed to benzene. Forty-three workers exposed to benzene (median = 31 ppm 8 h TWA) and 44 age- and sex-matched controls were studied in Shanghai, China. Hyperdiploid frequencies (mean ± S.D.) of chromosome 7 per 1000 scored interphase nuclei were increased from 0.009 ± 0.005 in controls ($n = 44$) to 0.012 ± 0.005 ($n = 21$, $p = 0.04$) in low exposed workers ($\leq$31 ppm) and to 0.016 ± 0.011 ($n = 22$, $p = 0.0003$) in highly exposed workers (>31 ppm). The hyperdiploid rates for chromosomes 8 and 9 were significantly elevated only in the high exposure group compared to controls ($p < 0.005$). Hypodiploidy also occurred more frequently in exposed workers. The absence of both copies of chromosome 7 was related to benzene exposure being 1/44 (2.2%) in controls, 2/21 (9.5%) in low exposed workers, and 8/22 (36.4%) in the high exposed group ($p = 0.0005$). These results confirm that benzene exposure causes aneuploidy of lymphocyte C-group chromosomes, with chromosome 7 being the most sensitive of those tested to lower levels of benzene exposure. This is the first application, to our knowledge, of FISH technology to studies of populations exposed to occupational carcinogens and shows that it may serve to compliment more labor-intensive methods of evaluating chromosome aberrations in exposed populations. (Supported in part by NIH grant RO1ES06721).

464 CHROMOSOMAL ABERRATIONS OF WELDING FUME EXPOSED WORKERS

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Welding fume contains various metals and hazardous gases and health effects from the inhalation are largely unknown. Genetic effect of welding fume has not been studied extensively. Currently we studied the composition of welding fume and its clastogenic effects on Korean welders. Individual air samples from the welding fume exposed workers were monitored in two shipbuilding plants and one container manufacturing plant. Chromosome aberrations (CA) were studied in the peripheral blood lymphocytes of 101 fume exposed workers. Total fume was weighed after processing sampled filter papers in microwave oven. Composition of metals was analyzed with an ICP (Inductive Coupling Plasma) analyzer, and other chemicals were evaluated by spectrophotometry. The mean durations of exposure were 12.6, 10.7, and 7.1 years in 3 plants, respectively. The average fume concentration of plant A (5.89 mg/m^3) was the highest among them and its value was a little higher than the permissible exposure level (PEL, 5 mg/m^3). However, the fume levels of other two plants (1.86 mg/m^3 and 1.62 mg/m^3, respectively) were below the PEL. ICP analysis showed that the levels of Fe, Cu, Mn, Pb, and Zn were not higher than PEL except Mn in plant A (1.57 mg/m^3). Cd, Ni and Cr were not detected. The concentrations of NO_2 and O_3 were also negligible. CA analysis of 101 welders showed negative incidence of CA (less than 5%) and only one worker had inconclusive incidence of CA (from 5% to less than 10%). Most types of observed CA were chromatid breakages and exchanges. None of chromosomal typed aberration was observed. CA of the welding fume exposed workers showed no increase in the frequencies of aberrant cells (mean = 0.91%) as compared with control groups.

465 CHROMOSOME ABERRATIONS OF STYRENE EXPOSED WORKERS

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We investigated the chromosome aberrations in peripheral blood lymphocytes of 21 styrene exposed workers in two reinforced plastic factories. Ambient styrene concentrations were measured and styrene levels of workers' blood were also analyzed. In addition, styrene metabolite, mandelic acid, in urine was examined. The mean exposure durations of workers were 48.0 ± 9.2 months for plant A and 44.8 ± 56.9 months for plant B. The ambient styrene concentrations were 32.14 ppm (plant A) and 14.88 ppm (plant B). Their values were lower than the threshold limit value (TLV, 50 ppm) of the American Conference of Governmental Industrial Hygienists (ACGIH). Styrene concentration in workers' blood were 0.52 mg/L (plant A) and 0.11 mg/L (plant B), and they were also below the biological exposure index (BEI) of ACGIH. The mandelic acid levels were also lower than the BEI of ACGIH. Chromosome aberrations of the styrene exposed workers showed no increase in the percentage of aberrant cells as compared with the control group. No correlation was found between the exposure levels and the frequencies of chromosome aberrations in workers. Smoking status also had no effect on the incidence of chromosome aberrations.

466 ARSENIC: AN EXAMPLE WITH VARIABILITY IN BOTH "DOSE" AND "RESPONSE"

C-J Chen, K G Brown. *National Taiwan University, Taipei, Taiwan; Kenneth G. Brown, Inc., Chapel Hill, NC.* Sponsor: D J Thomas

The primary source of evidence that inorganic arsenic in drinking water is associated with increased mortality from cancer at internal sites (bladder, liver, lung, and other organs) in a large ecologic study conducted in regions of Southwest Taiwan endemic to Blackfoot disease. While it is clear that cancer rates are markedly elevated at very high exposure levels, the nature of the dose-response relationship at relatively lower concentrations of arsenic is uncertain. Dose-response assessments typically assume that the data for dose is known without error so that statistical variability is introduced only through the data for response. For comparison with prior data from Taiwan used to estimate risk of skin cancer, the dose levels (arsenic concentrations in tested wells) for 60 villages were originally grouped into only three exposure levels. This grouping produced the appearance of a linear dose-response relationship suggesting very high risks at low concentrations as may be found in the U. S. Examining the data for all 60 villages with no grouping suggested that the dose-response curve may be quite nonlinear. Further examination of the test values for arsenic concentrations, however, revealed a high degree of within-village variability of arsenic concentrations thought to be largely attributable to different types and depths of wells. This example illustrates the potential significance of variability in dose, as well as response, to dose-response assessment of epidemiologic data.

467 PITFALLS OF USING EPIDEMIOLOGIC DATA TO ESTIMATE CANCER RISK DUE TO DIESEL-EXHAUST EXPOSURE

P A Valberg, J T Cohen, A Y Watson. *Gradient Corporation, Cambridge, MA.* Sponsor: B D Beck

A draft risk assessment for diesel exhaust was released in June, 1994 by California's Office of Environmental Health Hazard Assessment (OEHHA). Using job description and duration of employment in the railroad industry, OEHHA calculated historical diesel exposure either as lifetime average incremental atmospheric diesel concentration ($\mu g/m^3$) or lifetime incremental lung retention of diesel soot (mg). OEHHA took relative risk (RR) values from the Garshick *et al.* (1988) study of 55,407 railroad workers, in which the RR of lung cancer in "exposed" workers was reported relative to "unexposed" workers. For the entire cohort of exposed workers, OEHHA showed a positive correlation between diesel exposure as calculated by OEHHA and lung cancer RR as estimated by Garshick *et al.* Garshick *et al.* also reported RR's for a subset of workers (N = 28,510), *excluding* the most exposed groups (shopworkers and hostlers); we repeated the OEHHA analysis for this subset. Average levels of diesel exposure as calculated using OEHHA's methodology were substantially *lower* for this subset of workers than average exposure for the entire cohort; RR's for this subset of workers were slightly *higher*. Using RR's published by Garshick *et al.*, and the OEHHA methodology, we found an *inverse* dose-response relationship. Therefore, any unit risk estimate for diesel exhaust derived from these data must be viewed with skepticism; application of OEHHA's methodology also suggests that the RR's reported by Garshick *et al.* are likely due to causes unrelated to diesel-exhaust exposure.

468 USING HUMAN DATA TO DEVELOP BETTER CANCER SLOPE FACTORS: PCBS AS AN EXAMPLE

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Historically, cancer slope factors (CSFs) for chemicals have been derived almost exclusively from animal bioassays. For many animal carcinogens, a significant body of epidemiological evidence from highly-exposed individuals (e.g., in occupational settings) indicates either no apparent human cancer risk, or a risk vastly lower than that predicted from the animal bioassay-derived CSF. Under these circumstances, exaggeration of cancer risks from exposure to these chemicals can be minimized by using the human data to establish plausible upper bound estimates of the CSF in humans. To illustrate this, human CSFs for polychlorinated biphenyls (PCBs) were generated using data from studies of PCB-exposed workers. PCB daily doses received by these workers estimated using two different approaches — (1) using published measures of inhalation and dermal exposures; and (2) using published pharmacokinetic parameters for these workers — yielded nearly identical lifetime dosage estimates. Incremental risk rates for liver cancer and their 95% upper confidence limits (UCL) from two prominent studies were combined with the estimates of PCB dosages associated with these risks to derive human CSFs of 0.77×10^{-3} $(mg/kg/day)^{-1}$ (8.9×10^{-3}, 95% UCL) and 5.9×10^{-3} $(mg/kg/day)^{-1}$ (1.9×10^{-2}, 95% UCL). For comparison, the current EPA CSF for PCBs, based on liver carcinogenicity in rats, is 7.7 $(mg/kg/day)^{-1}$. This analysis indicates that if a human cancer risk for PCBs is assumed to exist, the upper bound estimate for that risk based on human data is more than two orders of magnitude lower than that predicted using the CSF from rodent bioassays.

469 ASSESSING EVIDENCE FROM MULTIPLE EPIDEMIOLOGIC STUDIES: QUANTITATIVE AND QUALITATIVE ASPECTS

K G Brown. *Kenneth G. Brown Inc., Chapel Hill, NC.* Sponsor: A H Marcus

A single epidemiologic study is seldom conclusive for a risk assessment objective because of both quantitative and qualitative limitations. Quantitatively, an extraordinarily large study may be required to detect even a moderately serious effect with statistical assurance. Qualitatively, many factors affect a study's soundness and credibility. Consequently, methods of meta-analysis, a recently popularized term that usually refers to the statistical aspects of how and when to combine study evidence, need to address qualitative as well as quantitative differences between studies of health effects. Qualitative characteristics of interest can be divided into those pertaining to general principles of good epidemiologic practice related to study design, execution, analysis, and interpretation, and to subject-specific items related to applicability for one's objective. Taken together, the qualitative characteristics determine its "utility". Measures of utility need to be treated as ordinals in a meta-analysis, whereas quantitative outcomes can be combined by statistical procedures, as judged appropriate to the situation. The approach used to assess the evidence from 30 epidemiologic studies for the EPA report on the risk of lung cancer from passive smoke is described and used as an example.

470 ABSORPTION AND DISTRIBUTION OF CADMIUM IN METALLOTHIONEIN-I TRANSGENIC MICE

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Metallothionein-I transgenic (MT-TG) mice have higher concentrations of MT-I in stomach (10x), small intestine (4x), large intestine (6x), liver (16x) and kidney (5x) than control mice. The purpose of the present study was to use MT-TG mice to determine whether MT affects cadmium (Cd) absorption and distribution. A single dose of ^{109}Cd was given to control and MT-TG mice iv (0.03–10 μmol/kg, 20 μCi/kg) or po (0.3–300 μmol/kg, 200 μCi/kg). Cd concentrations in 15 tissues were quantified 7 days later. Approximately 60% of the Cd administered iv was retained in the tissues; retention of Cd in MT-TG mice was similar to controls. In both control and MT-TG mice, only 0.3–0.5% of Cd administered po was retained, except for 3–4% at the highest dose. Although MT concentrations in liver, kidney and intestine were higher in MT-TG mice than controls, the concentrations of Cd in these tissues were not correspondingly higher, except for the MT-TG mice given the highest dose of Cd. Cd administered iv was primarily distributed to the liver (70%) and kidney (15%), and was independent of dose. In contrast, when administered po, distribution of Cd to liver increased from 40 to 75%, whereas distribution to kidneys decreased from 30 to 5%, as doses were increased from 0.3 to 300 μmol/kg. No differences in the organ distribution of Cd were observed between control and MT-TG mice. These data indicate that MT does not appear to play a significant role in either Cd absorption or distribution. (Supported by NIH grant ES-01142).

471 CADMIUM-METALLOTHIONEIN-INDUCED NEPHROTOXICITY IN METALLOTHIONEIN-I TRANSGENIC MICE

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Metallothionein-I transgenic (MT-TG) mice have higher concentrations of MT in tissues. The target organ of acute cadmium (Cd) exposure is the liver, and MT-TG mice are resistant to the hepatotoxicity and lethality produced by acute Cd exposure. The target organ of chronic exposure to Cd is the kidney, and this renal injury is also observed after acute administration of the Cd-metallothionein complex (CdMT). MT-TG mice have high MT levels in kidneys (5 times control mice). Thus, the purpose of this study was to determine whether MT-TG mice are also resistant to CdMT-induced nephrotoxicity. Mice were injected with CdMT (0.1–0.4 mg Cd/kg, *iv*), and kidney injury was evaluated 24 hr later by urinary protein and glucose excretion, as well as by histopathological examination. CdMT produced renal toxicity in a dose-dependent manner in both control and MT-TG mice. CdMT (0.4 mg Cd/kg)

produced approximately a 50-fold increase in both urinary protein and glucose excretion in control mice. Similar increases in urinary protein and glucose excretion were also observed in MT-TG mice. In addition, CdMT produced a similar extent of segmental necrosis of the epithelial lining cells in the proximal convoluted tubules (S1 and S2 segments) of both control and MT-TG mice. In conclusion, MT-TG mice are not resistant to CdMT-induced nephrotoxicity, suggesting that increasing intracellular MT concentrations does not protect against CdMT nephrotoxicity. (Supported by NIH grant ES-01142).

472 ALTERATION OF METALLOTHIONEIN (MT) LEVELS IN MICE EXPOSED TO ZINC CHLORIDE ($ZnCl_2$) AEROSOL

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MT is a low molecular weight protein that is important in metal detoxification. Previous studies on MT have often involved metals which were injected in animals or added to hepatic cell cultures. The objectives of the present study were 1) to determine MT levels in mouse lung, liver, and kidney following inhalation exposure to $ZnCl_2$ aerosol and 2) to correlate potential changes in pulmonary MT levels with pulmonary responses to the aerosol. Mice were exposed to 50 mg/m^3 of $ZnCl_2$ aerosol for 3 hr, with some animals euthanized after the 2nd and 3rd hr of exposure, and at 3, 6, 24, 48 hr, and 1 wk post-exposure. MT levels increased in the lung, liver, and kidney after 2 hr of exposure. The greatest increases in MT occurred in the liver, approximately 24 hr post-exposure. Between 24–48 hr post-exposure, liver MT levels decreased, and returned to control levels by 1 wk post-exposure. The pulmonary response to $ZnCl_2$ aerosol occurred post-exposure, with increases in respiratory frequency (f) peaking 3–4 days post-exposure and returning to control levels 14–21 days post-exposure. Thus, pulmonary responses to $ZnCl_2$ and pulmonary MT production did not appear to be correlated. Because MT increased in the liver and kidney following $ZnCl_2$ exposure, this suggested that Zn was cleared from the lung and distributed to these distal sites where detoxification and elimination probably occurred.

473 CHROMIUM INCREASES METALLOTHIONEIN CONTENT IN RAT PANCREAS

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Metallothionein (MT) content was studied in pancreas, liver and kidney of rats, 24 hours after a potassium dichromate (Cr VI) subcutaneous injection (10, 20, 30, 40, 50 and 57 mg/kg body weight). Cr VI caused a dose-dependent increase in pancreatic MT (up to 5-fold), as well as in serum pancreatic amylase activity and glycemia; these changes were accompanied by diffuse hyperplasia of Langerhans islets and a marked increase in zymogen granules in acinar cells. In liver, the MT increase (up to 3-fold) was observed only at 10 and 20 mg/kg, higher doses produced histopathological damage. In kidney, severe histopathological changes, including total loss of tubular epithelium were observed; renal MT content showed no significant changes. These findings suggest that Cr VI induces and binds pancreatic MT. Whether the increase in pancreatic MT contributes to avoid severe signs of toxicity in pancreas remains to be established. (Supported by the Mexican Council for Science and Technology grant to MJS and AA).

474 QUANTIFICATION OF METALLOTHIONEIN IN ENVIRONMENTAL SAMPLES USING CAPILLARY ELECTROPHORESIS

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Metallothionein (MT) has been widely used as a biochemical marker for metal exposure in bioindicator species. Capillary zone electrophoresis (CZE) is an useful analytical tool for protein separation and quantification. A relative quick and simple method using CZE to separate and quantify MT in environmental samples is developed. Caribou *Rangifer tarandus* liver and kidney samples were homogenized in 0.25 M sucrose solution and the MT was precipitated by addition of acetone (80%). The precipitate was re-suspended in water and injected (1 s by pressure) in an automated CZE with a 47 cm × 75 μm I.D.-polyamine-coated capillary. Running conditions were: buffer, 50 mM phosphate, pH 7.0; voltage, 20 kV; temperature, 25°C; U. V. detector at 200 nm. Two MT peaks were found in both liver and kidney samples. The retention time of MT-1 and MT-2 were 6.03 min and 6.87 min respectively which coincided with those of commercial MT standard from rabbit liver. Standard curves cover a linear range (r^2 = 0.999) over 5–1000 μg/ml for both MT isoforms. Sensitivity for MT-1 and MT-2 are 1 μg/ml. MT estimations by CZE were comparable to those by metal saturation method. Elevated levels of MT and Cd were found in the kidney samples. CZE can be used routine measurement of environmental samples.

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475 METALLOTHIONEIN AND NITRIC OXIDE IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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The formation of nitric oxide (NO) from streptozotocin (STZ) or by macrophages has been considered as one of the mechanisms of STZ induced diabetes. The protective role of metallothionein (MT) and glutathione (GSH) on blood glucose, NO synthase (NOS) activity and DNA damage was investigated in STZ induced (60 mg/kg BW i.p) diabetic rats. The synthesis of MT or GSH was induced by either a single injection of zinc (10 mg/kg, Zn) or N-acetylcysteine (700 mg/kg, NAC) about 12 hours before STZ injection. In another group, GSH levels were depleted by injection of Buthionine-sulfoxamine (4 mmol/kg, BSO). Plasma glucose levels increased significantly ($p < 0.001$) in STZ (245.4 ± 22.4), Zn + STZ (149.6 ± 20.8), NAC + STZ (159.6 ± 17.7), and BSO + STZ groups (298.5 ± 14.8) as compare to the control group (94.8 ± 7.1), but glucose levels in Zn + STZ and NAC + STZ group were significantly lower than STZ group ($p < 0.05$). MT levels in Zn and Zn + STZ groups and GSH levels in NAC + STZ were significantly increased in the pancreas and the liver. Enhanced NOS activity was found in the pancreas of STZ (2.1 fold) and BSO + STZ (2.2 fold) groups, but not in the liver. Pretreatment with Zn and NAC reduced the NOS activity to near control levels in STZ injected rats. Fluorescence density of DNA-ethidium bromide and DNA gel electrophoresis showed that STZ caused damage to pancreatic DNA. This damage could be protected by both Zn and NAC pretreatment. These results suggest that increased levels of GSH or MT partially prevented the development of diabetes after STZ injection and this may be due to formation of nitrosothiols with GSH or MT.

476 NITRIC OXIDE MAY ENHANCE CADMIUM TOXICITY BY DISPLACING THE METAL FROM METALLOTHIONEIN

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Cadmium (Cd) is a pulmonary carcinogen in humans and rodents. Although extensive evidence indicates that the toxicity and genotoxicity of Cd is mediated by binding to cysteine clusters in metallothionein (MT), the factors governing Cd release at intracellular target sites remain unknown. Nitric oxide (NO) is a pollutant gas and an important intercellular messenger involved in neurotransmission, platelet aggregation and the inflammatory immune response. In the presence of oxygen NO reacts with thiols in proteins and bases in DNA. To determine if NO is capable of displacing Cd from MT, purified rat liver protein was saturated with excess Cd (containing ^{109}Cd) and subsequently exposed to various concentrations of 1,1-diethyl-2-hydroxy-2-nitrosohydrazine (DEA/NO), an NO-generating sodium salt. Under these conditions dose-related increases in Cd displacement were observed at DEA/NO concentrations between 0.1 and 0.5 mM; Cd release plateaued at 3 mols Cd/mol MT when concentrations between 0.5 and 3 mM DEA/NO were used. When growing Chinese hamster ovary cells were treated for 24 hrs with 0, 0.5, 0.75, or 1 μM $CdCl_2$ followed by a 1 hr exposure to 0, 1, 1.5, or 2 mM DEA/NO, NO enhanced Cd-induced inhibition of colony forming ability in the absence of effect on mitochondrial function. Further examination of the impact of such treatment on proto-oncogene expression revealed a specific decrease in cellular levels of c-*myc* mRNA relative to β-actin mRNA suggesting that NO liberates Cd from MT and that free Cd forces cells into a period of apparent growth arrest. Overall our results indicate that NO enhances Cd cytotoxicity by displacing the metal from MT.

477 CHEMICAL MODULATION OF METALLOTHIONEIN (MT) I AND III IN MOUSE BRAIN

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MT-I and II are present in all tissues, while MT-III is only expressed in brain tissue. Regulation of MT-I and MT-III mRNAs was studied in brain and liver of control C57BL/6J mice and mice given the following MT-I and II inducers: lipopolysaccharide (LPS), zinc chloride (Zn), cadmium chloride (Cd),

dexamethasone (Dex), ethanol, and kainic acid (KA). Northern-blot analyses revealed that MT-I mRNA in liver was induced markedly (12–27 fold over control) by all the chemicals, while in brain only LPS produced an increase (2-fold). In contrast, MT-III mRNA in brain tended to be decreased approximately 30% by all inducers except for Zn. *In situ* hybridization was used to localize MT-I and III mRNA in the brains of these mice. MT-I mRNA signal was most abundant in the glial cells of the Purkinje cell layer of the cerebellum, which appeared to be enhanced by all treatments. MT-I mRNA signal in olfactory bulbs appeared to be increased by LPS and Cd. MT-III mRNA was localized predominantly in hippocampus, but was also found in glial cells of the Purkinje cell layer of the cerebellum. MT-III mRNA signals in hippocampus appeared to be reduced by KA, Dex and LPS, while in the cortical region, they appeared to be enhanced by KA, Cd, and ethanol. In conclusion, the hepatic MT-I and II inducers were much less effective at increasing brain than liver MT-I mRNA, and tended to decrease MT-III mRNA in brain. *In situ* hybridization of brain MT-I and III mRNA suggests that the inducers alter the regional expression of MT. (Supported by NIH Grants ES-01142 and ES-07079.)

478 INDUCTION OF METALLOTHIONEIN (MT) IN PRIMARY MURINE NEURON CULTURES

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MT-I and II are present in most if not all cells of the body and are readily inducible both *in vivo* and *in vitro*. MT-III is present only in brain, primarily in neurons, and is seemingly not inducible. Because it is difficult to determine whether chemicals reach neurons in sufficient concentrations *in vivo* to induce MT-III, the purpose of the present study was to determine whether MT-III is directly inducible in neuron cultures. Cultures were established from fetal (days 14–17) CF-1 mice. The cells were treated after they achieved a mature phenotype (around 6 days) with various concentrations of the following inducers: zinc (Zn), mercury (Hg), cadmium (Cd), or dexamethasone (Dex). MT protein was quantified by the Cd-hemoglobin assay at 24, 48, 72, 96, and 120 hrs. All inducers produced maximum increases in MT protein between 24 and 96 hrs. MT protein increased in a dose-dependent manner, and maximum (up to 3 fold over controls) increases were seen at 3, 30, 100, and 1000 μM for Cd, Hg, Zn, and Dex, respectively. The expression of MT-I, II, and III mRNA was examined by dot-blot analysis 6 hrs following addition of the maximum MT inducing concentrations of the four MT inducers. Zn and Cd produced 2.5–3.5-fold increases in MT-I and II mRNA, whereas Dex and Hg produced 1.5–2.5-fold increases. However, all inducers decreased MT-III mRNA about 50%. In conclusion, unlike MT-I and II, MT-III is not directly inducible in neurons, but rather is decreased by MT-I and II inducers. (Supported by NIEHS grants ES-01142 and ES-07079.)

479 METHYLMERCURY (MeHg)-INDUCED METALLOTHIONEIN (MT) EXPRESSION IN NEONATAL RAT PRIMARY ASTROCYTE CULTURES

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Astrocytes are known to act as a "sink" for MeHg in the CNS. Since MT induction in astrocytes is a potential mechanism of neuroprotection against MeHg toxicity, we measured both mRNA and protein levels following exposure of rat neonatal primary astrocyte cultures to MeHg. Western-blot analysis revealed a time-dependent increase in MT synthesis through 96 hrs of exposure (5 μM MeHg), the longest period of incubation performed to date. Since accumulation of MT is predominantly controlled at the transcriptional level, we also examined the accumulation of MT-1 mRNA following exposure to MeHg (2 μM). Total RNA was electrophoresed, blotted, and hybridized to a cDNA probe specific to MT-1 mRNA (a gift from Dr. Robert D. Andersen, UCLA). Expression of MT-1 mRNA in astrocyte monolayers exposed to 2 μM MeHg for 6 hours was increased over control levels (2 fg/μg total RNA). The induction of MT by MeHg was not as pronounced as induction by $CdCl_2$. The present study suggests that rat astrocyte cultures constitutively express MTs, and that MT induction by MeHg may serve to protect astrocytes and also, indirectly, juxtaposed neurons from its neurotoxic effects. (Supported by NIEHS 05223 and EPA 89190).

480 CADMIUM ($CdCl_2$)-INDUCED METALLOTHIONEIN EXPRESSION IN NEONATAL PRIMARY ASTROCYTE CULTURES

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Both mRNA and protein levels were monitored following exposure of rat neonatal primary astrocyte cultures to $CdCl_2$. MT mRNA was probed on Northern blots with a ^{32}P labeled synthetic cDNA probe specific for rat MT-I mRNA (a gift from Dr. Robert D. Andersen, UCLA). The probe hybridizes to a single mRNA with a size appropriate for MT, approximately 550 bases. Expression of MT-I mRNA in astrocyte monolayers exposed to 2 μM $CdCl_2$ for 6 hours was increased approximately 5-fold (9.7 fg/μg total RNA) over MT-I mRNA levels in controls (2 fg/μg total RNA). MT-I mRNA could also be detected in untreated cells, suggesting constitutive MT expression in these cells. Western-blot analysis revealed a dose-dependent increase in MT protein synthesis through 96 hrs. of exposure, the longest period of incubation performed to date. Consistent with the constitutive expression of MTs both at the mRNA level and protein level, we have also demonstrated a time-dependent increase in MT-immunoreactivity in astrocytes exposed to $CdCl_2$. The present study suggests that astrocytes constitutively express MTs and that MT induction by $CdCl_2$ may serve to protect astrocytes, and perhaps also indirectly, juxtaposed neurons from its neurotoxic effects. (Supported by NIEHS 05223 and EPA 89190).

481 IDENTIFICATION OF 3′-METHOXY-4′-NITROFLAVONE AS A PURE ARYL HYDROCARBON (Ah) RECEPTOR ANTAGONIST AND EVIDENCE FOR MORE THAN ONE FORM OF THE NUCLEAR Ah RECEPTOR IN MCF-7 HUMAN BREAST CANCER CELLS

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The competitive binding of 3′-methoxy-4′-nitro, 4′-amino-3′-methoxy, 4′-methoxy-3′-nitro, and 3′-amino-4′-methoxyflavone (**1** to **4**, respectively) to the rat cytosolic aryl hydrocarbon receptor (AhR) gave IC_{50} values of 2.27, 86.1, 872 and 19.4 nM. Flavones **3** and **4** were characterized as AhR agonists in MCF-7 human breast cancer cells and induced CYP1A1 gene expression whereas flavones 1 and 2 were inactive. All four compounds inhibited ethoxyresorufin *O*-deethylase activity induced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) by metabolically-mediated processes. In MCF-7 cells cotreated with flavones **1** or **2** (0.01–10 μM) + TCDD or [^{3}H]TCDD, there was a concentration-dependent inhibition of TCDD-induced CYP1A1 mRNA levels and formation of radiolabeled nuclear AhR complex. Velocity sedimentation analysis of nuclear extracts from MCF-7 cells treated with [^{3}H]TCDD + 1 or 10 μM concentrations of flavones **1** and **2** showed that an early-eluting specifically-bound nuclear AhR complex was present. However, under these same conditions the flavones inhibited TCDD-induced CYP1A1 gene expression. The apparent molecular mass of this nuclear complex was 190-kDa as determined by crosslinking to a [^{32}P]labeled bromodeoxyuridine-substituted consensus dioxin responsive element. Similar crosslinking results were obtained using the nuclear extract from MCF-7 cells treated with [^{3}H]TCDD alone. These data suggest that there are at least two forms of the nuclear AhR complex in MCF-7 cells; one of these forms appears to be transcriptionally-inactive and ligand binding with [^{3}H]TCDD is not competitively inhibited by flavones **1** and **2**.

482 RT-PCR ANALYSIS OF THE Ah RECEPTOR NUCLEAR TRANSLOCATOR (ARNT) GENE IN MDA-MB-231 HUMAN BREAST CANCER CELLS

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MDA-MB-231 human breast cancer cells express the aryl hydrocarbon (Ah) receptor and Ah receptor nuclear translocator (Arnt) proteins and treatment with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) gives a 6S nuclear Ah receptor complex. However, TCDD does not induce CYP1A1 gene expression in this cell line. The properties of the nuclear Ah receptor complex suggest variability in the Arnt protein and Northern analysis of Arnt mRNA levels revealed 1.1- and 2.6-kb transcripts. Reverse transcriptase-polymerase chain reaction (RT-PCR) methods were developed to examine *Arnt* transcripts from the MDA-MB-231 human breast cancer cell line. The following primers were utilized in the experiments:

PAS F (5′-ATAAGCCGTCTTTCCTCACTGA-3′, position 461),
PAS R (5′-GTTCTGGTCCTGTGGTTGTCAC-3′, position 1591),
Full F1 (5′-ACTGCCAACCCCGAAATGAC-3′, position 13),
Full R1 (5′-CTCCCCCACCCCTTATCCTC-3′, position 2402).

Amplification of the highly conserved Per-Arnt-Sim (PAS) region resulted in products of similar size (≈1.1-kb) in both MDA-MB-231 cells and MCF-7 cells. Amplification of a full-length *Arnt* transcript (≈2-kb) gave products of ≈1-kb and ≈2.6-kb in MDA-MB-231 cells and MCF-7 cells, respectively. Partial sequencing of the RT-PCR products in the MDA-MB-231 cell line revealed no deviation from a previously published sequence and a sequence of the full-length *Arnt* transcript is in progress.

483 SUPPRESSION OF ADIPOCYTE DIFFERENTIATION BY 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN (TCDD) IN TWO URINE 3T3 CELL LINES

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Among the pleiotropic effects associated with TCDD toxicity is alteration of cellular differentiation. We recently reported that TCDD suppresses conversion of 3T3-L1 fibroblasts into cells with the biochemical and morphological characteristics of differentiated adipocytes. In the current study, we compared the effect of TCDD on adipocyte differentiation of two murine fibroblast clonal cell lines, 3T3-L1 and 3T3-F442A. TCDD effectively reduced formation of differentiated adipocyte colonies at a dose of 1 nM as assessed by Oil Red O staining for triglyceride in 3T3-L1 cells. Expression of a group of adipocyte-specific genes was also suppressed in TCDD-treated cells compared to DMSO-treated controls. In contrast to 3T3-L1 cells, the 3T3-F442A cell line appeared to be much less sensitive to TCDD's effects with no observable suppression of differentiation even at a dose of 10 nM. We tested whether this difference in sensitivity to TCDD was related to an alteration of Ah-receptor (AhR) functionality. Comparison of the amount of AhR ([3H] TCDD sucrose gradient ligand binding assays) and the DNA binding activity of nuclear TCDD:AhR complexes (mobility shift analysis) revealed no quantitative differences in apparent receptor function between these two cell lines. Thus, we have identified two preadipocyte cell lines which respond differently to TCDD's effect on differentiation, but which are indistinguishable at the level of Ah-receptor functionality. These two cell lines may provide a useful model for probing the effect of TCDD on adipocyte differentiation.

484 CHARACTERIZATION OF Ah RECEPTOR BINDING FACTORS ISOLATED FROM RABBIT LIVER CHROMATIN

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Dioxin induces biological responses through interaction with the Ah receptor (AhR) and the subsequent interaction of the AhR with chromatin. We have purified AhR binding factors (AhRBFs) from rabbit liver chromatin protein (CP) by sequential DNA affinity chromatography, molecular sieve chromatography, preparative isoelectric focusing, and preparative SDS-PAGE. To assay for receptor binding factor activity a portion of CP was reconstituted to ds-DNA and binding to radiolabeled AhR was measured. Competition experiments with excess unlabeled AhR complexes demonstrated that binding of radio-labeled AhR to AhRBFs was >90% specific and AhRBFs are distinct from RBFs for the estrogen receptor. AhRBF activity has been attributed to two proteins, molecular weights 12 and 13 kDa. We envision that AhRBFs may act as docking proteins in chromatin which bind the AhR complex via protein-protein interaction and subsequently direct or stabilize AhR complex interaction with dioxin responsive elements.

485 RECOMBINANT MOUSE HEPATOMA CELL LINE BIOASSAY FOR DETECTION OF DIOXIN-LIKE CHEMICALS

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Exposure to halogenated aromatic hydrocarbons (HAHs), such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), produces a wide variety of toxic and biological effects. The presence of HAHs in environmental samples as complex mixtures has made it difficult to predict the biological and toxic potency of these chemicals. Taking advantage of the Ah receptor (AhR)-dependent nature of the mechanism of action of these chemicals, we have to developed a cell bioassay for detection of dioxin-like chemicals. A recombinant expression vector in which the firefly luciferase (LUC) gene is under HAH (TCDD)-inducible control of four dioxin responsive DNA enhancer elements has been constructed. HAH-inducible expression of LUC in mouse hepatoma (Hepa1c1c7) cells stably transfected with the vector occurs in a time- and dose-dependent manner. TCDD-inducible LUC activity was observed in as little as 2 h after chemical treatment, the limit of detection was between 0.1–1 pM TCDD and maximal induction observed at 1 nM TCDD. The specificity of LUC induction by several polycyclic hydrocarbons and HAHs was also determined. This bioassay system can readily be used for the detection and relative quantitation of AhR agonists. (NIH ES04911 and ES04699, Dutch Technol. Foundation).

486 CHARACTERIZATION OF THE ACTIVATED FORM OF THE ARYL HYDROCARBON RECEPTOR FROM NUCLEAR EXTRACTS OF HELA CELLS CULTURED WITHOUT EXOGENOUS LIGAND

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The Aryl hydrocarbon receptor (AhR) is known to mediate TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) induced toxic effects. In HeLa cells using a anti-AhR MAb Rpt9, laser-scanning confocal and indirect immunofluorescence microscopy it was demonstrated that the AhR is localized throughout the cell. However, upon TCDD treatment most of the cytoplasmic receptor is found in the nucleus in a time dependent manner. We observed a significant amount of AhR was tightly associated with the nuclear fraction of untreated HeLa cells. Under similar culture conditions Hepa 1 cells showed that, although, the relative amount of total AhR in these cells was about 10 fold higher than HeLa cells, the amount of the receptor in the nuclear fraction of untreated Hepa 1 cells was significantly lower than that of HeLa cells. Further characterization by sucrose density gradient analysis showed that the HeLa AhR is present in the 6S form. Using a anti-Arnt MAb 4G9 the AhR was immunoprecipitated which indicates that the receptor is present as a heterodimer with Arnt. In gel retardation assays using wild type DRE and two mutant DREs (with mutations in the core sequence) it was observed that the AhR is capable of specifically binding the DRE. Further, in super gel shift assays using a anti-Arnt MAb 2B10 it was established that the AhR binds DRE as a heterodimer with Arnt. The data would suggest that in untreated HeLa cells, the AhR associated with the nuclear fraction is a activated AhR-Arnt heteroduplex which is capable of binding the xenobiotic responsive elements independent of exogenous ligand.

487 IDENTIFICATION OF mRNA TRANSCRIPTS FOR Ah RECEPTOR AND ARNT PROTEIN IN MOUSE SPLEEN

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Suppression of humoral immune response by 2,3,7,8-tetrachlorodibenzo-p-dioxin is well established in a number of mouse models including in female B6C3F1 (C57BL/6 × C3H) mice. However, it is unclear whether this inhibition is mediated through the aryl hydrocarbon receptor (AhR), as demonstrated in a variety of other tissues, or even if this receptor is present and functional in mouse splenocytes. Function of the AhR is dependent on the AhR nuclear translocator (ARNT), which facilitates the binding of the receptor-ligand complex to the dioxin-responsive enhancer. The purpose of the present studies was to determine if mRNA transcripts for the AhR and ARNT could be detected in mouse spleen as indirect evidence for or against the existence of a functional AhR. For these studies, polyA RNA was isolated from mouse spleen and liver, which served as a positive control, and analyzed by Northern Blot. The isolated polyA RNA was probed with a 1.8 kb cDNA fragment excised from the 3′ end of pSport AhR (Burbach *et al.* PNAS 89:8185, 1992) and with a 75 bp oligomer (bp #361–426) derived from a cDNA clone of the human ARNT protein (Hoffman *et. al.* Science 252, 954–958, 1991). Northern analysis revealed a single band in both spleen and liver confirming the existence of mRNA transcripts for the AhR in mouse spleen. Transcripts for the ARNT protein were also detected as evidenced by the appearance of three bands in both the spleen and liver. These findings suggest that two key components essential for receptor-mediated dioxin toxicity are present in mouse spleen. (These studies were supported in part by the National Institutes of Health grant ES02520 and the Pharmacological Sciences Training grant GM07392).

488 SPECIFICITY OF DNA BINDING BY THE Ah-RECEPTOR, ARNT AND SIM

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The Ah-receptor (AHR), the Ah-receptor nuclear transporter (ARNT) and SIM are basic-helix-loop-helix proteins that also harbor PAS homology domains at their N-termini. In this study, we employed a DNA selection and amplification protocol to characterize the recognition sites and pairing rules for this family of proteins. We found that out of approximately 7×10^7 possible 13mer oligonucleotide sequences, the AHR/ARNT complex has the greatest affinity for the core, GCGTG. This sequence is identical to that previously described as an AHR/ARNT responsive enhancer element *in vivo*. Further analysis indicated that in addition to recognition of this core sequence, the AHR/ARNT complex has binding specificity for flanking nucleotides that spanned a region of at least 19 nucleotides. Once the DNA selection strategy was validated, we attempted to determine if previously unidentified homodimeric or heterodimeric combinations of the AHR, ARNT and SIM could exist, and if so, did they generate unique DNA binding specificities. We found that in addition to its ability to heterodimerize with the AHR, ARNT is capable of forming a homodimer that binds CACGTG, a sequence identical to the "E-box" that is recognized by a variety of other bHLH proteins such as myc/max, and is also capable of forming both a heterodimer with SIM recognizing GTGCGTG. Finally, metaanalysis of the data obtained from this study suggests that the half-site recognition sequences for the AHR, SIM and ARNT are TC/TGC, GTGC and GTG, respectively.

489 LOCALIZATION OF Ah RECEPTOR AND ARNT IN EMBRYONIC C57BL/6N MOUSE ADRENAL FROM GESTATION DAY 13 TO 16

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Exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) produces teratogenic effects in C57BL/6N embryonic mouse. TCDD binds to the Ah receptor (AhR) and heterodimerizes with the AhR nuclear translocator (ARNT) protein. This complex binds to the xenobiotic response element, regulating gene expression. This study examines the expression of AhR and ARNT in the developing adrenal gland of control and TCDD-exposed C57BL/6N embryonic mice. On gestation day (GD) 10 pregnant mice were dosed with corn oil or TCDD (12 μg/kg/5 ml). On GD 13, 14, 15, or 16 embryonic adrenals were dissected, fixed in 3% paraformaldahyde (PA), embedded in paraffin, and sectioned. The expression of AhR and ARNT was determined immunohistochemically with an affinity purified antibody against mouse AhR (G.Clark, 8 μg/ml) and human ARNT (M.R.Probst 1.1 μg/ml). In controls, the expression of AhR and ARNT changed during adrenal development, increasing with respect to both the number of stained cells and the level of protein detected. The cellular pattern of expression of AhR and ARNT shifts during development with increasing expression in the cytoplasm verses the nucleus. Also, during differentiation of the adrenal, the organization of the cortical cells becomes more linear, a subcapsular zone begins to appear, and small clusters of medullary cells are detected. Although there was a mixture of cellular expression types, AhR and ARNT were expressed at very high levels in the cytoplasm of cortical cells with little or no nuclear expression. The effects of TCDD treatment appeared to be minimal with respect to these proteins. With the strong cytoplasmic localization, AhR and ARNT have unusual expression patterns in the developing adrenal and it will be of interest to explore the role of these proteins in this tissue. (This abstract does not necessarily reflect EPA policy.)

490 ARNT EXPRESSION IN C57BL/6N EMBRYONIC MICE FROM GESTATION DAY 10 TO 16

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2,3,7,8-tetrachlorodibenzo-*p*-dioxin(TCDD) is developmentally toxic and teratogenic in mice. The Ah receptor (AhR) binds TCDD and heterodimerizes with the AhR nuclear translocator (ARNT) protein. This complex binds to upstream regulatory DNA sequences, xenobiotic response elements, thereby controlling gene expression and inducing biological effects. Since ARNT is required for transcriptional regulation by the TCDD-AhR complex, expression of ARNT was examined in C57BL/6N mouse embryos on gestation days (GD) 10 to 16 by immunohistochemistry and *in situ* hybridization. An affinity purified antibody against human ARNT (1.1 μg/ml) was incubated at 4°C for 26 hours and detected with avidin-biotin-peroxidase complex. ARNT mRNA was localized with a ^{35}S-RNA probe from pBM5/NEO-M1-1 (provided by O. Hankinson, UCLA, CA), which includes the complete coding sequence of human ARNT. Specific spatial and temporal patterns of ARNT expression were found. Tissues in which ARNT was localized immunohistochemically also showed mRNA expression. The GD10–11 embryos showed highest levels of ARNT in neural tissues (neuroepithelial cells of the neural tube, visceral arches, otic and optic placodes, and preganglionic complexes). The heart also had significant expression of ARNT with strong nuclear localization. After GD11 expression in heart and brain declined. In GD12–13 embryos expression was highest in the liver with relatively little ARNT elsewhere. Liver expression increased GD12–16 with strong nuclear localization emerging. At GD15–16 the highest levels of ARNT occurred in adrenal and liver, and moderate levels were also found in submandibular gland, ectoderm, tongue, bone, and muscle. In all of these tissues ARNT was cytoplasmic as well as nuclear, except in the cortical adrenal cells in which ARNT was strongly cytoplasmic with little or no nuclear localization. These specific patterns of expression suggest that ARNT has a role in embryonic development. (This abstract does not necessarily reflect EPA policy.)

491 PUTATIVE DIOXIN-RESPONSIVE ELEMENTS (DREs) IDENTIFIED DOWN-STREAM OF THE RAT PHOSPHOENOLPYRUVATE CARBOXYKINASE (PEPCK) GENE

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Studies have shown that transcriptional activation of genes can be stimulated by binding of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-Aryl hydrocarbon Receptor (AhR) complexes to *cis*-acting dioxin-responsive enhancers (DREs). In contrast, the mechanism by which TCDD inhibits transcription of the rat phosphoenolpyruvate carboxykinase (*PEPCK*) gene has not yet been elucidated. Inhibition of *PEPCK* transcription and thereby gluconeogenesis has been suggested to be a mechanism by which TCDD induces body weight loss, wasting, and lethality in rats. In this investigation, the rat *PEPCK* gene was searched for homology to the functional consensus sequence defined for DREs. Ten putative DREs were identified, six of these are located downstream of the gene in a unique overlapping arrangement within the 102-bp region suggested to be important in this genes regulation. Of these DREs, only two are expected to be functional in the stimulation of gene transcription. However, overlapping nonfunctional DREs with a higher relative binding affinity for TCDD-AhR may effectively block functional DRE binding. Therefore, the location, functionality, and relative binding of DREs associated with the rat *PEPCK* gene suggest a novel AhR-dependent mechanism by which TCDD may inhibit gene transcription. A similar analysis of the human *PEPCK* gene did not find DREs down-stream of the gene and suggests that humans are not responsive to TCDD inhibition of *PEPCK* gene transcription.

492 Ah-RECEPTOR AND ARNT PROTEIN DISTRIBUTION AND CONCENTRATION IN HEPATIC AND NON-HEPATIC CELL LINES FROM VARIOUS SPECIES

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Immunofluorescence microscopy was used to evaluate the subcellular distribution of the Ah-receptor (AhR) and Arnt protein in cell lines derived from hepatic and non-hepatic tissues of different species. Cells were fixed in 4% paraformaldehyde and stained with affinity purified IgG antibodies against bacterial expressed murine AhR or Arnt protein. The mouse embryonic fibroblast cell lines C3H/10T1/2 and NIH3T3, showed distributions of the AhR in both cytoplasm and nucleus. The staining pattern became predominately nuclear following treatment with TCDD. The rat H4IIE hepatoma line also showed nuclear and cytoplasmic AhR under basal growth conditions. Arnt was nuclear in all cell lines. In D-17 canine osteogenic sarcoma cells, both the AhR and Arnt staining was exclusively nuclear. Modifications of media, serum, cell density or substratum did not effect the nuclear distribution. Gel shift assays with D-17 nuclear extracts did not results in shifts, but shifts were detected when extracts were prepared from TCDD treated cells. The concentration of both AhR and Arnt protein were evaluated in each cell line by Western blots and *in vivo* binding assays with [^{3}H]-TCDD. All the cell lines expressed reduced AhR as compared to Hepa-1 cells, while Arnt expression was similar to Hepa-1. These results suggest that the subcellular distribution of the AhR is distinct in cells derived from different tissues and that "untransformed" AhRs reside in both the cytoplasmic and nuclear compartment. In addition, AhR/Arnt ratio may influence subcellular location.

493 OZONE-INDUCED 4-HYDROXYNONENAL FORMATION IN MOUSE SURFACE LINING FLUIDS

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Inspired O_3 is absorbed within the lung via a reaction-dependent process which, due to reaction substrate availability, is most likely localized to the pulmonary surface lining layer (SLL). O_3 reacts with unsaturated fatty acids (UFA) to produce distinct aldehydic products. However, alternative reaction pathways produce free radicals which initiate peroxidative processes and can generate hydoxylated aldehydes. To evaluate the formation of SLL-derived UFA products, we exposed mice (C57BL/6) *in vivo* and mouse bronchoalveolar lavage (BAL) *in vitro* to O_3 and assayed BAL fluid (BALF) and cells for 4-hydroxynonenal (HNE)-protein adduct formation via Western blotting using a polyclonal antibody against HNE adducts to his, lys, cys. *Results:* (1) *In vivo* air controls showed no adducts in BALF or cells. (2) 1.2 ppm O_3 (3 hr) *in vivo* resulted in BALF adducts (25–27 & 40–45 kD) with minimal detection in cells. (3) *In vitro* BAL exposure (0.6 ppm O_3, 30 min) produced HNE in BALF (50–55, 70–80 kD) but little in cells. (4) Direct HNE (1 μM) addition to BAL generated BALF adducts similar to *in vitro* exposures but also produced cellular adducts. *Conclusions:* The results suggest that substantial O_3 chemistry occurs within the SLL extracellular space. HNE formation may contribute to O_3-induced toxicity. Differences in adduct distribution between O_3 exposures and HNE addition may result from experimental discrepancies between well-mixed BAL and the native SLL. (CIAR 90-23, & NIH ES04952, ES04804, T32ES07254).

494 STOICHIOMETRY OF LINING LAYER NONANAL PRODUCTION IN O_3 EXPOSED ISOLATED LUNGS

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O_3 is absorbed from inspired air due to chemical reactions with constituents of the lung surface lining layer (SLL). While the preferential targets for O_3 reactive absorption have not been extensively defined, the prevalence of unsaturated fatty acids should facilitate direct reaction with the SLL. Previous *in vitro* and *in vivo* studies have demonstrated that aldehydes, specific to O_3-fatty acid reactions, are produced as a consequence of exposure. To investigate the stoichiometry between pulmonary O_3 absorption and nonanal production (derived from oleic acid), we exposed isolated lungs and subsequently quantified nonanal in bronchoalveolar lavage (BAL). Isolated lungs (unperfused; $V_2 \cong 2.4$ ml, f = 50/min; 37° C) were employed to enable control of O_3 delivery rate, measurement of O_3 absorption, and to limit perfusion-based removal of products. Aldehydes were quantified using O-pentafluorobenzylhydroxylamine HCL derivatization and gas chromatography/ECD. *Results:* A linear (r = 0.99) dose/response was observed. (1) 0.26 ppm, 60 min (74 nmol O_3 dose) = 69 nmol O_3 uptake & 126 pmol nonanal production. (2) 1.0 ppm, 30 min (154 nmol dose) = 141 nmol uptake & 243 pmol nonanal. (3) 1.0 ppm, 60 min (304 nmol dose) = 278 nmol uptake & 380 pmol nonanal. (4) Hexanal and heptanal were also detected. The results suggest that direct O_3/fatty reactions occur within the SLL. O_3-specific aldehydes as measured represent only a small fraction of the absorbed dose. (CIAR 90-23, HEI 91-7, NIH ES04952 & T32ES07254).

495 OZONE DEGRADES IMPORTANT LUNG PHOSPHOLIPIDS AND PROTEINS TO SUBSTANCES THAT INDUCE DNA SINGLE STRAND BREAKS

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We have previously shown that ozone (O_3) can degrade free arachidonic acid (AA) to the genotoxic agent hydrogen peroxide (H_2O_2). However, in the lung, the vast majority of unsaturated fatty acids (UFAs) such as AA are esterified in phospholipids located intracellularly and in epithelial lining fluid (ELF). Albumin is the most abundant protein in human ELF. Upon exposure to O_3, these biomolecules may be degraded to H_2O_2. In these studies, the effect of O_3 exposure on the degradation of arachidonyl-containing phosphatidylcholine (PC) (used as a model phospholipid), and human serum albumin were determined. Additionally, the effect of the ozonized PC on the induction of DNA single strand breaks (ssb) was examined. PBS vehicle, 30 or 60 μM PC, and 0.5 mg/ml albumin were exposed to 0.4 ppm O_3 (2 hr) and generated 3, 13, 19, and 44 μM H_2O_2, respectively. H_2O_2 formation was also exposure time-dependent. Incubation of human lung fibroblasts (CCD-32Lu) at 4°C (1hr) with 30 μM ozonized PC (0.4 ppm, 2 hr) or 25 μM H_2O_2 induced approximately 65 and 185 rad equivalents of DNA ssb, respectively. These data suggest that some proteins and UFAs esterified to phospholipids can be degraded by O_3 to genotoxic species. The production of H_2O_2 from free and esterified UFAs as well as some proteins may contribute to the possible lung tumorigenic potential of O_3. [Supported in part by CR81763 and ES04951. This is an abstract of a proposed presentation and does not necessarily represent EPA policy.]

496 NO_2-INDUCED SECONDARY MEMBRANE OXIDATION: POTENTIAL ANTIOXIDANT AND OXYGEN-DEPENDENCE

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NO_2-induced pulmonary injury is governed by the initial reactions between NO_2 and surface lining layer (SLL) target molecules. Since NO_2 *per se* is eliminated upon absorption, it is likely that SLL-derived products initiate the cytotoxic cascade. Previous studies demonstrated that SLL ascorbate (AA) and glutathione (GSH) are preferential NO_2 reactive absorption targets. We used a 3-compartment model to represent the lung surface and investigate the potential for these targets to initiate exposure-induced secondary oxidative processes. We covalently adhered red blood cell membranes (RBCM) to a glass substratum. Exposures (10 ppm NO_2, 20 min) were conducted by covering RBCM with model SLLs and cyclically tilting dishes to produce a thin film but preclude direct RBCM contact with the gas phase. RBCM oxidation was determined via thiobarbituric acid reactants (TBARS). *Results:* (1) NO_2 + 10 μM GSH, 25 μM AA, or rat lung lavage fluid significantly elevated TBARS production. (2) Air-only (+GSH or AA), or NO_2 (−GSH or AA) produced negligible TBARS. (3) NO_2 in N_2 (+GSH) reduced TBARS compared to air. (5) Superoxide dismutase (SOD) (+GSH) eliminated TBARS. *Conclusions:* GSH and AA may function as prooxidants during NO_2 exposure. The O_2-dependence and SOD effect suggest that $GS^{\bullet}$, produced from NO_2 + GSH, reacts to form $GSSG^{\bar{\bullet}}$ which reduces O_2 to $O_2^{\bar{\bullet}}$. Potentially, the extracellular production of $O_2^{\bar{\bullet}}$ within the SLL may function as an unrecognized mechanism of NO_2 toxicity. (CIAR 90-23, NIH ES04952 & T32ES07254).

497 REGULATION OF TYPE II PNEUMOCYTE PROLIFERATION FOLLOWING ACUTE EXPOSURE OF RATS TO OZONE

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Ozone is a highly reactive oxidant that causes necrosis of Type I epithelial cells in the proximal alveolar regions and proliferation of Type II pneumocytes which act as progenitors for Type I cells. In the present studies we analyzed mechanisms regulating Type II cell proliferation. Female SD rats were exposed to 2 ppm ozone or air for 3 h and the lungs isolated 12, 24 and/or 48 h later. Ozone inhalation resulted in proliferation of Type II cells which was evident at 12 h postexposure, as measured histologically and immunohistochemically by proliferating cell nuclear antigen expression. Type II cells isolated from ozone treated animals also incorporated significantly more ^{3}H-TdR than cells from control animals. Alveolar macrophages (AM) from both air and ozone exposed animals were found to be mitogenic towards Type II cells. AM from ozone treated rats caused a significantly greater increase in Type II cell ^{3}H-TdR incorporation. This effect required cell-cell contact indicating the involvement of cell surface molecules in the signaling pathway. These data suggest that AM participate in the repair of the alveolar epithelium after ozone-induced lung injury. (Supported by NIH Grant ES04738).

498 INFLAMMATORY AND MORPHOLOGICAL RESPONSES OF LACTATING AND POSTLACTATING RATS FOLLOWING OZONE EXPOSURE: A COMPARISON OF CONCENTRATION AND INHALED DOSE

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Ozone (O_3) has been shown to elicit an inflammatory response and induce morphological changes in the lung. We have previously shown that rats late in pregnancy and throughout lactation had enhanced inflammatory responses following acute exposure to a high concentration (1.0 ppm) of O_3 compared to those of age-matched, virgin female and postlactating rats. This study examined the inflammatory and morphological responses in lactating and postlactating rats following an acute exposure to a range of O_3 concentrations. Adult, primiparous Sprague-Dawley CD VAF rats at day 13 of lactation or day-10 postlactation were exposed in barometric plethysmographs to 0, 0.3, 0.5, or 1.0 ppm O_3 for 6 hours. Tidal volume and breathing frequency were measured

at regular intervals throughout exposure for calculation of inhaled dose. The rats were killed 18 hours after exposure, the right lung was lavaged, and the left lung was fixed for histopathology. Lactating rats had increases in total protein and neutrophils recovered in bronchoalveolar lavage fluid (BALF) compared to postlactating rats. Severity and size of lesioned areas and number of free inflammatory cells in the lung parenchyma were greater in lactating rats and were concentration dependent for both groups. Alveolar septal tip thicknesses in the periacinar regions were increased at 1.0 ppm only, and there was no difference between groups of rats. Lactating rats had greater minute volumes than postlactating rats at all O_3 concentrations. The results of this study show that lactating rats are more sensitive than postlactating rats in selected responses to acute exposures of O_3 and suggest that inhaled dose explains a portion of the difference between these groups of animals. (Supported by NIEHS Grants ES05939 and ES00260).

499 ADAPTATION OF RABBIT AIRWAYS AND PULMONARY MACROPHAGES TO SULFURIC ACID AND OZONE AFTER 20 DAY *IN VIVO* EXPOSURES

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Previously, we have shown that ozone (O_3) and sulfuric acid (SA) induce airway hyperreactivity and modify the pharmacologic regulation of H_2O_2 production by pulmonary macrophages (PM) of rabbits following a 5d, 3h/d exposure. In this study, rabbits were exposed to either 50 or 75 $\mu g/m^3$ SA, 0.1 or 0.3 ppm O_3, or a combination of O_3 and SA for 20 d, 3 hr/d. *In vitro* bronchial responses to cumulative doses of acetylcholine or histamine were not altered by pollutant exposures. This contrasts with results from 5d exposures of increased reactivity at both O_3 concentrations, and at 125 $\mu g/m^3$ SA. β-adrenergic modulation of H_2O_2 production by PM, measured by chemiluminescence, was significantly altered only by 50 $\mu g/m^3$ SA, which increased production. In contrast, H_2O_2 production was reduced by both 0.1 and 0.3 ppm O_3 and also by 50 $\mu g/m^3$ acid in 5d exposures. Pharmacologic regulation of H_2O_2 production by isoproterenol was modified only after exposure to 50 $\mu g/m^3$ SA, while after 5d exposures pharmacologic regulation was altered by 0.1 ppm O_3 and 125 $\mu g/m^3$ SA, but not by 50 $\mu g/m^3$ SA. Responses to combined 20d pollutant exposures were generally additive, while 5d exposures produced concentration-dependent synergistic or antagonistic responses. This study indicates that airways and PM may adapt to insults by O_3 and high SA concentrations, while environmentally relevant SA concentrations may induce greater effects over a longer exposure period. Thus, acute health effects induced by these pollutants may be mitigated in prolonged pollutant episodes. However, an adaptational response is not necessarily protective but, rather, may mask a secondary phase of injury. (EPRI contract #RP2155-2).

500 ENHANCED PRODUCTION OF NITRIC OXIDE BY HEPATIC PARENCHYMAL AND NON-PARENCHYMAL CELLS FOLLOWING ACUTE INHALATION OF OZONE

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Treatment of rats with ozone (2 ppm; 3 hr) results in epithelial cell injury. This is associated with an influx of activated macrophages into the lung. These cells release increased quantities of reactive mediators and pro-inflammatory cytokines which have been implicated in tissue injury. Previously we demonstrated that acute exposure of rats to ozone also results in changes in the liver including increased protein synthesis and nitric oxide production by hepatocytes. In the present studies we determined if hepatic endothelial cells and Kupffer cells were also sensitized to produce increased amounts of nitric oxide following exposure to ozone. Cells were isolated from rats 48 hrs after exposure to air or ozone by liver perfusion with collagenase and protease followed by centrifugal elutriation. Following ozone inhalation both cell types were found to produce increased amounts of nitric oxide when compared to control. Endothelial cells were more sensitive to the effects of inhaled ozone than were macrophages. These data demonstrate that brief inhalation of ozone limited to the lung induces alterations in liver parenchymal and non-parenchymal cells. Our observations of increased TNFα and IL-1 staining suggest that the extra-pulmonary effects of ozone are mediated by cytokines. (Supported by NIH grants ES04738 and ES07148).

501 MODIFICATION OF INFLAMMATORY FUNCTIONS IN THE RAT FOLLOWING OZONE EXPOSURE

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Several cell types and their soluble mediators are involved in an inflammatory response in the lung following ozone (O_3) exposure. In this study, we have examined the effects of the combination of antibodies to two important cytokines, interleukin (IL)-1α and tumor necrosis factor (TNF)-α, on the *in vivo* and *in vitro* responses to inhaled O_3. Male, Sprague-Dawley rats (250–300 g) were exposed, nose-only, to 0.8 ppm O_3 for 3 hrs and the *in vivo* and *in vitro* parameters were measured 8–12 hrs following exposure. In *in vitro* studies, BALF macrophages harvested from O_3-exposed rats exhibited a significant increase in adherence to cultured lung epithelial cells (ARL-14) as compared to the adherence of macrophages isolated from air-exposed controls. Furthermore, this adherence was significantly reduced in antibody-treated cells as compared to cells treated with pre-immune rabbit serum. The *in vivo* studies revealed increased recovery of neutrophils in bronchoalveolar lavage fluid (BALF), elevated transport of ^{99m}Tc-diethylenetriaminepentaacetate across tracheal epithelium, and higher concentrations of total protein and albumin in BALF following O_3 exposure. However, these effects were not significantly altered by treatment with the anti-IL-1α/anti-TNF-α combination. From these studies we conclude that O_3, affects the early stages of the inflammatory response, particularly with respect to macrophage activation and adherence to epithelial cells, and that this early response may be mediated by IL-1 and/or TNF. Supported by NIEHS grant ES03521.

502 PULMONARY BIOCHEMICAL CHANGES AFTER EXPOSURE TO URBAN PATTERNS OF THE OXIDANT AIR POLLUTANTS: NITROGEN DIOXIDE, OZONE AND THEIR COMBINATION

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Exposure to individual oxidant air pollutants such as nitrogen dioxide (NO_2) and ozone (O_3) at environmental or occupational levels was shown to cause pulmonary injury in both animals and humans. However, in urban cities where pollution is highest, oxidants are present continuously at low levels, and peak only periodically either individually or in combination. In this study, we examined the pulmonary response to such an urban pattern of exposure. We exposed Swiss Webster mice (6 mice/group) continuously to 1.6 ppm NO_2 for 7 days, and superimposed intermittently (8 h/day) peaks of 4.8 ppm NO_2, 0.48 ppm O_3 or a combined NO_2 + O_3 peak. After 7 days of exposure, all mice were euthanized and their lungs analyzed for a number of established biochemical indices of pulmonary injury involved in detoxification and repair that included, enzymes of the pentose phosphate cycle, and protein and nonprotein bound sulfhydryl (glutathione) contents. We found that the continuous exposure to low-level NO_2 had no significant effect on the parameters measured. Similarly, exposure to single peaks of either NO_2 or O_3 superimposed on the continuous low-level NO_2 were also not significant. Only the exposure to low-level NO_2 combined with the NO_2 + O_3 peak had a significant effect. We conclude that the lack of effect when the animals were exposed to NO_2 or O_3 peaks superimposed on low-level NO_2, may be due to low toxicity or to an acquired resistance (adaptation) to injury induced by the continuous low-level NO_2 exposure. The significant response resulting from continuous exposure to low-level NO_2 combined with the superimposed intermittent NO_2 + O_3 peak reflects a synergistic effect of the combined gases.

503 EFFECTS OF TEMPERATURE ON THERMAL EMISSIONS CHARACTERIZATION AND SENSORY IRRITATION IN MICE USING ETHYLENE METHYL ACRYLATE (EMA) COPOLYMER

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Thermal emissions (TE) are produced when polymeric materials are heated during manufacture and use. EMA was tested in a modified DIN 53 436 apparatus to characterize the major chemical components of TE and to evaluate sensory irritation in mice. EMA was tested over a range of typical use conditions: 300, 400, 450, 490, and 525°F. Air flows of 8 L/min were used. Chamber concentrations at 300 to 450°F were relatively consistent for total hydrocarbons (THC) (11 ± 2 mg/m^3), nonvolatile aerosols (NVA) (4 ± 3 mg/m^3), acrylic acid (AA) (<0.2 mg/m^3), methyl acrylate (MA) (0.5 ± 1 mg/m^3) and formaldehyde (FA) (<0.6 mg/m^3). Respiratory rates (RR) at these temperatures were within normal limits. At 490°F, NVA, AA and FA emissions began to increase. At 525°F, THC and MA increased 2 to 3-fold (28 and 1.0 mg/m^3) while NVA and AA increased 10-fold (44 and 1.8 mg/m^3). FA levels increased to 3.8 mg/m^3. Corresponding RR decreases were 51 and 68% at 490 and 525°F. Maximal RR decreases were observed within 10 min of exposure initiation and were sustained for the duration of the 30 min exposure period. Groups at 490 and 525°F had recovered to 14 and 24% of pre-test within 10

min post-exposure. Concentrations of the individual analytes were compared to published RD_{50} values or ACGIH TLVs. The results for EMA suggest that sensory irritation may be most closely associated with FA, NVA and perhaps other irritant aldehydes.

504 USE OF A NEW COMPUTER PROGRAM TO DETECT THE RESPIRATORY EFFECTS OF ZINC CHLORIDE ($ZnCl_2$) AEROSOL IN MICE

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Accidental exposure of humans to $ZnCl_2$ fumes has resulted in acute mortality, where autopsies revealed ulceration, hemorrhage, congestion, and edema of the respiratory tract. However, initial survival with delayed development of pulmonary fibrosis has also been reported. Recently, Schaper et al. (Am. Rev. Resp. Dis. 143, A697, 1991) evaluated acute respiratory reactions of mice exposed to $ZnCl_2$ aerosols, at concentrations ranging from 2–50 mg/m^3. Breathing patterns and respiratory frequency (f) of mice were evaluated using pressure-based body plethysmographs. After 3 hr exposures, concentration-dependent increases in f occurred, peaking 3–4 days post-exposure, and returning to control levels 14–21 days post-exposure. The objectives of the present study were 1) to more fully characterize these acute respiratory reactions via flow-based plethysmographs and a new computer program, and 2) to identify the possible onset of pulmonary fibrosis via display of sustained, rapid shallow breathing. Mice were exposed to 50 mg/m^3 $ZnCl_2$ for 3 hr, and then monitored for 10 wks post-exposure. Initial increases in f occurred, following a similar time course as seen previously. Also, there were accompanying increases in airflow (VD) and decreases in tidal volume (VT). No further changes in f, VD, or VT over the 10 wks suggested that the mice developed pulmonary fibrosis.

505 JUST DETECTABLE TOXICITY (JDT) AT THREE DIFFERENT LEVELS OF THE RESPIRATORY TRACT USING SINGLE ANIMAL DATA OR GROUPS OF FOUR ANIMALS DATA

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A computer program is available to analyze the breathing pattern of unanesthetized mice held in a body plethysmograph and to classify each breath as normal (N), sensory irritation effect on the upper respiratory tract (S), airflow limitation along the conducting airways of the lung (A) or pulmonary irritation at the alveolar level (P), (Vijayaraghavan et al., Arch. Toxicol. 68: 490, 1994). Using this program, 8 groups of 4 mice were used for sham exposures (water vapor) of 3-hr duration. The breath classification data (N, S, A or P) as well as the variables on which these classifications are based, time of braking (TB) for S, airflow at 0.5 tidal volume during expiration (VD) for A and duration of pause (TP) for P were analyzed using smoothing polynomial splines (SPS) and filtering methods. Filtering reclassifies S, A or P breaths to N, unless 3 (or more) of them occur in a row. This reduces the number of abnormal breaths due to body movements. A computer program was written for the SPS using a maximum likelihood estimate (MLE) approach to plot the data and the 95% C.I. The results indicate that the JDT for S, P or A would be an increase of only 5% in any classification when using group data and 15% when using single animal data. The 5% and 15% values (i.e. an increase in percentage of breaths in each classification above zero, as a "percent of total breaths" during each 15 sec of data collected) were arrived at by plotting the group data (8 curves + 95% CI for each curve) and individual data (32 curves + 95% CI for each curve) to obtain the entire range of values above zero observed during 3 hours of exposure. Thus, very low levels of effect can be reliably detected. Two examples will be presented for complex airborne mixtures. Supported by RO1-ESO2747, NIEHS.

506 SMOOTHING POLYNOMIAL SPLINES (SPS) TECHNIQUES COMBINED WITH BREATHING PATTERN ANALYSIS DATA TO EVALUATE THE EFFECTS OF MIXTURES OF AIRBORNE CHEMICALS AT THREE LEVELS OF THE RESPIRATORY TRACT

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Groups of 4 male Swiss Webster mice were exposed for 3 hrs to various concentrations of an aerosol of machining fluid G (MFG) or sham exposed while held in a body plethysmograph to which a pneumotachograph and a differential pressure transducer were attached to measure airflow (V). A computer program was used to analyze the breathing pattern and classify each breath as normal (N) or in one of 3 abnormal categories: sensory irritation of the upper respiratory tract (S), airflow limitation along the conducting airways of the lung (A) and pulmonary irritation at the alveolar level (P). These data were then analyzed using two SPS methods, the maximum likelihood estimate (MLE) and the generalized cross validation (GCV). Both methods were first evaluated via data simulation to determine their applicability for slowly developing effects and short duration effects. The results revealed that MFG induced all three types of effects: S, A and P. The MLE method was found to be very useful to follow the development of S followed by P effect and to separate individual animals in their primary response, S or P. The MLE fitted spline function with the 95% CI is also very useful in indicating the earliest time during exposure when a particular effect, S, A or P begins. The GCV spline revealed short periods of reaction better, particularly for S. This was consistent with the data simulation experiments showing that the structure of a data set was better highlighted by the GCV than the MLE method. Thus, the very large data sets created by the breathing pattern analysis program can be analyzed rapidly by the SPS program to reveal the effects of exposures to airborne chemicals. The programs are available. Supported by RO1-ESO2747, NIEHS.

507 THE INFLUENCE OF CIGARETTE DESIGN ON UPPER AIRWAYS IRRITATION IN THE MOUSE

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The mainstream smokes of seven different cigarettes or cigarette prototypes were evaluated by the Upper Airways Irritation assay. Breathing rates were measured during a 30 minute inhalation exposure period to mainstream cigarette smoke and were compared to pre-exposure control rates to determine the concentration of cigarette smoke that reduced the rates by 50% (RD50). The smokes were generated at Federal Trade Commission defined conditions of one 35 ml puff per minute, diluted to the desired concentrations and presented to the mice in nose-only exposure chambers. The concentrations of smoke, expressed as cigarettes per cubic meter, were compared to identify cigarette designs that correlated with reductions in upper airways irritation. Changes in the RD50 value for the smokes differed by as much as a factor of four. Chemical analyses of the mainstream smokes were also conducted and significant differences in classes of compounds were detected. Reductions in the concentrations of carbonyls were correlated with reductions in upper airways irritation.

508 CHAOS ON YOUR BREATH

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Mathematicians and physicists have developed recurrence analysis techniques which became popular in the 1970s and began to be applied to physiological systems data in the 1980s. These techniques, known under the general term of "chaos analysis" are quite powerful to search time series data sets to look for changes from "normal", however they require fairly large data sets to be effective. We have developed a computer program (Vijayaraghavan et al., Arch. Toxicol. 68: 490, 1994) for analyzing the breathing pattern of mice from plethysmographic recording of inspired and expired airflow ($\dot{V}I$ and $\dot{V}E$) and to classify each breath as normal (N) or in 7 different classes of abnormal breaths due to sensory irritation (S), airflow limitation (A) or pulmonary irritation (P) as well as the combinations of S + P, S + A, P + A and S + P + A. Since mice breathe at a frequency of 250/min, very large data sets can be accumulated. We have used a recurrence analysis program provided by C. Webber (J. Appl. Physiol. 76: 965, 1994) to analyze data from sham exposed mice vs. mice exposed to an aerosol of machining fluid G (MFG) which can induce S, A or P effects. Also we used a simple recurrence analysis where the data set (Xn) is plotted against itself with a lag of 1, i.e. Xn + 1 or other lag values. The results indicate that with large data sets, the beginning of a toxic effect can be illustrated by an increase in the variance of a measured variable rather than by a change in the absolute value of the variable from control values. This is different from the classical toxicological approach, requiring a concentration or dose dependent in the variables being measured. In effect, our analyses show that normal values and toxic levels values for variables have low variation, but the transition between normal and toxic levels is the area that can be described as "chaos", characterized as breathing patterns hunting between normal and abnormal. Supported by RO1-ESO2747, NIEHS.

509 SENSORY IRRITATION (S), PULMONARY IRRITATION (P) AND AIRFLOW LIMITATION ALONG THE CONDUCTING AIRWAYS OF THE LUNG (A) IN GUINEA PIGS USING DIGITIZATION OF AIRFLOW ($\dot{V}$) DURING INSPIRATION ($\dot{V}I$) AND EXPIRATION ($\dot{V}E$) OBTAINED FROM A BODY PLETHYSMOGRAPH

M F Stock, J E Luo, L A Boylstein, R D Thompson, Y Alarie. *University of Pittsburgh, Pittsburgh, PA*

A computer program is available to analyze the breathing pattern of unanesthetized mice held in a body plethysmograph, to recognize S on the basis of duration of breaking at the beginning of expiration (TB), P on the basis of duration of a pause at the end of expiration (TP) and A on the basis of a decrease in $\dot{V}E$ at 0.5VTE ($\dot{V}D$) (Vijayaraghavan et al., Arch. Toxicol. 68: 490, 1994). This computer program is being modified to process data with guinea pigs. In this species there is a strong first phase of pulmonary irritation (P1) which is characterized by a shorter duration of inspiration (TI), shorter duration of expiration (TE) and lower VT, i.e., rapid, shallow breathing, before A or P occur. Recognition of P1 is important in this species since it occurs during immediate pulmonary hypersensitivity reactions (Alarie et al., Am. Coll. Toxicol. 9: 407, 1990). Each breath of each animal was collected for a period of 15 min to establish control values for TB, TP, VD, TI, TE and VT and increases or decreases in these values were then used to classify each breath as normal (N), or S, A, P1 or P during exposure. The results indicate tlat a P1 effect can be easily detected, as induced by low exposure concentrations of histamine or propranolol aerosols. An S effect was also easily recognized during exposure to chlorobenzylchloride (CBC). However, guinea pigs were about 7–10 times less sensitive than mice to the effect of CBC. For A effects the program does not yet appropriately recognize this pattern. Similarly the program overclassifies the P effects. Thus, so far, only P1 and S effects can be detected. Supported by RO1-ESO2747, NIEHS.

510 INHALATION TOXICITY IN RATS EXPOSED TO p-PHENYLENE DIISOCYANATE AND CYCLOHEXANE DIISOCYANATE FOR TWO WEEKS

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Isocyanates have numerous industrial uses and are also noted respiratory tract irritants. Two diisocyanates with ring structures, p-phenylene diisocyanate (PPDI), an aromatic, and cyclohexane diisocyanate (CHDI), an aliphatic, were examined in 2-wk (6 hrs/day; 5 days/wk) studies with rats. The solid test materials were heated, and vapor sublimation produced mixed particulate/vapor exposure atmospheres. Design concentrations were 0, 0.5, 2, or 8 mg/m^3 PPDI and 0, 1, 3, or 10 mg/m^3 CHDI. Reduced body weight gains, lung noise and/or nasal discharge were observed. Respiratory tract irritation was noted microscopically after the last exposure. At the lowest levels, lesions were limited to the anterior nasal cavity; at the intermediate and high levels, lesions extended into the lower airways. Anterior nasal lesions from both isocyanates included rhinitis and hyperplasia, metaplasia and/or denudation of epithelial cells. Lower airways had inflammation and hyperplasia from both compounds; metaplasia and epithelial cell denudation with CHDI. After a 2-wk recovery, PPDI-exposed rats were free of lesions; CHDI-exposed rats (3 or 10 mg/m^3) had mild lesions in the nasal cavity. Under study conditions there was no no-observed-adverse-effect level for either isocyanate; however, irritant effects were slightly more severe with CHDI than with PPDI.

511 FORMALIN INDUCES SPONTANEOUS OSCILLATIONS OF MEMBRANE POTENTIAL IN HUMAN AIRWAY SMOOTH MUSCLE TO SUBSTANCE P

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Previous studies from our laboratory have shown that formaldehyde, a ubiquitous indoor environmental contaminant and respiratory irritant, produces alterations in several aspects of the electromechanical properties of human airway smooth muscle (ASM) including: 1. the production of transient contractions, 2. depolarisations of ASM cell membranes over the concentration range of 0.1–10 ppm formalin, and 3. an apparent increase in sensitivity to the contractile agonist, acetylcholine. The present study was conducted to determine if acute formaldehyde exposure of human ASM *in vitro* altered the transmembrane resting potential during the subsequent exposure to the neurotransmitter Substance P. Preparations were studied at 37°C using a specially constructed superfusion system and 3 M KCl-filled microelectrodes. We observed that 2 hours of exposure to 1 ppm formaldehyde (as formalin), produced spontaneous electrical oscillations of membrane depolarizations and repolarizations in the presence of 1.0×10^{-12} M Substance P. At this concentration of Substance P we were unable to demonstrate any changes in membrane potential in the absence of formalin. These results suggest that increased sensitivity to multiple neural contractile agonists may result from short-term exposures to moderate levels of formaldehyde. (Supported in part by a grant from The Center for Indoor Air Research, Inc.).

512 ASSESSING THE PENETRATION OF INHALED NITRIC ACID VAPOR IN THE RESPIRATORY TRACT

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Nitric acid (HNO_3) vapor is a component of ambient photochemical pollution. Because of its high water solubility and reactivity, it would be anticipated to undergo significant removal within the upper respiratory tract (URT). However recent studies show that inhaled vapor can alter bronchial responsiveness and other functions of the lower respiratory tract (LRT). The penetration of HNO_3 into the lungs was examined using a physical model which mimiced the URT of the animal used in this laboratory for toxicologic studies, namely the rabbit. The system allowed for mixing of precisely metered HNO_3 with ammonia (NH_3) at concentrations present in the URT, under conditions of controlled relative humidity (RH). The penetration of HNO_3 is determined by measuring the concentration of particles and vapor downstream from the mixing port. The penetration of HNO_3 through the URT increased with increasing relative humidity and NH_3 concentration. The portion of HNO_3 that penetrated was found to be present as particles. In an NH_3-free atmosphere at 77% RH, ultrafine particles at 0.003 μm were formed. When NH3 was added, the particle size increased to 0.15 μm. While there was greater production of particles when NH_3 was present at various levels of RH, even in the absence of NH_3, almost 50% of HNO_3 formed particles at high RH. We also examined the ability of other particles (saline) to act as vectors for adsorbed HNO_3, which could then be carried into the LRT. The results indicate that HNO_3 vapor is likely transformed into particle form at some point following inhalation. Endogenous ammonia may react with inhaled HNO_3, producing particulate ammonium nitrate. Once formed, these particles may then also serve as absorption surfaces, and additional vectors, for HNO_3 delivery to the LRT. Supported by CARB.

513 MOLECULAR AND MORPHOLOGIC DETERMINANTS OF ORGANIC DUST EXPOSURES IN ALVEOLAR MACROPHAGE (AM) CELLS

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To understand the pathology of pulmonary diseases after exposures to airborne dusts, we have previously measured acute-phase gene induction in rat/mouse lung after inhalation of respirable particulates. We have now measured *in vitro* responses of target AM cells to grain dust (*GD*) extracts and specific etiologic agents (bacterial endotoxin and fungal mycotoxins) found in elevator wheat samples. Area samples of respirable wheat dust were collected onto filters by personal pumps during routine elevator operations. Endotoxin levels were quantitated by chromogenic *LAL* assay, and fungi identified by standard mycologic culture. Rat AM cell line NR8383 displayed greatly reduced cell growth and elevated toxicity in response to purified endotoxin vs. *Alternaria* mycotoxins. When cells were exposed to GD extracts, dose-responses most closely resembled those of endotoxin alone, suggesting a pivotal role for this febrile agent in the pathophysiology of dust-related pulmonary diseases. Induction of the metallothionein gene was also detected by Northern blot analyses. Further acute-phase gene induction and oxidative burst analyses are in progress. Supported by NIOSH #U07/CCU807121-02.

514 BIOCHEMICAL RESPONSES OF INHALED CARBON BLACK IN RATS

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The objective of this study was to establish mechanisms of response of lung to low toxicity particles after subchronic inhalation. Fischer-344 rats were exposed to carbon black for 3 months at 6h/day, 5 days/week at concentrations of 1.7 and 50 mg/m^3. At intervals during the exposure and up to 6 months post-exposure the pulmonary response was assessed by biochemical analysis of lavage fluids for pulmonary surfactant and fibrogenic growth factors. In addition, cells recovered from lavage were assayed *in vitro* for production

of similar growth mediators. Proinflammatory cytokine gene expression was also assessed by *in situ* hybridization on paraffin sections. Statistically significant increases in pulmonary surfactant were observed only in the highest dose group. This was present during exposure and persisted through 6 mos. post-exposure. Expression of MIP-1 mRNA was found associated in cells in the alveolar spaces. MIP expression was seen in the high dose group where it persisted for up to 1 yr. Initially, expression of MIP was observed independent of cellular particle content, while at longer times only particle laden cells were positive. Growth factor production *in vitro* showed minor alterations at all doses but persistent effects only at the highest dose. These results suggest a pattern of persistent responses after inhalation exposure at high lung burdens and is consistent with a threshold for adverse effects.

515 PULMONARY RESPONSES TO AMIODARONE IN HAMSTERS: COMPARISON OF INTRATRACHEAL AND ORAL ADMINISTRATIONS

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Amiodarone (AD) produces a transient pulmonary fibrosis in hamsters after intratracheal (i.t.) instillation. The goal of this study was to explore the mechanism of AD-induced pulmonary fibrosis in the hamster model. Bronchoalveolar lavage (BAL) parameters during the development of fibrosis following i.t. AD in hamsters were examined; the responses to oral AD in hamsters were examined for comparison to responses to i.t. AD in an effort to explore the roles of inflammation, phospholipidosis, and lung drug burden in AD-induced pulmonary disease. Two i.t. instillations of AD on days 0 and 7 in hamsters produced variable increases in lavage macrophage, neutrophil, and eosinophil number through day 28. I.T. AD also increased the permeability of the alveolar-capillary barrier as evidenced by an increase in BAL fluid albumin on day 8. Pulmonary phospholipidosis was not induced by i.t. AD and only small amounts of AD and its metabolite desethylAD (dAD) were detected in lung tissue through day 10 after instillations on days 0 and 7. The repeated oral administration of AD did not result in pulmonary fibrosis during the 35 day course of this study. Oral AD did cause a sustained increase in BAL fluid neutrophil number; other BAL cells were only slightly affected. Oral AD did not increase BAL fluid albumin content but a prominent BAL cell phospholipidosis was noted. Measurement of AD and dAD in lung tissue demonstrated a substantial sequestration of drug and metabolite after oral treatment with AD. The results of this study indicate that lung drug burden, pulmonary phospholipidosis, and lung neutrophil influx are not crucial factors in the development of AD-induced pulmonary fibrosis in hamsters. This study supports the involvement of physical damage to the lung and/or pulmonary eosinophilia in the generation of AD-induced pulmonary fibrosis in hamsters. (NIH/NIGMS Training Grant T3GM07039).

516 PARTIAL PROTECTION FROM ACUTE SILICA TOXICITY BY DEXAMETHASONE IN RATS

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The generation of silica dust and human exposure to this dust is of great occupational concern because it causes pulmonary inflammation/damage that ultimately proceeds to fibrosis. The initial phase of the toxic response occurs within 2 hours of exposure although the "classical" sign of inflammation (neutrophil influx) is absent. The purpose of this study was to differentiate between the toxicities due to the intrinsic properties of silica, and those due the cellular infiltration. Male Fischer 344 rats were intratracheally (i.t.) instilled with silica (10 mg/100 g bw) or saline vehicle. Two hours post-instillation, half of each group received either steroid (dexamethasone 8 mg/kg, i.p.), in an attempt to suppress cellular infiltration and function, or saline vehicle. Post-treatment continued on alternate days. Animals were killed on day 2 or 8 prior to that day's scheduled injection. Cellular (total and differential cell counts) and biochemical (β-glucuronidase [β-glu] and albumin) parameters of inflammation/damage were evaluated in the broncho-alveolar lavage (BAL). At 2 days, silica caused a neutrophilia that was reversed by continued steroid injections up to day 8. BAL albumin and β-glu were significantly elevated in response to silica at both times despite the presence of steroid. These data suggest the intrinsic properties of silica play a primary role in the toxicities following i.t. instillation. However, the transient cellular influx at day 2 may initiate the inflammatory cascade. To eliminate the early influx of cells, a steroid pre-treatment was employed in addition to the post-treatment. Rats received i.p. steroid or saline on days −5, −3, and −1. On day 0, rats from each group received i.t. silica or saline. Two hours after the instillations and on alternate days after, rats received i.p. steroid or saline identical to pre-treatment. On days 2 and 8 the cellular response to silica was suppressed by steroid, but β-glu activity was significantly elevated. Although elevated over control levels, BAL albumin content of the steroid/silica group, at days 2 and 8, was markedly suppressed when compared to values from the steroid post-treatment protocol. The addition of the steroid pre-treatment seems to afford a greater protective effect than does the post-treatment alone, implicating the cells as well as the silica itself contribute to the toxic response. (Supported by NIOSH Grant U60/CCU306149-03).

517 *IN VIVO* PNEUMOTOXICITY OF RESPIRABLE WELDING FUME PARTICLES IN A RAT BIOASSAY MODEL

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Using an *in vivo* rat bioassay model, we compared the potential of three welding fume samples generated during electric arc welding to elicit lung inflammation or injury. Particles were collected from conventional spray (mild steel or stainless steel electrode wire) or pulsed current (mild steel electrode wire) welding. The samples were sized by laser scanning confocal microscopy and were measured to be of respirable size with mean diameters <2 μ. CD/VAF rats were dosed intratracheally with the three welding fume samples, and with a relatively inert dust (iron oxide), a pneumotoxic, inflammatory dust (silica), and a vehicle control (saline) at doses of 0.2, 1.0, and 5.0 mg/100 g b wt. Bronchoalveolar lavage (BAL) was performed 1, 7, and 14 days post-instillation, and indicators of pulmonary damage (neutrophils, albumin, lactate dehydrogenase and β-n-acetyl glucosaminidase activities) were assessed in the BAL fluid. For all three doses, no consistent differences were observed in the pulmonary response of the three welding fume samples 1 day post-exposure. However, by 14 days, animals instilled with stainless steel (1.0 mg/100 g b wt) had significant elevations ($p < 0.05$) in lung damage when compared with the two treatment groups exposed to mild steel welding fume. Over the observation period, the results observed in the stainless steel group closely followed the elevated response ($p < 0.05$) seen with the silica group, and by 14 days, there were no differences in any of the parameters measured among the vehicle control, two mild steel, and iron oxide groups. We have demonstrated that welding fume particles generated from wires of different composition produce different pulmonary responses. However, in comparing particles generated from the mild steel wire using different power supplies, no differences were observed. (Supported by NIOSH grant #U60/CCU109979-01).

518 STRUCTURAL INTEGRITY OF TRACHEAL EPITHELIAL CELLS IN SEPTIC SHOCK AND ARDS CAN BE MAINTAINED BY BLOCKADE OF THE INTERLEUKIN-1 RECEPTOR

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We have previously shown *in vivo* morphological damage to tracheal epithelial cells by endotoxin (ENDT). Comparison of trachea from anesthetized male Sprague-Dawley rats receiving only *Escherichia coli* ENDT (6 mg/kg) was made with those receiving interleukin-1 receptor antagonist (IL-1ra) (0.2 mg/kg/min IV) beginning one hour prior to ENDT. When the mean arterial pressure (MAP) dropped to 40 mmHg (4 to 6 hrs.) in those animals receiving only ENDT, the tissues were taken and processed for electron microscopy (EM). Since MAP did not drop significantly in those animals receiving IL-1 ra + ENDT tissues were taken 6 hrs after ENDT and processed for EM. Extensive damage of tracheal epithelial cells was noted with ENDT alone but no damage was noted in animals receiving IL-1 ra. Subsequent *in vitro* studies of tracheal (3 × 6 mm strips) tissue was incubated *in vitro* in Dulbecco's Modified Eagle Medium with and without IL-1ra (1 mg/ml) over a 5 hr period for the following groups: 1) tracheal tissue alone, 2) tracheal tissue plus ENDT (1–250 μg/ml), 3) tracheal tissue with (neutrophils) PMN (separated by centrifugation and Ficoll layering from blood of the same rat), and 4) tracheal tissue plus PMN plus ENDT. The tissue was then processed for electron microscopy. The *in vitro* assessment showed no damage to epithelial cells in groups with and without IL-1ra in tracheal tissue alone, tracheal tissue plus ENDT, or tracheal tissue incubated with PMN. Tracheal tissue plus PMN plus ENDT showed epithelial cell damage without IL-1ra and not with IL-1ra. Thus the *in vitro* model as well as the *in vivo* model demonstrates IL-1ra affords protection in this rat model of sepsis and ARDS.

519 RETINOL PROTECTS AGAINST CHEMICAL-INDUCED PULMONARY TOXICITY IN MALE SPRAGUE-DAWLEY RATS

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We have previously shown that retinol limits the amount of pulmonary injury caused by 1-nitronaphthalene (1-NN) in male Sprague-Dawley rats. The objective of this study was to determine if retinol could protect the lung from the toxicity of other pneumotoxicants. Rats were administered *all-trans*-retinol (75 mg/kg/d, *po*) for one week. Twenty-four hr after the last dose of retinol, animals were given 1-NN (100 mg/kg, *ip*), 2-nitronaphthalene (2-NN; 100 mg/kg, *ip*), or paraquat (PQ; 25 mg/kg, *ip*). Pulmonary injury was evaluated at 24h (1-NN or 2-NN) or 48h (PQ) using bronchioalveolar lavage (BAL) and morphological techniques. The most overt difference between retinol and vehicle pretreated animals, after receiving 1-NN, 2-NN, or PQ, was the lack of BAL protein, BAL LDH activity, pulmonary edema and inflammation in retinol animals. In the case of 1-NN or 2-NN, retinol pretreated rats possessed only limited bronchiolar cell necrosis, restricted to the nonciliated bronchiolar epithelial cells. Vehicle pretreated rats displayed necrosis and exfoliation of ciliated and nonciliated bronchiolar epithelium. Rats treated with PQ elicited increases in alveolar edema, inflammatory cell content, as well as damage to the alveolar parenchyma. However, animals which received retinol lacked alveolar edema and inflammatory cell infiltration following treatment with PQ. From these experiments, we concluded that retinol pretreatment decreased the severity of 1-NN, 2-NN, and PQ-induced pulmonary toxicity apparently by inhibiting the inflammatory responses associated with the progression of toxic injury. (Supported in part by NIEHS center grant, P30 ES 06694 and DuPont Graduate Fellowship).

520 CYTOTOXICITY OF AGRICULTURAL DUSTS

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Exposure to agricultural dusts generated during farming may cause pulmonary disease. A spectrum of diseases caused by diverse etiologic agents are well recognized in agricultural workers. This study was designed to determine whether the mineral dusts generated during agricultural work play a role in the cytotoxicity which leads to lung disease in workers. To investigate this we studied the cytotoxic potential of two agricultural dusts collected during harvest seasons from northern California grape and citrus farms. Samples were analyzed by plasma emission spectrometry, scanning electron microscopy, and x-ray spectrometry to determine the concentration of inorganic minerals and crystalline silica. Rat alveolar macrophages were exposed to these two well characterized agricultural dusts and the release of lactate dehydrogenase, B-N-acetyl glucosaminidase, superoxide dismutase, glutathoine peroxidase and hydrogen peroxide into the medium was monitored as an index of cytotoxicity. In all the bioassays, a dose dependent greater cytotoxicity was found for grape farm dust compared to citrus farm dust. Antioxidant enzymes (SOD, GPx) in grape dust exposed alveolar macrophages were significantly quenched, indicating a potential of the dust to impair defense mechanisms. Total mineral concentration and crystalline silica content in the grape farm dust was significantly greater (21%) than that in the citrus farm dust (15%). These results correlate with pulmonary function impairments recently reported in the California grape farm workers.

521 DEVELOPMENT OF ROUTINE HEALTH MONITORING METHODS FOR USE WITH AN AQUATIC MODEL (ORY ZIAS LA TIPES) USED IN IMMUNOTOXICOLOGICAL TESTING

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We are currently developing and standardizing a battery of assays for detecting potential immunotoxic compounds in the Japanese medaka fish with the goal of using it as a predictive model of immunotoxicity in mammalian and non-mammalian species. In order to use a new species for toxicological studies, it is critical to establish baseline health endpoints. In mammalian models, procedures for monitoring the health status of test animals have been established and in use for many years; in many aquatic models, including medaka, much of this routine health screening has not been documented. Thus, the purpose of this study was to assess the normal variability in routine health parameters in medaka, as well as to develop parameters specific for demonstrating the suitability of animals for use in immunotoxicological studies. The endpoints were assessed on a quarterly basis and included histopathology (31 organs), identification of endogenous bacterial flora and, gross necropsy including body weight, length, hematocrit, leukocrit, and plasma immunoglobulin levels. Additional parameters assessed in animals for future immunotoxicological testing included anterior kidney (the teleost bone marrow equivalent) weight and cell yields plus superoxide anion production. Results of this study demonstrate that multiple strains of *A eromonas, Pseudomonas* and *A cinetobacter* were the predominant internal flora. Hematocrit, leukocrit and plasma IgM levels were within the normal range for teleost species. The most striking histological finding to date was the presence of granulomas, which correlated with age. Supported by U.S. Army Medical Research and Materiel Command contracts DAMD17-93-C-3006 and DAMD17-17-93-C-3059.

522 IMMUNOLOGICAL BIOMARKERS IN FISH: INFLUENCE OF HOST AGE

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Because of increasing social and political pressure to use non-mammalian (alternative) models for predicting human health risks, as well as to develop markers for assessing the biological effects of environmental stress, studies that utilize less traditional animal species are increasing. In assessing the usefulness of a particular biological response to serve as a biomarker, it is important to understand the factors that may influence the sensitivity/responsivity of the selected endpoint. This study was designed to determine whether macrophage-mediated immune functions being developed as indicators of pollutant immunotoxicity in fish were influenced by host age. Three and 14 month old medaka (*Oryzias latipes*) were exposed to cadmium (Cd), a well-studied immunotoxicant and major aquatic pollutant, for 3 days and the immunotoxic effects on collected kidney cells (bone marrow-equivalent cells) were evaluated. While Cd at 10 ppb accumulated in body tissues, no effects were observed on fish body weight, cell viability, recovered cell number, or leukocrit levels in either aged fish. In the young fish, Cd exposure significantly increased hematocrit, total plasma protein, and immunoglobulin levels; greater splenic Cd burdens in the juvenile fish may explain these results. Conversely, macrophage function (i.e., hydrogen peroxide and superoxide production) was only altered in the older fish. This study demonstrates that macrophage responses of medaka are useful for predicting the immunotoxic effects of Cd on directly-exposed aquatic species, and that fish age at the time of pollutant exposure, like that observed for mammals, influences the immunotoxicity of environmental contaminants. Supported by U.S. Army Medical Research, and Materiel Command. Contract No. DAMD-17-93-C-3059.

523 FLOW CYTOMETRIC ASSAY FOR VIABILITY OF LEUKOCYTES FROM SEVERAL SPECIES USING FLUORESCENT PROBES

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A rapid, sensitive two-color flow cytometric assay to evaluate viability and potential cytotoxicity is described for leukocytes of chicken, cattle, mouse, rat, sheep, trout, and tree swallow. Ethidium homodimer-1 (EthD-1), a red fluorescent (600 nm) DNA stain passes through compromised plasma membranes of degenerating or dead cells. Calcein-AM, a fluorogenic esterase substrate is cleaved in viable cells to calcein, a green fluorescent product (530 nm). Both dyes are nonfluorescent prior to cell exposure. Concentration grids were used on each species and cell type to determine optimal conditions. Dye concentrations varied with species and cell type. Mouse leukocytes required 0.1 uM calcein and 0.25 uM EthD-1, while cattle needed 0.25 uM calcein and 1.0uM EthD-1. Simultaneous viability and cell number were determined on a Coulter EPICS XL-MCL flow cytometer equipped with stop volume. There was good correlation between this flow cytometry (FC) and trypan blue (TB) viability methods. Leukocyte viability (mean percent ± standard deviation) for cattle (97.8 ± 1.6 (FC); 96.5 ± 1.4 (TB), trout (96.0 ± 1.2 (FC); 88.7 ± 5.7 (TB), chicken (91.2 ± 4.39 (FC); 94.2 ± 2.0), rat (97.3 ± 1.0 (FC); 97.6 ± 1.42 (TB), mouse (96.2 ± 0.75 (FC); 97.8 ± 0.75 (TB). Mammalian RBCs and cell debris were easily discriminated out making results reproducible. RBCs in trout and birds are nucleated and overlapped in size with leukocytes, representing a challenge. This method may replace the trypan blue test and the toxic ^{51}Cr release assays. This cytotoxicity assay will be used to assess the immunotoxicity of chemicals introduced to these species.

524 THE USE OF FLOW CYTOMETRY TO MEASURE THE PHAGOCYTIC CAPABILITIES OF LEUKOCYTES IN CATTLE, TROUT AND CHICKEN

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A rapid flow cytometric method to measure phagocytosis by leucocytes in different species is described. Phagocytes isolated included neutrophils (cattle), heterophils (chicken) and macrophages (trout). Phagocytosis was quantitated by number of cells ingesting fluorescent beads and the distribution of beads within phagocytes. Cytometer parameter settings were adjusted to accommodate different cell morphologies and a discriminator, based on size, eliminated free bead fluorescence. Non-phagocyte contamination in chicken and trout cell preparations was reduced by gating on the different cell populations based on their forward scatter and side scatter. The gate on trout macrophages was adjusted due to increasing granularity with phagocytosis over time. Bead size, incubation temperature, rate and phagocytic capability varied between species. The maximum % of phagocytes were 95.57 mean ± 4.43 standard deviation (bovine), 71.35 ± 7.81 (chicken) and 33.70 ± 2.83 (trout). This phagocytic function assay will be used to assess the immunotoxicity of chemicals introduced to these species.

525 DEVELOPMENT OF A MOUSE INTRANASAL MODEL TO ASSESS THE IMMUNOGENICITY OF PROTEINS

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The goal of these studies was to develop a mouse model to determine the relative immunogenic potency of proteins as a measure of allergenicity. Intratracheal (i.t.) administration of various enzymes has been demonstrated to produce a good IgE and IgG1 response. However, a less labor intensive method was needed. In this study, the intranasal (i.n.) route of administration of protein to anesthetized mice was investigated. Studies were conducted to determine the best dosing volume, dosing regimen, endpoint, and mouse strain (inbred, F1 hybrid and outbred). Specific IgG1 levels in serum, as determined by ELISA, were found to be a more sensitive endpoint than specific IgE or lymph node cell numbers. A dose-related IgG1 response (titers > 1:1,000 at high doses) was obtained in several strains of mice when Alcalase (protease; 0.03–1 μg/mouse) was administered i.n. with detergent matrix (30 ug/mouse) in a volume of 10 ul, three times over a two week period (days 1, 3, and 10; serum collected on day 15). Using these conditions, the relative potency of three other enzymes were compared against Alcalase in BDF1 mice. Enzyme B (amylase) was found to more potent than Alcalase, Enzyme C was of equal potency and Enzyme D was less potent. These potency differences are consistent with findings of specific IgG1 levels in guinea pigs administered the same enzymes via the i.t. route. Thus, the mouse i.n. model appears to be a promising approach to assess the allergenic potency of proteins. Further validation of the model will involve comparing Alcalase to other enzymes.

526 DISTRIBUTION OF PROTEIN ANTIGEN FOLLOWING INTRANASAL INSTILLATION IN BDF1 MICE

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Our goal is to develop a mouse model to determine the immunogenicity of proteins. Intranasal (i.n.) administration of proteins to BDF1 mice has been found to produce a robust specific IgG1 response when administered in volumes of 10 and 60 ul. However, we did not know if the IgG1 response was attributed to the distribution of antigen in the upper respiratory tract and/or the lungs. To examine this question, fully anesthetized female BDF1 mice were i.n. dosed with 1 μg Iodine-125 labeled bovine serum albumin (1 μCi) in a volume of either 10 or 60 μl (5 or 30 μl/nostril). In addition, mice were dosed in the upright position (up) or on their backs with the nose angled down (down). Mice were exsanguinated and necropsied after 30 minutes or two hours. Blood, trachea, esophagus, lungs, stomach, liver, kidneys & spleen, mouth, nose, head, intestines and carcass were counted separately. The results, shown as percent of total dose, indicated that with a 10 ul dose (down position), most of the protein stayed in the nasal area (62% after 30 min and 35% after 2 hr) with limited distribution to the lung (0.035% after 30 min and 0.32% after 2 hr). In contrast, a 60 ul dose volume resulted in an equal distribution after 30 min between the nasal area (34%) and the lung (35%). The up position of the mouse (10 ul dose) was found to result in greater distribution to the stomach (11.8% after 30 min and 46.27% after 2 hr) in comparison to mice placed in the down position (0.27% after 30 min and 23.67% after 2 hr). These results indicate that dose volume and position affect distribution of antigen following i.n. dosing and therefore may determine the induction site of the IgG1 response.

527 SYSTEMIC ANAPHYLAXIS MODEL TO OVALBUMIN IN GUINEA-PIGS

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Ovalbumin-induced systemic anaphylaxis is a prototypic even though poorly standardized model of anaphylactic reaction in the guinea-pig. In this study, 255 adult male Dunkin-Hartley guinea-pigs, allocated to groups of 5 animals received one or two sensitizing ip, sc or id doses at day 1, or 1 and 8, or 1 and 15, and one iv challenge dose of standard ovalbumin at day 22. Anaphylactic response was scored from 0 (no symptoms) to 4 (death). The most severe anaphylactic responses, namely 4 deaths and one severe reaction (score 3) were triggered by 2 sensitizing doses of 2 μg/animal, be they sc or id, at day 1 and 8. Milder or no responses were obtained when the animals received only one sensitizing dose, sensitizing injections via ip route, both lower (0.2 μg) and higher (20 μg) sensitizing doses, or two sensitizing doses at day 1 and 15. Interestingly, the anaphylactic responses were found to be dependently related to the challenge dose injected at day 22 with no or mild response following 2 μg/animal, and increasing death rate and severity of clinical reactions from 10 to 100 μg.

These results show that several factors critically affect the systemic anaphylactic response of guinea-pigs to ovalbumin, a finding that should be borne in mind when conducting preclinical anaphylaxis testing of new macromolecules.

528 INTERLABORATORY VALIDATION OF AN ELISA FOR THE MEASUREMENT OF SPECIFIC GUINEA PIG IGG1: AN ALTERNATIVE TO THE PASSIVE CUTANEOUS ANAPHYLAXIS ASSAY

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The guinea pig passive cutaneaous anaphylaxis (PCA) assay is the standard method to measure specific IgG1 from guinea pigs. We have developed and validated an antibody (Ab) capture ELISA to replace the PCA for 2 enzymes, Alcalase (protease) and a fungal derived Enzyme B. The ELISA design is as follows: (1) rabbit anti-enzyme, (2) enzyme, (3) guinea pig anti-enzyme sera, (4) goat anti-guinea pig IgG1, (5) rabbit anti-goat IgG conjugated to alkaline phosphatase, (6) p-nitrophenyl phosphate substrate. Prior to validating the ELISA, the specificity of the goat anti-guinea pig IgG1 preparation was confirmed by evaluating the cross reactivity with IgG2. This was accomplished using purified IgG1 and IgG2 from guinea pig anti-Ovalbumin sera. Validation involved 3 replicate ELISAs by each of 3 laboratories and 3 replicate PCA analyses by 1 laboratory on sera samples of varying titers from guinea pigs immunized with either Alcalase or Enzyme B. Good correlations were observed between the ELISA and PCA assays for Alcalase and Enzyme B ($r^2 \approx 0.792$ and 0.873 respectively). Additionally, ELISA reproducibility between pairs of the 3 laboratories were good for both enzymes: Alcalase r^2 values ranged from 0.814 to 0.962 and Enzyme B r^2 values ranged from 0.773 to 0.802. These data indicate the Ab capture ELISA is reproducible and a valid replacement to the *in vivo* PCA assay. Advantages of the ELISA over the PCA include a decrease in animal usage, significant cost savings, and a faster, reproducible assay.

529 EVALUATION OF THE MYCOBACTERIUM AVIUM-INFECTED MOUSE MODEL FOR DETECTION OF IMMUNOMODULATORY DRUG ACTIVITY

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Mycobacterium avium complex (MAC) is the most common cause of bacteremia in AIDS patients. C57BL6 mice with the *beige* (*bg*) mutation (beige mice) are susceptible to MAC infection, apparently due to decreased NK cell and macrophage function. We have determined that littermates of beige mice which are heterozygous for the *bg* mutation (heterozygous littermates) are both immunocompetent and susceptible to MAC infection. MAC-infected heterozygous littermates demonstrated hepatosplenomegaly and progressively increasing tissue burdens of *Mycobacterium avium* over time. Serum levels of tumor necrosis factor α (TNFα) were substantially increased, an effect that was suppressed by treatment of mice with dexamethasone. Splenocytes obtained from MAC-infected animals demonstrated high levels of spontaneous TNFα secretion, an effect that was suppressed by addition of dexamethasone to cultures. Dexamethasone treatment also reduced hepatosplenomegaly in infected animals. Preliminary results suggest that this model is effective for demonstrating immunomodulatory drug activity.

530 EVALUATION OF VIRAL LOAD IN HCV AND HIV-1 INFECTED CHIMPANZEES BY BRANCHED DNA (BDNA) SIGNAL AMPLIFICATION METHOD

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Development of bDNA technology has allowed for the rapid quantitation of viral nucleic acids from blood and tissue of infected animals. Recent applications of this technology have brought about an efficient and effective method for monitoring the reduction of viral load by antiviral and chemotherapeutic agents. Reduction in viral load is the most effective means available to quantitate efficacy of experimental therapeutics in the chimpanzee model of human HIV, HCV and HBV infections.

We evaluated bDNA technology in HCV (2) and HIV (2) infected and naive (2) chimpanzees. Virally infected animals were evaluated pre and post inoculation for the presence of viral RNA. Due to the intermittent viremic state of HCV infection, it was necessary to evaluate the presence of HCV RNA from liver biopsy extracts by bDNA as well. Preliminary data suggests that quantitation of nucleic acid by bDNA assay pre and post infection is a reliable and quantitative indicator of viral load. This assay holds promise for use in preclinical trails to assess dose response of antiviral therapeutics and replace tedious and time consuming semiquantitative assays (e.g. RT-PCR) currently in use.

531 APPLICATION OF THE HU-PBL-SCID MOUSE MODEL TO ANALYSIS OF IMMUNOTOXICITY

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Immunodeficient C.B.17 *scid/scid* (SCID) mice were reconstituted with human peripheral blood lymphocytes (PBL, 5×10^7 cells/animal) and the resulting hu-PBL-SCID mice were analysed for their immune functions and for the responsiveness to known immunosuppressants. At 3 weeks after reconstitution, the serum concentrations of human IgG were ranged from 740 to 2599 ug/ml and IgM from 7.7 to 22.9 ug/ml measured with ELISA. Spleen weight of hu-PBL-SCID mice was relatively increased compared with control SCID mice. These results showed that human PBL was successfully transplanted into SCID mice. Eventhough freshly isolated human PBL responsed to mitogens (PHA, ConA and PWM), splenocytes isolated from hu-PBL-SCID were quite refractory to mitogenic stimulations. In contrast to normal mice like BALB/c, hu-PBL-SCID and freshly isolated human PBL did not showed any response to T-dependent antigenic stimulation. Cyclophosphamide (CP, 200 mg/kg) and cyclosporin A (CsA, 100 mg/kg) were treated at 4 weeks after reconstituted with human PBL. Chemical treatment resulted in a significant decrease of IgM levels in hu-PBL-SCID mouse, whereas the level of IgG were moderately decreased. These shows that the metabolic activation of CP could be occurred in hu-PBL-SCID mouse. CsA (0.5–50 μg/ml) strongly suppressed the mitogenic responses of freshly isolated human PBL. Eventhough CP and CsA were treated *in vivo* or *in vitro*, it was quite difficult to show any immunosuppression in hu-PBL-SCID. Owing to unresponsiveness of hu-PBL-SCID and human PBL to T-dependent antigen, it was impossible to observe the immunosuppression induced by chemicals in primary antibody response. In conclusion, the immunosuppression induced by toxic chemicals in hu-PBL-SCID model could be observed only in selected parameter like serum Ig concentration.

532 A MULTI-INDICANT PREDICTIVE MODEL OF IMMUNOSUPPRESSION BY DEXAMETHASONE IN B6C3F1 MICE

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Quantitative analysis of immune parameters and host resistance is an important issue in immunotoxicology. Multivariate statistical methods can be applied to immunotoxicology to determine relationships between immune parameters and host resistance. These statistical methods are commonly used in other disciplines such as ecology where interactions and relationships among several variables can be considered. However, a large number of observations are required for such analysis. This has been addressed by developing a method that permits a random composite of observations from several experiments assuming defined conditions. This random data set eliminates any systematic bias within the data for reliable resolution of relationships among variables. Among the immune parameters included in this model were NK cell activity, subpopulation evaluation of spleen, thymus and peritoneal cells, complete blood counts and differentials, peritoneal macrophage function assays, cytotoxic T cell assay, complement levels, antibody production, and delayed-type hypersensitivity. Immune parameters were evaluated at 3, 6, or 16 days where applicable. In addition, 5 host resistance models were used including resistance to B16F10 tumors, *Listeria monocytogenes*, mouse malaria, *Trichinella spiralis* and *Streptococcus agalactiae* (type B strain 090). Dexamethasone was used as prototype immunotoxicant for these studies. Initial analysis has determined that of the immune parameters measured after 16 days of dexamethasone exposure, the following are significantly predictive of resistance to B16F10 tumor (1×10^5 tumor challenge): dose of dexamethasone, spleen weight, thymus weight, NK cell activity, peritoneal macrophage nitrite production after interferon-γ stimulation, and absolute number of peritoneal and spleen $B220^+Ia^-$ cells. Data sets measuring the same immune parameters after 3 and 6 days will also be analyzed. An initial survey of data from other labs suggest that predictive models may tend to be immunotoxicant-specific. However, this model will be evaluated using other immunotoxicants. This technique represents an attempt to elucidate fundamental relationships between immune parameters and host resistance that could be useful in risk assessment.

533 THE LOCAL LYMPH NODE ASSAY: AN INTERNATIONAL TRIAL AND COMPARISON OF MODIFIED PROCEDURES

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The murine local lymph node assay is a predictive test for the identification of skin sensitizing chemicals. In the present investigation the method has been evaluated further in the context of an international study comprising 5 independent laboratories. In addition, the influence of minor modifications to test procedure (including exposure for 4, rather than 3, consecutive days and the use of an alternative isotope) on assay performance has been examined. Irrespective of whether the standard or a modified protocol was used, each laboratory identified accurately, and with comparable sensitivity, potassium dichromate and 2,4-dinitrochlorobenzene as skin sensitizers. On the basis of standard criteria (a stimulation index of 3 or greater indicating sensitizing activity), methyl salicylate, a non-sensmtizer, failed to induce a positive response in any laboratory. In some participating laboratories lymph nodes pooled from individual mice were analyzed and statistical evaluation of responses compared with the use of a standard stimulation index for the identification of skin allergens. There are insufficient data presently to draw firm conclusions regarding the relative value of statistical evaluation. These studies indicate that the local lymph node assay is sufficiently robust to accommodate minor procedural modifications without material changes in test performance.

534 MICE OF H-2^s AND H-2^d HAPLOTYPES DIFFERENTLY RESPOND TO PENICILLIN AS RELATED BY ANTI-PENICILLIN IMMUNOGLOBULIN ISOTYPE PROFILES AND CYTOKINES mRNAs EXPRESSION

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Allergies to penicillin show various types of signs, probably reflecting preferential activation of Th1 or Th2 types of helper T cells. We evaluated how two different mouse strains bearing different haplotypes: SJL (H-2^s), and Balb/c (H-2^d) could develop different responses to subcutaneously-injected benzylpenicillin coupled to tetanus toxoid (BPO-TT). SJL mice, that have no detectable total IgE when naive, produced BPO-specific IgE 10 days following immunization with BPO-TT, showed a high anti-BPO specific IgG1 response that peaked on day 15, and a low IgG2a response. Balb/c mice, that have detectable total IgE when naive, showed no BPO-specific IgE response, an early IgG2a response, and a low IgG1 production. These responses can be identified as Th1 and Th2-like responses in Balb/c and SJL mice respectively. Cytokines mRNAs expression in sensitized lymph node cells was assessed by semi-quantitative RT-PCR. Both strains expressed IL-2, IL-4, and IFN-γ mRNAs, with a higher expression of IFN-γ mRNA in Balb/c mice on day 4. These preliminary data, in agreement with antibody responses described above, will provide help to further characterize key factors involved in differential responses to haptenic compounds. They show the complementarity of specific antibody responses and cytokines mRNAs measurement in hypersensitivity assessment.

535 DOSE-RESPONSE RELATIONSHIPS IN THE MOUSE IGE TEST

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The mouse IgE test is a method used for the prospective identification of chemicals with the ability to cause sensitization of the respiratory tract. Respiratory sensitization potential is measured as a function of induced increases in the serum concentration of IgE following topical exposure to mice to the test chemical. In the present study dose-response relationships have been examined using both known chemical respiratory allergens (toluene diisocyanate, diphenylmethane diisocyanate, hexamethylene diisocyanate and trimellitic anhydride) and contact allergens known or suspected not to cause respiratory hypersensitivity (2,4-dinitrochlorobenzene and oxazolone). In each instance, exposure of mice to chemical respiratory allergens induced a substantial dose-related increase in the serum concentration of IgE measured 14 days following the initiation of treatment. In contrast, treatment of mice with the contact allergens (under conditions of exposure which resulted in positive local lymph node assay responses) caused no change in serum IgE concentration or, in the case of oxazolone, only a comparatively modest increase. These data provide further confirmation that the mouse IgE test is able to discriminate accurately between different classes of chemical allergen and support the use of this method for the predictive assessment of respiratory sensitizing potential.

536 THE MURINE SERUM IGE TEST FOR ASSESSING POTENTIAL RESPIRATORY SENSITIZERS: EVALUATION OF MOUSE STRAIN AND RESPONSE KINETICS

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The murine serum IgE test (MSIT) is currently being evaluated as a means of identifying chemicals with respiratory sensitizing potential. This potential is assessed following the topical application of a chemical by quantifying the total amount of serum IgE antibody using a sandwich ELISA. Two strains of female mice were examined: BALB/c (inbred) and CD-1 (outbred). Mice (n = 7) were untreated or exposed once (50 μl) to either vehicle (VH); 2,4-dinitrochlorobenzene (DNCB) or toluene diisocyanate (TDI); oxazalone (OX) or trimellitic anhydride (TMA) on each shaved flank. TDI and TMA are human respiratory sensitizers. After 7 days, 25 μl of the above solutions were applied to respective animals on the dorsum of both ears. One or two weeks post-challenge, blood was obtained by retro-orbital eye bleed or cardiac puncture, respectively, and serums analyzed for IgE. Stimulation Indexes (SI's) were determined. One week post-challenge, SI's of 0.6 and 2.8 for DNCB and 3.6 and 45 for TDI were observed with BALB/c and CD-1 mice, respectively. At two weeks, SI's of 0.3 and 2.4 for DNCB and 1.6 and 17 for TDI were observed with BALB/c and CD-1 mice. SI's of 5.6 and 1.6 for OX and 7.4 and 11 for TMA were observed one week post-challenge with BALB/c and CD-1 mice, respectively. At two weeks, SI's of 2.1 and 3.1 for OX and 2.5 and 5.8 for TMA were observed with BALB/c and CD-1 mice, respectively. IgE responses to VH were higher in BALB/c mice compared to CD-1 mice. Data for the tested chemicals indicate that the IgE response peaks one week post-challenge. Additional studies with other chemical allergens are needed.

537 IL-4 AND IFN-γ ARE KEY FACTORS IN DRIVING ANTI-PENICILLIN IMMUNE RESPONSES IN MICE

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Different mouse strains are able to develop various types of immune response to penicillin as defined by specific antibody isotype profiles and cytokines mRNAs expression patterns. SJL mice, when immunized subcutaneously into the hindfootpad with benzylpenicilloyl-tetanus toxoid (BPO-TT) develop a response characterized by anti-BPO specific IgE production 10 days following immunization, specific IgG1 and IgG2a production, with a ratio IgG1/IgG2a reaching 68 on day 15 post-immunization. Since IL-4 has been described as playing a dominant role in the differential development of Th0 cells in Th1 and Th2 cells, we evaluated how IL-4 participate into the "Th2-like" response we described in SJL mice. When SJL mice were injected with 10 mg 11B11 (a rat IgG1 anti-mouse IL-4 antibody), IgG1/IgG2a ratio went down to 5.8 as compared to 30.2 in non-treated mice on day 10. This modification of the response was not due to a decrease of IgG1 production, but to an increased BPO-specific IgG2a production. By using RT-PCR, we observed that this effect was related to an increase of IFN-γ mRNA expression following 11B11 treatment. This illustrates the importance of the IL-4/IFN-γ balance in determining the type of response triggered by haptenic compounds.

538 THE IMMUNOTOXIN WORTMANNIN SELECTIVELY BLOCKS EOSINOPHIL GRANULE PROTEIN-INDUCED PLATELET DEGRANULATION

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The activation of extravascular platelets in the lungs may contribute to the pathogenesis of many pulmonary inflammatory diseases including asthma. The presence of degranulated platelets in the broncheoalveolar lavage fluid of allergen-challenged asthmatics and the close association between platelets and eosinophils in bronchial smooth muscle of allergen-challenged animals are consistent with the hypothesis that extravascular platelet activation may play a role in the pathogenesis of asthma. In these studies, we demonstrate that the cationic eosinophil granule proteins eosinophil peroxidase (EPO) and major basic protein (MBP) dose-dependently induce human platelet degranulation. Both agonists also stimulate PI turnover and the tyrosine phosphorylation of several proteins distinct from those induced by thrombin (Tb). The sterol-like fungal toxin wortmannin (WT) has previously been shown to inhibit histamine secretion in the mast cell/basophil model cell line by blockade of the enzyme phosphatidylinositol-3 kinase (PI3K). WT dose-dependently suppressed platelet PI3K activity (IC_{50} = 3 nM). Pre-treatment of platelets with WT dose-dependently blocked (IC_{50} = 3 nM) both EPO- and MBP-, but not Tb-induced degranulation. Similar results were observed on agonist-induced phosphorylation and PI turnover. These data implicate PI3K in the signaling pathway leading to degranulation and suggest that WT may have clinical applications in the treatment of atopic asthma. Supported by NIH AI34577-P3 & GM16633.

539 TRIMELLITIC ANHYDRIDE (TMA)-INDUCED DECREASE IN PLATELETS DOES NOT ALTER LUNG INFLAMMATION

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Intratracheal instillation of TMA conjugated to guinea pig serum albumin (TMA-GPSA) causes an immediate decrease in the circulating platelets in sensitized Hartley guinea pigs supplied by Sasco Inc. Lung inflammation occurs 24 hours post challenge in Hartley guinea pigs from Charles River Lab. Both TMA-induced phenomena are partially dependent on complement system activation. The purpose of this study was to determine whether this immediate TMA-induced decrease in circulating platelets was necessary for the TMA-induced white blood cell (WBC) infiltration into the bronchoalveolar lavage (BAL) at 24 hrs. Actively sensitized guinea pigs from Charles River Lab were challenged by intratracheal instillation with TMA-GPSA. Controls were challenged with GPSA. At 2 min before and at 2, 7, and 21 min after challenge, platelets/ml blood were $5.2 \pm 0.1 \times 10^8$, $3.2 \pm 1.1 \times 10^8$, $3.2 \pm 0.9 \times 10^8$, and $3.0 \pm 0.8 \times 10^8$, respectively. No significant decrease in circulating platelets resulted from TMA-GPSA challenge when compared to control animals. However, TMA-GPSA challenge did result in lung inflammation at 24 hrs as indicated by a significant increase in the number of WBC in the BAL ($11.1 \pm 1.6 \times 10^6$ vs. $24.2 \pm 2.3 \times 10^6$). Although complement system activation has a significant role in TMA-induced decrease in circulating platelets and in lung inflammation, these data suggest that the initial platelet decrease in the blood after TMA-GPSA challenge is not required for the subsequent WBC infiltration into the BAL. (Supported by Minnesota Medical Fdn).

540 ASSESSMENT OF PEAK EXPOSURES AND SENSITIZATION TO ENZYME ALLERGEN IN THE GUINEA PIG INTRATRACHEAL (GPIT) TEST

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The GPIT model was used to better understand the relationships among exposure to low levels of enzyme, peak exposures, sensitization and elicitation of allergic symptoms in the workplace. Guinea pigs were IT exposed once/week to high levels (3.0ug) or low levels (0.05 ug) of Alcalase allergen over a 14 week time course. Another group of animals was exposed to 0.05 ug Alcalase with peak exposures (3.0 ug or 60-fold peak) at selected time points. A fourth group was exposed to the peak exposures alone and a fifth group was exposed to a single high level (12.0 ug) of Alcalase. Those animals exposed to the low dose of Alcalase with the 60-fold peaks made high titers of antibody faster than those animals exposed to either 0.05 ug of Alcalase or just to the peak exposures. These titers were comparable to the titers made by animals exposed weekly to 3.0 ug Alcalase. Only 20% of animals exposed to 12.0 ug of enzyme made antibody. Between 40% to 80% of animals exposed to 0.05 ug Alcalase

with the peaks had allergic symptoms only during the weeks they were exposed to the peaks. In contrast, only 10% of animals exposed to 0.05 ug Alcalase had symptoms which were apparent during the later weeks of the study. Although exposure to peaks or low levels of allergen will sensitize animals, these data suggest that intermittent exposures to high levels of allergen in combination with low level exposure will sensitize animals faster than exposure to peaks alone or to low levels alone. Also, the data suggest the peaks may be more important for elicitation of symptoms.

541 PHENYL ISOCYANATE INDUCED RESPIRATORY HYPERRESPONSIVENESS: A COMPARISON OF ROUTES OF INDUCTION AND AGENTS USED FOR CHALLENGE

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Groups of guinea-pigs of the Hartley strain were sensitized to phenyl (mono)isocyanate (Phi) by (1) repeated intradermal injections, (2) by a single, brief high-level inhalation exposure of 1 × 15 minutes, or (3) by repeated inhalation exposures of 3 × 3 hours. An additional group (4) of guinea-pigs was sensitized both intradermally and by inhalation. Starting with day 21, guinea pigs were challenged with a slightly irritant concentration of the free Phi and, one week later, also to the Phi-protein (GPSA) conjugate. An additional ramped exposure to acetylcholine (ACh) aerosol served as means to detect non-specific airway hyperresponsiveness. Evaluations were based on immediate-onset respiratory responses evoked during or following the various challenge exposures. In intradermally sensitized animals and in those sensitized by repeated 3 × 3 hours inhalation exposures during challenge with Phi as well as with the Phi-conjugate respiratory responses of weak intensity occurred. No response was observed in guinea pigs sensitized by the single high-level exposure regimen. During the ACh bronchoprovocation, however, a marked difference between the protocol 1 (API = 38), 3 (API = 150), and 4 (API = 350) was observed [API: ACh provocation index]. In vehicle controls and group 2 the API was 25. These findings suggest that for evaluation of phenyl isocyanate induced respiratory hypersensitivity, the combination of the intradermal and inhalation routes was particularly efficient for induction. Elicitation of non-specific airway hyperresponsiveness was achieved best by ramped acetylcholine provocations. It appears, that the acetylcholine bronchoprovocation assay is a useful means to identify airway hypersensitivity. An additional advantage of the acetylcholine provocation assay is that the type of change in breathing pattern is highly predictable which is not the case when challenged with the hapten.

542 DETECTION OF A TOLUENE DIISOCYANATE (TDI)-HEMOGLOBIN ADDUCT IN ERYTHROCYTES FROM GUINEA PIGS EXPOSED TO THE CHEMICAL VAPOR

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TDI is a leading cause of occupational asthma. Although extensive investigations have indicated the inflammatory and immunologic potency of this highly reactive industrial chemical, the mechanism of sensitization remains unclear. We have reported development of a sensitive and specific rabbit antiserum to TDI and its use in identifying TDI-protein adducts in biological tissues and fluids obtained from guinea pigs following exposure to the chemical. In the current study we investigated the occurrence of TDI-hemoglobin adducts since such materials would have the potential to function as biomarkers of previous exposure. Animals were sensitized by exposure to 1 ppm TDI for 3 h per day on 5 consecutive days. On the 24th day of the experiment, blood was drawn and collected in heparinized tubes. The red blood cells (rbc) were isolated, washed thoroughly, then lysed by a freeze, thaw procedure. The hemoglobin was isolated by centrifugation at 10,000g for 20 min. Analysis of the product, using SDS-PAGE and reducing conditions, indicated 3 bands. The major band had a migration mass equivalent to 16 kD. Minor bands were located at 32 kD and 48 kD. The presence of a TDI-hemoglobin adduct was investigated using the rabbit anti-TDI antiserum, and the Western blot procedure. Results indicated an adduct migrating with a molecular mass of 32 kD. Hemoglobin isolated from control animals gave no reaction with the rabbit anti-TDI antiserum. The molecular structure of the TDI-hemoglobin adduct is being studied using affinity chromatography with a rabbit antiserum to guinea pig hemoglobin made in this laboratory. Such studies will enable identification of protein sites modified by TDI and permit use of TDI-adducts to gain understanding of the *in vivo* molecular reactions of this volatile, potent chemical sensitizer. Supported by NIH ES05651.

543 HUMORAL IMMUNE RESPONSE AND HEPATOTOXICITY AFTER MULTIPLE-HALOTHANE EXPOSURES IN GUINEA PIGS

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Halothane hepatitis (HH) patients have antibodies specific for the trifluoroacetyl (TFA) protein adduct of halothane. HH almost always occurs after multi-exposures. Guinea pigs generate anti-TFA antibodies after a single halothane exposure, while repeated exposures at short time intervals suppressed the secondary antibody response. Thus, longer intervals between multiple exposures of halothane (or a hepatotoxin) were examined. First the humoral immune responses in guinea pigs was characterized after the halothane exposure (1%, 4 hr) Anti-TFA IgG titers were seen in 1 wk with the peak level at day 21 and a return to baseline level by day 40. Thus a 40 day interval was utilized for subsequent studies. Guinea pigs exposed to halothane (3x) or halothane-CCl_4-halothane were profiled for anti-TFA IgG levels and ALT after each exposure. In the multiple halothane exposed group, after the second exposure the peak IgG titer remained maximal between day 21 until day 28. In addition the 48 hr ALT level was higher following the 2nd exposure. After the 3rd halothane exposure, anti-TFA IgG titer profile were similar to the flint exposure however the increase in ALT was again evident. The anti-TFA IgG and ALT after 3 exposures were the same as an age related set of guinea pigs exposed only once to halothane. The effect of a hepatotoxicant (CCl_4) was examined to determine if the response to multiple halothane exposure resulted from the halothane adducts or any hepatotoxic insult. In the halothane-CCl_4-halothane group, CCl_4 did not produce anti-TFA IgG even with 48 hr ALT levels similar to those after two halothane exposures. Also CCl_4 did not affect anti-TFA titers or ALT levels in a subsequent halothane exposure. These data suggest that long intervals (40 days) may be needed for full expression of humoral immune responses, however, this did not increase hepatotoxicity or circulating antibody titer.

544 TRIFLUOROACETYLATED (TFA) PROTEINS AND KUPFFER CELLS SENSITIZE THE IMMUNE SYSTEM IN HALOTHANE EXPOSED GUINEA PIGS

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Halothane has been implicated as a causative agent for hepatic injuries that range from mild, acute insults, to a rare, fulminant hepatitis. The hepatitis is due to an idiosyncratic autoimmune reaction brought about by the formation of neoantigens that have been generated by covalent binding of a halothane biotransformation intermediate. The guinea pig is being developed as a sensitive animal model to investigate the autoimmune reaction. Female Hartley guinea pigs were exposed to 1.0% halothane, 40% oxygen for 4 hr. Kupffer cells and splenocytes were isolated from animals on days 4–35 post-exposure. Splenocytes were co-cultured in a lymphocyte transformation test (LTT) with various concentrations of TFA-antigens for 7 days and proliferation measured by ^{3}H-thymidine incorporation. In a second experiment, Kupffer cells were co-cultured with autologous or allogeneic splenocytes and concavalin A to determine whole cell sensitization and accessory function by Kupffer cells from exposed animals. The LTT showed a four-fold increase in splenocyte proliferation in response to TFA-guinea pig albumin (GSA). Peak levels of sensitization were observed in splenocytes isolated from guinea pigs 28 days after the animals were exposed to halothane. No significant increase in proliferation could be detected with TFA-lysine or GSA. A two-fold increase in splenocyte proliferation also occurred in response to Kupffer cells from halothane exposed animals and these Kupffer cells had an increased accessory function with respect to splenocyte proliferation in response to mitogen stimulation. Autologous splenocytes demonstrated more of a response from treated vs control animals indicating possible involvement of MHC II antigens. These results indicate sensitization of the immune system by TFA-antigens in halothane exposed guinea pigs. In addition Kupffer cells appear to play an important role in the sensitization of splenocytes. This study provides good evidence that halothane hepatitis is mediated by a cellular immune response.

545 ENHANCED LYMPHOCYTE ADHESION TO KUPFFER CELLS CO-CULTURED WITH TRIFLUOROACETYLATED (TFA) PROTEIN

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Studies have found an increased adhesion of lymphocytes to hepatocytes in autoimmune liver disease. These ligands mediate various cell interactions, including neutrophil extravasation and lymphocyte-mediated cytotoxicity. This

study was done to investigate cellular adhesion to Kupffer cells in response to drug injury, specifically halothane metabolism to TFA-proteins, as an immune-mediated mechanism for halothane hepatitis. Kupffer cells were isolated from guinea pigs and cultured with various TFA-protein adducts. Cultures were also treated with silica beads and guinea pig liver homogenates to determine if activation of Kupffer cells could result in an increase in cellular adhesion. An adhesion assay was performed using unstimulated lymphocytes and lymphocytes stimulated with phorbol myristate acetate. Lymphocytes which had been surfaced-labelled with biotin were added to treated Kupffer cells and lymphocyte adhesion was measured. The number of lymphocytes which became bound to the Kupffer cells was calculated from the amount of biotin which remained associated with the monolayer after extensive washing to remove non-adherent cells. Colorimetric detection, using a streptavidin-peroxidase reagent, showed a noticeable increase in lymphocyte adhesion to Kupffer cells previously treated with TFA-guinea pig albumin. Stimulation of lymphocytes also increased their adhesion. TFA-lysine, TFA-rabbit albumin and guinea pig albumin did not induce lymphocyte adhesion. Liver homogenate from allogeneic animals and silica beads also had no effect on cellular adhesion. These results demonstrate that the halothane TFA-guinea pig albumin antigen enhances the adhesion of lymphocytes to cultured Kupffer cells by possible up-regulation of the adhesion molecule expression on the surface of these cells. This mechanism may play an important role in the pathogenesis of halothane hepatitis.

546 EFFECT OF DIETARY VITAMIN A (VAA) ON UVR INDUCED SUPPRESSION OF CONTACT HYPERSENSITIVITY (CH)

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Decreases in stratospheric ozone have raised concern over the health effects of ultraviolet radiation (UVR) exposure which include sunburn, skin cancer, cataracts and immune suppression. The induction of immune suppression is associated with release of cytokines, a defect in antigen presentation, induction of suppressor cells and suppression of CH, which is assessed via the mouse ear swelling test (MEST). Studies have shown that dietary supplementation with VAA enhanced CH assessed by the local lymph node assay and MEST, possibly because VAA increases the number of Langerhans' (antigen presenting) cells. In contrast, UVR decreases Langerhans' cells. The MEST was used to test the hypothesis that VAA reduces UVR immunosuppressive effects. Mice on the VAA (0.477 g/kg) or normal diet were exposed to 9 KJ/M^2 UVR and then sensitized to 2,4,-dinitrofluorobenzene after 3 days and challenged 4 days later. Post challenge MEST differences were compared (24 and 48 hrs). Differences ($p < 0.01$) in the percent suppression caused by UVR were 41.4% suppression for VAA and 83.2% for the normal diet at 24 hrs and 58.2% and 88.3%, respectively, at 48 hrs post challenge. Results indicate that VAA restores UVR induced CH suppression to a pre-UVR level and may modulate some of the damaging effects of UVR on the immune system (CH). (This abstract does not reflect EPA policy.)

547 UVB CAN INDUCE IMMUNOTOXICITY RESULTING IN DECREASED RESISTANCE TO INFECTIONS

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As a result of depletion of the ozone layer by industrial waste compounds, all living organisms on the earth's surface may be exposed to increased amounts of UVB radiation. In man UVB radiation can cause deleterious effects on the skin and the eyes. During the last years it has become clear that UVB can affect the immune system also. It is demonstrated that not only the genotoxic effects of UVB, but also UVB induced immunosuppression plays a role in the induction of skin cancer. Because UVB can affect the immune system, also the resistance against infectious diseases are likely to be affected. It is demonstrated that UVB can inhibit the immune response against skin-associated infectious diseases. However recently it became clear that UVB can also induce immunosuppression at other loci than the exposed skin. Hence, also the immunological resistance against non-skin associated infectious diseases and tumors can be affected. Recently we demonstrated that low doses of UVB (sub-erythemal) can induce immunosuppression in the rat and man. This suppression was not restricted to the exposed skin. Finally we demonstrated that this UVB induced immunosuppression leads to a significant suppression of the resistance against non-skin associated infections in the rat. It is noteworthy that the resistance against bacterial (Listeria monocytogenes), viral (cytomegalo virus) as well as parasitic (Trichinella spiralis) infections was inhibited and that this inhibition was correlated to a suppression of the cellular immune system. Because these data demonstrate that low doses of UVB can affect the immune system in man and rodents and because animal studies showed that this immunosuppression inhibits the resistance to infections it is worthwhile to analyse the risk for increased UVB levels with respect to infections in man.

548 THE EFFECT OF ULTRAVIOLET RADIATION (UVR) ON SUSCEPTIBILITY OF MICE TO MURINE CYTOMEGALOVIRUS (MCMV) AND INFLUENZA VIRUS

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Depletion of stratospheric ozone caused by industrial waste products has caused concern over health effects which may result from potential increases in exposure to UVR. Suppression of immune responses including delayed type hypersensitivity (DTH) and natural killer (NK) cell activity has been demonstrated in laboratory rodents and humans following UVR exposure; UVR has also been shown to enhance susceptibility to certain bacterial and parasitic infections in rodents. In this study we examined the effects of UVR on two viral infectivity models. Enhanced susceptibility of C3H/HeJ mice to MCMV infection is strongly associated with suppression of virus-augmented NK activity. Enhanced mortality was not observed in C3H/HeJ mice infected with a sublethal dose of MCMV on day 3 or 6 post exposure to 9 kJ/M^2 UVR, nor were spontaneous or virus-augmented NK cell activities suppressed. MCMV in key organs on days 3, 6, 10, and 13 post infection were also unaffected. T cell responses are thought to play an important role in defense against influenza. There was a dose dependent increase in mortality following influenza infection of Balb/C mice 3 days post exposure to 3.3–10 kJ/m^2. These same exposures had no effect on virus titers in the lung or dissemination of virus to other organs. There was no difference in the level of protection when spleen cells from UVR or untreated infected mice were adoptively transferred to naive mice that were subsequently challenged with the virus. Our data indicate that UVR does not affect susceptibility of C3H/HeJ mice to MCMV infection. Enhanced mortality following influenza infection was observed but does not appear to be linked to suppression of virus specific immune defenses. (This abstract does not reflect EPA policy.)

549 DISSIMILAR MODULATION OF CYTOTOXIC T LYMPHOCYTE (CTL) AND ANTIBODY RESPONSES TO P815 TUMOR CELLS FOLLOWING TCDD EXPOSURE

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Previous studies in our laboratory have described the dose-dependent suppression of the peak (day 10) CTL response to allogeneic P815 tumor cells in C57Bl/6 mice treated with a single dose of TCDD one day prior to tumor cell challenge. The present studies directly compared the ability of TCDD to suppress the CTL response and the alloantibody response in the same animals. Cytotoxic antibody titers in serum were measured by complement-dependent lysis of ^{51}Cr-labeled P815 tumor cells in a 45′ assay. CTL activity was measured in a standard 4-hr ^{51}Cr-release assay. Results showed that doses of TCDD that suppressed the CTL response also suppressed antibody titers. However, the degree of suppression of CTL activity was more severe at any given dose of TCDD. In a time course study, mice were treated with 15 μg TCDD/kg on day −1 and then killed on days 4–10 relative to tumor injection. Both CTL and antibody responses were first detectable on day 6, at which time the responses of TCDD-treated mice were already lower than vehicle-treated mice. Both CTL and antibody responses increased in control mice through day 10; both responses were significantly suppressed by TCDD on all days examined. In order to determine if there was a window of susceptibility of the allograft responses to TCDD, mice were treated with 15 μg TCDD/kg on days 1–5 *after* P815 tumor injection and peak responses were measured on day 10. TCDD given on day +1 or +2 suppressed the CTL response to a similar degree as day −1 treatment. Suppression of CTL activity diminished with treatment on days +3, +4 and +5. In contrast, the degree of suppression of the antibody response was essentially the same whether TCDD was given on day −1, +1, +2, +3, +4, or +5. These results suggest dissimilar mechanisms for suppression of humoral and cell-mediated responses to alloantigens by TCDD.

550 IMMUNOSUPPRESSIVE EFFECTS OF TCDD CORRELATE WITH ALTERED B7 EXPRESSION ON ANTIGEN-PRESENTING CELLS (APCs)

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Exposure of C57Bl/6 mice to Ah-receptor binding halogenated aromatic

hydrocarbons (HAH) dose-dependently suppresses cytotoxic T lymphocyte (CTL) activity following the injection of allogeneic P815 tumor cells. We have used flow cytometry to characterize changes in splenic subpopulations during allograft rejection in mice treated with an immunosuppressive dose of TCDD (15 μg/kg) in hope of providing insight into the cellular basis of this immunosuppressive effect. In vehicle-treated mice, CTL activity correlated with the appearance of a population of CTL effector cells (CTLe) defined by the phenotype $CD8^+CD44^{hi}CD45RB^{lo}$. In TCDD-treated mice, CTLe were absent. Activated T helper cells defined by the phenotype $CD4^+CD44^{hi}CD45RB^{hi}$ were also absent in TCDD-treated mice. Because the interaction of T cells with APCs via the CD28/B7 costimulatory pathway is critical for T cell activation, we examined B7 expression on both $B220^+$ and Mac-1^+ cells. In vehicle-treated mice, the total number of $B220^+$ cells increased on days 5–9 following P815 injection, coincident with an induction of expression of both B7-1 and B7-2 molecules. The number of Mac-1^+ cells did not change in vehicle-treated mice in response to alloantigen, however, an increased fraction of the cells transiently expressed B7-1 and B7-2 on days 5–7. In TCDD-treated mice, the number of $B220^+$ cells did not increase, resulting in significantly fewer $B220^+$ cells as well as a smaller proportion that expressed B7-1 or B7-2 on days 5–9. Mac-1^+ cells from TCDD-treated mice had decreased B7-2 but not B7-1 expression on days 5–7. Beginning on day 7, a large increase in Mac-1^+ cells was seen in the spleen of TCDD-treated mice. B7-2 expression decreased over the same time period suggesting that these Mac-1^+ cells represent inflammatory macrophages rather than APC. Their role in TCDD immunotoxicity is unknown. Supported by NIH Grant ES00040.

551 EFFECTS OF PERINATAL 2,3,7,8-TCDD EXPOSURE ON THE DEVELOPING IMMUNE SYSTEM OF THE F344 RAT

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Since our previous studies showed that prenatal TCDD exposure produced alterations in fetal thymocyte subpopulations, this study was designed to determine the persistence and functional significance of these effects. Pregnant F344 rats were dosed with 0, 1, or 3 μg TCDD/kg by gavage on GD15. One day after birth litters were cross-fostered to produce control, placental-only, lactational-only, and placental/lactational exposure groups. The organ weights and thymic and splenic phenotypes of these pups were assayed 1 day or 1, 2, or 3 weeks post-birth. An increased liver/body weight ratio, a decreased percentage of thymic CD3+/CD4−CD8− cells, and an increased percentage of thymic CD3+/CD4−CD8+ cells were seen through 3 weeks of age in both genders. The severity of the effects was related to the time of exposure (i.e. placental/lactational > lactational > placental). Immune function was assessed in separate placental/lactational exposure groups at 14–17 weeks of age. In both male and female offspring the delayed type hypersensitivity response (DTHR) to bovine serum albumin was suppressed while the antibody response to sheep red blood cells was not affected. Examination of the organ weights and splenic phenotypes showed an increased spleen/body weight ratio, a decreased thymus/body weight ratio, and a decreased percentage of splenic CD3+/CD4−CD8− cells in both genders. At 8 months of age the DTHR was suppressed only in the males. These results suggest that the immunotoxic effects of perinatal TCDD exposure of rats persist into adulthood. (Supported by EPA Training Grant a/c 5-35448. This abstract does not reflect EPA policy.)

552 HOST RESISTANCE TO *TRICHINELLA SPIRALIS* INFECTION FOLLOWING 2,3,7,8-TETRACHLORO- DIBENZO-p-DIOXIN (TCDD) EXPOSURE: SUSCEPTIBILITY OF MALE RATS

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We have determined that resistance to *T. spiralis* (Ts) infection is not altered in female F344 rats exposed to TCDD. However, resistance to infection is greater in female than in male rats, as measured by rate of adult parasite expulsion, female parasite fecundity and numbers of larvae that survive to encyst in host tissue. This study was done to determine whether lower constitutive resistance to Ts infection rendered male rats more susceptible to alteration of host resistance following TCDD exposure. Male rats were given a single intraperitoneal injection to 0, 1, 10 or 30 μg TCDD/kg body weight 7 d before infection with 1000 Ts larvae. Intestinal parasite counts revealed a delay in onset of expulsion and an altered distribution of parasites in the high dose group. Parasite fecundity was increased at the high dose, as were numbers of larvae/g of muscle. However, as observed in female rats, proliferative responses to parasite antigens or mitogen were increased in splenocytes 7 and 14 d after infection and only slight alterations were observed in the response of mesenteric lymph node cells (MLNC) to stimulation. These results suggest that the lower constitutive level of resistance to Ts infection in male rats is associated with decreased host resistance following TCDD exposure. (This abstract does not reflect EPA policy.)

553 TOPICAL CHLORDANE EXPOSURE REDUCES THE CONTACT HYPERSENSITIVITY RESPONSE IN BALB/c MICE

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Chlordane is an organochlorine pesticide with immunotoxic properties. Previous studies have shown that prenatal exposure to chlordane significantly decreases the ear swelling response in BALB/c mice to the contact allergen oxazolone as well as altering macrophage function in the efferent arm of the contact hypersensitivity response. Additionally, granulocyte-macrophage colony forming units were significantly decreased. In the current study, chlordane was applied topically and the effects on oxazolone-induced contact hypersensitivity were determined. Initially, the reduction in oxazolone-induced ear swelling in BALB/c female mice prenatally exposed to chlordane was compared to topically-treated mice sensitized and challenged with oxazolone. Prenatal chlordane treatment induced a 36% reduction in ear swelling while topical exposure reduced ear swelling by 60% when the treatment occurred at challenge. Topically-applied chlordane also reduced the oxazolone-induced ear swelling by 40% when applied at the time of sensitization. When applied at both sensitization and challenge with oxazolone, ear swelling was reduced by 71%. In a time-course study, it was determined that chlordane must be applied at the time of sensitization, challenge or both or within one hour postchallenge to significantly reduce the contact hypersensitivity response, suggesting alterations in the afferent arm of the response. A dose response study showed that the lowest concentration of chlordane resulting in a significantly reduced ear swelling response was 20 μg. This research was supported in part by LEQSF Grant #(RF/1993-95)-RD-A-44.

554 IMPLICATION OF IP3 AND TYROSINE KINASES IN DMBA-INDUCED CALCIUM RELEASE IN T-LYMPHOCYTES

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The prototypic polycyclic aromatic hydrocarbon 7,12-dimethylbenz(a)anthracene (DMBA), a potent carcinogen, was evaluated for its ability to interact with murine T lymphocytes calcium homeostasis. To evaluate intracellular calcium (Ca^{2+}IC) level, we used murine T lymphocytes loaded with fluo-3, a fluorescent indicator of free calcium, and cytofluorometry or confocal microscopy. DMBA (40 μM) induced a rise in Ca^{2+}IC with a peak occuring 2 mn after its addition. Using calcium-free medium, we demonstrated that this rise was due to the release of calcium from intracellular stores. However, DMBA did not entirely deplete internal stores, since subsequent addition of Thapsigargin (TG), an inhibitor of endomembrane calcium-ATPase still increased Ca^{2+}IC. DMBA induced inositol-1,4,5-triphosphate (IP3) production with a peak occuring 1.5 mn after its addition which then declined quickly. This kinetic pattern was correlated to the peak of intracellular calcium. Finally, these two events were tyrosine kinase dependent, since tyrosine kinase inhibitors block Ca^{2+} rise and IP3 production.

555 INHIBITORY EFFECT OF HYDROQUINONE ON IL-2 DEPENDENT T CELL PROLIFERATION

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Benzene, an environmental pollutant found in car exhaust and cigarette smoke, is known to induce immune suppression. Benzene is not immunosuppressive until metabolized to hydroquinone (HQ) or benzoquinone (BQ). Although BQ is the more potent inhibitor of T cell activation in vitro, HQ has been shown to suppress PHA stimulated rat spleen cell blastogenesis without loss of viability. In order to assess the immunosuppressive effect of HQ, we compared its effects on IL-2 production and IL-2-mediated proliferation with human T lymphoblasts (HTLs). HTLs were derived from PBMC stimulated with PHA and cultured for 15–18 days; they produce IL-2 in response to A23187 (1 μM) and PMA (3 nM) and proliferate in response to recombinant IL-2 (20 units/ml). They do not spontaneously produce IL-2 nor do they proliferate in the absence of IL-2. IL-2 production was determined by stimulating the cells with A23187 + PMA for 24 hrs and assaying the supernatants on

the HT-2 cell line. IL-2 dependent proliferation was determined by culturing HTLs in the presence of 20 units/ml IL-2 for 24 hrs and then pulsing the cells with ^{3}H-TdR for 6 hrs. HQ did not inhibit IL-2 production by HTLs, nor did it affect viability at concentrations up to 20 μM. In contrast, HQ suppressed the response of HTLs to IL-2 by 65% ± 9%.

556 EXPRESSION OF THE PERIPHERAL CANNABINOID RECEPTOR, CB2, IN B6C3F1 MOUSE SPLENOCYTES

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The major psychoactive component of marijuana, Δ9-THC, and a number of structurally-related analogs have been shown to be immunosuppressive. Recently two distinct cannabinoid receptors have been cloned both of which are negatively coupled to adenylate cyclase. CB1, the first characterized, is primarily located in neuronal tissue while CB2 is found predominantly in spleen. The objective of the present studies was to investigate whether B6C3F1 mouse splenocytes express functional CB2 receptors. Poly-A purified mRNA demonstrates CB1 expression mainly in brain with only slight hybridization in the spleen and thymus. CB2 expression, however, predominates in mouse spleen and thymus, but is not present in the brain. This CB2 transcript was determined to be 4.4 kb in length. Immune function studies using cannabinol (CBN), a non-CNS active CB2 selective ligand, revealed that the expressed CB2 is immunomodulatory as demonstrated by a ≥50% inhibition of proliferative responses to anti-CD3, LPS, and PMA/Io mitogens in the presence of CBN at 12, 22, 32 μM concentrations. The IgM SRBC antibody forming cell response also exhibited modest inhibition by 22 μM CBN. Consistent with signalling through Gi coupled receptors, CBN produced a dose related inhibition in forskolin-stimulated intracellular cAMP accumulation. Additionally, competitive binding studies using [^{3}H]-CP-55,940 revealed an IC 50 of 4.5 nM for CBN in mouse splenocytes. Taken together, these results suggest functional expression of CB2 receptors in mouse splenocytes which are involved in immune modulation by cannabinoid compounds. (This work was supported in part by NIH RO1DA07908).

557 EXPRESSION OF FUNCTIONAL GLUCAGON RECEPTORS ON MOUSE SPLENOCYTES AND THEIR ABILITY TO REVERSE IMMUNE INHIBITION BY Δ^9-THC OR 2′,3′-DIDEOXYADENOSINE

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The objective of the present studies was to determine whether the existence of functional glucagon receptors could be established on mouse lymphoid cells. The glucagon receptor, which positively regulates adenylate cyclase, is a member of the superfamily of seven transmembrane domain G-protein coupled receptors. Previously reported specific binding with [^{125}I]-glucagon to a variety of lymphoid and myeloid cell preparations suggests that glucagon receptors are expressed within the immune system. In the present study, northern analysis of polyA RNA isolated from primary mouse and rat derived lymphoid tissues and EL-4 and Jurkat cell-lines was probed with a ^{32}P-labelled human hepatic glucagon receptor. Mouse spleen and thymus and rat spleen all possessed a single 1.5 kb fragment which hybridized to the glucagon receptor cDNA probe, as compared to mouse liver which exhibited a 2.8 kb fragment. EL-4 and Jurkat cells possessed a 3.7 kb fragment and with Jurkat cells an additional 2.75 kb band was present. Treatment of mouse splenocytes with glucagon (50 nM) produced a marked enhancement in intracellular cAMP which was maximal at 5 min post treatment followed by a gradual decline. Direct addition of glucagon to spleen cell cultures over a broad concentration range produced no effect on either lymphoproliferation following stimulation with anti-CD3 mAb, PMA/ionomycin, or LPS nor on the antibody forming cell (AFC) response to SRBC. Conversely, glucagon effectively reversed the suppression of the SRBC AFC response produced by Δ^9-tetrahydocannabinol, both of which are potent inhibitors of adenylate cyclase. These study confirm the expression of functional glucagon receptors on lymphoid cells. (These studies were supported in part by NIH grants DA07908 and DA08351.)

558 COMPARISON OF THE EFFECTS OF DIAZINON AND β-IONONE ON COCAINE IMMUNOTIXICITY

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Cocaine has been shown to modulate the immune system in both humans and mouse models. Part of this modulation has been proposed to be through the cytochrome P-450 system. To test this hypothesis, B6C3F1 female mice were dosed daily for seven consecutive days with cocaine (30 mg/kg i.p.) 30 min after administration of diazinon (20 mg/kg), an esterase inhibitor, or β-Ionone (600 mg/kg), a cyt. P-450 inducer used to study cocaine-induced hepatotoxicity. Mice were sensitized with sheep red blood cells (sRBC) and upon sacrifice the spleens were removed and analyzed for the primary T-dependent antibody-forming cell (AFC) response. Results showed that diazonin, β-Ionone, or cocaine alone did not suppress the antibody response. Cocaine administration in diazinonpretreated mice resulted in an 80% reduction in the AFC response, while cocaine treatment in β-Ionone pretreated mice showed an AFC suppression of 50%. SGPT levels in sera (a marker for liver damage), were dramatically elevated in mice pretreated with diazinon and cocaine. β-Ionone pretreated mice receiving cocaine also showed increased SGPT levels, though not as high as mice pretreated with diazinon. Serum was also analyzed for TGF-β, a suppressive serum factor released upon hepatic damage. TGF-β elevations occurred in serum of mice treated with cocaine when pretreated with either diazinon or β-Ionone, although higher increases were seen in diazinon-pretreated animals. Serum was added *in vitro* to naive female B6C3F1 splenocytes, and analyzed for the primary AFC response to sRBCs. Serum from mice treated with cocaine and pretreated with diazinon or β-Ionone suppressed the *in vitro* AFC response, and antibodies to TGF-β reversed the suppression. Taken together, these results suggest that cocaine-induced immunosuppression is partially mediated through the cytochrome P-450 system, and that TGF-β released upon cocaine administration is a factor in this immunotoxicity. (Supported by NIH Grant DA 08161 and by NIH training grants ES 07087).

559 COCAINE INDUCED IMMUNOENHANCEMENT: POTENTIAL INVOLVEMENT OF NEURO-ENDOCRINE FACTORS

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Previous studies in our lab have shown an enhancement in the antibody response to SRBC following acute cocaine exposure. This enhancement has been shown to occur when cocaine (30 mg/kg × 2) is administered at 24 or 48 hours prior to sensitization. The enhancement is also restricted to mice older than 16 weeks and is not observed in mice at 8–10 weeks of age. Acute cocaine has been show to elicit a rise in plasma corticosterone levels via stimulation of the Hypothalamic-Pituitary-Adrenal axis. Administration of exogenous corticosterone under similar conditions as cocaine also causes a similar immunoenhancement. The T-independent antibody response was not enhanced following exposure to either cocaine or corticosterone, thus suggesting the T cell as a target. Current studies include using various antagonists to assess the relative contribution of endocrine vs. adrenergic stimulation in the enhancement. Future studies will involve measuring changes in the balance of Th1/Th2 cytokine production and investigating selective targeting of these T-cell subsets. (Supported by NIH Grant DA-08161 and NIH Training Grant ES-07087).

560 INHIBITION OF ETHANOL-INDUCE SUPPRESSION OF MOUSE NATURAL KILLER CELL ACTIVITY BY A SELECTIVE BENZODIAZEPINE INVERSE AGONIST

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Our previous studies demonstrated that ethanol (EtOH) can suppress natural killer (NK) cell activity *in vivo*. The mechanisms by which EtOH affects NK cell activity are unclear. This suppression could be due to direct or indirect action of EtOH on NK cells. B6C3F1 female mice were given vehicle (water) or a single dose of EtOH (32% in water) at 6.0 g/kg by gavage. NK cell activity was determined 12 hrs after EtOH administration using a 4-hr chromium release assay with YAC-1 target cells. *In vitro* experiments were conducted in which the concentration and duration of exposure to interlukin-2, EtOH, and the major metabolites of EtOH (acetaldehyde and acetate) were designed to match *in vivo* conditions. These experiments demonstrated that the direct effect of EtOH and its metabolites account for 20% or less of the suppression of NK cell activity noted after administration of EtOH *in vivo*. The role of indirect neuroendocrine mediators was investigated with the following an-

tagonists *in vivo*: naltrexone (10 mg pellet/mouse), phentolamine (0.1, 0.5, 1 mg/kg), propranolol (2 mg/kg), and RU 486 (100 mg/kg). None of these agents blocked EtOH-induced suppression of NK cell activity. In contrast, the benzodiazepine inverse agonist, Ro15-4513 (25 mg/kg), was given by intraperitoneal (i.p.) injection and significantly blocked the suppression of NK cell activity in EtOH-treated mice. Ro15-4513 did not affect blood EtOH levels or the rate of clearance. However, another benzodiazepine inverse agonist, FG 7142 (up to 80 mg/kg, i.p.), failed to block EtOH-induced suppression of NK cell activity. This suggests there is a novel interaction of Ro15-4513 with the benzodiazepine receptor complex. These results indicate that the major mechanism by which EtOH decreases splenic NK cell activity in this model is through an indirect effect mediated through benzodiazepine receptors. A minor role for direct action of EtOH on NK cell activity was also indicated.

561 A ROLE FOR NEUROPEPTIDES IN THE EARLY SYMPTOMS OF CHEMICAL CONTACT IRRITATION

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Experiments were designed to address the role played by neuropeptides in the early symptoms of chemically-induced skin irritation. Our hypothesis was that specific pharmacological antagonists of Substance P (SP) and Neurokinin A (NKA), capsaicin-induced sensory denervation, and pentobarbital pretreatment, would delay or abolish the early inflammatory symptoms (i.e., erythema, post-venule extravasation, tissue swelling) associated with chemical contact irritation. Acute injections of the non-peptide SP antagonist CP-96,345 (10 mg/kg) or the NKA antagonist SR 48968 (1 mg/kg) were administered to female BALB/c mice (n = 8/group) before the ear was "painted" with a highly irritating dose (0.5%) of DNFB. Ear swelling, a conventional response to this chemical irritant, was measured with an Otitest 1000 hand-held caliper at 0, 0.5, 1, 4 and 24 hr post-exposure to DNFB. Each antagonist reduced DNFB-ear swelling at early time points (<4 hr post-exposure) by 35–55% but when used in combination, depressed ear swelling >90% for over 24 hr.

Subsequent experiments examined the effect of capsaicin-induced sensory fiber denervation anesthesia on the ear swelling associated with DNFB irritation. Incremental doses (0.25, 0.5, 1.0%) of capsaicin were administered over 2–3 d to groups of BALB/c mice (n = 6–12/group) using various exposure routes (e.g., topical, IP, and SC). Ear thickness measurements taken at 4 and 24 hr post-exposure to 0.5% DNFB indicated that IP and SC routes of exposure depressed DNFB-ear swelling by 16%, 53% (IP) and 47%, 59% (SC) at 4 and 24 hr post-exposure, respectively. Topical administration (ear "painting") depressed ear swelling by 32% at 4 hr, but caused a 30% increase in swelling over control values at the 24 hr time point.

A final experiment attempted to block neurotransmitter release with pentobarbital. Mice (n = 12/group) were anesthetized with Nembutal (1 ml/kg) for 0.5 hr while the ears were "painted" with 0.5% DNFB. Ear measurements, taken at 4 and 24 hr post-exposure showed depression of ear swelling in anesthetized mice by 63% and 72% compared to DNFB-treated, unanesthetized animals. Taken in concert, these data suggest an early and decisive role for neuropeptides in chemical-contact irritation.

This abstract does not necessarily reflect EPA policy.

562 MULTIORGAN INFLAMMATION AND HEMATOPOIETIC ABNORMALITIES IN MICE WITH A TARGETED DISRUPTION OF THE Rel/NF-κB FAMILY MEMBER RelB

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RelB, a member of the Rel/NF-κB family of transcription factors, has been implicated in the constitutive expression of κB-regulated genes in lymphoid tissues. We have generated mice carrying a germline mutation of the *rel*B gene that results in the absence of RelB protein and in a dramatic reduction of constitutive κB-binding activity in the thymus and spleen. Mice homozygous for the disrupted *rel*B locus, *rel*B$^{-/-}$, lacked phenotypic abnormalities at birth, but had marked differences from their wild-type and heterozygous littermates as early as 10 days of age. Morphological changes included a severe, mixed inflammatory cell infiltrate, predominantly involving the liver, lung, and lymph nodes, myeloid hyperplasia of the bone marrow, splenomegaly resulting from extramedullary hematopoiesis, and a reduced population of antigen-presenting dendritic cells in the thymus. The inflammatory infiltrate in *rel*B$^{-/-}$ mice increased in severity over time, resulting in organ failure and death in some animals. The results of this study indicate that RelB plays an important role in the regulation and balance of the hematopoietic system, and the absence of RelB cannot be functionally compensated by any other member of the Rel/NF-κB family. The histopathological changes observed in this transgenic mouse line were similar to that observed in several human immune and inflammatory disorders, which suggests that *rel*B$^{-/-}$ mice may be a useful model for these disorders.

563 EFFECT OF TRIBUTYLTIN OXIDE (TBTO) EXPOSURE *IN VIVO* ON THE EXPRESSION OF CYTOKINE mRNAs IN CULTURED RAT SPLENIC LYMPHOCYTES

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Analysis of cytokine mRNA expression within defined immune cell populations has been proposed as a sensitive, specific technique for predicting the immunomodulatory potential of drugs and chemicals. We have studied the effects of the organotin compound TBTO *ex vitro*. Exposure to TBTO is known to result in immune suppression in the rat at doses that do not cause general toxicity. Wistar rats were exposed to TBTO (0, 5, 20 or 80 mg/kg diet) for 6 weeks. Purified splenic lymphocytes were cultured in the presence of Con A (1 μg/ml) for 20h. The cells were lysed and total RNA was isolated. Total RNA samples were dot-blotted and hybridised with cDNA probes for the cytokines IL-2, IL-2 receptor (IL-2R), IL-4 and IFNγ, and for the house-keeping gene actin.

For IL-2 there was a 32% increase at 20 mg/kg diet and a 57% increase at 80 mg/kg diet. For IL-2R there was a 33% decrease at 5 mg/kg diet, a 50% decrease at 20 mg/kg diet and a 52% decrease at 80 mg/kg diet. For IL-4 there were no significant treatment induced changes. For IFNγ there was a 16% increase at 5 mg/kg diet, a 32% increase at 20 mg/kg diet and a 50% increase at 80 mg/kg diet. There were no differences in the relative levels of actin. These results suggest that immune suppression following exposure to TBTO may be mediated via decreased expression of IL-2R mRNA. In terms of predictive testing, our results suggest that analysis of the relative levels of mRNAs for key immunoregulatory cytokines may be indicative of immunotoxic potential. Current research is focused on correlating these results with cytokine mRNA profiles in lower numbers of lymphocytes from peripheral blood of exposed animals using more sensitive RT-PCR technique.

563a CYTOKINES AND GROWTH FACTORS IN ARSENIC CARCINOGENICITY

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Although inorganic arsenicals are established human carcinogens, there are currently no well-defined mechanisms known for arsenic-induced neoplasia or reliable animal models for its study. Increased gene expression and secretion of keratinocyte growth factors, including GM-CSF and TGF-α, as well as *c-myc* expression, occurred in primary human epidermal keratinocytes cultured in the presence of sodium arsenite. The influence of arsenic on mouse skin tumor development was studied in transgenic TG.AC mice which carry a v-Ha-*ras* oncogene. Following low-dose TPA application, a marked increase in the number of skin papillomas occurred in transgenic mice receiving arsenic in the drinking water as compared to controls, while no papillomas developed in arsenic-treated transgenic mice that had not received TPA or wild-type FVB/N mice.

Furthermore, increases in GM-CSF mRNA transcripts and TGF-α immunostaining were found in the epidermis of arsenic-treated mice compared to controls within 6 and 14 weeks, respectively, following initiation of exposure. These results suggest that arsenic stimulates the production of selected keratinocyte growth factors, which in turn, act to enhance papilloma formation.

564 USE OF MICRO-PROTON INDUCED X-RAY EMISSION (μPIXE) FOR HEAVY METAL ANALYSIS

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Heavy metals are common contaminants found at waste disposal sites. Past

work in our laboratory has successfully utilized a multi-element analysis technique known as proton induced x-ray emission (PIXE) in the conduct of a number of heavy metal (Hg, Cd, Cr, As) toxicity studies in precision-cut rabbit renal cortical slices (0.6 cm diameter; 275 μm thick). Renal slices were exposed to metal salts *in vitro*, sandwiched in mylar film and placed in the path of a 2 MeV proton beam. The resulting x-rays emitted by the sample were recorded and the raw data processed by a special computer program (GUPIX). Beam diameters were on the order of ≈4000 μm and allowed an overall concentration of the metal(s) of interest in the tissue to be determined. We now have access to a newly constructed derivative of this analytical method (μPIXE) that produces proton beam diameters of <10 μm and includes the ability to raster this beam over a selected area (120 × 170 μm). Positive control slices of iron oxide-perfused kidney (compound selectively collects in the glomeruli) were analyzed using μPIXE to confirm resolution. Capillaries filled with Fe were clearly visible and well defined. Renal slices that had accumulated 250 ppm As or 500 ppm Hg were also subjected to the micro-beam to localize concentrations of these metals. Arsenic did not appear to generally localize at this concentration but Hg did show signs of differing concentrations in separate scanned areas of a slice. The scanning ability of μPIXE allows for the precise location of artifacts and contaminants that would otherwise be overlooked in PIXE. The improved resolution of μPIXE promises to be a useful analytical technique in the study of heavy metal toxicity in the kidney. (NIH P42 ES 04940).

565 VALIDATION OF THE USE OF 6,6′-DITHIODINICOTINIC ACID (DTNA) AS A CHROMOGEN IN THE ELLMAN METHOD FOR CHOLINESTERASE DETERMINATIONS

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The Ellman method for cholinesterase (ChE) determination is a spectrophotometric method which entails the use of 5,5′-dithiobis-(2-nitrobenzoic) acid (DTNB) as a chromogen and records the level of ChE activity as change in absorbance at 412 nm. Although this procedure commonly poses no problem, an exception arises when analyzing tissues rich in hemoglobin (Hb), because Hb also optimally absorbs light at 400–430 nm. Use of 6,6′-dithiodinicotinic acid (DTNA) might be a solution because, like DTNB, it also is a chromogen for sulfhydryl groups, but with an optimal absorption wavelength of 340 nm (i.e., removed from the Hb absorbance maximum). Our validation studies indicate that (1) DTNA can be easily substituted for DTNB as the optimal concentration is identical and because DTNA yields similar activity and % inhibition when applied to control and to treated tissues. Even though (2) DTNA is a slightly less efficient indicator of sulfhydryl group concentration, (3) the DTNA assay is markedly more sensitive for Hb-rich tissues because more tissue can be used in the assay, allowing higher ChE activity to be recorded. Since the advantages of the DTNA method far outweigh the disadvantages, it should be regarded as a sensitive and convenient procedure for determining ChE activity in Hb-rich tissues.

566 RAT CORTICOSTERONE LEVELS WITH REPEATED ACTH CHALLENGE

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Several agents are known to affect the hypothalamic pituitary adrenal axis (HPA). One method of assessing these influences is to compare corticosterone (C) release in response to a known stimulatory challenge during concomitant exposure to test compound. Possible outcomes include alterations of peak C levels or response time course. We measured C via RIA in rats dosed with adrenocorticotropic hormone (ACTH) to determine the optimal challenge dose, time course and the reproducibility of the response over several trials. Male and female rats were bled retro-orbitally under CO_2 anesthesia at baseline and once again at 1, 2, 4, or 8 hours post ACTH challenge. Challenges were repeated three times (day 1, 22, and 36). During trial 1 groups were dosed with vehicle 0.2, 2.0 and 20 IU/kg of ACTH. During trial 2 and 3, groups were dosed with vehicle 20, 50 and 100 IU/kg of ACTH. The same groups of animals received vehicle and 20 IU/kg challenge during all three repetitions. Pre-challenge C levels were not significantly different between groups. Female rats demonstrated higher variability at all measured time points (Male pre-challenge 62 ± 114; Female pre-challenge 277 ± 212 μg/dL). Rats dosed with 20, 50 and 100 IU/kg of ACTH showed nearly the same peak C levels at 1 hour. Rats dosed with 50 and 100 IU/kg maintained elevated C levels for eight hours while C levels in those given 20 IU/kg returned to baseline by eight hours. Responses to the 20 IU/kg doses were consistent over the three repetitions. We conclude that rats challenged with 20 IU/kg of ACTH demonstrate a consistent, reproducible pattern of time- and dose-dependent changes in C under conditions similar to those found in a standard toxicology study. ACTH challenge may be suitable for assessing changes in the HPA resulting from test compound exposure.

567 TIME COURSE OF CORTISOL (C) LEVELS WITH ADRENOCORTICOTROPIC HORMONE (ACTH) AND CORTICOTROPIC-RELEASING HORMONE (CRH) CHALLENGES IN DOGS

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Several agents are known to affect the hypothalamic pituitary adrenal axis (HPA). One method of assessing these influences is to compare C response to a known stimulatory challenge during concomitant exposure to test compound. Possible outcomes include blunting or enhancement of peak C response or alteration of response time course. C levels were measured (0. 0.5, 1, 2, 4, 6 hrs post-challenge) by RIA in Beagle dogs (7/dose) challenged with ACTH (0.2, 2, 20 IU/kg, i.m.) or CRH (1, 4, 16 ug/kg, i.v.) to determine the optimal dose and time course of response. All challenge doses were well tolerated. C levels were consistent in vehicle treated dogs (2.3 ± 1.3 ug/dl, mean ± S.D.). ACTH challenge resulted in peak C levels (≈4–7 × baseline) of 9.2 ± 1.3, 14.4 ± 3.8 and 16.1 ± 2.7 ug/dl at 0.2, 2 and 20 IU/kg, respectively, within 0.5 hr (0.2 IU/kg) to 2 hrs (2 and 20 IU/kg) post-challenge. C levels returned to baseline by 4–6 hrs at 0.2 and 2 IU/kg, but remained elevated at the final sampling time (6 hrs) at 20 IU/kg. CRH challenge resulted in peak C levels (≈2–5 × baseline) of 3.6 ± 1.8, 11.1 ± 2.1, and 11.5 ± 1.6 ug/dl at 1, 4 and 16 ug/kg, respectively, within 0.5 hr (1 ug/kg) to 1 hr (4 and 16 ug/kg). C levels returned to baseline by 2 hrs (1 ug/kg) to 4 hrs (4 and 16 ug/kg) post-challenge. These results demonstrate that ACTH and CRH challenges result in a consistent pattern of dose- and time-dependent changes in C levels under conditions similar to those in a standard toxicology study. Challenges with ACTH or CRH may be suitable to assess changes in the HPA resulting from test compound exposure.

568 CONTINUOUS INTRAVENOUS INFUSION METHODS IN RATS AND DOGS

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Short-term continuous intravenous infusion techniques were developed for rats and dogs that provide accurate drug delivery while allowing the animal freedom of movement within its cage. The development of these techniques was prompted by the need to assess the preclinical safety of drug candidates by the same delivery method in the clinic. Drug was administered to rats and dogs using a state-of-art infusion pump and a surgically implanted jugular cannula. Animals were surgically prepared at least 2 weeks prior to the initiation of drug delivery using a procedure that was completed within 0.5 hr in rats and 1 hr in dogs. Rats were attached to the delivery pump using a rodent jacket and tethering system that consisted of a flexible stainless steel tethering spring and an infusion swivel. The swivel apparatus allowed the rat unrestricted access to its cage. Dogs carried the battery-operated programmable pump in a pocket of a canine jacket. Dosing cannulas remained in place and patent for up to 3 weeks in rats and 7 weeks in dogs. Cannula placement in both species was determined in-life by resistance-to-flow and at necropsy by observation. Health status in both species was assessed by monitoring physical appearance, body weight, food intake, serum chemistry, hematology and histopathology endpoints. Health status changes in dogs were limited to histologic findings in the jugular vein surrounding the cannula that were characteristic of those associated with a chronic indwelling catheter. Rats had similar histologic changes plus transient decreases in body weight and increases in total white blood cell count that were considered secondary to the surgical procedure. Drug was successfully delivered using these methods in toxicology studies that supported regulatory submissions.

569 HEMATOLOGY, CLINICAL CHEMISTRY, BODY WEIGHT, AND FOOD CONSUMPTION IN CONTROL BABOONS DURING 28 DAYS OF INTRAVENOUS D5W INFUSION

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Six male and six female baboons served as controls over a period of 28 days in an intravenous infusion dosing scenario. Following acclimation to the jacket portion of the tether system, the baboons, which weighed an average of 13 kg, were implanted with a catheter in the jugular vein which was exteriorized in

the mid-back and brought to the outside of the cage through an armored conduit. For approximately one week following surgery, the animals were infused with a heparinized saline (5 units per ml) at a low flow rate. During the test period, the animals were infused with D5W at a rate of 1.5 ml/kg/hour for 28 days. Blood samples were taken for clinical chemistry and hematology on days −4, −1, 3, 5, 7, 14, 21, and 28. Food consumption and body weight were measured weekly and urine specimens were collected overnight preceding each blood sample. A comprehensive set of tissues was collected at necropsy and processed for histopathologic examination. Under the conditions in this study, toxicity in the endpoints examined attributable to the D5W per se were not observed. Blood vessel irritation and thrombus near the end of the catheter was observed in most animals.

570 MICROTITER PLATE ASSAY (MPA) FOR LEUKOCYTE ASCORBIC ACID (AA)

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Exposure to environmental toxicants and xenobiotics often result in altered ascorbic acid (AA) status. Leukocyte (LEU) content of AA best reflects AA status; however, methods for the isolation of LEU and for AA analysis are laborious and costly. We have overcome such concerns by developing a microtiter plate assay (MPA, using a BIO-TEK Model EL340) to measure the AA-dinitrophenylhydrazine derivative (515 and 562 nm) from LEU isolated by differential sedimentation. LEU were isolated from whole blood components using Histopague 1107 and 119 (Sigma Chemical, Co). AA contribution from red blood cells was <0.15%. Incubation time for AA DNPH derivative formation was reduced to 50% with no loss in recovery (>90%). LEU AA values by our method using human subjects were in agreement with published values (0.506 mg/dl, range of 0.324–0.627 mg/dl). The AA MPA has explicit benefits over traditional methods, because it can handle small sample volumes and multiple samples simultaneously, requires the use of less hazardous reagents, and can be done on 3 ml of blood.

571 A UNIQUE, NON-INVASIVE METHOD FOR ACCURATELY IDENTIFYING RABBIT PARAVERTEBRAL INTRA-MUSCULAR INJECTION SITES FOR SUBSEQUENT HISTOPATHOLOGICAL ANALYSIS

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The localized effects of drugs and vaccines administered intramuscularly is an important aspect of preclinical toxicity screening. It is often difficult to accurately locate intramuscular injection sites weeks or months after the original injection. To help overcome this difficulty, we have developed a polyethylene intramuscular injection template that is utilized during the injection procedure as well as for excision of injection sites for histopathology. The template is placed over the dorsal back area of the rabbit and aligned with the ends of the left and right caudal rib and the vertebral column. One or more injection sites are chosen and the material under study is injected to a constant depth. Excision of the injection sites requires a similar procedure whereby the animal is euthanized and the dermal tissue above the dorsal back area is removed. The template is then placed over the muscle facia and aligned in the same manner as was used for the injection procedure. The underlying block of muscle tissue is then excised for histopathological examination. Use of the template by our lab has proven to be a significant improvement in accurately relocating intramuscular injection sites.

572 COMBINED TOXICOLOGY AND TOXICOKINETIC STUDIES IN NONHUMAN PRIMATES: CHOICE AND TIMING OF SELECTED CLINICAL PATHOLOGY MEASUREMENTS

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To maximize information and minimize costs in nonhuman primate studies experimenters combine multiple research objectives into the same protocol. Inclusion of toxicokinetic/pharmacokinetic measurements into subchronic and chronic target organ toxicity studies is common. This apparently prudent approach can create interpretation problems with clinical pathology measurements that potentially overshadow meaningful objectives of the toxicologic portion of the study.

To demonstrate how this might occur, normal chimpanzees were subjected to experimental procedures simulating a combined protocol. Moderate to severe transient elevations in serum creatine phosphokinase (CPK; > 15,000 U/L), lactate dehydrogenase (LDH; >4,500 U/L), aspartate aminotransferase (AST; > 200 U/L) and alanine aminotransferase (ALT; > 100 U/L) were observed. Serum CPK and LDH isoenzyme electrophoresis were helpful in eliminating apparent cardiac muscle damage as a possible contributing factor but not skeletal muscle or liver damage. Suggestions on experimental design for these combined protocols are offered.

573 DYNAMIC ORGAN CULTURE IS SUPERIOR TO MULTIWELL PLATE CULTURING SYSTEMS FOR MAINTAINING LIVER SLICE VIABILITY

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The increase in the use of tissue slices for metabolism and toxicity studies has resulted in a number of laboratories developing and applying various incubation systems and techniques to the slices. For data to be comparable from one laboratory to another a reliable and consistent incubation system must be used that will give the researcher the most optimal slice viability levels. This study compares and contrasts the dynamic organ culture system (surface culture) and the multiwell plate culture system (submersion culture). Rat liver slices were produced using the Brendel/Vitron tissue slicer under oxygenated and ice cold V-7 preservation solution. The slices (200 um thick) were incubated in Waymouth's medium + 10% FCS containing either $NaHCO_2$ or HEPES and gassed with either 95% O_2 or air and 5% CO_2. Slice viability was assessed for up to 72 hrs using ATP content, K+ retention, protein synthesis, ALT and LDH leakage, and MTT reduction. 7-Ethoxycoumarin metabolism was used to assess the slice's metabolic capability. The results indicated that the dynamic organ culture system maintained the rat liver slices at a higher level of viability than the multiwell plate culture system and that Waymouth's medium gassed with 95% O_2 5% CO_2 was the best incubation condition for both systems. It is essential to optimize slice viability in order to obtain reliable and consistent scientific data which will ultimately be used to predict what will be observed in humans. (supported by NIEHS RO1-ES-05790)

574 MEASUREMENT ALGORITHM USED FOR COMPARISON OF ANATOMICAL CHARACTERISTICS OF THE SKIN FOR SEVERAL LABORATORY ANIMALS

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Defining anatomical characteristics that influence chemical penetration through the epidermis can be aided by computer-based image analysis (IA). The algorithm presented was used to measure the thickness of the stratum corneum (SC), the stratum granulosum (SG) and the remaining underlying viable epidermis (VE). This algorithm was developed using an IA system (Quantimet 570c, Leica, Inc., Deerfield, IL) and routine histologic sections stained with hematoxylin and eosin. Following vertical alignment of the tissue with the epidermal surface on the left, the initial step involved determination of the background lighting intensity. The second step was detection of the SG layer by thresholding the digitized image in a video image plane, thus storing its position and size. The third step was a similar detection of the total epidermis in an image plane. Individual SC, SG and VE measure-ments were obtained along a row of pixels (i.e., a chord) horizontally and each was repeated at ten pixel intervals in a vertical direction. The thickness of the SC was equal to the length of a horizontal chord between the total epidermal surface and the superficial border of the SG layer. In the loosely compacted SC, the area between cornified layers that was recognized as background lighting intensity was subtracted from the SC thickness. The thickness of the SG was measured directly from the thresholded image. The length of a horizontal chord from the deep SG border to the deepest border of the total epidermis equals the thickness of the underlying VE. This automated method allows rapid measurement of the thickness parameters in various skin samples. The method is more accurate and quicker than manual methods using a calibrated ocular device. This method can be used to quantitate regional and species differences in skin layers.

575 AN IMPROVED METHOD FOR DETERMINATION AND SEPARATION OF ORGANOCHLORINE PESTICIDES AND POLYCHLORINATED BIPHENYLS IN HUMAN BREAST ADIPOSE TISSUE

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Extensive literature exists supporting the accumulation of organochlorine compounds such as Dichloro-Diphenyl-Trichloro-ethane and Polychlorinated

Biphenyls (PCBs)in human adipose tissue. Recently, debate has surfaced concerning the link between these compounds and human breast cancer. Studies reported here detail development and validation of an improved method for determination of organochlorine pesticides and polychlorinated biphenyl isomers in human breast adipose tissue. The method involves extraction of the compounds of interest from breast adipose samples, clean-up of the residues from the extract using Florisil chromatography, separation of the PCBs from the organochlorine pesticides via silica gel chromatography, and compound identification and quantification using gas chromatography. This method yields approximately 78–94% spike recoveries and acceptable separation of the organochlorine pesticides and the PCBs. These results eliminate the possibility of interference between the two classes of compounds. This method enables distinction between organochlorine pesticides and PCBs as possible factors in breast cancer.

576 DETECTING METABOLITES IN TISSUES OF RATS EXPOSED TO ^{13}C-LABELED ACRYLAMIDE USING NMR SPECTROSCOPY

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Elucidation of pathways involved in toxicant metabolism in tissues is important in understanding the correlation between tissue susceptibility and adverse effects. Studies in our laboratory have shown significant advantages in characterization of urinary metabolites using ^{13}C-labeled toxicants and NMR spectroscopy. We are examining the feasibility of direct detection of metabolites in tissues of rodents administered ^{13}C-labeled toxicant using NMR. Male Fischer-344 rats were administered, by gavage, 100 mg/kg [1,2,3-^{13}C]acrylamide (AM). According to previous disposition studies using ^{14}C-labels, this dosing regimen should result in tissue metabolites at concentrations feasible for NMR detection. Brain, liver, kidney, testis, blood and urine were collected at 30 min, 1 hr, 3 hr and 6 hr after administration. ^{13}C NMR spectra were acquired on tissue homogenates, plasma and urine. AM and a metabolite derived from direct conjugation of AM with glutathione were detected in the tissue homogenates, plasma and urine. Glycidamide, the epoxide of AM, and metabolites derived from direct conjugation of glycidamide with glutathione at the 2 or 3 carbon position were detected in plasma and urine. The hydrolysis product of glycidamide was detected in urine. Studies are underway to compare the method of characterizing and quantitating metabolites in tissue homogenates and water-soluble tissue extracts using solid state and solution NMR. Assignment of metabolites directly in tissues without the requirement for synthetic standards will provide a complementary approach to conventional studies.

577 COMPARATIVE EFFICACY OF THREE METHEMOGLOBIN FORMERS IN PREVENTING CYANIDE INTOXICATION

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Current therapy for cyanide (CN) intoxication consists of the intravenous administration of sodium nitrite and sodium thiosulfate. A prophylactic treatment is needed to overcome constraints of the current therapy. The present study was designed to compare the anticyanide effects of three candidate pretreatment methemoglobin (MHb) forming compounds in an anesthetized animal model using a nonlethal endpoint. The experiment was conducted in five periods, with each of nine animals receiving the vehicle control in the first period, 0.2 mg/kg p-aminopropiophenone (PAPP) in the second period, and 2.5 mg/kg WR242511AE in the third period. Four of the animals received the vehicle control in period four followed by 7 mg/kg p-aminoheptophenone (PAHP) in period five, and five animals received 7 mg/kg PAHP in period four followed by the vehicle control in period five. NaCN infusion for PAPP, PAHP and WR242511AE experiments was initiated when the predicted MHb level was approximately 5 percent. Infusion was stopped ten seconds after cessation of functional breaths (respiratory arrest). Scheduled blood samples were drawn for hemoglobin (Hb), MHb, and total blood CN^- levels. The primary response parameters were time to respiratory arrest, percent MHb, and NaCN dose. Other data collected included heart and respiratory rates. All pretreatment regimens effectively mitigated the effects of NaCN poisoning when compared to the vehicle control ($p < 0.05$). There were no discernable differences between protection among the three compounds. These data show that the protective effect is related to the MHb level rather than specific pretreatment drug class. [Supported by USAMRDC DAMDI7-89-C-9050]

578 EVALUATION OF A NON-LETHAL ANESTHETIZED CANINE MODEL FOR EFFICACY EVALUATION OF CANDIDATE ANTICYANIDE THERAPY

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Efficacy evaluations of anticyanide therapeutic compounds historically have used lethality in unanesthetized animals as the toxic endpoint. In 1993, von Bredow *et al.* (Medical Defense Bioscience Review, 1993) reported a nonlethal, repeated-testing, anesthetized canine model. Time to respiratory arrest (RA) induced by a continuous, slow intravenous (IV) infusion of sodium cyanide (NaCN) was shown to be a consistent, well-defined endpoint in anesthetized canines. Thirty seconds after RA, an IV bolus of a methemoglobin-forming compound reversed the respiratory effects. This study was designed to determine the variability in time to RA, survivability of anesthetized dogs repeatedly infused with NaCN and treated with hydroxylamine, replicability of the procedure within an animal and between animals, and development of trends in data. Four animals were anesthetized, intubated, catheterized, and instrumented to record respiratory and heart rates. Immediately following baseline data collection, a 4 mg NaCN/mL saline solution was infused at a rate of 2 mL/min. Following a 10-second period without functional respiration, infusion was stopped. Hydroxylamine therapy was administered 30 seconds after RA. Inter-animal variability in time to RA was significant ($p = 0.04$). The average percent variability in time to RA within an animal was less than 10 percent. Baseline heart and respiratory rates were not altered significantly. Data confirm, by replicate testing, the utility of this survival model using a non-lethal endpoint. [Supported by USAMRDC DAMD17-89-C-9050]

579 COMPARISON OF ALTERNATIVE ANIMAL MODELS FOR EVALUATION OF EMETIC ACTIVITY

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The emetic potential of a consumer product is one of the parameters used in assessing its risk following accidental ingestion. Historically, the animal model used to assess this potential is the dog. Since the ferret and pigeon are sensitive to a variety of therapeutic agents for emesis, the objective of this study was to determine if they could be used as an alternative animal model for laundry and cleaning products. In the present study, varied doses of selected products were gavaged undiluted or as a solution or suspension. Control animals were dosed with water (20 ml/kg). In-life observations were made periodically during the 24 hr post-dose period. For the historical model, the ED50 for inducing emesis ranged from 4 mg/kg (laundry detergent) to 4 g/kg (fabric conditioner), with emesis occurring primarily within the first hour post-dose. For ferrets, ED50 values ranged from 5.6 mg/kg (laundry detergent) to ≈3 g/kg (fabric conditioner), with emesis occurring almost exclusively within the first hour post-dose. For pigeons, ED50 values ranged from 220 mg/kg (liquid laundry detergent) to ≈13 g/kg (fabric conditioner). For dishwashing products, the pigeon ED50 values were ≈10 times higher and the mean onset time was consistently longer as compared to the ferret and the historical model, but still occurred from ≈30 to 60 minutes post-dose. For hard surface cleaners, the relative responses were similar for all animal models (≈2 g/kg). These data demonstrate that both the ferret and pigeon may be appropriate animal models for assessing the emetic property of laundry and cleaning products.

580 COMBUSTION PRODUCTS OF ADVANCED COMPOSITE MATERIALS (ACM): EVOLUTION OF THE UPITT II METHOD

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The development of methods to evaluate the composition and toxicity of smoke from burning materials is an evolving science. Using new synthetic materials, such as ACM, raises concerns about the potential environmental and human health risk resulting from exposure to the chemically complex smoke produced by burning these materials. We are using methods we developed to evaluate the characteristics of smoke produced from controlled combustion of test materials. Our combustion apparatus is an evolved form of the cone heater combustion module from the UPITT II method developed at the University of Pittsburgh. The salient features of this apparatus are: (a) continuous plume temperature recording, (b) atmospheric dispersion modeling data, (c) plume gas FT-IR Spectrometer analysis, (d) TGA-FTIR of the ACM sample, and (e) sample ports to obtain soot for analytical chemistry and morphologic evaluation of particulate material using light and electron microscopy, combined with

computer-based image analysis. Endpoints obtained by this method include: FT-IR spectroscopic evolution of CO, CO_2, and H_2O; IR spectroscopy of cold trapped compounds; GC separation and mass spectroscopy identification of organic compounds extracted from soot; emission rate and size distribution of the aerosol. This testing method significantly advances the ability to determine chemical and morphologic characteristics of the combustion products.

581 44 DAYS OF CONTINUOUS INFUSION INTO THE INTRATHECAL SPACE OF BEAGLE DOGS USING AN INDWELLING CATHETER

S Cornell, V Mendenhall. *TSI Mason Laboratories, Worcester, MA.* Sponsor: F J Mecler

Ambulatory, continuous intravenous infusion of test articles in dogs is frequently used for preclinical safety evaluation studies. Similar infusion into the intrathecal space has not previously been reported. This technique was successfully validated in 8 Beagle dogs, and subsequently used in a group of 36 dogs on study. A specially prepared silastic catheter was surgically inserted through a cervical durotomy into the intrathecal space and advanced caudally to the lumbar area. The catheter was subcutaneously stabilized and brought through the skin at the interscapular area and the animals were placed into a jacket and tether system. All animals were continuously infused for 44 days with either saline or the test article in the subsequent study, at a rate of 0.1 mL/hour. Conventional observations, neurological evaluation and various physiologic parameters were monitored at selected times throughout the studies. Cerebrospinal fluid was collected and examined immediately prior to euthanasia and the subsequent complete necropsy and histopathological evaluation. All animals tolerated the procedure quite well. Continuous infusion of the control and test articles for the full period of study was accomplished, with no clinical signs or gross or histologic evidence of toxicity in the validation or control group of animals. Attention to detail at the surgical procedure and meticulous care of the catheter exit site during the test period were deemed essential to insure success of the technique.

582 28 DAYS CONTINUOUS INTRATHECAL INFUSION IN DOGS USING AN INDWELLING CATHETER

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Intrathecal infusion via an indwelling catheter is used to administer some compounds clinically. To assess these compounds in preclinical safety evaluation studies, the same route should be used in animals.

To determine the feasibility of intrathecal infusion in dogs, 6 animals were anesthetized, an epidural needle was inserted in the lumbar sacral area and a catheter fed through the needle so that the tip lay in the intrathecal space at the lumbar area. Following removal of the epidural needle, the catheter was sutured in place, passed subcutaneously and exteriorized in the scapular region. The catheter was connected to an external pump using a jacket and tether system which allowed the animal free range movement within the cage. Dogs received 0.9% sterile saline by continuous infusion at a rate of 0.1 ml/hr for 28 days.

Radiography, using an imaging agent, was performed post surgery and preterminally to confirm placement of the catheter. Clinical observations, neurological examinations, bodyweight gains, food intake, ophthalmoscopy, electrocardiograms, blood pressure and clinical pathology revealed no adverse effects. Gross and histopathological examinations after 28 days showed only minor findings which were considered to be a normal tissue response to the presence of an indwelling catheter.

It was concluded that intrathecal infusion in dogs is a viable route for preclinical safety assessment for up to 28 days.

583 AN INVESTIGATIVE STUDY TO ASSESS THE SUITABILITY OF THE MICRO-PIG FOR SUBCHRONIC INTRAVENOUS INFUSION TOXICITY STUDIES

V Badalone, L Pouliot, M Lafleur, C J Perkin. *Bio-Research Laboratories Ltd. Senneville, Quebec, Canada*

Continuous intravenous infusion via an indwelling catheter is frequently used in preclinical safety evaluation studies to mimic the proposed clinical route. Control data at our laboratory was evaluated to determine any effects of the presence of a catheter in the abdominal *vena cava* and of continuous intravenous infusion in the micro-pig. A medical grade silastic catheter was surgically implanted under general anesthesia in the *vena cava* via the saphenous or femoral vein. Animals were infused with saline for 24 hours a day for 42 days at a rate of 1.0 mL/kg/h using a jacket and tether system. Minor gross and histopathological findings which are commonly seen in infusion studies in other laboratory species were noted. There were no effects on body weight, food consumption, ophthalmoscopy, cardiovascular parameters, clinical pathology parameters, respiration rate or body temperature.

It is concluded that the micro-pig is a suitable non-rodent species for use in subchronic intravenous infusion toxicity studies via an indwelling catheter with only minor changes consistent with the presence of a foreign body.

584 A FUNCTIONAL APPROACH TO THE VALIDATION OF A COMPUTERIZED ELECTROCARDIOGRAM ANALYSIS SYSTEM

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GLP regulations require that a validation be performed for computer systems. A computer system designed to collect and analyze electrocardiograms (ECG) in the dog was purchased on the basis of a predetermined Functional Requirements Document, which served as the basis for this validation. The System included the VecgLAB software, a personal computer and an ECG recorder. The VecgLAB software contained several functional components: VecgLAB Study Editor (study definition), VecgLAB Data Collection module and Studyit (data management). The System was set in a minimal configuration to report heart rate (HR), sinus arrhythmia index (SA), P, PR, QRS and QT duration from a single lead (aVF). The ECG recorder was configured to record and transmit simultaneously a recording of a 10 lead ECG and a rhythm strip. The validation was designed to examine the functionality of the System on several levels: user interface, data collection/analysis, data transfer and effectiveness under stress conditions. The user interface testing involved a full evaluation of the function of the user entry screens and the System's ability to create the appropriate files under the conditions of a standard study. The data collection and analysis testing phase involved the comparison of the computer output to manual measurements on ECGs from control dogs and dogs treated with various compounds. In addition, this phase also evaluated the System's ability to transfer data internally (Studyit) and externally, to a statistical package (SAS). The stress test examined how the System would handle a study that is beyond a "normal" size. The System was successfully validated using this approach and found to be suitable for use in toxicity studies.

585 VALIDATION OF COMPUTER BASED DATA ACQUISITION SYSTEMS IN A GLP ENVIRONMENT

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Computer based systems are used in our laboratory for real time data acquisition in Safety Pharmacology studies. These studies are conducted according to GLP, requiring that data acquisition systems be tested and controlled (ie. validated) to assure the quality and integrity of the data and proper functioning of the system over time. In our laboratory, validation has focused on "functional testing" to ensure the operational reliability and control within the GLP compliant process of data collection and manipulation.

The FDA has recently indicated that, in addition to functional testing, computer validation should include "structural testing" (ie. line by line verification of source code), along with a vendor assessment accompanied by documentation that quality development activities have been accomplished.

A proposal for validation of data collection and analysis systems for Safety Pharmacology studies will be discussed within the framework of the "system life cycle", beginning with inception through development, purchase and implementation, and ending with the removal of an obsolete system from production.

586 A NON-INVASIVE METHOD FOR DISTINGUISHING CENTRAL FROM PERIPHERAL NERVOUS SYSTEM EFFECTS OF RESPIRATORY DEPRESSANT DRUGS IN CONSCIOUS RATS

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A simple, non-invasive method for distinguishing the central from peripheral effects of respiratory depressant drugs was developed in conscious rats. The method involves exposing rats for 5 minutes to an air mixture containing 8% CO_2 (central stimulant) followed by an i.v. bolus injection of 300 mcg/kg sodium cyanide (peripheral stimulant) and comparing the changes in minute volume and mean inspiratory flow (respiratory drive) before and after drug

treatment. The effects of the centrally acting respiratory depressant drug morphine and carotid body denervation (peripheral depression) on the CO_2- and NaCN-induced changes in ventilatory parameters demonstrated that changes in minute volume and mean inspiratory flow could distinguish the central effects of morphine from the peripheral effects of carotid body denervaton (CBD). The CO_2-induced increases in the minute volume and mean inspiratory flow were inhibited by morphine sulfate, but not by CBD, whereas the NaCN-induced stimulation of these parameters was inhibited by CBD, but not morphine. In fact, morphine enhanced the NaCN stimulation of mean inspiratory flow. Evaluation of the effects of the centrally acting respiratory depressant drugs L-2-phenylisopropyladenosine (L-PIA), γ-hydroxybutyric acid (GHBA), xylazine and phenobarbital on the CO_2- and NaCN-induced increases in minute volume and mean inspiratory flow further demonstrated that centrally acting respiratory depressant drugs inhibit the CO_2-induced stimulation of minute volume and enhance the NaCN-induced stimulation of mean inspiratory flow.

587 A NEW INCUBATION SYSTEM FOR VOLATILE TEST AGENTS IN PRECISION CUT LIVER SLICE CULTURE

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Tissue slices provide a convenient, economical method of determining ischemic or toxic effects on human or animal liver cell viability and metabolism. Precision cut liver slices provide an *in vitro* system which resembles *in vivo* systems more closely than isolated cell cultures because of the differentiation and functional heterogeneity of the slices. Organ slices retain the normal three dimensional configuration found in the whole organ and natural adhesion and signaling systems between cells are not lost. Precision cut organ slices allow for many replicate cultures per organ, thereby reducing the number of animals needed. Liver slices have been maintained in traditional rolling incubation systems for up to 5 days. However, these systems do not readily allow for testing volatile substances at low concentrations. Recently, a new incubation system has been developed which accommodates volatile substance studies. Using closed bottles, incubating slices are exposed to one liter of gas as opposed to only 22 ml in traditional scintillation vial systems. A comparison was made of liver slice viability (K^+ content and LDH leakage) over time in several incubation systems in the presence of ambient air or 95% O_2/5% CO_2. The three systems tested included the traditional "Hot Dog" Roller system, the Continuously Gassed Roller Culture Incubator with slices in scintillation vials and the Volatile Substance Incubator designed especially for volatiles. Slices in all systems incubated in the absence of 95% O_2/5% CO_2 showed significantly decreased viability. In the presence of carbogen, all three incubation systems resulted in comparable liver slice viability up to 16h. The gassed "Hot Dog" Roller and Volatile Substance Incubator resulted in similar slice viability up to 36h without regassing. At later time points, slices in the Volatile Substance Incubator retained significantly greater viability, required regassing at less frequent intervals and lower concentrations of volatile test agents could be achieved in a sterile environment. (Supported by the Arizona Elks.)

588 QUANTITATIVE RT-PCR ON HETEROLOGOUS NUCLEAR RNA: A SURROGATE FOR THE *IN VITRO*, TRANSCRIPTION RUN-ON ASSAY

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Biological responses to toxicants frequently involve changes in gene transcription. Northern analysis of mRNA content provides only an indirect assay for transcriptional changes. Direct measurements are possible with the *in vitro* transcription run-on assay. The assay however, is time consuming, laborious and requires *large* numbers of nuclei. Furthermore, many experimental systems are not conducive to the isolation of sufficient nuclei for the run-on assay. As a surrogate for the transcription run-on assay, we developed a quantitative Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) assay to amplify hnRNA transcripts encompassing the first exon-intron boundary of the *Cypla-1* gene. Using Hepa 1c1c7 cells, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin induction of *Cypla-1* examined by both methods generated quantitatively equivalent results. The RT-PCR method is rapid, and requires only nanogram quantities of total cellular RNA. This represents RNA from about a thousand cells, each containing fewer than a hundred copies of the target sequence. Hence, the RT-PCR assay represents a very sensitive tool for the study of transcriptional events.

589 A SEMI-AUTOMATED AND PRECISE CHOLINESTERASE (CHE) METHOD FOR CNS REGIONAL TISSUE SAMPLES

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A highly efficient and precise ChE method for rat CNS regional tissue samples was developed for the Monarch® Chemistry System of Instrumentation Laboratory (IL). Tissue samples, which were collected, weighed, and then frozen in 14-ml polypropylene tubes, were processed in sets of 35. The thawed samples were rapidly homogenized in the 14-ml tubes with ice-cold buffer using a Brinkmann Polytron® and a fixed volume of each sample was then transferred to a sample cup. Large tissue fragments, which could adversely affect data reproducibility and even cause clogging problems for automated instruments, were disintegrated by adding an extraction solution consisting of Triton-X 100 and NaCl (final levels of 1% and 0.25 M). Acetylcholinesterase was also effectively solubilized at these tissue concentrations (w/v) of 2.5% (cerebral cortex), 2% (cerebellum), 1.5% (hippocampus), 1% (thoracic spinal cord), and 0.5% (striatum). Cholinesterase activity was measured simultaneously for multiple samples (up to 35) by monitoring absorbance at 405 nm for 75 sec. using 4 μl of extract mixture, acetylthiocholine iodide (ACHI), and dithiobisnitrobenzoic acid (DTNB). Accurate ChE values were generated by the automatic subtraction of reagent blank (no sample) and individual sample blank (no ACHI) from total activity. The assay has excellent linearity; data reproducibility was also superior in comparison with the original Ellman method (a manual procedure using the crude homogenate). Sample throughput was at least 4-fold higher than by using a procedure recommended by IL.

590 FLUID HOMEOSTASIS DURING CONTINUOUS INTRAVENOUS INFUSION OF SALINE TO SPRAGUE-DAWLEY RATS

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A study was performed to validate an improved system for continuous intravenous administration of compounds to rats on toxicity studies. The study included 4 groups of 5 male and 5 female Sprague Dawley rats treated during a 28 day period. Surgically prepared rats were individually housed in HRC designed and purpose-built caging, and were continuously infused using dedicated programmable infusion devices with 0.9% NaCl at dose rates of 1, 2, 3, or 4 ml/kg/hr.

There were no adverse effects of the treatment on weight gain or food consumption. Haematological values remained within normal limits. Pathological changes were minimal and associated with tail attachment of the tether. Changes considered to be associated with fluid homeostasis were seen mainly at the two highest infusion rates and consisted of: marginal reductions in water intake and increased urine output, marginal dose rate dependant reductions in urinary specific gravity, increases in urinary levels of Na and Cl and marginally increased kidney weights in some animals. It is concluded that fluid homeostasis can be maintained during continuous infusion of 0.9% NaCl at dose rates of up to 4 ml/kg/hr for at least 28 days without tompromising renal integrity.

591 A NOVEL NONCHROMATOGRAPHIC METHOD TO ABSORB POLYCLONAL ANTIBODIES MAKING THEM MONOSPECIFIC

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Traditionally, in order to back absorb a polyclonal antibody and produce monospecific antibody the heterologous antigens are coupled to a solid support or cross-linked to render them insoluble. This step is necessary to allow for separation of the antibody recognizing the cross reacting epitopes from those antibodies to unique epitopes not present on the cross-reacting material. It occurred to us that microsomes alone might be suitable for use as an absorbant because the putative cross-reacting antigens (present in microsomal preparations) were already insoluble by virtue of their being incorporated into microsomal vesicles. We tested this hypothesis by evaluating whether we could produce monospecific anti-P4503A2 from anti-P4503A2 raised in rabbits by absorbing with liver microsomes from dexamethasone treated female rats; a source rich in the major cross-reacting cytochrome P4503A1 but almost devoid of P4503A2. After incubating at room temperature and centrifuging to remove the microsomes and bound antibody, the supernatant was concentrated and tested for recognition of cytochrome P4503A1. Little or no inhibition of testosterone 6B hydroxylation was detected using this absorbed antibody in female liver microsomal preparations from rats treated with dexamethasone.

The absorbed antibody could still inhibit testosterone 6B hydroxylation catalyzed by control adult male rat liver microsomes which have P4503A2 but not P4503A1. This work was funded by grant R01-GM44982.

592 URINARY PROTEIN CHARACTERIZATION BY HIGH PERFORMANCE CAPILLARY ELECTROPHORESIS (HPCE)

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Chemical-induced proteinuria is associated with changes in glomerular permeability/integrity and/or changes in proximal tubule reabsorptive capacity. The origin of proteinuria may be differentiated by examining the relative urinary protein distribution based on molecular weights. The present study combined traditional SDS-PAGE with HPCE to differentiate urinary proteins based on molecular weight. Using a gel-filled coated capillary with on-line detection using a diode-array detector at a wavelength of 214 nm, proteins of various molecular weight, consisting of β_2-microglobulin (11.8 kD), a-lactalbumin (14.2 kD), carbonic anhydrase (29 kD), ovalbumin (45 kD), bovine serum albumin (66 kD), phosphorylase b (97.4 kD), β-galacotosidase (116 kD) and myosin (205 kD) can be analyzed by HPCE. Electrophoretic mobility is linear over a wide molecular weight range of 11.8 to 205 kD, with a correlation coefficient of 0.997. Lower limits of quantification (LLOQ) for representative protein markers of interest were 250 μg/ml for β_2-microglobulin, 100 μg/ml for albumin, and 100 μg/ml for IgG. Urinary proteins can be further identified by comparing electropherograms before and after reduction with 2-mercaptoethanol, which dissociates subunits of proteins held together by disulfide bonds. This novel application may be very helpful in differentiating proteinuria of glomerular versus tubular origin.

593 SYNTHESIS OF BIOTINYLATED SAXITOXINOL (STXOH) FOR USE AS A SCREENING MOIETY FOR SAXITOXIN (STX) PEPTIDE BINDING

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STXOH has been attached to biotin in an effort to study phage displayed peptide binding to STX. Using a modification of Koehn, *et al.*, Biorg. Chem. 10: 312–328 (1981), STX hydrated ketone (1 μmol in 100 μM acetic acid) was reduced in a N_2 atmosphere to STXOH using 3 mol eq $NaBH_4$ in water at 4°C. The water was removed under reduced pressure, the solids were dissolved in CH_3OH and chromatographed on a 1 × 20 cm Sephadex LH-20 column. Fractions were dried in a vacuum centrifuge at 55°C overnight, resuspended in phosphate buffer, pH 5.0, and electrophoresed in an open-bore capillary column using sodium borate buffer, pH 8.0 on a Beckman CE 5500 equipped with a photodiode array detector. Migration time of STX and STXOH were 2.5 and 2.0 min at 30 kV, respectively. STXOH fractions were combined and reacted with 3 × 2h additions of 1 mol succinic anhydride while the temperature and pH were maintained at 10°C and 5.5, respectively. Products were fractionated on a 1 × 37 cm Sephadex G-15 using water, concentrated, analyzed on CE using borate buffer followed by drying and fractionation on LH-20 in CH_3OH. Fractions exhibiting STXOH-succinate UV absorption were combined, concentrated to 0.75 ml, and reacted by activation with carboxyldimidazol with 3 mol eq of biotin hydrazine. The reaction was fractionated on G-15 followed by CE analysis of products before and after addition of strepavidin agarose for product determination. Biotinylated STXOH applied to neutravidin-coated microtiter plates will be used to isolate STX binding peptides from a phage-display library.

594 USE OF NONMEM IN DESTRUCTIVE SAMPLING STUDIES

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Destructive sampling studies are often performed to determine the pharmacokinetic (PK) profile of a new drug. The data are usually analyzed by the Naive Averaging of Data (NAD) method which estimates the PK parameters for the population using the mean plasma concentration of each animal at each time point. Another approach that may be used is Nonlinear Mixed Effect Modeling by using a program such as NONMEM. In this method the mean plasma concentrations are used; however, NONMEM preserves the distinction between individual and population parameters through the characterization of inter individual variance across the population. Using computer simulated data for various study designs there is high precision in the estimation of Vdss and Cl by NONMEM and the accuracy of these estimates compared to NAD suggest that the parameters are not significantly different. The use of NONMEM may allow for the population parameters from destructive sampling to be obtained with fewer animals. The purpose of this study was to evaluate NONMEM in the estimation of PK parameters in destructive sampling studies. Male rats were dosed with 6 mg/kg theophylline. Serum concentrations of theophylline were determined by reversed-phase HPLC. One sample was obtained from three animals at each time point. This sample is equivalent to the "destructive" sample. Additional sampling was then continued for each animal. NONMEM was used to estimate the PK parameters (e.g., Vdss and Cl) using profiles generated for each animal. These parameters were compared to the parameters estimated from the "destructive" sample. Initial results show that the population analysis accounts for differences that are seen in the individual subjects. Advantages of NONMEM include its ability to use data from individual subjects and to differentiate the PK parameters of the population from the inter subject variability and random residual error.

595 THE NECESSARY PERIOD FOR RE-ENTRAINMENT IN F344 RATS AFTER REVERSING THE LIGHT-DARK CYCLE — A METHOD FOR DETERMINING CONCENTRATIONS OF A TEST SUBSTANCE IN THE BLOOD

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When blood concentrations are examined in a study by administering food mixed with a test substance to rats, blood sampling during dark period (nighttime) is needed because the test substance is taken mainly in the dark period. However, if blood is collected from the animals at the same time in two rooms controlled separately with normal and reverse light-dark conditions, this will be able to clear the change in blood concentrations by sampling only in the light period. In this case, animals need to entrain in the reverse light-dark condition before initiation of sampling. In the present study, we confirmed the necessary period for re-entrainment of behavioral parameters and estrus cycles in F344 rats after reversing the light-dark cycle. In addition, the necessary period for re-entrainment of cytochrome P450-dependent oxygenase system (P450) and glycogen contents in the liver were investigated. Each behavioral parameter was confirmed to be reversed approximately 1 week after reversing the light-dark cycle. The estrus cycles by observations of the smear cycles hardly changed after reversing the light-dark cycle. The variations in P450 and glycogen were also reversed 1 week after reversing the light-dark cycle. From these results, it is suggested that the blood concentrations of a test substance could be determined by the present method after acclimatization in the reverse light-dark condition for 2 weeks at least.

596 ANALYSIS OF BLOOD AND TISSUE SAMPLES FOR TRACE LEVELS OF LEAD AND CHROMIUM

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The authors have developed sensitive, atomic absorption spectrophotometric (AAS) analytical methods for the analysis of trace levels of lead and chromium in whole blood, brain, kidney, bone, and liver tissue samples. The blood method combines aqueous calibration standards and sample preparation with a simple dilution using a matrix working modifier and partition wall tube atomization. The tissue method utilizes hot acid under pressure in Teflon^R PFA vessels, microwave digestion, and standard addition calibration with the use of a Pallidium modifier. The methods elucidate the advantages of microwave digestion, the use of matrix modifiers to stabilize the analytes to higher temperatures, and partition wall tube atomization. Higher temperatures can reduce background absorbance and chemical interferences, which in turn can greatly improve the determination of volatile elements in difficult sample matrices. The method's detection limit (DL) for blood is 15 ppb for lead and 20 ppb for chromium. For tissue samples the DL is 50 ppb for both elements. Percent recoveries ranged from 75–133% depending on the matrix (either blood or tissue).

597 DETECTION OF CYTOCHROME P450 INDUCTION IN RAT HEPATOCYTES BY FLOW CYTOMETRY

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Cultured hepatocytes are increasingly used to detect the enzyme induction potential of new drug candidates. However, the methods currently employed (measurement of enzyme activities, Western blotting) are complex, time consuming and necessitate large cell samples. The objective of this study was to

evaluate the use of flow cytometry to detect cytochrome P450 (P450) induction. The expression of P450 was studied in isolated hepatocytes from rats treated *in vivo* and in cultured rat hepatocytes treated *in vitro*. Compounds tested included P450 inducers 3-methylcholanthrene (3-MC) (1A), phenobarbital (PB) (2B) and dexamethasone (DXM) (3A), and the P450 synthesis inhibitor $CoCl_2$. After permeabilization, 1-2 × 10^7 hepatocytes were labeled with mouse monoclonal anti-rat P450 1A ($KO_{)8}$) and rat P450 2B (KO_1) antibodies followed by FITC-labeled anti-mouse IgG. Hepatocytes were analyzed on an ATC 3000 cytometer. Cell viability (propidium iodide) was always >85%. Cell fluorescence was significantly increased following treatment *in vivo* and *in vitro* with 3-MC and PB when hepatocytes were labeled with anti-1A and 2B respectively. No increase was evidenced with DXM. A significant decrease in cell fluorescence was observed after treatment with $CoCl_2$. Results obtained with flow cytometry correlated well with those obtained by Western blotting and by measurement of specific enzyme activities (EROD, PROD, EMND) on hepatocyte microsomes. In conclusion these data show that P450 induction can be detected by flow cytometry in a small sample of rat hepatocytes (1–2 × 10^7). This approach could be used to screen new drug candidates at an early stage of development.

598 ACTIVITY OF HEPATIC DRUG METABOLIZING ENZYMES AFTER NALIDIXIC ACID DOSED FEED TREATMENT IN RATS AND MICE

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Nalidixic acid, commonly prescribed to treat urinary tract infections, has been found to cause tumors in the preputial and clitoral glands of male and female rats, respectively. Although preputial and clitoral glands are not found in humans, analogous tissues are found. The potential of nalidixic acid to induce enzymes and the role of induction in tumorigenesis has not been evaluated. In this study, groups of male F344 rats and B6C3F1 mice were given nalidixic acid dosed feed at 0, 500 (therapeutic-equivalent), and 2000 (carcinogenic) ppm for 7 days. In liver homogenates total hepatic P450 along with the enzymatic activities of the isozymes CYP1A, CYP3A, CYP2B, and CYP2E were quantitated. Levels of glucuronosyl transferase and GSH-S-transferase were also assayed. Slight but significant increase in protein content resulted in slight dose-dependent elevations in the activities of most drug metabolizing enzymes. GSH-S-transferase levels increased significantly in mice receiving 2000 ppm and in rats receiving 500 and 2000 ppm nalidixic acid. Nalidixic acid was determined to be a weak (2X elevations) but selective inducer of CYP2E and CYP1A in mice. Although the results show a slight increase in the ability to metabolize xenobiotics, minimal differences between the carcinogenic and therapeutic doses and between susceptible F344 rats and non-susceptible B6C3F1 mice indicate that induction of drug metabolizing enzymes plays little or no role in the tumorigenicity of nalidixic acid. However even slight induction of specific cytochrome P450 isozymes could be of clinical relevance with regard to drug interactions.

599 PITUITARY REGULATION OF RAT HEPATIC MICROSOMAL CYP 2E1

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The major alcohol-inducible cytochrome P450, CYP 2E1 is among the most clinically important P450 isozymes because of its extensive capacity to metabolize drugs, solvents and procarcinogens to cytotoxic compounds and carcinogenic metabolites. The expression of CYP 2E1 is under the complex regulation and highly influenced by hormonal and nutritional factors. In the rat, growth hormone (GH) plays a key role in the regulation of liver secretory proteins, hormone receptors and enzymes of xenobiotic and endogenous compound metabolism, which include cytochrome P450s. We have demonstrated that chronic ethanol treatment resulted in a disruption of GH secretion accompanied by a 2-step induction of CYP 2E1 at the post-transcriptional and transcriptional levels. The current study was conducted to investigate the role of pituitary hormones (especially GH) in the regulation of CYP 2E1 using a hypophysectomized rat model. Hypophysectomy (Hx) of male rats resulted in a 6-fold increase of CYP 2E1 apoprotein as determined by Western blot analysis. Pituitary hormones were administered using either a rat pituitary extract or recombinant human GH via a subcutaneously implanted mini pump which released at a relative constant rate in order to approximate the GH secretion pattern of female rats. This constant plasma GH concentration reduced the level of CYP 2E1 apoprotein in Hx rats towards the level of sham-operated rat. The increase in CYP 2E1 apoprotein in Hx rats was accompanied by a 4-fold elevation of CYP 2E1 mRNA. Regulation of CYP 2E1 appears to be primarily at the level of transcription as our results showed a 2–3 fold increase in nuclear run-on analysis. Although Hx of male rat increased CYP 2E1 mRNA and protein levels, enzyme activity (PNP hydroxylation) was not changed. The discrepancy between activity and apoprotein in liver microsomes from Hx male rats can be best explained by decreased NADPH-P450 reductase activity associated with Hx, which then becomes the rate-limiting factor in monooxygenase activity. These data suggest that pituitary hormones (in particular the female pattern of GH secretion), suppress CYP 2E1 at the transcriptional level and that disruption of GH secretion by chronic ethanol treatment may contribute to CYP 2E1 induction. Supported by AA08645 and AA08213 (T.M.B.).

600 DEVELOPMENT OF AN RT-PCR APPROACH FOR EVALUATING RAT P450 2B1 AND 2B2 INDUCTION *EX VIVO* AND *IN VITRO*

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An induction, or increase of metabolic enzymes after an animal is exposed to a dietary, hormonal, or chemical stimulus has many implications for pharmaceutical development and treatment. If a compound designed for pharmaceutical purposes can undergo enzymatic transformation, than an alteration in the levels of those enzymes may result in increased activity, toxicity, clearance, or a combination of these. Toward the development of an *in vitro* assay for metabolic enzyme induction we have explored the use of RT-PCR (reverse transcriptase–polymerase chain reaction, also called RNA PCR) in measuring the levels of rat cytochrome P450 2B1 and 2B2, the major phenobarbital inducible forms in the rat liver. We describe here design of PCR primers that span exons 1–3 of both the Cyp 2B1 and 2B2 genes and amplify a 341 bp cDNA fragment from reverse transcribed mRNA. Although the amplified cDNA sequences from Cyp 2B1 and Cyp 2B2 are different at only 2 nucleotide positions, these base changes result in changes in restriction enzyme sites, such that the ratio of the two isoenzyme's mRNA may be determined following restriction enzyme digestion. P450 2B1 and 2B2 mRNA can be easily detected in rat liver RNA; preliminary results indicate that under conditions of extreme amplification the message can be detected in the IEC-18 rat intestinal cell line, Clone 9 rat liver cells, and the H4-II-E rat hepatoma cell line. Experiments to quantitate mRNA in control and phenobarbital-treated cells are in progress.

601 EFFECTS OF COMMONLY USED P450 INHIBITORS ON COUMARIN 7-HYDROXYLASE (CYP2A6) ACTIVITY IN HUMAN LIVER MICROSOMES

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Several solvents and commonly used P450 inhibitors were examined for their effects on coumarin 7-hydroxylase (CYP2A6) activity in human liver microsomes. Of nine organic solvents examined (final concentration 1%), only methanol had no effect on the 7-hydroxylation of coumarin (0.5 to 50 μM) by human liver microsomes. Dioxane and tetrahydrofuran, which are structurally related to coumarin, were the most inhibitory solvents examined. At a 200-fold molar excess, the following chemicals caused little or no inhibition of CYP2A6: caffeine, cimetidine, diazepam, fluconazole, hexobarbital, mephenytoin, naringenin, naringin, nifedipine, quinidine, ranitidine, sulfaphenazole, sulfinpyrazone, and tolbutamide. The following chemicals were classified as weak inhibitors of CYP2A6 (because at 100 μM they inhibited the 7-hydroxylation of coumarin only at substrate concentrations below 50 μM): ketoconazole, 4-methylpyrazole, α-naphthoflavone, nicotine, and testosterone. The following chemicals were classified as strong inhibitors of CYP2A6 (because at 100 μM they inhibited the 7-hydroxylation of coumarin more than 50% at a substrate concentration of 50 μM): clotrimazole, diethyldithiocarbamate, 8-methoxypsoralen, miconazole, omeprazole, papaverine, and tranylcypromine. Several of the chemicals that strongly inhibited CYP2A6, such as diethyldithiocarbamate and tranylcypromine, are often used with the intention of selectively inhibiting human P450 enzymes other than CYP2A6. The results of this study illustrate the need for a systematic evaluation of the specificity of commonly used P450 inhibitors. Supported by NIH grants ES03765 and ES07079.

602 EFFECT OF INDUCERS ON HEPATIC TISSUE FROM MALE AND FEMALE BEAGLE DOGS

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New chemical entities (NCEs) undergoing preclinical development must be

assessed for safety in both a rodent and a non-rodent species. Induction of hepatic drug-metabolizing enzymes by the NCE is an important parameter that is most often assessed only in the rat. Often the rodent is a poor model for induction in humans, and it is possible that a non-rodent species, such as the dog, would provide a better correlation. A database for the type and potency of induction by standard inducers in the dog must be established before evaluation of the correlation can be made. Male and female dogs were treated orally over 14 days with the following inducers: β-naphthoflavone (β-NF) for CYP (cytochrome P450) 1A1 and CYP1A2 induction, phenobarbital (PB) for CYP2B induction, and dexamethasone (Dex) for CYP3A induction. Clinical signs and body weights were recorded, and blood and urine samples were collected for clinical pathology evaluations. All dogs were examined macroscopically, organ weights were recorded, and the liver of each dog was examined microscopically. Microsomes were prepared from a portion of the hepatic tissue and assayed for cytochrome P450 enzyme activity. Evidence of induction included increased liver enzymes, and macro- and microscopic liver changes. Increased serum concentrations of alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase; increased liver weights; and hepatocellular vacuolation and enlargement were noted for dogs treated with Dex or PB. In β-NF and PB treated animals increases in CYP1A and CYP2B enzyme activity, respectively, and increased levels of cytochrome P450 in both groups gave further evidence of induction. Although clinical changes were seen with Dex treated animals, preliminary results indicate limited induction of CYP3A activity. The information generated will serve as a reference point for evaluating inductive effects of NCEs in dogs.

603 REGULATION OF HEPATIC DRUG METABOLIZING ENZYMES IN CD-1 MICE AND SPRAGUE DAWLEY RATS

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Polychlorinated biphenyls (PCBs), have been shown to be powerful inducers of hepatic microsomal enzymes systems in rats. The present study was designed to evaluate the constitutive and induced levels of several drug metabolizing enzymes, using enzyme activity as a measure of the amount of enzyme present, in CD-1 mice and Sprague Dawley rats. Male and female CD-1 mice were treated with Aroclor 1254 (IP, 500 mg/kg) and sacrificed four days later. Livers were removed and homogenates and microsomes were prepared. Microsomes from male and female CD-1 mice treated with Aroclor showed a significant increase in benzyloxyresorufin O-dealkylase activity, indicative of an increase in cytochrome P-450 2B isozymes. Lauric acid hydroxylase activity (associated with cytochrome P-450 4A isozymes) was also increased in male and female mice exposed to Aroclor 1254. In rats only peroxisome proliferators increased cytochrome P-450 4A activities. Aroclor is not a peroxisome proliferator in rats or mice. Since coplanar PCBs lower hepatic peroxisomal enzymes and cytochrome P-450 4A, the increase in lauric acid hydroxylase in the mouse is of special interest (supported by NIH F-33-ESO5565).

604 CHARACTERIZATION OF A NEW TCDD-INDUCIBLE CYTOCHROME P450 FOM MOUSE AND RAT DESIGNATED CYP1B1

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A 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-inducible cytochrome P450 (CYP1B1) which is very active in polycyclic aromatic hydrocarbon metabolism has been cloned from C3H10T1/2 mouse embryo fibroblasts [Savas et al. *J. Biol. Chem.* 269:14905 (1994)]. The 5.2 kb CYP1B1 mRNA was equally induced by BA (10 μM) and TCDD (10 nM) in C3H10T1/2 cells, consistent with the involvement of the aryl hydrocarbon receptor in this induction process. The deduced amino acid sequence (543 amino acids), the longest of any known cytochrome P450, exhibits 41% identity to mouse CYP1A1, 92% identity to rat CYP1B1 cloned from rat adrenal glands, and 82% to human CYP1B1, which was cloned from skin carcinoma cells [Sutter et al. *J. Biol. Chem.* 269:13092 (1994)]. There are five extended regions of rat and mouse CYP1B1 which show ≥60% identity to CYP1A1: *a*, 51–118; *b*, 199–222; *c*, 326–343 [I-helix, O_2-binding threonine]; *d*, 357–430; and *e*, 459–484 (heme-binding cysteine), while other regions show <10% identity. Hybridization of mRNA and immunoblot analyses of microsomes both demonstrated β-naphthoflavone inducibility of *CYP1B1* expression in mouse and rat lung, liver, and uterus although at lower levels relative to *CYP1A1*. *CYP1B1* was expressed in ovaries and testis from both rats and mice, however only in adrenal glands from rats where expression was relatively insensitive to adrenocorticotropic hormone. Mouse CYP1B1 cDNA was expressed in *E. coli* as a chimeric protein in which the membrane anchor N-terminal portion was substituted by the N-terminus of human CYP17. The characteristics of this protein will be presented. Supported by NIH grant #CA 16265.

605 REGULATION OF RODENT P4501A EXPRESSION BY METHYLENEDIOXYBENZENES: ROLE OF THE ARYL HYDROCARBON RECEPTOR (AhR)

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The role of the AhR in the mechanism(s) by which the family of naturally occurring methylenedioxybenzene (MDB) compounds induce CYP1A has been unclear. A series of 4-*n*-alkyl-substituted MDBs (n = 0–8) was utilized to treat wild type (WT), mutant $BP^r c1$ (ARNT-deficient) and $BP^r c4$ (AhR deficient) Hepa-1 mouse hepatoma cells in culture. A dose-(10–150 uM) and structure-dependent increase in CYP1A1 apoprotein and was observed in WT but not mutant cells. AhR antagonists (α-naphthoflavone [1 uM] or 2,2′,5,5′-PCB [60 uM] blocked increases in WT cells. Time-course studies indicated a peak in CYP1A1 protein and mRNA 8–12 and 1 hour respectively, post treatment with 6 uM βNF or 100 uM C6-MDB. MDB treatments of Hepa-1 cells transfected with expression vectors containing the firefly luciferase (LUC) gene under the control of several AhR-binding regulatory sequences (XREs) resulted in MDB-induced expression of LUC similar to that of CYP1A1. Gel retardation assays (GRA) revealed MDBs activated binding of cytosolic AhR to consensus XRE sequences. Binding specificity was confirmed by competition by WT but not mutant DRE oligonucleotides for binding, and sucrose density gradient assays indicating competition between MDBs and TCDD for AhR binding. These results indicate transcriptional activation of the CYP1A1 gene via binding of MDBs to the AhR. (NIH ES05311[1] and ES04699[2]).

606 INDUCTION OF CYP1A1 IN MURINE KERATINOCYTES *IN VITRO* IS DEPENDENT ON TERMINAL DIFFERENTIATION

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Previous studies from this laboratory demonstrated that the induction of *Cyp1a-1 in vivo* occurs primarily in differentiating keratinocytes. We sought to establish an *in vitro* system to examine the molecular basis of this phenomenon. Murine keratinocytes maintained in low-calcium medium (0.05 mM) only differentiate in response to a calcium shift (0.6 mM). Both basal and differentiating cells maintained in this system fail to demonstrate inducible ethoxyresorufin O-deethylase (EROD; an indicator of CYP1A1) activity following exposure to dioxin (i.e., TCDD). We have established a culture system in which keratinocyte differentiation is independent of changes in extracellular calcium concentration. Primary cultures of murine basal cell keratinocytes were established in a serum-free, chemically defined medium (SPRD-111 containing 0.9 mM calcium). Unlike keratinocytes maintained in the low-calcium system, keratinocytes maintained in SPRD-111 begin to differentiate and stratify upon reaching confluency. This is demonstrated by phase contrast- and electron microscopy, and trichromic staining. Non-differentiating basal cell keratinocytes maintained in this medium lack inducible EROD activity. However, EROD activities were markedly increased following treatment of differentiating cultures with dioxin. These results indicate that 1) this system supports normal keratinocyte growth and differentiation and 2) *Cyp1a-1* inducibility is a function of keratinocyte differentiation.

607 THE ANTIESTROGEN TAMOXIFEN IS A WEAK PHENOBARBITAL-TYPE INDUCER OF HEPATIC ENZYME EXPRESSION

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Tamoxifen administration to rats for seven days produced a dose-dependent increase in the expression of certain hepatic xenobiotic metabolizing enzymes. Interestingly, the pattern of enzyme induction produced was very similar to the pattern produced by phenobarbital. Increases in CYPIIB1, CYPIIB2, CYPIIA1, and EH protein levels in males and females were observed by western blotting. Also, mRNA levels for CYPIIB1, CYPIIB2, CYPIIIA, EH, and ALDH were increased in both sexes as assayed by northern blot analysis. The expression of CYPIA1, CYPIA2, and GGT mRNA were not significantly affected by tamoxifen treatment. Tamoxifen was approximately ten-times less potent an inducer of combined CYPIIB1/2 mRNA than phenobarbital when the two drugs were administered at equimolar doses. Taken together, these

results suggest that tamoxifen is a weak phenobarbital-type inducer. However, there are significant differences in the induction profiles produced by the two drugs. Most significant of these differences was the stronger induction of CYPIIB2 than CYPIIB1 by tamoxifen. Also, females were often more sensitive than males to tamoxifen, especially at low doses. These tamoxifen-induced increases in enzyme expression could be significant for human exposure considering effects were seen in rats at doses which are comparable to the doses women receive for disease management.

608 CELL SPECIFIC EXPRESSION AND TCDD INDUCIBILITY OF CYP1B1 IN MOUSE EMBRYO FIBROBLASTS AND CULTURED MOUSE EMBRYOS

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CYP1B1 is by far the dominant PAH-metabolizing cytochrome P450 in C3H10T1/2 cells [Savas, U., *et al* (1994) J. Biol. Chem. 269;14905.] Primary cultures of C3H, C57B6, and DBA mouse embryo fibroblasts similarly express CYP1B1. The 5.2 kb CYP1B1 mRNA is detected by Northern Hybridization, and the 56 kD protein is detected by Western Immunoblot. Little 2.6 kb CYP1A1 mRNA is present and no protein is observed. TCDD and many PAHs induce P450s through the Ah receptor. 6-methyl-2,3,8 trichlorodibenzofuran, an Ah receptor antagonist, blocks TCDD and PAH induction of CYP1B1 demonstrating Ah receptor regulation of CYP1B1. Ah receptor regulation is also be shown by linking TCDD induction response to Ah receptor phenotype. We measured mRNA levels and enzyme activity from primary embryo cultures generated from congenic C57B6 mice that differed at the Ah locus. Fibroblasts cultured from phenotypically responsive mice expressing the *b* allele exhibited a five-fold lower EC_{50} for TCDD as compared with non-responsive mice carrying the low-affinity *d* allele. The expression of CYP1B1 was dependent on the differentiation status of the primary cultures. The onset of proliferative crisis, as marked by the presence more differentiated cells (adipoblasts, myoblasts, and osteoblasts), loss of uniform fibroblast morphology and low passage efficiency suggest a cell-type specific expression of CYP1B1. Restoration of CYP1B1 expression occurred when cells escaped from senescence, as marked by uniform cell shape and ready passage. The immortalized cells retained the differences in TCDD responsiveness associated with the distinct Ah receptor phenotypes. Other studies demonstrate CYP1B1 expression in primary stromal fibroblast cell cultures from mammary gland, cell lines from mouse prostate and uterus, and high expression of CYP1B1 in steroidogenic tissues, particularly the rat adrenal. These observations are consistent with a pattern of cell-specific expression of CYP1B1 in which undifferentiated cells of mesodermal origin predominantly express CYP1B1. *In situ* hybridization studies in mouse embryos are being carried out to support this hypothesis of mesenchymal derived cell-specific expression of CYP1B1. This work sponsored by NIH grant #CA 16265 and NIHES grant #ES07260.

609 CELL-SPECIFIC REGULATION OF CYP1B1 IN RAT CELLS

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This laboratory has cloned a new class of polycyclic aromatic hydrocarbon (PAH)-metabolizing cytochrome P450 from mouse embryo fibroblasts [Savas *et al.* (1994) *J. Biol. Chem.* 269: 14905.] and rat adrenals [Bhattacharyya *et al.* (1994) *J. Biol. Chem.* submitted] which, based on the sequence relationship to CYP1A1, has been designated CYP1B1. The 57 kDa CYP1B1 protein has been shown to be hormonally regulated in the intact rat adrenal gland by adrenocorticotropic hormone (ACTH) [Otto *et al.* (1991) *Endocrinology* 129: 970.]. Activity and expression of CYP1B1 is elevated 4-fold in primary cultures of rat adrenocortical (RAC) cells following 24 h of ACTH treatment as measured by selective 7,12-dimethyl-benz[*a*]anthracene metabolism and Northern blot analysis, respectively. Maximal stimulation of CYP1B1 by ACTH has been duplicated in RAC cells by the cyclic adenosine 3′,5′-monophosphate (cAMP) analogue, 8-bromo-cAMP, and by the adenylyl cyclase agonist, forskolin, suggesting that cAMP mediates induction of CYP1B1 by ACTH. RAC cells respond to PAHs such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) with a lower induction of CYP1B1 (1.8-fold). Conversely, CYP1B1 is induced 6-fold in rat embryo fibroblast cells by TCDD, presumably through the cytosolic Ah receptor, but is insensitive to cAMP treatment. In cultures of rat mammary cells, CYP1B1 exhibits cell-type specific expression. It is constitutively expressed in fibroblast cells and is highly induced by TCDD. Mammary epithelial cells, however, do not appear to express CYP1B1, and instead selectively induce CYP1A1 to high levels following TCDD treatment. CYP1B1 and CYP1A1 are suppressed by glucocorticoids in mammary fibroblast and epithelial cells, respectively. CYP1B1 expression is increased about 2-fold in mammary fibroblast cells after treatment with 17β-estradiol. Involvement of the estrogen receptor in regulation of CYP1B1 is currently under investigation by use of the anti-estrogen, ICI 182,780. Thus, CYP1B1 exhibits cell-specific regulation, representing the influences of both endogenous (hormonal) and exogenous (PAH) factors. The CYP1B1 response to hormonal stimuli suggests that this protein may have an important physiological role in the steroidogenic and steroid-responsive cells where it is constitutively expressed (adrenal, testis, ovary, mammary, and uterus). Supported by NIH Grant CA16265 and DAMD Grant 17-94-J-4054.

610 INDUCTION OF A PUTATIVE NOVEL ISOZYME OF CYTOCHROME P450 3A IN FEMALE RATS AND THE INHIBITORY EFFECT OF ALCOHOL ON ERYTHROMYCIN N-DEMETHYLATION

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Previous work in this laboratory identified a constitutive cytochrome P450-related catalytic activity in adult female rats, which has high affinity for erythromycin N-demethylation. We found that although this presumptive female specific isozyme is resistant to being induced by several compounds known to induce other cytochrome P450 isozymes, it could be increased by pregnancy and lactation. The highest induction of this enzyme is found in rats treated with 17α-ethinyl estradiol, where about a 5 fold increase in erythromycin N-demethylation was found, but no obvious increase in testosterone 6β-hydroxylation activity, another commonly used marker for 3A isozymes. Immunoblots with polyclonal anti-P450p (3A1) and anti-P4501 (3A2) antibodies show a single band which has the same mobility as that of P450 3A1/3A2, however MAbs specific for P450 3A1 and 3A2 show no band in liver microsomes from untreated female rats or those from rats treated with 17α-ethinyl estradiol. The high affinity erythromycin N-demethylase is also inhibited by anti-P450p or anti-P4501, but not by an inhibitory MAb that recognizes both P450p and P4501. Together, this evidence suggests that this high affinity erythromycin N-demethylase is a 3A related isozyme distinct from P450p or P4501. In addition we found that alcohols (methanol, ethanol) are inhibitory to erythromycin N-demethylase at low concentration, which are usually used as vehicles to introduce substrate into catalytic assays. These results suggest that caution should be taken when these solvents are used to dissolve and introduce chemicals into enzymatic assays.

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611 EFFECTS OF OXANDROLONE TREATMENT ON HEPATIC CYTOCHROME P450 LEVELS IN SPRAGUE-DAWLEY RATS

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Oxandrolone (OXA) (5α-androstan-2-oxa-17α-methyl-17β-ol-3-one) is a clinically useful, synthetic, anabolic androgen steroid hormone. It is related to methyltestosterone, but differs chemically from this compound by the substitution of a lactone ring for the androstane nucleus (A ring). OXA was administered to rats *p.o.* twice daily for three days at 75 mg/kg in order to study the effect on hepatic cytochrome P450 isozymes. Microsomal proteins were separated by SDS-PAGE and were electrotransferred to nitrocellulose membranes, which were probed with monoclonal antibodies raised against various rat hepatic P450 isozymes. Microsomes were also tested for catalytic activity in a testosterone metabolism assay. The data from western blots revealed that in female rats, there were increased levels of immunoreactive P450 2C11 and 3A2, which are known to be male specific, as well as P450 2B1, 2B2, and 3A1. In contrast, male rats showed little or no change in expression of these P450 isozymes after OXA treatment. The 6β-hydroxylation of testosterone, which is related to P4503A1 and 3A2, increased approximately 9 fold in female rats after treatment with OXA (from 0.05 to 0.45 $\pm$ 0.09 nmol/min/mg protein) but only relatively small changes were seen in the male rats (from 1.02 to 1.38 $\pm$ 0.07 nmol/min/mg protein). This data indicates that OXA induces cytochrome P450 isozymes in the female but not in the male rat, and some of these induced isozymes are male specific. Whether these changes are clinically important is not yet known. This work funded in part by grants T32-ES07148 & R01-GM44982.

612 EFFECTS OF ZANOTERONE, A STEROIDAL ANDROGEN RECEPTOR ANTAGONIST, ON CYTOCHROME P450 ISOZYME EXPRESSION IN RATS

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This study was conducted to assess the potential for Zanoterone (Z), a steroidal androgen receptor antagonist, to modulate hepatic cytochrome P450 (CYP) protein levels and activities. Sprague-Dawley rats (6/sex/group) were fed the clinical formulation of Z at doses of 6.25, 25, 100 or 400 mg/kg/d for 14 days. Previously, enzymologic analysis of rat liver microsomes showed that Z induced testosterone 6-B-hydroxylase activity, a probe for the CYP 3A subfamily. To verify the biochemical results, we conducted detailed analysis of CYP isozyme expression by Western immuno-blots for 1A1, 2B1/2, 2E1, 3A and 4A. Changes in protein expression were noted for 2E1 and 3A in the Z treated rats compared to excipient controls. Significant induction of 3A (3-fold increase) occurred for males in both the 100 and 400 mg/kg dose groups. Females, however, showed a 3-fold increase in 3A protein for the 100 mg/kg group and a significantly higher level (4-fold increase) in the 400 mg/kg dose group compared to excipient control. Interestingly, constitutive levels of 3A protein were similar for both males and females. Levels of 2E1 were lower (2-fold decrease) in the 100 and 400 mg/kg male rat groups, while no change in protein levels of 2E1 were detected in the female rats. These results confirm the CYP induction responses that were suggested by the previous enzymologic assays.

613 INDUCTION OF CYTOCHROME P450 ISOZYMES IN DOG LIVER MICROSOMES BY DIFFERENT CYTOCHROME P450 INDUCERS

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The purpose of this study was to determine the effect of oral treatment with phenobarbital (PB), β-naphthofalvone (βNF), dexamethasone (DEX) or isoniazid (IZD) on dog liver P450 isozymes. Male beagle dogs were treated with PB (10 mg/kg for 2 days and 30 mg/kg for the following 5 days), βNF (50 mg/kg for 5 days) or IZD (10 mg/kg for 2 days and 30 mg/kg for the following 5 days). Female beagle dogs were treated with DEX (50 mg/kg for 5 days). Treatment of dogs with PB and DEX produced 33% and 53% increases in the liver/body weight ratio, respectively. Total cytochrome P450 content was increased after treatment with PB (164%) and βNF (86%) while it decreased after treatment with IZD (46%) or DEX (26%). Treatment of dogs with PB produced 84%, 279%, 110%, 95% and 103% increases in 6β-, 16α-, 16β-, 1β and 2β-testosterone hydroxylase activities, respectively. Using western blot analysis with specific antibodies directed against rat liver P450 isozymes, PB treatment produced induction of P450 3A and 2B1/2B2-related protein. Although treatment with DEX resulted in a large increase in liver weight, no significant increase in P450 3A or 6β-hydroxylase activity was detected. However, DEX and IZD treatment produced 46% and 54% increases in chlorzoxazone hydroxylase activity, respectively. Treatment with IZD induced P450 2B1-, 2B2- and 2E1-related protein. Treatment with βNF produced 591% and 257% increases in EROD and chlorzoxazone hydroxylase activities, respectively. Both P450 1A1 and 1A2-related protein were induced to the same extent by βNF. Thus, the response of the dog to P450 inducers differs from the rat in some cases.

614 FLUVASTATIN IS AN EFFECTIVE INDUCER OF CYP2B mRNA IN PRIMARY CULTURED RAT HEPATOCYTES

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Lovastatin (LOVA), an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, was previously demonstrated to induce the mRNAs for several cytochromes P450 in primary cultures of rat hepatocytes (*Toxicol. Appl. Pharmacol.* 120: 298–307, 1993). To test whether other drugs of this class are P450 inducers, the effects of three additional HMG-CoA reductase inhibitors, simvastatin (SIMVA), pravastatin (PRAVA) and fluvastatin (FLUVA), were tested on P450 mRNA expression in primary rat hepatocytes cultured on Matrigel-coated dishes in serum-free Williams Medium E containing 0.25 U/ml insulin. Cultures were incubated with drugs during the 48 to 72 hr interval following placement of hepatocytes into culture, and CYP1A, 2B, 3A and 4A mRNA levels were assessed by Northern blot analysis. Consistent with previous findings, treatment of hepatocytes with 3×10^{-5} M LOVA produced modest increases in CYP2B and CYP4A and a slight increase in CYP3A mRNA levels. Treatment with 3×10^{-5} M SIMVA produced effects nearly identical to those of LOVA, while PRAVA treatments were ineffective. In contrast, 3×10^{-5} M FLUVA induced CYP2B mRNA to levels that were ≈2- to 5-fold greater than those produced by 10^{-4} M phenobarbital (PB). FLUVA also induced CYP4A mRNA to higher levels than did LOVA or SIMVA, although the maximum induction by FLUVA was only ≈50% of that produced by 10^{-4} M ciprofibrate. FLUVA induced CYP3A mRNA only slightly, as observed for LOVA and SIMVA. CYP2B induction by FLUVA was not affected by the presence of up to 10^{-8} M dexamethasone (DEX) in the medium, but was suppressed by higher concentrations of DEX. In contrast, CYP2B induction by PB was progressively potentiated by 10^{-8} to 10^{-7} M DEX. These results demonstrate that FLUVA is an effective inducer of CYP2B and CYP4A mRNAs in cultured rat hepatocytes, and suggest that the mechanism of CYP2B induction by FLUVA may differ from that of PB. Supported by NIH grant HL50710.

615 ETHYLMORPHINE N-DEMETHYLASE ACTIVITY IN MICROSOMES FROM MALE AND FEMALE RATS TREATED WITH PREGNENOLONE-16α-CARBONITRILE, ETHANOL, PHENOBARBITOL, β-NAPHTHOFLAVONE, CLOFIBRATE AND ISONIAZID

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The purpose of this study was to compare the potential of ethanol (EtOH), pregnenolone-16α-carbonitrile (PCN), phenobarbitol (PB), β-naphthoflavone (β-NF), clofibrate (CF), and isoniazid (ISO) to induce ethylmorphine N-demethylase (EMD) activity in hepatic microsomes. Male and female Sprague-Dawley rats were dosed with either PCN (50 mg/kg/day for 5 days), EtOH (500 mg/kg/day for 8 days), PB (100 mg/kg/day for 4 days), β-NF (80 mg/kg for 1 day), CF (300 mg/kg/day for 14 days), or ISO (100 mg/kg/day for 3 days). Microsomes were isolated from liver homogenates, frozen, and subsequently assayed for protein and EMD. In males and females, significant ($p < 0.01$) elevations in EMD activity were observed in microsomes from PB- and PCN-dosed animals compared to controls. In males, a lesser elevation was noted in microsomes from EtOH-dosed rats. In contrast, microsomes from β-NF-dosed males showed a reduction in enzyme activity when compared to control microsomes; CF and ISO caused no change in enzyme activity. In females, no significant changes were noted in microsomes from β-NF, ISO, CF, or EtOH-dosed animals. In conclusion, the greatest elevations in EMD activity were observed in microsomes from rats dosed with PCN or PB in both sexes. Immunoblotting procedures clearly detected the induction of cytochrome P450 IIIA in these microsomal preparations from livers of male rats treated with PCN and PB, but not in liver microsomes from rats treated with EtOH, CF, or β-NF, inducers of cytochromes IIE, IVA, and IA1, respectively. Thus, in addition to the known elevation of EMD by PCN, these data clearly indicate that EMD can be used as a selective indicator of rat hepatic cytochrome P450 IIIA activity in either sex.

616 INDUCTION OF 7-PENTOXYRESORUFIN O-DEALKYLATION (PROD) IN MICE TREATED WITH CYP1A ENZYME INDUCERS

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Induction of rat CYP2B by phenobarbital (PB) results in a marked increase (>20 fold) in liver microsomal PROD activity, whereas induction of CYP1A by β-naphthoflavone (BNF), 3-methylcholanthrene (3MC) or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) results in a marked increase (>20 fold) in liver microsomal EROD activity. Treatment of rats with PB increases EROD activity up to 3 fold, hence, EROD distinguishes between PB and TCDD with a *discrimination factor* of at least 7. Similarly, treatment of rats with BNF, 3MC or TCDD causes up to a 2-fold increase in PROD activity, hence, PROD has a discrimination factor of at least 10 in rats. However, in B6C3F1 and CD-1 mice, the discrimination factor for PROD activity was only 2 or 3, meaning that PROD activity did not readily distinguish CYP2B induction from CYP1A induction. For example, treatment of B6C3F1 mice with TCDD caused a 4- to 6-fold increase in PROD activity, which was similar in magnitude to the 10- to 15-fold increase caused by PB. The discrimination factor for EROD activity was just as high in mice as it is in rats. For example, TCDD increased mouse EROD activity 22 fold, whereas PB caused only a 1.9-fold increase (discrimination factor = ≈12). In the case of mouse PROD activity, a low discrimination factor was observed regardless of substrate concentration (over the range 2 to 50 μM 7-pentoxyresorufin). PROD activity in mouse liver microsomes was 1.5- to 2-times higher when the substrate was added in methanol rather than DMSO, but solvents had no major effect on the discrimination factor. These results suggest that, in terms of PROD activity, mouse CYP2B may be only 2- to 3-times more active than the mouse

CYP1A enzymes, which differs from the situation in rats. Supported by NIH grant ES03765.

617 DELAYED INDUCTION OF PULMONARY CYP2B IN MICE AFTER COADMINISTRATION OF NITROSO-DIMETHYLAMINE (NDMA) AND ETHANOL

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Ethanol coexposure potentiates tumorigenesis in mouse lung by NDMA, probably by inhibiting hepatic clearance. Exploration of biochemical correlates of this led to an unexpected finding. Male Swiss mice were exposed to 1 or 5 ppm NDMA plus 1% ethanol in drinking water for 2 weeks. After cessation of treatment, mice were killed at 1, 4, or 10 weeks. In lungs of mice given 5 ppm NDMA/ethanol, the activity of cytochrome (cyp) P450 2B-specific benzyloxyresorufin O-dealkylase (BenzROD) increased progressively over the 10 week period, achieving significance at the 4- and 10-week endpoints. This effect was confirmed by Western immunoblotting, with a 2-fold increase in reactive protein. Preliminary Northern blot results indicate an increase in gene expression. Significant effects were also seen after 5 ppm NDMA was given alone at the 1 and 4 week time points. No effects were seen with 1 ppm NDMA with or without alcohol or alcohol alone. Thus the effect seems to be mediated by NDMA, and the ethanol may act primarily to increase the NDMA dose to the lung. This delayed, NDMA-mediated stimulation of pulmonary cyp2B has interesting toxicological implications. Its mechanism is unknown at present.

618 REGULATION OF HEPATIC CYTOCHROME P450 3A1 AND 3A2 ISOZYMES IN RAT FOLLOWING TREATMENT WITH DEXAMETHASONE

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Regulation of CYP P4503A1 and 3A2 was studied in rat liver following treatment with dexamethasone. Adult male and female rats were injected with a single high dose of dexamethasone (100 mg/kg, i.p.), and a time dependent increase of CYP3A1 and 3A2 was studied both at the protein and mRNA level by Western blots using isozyme-specific monoclonal antibodies, and Northern blots, respectively. Dexamethasone at the above dose increased 3A1 mRNA significantly over controls at 24 hr and 48 hr time points in both males and females. Interestingly, 3A1 protein could be detected as early as 12 hr in both sexes, and in females the level appeared to be little higher than that in males. Both CYP3A2 mRNA and protein in male rats were increased as early as 3 hr after treatment. This elevated level of both mRNA and protein was maintained as late as 48 hr, after which both started to decline. In female rats, no increase in CYP3A2 mRNA was detected by Northern blot analysis using total RNA. Additionally, while for 3A2 both mRNA and protein increased rapidly in males at 3 hr and remained high as late as 96 hr, for 3A1 both mRNA and protein increased sharply at 24, further increased at 48 hr and then rapidly decreased therafter in both males and females. In conclusion, the profiles of increase of 3A1 and 3A2 mRNA as well as protein following dexamethasone treatment, were different. This suggests the existence of divergent regulatory mechanisms of these two genes. (Supported by NIH Grant RO1-GM44982).

619 HEPATIC DIFFERENTIALLY DISPLAYED GENES AFTER DEXAMETHASONE TREATMENT IN RATS

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Steroid hormones are derived in specific tissues from cholesterol requiring the common intermediate δ^5-pregnenolone. Many steroid hormones (from natural or synthetic sources) are commonly prescribed for different medical conditions, among these are asthma and allergies. Dexamethasone (DEX) is a typical example of a synthetic steroid hormone used for these purposes. This steroid has an inhibitory effect on the immune system and affects carbohydrate, lipid and protein metabolism. Steroids have also been described as catatoxic agents due to their protective action by reducing the toxicity of xenobiotics agents through gene induction. It has been reported that steroid treatment produces increases in NADPH-cytochrome-c-reductase and cytochrome P450. This effect could result either by control of mRNA templates for enzyme synthesis or by changes in translational efficiency of mRNA. This research studies the effect of DEX on gene induction or inhibition. Sprague-Dawley female rats were treated with a carrier or DEX at 85 mg/kg for two consecutives days. RT-PCR differential display was employed for the isolation and the identification of genes affected by DEX. cDNA fragments were identified using slight modifications to the previous described technique of Liang, et. al. A total of fifty DEX induced and nine inhibited cDNA fragments were identified and isolated by this technique. Thus far we have positively identified five of these cDNA fragments as recognizing differentially expressed mRNAs. The size of these fragments have been estimated between 2–5 hundred base pairs. True positives were identified on Northern blots using randomly labeled probes. The identification and characterization of genes affected by commonly prescribed drugs is the first step in order to understand and study their regulation which can aid in the development of new ways for therapeutic treatment. Cloning of these fragments is currently being conducted for specific cDNA sequencing.

620 MUSK XYLOL (MX) INDUCES AND INHIBITS MOUSE CYTOCHROME P-450 2B ENZYMES

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The purpose of this work was to characterize the profile and dose-response relationship of microsomal enzyme induction following exposure to MX (1,3,5-trinitro-2-*t*-butylxylene). MX was dosed by gavage to male B6C3F1 mice for 7 days at 0, 1, 5, 10, 20, 50, 100 and 200 mg/kg, and microsomes were prepared. At 200 mg/kg, MX increased liver weight by 65%, caused hepatocellular hypertrophy and increased total cytochrome P-450 2-fold over control. MX increased EROD and MROD activity 4- and 2-fold, results consistent with increased 1A1 and 1A2 protein levels determined by Western blotting. The NOEL for CYP1A induction was 100 mg MX/kg. No increase in PROD activity was seen, but MX treatment did increase CYP2B protein levels (NOEL of 10 mg/kg). In vitro studies showed that MX competitively inhibited mouse CYP2B enzymes (IC_{50} = 1 μM), but did not cause mechanism-based inactivation of these enzymes. To study inhibition of CYP2B enzymes in vivo, mice were treated with pheno-barbital (PB; 0.05% in drinking water for 5 days), then given a single dosage of corn oil or MX (200 mg/kg) 18 hours before necropsy. PROD activity in PB/MX treated mice (92 ± 16 pmol/min/mg) was decreased 90% relative to PB/control (1022 ± 60 pmol/min/mg). However, in mice dosed orally with antibiotics throughout the study, MX did not inhibit PB-induced PROD activity. Thus, MX causes hepatic changes similar to classical CYP2B inducers. However, MX also inhibits CYP2B enzymes, an action which likely results from reduction of the nitro function by intestinal microflora.

621 EFFECTS OF LOW LEVEL DIETARY EXPOSURE TO AROCLORS 1242 OR 1260 ON HEPATIC CYTOCHROMES P450 IN FEMALE F344/NCR RATS

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Female F344/NCr rats were fed various concentrations (1–48 ppm) of Aroclors continuously in the diet for 84 days or to 48 ppm dietary Aroclor for various durations of time. Aroclors 1242 and 1260 each caused time-dependent increases in hepatic *O*-dealkylation activities. Exposure for 84 days to Aroclor 1260 resulted in a concentration-dependent phenobarbital-type induction of CYP2B, CYP3A, epoxide hydrolase and hepatocytomegaly, while more minimally inducing the CYP1A subfamily. In contrast, an 84-day exposure to Aroclor 1242 resulted primarily in a TCDD-type response, with relatively high induction of the CYP1A subfamily and limited induction of CYP2B. The sensitive nature of the alkoxyresorufin *O*-dealkylation assays allowed one to detect statistically significant induction at dietary concentrations as low as 1 ppm (Aroclor 1242) or 3.3 ppm (Aroclor 1260). These results will be compared with our published results concerning exposure of female F344/NCr rats to Aroclor 1254.

622 EFFECTS OF OCTAMETHYLCYCLOTETRASILOXANE (D4) ON LIVER SIZE AND CYTOCHROME P450 IN FISCHER RATS: A 28 DAY WHOLE BODY INHALATION STUDY

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Previous studies suggest that D4 causes liver enlargement and proliferation of hepatic endoplasmic reticulum. Therefore, the primary objective of this study was to examine the effects of D4 on liver size and P450 induction over time and dose. Male and female rats were exposed to 0, 70, and 700 ppm D4 in whole body inhalation chambers for 28 days, 6 hr/day, 5 days/week. Recovery groups were also included. Animals were sacrificed at various times during the study. Liver:body weight ratios were determined and compared to controls. Hepatic

microsomes were prepared and analyzed for total P450 and ethoxycoumarin-O-deethylase (ECOD) activity. Both males and females exposed to 700 ppm D4 had increased liver size. Females ranged from approximately 3% on day 3, to 20% on day 28. In contrast, male liver size in this dose group was increased by approximately 12% on day 3, and 14% on day 28. Hepatomegaly was less evident in the 70 ppm groups, but a similar trend was observed. Both male and female liver size decreased by day 14 of recovery. Total P450 in the 700 ppm groups increased 54% in females and 43% in males, while ECOD activity increased 126% in females and 109% in males on day 14. In the 70 ppm groups, ECOD was increased 25% in females and 48% in males on day 14. These findings suggest that exposure to D4 increases liver size and induces P450 enzymes in a dose and time-dependent manner.

623 EFFECTS OF DOXYLAMINE SUCCINATE (DOX) ON THYROID HORMONE BALANCE AND ENZYME INDUCTION IN MICE AND RATS

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DOX is a nonprescription antihistamine and hypnotic. Recent two-year carcinogenicity bioassays have shown benign hepatocellular adenomas and benign thyroid follicular cell adenomas in DOX-treated B6C3F1 mice. To determine whether the thyroid adenomas seen in the bioassays could be attributed to alterations in serum thyroid hormone and thyroid stimulating hormone (TSH) concentrations secondary to hepatic enzyme induction, we have studied the effects of DOX on thyroid hormone balance in mice and enzyme induction in mice and rats. B6C3F1 mice were given up to 1500 ppm DOX in the diet (350–450 mg/kg/day) for up to 15 days. Fischer 344 rats were given up to 2000 ppm DOX in the diet (≈135 mg/kg/day) for 8 days. In both rats and mice, DOX showed a pattern of enzyme induction similar to phenobarbital, a CYP 2B inducer. DOX was equivalent in potency to phenobarbital in the mice, but was less potent in the rats. Large increases in the O-dealkylation of 7-pentoxyresorufin and in testosterone 16β-hydroxylation were indicators of CYP 2B induction. Antipyrine plasma clearance measured in the rat was also increased with DOX. Hepatic thyroxine glucuronidation activity and serum TSH levels were increased and serum thyroxine levels were decreased in the mice. Since previous studies have shown DOX is nongenotoxic, it appears similar to other nongenotoxic phenobarbital-type inducers that produce liver tumors in rodents. The thyroid tumors likely arose via TSH stimulation of the thyroid secondary to increased hepatic metabolism of thyroxine.

624 RIFAMPICIN TREATMENT ELICITED CHANGES IN RABBIT LIVER DRUG METABOLIZING ENZYMES

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The effect of daily treatment of adult male N.Z. White rabbits with rifampicin (10–100 mg/kg, i.p. in corn oil for five days) on drug metabolizing enzymes was investigated. Cytochrome P450 concentration showed dose dependent increases of 50 to 100% and no evidence of the presence of any P450 metabolic-intermediate (MI) complex. 7-Ethoxy- (isozyme 1A) and 7-pentoxy- (isozyme 2B) resorufin dealkylase and 4-nitrophenol hydroxylase (isozyme 2E) activities were not increased. Accompanying the elevation in isozyme 3A, monitored by increases in erythromycin demethylase activity and *in vitro* troleandomycin (TAO) P450 MI complex formation, was an increase in the P450 MI complex formation rate from other amines. The rate for fluoxetine was similar to that for TAO, rates for norpropoxyphene and norbenzphetamine were 50% higher while those for acetylmethadol, SKF 8742A and SKF 525A were 200% greater. The rate for SKF 525A was comparable with that seen in phenobarbital induced microsomes while the rate for norbenzphetamine was much less. Amine P450 MI complex formation was absent from microsomes of untreated animals. UGT activities towards both 1-naphthol and morphine were induced up to 100% by rifampicin treatment. Cytosolic enzyme activities, glutathione S-transferase (CDNB), NAD(P)H quinone oxidoreductase and sulphotransferase (4-nitrophenol), were unchanged.

625 THE EFFECT OF TRICHLOROETHYLENE ON CYTOCHROME P450 FORM-SPECIFIC ACTIVITIES OF MOUSE, RAT AND HUMAN MICROSOMES

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Trichloroethylene (TCE) is a commonly used industrial solvent and a frequent environmental contaminant; therefore exposure to TCE may frequently occur in certain human populations. TCE has been found to cause hepatocellular carcinoma when administered by gavage to B6C3f1 mice, but not rats; this observation implies that TCE is metabolized differently in the two species. As part of an investigation of TCE metabolism, we investigated the effect of TCE on form specific P450 activities in microsomes from mouse, rat and human. TCE (1000 ppm), added to the headspace of reaction vessels, inhibited CYP2E1 dependent activity (N-nitrosodimethylamine N-demethylase [DMN] and p-nitrophenol hydroxylase, [PNPOH]) in all three species and CYP3A activity (BROD) in mice and rats; TCE (1000 ppm) increased CYP1A1/1A2 activity (EROD and phenacetin O-deethylase), and had no effect on CYP2A activity (coumarin hydroxylase). Inhibition kinetic studies using mouse microsomes demonstrated that TCE was a competitive inhibitor of PNPOH and BROD with *Ki* of 50 and 250 ppm, respectively. Preincubation of microsomes with TCE and a source of NADPH resulted in decreased absorbance of CO-bound P450, which correlated with the NADPH-dependent uptake of TCE from the incubation-vessel headspace. This work demonstrates that TCE is likely metabolized by CYP2E1 in all three species and by CYP3A in mouse and rat, and that TCE can interact with other form-specific activities in ways other than inhibition.

626 RUNNING EXERCISE INHIBITS HEPATIC CYP2E1 FUNCTION IN FISCHER 344 RATS

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We have previously shown that a moderate running exercise regimen inhibits hepatic CYP2E1 in male Fischer 344 rats, evidenced by lower activities towards the p-hydroxylation of aniline by hepatic microsomes of exercised rats compared to those of sham-treated rats. The exercise effects appear to be selective since the same running protocol did not change ethoxyresorufin metabolism. Furthermore, using CCl_4 which is bioactivated by CYP2E1, we showed that hepatocytes prepared from exercised rats were less sensitive to CCl_4-mediated toxicity indicating that the exercise effects on CYP2E1 activity were functional at the cellular level. The present studies were therefore initiated to determine the mechanisms that could account for the decreases in CYP2E1 activity with moderate running exercise. Male Fischer 344 rats were exercised on a motorized treadmill, 24 m/min, 60 min/day 4 times per week for 2 months. Sham-treated rats were placed on the treadmill at 8 m/min for 5 min periods once a week for 2 months. In order to determine if running exercise altered the levels of CYP2E1 protein, hepatic microsomes were prepared and Western immunoblot analysis effected using a monoclonal antibody selective for the CYP2E1 isoform. Detection of the antigen-antibody complexes was by enhanced chemiluminescence. The results of immunoblot studies show that the exercise regimen caused about 30% reductions in CYP2E1 protein levels. Thus, reductions in CYP2E1 protein with exercise may explain exercise-associated decreases in aniline metabolism and CCl_4 toxicity. We also performed Northern blot analysis of RNAs extracted from the livers of exercised and sham-treated rats so as to assess whether moderate running exercise alters the steady-state levels of CYP2E1 mRNA. The results of our preliminary work using CYP2E1 cDNA as a probe indicate that exercise attenuates the level of CYP2E1 mRNA levels. The exercise-associated decreases in CYP2E1 mRNA could be mediated by reductions in the rate of transcription of the CYP2E1 gene and/or decreased stability of that mRNA.

627 MAINSTREAM CIGARETTE SMOKE EXPOSURE ALTERS CYTOCHROME P4502G1 EXPRESSION IN F344 RAT OLFACTORY MUCOSA

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Inhalation of mainstream cigarette smoke (MCS) by rats results in multifocal rhinitis, mucous hypersecretion, nasal epithelial hyperplasia and metaplasia, and focal olfactory mucosal atrophy. In humans, cigarette smoking causes long-term, dose-related alterations in olfactory function. Cytochrome P4502G1 (2G1) is expressed exclusively in olfactory mucosa and may play a role in olfactory function by activating or inactivating inhaled odorants. To examine the effect of MCS exposure on 2G1 protein expression, male CDF (F344)/CrlBR rats were exposed to filtered air or 250 mg/m^3 MCS, 6 h/day, 5 days/wk, for 10 wk or 30 wk (n = 5/group). Relative levels of immunoreactive 2G1 protein in the olfactory surface epithelium were assessed by microdensitometric analysis of immunohistochemically stained nasal tissue sections. Compared to filtered air controls, MCS exposure reduced the amount of immunoreactive 2G1 protein in the surface epithelium by 30% and 58% in rats

exposed for 10 wk and 30 wk, respectively. If 2G1 does play a role in olfaction, its decreased expression in olfactory neuroepithelium following MCS exposure may contribute to alterations in olfactory function experienced by chronic human smokers. (Research sponsored by the Office of Defense Programs and the U.S. DOE/OHER under Contract No. DE-AC04-76EV01013.)

628 EFFECT OF CYTOKINE TREATMENT ON CYTOCHROME P450 CONTENT AND ACTIVITY IN CULTURED RAT HEPATOCYTES

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Liver injury due to chemical exposure is a two stage process in which injury initiated by a chemical is propagated and/or ameliorated by endogenous mediators such as macrophage derived cytokines. Cytokines have been shown to down-regulate cytochrome P-450 (P-450) levels and decrease certain P-450 activities in hepatocytes and *in vivo* (e.g., Abdel-Razzak et al., Mol. Pharm. 44:707–715, 1993; Ghezzi, et al., BBRC 136:316–321, 1986). To understand the extent and mechanisms of cytokine mediated P-450 regulation, we have examined the effects of direct cytokine treatment on constitutive P-450 content and activity in hepatocyte culture. A combination of tumor necrosis factor-α, interleukin-1β, and interferon-γ was found to decrease P-450 content in a dose dependent manner. In contrast, the O-dealkylation activity of 3-cyano-7-ethoxycoumarin was found to increase dose dependently in response to the cytokine treatment. Cytokine treatment apparently exerts selective regulation of various P-450 enzymes, and while P-450 is, in general, down-regulated, it appears that an enzyme that mediates 3-cyano-7-ethoxycoumarin dealkylation is maintained or induced. The cytokine mixture also induced nitric oxide synthase, and it has been proposed that nitric oxide is an important regulator of P-450 *in vivo* (Khatsenko et al., PNAS 90:11147–11151, 1993). Inhibition of nitric oxide formation with N^G-monomethyl-L-arginine and N^W-nitro-L-arginine methyl ester significantly prevented the decrease in P-450 content, suggesting that nitric oxide may be involved in the cytokine mediated down-regulation of P-450. Supported by NIH grant DK44755.

629 EFFECTS OF LIFETIME LEAD EXPOSURE ON CYTOCHROME P450 EXPRESSION AND TESTOSTERONE METABOLISM IN RAT LIVER, KIDNEY AND BRAIN

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Acute lead exposure is well known to produce suppression of hepatic oxidative drug metabolism and loss of cytochrome P450 content. This has been suggested to be associated with decreased heme biosynthesis due to inhibition of δ amino laevulinic acid synthetase. Much less is known of the chronic effects of lead exposure on this system and on P450 expression in extra-hepatic tissues. In the current study, either lead acetate at 0.6% (v/v) in drinking water or an equivalent amount of acetic acid was administered to time impregnated female Sprague Dawley rats (N = 6–9/group) beginning on gestational day 4. Exposure was continued in the pups following weaning until age 85 d. At sacrifice liver, kidney and brain and plasma were collected from N = 8–12 male and female pups/treatment group. Microsomes were prepared from liver and kidney and assayed spectrophotometrically for cytochrome P450 content. In addition, metabolism of [^{14}C]testosterone, a model monooxygenase substrate, was measured in liver and kidney microsomes and in brain homogenates, male specific CYP 2C11 apoprotein assayed by Western blot analysis and plasma testosterone concentration measured. Spectral P450 content was significantly higher in control male than female microsomes from both liver and kidney ($p < 0.05$). Lifetime lead treatment significantly reduced spectral P450 content in male liver and kidney microsomes 35–50% ($p < 0.05$), but resulted in a much smaller 25% decreases in P450 content in female organs. In addition, metabolism of testosterone was found to be sexually dimorphic in both liver and kidney but not in brain. Lifetime lead treatment had no effect on testosterone metabolism in liver microsomes or brain homogenate in spite of a suppression of circulating testosterone to castrate levels in lead-treated males. However, male predominant testosterone 2α- and 16α-hydroxylation were suppressed in male kidney microsomes accompanied by a loss of immunoreactive CYP 2C11 apoprotein. This suggests selective effects of lead exposure on extra hepatic androgen metabolism secondary to alterations in circulating sex steroids rather than a generalized loss of P450 due to alterations in heme biosynthesis. Supported by R01 ES06684 (MJJR).

630 DIFFERENCES IN THE P450 REGULATORY EFFECTS OF CHLORAMPHENICOL AND CYCLOPHOSPHAMIDE BETWEEN SPRAGUE-DAWLEY AND FISCHER 344 RATS

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Chemically-mediated P450 suppression or induction is frequently characterized by species dependence. When Sprague-Dawley (SD) rats were given either chloramphenicol (CAP) or cyclophosphamide (CPA), suppression of P450 2C11 (2C11) was observed 6 days after a single i.p. dose. However, CAP caused no loss of 2C11 protein in Fischer 344 (F344). Treatment of rats with 130 mg/kg CPA produced non-specific P450 suppression in both strains, with F344 rats being more sensitive than SD as indicated by a greater loss of total P450. Loss of 2C11 was the most pronounced change in P450 in both strains with a significantly greater decrease in 2C11 occurring in F344 than SD at both 50 and 130 mg/kg. Additionally, CPA elicits induction of immundetectable P450 1A1 in SD but not F344 rats. These data suggest that chemically-mediated P450 regulation in rats may involve mechanisms that are strain-specific. In individual rats, plasma thyroxine (T_4), a factor known to regulate several P450 enzymes, was found to be correlated with the decline in 2C11 produced by CPA at 130 mg/kg in SD whereas the same relationship was observed at 50 mg/kg in F344 rats. CPA (130 mg/kg) caused a loss of both testosterone and T_4 in F344 rats to levels below the limits of detection, while no loss of testosterone was detected in SD at the same dose. CAP-treated SD rats also displayed a decline in T_4 proportional to the loss of 2C11. Correspondingly, no loss of T_4 was observed in CAP-treated F344 rats, which are refractory to 2C11 suppression by CAP. In CPA-treated animals the decrease in T_4 was proportional to the lack of weight gain over the 6 day period after dosing and was likely to have been the result of inadequate dietary caloric intake. This commonly occurs in animals treated with alkylating anti-neoplastics. No deviation from normal weight gain was found in animals treated with CAP, suggesting that CAP and CPA bring about T_4 loss by different mechanisms. Whether T_4 loss is causally linked to 2C11 suppression by these agents has yet to be determined. These studies demonstrate that strain as well as species appears to be of importance in assessing the changes in P450 levels produced by exogenous compounds. Supported by Grants ES03619 (JRH), Core Center Grant to the University of Arizona ES06694, GM46897 (ETM) from the National Institutes of Health and National Research Service Award F32 ES05613 (JCK).

631 ACUTE EFFECTS OF THE COLON CARCINOGEN 1,2-DIMETHYLHYDRAZINE ON CYTOCHROME P-450 2E1 AND 1A1 AND ALCOHOL DEHYDROGENASE IN INBRED MICE

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1,2-Dimethyldrazine (DMH), an organotropic colon carcinogen, is metabolized to reactive species that methylate DNA. Indirect evidence supports cytochrome P4501A1 involvement in DMH metabolism in the rat model while CYP2E1 and alcohol dehydrogenase (ADH) have been shown to metabolize DMH intermediates directly. To evaluate the acute effects of DMH on these metabolic enzymes in AKR/J (colon tumor resistant) and SWR/J (susceptible) mice, subcutaneous injections of 40 mg/kg DMH were given 20 hours prior to sacrifice. Quantitation of P450 isoforms by Western analysis revealed a significant decline in hepatic-CYP1A1 in both strains treated with DMH. Liver CYP2E1 was also lowered after treatment, but only in SWR/J mice. Immunoreactive levels of colonic CYP1A1 and CYP2E1 were unchanged in either strain after DMH treatment. However, the levels of CYP2E1 in the colons of SWR/J mice were higher than in AKR/J, both before and after carcinogen treatment. The activities of hepatic CYP1A1 and CYP2E1 were measured by O-deethylation of ethoxyresorufin and hydorxylation of p-nitrophenol, respectively. Functional activity correlated with levels of immunoreactive protein. ADH activity, measured by oxidation of ethanol to acetaldehyde, was significantly reduced in livers of both strains treated with DMH. No change in colonic ADH activity was observed in either strain. These data suggest that tumor susceptibility to DMH may be partially explained by acute strain-specific alterations in metabolic enzymes particularly CYP2E1, that affect disposition of reactive metabolites to target tissue.

632 THE PROFILE OF DRUG METABOLIZING ENZYMES INDUCED BY HETEROCYCLIC NAPHTHALENE AND PHENANTHRENE DERIVATIVES CONTAINING A SINGLE NITROGEN ATOM

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Induction of drug metabolizing enzymes by phenanthrolines has shown that when induction occurs these compounds selectively affect phase II (conjugation) activities. In the present study the effect of quinolines, benzoquinolines and phenanthridine on hepatic drug metabolizing enzymes was investigated. Male rats were treated with quinoline (Q), isoquinoline (IQ), 5,6- (5,6-BQ) or 7,8- (7,8-BQ) benzoquinoline or phenanthridine (P) at 75 mg/kg, i.g. for 3 days. Induction of conjugation activities was seen with all compounds. The agents Q, 7,8-BQ, and P all showed 2–3 fold increases in UGT activity towards 4-nitrophenol, 1-naphthol, and morphine. Changes in these activities following treatment with IQ and 5,6-BQ were much less. Changes in UGT activity towards estrone and testosterone were minor for all compounds. Glutathione S-transferase (CDNB) activity increased only 25–50% for the agents investigated. Only 7,8-BQ caused a dramatic elevation in NAD(P)H quinone oxidoreductase activity. Accompanying this unique property, 7,8-BQ also produced a major increase in P450. This increase appears to be in the IA isozyme(s) since the CO absorbance maximum showed a 1.5 nm hypsochromic shift and 7-ethoxyresorufin deethylase activity increased from non-dectectable in untreated rats to 1.3 nmole/mg/min. No changes in pentoxyresorufin dealkylase (2B isozyme), 4-nitrophenol hydroxylase (2E isozyme) or erythromycin demethylase (isozyme 3A) activities were observed. Thus, heterocyclic naphthalene and phenanthrene derivatives containing a single nitrogen show induction profiles ranging from low to high magnitude conjugation-selective, but include a compound, (7,8-BQ) which also acts in a polycyclic hydrocarbon-like manner to induce the P450 IA.

633 EFFECTS OF TOTAL ENTERAL NUTRITION AND CHRONIC ETHANOL TREATMENT ON EXPRESSION OF CYP 4A ISOZYMES IN COLON OF MALE RATS

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Arachidonic acid metabolites generated by the action of cytochrome P450-dependent microsomal monooxygenase are known to play an important role in modulation of endogenous physiological processes such as regional blood flow and epithelial water and electrolyte transport. A recent report demonstrated P450-dependent metabolism of arachidonic acid to the ω and ω-1 metabolites, 20 and 19-HETE (known to be catalyzed by isozymes in P450 family 4A), in the rabbit intestine. We examined distribution of P450 isozymes in gene family 4A in the rat colon and their responses to total enteral nutrition (TEN) and ethanol, treatments known to produce significant alterations in large intestinal function. Groups of 300 g male Sprague-Dawley rats (N = 6–10) were fed either standard rat chow *ad libitum* or infused with defined diets via a TEN delivery system for 35 days. In some TEN diets, 39% carbohydrate calories were replaced by ethanol. Following sacrifice, colon was dissected from the intestine and microsomes prepared by ultra centrifugation. Western blot analysis using a well characterized sheep anti-rat CYP 4A1 antibody indicated the presence of three cross-reactive P450 apoproteins in colon of which the two slowest migrating appeared to correspond to rat liver CYP 4A3 and CYP 4A1. TEN produced a significant ($p < 0.05$) decrease in all three apoproteins compared to chow-fed animals. In addition, chronic ethanol treatment produced a significant ($p < 0.05$) increase in the band corresponding in mobility to CYP 4A1. Thus, diet and ethanol treatment are capable of altering CYP 4A expression in the colon. These results may be significant in relation to GI function. For example, alterations in CYP 4A expression may result in significant alterations in large intestinal motility, absorption and blood flow via alterations in patterns of P450-dependent arachidonic acid metabolites. Supported by AA08645 (TMB).

634 A RAT PROXIMAL TUBULE CELL CULTURE MODEL FOR ASSESSING CELL PROLIFERATION

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An *in vitro* model using rat proximal tubule (RPT) cultures was evaluated for the capability to determine the mitogenic potential of xenobiotics. RPT cells were isolated using collagenase digestion and cultured in a basal medium consisting of Hams F12:DME cell culture media (50:50) supplemented with 0.1% BSA for three days. The cultures formed colonies of monolayer cells retaining differentiated RPT cell functions as evidenced by high levels of sodium dependent glucose transport and the RPT marker enzymes alkaline phosphatase and gamma glutamyltranserfase. RPT cultures were treated with 1,4-dichlorobenzene (DCB) at 30, 100 and 300 μg/ml, gentamicin at 10, 30 and 100 μg/ml or epidermal growth factor (EGF) at 1, 10 and 30 ng/ml in basal media or in basal media supplemented with 10 ng/ml insulin and cholera toxin. Cell proliferation, as measured by increased cell number and thymidine incorporation, was assessed at 24, 48 and 72 hr. Gentamicin had no effect, while EGF and 1,4-DCB induced moderate cell proliferation. Thymidine incorporation in basal media was maximally increased 31% and 58% for 100 μg/ml DCB and 10 ng/ml EGF, respectively. Thymidine incorporation in supplemented basal media was maximally increased 80% and 100% for 30 μg/ml DCB and 10 ng/ml EGF, respectively. Cell numbers were maximally increased 47% and 90% for 100 μg/ml DCB and 10 ng/ml EGF in basal media, respectively. Cell numbers were maximally increased 68% and 105% for 30 μg/ml DCB and 10 ng/ml EGF in supplemented basal media, respectively. Although moderate cell proliferation was observed in these experiments further studies are necessary to optimize and validate the utility of this model.

635 STABLE ALTERATIONS IN THE COMPOSITION OF THE NUCLEAR MATRIX AND INTERMEDIATE FILAMENT PROTEINS AFTER LONG-TERM EXPOSURE OF LLC-PK1 CELLS TO LOW DOSES OF THE NEPHROTOXIN DICHLOROVINYLCYSTEINE

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LLC-PK1 cells were exposed for 7 weeks to low, non toxic doses (1 and 5 μM) of dichlorovinylcysteine, the key metabolite of the nephrocarcinogen trichloroethene. Thereafter morphologically altered clones were picked under the microscope and cultured in absence of dichlorovinylcysteine for four to eight weeks. In stable clones that retained their altered morphology after this drug-free period, we investigated cell proliferation, glucose transport, dome formation as well as composition of nuclear matrix and intermediate filament proteins by two-dimensional gel electrophoresis. Compared with the physiological cuboidal shape of these renal cells, the treated clones consisted mainly of elongated, spindle-shaped cells exhibiting the tendency to form polymorphic borders. Although treated clones and control cells revealed nearly the same proliferative capacity, glucose transport and dome formation were severely impaired in the 1 μM and 5 μM clones in a dose dependent-manner, indicating loss of membrane polarity. In addition, the two-dimensional gel electrophoresis of the isolated nuclear matrix and intermediate filament proteins showed that treatment resulted in an altered protein composition, mainly reflected as a reduction in the number of proteins in the 1 μM and more so in the 5 μM clones. The possible role of these stable changes in cell biochemistry in the renal specific long-term effects of dichlorovinylcysteine will be discussed.

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636 DEVELOPMENT AND CHARACTERIZATION OF A PRIMARY CULTURE OF PROXIMAL TUBULAR CELLS FROM PORCINE KIDNEY TO STUDY NEPHROTOXICITY *IN VITRO*

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Most nephrotoxic agents exert their effect mainly on proximal tubular cells (PTC) of the kidney. Until now, most in vitro studies have been performed with renal slices, isolated tubules or proximal tubular cells from rat and rabbit kidney. Since the structure and function of pig kidneys are more related to man than rat or rabbit kidney, porcine proximal tubular cells (PPTC) can provide an in vitro system, more related to man.

Porcine kidneys were degraded enzymatically with collagenase and further purification of proximal tubular cells was obtained by means of a discontinuous density gradient. One kidney yielded approximately 22.10^6 viable cells, of which more than 80% displayed GGT- and NE activity, marker enzymes of the proximal tubule. Cells in culture displayed epithelial morphology, i.e domes and microvilli and could be grown to confluence on collagen within 3–4 days. Screening with a panel of antibodies showed that the cells were of epithelial origin and excluded the presence of endothelial or distal tubular cells.

Furthermore, the validity of the freshly isolated porcine PTC (PPTC) to study nephrotoxicity was investigated by studying the effect of nephrotoxins on the viability and mitochondrial membrane potential. The freshly isolated PPTC responded time and dose dependently to cisplatin, mercuric chloride, para-aminophenol and halogenated hydrocarbons and are currently used in our laboratory for more detailed studies on cisplatin-induced nephrotoxicity.

637 THE EFFECTS OF VANADATE (VO_3^-) AND CEPHALORIDINE (CEPH) ON THE TRANSPORT OF AMINO ACIDS BY LLC-PK_1 CELLS

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VO_3^- is a potent inhibitor of Na^+/K^+-ATPase which facilitates Na^+-dependent reabsorption of amino acids in renal tubules. LLC-PK_1 cells were used to examine the influence of VO_3^- upon the transport of aminoisobutyric acid (AIB) and aspartic acid (ASP). CEPH was used as a negative control for Na^+/K^+-ATPase inhibition, since, unlike VO_3^-, has not been characterized as an inhibitor of the enzymatic pump, but produces its toxic effects by other mechanisms. The LC10 and LC50 for VO_3^- and CEPH were determined utilizing 0.01 μM–50 mM VO_3^- or 0.01 – 50 mM CEPH. Uptake of AIB and ASP, and ATP and protein contents were determined after exposure of the cells to the LC10 and LC50 of VO_3^- (0.003 mM and 1.5 mM, respectively) or CEPH (0.05 mM and 6 mM respectively). Na^+-dependent uptake of AIB and ASP in cells treated with the LC50 of VO_3^- was significantly lower (131 ± 16 and 3289 ± 442 fmol/mg protein/30 min., respectively) than for control cells (296 ± 55 and 5212 ± 478). CEPH had no major influence upon AIB or ASP transport at either the LC10 or LC50. VO_3^- treatment reduced ATP concentration by 16–84%, and protein by 30%. CEPH reduced ATP concentration by 20–70%, and protein by 33%. In summary, VO_3^- but not CEPH reduced the uptake of AIB and ASP by LLC-PK_1 cells. However, both reduced the concentration of ATP in the cells. This implies that a reduction in ATP is not the determinant factor responsible for the decrease in amino acid transport in cells treated with VO_3^-. Since Na^+/K^+-ATPase has been proven to be inhibited by VO_3^- and not CEPH, inhibition of Na^+/K^+-ATPase might be the possible mechanism by which amino acid transport is reduced by VO_3^-.

638 RELATIONSHIP BETWEEN MERCURY ACCUMULATION AND TOXICITY IN CULTURED RENAL CELLS EXPOSED TO ORGANIC AND INORGANIC MERCURIALS

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This study examined the relationship between mercury(Hg) accumulation and toxicity in cultured renal cells treated with the mercuric compounds methylmercury (CH_3HgOH), mercuric chloride ($HgCl_2$) or parachloromercuribenzene sulfonic acid (PCMBS). LLC-PK_1 cells were used in the assay to determine Hg cytotoxicity. The LC10 and LC50 were determined from dose-response curves. On the basis of vital dye exclusion, CH_3HgOH was more toxic to LLC-PK_1 cells than $HgCl_2$, or PCMBS with mean LC50 values of 4.6 uM for CH_3HgOH, 31.4 uM for $HgCl_2$ and 115.8 uM for PCMBS. Total cellular mercury accumulation was $6.7 \pm 2.46 \times 10^{-6}$, $4.0 \pm 1.85 \times 10^{-6}$ and $3.3 \pm 0.32 \times 10^{-6}$ mmol Hg/mg protein, respectively. For further studies, selenium (NaSe) was used to bind Hg extracellularly in order to investigate whether cytotoxicity could be reduced proportionately to decreased Hg accumulation. LLC-PK_1 cells were exposed to the LC50 of CH_3HgOH, $HgCl_2$ or PCMBS ± SE. In the presence of NaSE a significant increase in cell viability was noted compared to $HgCl_2$ or PCMBS alone, as well as a decrease in cellular mercury accumulation. However, NaSe reduced Hg accumulation following exposure to CH_3HgOH but there was no change in cell viability. In conclusion, it can be suggested that there is a correlation between the cytotoxicity of mercurials and cellular Hg accumulation; however, it is likely that NaSe protects against $HgCl_2$ or PCMBS toxicity by another mechanism.

639 USE OF FLUORESCENT DYE TO EXAMINE Hg AND Sn DISRUPTION OF MDCK JUNCTIONS

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Madin-Darby canine kidney (MDCK) cells have structural, functional, and enzymatic properties that make them potentially useful for identification of compounds that disrupt the blood-brain barrier (BBB). Therefore, passage of fluoroisothiocyanate dye (MW 4300 + 50700) though a confluent layer of MDCK cells grown on 24-well insert plates was used to examine interruption of intercellular junctions by mercuric chloride (Hg) and triethyltin bromide (Sn). Hg (10, 25 and 50 μM) and Sn (1, 5 and 10 μM) were applied to serum-free cells for up to 94 hr. Barrier disruption was indicated by concentration-dependent increases in fluorescence that accumulated in the media under the cell layer within 7–12 hr after exposure to 50 μM Hg. Fluorescence increased as exposure increased to 94 hr. Concentration-dependent barrier disruption was also noted when MDCK cells were incubated with Sn, with significant increases in fluorescence detected 22–70 hr after exposure. Cells were, however, still viable, as indicated by typan blue dye exclusion, 94 hr after exposure to Hg and Sn. Dye penetration, therefore, was useful for detection of cell barrier disruption in the absence of cell death. (Supported by US EPA HERL).

640 ADRENAL TOXICITY INDUCED BY A NOVEL ACAT INHIBITOR

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Adrenal toxicity has been observed in our newly synthesized Acyl-CoA: Cholesterol acyltransferase (ACAT) inhibitors in the early stage of drug screening. We developed an *in vitro* screening system for detecting the adrenal toxicity induced by an ACAT inhibitor. Adrenal glands were removed from adult female SD rats and cortical cells were isolated by the collagenase digestion method. The isolated cells were plated in 24 well-microplates and cultured for 72 hrs until use. The cultures were exposed for 6 hrs to the ACAT inhibitors newly synthesized by our company, and LDH activities and corticosterone (CB) concentrations generated in the media were then measured. The adrenal toxicity was also histopathologically examined in the rats treated with these inhibitors for 7 days. The results were as follows: (1) All the compounds examined had no effect on the release of LDH into the medium. (2) For the chemicals tested there was a correlation between the extent of the inhibition CB secretion *in vitro* and the grade of adrenal morphological changes. (3) There was no correlation between the potency of ACAT inhibition and the extent of the CB secretory inhibition *in vitro* induced by the tested chemicals. (4) Almost all of the chemicals that exhibited adrenal toxicity had the same moiety in their chemical structure. These results suggest that this *in vitro* system is useful for detecting potential adrenal toxicity, and that adrenal toxicity by ACAT inhibitors may be due to the portion of their molecules but not the ACAT inhibition itself.

641 SCREENING WITH INTESTINAL SLICES TO IMPROVE ORAL BIOAVAILABILITY OF PEPTIDE-LIKE INHIBITORS OF INTERLEUKIN-1β (IL-1β) CONVERTING ENZYME

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Inhibitors of IL-1β converting enzyme may be useful in treating some inflammatory diseases. Many inhibitors were made with peptide backbones of Ala-Asp, Val-Ala-Asp, or mimetics of these peptides, with various modifiers on the N- (Z-group) and C-termini. Hydrolysis by intestinal peptidases can be a significant barrier to oral bioavailability, so compounds were routinely screened for stability by incubating with male dog intestinal slices. Compounds were incubated with one slice (8 mm core of duodenum) in a roller system for 6 hr at 37°C in Krebs-Henseleit buffer. Compound remaining was quantitated using specific HPLC assays. Dipeptides generally exhibited stability of 70–100%. Tripeptides were not as stable, although applying information from the slice screens resulted in several tripeptide mimetics with stability in the range of 90–100%. The mimetics were structurally based on unique pyrimidone backbones, or contained amino acid mimics. Stability of a subset of compounds in human duodenal slices correlated well with dog slices. WIN 68580, a tripeptide with low oral bioavailability, was highly metabolized in dog intestinal slices to Z-Val and Z-Val-Ala, which were predominant metabolites following oral administration to dogs. These were the major metabolites produced during incubation with human intestinal slices, although the amounts were inverse of the dog. WIN 69232, a dipeptide with oral bioavailability (dog) of approximately 47%, was essentially stable in dog and human intestinal slices. Stability in liver slices was generally high for most compounds and did not correlate with intestinal stability or bioavailability. Metabolic stability of inhibitors of IL-1β converting enzyme during incubation with intestinal slices was found to be essential for good oral bioavailability.

642 AN *IN VITRO* MICROSOMAL/CELL LINE CO-INCUBATION SYSTEM TO DETECT POTENTIAL HEPATOTOXICANTS. VALIDATION WITH CYCLOPHOSPHAMIDE (CP) AND ACETAMINOPHEN (AP)

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Species specific and/or novel mechanistic hepatotoxins often become apparant relatively late in the drug development process and can incur significant costs. A rapid, robust, modified microsomal incubation system has been developed utilizing an adherent cell line (3T3 mouse fibroblast derived) as an indicator of

toxicity. Previous, similar studies using suspension cells and microsomes have successfully shown NADPH dependent toxicity of carbamazepine, phenytoin, mianserin and others but can be cumbersome, time consuming and microsomes themselves can be toxic to the cells. The 3T3 cells were plated 24 hrs before use in 24 well plates and subsequently coincubated with rat phenobarbital (PB)-induced or control liver microsomes or human microsomes, an NADPH regenerating system and between 1 μM and 1 mM of CP or 1 to 20 mM APAP for 2 hrs. After a further 22 hrs incubation in drug and microsome free media there was significant NADPH dependent toxicity at 1 mM of CP with control rat microsomes (19 ± 11% live cells $P < 0.01$ versus 87 ± 13% in the absence of NADPH, by MTT) which was detected at over ten fold lower concentrations of CP with PB-induced rat microsomes. AP-induced NADPH dependent toxicity was detected only with rat PB-induced microsomes but could be inhibited by addition of SKF525A (15 μM). The system was thus able to indicate toxicity as a result of hepatic P450 dependent metabolism of CP and AP. The toxicity of the microsomes alone, to the cell line precluded incubations of longer than 2–4 hrs. Despite limitations such that false negatives could occur, this assay can utilize human microsomes where alternate in vitro human systems (slices or hepatocytes) are difficult to obtain.

643 THE EFFECT OF SURFACTANTS ON THE HYDROLYSIS OF FURA-2 ACETOXYMETHYL ESTER EVALUATED IN PRIMARY CULTURES OF RABBIT CORNEAL EPITHELIAL CELLS

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In a previous investigation, the effects of benzalkonium chloride (BzCl) and sodium dodecyl sulfate (SDS) on intracellular calcium ($[Ca^{2+}]$) in rabbit corneal epithelial cells were investigated using fura-2 to measure $[Ca^{2+}]$ (Toxicologist 14: 257, 1994). In this investigation, the effects of the surfactants on the hydrolysis of fura-2 acetoxymethyl ester (AM) were studied. If fura-2 AM was incompletely hydrolyzed, a calcium insensitive derivative of fura-2 would be formed. We used fura-PE3, a zwitterionic derivative of fura-2 that is retained in cells, to preload the cells before a 1 hr treatment with the surfactants. $[Ca^{2+}]$ was measured 1 and 3 hr after removal of the suffactants. Using PE3, BzCl-treated cells had a significant rise in $[Ca^{2+}]$ 1–3 hr post-exposure when compared to control cells (88 ± 10 nM for controls vs. 148 ± 6 nM for BzCl-treated cells), whereas the use of fura-2 in BzCl-treated cells did not show any significant differences from control cells. SDS treatment produced a significant decrease in $[Ca^{2+}]$ 1–3 hr post-exposure using either PE3 or fura-2. Twenty four and 48 hr after surfactant exposure, treated cells loaded with fura-2 AM were compared to control cells after treatment with 20 μM ionomycin and 10 mM $MnCl_2$. BzCl-treated cells, even at 24–48 hr post-exposure, incompletely hydrolyzed fura-2 AM, whereas SDS-treated cells showed no significant differences from control cells. We conclude that fura-2 AM is not completely hydrolyzed to free fura-2 in cells that have been treated with BzCl. Treatment of cells with SDS, however, did not affect the ability of the cells to hydrolyze fura-2 AM.

644 EVALUATION OF A HUMAN CORNEAL EPITHELIAL CELL LINE AS AN *IN VITRO* MODEL FOR PREDICTING OCULAR IRRITATION

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A three-dimensional *in vitro* model of the human corneal epithelium has been developed using a human corneal epithelial cell line, HCE-T, to serve as a tool for the prediction of ocular irritation. These cells form a stratified culture when grown at the air-liquid interface on a collagen membrane in serum-free medium. Cellular alterations in the HCE-T model were measured following 5 minute exposures to 20 raw chemicals (listed in the European Center for Ecotoxicology and Toxicology of Chemicals (ECETOC) Data Bank) and 25 surfactant-based personal care product formulations (utilized in the Cosmetic, Toiletry, and Fragrance Association (CTFA) Alternatives Program Phase III). *In vitro* assays used were transepithelial permeability to sodium fluorescein (TEP) and transepithelial electrical resistance (TER). *In vitro* assay data, averaged from triplicate cultures, were compared to Draize rabbit eye irritation data from the publicly available ECETOC and CTFA databases using linear regression with Pearson's correlation analysis. For raw chemicals, the correlation coefficients, r, for comparisons of TEP data to Draize maximum average scores (MAS) and Draize corneal subscores (CS) were 0.75 and 0.75, respectively, while those for TER data were 0.55 and 0.58, respectively. For product formulations, the correlation coefficients for comparisons of TEP data to MAS and CS were 0.89 and 0.83, respectively, while those for TER data were 0.78 and 0.74, respectively. Reasonable correlations among the data suggest that the HCE-T model may be useful for predicting Draize eye irritation. This limited study indicated that raw chemicals were effectively evaluated only by TEP analysis, but product formulations were reasonably evaluated by analysis with TEP and TER.

645 VALIDATION OF THE BOVINE CORNEAL OPACITY AND PERMEABILITY TEST WITH SURFACTANTS

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Previous evaluations of the Bovine Corneal Opacity and Permeability Assay (BCOP) demonstrated correlation with the Draize Eye Irritation Evaluation performed in rabbits. In this validation study, we evaluated the performance of the BCOP relative to an abbreviated 3 rabbit Draize eye test using surfactant dilutions. The surfactants chosen for analysis were lauryl sulfate–sodium salt; benzalkonium chloride; polyoxyethlene 9 lauryl ether; dioctyl sulfo-succinate and Tween 80. The dilutions for each surfactant were chosen based on their ability to produce a range of responses from very low to moderate ocular irritation. No severely irritating concentrations were selected to insure no unnecessary pain was inflicted on the rabbits. The calculated *in vitro* scores from the BCOP assay were compared with the day 1 weighted Draize mean scores for the 3 animal eye irritation evaluations.

BCOP *in vitro* scores ranged from less than 0 (zero) to approximately 30. Day 1 Draize mean scores ranged from 0 to greater than 50. The results indicated that increases in *in vitro* scores using the BCOP were associated with increasing Draize scores for benzalkonium chloride, polyoxyethlene 9 lauryl ether, and dioctyl sulfo-succinate concentrations. An inverse relationship was noted for sodium lauryl sulfate. There was some indication the opacity and permeability scores may be used independently to predict ocular irritation.

646 THE COMBINED USE OF THE BOVINE CORNEAL OPACITY AND PERMEABILITY (BCOP) ASSAY AND THE CAMVA ASSAY FOR THE PREDICTION OF POTENTIAL OCULAR IRRITANCY FROM PHARMACEUTICAL INTERMEDIATES

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During the manufacture of many pharmaceutical products, plant workers may be exposed to high concentrations of chemical intermediates. There is often very little historical toxicology information for these intermediates, and therefore manufacturers need to asses the potential toxicity of such materials so that workers can be informed of safe handling practices. To reduce reliance on animal testing for this purpose, we have investigated the use of a combination of two *in vitro* tests to predict potential ocular irritancy. Previous investigations have suggested that the BCOP assay (Gautheron, et al., 1992. Fund. Appl. Toxic. 18: 442–449) and the Chorioallantoic Membrane Vascular Assay (CAMVA [10-day]) are useful assays to use with pharmaceutical intermediates which are very often solid, sparingly water-soluble, materials. For the BCOP assay, normally discarded cow eyes were obtained from a local slaughterhouse. The corneas were excised and exposed to test materials for ten minutes (liquids) or four hours (solids) before directly measuring opacity and corneal damage. The CAMVA test utilized 10-clay old embryonated eggs with an exposure period of 30 minutes. We compared the *in vitro* data to the Draize Maximum Average Score, individual tissue scores and the Kay and Calandra evaluation system. Results from over 60 materials indicated a good correlation between the results of the BCOP assay and the *in vivo* scores. Severely irritating materials were consistently identified, although a few false negative materials were observed. The CAMVA was able to identify as irritant some of these false negative materials. We believe that a combination of the BCOP and CAMVA assays may be used to identify and classify severely irritating materials without further animal testing.

647 EVALUATION OF THE BCOP AND CAM-VA AS ALTERNATIVES TO OCULAR IRRITATION TESTING FOR PHARMACEUTICAL INTERMEDIATES

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Chemical intermediates created during the synthesis of pharmaceutical products are routinely tested using the *in vivo* Draize Ocular Irritation Test (DOIT). Twenty chemical intermediates were surveyed using two *in vitro assays:* the Bovine Corneal Opacity and Permeability Assay (BCOP), and the Chorioallantoic Membrane-Vascular Assay (CAM-VA). The BCOP assay measures the ocular irritation potential of isolated bovine cornea as changes in the opacity

and permeability. The CAM-VA measures damage to the vascular membrane in chicken eggs as an indicator of potential irritancy. *In vivo* findings of compounds ranged in irritation potential from non-irritating/mild (n = 12), moderate (n = 3), to severe/extremely irritating (n = 5). The results of the in vitro tests were compared to different endpoints of DOIT, and analyzed using the Pearson Rank Correlation Coefficient. A greater than 70% predictive correlation for potential ocular irritation was found for the BCOP the average Draize score and CAM-VA vs. corneal opacity, corneal area, iris inflammation, and average Draize score. When compared to *in vivo* data, the BCOP incorrectly predicted one moderate compound as extreme and one extremely irritating compound as mild. The CAM-VA inaccurately predicted the same moderate compound as extreme and the same extreme compound as moderately irritating. All other compounds were correctly reported. As a prescreen to *in vivo* testing any compound found to be moderate/extremely irritating using these *in vitro* assays would be reported as such, compounds found mildly/non-irritating would be tested *in vivo* to insure against false negative results. This would reduce the number of animals used without compromising employee safety.

648 A COMPARISON OF THE CYTOTOXICITY OF BENZLAKONIUM CHLORIDE IN TWO CELL MODELS; PRIMARY CULTURES OF THE SKIN AND EYE

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Benzalkonium chloride (BzCl), a cationic surfactant, is commonly used as a preservative in ophthalmic preparations, topical preparations, and cosmetics. The toxicity of BzCl was evaluated using an *in vitro* model of primary cultures of rabbit corneal epithelial cells (CE) and neonatal rat keratinocytes (KC). Cell viability was evaluated over a concentration range of 5–50 μg/ml BzCl, following a 1 hour exposure by the following methods: lactate dehydrogenase (LDH) leakage; 3-(4-5-dimethylthiazol-2yl-)-2,5 diphenyl tetrazolium bromide (MTT) reduction; neutral red (NR) uptake; and nuclear propidium iodide (PI) staining. To assess the involvement of intracellular calcium ([Ca]i) in BzCl cytotoxicity, fluo-3, a fluorescent calcium indicator, was used by scanning cells on a fluorescence microplate reader. The results for LDH and PI were similar between the two cell types. Organellar toxicity predicted by MTT and NR was similar within each cell type, but showed toxicity at lower concentrations in CE than KC. MTT and NR may be more sensitive predictors of cell damage than LDH or PI as distinguished by both cell types. The increased sensitivity of MTT and NR assays may be due in part to the lipophilic nature of BzCl binding. Fluo-3 showed increased [Ca]i at higher concentrations for both cell types, with CE responding at lower concentrations of BzCl and earlier time periods. Increased [Ca]i at concentrations higher than 20 μg/ml suggest that a rise in [Ca]i may not be an early event of cell injury, but may be associated with membrane damage. (Supported by a grant from Johnson & Johnson.)

649 DERMAL IRRITANCY INDUCED BY SDS AND TWEEN-20, ANIONIC AND NON-IONIC SURFACTANTS, IN RAT KERATINOCYTE CULTURES

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The objective of this study was to investigate the cytotoxicity induced by sodium dodecyl sulfate (SDS) and Tween-20 (TW-20), as anionic and nonionic surfactants, respectively, in kerationcyte cultures as an *in vitro* model. Three days after seeding, keratinocyte cultures were exposed to different concentrations of SDS and TW-20 for 5, 10, 15, and 20 min. Cytotoxicity was monitored by lactate dehydrogenase (LDH) leakage, neutral red (NR) uptake, rhodamine 123 (Rh 123) uptake, and changes in intracellular pH using the molecular probe BCECF. The uptake of Rh 123 and the change in intracellular pH were monitored by digitized fluorescence imaging. Cytotoxicity induced by SDS and TW-20 was concentration- and time-dependent as indicated by an increase in LDH leakage and a reduction in NR and Rh123 uptake. Although both surfactants induced toxicity, SDS was more toxic than TW-20. The intracellular pH was reduced as early as 1 min after addition of SDS or TW-20 and continued its decrease to about 6.7 at 20 min. In conclusion, the cytotoxicity induced by SDS was significantly higher than that induced by Tw-20 and this may be related to its anionic nature. (Supported by a grant from Johnson & Johnson Consumer Products, Inc.)

650 DEVELOPMENT OF HISTOPATHOLOGIC CRITERIA FOR ASSESSING *IN VITRO* SKIN CORROSION

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The United States Department of Transportation (DOT) recently granted an exemption for use of the *in vitro* Skin^2 Model ZK1350 Corrosivity Assay as a replacement to rabbit skin corrosion testing for 6 chemical classes. The *in vitro* skin corrosion test is based on methods we previously developed in which test substances are applied topically to the stratum corneum surface of human skin cultures and damage is assessed using a cell viability assay (MTT metabolism). The DOT exemption specifies that if chemicals are found to be corrosive in the *in vitro* test using the MTT endpoint, they may be labeled as corrosive. However, a non-corrosive classification requires confirmatory histopathologic analysis. If standard histopathology using Hematoxylin & Eosin (H&E) is not used or indicates a material causes alterations, then a standard rabbit skin test must be performed. We have established histopathologic criteria to evaluate human skin equivalent cultures in order to distinguish corrosive chemicals from non-corrosives. Treated and control Skin^2 and EpiDerm human skin cultures were graded for epithelial degeneration and necrosis. The grades ranged from 0, which was considered normal human skin culture epithelium, to a grade of 5, which represented severe degeneration/necrosis of the epithelium. The histologic appearance (grade) of skin cultures treated with non-corrosive chemicals was statistically indistiguishable from control Skin^2 and EpiDerm cultures. However, Skin^2 and EpiDerm cultures treated with corrosives showed severe histologic changes with grades that were significantly different from controls ($p \leq 0.003$). Standardized histopathologic grading criteria will provide for definitive analysis of degenerative changes in human skin cultures, and aid in interpretation of *in vitro* data for regulatory classification.

651 AN INTRALABORATORY VALIDATION STUDY WITH CORROSITEX™

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Corrositex™ is a standardized *in vitro* corrosivity test. The model is based on the time required for a test compound to pass through a biobarrier membrane and alter a Chemical Detection System (CDS). Alterations in the CDS may include color changes, flaking, or precipitation. Based on the time required to alter the CDS, the sample is classified as either corrosive or not and also placed in Packing Groups I, II, III, or Noncorrosive as described by the United Nations and U.S. Department of Transportation Guidelines. The objective of this study was to evaluate Corrositex™ for its ability to assess the corrosivity of several compounds previously evaluated *in vivo*. The following compounds were examined: 70% glycolic acid; a commercial yellow ink; 50% hydrogen peroxide; gold chloride; palladium chloride; 10 and 40% buffered glycolic acid. Sodium hydroxide was used as the positive control. Samples were initially classified into categories to determine how Packing Groups would be assigned using the Corrositex™ Screening Test. Corrositex™ correctly identified 87% of the compounds as either corrosive or noncorrosive when compared to *in vivo* data. One compound was identified as noncorrosive which previously had been labeled as corrosive *in vivo*. The correct packing group was assigned for 86% of the compounds. The remaining test compound was under predicted by 1 packing group (classified as less corrosive than *in vivo* findings). These data indicate that the Corrositex™ test system is a useful animal alternative model for screening the skin corrosivity potential of compounds.

652 SYNERGISTIC AND ANTISYNERGISTIC EFFECTS IN MIXTURES OF SURFACTANTS, SOLVENTS, ACIDS AND ALKALAIS ON THE CORROSIVITY POTENTIAL OF SAMPLES

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A new *in vitro* test method, CORROSITEX, was used to evaluate mixtures of acids and bases with solvents, surfactants, silicates and other materials. This method provides a dermal biobarrier constructed of keratin and collagen as the substrate. Test samples or mixtures are applied directly and the time to corrode the dermal biobarrier quantitated. A study of 374 pure chemicals and then mixtures were studied in the CORROSITEX System. Synergistic and antisynergistic effects were observed and were confirmed with *in vivo* testing on 60 samples. Two noncorrosive samples can be mixed and become corrosive and this can be observed *in vitro* and *in vivo*. Two corrosives can be mixed and become noncorrosive. NaOH at 1% can be combined with 20% cellusolve, or 10% SDS, or 10% sodium metasilicate and become a corrosive mixture.

The second component produces changes in the *in vitro* biobarrier. The *in vivo* studies demonstrate increased corrosivity of the NaOH in the mixture. Similar studies have been performed with acid mixtures. CORROSITEX provides a rapid, quantitative tool to predict the effect of adding additional components to pure chemicals and their dilutions.

653 PREDICTING THE DERMAL IRRITATION POTENTIAL OF FIFTY SURFACTANTS AND SURFACTANT-BASED FORMULATIONS USING THE *IN VITRO* SKINTEX MODEL

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Fifty surfactants, surfactant-based formulations, soaps and detergents have been evaluated in an *in vitro* model, SKINTEX to determine their dermal irritation potential. This model has been optimized to produce results comparable to *in vivo* human patch test results. *In vivo* tests on these test materials ranged from nonirritating to severely irritating. SKINTEX scores were used to rank order the test materials as well as classify and group them according to their dermal irritation potential to humans. These rankings were very concordant to the *in vivo* rankings. The concentration response curves of these test samples could be used to determine the "threshold concentration" for irritancy and as a tool for formulations. This model and protocol appear to be promising *in vitro* tolls to classify surfactants and surfactant formulations and optimize lower irritation formulations.

654 PILOT VALIDATION STUDY ON SURFACTANT SKIN IRRITATION UTILIZING A HUMAN TISSUE ANALOG

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A pilot interlaboratory validation study was conducted to evaluate the ability of Advanced Tissue Sciences' ZS1301 co-culture skin analog for predicting the skin irritation potential of diluted surfactants. This protocol was designed as a quantitative prescreen to clinical patch studies. Sixteen test materials, which represented various surfactant categories and ranges of irritation potential, were tested at 4 participating laboratories. The endpoints used in this study were percent of untreated control (%UC) response by treated tissues following specific exposure periods and T_{50}, the time required to reduce the viability to 50% of untreated control as measured by MTT reduction. Cytokine release in previously frozen supernatants was used to further evaluate non-toxic surfactants. Statistical evaluation indicated that the 30 minute %UC best predicted the 24 hour clinical patch scores. Statistical analysis resulted in a Pearson's correlation coefficient of 0.72 for two of the individual laboratories, 0.67 for one laboratory, and 0.59 for one laboratory. No statistically significant interlaboratory variability was shown. When non/mild and moderate/severe irritant categories were assigned according to *in vitro* scores, only one false negative was exhibited. With the encouraging correlation seen in this pilot validation study, this test demostrates utility as a screening method for clinical patch studies.

655 USING *IN VITRO* METHODS FOR UVA-PF AND SYSTEMIC PHOTOTOXICITY ASSESSMENT

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The effectiveness of existing methodology for the determination of UVA protection factor (UVA-PF) and systemic drug phototoxicity has recently been the subject of debate. Innovative in vitro methods have been developed using a three-dimensional human skin analog which show promise in their ability to assess UVA-PF and systemic phototoxicity. The Skin^2 system utilizes a laboratory grown skin co-culture, consisting of both dermal and epidermal components which allow for the mechanistic detection of human biochemical and physiological responses. For the determination of UVA-PF, values are calculated from comparisons of 540 nm optical density readings of sunscreen treated tissues against a standard curve generated from the UVA exposed and neutral density filtered tissues. UVA-PF predictions of the CTFA round robin standards exhibited high degrees of correlation with clinical data. For systemic phototoxicity, photoreactive and non-photoreactive antibiotics were applied in the culture medium to mimic systemic application. Tissues were then treated with 2.9 J/cm^2 UVA and later assayed for viability. A 100% correlation has been shown with 6 different antibiotics. This model shows promise as a powerful screening tool for UVA-PF as well as a method for the determination of systemic drug phototoxicity.

656 MEASUREMENT OF $[Ca^{2+}]_i$ AND INDUCTION OF *c-fos* TO PREDICT SKIN TOXICITY *IN VITRO*

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Prediction of cutaneous toxicity using *in vitro* methods can potentially avoid the use of animal *in vivo* studies and/or permit rapid screening of potential drugs and other chemicals. As both $[Ca^{2+}]_i$ and *c-fos* induction represent very rapid responses to injury, we have compared their responses in the JB6 mouse keratinocyte cell line (clone 41) with assays of cell death (propidium iodide and trypan blue) and morphology as seen by LM and EM, and published results on *in vivo* skin toxicity. The following toxins were studied at 100 μM and are listed in order of decreasing *in vivo* toxicity in the Registry of Toxic Effects of Chemical Substances: ionomycin > $HgCl_2$ > formaldehyde > sodium lauryl sulfate (SLS) > benzoyl peroxide (BOP) > DMSO. When cell viability was measured with trypan blue, the rank order was the same. Early increases of $[Ca^{2+}]_i$ were seen following addition of all toxins. When ranked by the fold-increase $[Ca^{2+}]_i$, the order was the same except for SLS which showed an increase of $[Ca^{2+}]_i$ nearly equal to that of ionomycin. However, when studied at the cell density used for measurements of $[Ca^{2+}]_i$, SLS toxicity was similar to ionomycin. Since *c-fos* induction is partially dependent on $[Ca^{2+}]_i$, we are in the process of correlating this with the other parameters. There was good correlation in the cases of ionomycin and $HgCl_2$, but the induction was greater than expected for BOP. This my indicate a role for oxidant-DNA damage in addition to increase of $[Ca^{2+}]_i$ in mediating *c-fos* induction. (Supported in part by NIH DK15440, JHU/CAAT, & ACS/IRG147L)

657 EVALUATION OF PHARMACO-TOXICOLOGICAL EFFECTS OF ALUMINUM AND SUPEROXIDE DISMUTASE BY THE BRAIN REAGGREGATE CULTURE MODEL

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To establish a neuro-toxicological screening assay which be applicable easily and practically *in vitro*, pharmaco-toxicological effects of aluminum chloride(Al) and superoxide dismutase(SOD) were evaluated using by brain reaggregate culture. Fetal brain reaggregating cultures, from Sprague-Dawley rats exposured by a neurotoxicant (Al) (0.1%, intragastric administration) with/without a possible interactive agent (SOD) (6.8, 13.6 I.U./kg body weight, intraperitoneally) during pregnancy, were grown essentially by the method of Honegger and Lenoir (1980) and as described by Atterwill *et al.* (1987) without any more treatment for the period of culture in order to evaluate pretreatment effects of Al and/or SOD on brain cell culture. Fetal brain reaggregating cultures from non-exposed rats were also conducted to evaluate the toxico-pharmacological interaction between Al (0.1, 0.25, 0.5, 0.75, 1.0 mM) and SOD (2.0, 4.0, 6.0, 8.0, 10.0 mM). Although Al induced maternal toxicity and mild teratogenicity, no difference in fetal whole brain reaggregating cultures from pre-exposed rats was found. However, Al (>0.5 mM) inhibited significantly the growth rate of fetal brain cell collected from no treatment rats in a manner of dose-dependence. SOD (>6.0 mM) promoted the cell growth and decreased the inhibited effect of Al on fetal brain reaggregating cultures.

658 USE OF AUTOMATED METHODS OF IMAGE ANALYSIS TO EVALUATE NEUROTOXIC EFFECTS *IN VITRO*

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Many drugs induce neuropathies which may not be discovered until late in the development process. The objective of the present study was to utilize image analysis to evaluate xenobiotic-induced neurotoxicity in neonatal rat dorsal root ganglion (DRG) cultures. DRG cultures consist of foci of neuronal cells, Schwann cells, and fibroblasts. Image processing and fluorescence microscopy were used to distinguish and enumerate neuronal and non-neuronal cells. Endpoints included Morphology, Percent Dead Cells (PDC), Area of Neuronal Cells (ANC), and Mean Foci Size (MFS). The concentration reducing an endpoint by 50% control values (IC_{50}) was determined for all endpoints excluding morphology. Two selective neurotoxins, paclitaxel and vinblastine, were compared with a non-selective, pantoxic agent, oligomycin. Paclitaxel induced a reduction in cell body size with convoluted and abnormally thickened neurites. Vinblastine induced flattening of neuronal cell bodies with decreased number of neurites. Oligomycin induced marked necrosis of all cell types. Paclitaxel and vinblastine both adversely affected neuronal morphology, but paclitaxel alone preferentially killed Schwann cells and fibroblasts without killing neurons. PDC was the most sensitive indicator of paclitaxel exposure (IC_{50}: 272.05 nM), while MFS was reduced only at higher concentrations

(IC_{50}: 787.23 nM). ANC was not affected by paclitaxel up to a concentration of 5 μM. PDC and ANC IC_{50} values, were 57.2 and 57.4 nM, respectively, after vinblastine exposure. MFS was reduced by vinblastine only at higher concentrations (IC_{50}: 87.22 nM). MFS, PDC and ANC IC_{50} values were similar after oligomycin exposure (19.54, 20.01, 28.82 nM, respectively). It is concluded that image analysis is an effective technique to evaluate potential neuropathic agents and distinguish between them on the basis of their relative toxicity for different cell types in mixed cell cultures.

659 *IN SITU* ACTIVATION OF A TETRAHYDROPYRIDINE ANALOG OF MPTP IN NEUROBLASTOMA CELLS

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A nonfluorescent tetrahydropyridinium compound, *trans*-1-methyl-4-[4-(dimethylaminophenyl)ethenyl]-1,2,3,6-tetrahydropyridine (**1**) was incubated with human neuroblastoma cells (SH-SY5Y) and found to be metabolized to the fluorescent pyridinium species (**2**), which exhibited maximal excitation/emission wavelengths of 485/620 and 530/620 nm. Fluorescent microscopy and fluorescent measurements indicated that **2** was produced within 1 hr when cells were incubated with concentrations of **1** as low as 1×10^{-5} M. The production of **2** from **1** was inhibited by inclusion of MAO inhibitors (pargyline, clorgyline, or deprenyl) in incubates containing **1** and cells. Viability, as indicated by trypan blue dye exclusion, and mitochondrial integrity, as indicated by MTT reduction, were not affected in cells incubated with concentrations of **1** as high as 10^{-3} M for 4 hr. Concentrations of **1** that were $>10^{-4}$M, however, may cause damage to cell membranes, as there was some leakage of **2** from the cells. This study indicates that **1**, an analog of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydro-pyridine), can, like MPTP, be oxidized within living cells to a potentially neurotoxic metabolite.

660 INHIBITION OF TARGET ESTERASES IN HUMAN (SH-SY5Y) AND MURINE (NB41A3) NEUROBLASTOMA CELL LINES BY ORGANOPHOSPHORUS COMPOUNDS (OPS)

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The inhibition of acetylcholinsterase (AChE) and neuropathy target esterase (NTE, neurotoxic esterase) by OPs results in different neurotoxicological consequences, depending on the species used. Species differences were examined *in vitro* using human (SH-SY5Y) and rodent (NB41A3) neuroblastoma cell lines. AChE and NTE activites were in both types of cells, but specific activities were different in SH-SY5Y and NB41A3 cells. Relative activities of NTE and carboxylesterase (CbxE, a detoxifying enzyme) in human cells were similar to those activities in hen brain, the conventional test species used to identify neuropathy-inducing, NTE-inhibiting OPs. AChE and NTE activities could be inhibited by OPs in SH-SY5Y and NB41A3 cells, with some species differences, but neuropathy producing OPs could be identified in both human and rodent cells by their ability to inhibit NTE, suggesting both might be considered for use in OP screening. Species differences in AChE, NTE and CbxE inhibition were noted, however, for about 20% of the OPs tested. (Supported by HERL, USEPA).

661 DIKETONES DISRUPT INTERMEDIATE FILAMENT NETWORKS IN A DOSE DEPENDENT MANNER

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We have studied the restructuring of the intermediate filament (IF) network in fibroblasts exposed to the neurotoxic diketones, 2,5-hexanedione (HD) and 3,4-dimethyl-2,5-hexanedione (DMHD), and contrasted these findings with those of their non-neurotoxic analogs. Using immunofluorescence to study the distribution of cytoskeletal proteins, the earliest changes observed in cultured fibroblasts included selective, perinuclear accumulation, coiling, and peripheral depletion of vimentin filaments. The appearance of microfilaments and microtubules was unaffected, even when there were distinct alterations in vimentin. The perinuclear redistribution and coiling of the IF lattice was progressive up to the longest time points (96 h) and highest concentrations (HD 12 mM; DMHD 0.2 mM) examined and showed a striking dose-response relationship. DMHD was 70-fold more potent in inducing alterations in the IF network; the non-toxic analogs, acetone and 3-acetyl-2,5-hexanedione, did not affect vimentin organization. These findings indicate that the disruption of the IF network in fibroblasts shares temporal, dose-response, and structure-activity relationships with the accumulation of axonal neurofilaments, implying that alterations in axonal and fibroblast IFs share a common molecular mechanism. A model of IF remodeling which involved the dynamic exchange of IFs at sites of network incorporation would accommodate the finding of focal IF accumulations in both fibroblasts and axons.

662 INTRACELLULAR CALCIUM RESPONSE TO ISOPROTERENOL IN CULTURED CARDIAC MYOCYTES FROM RATS AND HUMANS

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A comparative investigation was conducted on the intracellular calcium ($[Ca^{2+}]_i$) response of neonatal-rat and fetal-human cardiac myocytes to isoproterenol. Neonatal-rat cardiac myocytes were cultured using an established overnight trypsin-digestion procedure. Fetal-human hearts, procured from the Anatomic Gift Foundation (Laurel, MD), were shipped overnight to this laboratory in a Krebs-bicarbonate buffer on wet ice. Upon arrival, hearts were minced and serially digested with collagenase in a Krebs-HEPES buffer. All cardiac myocytes were cultured on quartz cover glasses in M199 supplemented with 10% newborn calf serum. The yield of human myocytes per heart varied considerably and was well below that achieved from the rat heart. Both human and rat cultures contained spontaneously beating myocytes which were used for $[Ca^{2+}]_i$ assessment 2–3 days (rat) or 4–5 days (human) after plating. $[Ca^{2+}]_i$ was estimated spectrofluorometrically in myocytes loaded with fura-2. Basal $[Ca^{2+}]_i$ levels and responses to high calcium (5 mM) or to isoproterenol (1 nM–10 μM) were, in general, comparable between rat and human cardiac myocytes. Specific differences were observed at peak systolic calcium, which averaged 420 nM in rat myocytes and 600 nM in human myocytes. Rat cardiac myocytes also tended to have a slightly higher sensitivity to isoproterenol than did human cardiac myocytes. Results demonstrate that cardiac myocytes obtained from fetal-human heart have the potential to be used routinely for experimental investigation. Results also demonstrate that the rat model is a good predictor of the human model, with respect to the variables examined here.

663 CYTOTOXICITY ASSESSMENT OF SMOKE FROM TEST CIGARETTES WITH A NOVEL CARBON FILTER

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A novel carbon filter, which significantly reduces carbonyls and other volatiles in mainstream smoke has recently been developed. The neutral red assay was used to assess the cytotoxicity of the smoke condensates (particulate phase) and the whole smoke (vapor and particulate phases) from the test cigarettes in comparison with several commercially available cigarettes. Smoke condensates were obtained by solvent extraction of filter pads which had been placed in the cigarette smoke stream. The toxicity of whole cigarette smoke was assessed utilizing a cellular-smoke exposure technique which permits direct exposure of cells to whole smoke. There were no statistically significant differences in the cytotoxicity of the smoke condensates from the test and commercially available cigarettes. However, whole smoke from the test cigarette exhibited a 50% decrease in the cytotoxicity observed with the whole smoke of the commercially available cigarettes. Vapor phase chemistry is the primary contributor to reduced cytotoxicity since the only significant differences between test and commercial cigarettes were from whole smoke (which includes vapor phase) and not smoke condensate. In conclusion, this novel carbon filter, which significantly reduces the amount of carbonyls and other volatiles in the smoke, resulted in a 50% decrease in the cytotoxicity of the smoke.

664 COMPARISON OF THE TOXICITY OF 30 CHEMICALS AS MEASURED BY 85 DIFFERENT *IN VITRO* TOXICITY TESTS

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In the MEIC (Multicenter Evaluation of In Vitro Cytoxicity) project, we have made a comparative toxicity analysis of the results of the first 30 reference

chemicals tested in 68 different in vitro toxicity tests (15 with human cell lines, 3 with human primary cultures, 18 with animal cell lines, 21 with animal primary cultures and 11 ecotoxicological tests). Principal component as well as analysis of variance (ANOVA) according to Turkey indicated an 80% similarity of all results. The influence of five key methodological factors on the general variability of the data was then studied by linear regression as well as by linear contrast ANOVA: 1. Exposure time (3–168 h) increased toxicity (>10 times) for 9 chemicals. 2. Human cytoxicity was well predicted by animal cell tests (except for two compounds), and relatively well by ecotoxicological tests. 3. One organotypic toxicity criterion, i.e. contractility of muscle cells, gave different results compared with variability criteria. 4. All twelve comparisons of identical assays using different cell types showed a similar toxicity regardless of cell type. 5. All nine comparisons of identical assays (20–24 h exposure) using different viability endpoints, resulted in similar toxicity regardless of the endpoint used. Thus, factors 1–3 contributed to the 20% dissimilarity, while factors 4 and 5 explain the remarkable similarity of the total results. Recently, results from 17 other assays have been integrated in this analysis, not changing the earlier results much. The findings support the basal cytotoxicity concept and will improve future in vitro toxicity testing (fewer cell types and viability endpoints, several standard exposure times).

665 OSTEOPONTIN GENE EXPRESSION IS CENTRAL TO THE INDUCTION OF A PROLIFERATIVE/SYNTHETIC PHENOTYPE IN VASCULAR SMOOTH MUSCLE CELLS BY ALLYLAMINE

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Repeated cycles of vascular injury by allylamine (AAM), an atherogenic amine, modulates rat aortic smooth muscle cells (SMCs) from a quiescent to a proliferative/synthetic phenotype. This altered state is preserved as a function of serial passage and involves changes in the secretion and deposition of extracellular matrix proteins. In the present study, the expression osteopontin (OPN), a gene which encodes for a 45 kDa phosphoprotein that regulates cell-matrix interactions through the $\alpha_v\beta_3$ integrin, and is a component of atherosclerotic plaques in humans, was investigated. Steady state mRNA levels of OPN were elevated in AAM cells during the early phases of cell cycle transit relative to controls. Addition of exogenous thrombin, a protease inhibitor known to cleave OPN, to the conditioned media of [^{35}S]-methionine labeled AAM cells was associated with a decrease in the intensity of a 45 kDa protein and enhancement in the intensity of a 30 kDa band. An antibody to the α_v integrin subunit decreased DNA synthesis in AAM SMCs, but was without effect in control cells. These findings implicate changes in OPN gene expression in the induction of a proliferative phenotype by AAM and emphasize the fidelity of this *in vivo/in vitro* model of chemical injury in studies of atherogenesis. (Supported by NIH grant ES 04849).

666 PHENOTYPIC HETEROGENEITY IN CLONAL POPULATIONS OF VASCULAR SMOOTH MUSCLE CELLS (SMCS): IMPLICATIONS IN STUDIES OF CARCINOGEN-INDUCED ATHEROGENESIS

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Vascular SMCs within the vessel wall represent a mosaic of cells expressing considerable phenotypic heterogeneity in terms of synthetic and proliferative potential. This phenotypic spectrum may be critical to the ability of chemical carcinogens to "initiate and/or promote" the atherogenic process. In the present study, experiments were conducted to evaluate the mitogenic responses of clonal populations of rat aortic SMCs to serum and selected growth factors. To determine if carcinogen-induced modulation of inherent proliferative potential was influenced by clonal expansion *in vitro*, the properties of 4 different cell strains isolated from vehicle (MCT oil)- and benzo(a)pyrene (BaP)-treated (80 mg/kg cumulative dose) animals were examined. A considerable degree of heterogeneity in proliferative behavior was found in all clones irrespective of their predetermined phenotypic status. Highly proliferative clones were obtained from both vehicle and BaP strains, although the frequency was consistently greater in the latter group. Transforming growth factor $\beta 1$ (0.01–100 pM) exerted an antiproliferative effect in all clones. This inhibitory response was reversed by epidermal growth factor (1 and 10 nM) only in control clones. These findings suggest that heterogeneity of SMCs within the vessel wall may influence the responses of vascular SMCs to carcinogen challenge. (Supported by NIH grant ES 04849 and ES 00213).

667 ALTERATIONS IN MACROPHAGE FUNCTION BY EXPOSURE OF MICE TO CS_2 ALONE OR IN CONJUNCTION WITH AN ATHEROGENIC HIGH FAT DIET

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The toxic and carcinogenic effects of environmental chemicals have been extensively studied, but the effects of chemical modmfication of fluid phase proteins, and their subsequent uptake and metabolism, on disease have received less attention. We hypothesize that chemicals, such as CS_2, modify (cross link) low density lipoproteins (LDL) such that they are taken up by macrophages (MØ), thus enhancing the formation of foam cells and progression of early atheromatous lesions. We have previously shown that exposure of cultured MØ to modified LDL (oxidation or CS_2) induces foam cells and a set of alterations that may promote atherogenesis. In these studies we exposed C57BL/6 mice to CS_2 by inhalation to 0, 50, 500, & 800 ppm 6h/day 5d/week. Thioglycolate peritoneal macrophages were harvested and tested for killing of tumor cells, induction of class II MHC (Ia), LFA-1, and secretion of NO•. Exposure to CS_2 alone inhibited the expression of Ia but addition of a high fat diet (HFD) reversed this effect. LFA-1 was markedly inhibited by CS_2 and the addition of HFD had an additive effect. The HFD alone inhibited NO• release and exposure to CS_2 enhanced this effect in a dose dependent manner. There was no effect in any group on the killing of tumor cells indicating the overall health of the cells and suggesting selective effects of CS_2 and/or the HFD. These observations correlate with the *in vitro* studies and suggest that CS_2 alters MØ by modifying plasma proteins including LDL.

668 CROSS-LINKING OF APOLIPOPROTEIN B (APOB) IN THE ACCELERATION OF ATHEROSCLEROSIS BY CARBON DISULFIDE

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There is growing evidence that oxidation of low density lipoprotein (LDL) is the critical alteration that leads to macrophage uptake and foam cell formation in the pathogenesis of atherosclerosis. LDL oxidation results in the peroxidation of unsaturated fatty acids and in the production of aldehydes, particularly malondialdehyde (MDA) and 4-hydroxy-2-nonenal (4HN). When human LDL was incubated with MDA or 4HN, covalent cross-linking of the apoB protein moiety of LDL was seen. Thus, it is reasonable to hypothesize that toxicants that result in protein cross-linking may play a role in the pathogenesis of atherosclerosis. Following the observations that occupational exposure to excessive levels of CS_2 results in accelerated atherosclerosis, and that both rabbits and rats fed high fat diets show enhanced atherosclerosis when exposed to CS_2, human LDL was incubated with diethyldithiocarbamate (DDTC) as a source of CS_2. Cross-linking of apoB was demonstrated by SDS-PAGE. Incubation of LDL with both DDTC and 10 mM N^{α}-acetyl-L-lysine (NAL) resulted in binding of NAL to apoB monomers and dimers, while inclusion of 100 mM NAL reduced the quantity of dimer formed. These studies suggest that lysyl amines participate in this reaction through the formation of stable thiourea cross-links, paralleling the amine-directed cross-linking effected by MDA and 4HN. Other cross-linking toxicants may also participate in the pathogenesis of atherosclerosis through this mechanism.

669 GLUTATHIONE-S-TRANSFERASE (GST) 8-8 IS LOCALIZED IN SMOOTH MUSCLE CELLS OF RAT AORTA AND IS INDUCED IN AN EXPERIMENTAL MODEL OF ATHEROSCLEROSIS

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Allylamine (AA) is an electrophilic amine that has been utilized in a variety of experimental models attempting to mimic human atherosclerotic lesions and vascular injury. Metabolism of AA to the aldehyde (acrolein), oxidative stress, and the resultant increase in lipid peroxidation, generation of •OH radicals, and depletion of glutathione (GSH) may be factors in AA-induced vascular lesions. GST8-8 belongs to a distinct subgroup of GST isozymes involved in the detoxification of products of lipid peroxidation; hence, we designed studies to examine the effects of AA exposure on this GST isoform in rat aorta using Western blotting and immunohistochemical techniques. GST8-8 is expressed in rat aorta and is induced upon AA exposure. GST8-8 was localized in the smooth muscle cells of the vascular media, believed to be the site of metabolism of AA. A significant increase in Gamma-Glutamyl-Cysteine-Synthetase activity and GST activity towards 4-hydroxynonenal and acrolein,

preferred substrates of GST8-8, was seen as early as three days following AA treatment. Aortic defense mechanisms respond early with induction of GSH biosynthesis and GST8-8. The presence of GST8-8 in the vasculature, which is constantly exposed to products of lipid peroxidation, and its induction by AA, suggest that GST8-8 plays a key role in blood vessels in protection against oxidative stress. (Supported by HL26189 from the NIH).

670 BUTADIENE INHALATION ACCELERATES ARTERIOSCLEROTIC PLAQUE DEVELOPMENT

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1,3 Butadiene (BD) is a reactive gas used widely in the synthetic rubber industry. It is also a component of cigarette smoke and of gasoline exhaust. The threshold limit value (TLV) for BD is 10 ppm. There is wide susceptibility across species to BD-associated cancers with few or no excess cancers reported in BD workers. The atherogenicity of BD has not been investigated in animal models. However, an elevated standardized mortality ratio for deaths from arteriosclerotic heart disease in black workers in the rubber industry has been reported. We reported recently that inhalation exposure to cigarette sidestream smoke at environmentally-relevant levels causes significant acceleration of arteriosclerotic plaque development in cockerels (Circulation, 90: 1363–67, 1994). Here, we asked whether cockerels inhaling BD also exhibit accelerated plaque development. Thirty-two cockerels were exposed to 20 ppm BD, 6hr/day, 80 days. Eight controls (AIR) were exposed to filtered air. All exposures were carried out in dynamic exposure chambers. Blinded measurements of plaque localization in the abdominal aorta, plaque frequency and size (Plaque Index; PI) were made in both groups. There were no differences between groups in plaque location or frequency. PIs were lognormally distributed in both groups. PIs were significantly larger in BD than in AIR. (The median PI was 50%> for BD than for AIR). Nearly identical results were found earlier for sidestream smoke and AIR. The results suggest that BD may contribute to the atherogenicity of cigarette sidestream smoke and indicate that the current TLV for BD may be too high.

671 VASCULAR ENDOTHELIUM-DERIVED MATRIX PROTEINS IN PLASMA OF RATS TREATED WITH MONOCROTALINE PYRROLE (MCTP)

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A single, iv injection of MCTP causes lung vascular injury, sustained pulmonary hypertension, and right cardioventricular hypertrophy in rats. We hypothesized that changes in the plasma concentration of two endothelium-derived matrix proteins, cellular fibronectin (cFn) and von Willebrand factor (vWF), would correlate with markers of pneumotoxicity and cardioventricular hypertrophy in this model of pulmonary vascular disease. Rats treated with 3.5 mg MCTP/kg had delayed and progressive lung injury. The lung-to-body weight ratio and lactate dehydrogenase activity and protein concentration of cell-free bronchoalveolar lavage fluid (BALF) were increased by 5 days posttreatment. Values for these markers were further increased at 8 days and reached a plateau thereafter. MCTP-treated rats had increased total nucleated cells within the BALF at 8 and 14 days. Right cardioventricular hypertrophy, an indirect marker of pulmonary hypertension, was observed at 14 days. The cFn concentration in plasma of rats treated with MCTP was increased at 8 and 14 days. There was no difference between the vWF concentration in plasma of rats treated with MCTP and those treated with vehicle at any time. These results indicate that an increase in plasma cFn concentration occurs prior to the onset of right cardioventricular hypertrophy and suggests the possibility that increased cFn synthesis may play a role in the genesis of vascular remodeling and pulmonary hypertension in the MCTP-treated rat. The pneumotoxicity, pulmonary vascular injury and sustained pulmonary hypertension in this model are not reflected in altered vWF concentration in the plasma. (Supported by NIH Grant ES02581.)

672 CHANGES IN THE BASAL- AND HISTAMINE-STIMULATED LEVELS OF INOSITOL PHOSPHATES IN RABBIT CORONARY ARTERY AFTER RICIN ADMINISTRATION *IN VIVO*

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Ricin, a toxic lectin from the castor bean, causes enhanced contractions of the coronary artery to some vasoactive agents such as histamine. To further investigate intracellular mechanisms, the basal and histamine stimulated levels of inositol phosphates of rabbit coronary artery were measured after ricin administration. Rabbits were given a minimal lethal dose, 0.22 μg/kg of ricin *iv* or sham injected and euthanized 48h later. Coronary arteries were removed, equilibrated for 1 hour, incubated with [$_3$H] myoinositol for 2h, then further incubated with or without histamine for 30 min. Labeled inositol 1-phosphate (IP_1), inositol 1,4-biphosphate (IP_2) and inositol 1,4,5-triphosphate (IP_3) were isolated by anion exchange chromatography and measured by liquid scintillation counting. Ricin increased the basal IP_1 and IP_2 levels but decreased the IP_3 level. Stimulation by 10^{-6} and 10^{-4}M histamine raised the IP_3 levels 1.91 and 2.34 fold, respectively, in control rabbits. Histamine, 10^{-6}M, caused a slight increase in IP_3 in ricin administered rabbits, but after 10^{-4}M histamine stimulation the IP_3 level was below the basal level. Thus, the inositol phosphate messenger system in rabbit coronary artery was affected by ricin administration. The decreased IP_3 after 10^{-4}M histamine stimulation may result from an inhibitory feedback due to increased intracellular calcium which increases contractions of coronary artery. Supported by DOD Contract DAMD 17-90-C-0109.

673 TOXICITY OF DIMETHYLBENZANTHRACENE (DMBA) TOWARD CULTURED AORTIC ENDOTHELIUM

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DMBA is a polycyclic, aromatic hydrocarbon that has been shown to enhance the development of atherosclerosis in chickens and other susceptible species. This effect has been attributed primarily to direct, mutagenic effects on aortic smooth muscle cells. However, as endothelial cell (EC) perturbation, leading to sustained alterations in cell structure or function, appears to play a critical role in the initiation and/or progression of atherosclerotic lesions, these studies were designed to determine the effects of DMBA on cultured ECs. Porcine aortic ECs (PECs) were treated with various concentrations of DMBA (0.001–10 μM) or vehicle for up to 14 days. Concentrations less than 10 μM caused no overt cytotoxicity to confluent monolayers of PECs. Cell numbers and total protein/well increased slightly over the treatment period in vehicle controls, and these increases were paralleled in cells treated with $\leq$1 μM DMBA. Cells treated with 10 μM DMBA did not increase in number, and some cell detachment, vacuolization and enlargement was evident in this treatment group only. Significant release of lactate dehydrogenase was not evident in any of the treatment groups. Despite the absence of overt cytotoxicity in cells treated with low concentrations of DMBA, PECs treated with $\geq$0.1 μM DMBA demonstrated a concentration-dependent decrease in colony-forming efficiency and an increased tendency to take up modified low-density lipoprotein. These results suggest that exposure to low concentrations of DMBA may influence various aspects of cultured EC function. Similar changes in EC activity after exposure *in vivo* may contribute to the pro-atherogenic effects of this compound. [Supported by the Faculty Research and Creative Activities Support Fund (WMU) and a Grant-in-Aid from the American Heart Association of Michigan].

674 THE ROLE OF DT-DIAPHORASE IN MITOMYCIN C TOXICITY TO PULMONARY ARTERY ENDOTHELIUM

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Mitomycin C (MMC) is a cancer chemotherapeutic agent that has been implicated in causing pulmonary vascular injury, including injury to vascular endothelium, in cancer patients. MMC can be metabolized by several enzyme systems, resulting in either a toxic metabolite or the formation of oxygen radical species. DT-diaphorase (DTD; NAD(P)H: quinone oxidoreductase) is capable of metabolizing MMC to a toxic metabolite. Lysates of porcine pulmonary artery endothelial cells (PECs) demonstrate DTD activity which is inhibited by small concentrations of dicumarol and increased by exposure of cells to dimethyl fumarate. This study was designed to explore the role of DTD in the toxicity of MMC to pulmonary endothelial cells. 10 μM MMC was markedly cytotoxic to PECs (50% release of lactate dehydrogenase), PECs exposed to 50 μM dicumarol were protected from MMC toxicity. Treatment with 10 μM dimethyl fumarate for 24 hours prior to MMC resulted in a significant increase in cytotoxicity. Bovine pulmonary artery endothelial cells (BECs) have 10-fold less DTD activity than PECs and were less sensitive to the cytotoxic effects of MMC. These results support the hypothesis that DTD bioactivates MMC in pulmonary artery endothelium and suggest the possibility that vascular endothelial cell DTD may play an important role in pulmonary vascular toxicity caused by MMC in vivo. (Supported by NIH grant ES02581.)

675 CONSTITUTIVE OVEREXPRESSION OF CATALASE SPECIFICALLY IN THE HEART OF TRANSGENIC MICE

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The heart has a weak antioxidant system relative to other tissues. In particular, catalase activity per g heart tissue is very low, being only about 2% that of liver in the mouse. Because catalase is a major enzyme involved in detoxification of hydrogen peroxide (H_2O_2) in mammalian cells, this relative deficit in the heart in its ability to dispose of reactive oxygen species may be responsible for the unusual sensitivity of the heart to oxidative injuries. The objective of this research is to determine whether elevation of catalase activity specifically in the heart of transgenic mice can provide protection against oxidative cardiotoxicity. A MyCat transgene for overexpression of catalase in the heart of transgenic mice was constructed. This transgene contained fragments from the rat catalase cDNA ligated behind the alpha cardiac myosin heavy chain promoter. The transgenic mice were identified using Southern and Dot blot assays and PCR procedure. Catalase activities and its mRNA levels in the heart and other organs were measured. These analyses revealed that we have produced 15 transgenic mouse lines in which only catalase, not other antioxidant components including superoxide dismutase, GSH peroxidase, GSH reductase, GSH, and metallothionein, is overexpressed in the heart, both atria and ventricles. Each transgenic line exhibits a stably elevated catalase activity, ranging from 2- to 500-fold higher than normal. This enzyme activity is not altered in any other organs including liver, kidneys, lungs, and skeletal muscles. The model is ideal to study the role of catalase in protection against oxidative injuries. Supported by NIH Grant CA63752 and A Grant-In-Aid from the American Heart Association 94007070.

676 EFFECTS OF RICIN ON PHOSPHOINOSITIDE HYDROLYSIS AND CALCIUM FLUX IN ISOLATED RABBIT PAPILLARY MUSCLE AND CALCIUM UPTAKE INTO MICROSOMES FROM RABBIT HEART

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Ricin, a toxic lectin from the castor bean, affects the cardiovascular system including decreasing blood pressure. Because calcium plays an important role in the regulation of myocardial contraction and because phosphoinositide hydrolysis can affect intracellular calcium, the present studies were carried out to investigate the effects of *in vivo* ricin administration into rabbits on basal phosphoinositide hydrolysis and calcium efflux across papillar muscles. In addition, calcium uptake into microsomes from the heart was also studied. Rabbits were given a minimum lethal dose, 0.22 μg/kg of ricin or sham injected and euthanized 48h later. Ricin did not alter the levels of tritiated inositol 4-phosphate, inositol 4,5-biphosphate, and inositol 1,4,5-triphosphate in isolated papillary muscle. Although ricin did not alter basal calcium efflux in isolated papillary muscle, it inhibited norepinephrine (NE)-induced calcium efflux. Ricin also depressed basal calcium uptake into isolated papillary muscle (mean $\pm$ SEM, μmol/g protein) (control: 3.68 ± 0.57; ricin: 2.31 ± 0.28, $p = 0.045$, $n = 6$) and microsomes (mean $\pm$ SEM, μmol/g wet weight) (control: 9.9 ± 1.9; ricin: 3.1 ± 1.9, $p < 0.05$, $n = 6$). Thus, ricin treatment did not affect phosphoinositide hydrolysis and basal calcium efflux in isolated papillary muscle, but depressed calcium uptake into isolated papillary muscle and microsomes and NE-stimulated calcium efflux from isolated papillary muscle. Supported by DOD Contract DAMD 17-90-C-0109.

677 POLYCHLORINATED BIPHENYLS (PCBs) INCREASE DT-DIAPHORASE AND EROD ACTIVITIES IN PULMONARY ARTERY ENDOTHELIUM *IN VITRO*

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Certain PCBs and other polycyclic aromatic hydrocarbons (PAHs) of planar structure interact with a cytosolic arylhydrocarbon (Ah) receptor in cells resulting in increased expression of specific proteins, including DT-diaphorase (DTD: NAD(P)H:quinone oxidoreductase) and cytochrome P450 1A1 (P4501a1). In animal models, exposure to PAHs produces vascular changes. We tested the effects of PCB exposure on DTD and ethoxyresorufin-O-deethylase (EROD) activities in cultured porcine pulmonary artery endothelial cells (PEC). Exposure of PECs to the coplanar PCB congeners 3,3′,4,4′-tetrachlorobiphenyl (TCB). and 3,4,3′4′,5 polychlorobiphenyl, increased DTD activity in a dose dependent manner. Significant induction by 3,4,3′,4′-TCB and 3,4,3′,4′,5-PCB occurred after 48 hours of incubation at doses of 100 and 1 ng/ml. Treatment of PECs with a nonplanar congener, 2,4,2′,4′-TCB, which does not bind to the AH receptor, failed to increase DTD activity at doses as high as 10 μg/ml. EROD activity was assessed as a marker of P4501A1. EROD activity was not detected in vehicle-treated PECs. Incubation of PECs for 48 hours with coplanar PCBs increased EROD activity and 3,3′,4,4′,5-PCB was more effective than 3,3′,4,4′-TCB. The increased activity by PCBs was reduced by cotreatment of PECs with α-naphthoflavone. Treatment of PECs with 2,2′,4,4′-TCB failed to increase EROD activity. These results suggest that PCBs increase DTD and EROD activities in vascular endothelial cells by an Ah receptor-mediated mechanism. (Supported by NIH grant ES02581).

678 CARDIOVASCULAR EVALUATION OF FLUORESCEIN IN COMBINATION WITH RMP-7, A BLOOD-OCULAR-BARRIER PERMEABILIZER

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RMP-7, a peptide bradykinin agonist, is being developed to increase the blood-ocular permeability of diagnostic and therapeutic agents. Vitreous fluorophotometry with IV fluorescein will be used in RMP-7 dose ranging studies of permeability in man. However, fluorescein has been associated with a low incidence of anaphylactoid reactions, i.e. mild (nausea, vomiting) to severe (respiratory, cardiac or neurological) side effects. In comparison, acute RMP-7 effects consist of transient hemodynamic changes secondary to vasodilation.

To support the clinical study, a cardiovascular safety study was conducted in dogs. Cardiovascular changes were evaluated following IV bolus injection of 30 mg/kg fluorescein alone, 100 ng/kg RMP-7 alone or fluorescein (30 mg/kg) in combination with RMP-7 (20 or 100 ng/kg). Animals were monitored continuously during a 30-minute baseline period and for up to 1 hour following dosing. Animals dosed with 100 ng/kg RMP-7 showed a significant decrease in blood pressure ($\approx$20–30%) and increase in heart rate (>100%) within $\approx$2 minutes of dosing; these alterations returned to baseline values within 10 minutes. Animals treated with RMP-7 in combination with fluorescein had cardiovascular profiles similar to those in the RMP-7 alone group.

It is concluded that RMP-7 exhibited moderate transient alterations in cardiovascular parameters and addition of fluorescein did not potentiate or attenuate blood pressure or heart rate effects.

679 CARDIOVASCULAR EFFECTS AND PHARMACOKINETICS OF SDZ WAG 994 IN DOGS

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Beagle dogs were surgically implanted with radiotelemetry transmitters to measure systemic blood pressure and ECG data. The dogs were treated orally with multiple rising doses of SDZ WAG 994, an adenosine agonist, at dose levels of 0, 0.1, 0.3, 1.0, 3.0, 6.0 and 9.0 mg/kg/day. Dogs were treated with 7 daily doses followed by a 7 day wash out period. The 9 mg/kg dose level was given twice consecutively and an untreated dog was given 6 mg/kg. Blood samples were taken on day 1 of each level for pharmacokinetic analysis. Dose related acute hypotension and tachycardia were observed at dose levels of 0.3 mg/kg and higher. At 9 mg/kg acute hypotension and tachycardia was followed by hypertension later in the week. Clinical signs of lethargy were observed at 9 mg/kg. There was evidence of adaptation since the effects diminished during the 7 days of treatment and the clinical signs in the dog treated for two days with 6 mg/kg were more profound than those treated with 9 mg/kg in the multiple rising dose group. However, there were no significant differences between the week 1 and 2 of dosing at the 9 mg/kg level. AUC increased overproportionally with dose. Heart rate increased with dose; blood pressure decreased. Blood pressure troughed at blood concentration peaks. There were no consistent dose related effects on ECG data.

680 *IN VITRO* AND *IN VIVO* EFFECT OF PHENCYCLIDINE HYDROCHLORIDE ON CALCIUM ATP_{ASE} IN RAT HEART SARCOPLASMIC RETICULUM

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Phencyclidine hydrochloride (PCP) also known as Angel Dust is a very potent psychomimetic drug of abuse. PCP produces a number of adverse effects in a variety of tissues including the cardiovascular system. The present studies were initiated to study the changes in cardiac Ca^{2+} ATP_{ase} activity in rats treated with PCP. For *in vitro* studies the cardiac sarcoplasmic reticulum(SR) from

normal rats were used, whereas, for *in vivo* studies the SR from rats treated with PCP (10 mg/kg single dose,IP) were used. PCP reduced the Ca^{2+} ATP_{ase} activity both *in vitro* and *in vivo*. A 50% inhibition of the enzyme was obtained with 100 μM/PCP *in vitro*. A significant reduction of the enzyme was evident as early as one hour after treatment of rats with PCP. The reduction was irreversible even at 12 hr. after treatment. PCP was found to be a competitive inhibitor of Ca^{2+} ATP_{ase} with respect to the substrate, ATP. These results indicate that PCP alter the Ca^{2+} homeostasis in cardiac SR in rats which may result in the impairment of contraction and relaxation process in the myocardium. (Supported by in part MIRTP. NHLBI Grant #T32HL07635).

681 EMETINE CARDIOTOXICITY: *IN VIVO* AND *IN VITRO* EFFECTS IN THE SAME RATS

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Chronic abuse of emetine in ipecac syrup can result in cardiac injury. We have recently shown that LDH release occurs in isolated rat hearts perfused with emetine (*Toxicologist* 13, abstr 1198, 1993). In addition, we have shown that emetine cardiotoxicity in our system may be related to inhibition of glycolysis (*Toxicologist* 14, abstr 1485, 1994). It was the purpose of this study to measure the *in vivo* toxic effects (EKGs and body weights) of emetine given ip to male rats, and to take the hearts from the same rats in order to measure the *in vitro* parameters of heart rates, contactility, and coronary flows. Male SD rats initially weighing 330–350 gm were injected ip with 2 mg/kg of emetine diHCl daily for 5 days/week. Control animals received equivalent volumes of saline. After 3 weeks the animals were anesthetized with 80 mg/kg of sodium pentobarbital and their hearts were perfused in the Langendorff mode. During the study, the control rats continued to grow, while the emetine rats lost weight. By the end of the study, the heart rates in the treated animals were less than those of the controls. The QRS-waveforms generally were less complex in the treated animals, but their durations were increased. The PR-intervals were not changed. *In vitro* in our Langendorff system, the emetine treated hearts had lower heart rates than those from controls. In addition, the emetine treated hearts had greatly reduced contractility as measured by pulse pressure, +dP/dt, and −dP/dt. The coronary flow rates were much less in the hearts of the rats given emetine *in vivo*. The *in vivo* EKG changes only gave a slight hint of this greatly reduced functionality that we found *in vitro*.

682 BIOCHEMICAL AND HISTOPATHOLOGIC CHANGES IN AORTIC TISSUE INDUCED BY IV COCAINE IN RABBITS

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The ability of IV cocaine administration to cause an inflammatory injury to arterial tissues was investigated in rabbits. Male New Zealand rabbits were given daily injections of cocaine (5 mg/kg) for 14 days. Samples for histology and cyclic AMP (cAMP) content were taken from the aortic arch and the thoracic aorta 24 hours after the last injection. The remaining tissues were then incubated in ^{14}C-proline and assayed for collagen and noncollagen synthetic rates. Calcium content of the tissues was also measured. In the aortic arch only cAMP levels and protein synthetic rates were elevated. In the thoracic aorta all of the biochemical parameters measured were significantly elevated. Histologically, IV cocaine caused extensive medial damage characterized by disruption and fragmentation of elastic lamella which in the most severe cases resulted in the formation of raised lesions. Both the histopathologic and biochemical data are consistent with the hypothesis that IV cocaine can induce an inflammatory injury to aortic tissue. Further the data suggests that the thoracic aorta may be more sensitive than the aortic arch to the cocaine induce toxicity. In a second study reduced and oxidized glutathione levels and lipid peroxidation were found to be at control levels in aortic arch and thoracic aortic samples from cocaine treated rabbits. These data suggest that cocaine induced aortic injury is probably not the result of cocaine causing oxidative damage to the vessel wall. (Supported in part by NIDA Grant DAO3846 and UCRF).

683 CARDIAC ACTIONS OF DICHLOROMETHANE *IN VIVO* CORRELATE WITH DEPRESSION OF CALCIUM DYNAMICS IN CULTURED CARDIAC MYOCYTES

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Previous in vitro studies with cardiac myocytes have shown that inhibition of contractility by halothane or 1,1,1-trichloroethane correlates with depression of fluctuations in cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$). In the present study, spectrofluorometric analysis of fura-2-$[Ca^{2+}]_i$ binding in neonatal rat cardiac myocytes revealed that cumulative exposure to 0.6–41.0 mM DCM resulted in a concentration-dependent and reversible decrease in the height of electrically induced $[Ca^{2+}]_i$ transients. IC_{10} and IC_{50} values were 3.2 and 18.1 mM, respectively. Total inhibition of $[Ca^{2+}]_i$ transients and cessation of beating were observed at 41.0 mM DCM. Suffusion with DCM for 40 min did not cause morphological alterations of the myocytes. In a urethane-anesthetized rat model, p.o. administration of 3.1, 6.2 or 12.4 mmol DCM/kg resulted in DCM blood concentrations of 0.98 ± 0.11, 1.54 ± 0.08 and 1.6 ± 0.1 mM, respectively, after 15 min. A dose-dependent decrease of contractility and heart rate without influence on blood pressure and ECG parameters was observed. DCM administration also provided protection against arrhythmia development induced by $CaCl_2$ infusion. Present data are consistent with the conclusion that cardiac effects of DCM are mediated by alterations in Ca^{2+} dynamics during excitation-contraction coupling.

684 ELEVATION OF NITRITE AND NITRATE LEVELS IN THE SERUM OF RATS TREATED WITH CARBONTETRACHLORIDE AND GALACTOSAMINE

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Carbon tetrachloride (CCl_4) and Galactosamine(Gal-N) are known to produce cellular toxicity in various organs. Nitric oxide has been shown to be involved in a variety of physiological functions. The present studies were initiated to investigate the *in vivo* effects of CCl_4 and Gal-N on nitrite(NO_2) and nitrate (NO_3) levels in serum of rats treated with the toxicants. NO_2/NO_3 levels were estimated (by using griess reagent) in serum of rats receiving 0.5 ml.kg wt of CCl_4 in corn oil or 400 mg/kg wt of gal-N in saline for 24 hours. The serum devoid of proteins showed elevated levels of both NO_2/NO_3 in CCl_4 and Gal-N treated rats. The increase of NO_2/NO_3 may partly be due to the increased degradation of nitric oxide or from other amino acids.

685 THE COMBINED EFFECTS OF ANABOLIC-ANDROGENIC-STEROIDS & COCAINE ON PRIMARY MYOCARDIAL CELL CULTURES

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Unfortunately, the abuse of anabolic-androgenic steroids (AAS) has been found to be closely associated with the abuse of cocaine. The term "polydrug" abuse has been used to describe this type of drug abuse. Due to toxic effects induced by AAS and cocaine on heart cells, it has been speculated that individuals who simultaneously abuse AAS and cocaine may be at greater risk of adverse cardiovascular events then when either drug is abused alone. This study assesses the additive or synergistic toxic effects of the popular, AAS, oxymetholone and cocaine on the heart by using an *in vitro* system of spontaneously contracting myocardial cells. The myocardial cell cultures, obtained from 3–5 day old Sprague Dawley rats, were exposed to doses of cocaine hydrochloride, at concentrations of 5 μM, 50 μM, or 500 μM, or oxymetholone, at levels of 1 μM, 30 μM, or 100 μM, and their combinations. Indices of toxicity were determined at 1, 4, & 24 hours after treatment. The release of the cytoplasmic enzyme lactate dehydrogenase (LDH) was measured to determine plasma membrane damage, and retention of neutral red dye was measured to determine cell viability. None of the cocaine toxicity levels were significantly different from that of the controls. Oxymetholone was toxic a level of 30 μM at 24 hrs, whereas 100 μM oxymetholone was toxic at 4 hrs. None of the combinations were significantly different from the individual drugs. Thus, the data demonstrated that combining oxymetholone and cocaine at the studied concentrations does not have any additional adverse effects, as compared to using them alone.

686 COCAINE-INDUCED IMPAIRMENT OF CARDIAC MITOCHONDRIAL FUNCTION *IN VITRO*

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Cocaine abuse is associated with many cardiac disorders. It has been reported that chronic treatment of rats with cocaine caused morphological disruption of heart mitochondria. However, whether cocaine has a direct effect on mitochondria remains unknown. The present study was designed to examine the

effect of cocaine on mitochondrial function in cultured rat myocardial cells and in preparations of isolated rat heart mitochondria. Cocaine (10^{-5} to 10^{-3} M) was incubated with myocardial cells for 12, 16, 20 and 24 hrs. Cocaine (10^{-3} M) markedly decreased cellular ATP levels at 24 hr. Experiments with digitized fluorescence imaging revealed that cocaine (10^{-3} M) significantly reduced mitochondrial membrane potential in cells after 24 hr. In contrast, cocaine moderately increased LDH leakage at 24 hr. In preparations of isolated heart mitochondria, cocaine (10^{-3} M) significantly decreased respiratory control ratios (RCR) and state 3 respiration when glutamate/malate were used as substrates. But, cocaine did not affect succinate-mediated respiration, indicating that cocaine may inhibit complex I of the electron transport chain. These data suggest that cocaine may have direct actions on mitochondrial functions, and mitochondrial impairment may be a mechanism of cocaine-induced cardiotoxicity. (Supported by NIH grant: DA07918-01A2).

687 NITROGEN DIOXIDE-INDUCED EXPRESSION OF A 78 kDa GLUCOSE REGULATED PROTEIN IS ASSOCIATED WITH INHIBITION OF GLYCOSYLATION IN PULMONARY ARTERY ENDOTHELIAL CELLS

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We recently reported that exposure to nitrogen dioxide (NO_2) results in upregulation of a number of cellular proteins including a 78 kDa glucose-regulated protein (GRP 78) in cultured pulmonary artery endothelial cells (PAEC) (Am. J. Respir. Crit. Care Med. 149: A454, 1994). Because upregulation of the GRP family is coordinately induced under altered physiologic conditions, including inhibition of glycosylation, we sought to determine whether NO_2-induced expression of GRP 78 is associated with inhibition of glycosylation. To test this, confluent monolayers of porcine PAEC were exposed to 5 ppm NO_2 or air (control) in 5% CO_2 for 24 hr at 37°C or to tunicamycin (10 μg/ml), an inhibitor of glycosylation, at 37°C for 12 hr. Expression of GRP 78 was identified following ^{35}S-methionine labeling, two-dimensional gel electrophoresis, and autoradiographic analysis as well as by immunoblot using rabbit GRP 78 antibody. Protein glycosylation was measured by ^{3}H-fucose incorporation into TCA precipitable fraction. Exposure to NO_2 resulted in 2–3-fold increased expression of GRP 78 compared to controls. Similarly, exposure to tunicamycin also resulted in 3–4-fold increased expression of GRP 78. Exposure to NO_2 resulted in a 37% ($p < 0.05$) reduction of ^{3}H-fucose incorporation into proteins compared to controls. These results demonstrate that increased expression of GRP 78 in NO_2-exposed cells was mimicked by the glycosylation inhibitor, tunicamycin. NO_2induced inhibition of protein glycosylation appears associated with increased expression of GRP 78 in PAEC.

688 NATIONAL TOXICOLOGY PROGRAM STUDIES: PRINCIPLES OF DOSE SELECTION AND APPLICATIONS TO MECHANISTIC BASED RISK ASSESSMENT

G W Lucier *NIEHS, RTP, NC*

The National Toxicology Program (NTP) was chartered in 1978 to coordinate toxicological research and testing activities within the Department of Health and Human Services. The NTP has characterized the toxicity of hundreds of chemicals primarily in rodents, under relatively standardized test conditions. The doses selected for use in these studies have generally been set sufficiently high to maximize the ability of the various assays to detect an adverse health effect. The use of animal data collected for hazard identification purposes in the derivation of quantitative risk assessments for both cancer and non cancer endpoints has created discomfort with the assays, and stimulated attempts to restructure the tests to produce data more appropriate for this new purpose. Aside from the need to better define the lower end of the dose response relationship, the criteria for selection of the top most dose are also continually questioned. The merits of making basic changes in the toxicology study paradigm to accommodate the increasing use of these data for risk assessment are worthy of further debate, and other approaches to these problems need to be considered. The purpose of this workshop is to provide an overview of factors that are currently considered important in selection of doses for NTP studies, to describe some of the confounding factors that can result from the indiscriminate use of bioassay data in quantitative risk assessment, and to suggest ways in which information from mechanistic studies or studies of biomarkers of exposure or effect might be used to better advantage in risk assessment.

689 IS INGESTED INORGANIC ARSENIC (As_i) A "THRESHOLD" CARCINOGEN?

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Ingested As_i is a carcinogen; it has been proposed to act via a threshold or nonlinear mechanism. This Roundtable will discuss the US and Canadian As_i risk assessments. It will consider the hypothesis that there is a threshold for As_i and implications for regulatory purposes. At the present time, the health effects and toxicological mechanisms of As_i are under intense scrutiny as the US EPA is in the process of developing a regulation for As_i in drinking water that could impact the regulation of As_i, which is ubiquitous in the environment. Some epidemiological evidence suggests that there is a threshold for As_i. These preliminary reports show no increase in cancers until the drinking water contains relatively high levels of As_i. Additional support is provided by metabolic studies which report that there is a saturation of detoxification pathways at high As_i exposure levels. There are also questions on the applicability of the Taiwanese data to the US population. These topics will be covered and open for discussion.

690 MORBIDITY AND MORTALITY FROM ACUTE INCREASES IN URBAN PARTICULATE: INTRODUCTION

J L Mauderly. *Inhalation Toxicology Research Institute, Albuquerque, NM*

Recent epidemiological studies have raised questions about the effects of environmental particle exposures for which we presently have few answers. Several studies have demonstrated associations between short-term fluctuations in ambient urban particle concentrations and daily mortality rates. Other studies have shown associations between ambient particle concentrations and changes in respiratory function and other indicators of respiratory morbidity. Cause-effect relationships, in contrast to indirect associations, have not been proven, but the current weight of evidence tends to implicate particles directly. Although heavy particle exposures are known to present health hazards, the concentrations associated with these newly-recognized effects are often within current air quality standards and the magnitude of the concentration fluctuations associated with the effects are small and common. Previous human exposures and toxicological studies have not predicted these effects, nor does our present knowledge explain causal mechanisms. The public health and regulatory implications of these issues are huge, particularly in view of EPA's impending review of the National Ambient Air Quality Standard for PM_{10}. This issue demands an immediate interactive investigative response from the epidemiology and toxicology communities. This symposium will familiarize attendees with the issues, current knowledge, and research opportunities.

691 WHAT ARE THE SIGNALS FROM EPIDEMIOLOGY?

D W Dockery. *Harvard School of Public Health, Boston, MA*

Recent epidemiological studies show that daily mortality is associated with particulate pollution at currently observed concentrations. Increased mortality implies that morbidity must also be increased. Among potentially responsive subjects (such as asthmatics), we expect increased symptoms, reduced lung function, increased medication use, and higher use of hospital services. Present evidence suggests a *coherence* of effects across related health outcomes and a *consistency* of effects among independent analyses. Time-series studies show total mortality increasing by 1.0% per 10 $\mu g/m^3$ increase in PM_{10}. Similar associations are found for respiratory hospital admissions and emergency visits, stronger associations are found for cardiovascular and respiratory mortality, lower respiratory symptoms and cough, hospital treatment of asthma, asthma attacks, and use of bronchodilators. Smaller associations are found for upper respiratory symptoms and lung function. Thus, current evidence is consistent and coherent in suggesting adverse effects at current pollution levels.

692 WHAT DO WE KNOW ABOUT THE EXPOSURES?

J D Spengler. *Harvard School of Public Health, Boston, MA.* Sponsor: J L Mauderly

While some recent epidemiological studies of particulate matter less than 10 μm in diameter (PM_{10}) suggest an association with mortality and morbidity, it is not yet clear what portion or characteristic of PM_{10} may be responsible.

For example, there is evidence that some of the more toxic elements (e.g., Pb and As) and aerosol strong acidity (H+) are found predominantly in the submicrometer fraction. Some transition metals, nitrate compounds and most aero allergens are in the larger fraction. Thus, if the size and compositional characteristics of aerosols vary spatially and over time, then the present PM_{10} NAAQS permits varying levels of public health protection. Moreover, efforts to assess the health effects of PM_{10} via epidemiology can be confounded if the character of PM_{10} varies substantially within and across study communities. In the presentation, the regional characteristics of ambient particulate pollutants are contrasted. However, individuals are exposed to a complex mixture of gases and particles from indoor and outdoor sources. Several studies have demonstrated that for particle mass, personal exposures are typically higher than the concentrations recorded in ambient locations. Tobacco smoke, resuspended indoor dust, transportation sources and a variety of personal activities result in substantial between-subject variation in particle exposure for both mass and composition. Accounting for activity patterns, chemical reactivity along with ambient and indoor concentrations can yield reliable prediction models for specific components of ambient aerosols.

693 COULD ULTRAFINE PARTICLES BE THE CULPRIT?

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It has been demonstrated that inhalation of particles of low intrinsic toxicity can induce severe adverse pulmonary effects in experimental animals. However, the inhaled concentrations used in those studies were in the several mg/m^3 range, and exposures had to be of chronic or subchronic duration, far removed from the acute nature and orders of magnitude lower concentrations associated with effects in the epidemiological studies, including acute morbidity and even mortality. Studies in our laboratory have shown that rather inert particles, when inhaled as ultrafine particles, have a significantly greater pulmonary toxicity than larger-sized (i.e., >200 nm) particles. Our most recent studies have shown that ultrafine particles (i.e., 26 nm Teflon® fume particles), when inhaled for 15 mins. as singlet particles at concentrations below 100 $\mu g/m^3$, can indeed cause severe acute effects and even mortality in healthy laboratory rats. Mechanistically, the integrity of the alveolo-capillary barrier appears to be affected, potentially augmented by reactive oxygen radicals released from inflammatory cells. Singlet ultrafine particles, although not very stable, are always present in the urban atmosphere. Recent measurements showed that their ambient concentration is about 1/2 of the concentration used in our rat experiments. There is, therefore, a need to characterize the physicochemical characteristics of the ambient ultrafine particle phase. People with a compromised respiratory system may be more likely to be affected by such particles.

694 ARE PARTICLE SURFACE REACTIONS A PLAUSIBLE MECHANISM?

D L Costa. *U.S. E.P.A., Research Triangle Park, NC*

The collective surface of an inspired aerosol comprises the initial interface encountered by lung fluids and cells. Most anthropogenically-derived particles are found in the fine phase ($d \leq 2.5\ \mu m$) of the ambient PM_{10} and are readily respirable. The surface of these particles consists of a complex array of polycyclic organics, various silicates, and metal compounds. The organic fraction of the particle has been linked to the carcinogenic potency of ambient particles, but has as yet uncertain acute toxicity. Sulfuric acid layered on the surface of a prototypic combustion particle has been shown to elicit much greater lung irritancy and toxicity than as does the acid alone. Metals, on the other hand, may exist as oxides or salts; the latter often as acid sulfates. Each can vary in toxicity in part due to its persistence, solubility, or bioavailability for interaction with the lung. Many combustion-derived metals are transition metals and by virtue of their ability to redox between valence states, they can generate reactive oxygen species when they coexist with precursor oxidants and reducing agents. Ionic Fe, V, Ni, and Cu, as well as other metals, are common to ambient aerosols and are capable of redox interactions with H_2O_2, a common and weak cellular oxidant, to yield highly toxic hydroxy ion, ·OH. Surface-derived mechanisms may contribute to the apparent generic toxicity of ambient particulate matter. [This does not reflect EPA policy.]

695 RECONCILING THE OBSERVATIONS WITH PRESENT KNOWLEDGE AND REGULATORY IMPLICATIONS

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Several recent epidemiological studies suggest increased morbidity and mortality associated with increased levels of urban atmospheric materials at levels frequently found in the U.S. As with all epidemiological studies, the exposures are uncontrolled and involve a mixture of materials in the environment and populations whose exposures, lifestyles and medical status are not well documented. This makes it difficult to identify associations between exposure to single agents and specific responses. This weakness of individual studies is overcome to some extent when multiple studies give similar results. In contrast to the human data, animals exposed to well-defined particulate atmospheres do not reveal an association between particulate exposure concentration and health outcome until exposure concentrations are typically much higher than experienced by urban populations. The results of the epidemiological and experimental animal studies will be reviewed in an attempt to understand their strengths and weaknesses for establishing national ambient air quality standards and occupational exposure limits for particulate material and related research.

696 MOLECULAR MARKERS IN THE EVALUATION OF CHEMOPREVENTIVE AGENTS

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Chemopreventive agents against various cancer types have been identified in laboratory animal models of carcinogenesis. These agents are awaiting evaluation in human clinical studies. Also the efficacy needs to be determined for numerous other potential cancer chemopreventive agents that are either analogues of already identified compounds or active in *in vitro* screening assays. The testing of these agents in human clinical studies can best be accomplished by evaluation of their modulation of molecular markers, biomarkers and/or intermediate endpoints of the carcinogenic progress. Hence, agents could be distinguished that prevent or reverse the carcinogenic process prior to the occurrence of cancer. Molecular markers and biomarkers are required in laboratory efficacy studies so as to have the identification of new chemopreventive agents driven by mechanistic considerations. The use of intermediate and precancerous endpoints would allow the preclinical studies to identify agents that will be evaluated in human studies using similar precancerous endpoints. The first presentation will discuss the application of surrogate endpoint biomarkers in chemoprevention studies, the next two will give examples in animal models and the last two will present discussions of clinical studies.

697 SURROGATE ENDPOINTS BIOMARKERS (SEB) IN CHEMOPREVENTION STUDIES: USE OF MOLECULAR MARKERS

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Development of SEBs, as alternatives to cancer incidence endpoints, is a primary goal in the field of chemoprevention. SEBs included 1) Assays of specific biochemicals: PGE levels 2) Assays for inducible enzymes or genes: GST Yc2 3) Levels of DNA or protein adducts: Aflatoxin adducts in urine or target tissue 4) Detection of mutated oncogenes: Ki ras in colon 5) Altered genome: Loss or amplification of specific chromosomal sites or aneuploidy 6) High expression of genes or gene products associated with neoplasia in specific tissues: Cadherin, cell cycle proteins, CD-44 7) Genetic polymorphisms that determine susceptibility to cancer: FAP, Brac-1. Specific SEBs can be measured in neoplastic cells, normal cells or body fluids. The ideal SEB can be 1) quantitated in readily accessible tissues 2) modulated by treatment with chemopreventive agents and 3) modulation of the SEB is closely associated with a decrease in tumor incidence. Certain of the molecular markers may not meet all of these criteria but may help in defining target neoplastic tissues and populations at high risk.

698 MODULATION OF MOLECULAR, BIOCHEMICAL AND MORPHOMETRIC BIOMARKERS BY ISOTHIOCYANATES IN MOUSE LUNG AND RAT ESOPHAGUS

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Our laboratory is evaluating the use of oncogene mutations, carcinogen:

DNA adducts, proliferating cell nuclear antigen (PCNA), DNA ploidy and nuclear/nucleolar morphology as surrogate endpoint biomarkers in chemoprevention studies of mouse lung and rat esophagus. A/J mice and F-344 rats are administered phenethyl isothiocyanate (PEITC) by gavage or in the diet and then treated with either 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) or N-nitrosomethylbenzylamine (NMBA), respectively. Lung and esophageal tissues are collected at various periods after treatment and the effects of PEITC on the biomarkers are determined. PEITC causes a dose-dependent decrease in the formation of O^6-methylguanine adducts in the DNA of mouse lung and rat esophagus. In addition, using the double mismatched amplification mutation assay, we found that PEITC reduced the frequency of NNK-induced mutations in the K-*ras* gene of mouse lung tissue. Preliminary studies indicate that PEITC reduces the rate of cell proliferation and the incidence of DNA ploidy in rat esophagus treated with NMBA. These results indicate that the above-described markers are suitable for evaluating the efficacy of chemopreventive agents in mouse lung and rat esophagus.

699 MODULATION OF MOLECULAR MARKERS AND INTERMEDIATE ENDPOINTS IN CHEMOPREVENTION STUDIES IN RAT COLON AND MAMMARY GLAND

M A Pereira, K A Crist, M You. *Medical College of Ohio, Center for Environmental Medicine, Toledo, OH*

Molecular markers and intermediate endpoints are being developed in the azoxymethane (AOM)-induced colon and methylnitrosourea (MNU)-induced mammary gland carcinogenesis models. Chemopreventive agents used to evaluate these markers included non-steroidal anti-inflammatory drugs (NSAID) especially piroxicam, in the colon model and dihydroepiandrosterone (DHEA) in the mammary gland model. In the colon the intermediate endpoints of foci containing aberrant, GST-π-positive and/or hexosaminidase-negative crypts were evaluated and compared to the tumor yield and phenotype. Piroxicam was the most efficacious NSAID in reducing foci and tumor yield. Other molecular endpoints evaluated in the colon included proliferating cell nuclear antigen (PCNA)-labeling index, mitotic index, and activation of the K-*ras* proto-oncogene. In the mammary gland, molecular markers evaluated were PCNA, estrogen receptor, and activation of K-*ras*, and loss of Rb. The modulation by chemopreventive agents of the molecular markers in early and predictive lesions for cancer yield suggest that markers can be validated for use in the screening for chemopreventive agents and to determine their efficacy.

700 EFFECT OF TOPICALLY ADMINISTERED α-DFMO IN PATIENTS WITH ESTABLISHED SOLAR KERATOSES IN A RANDOMIZED CLINICAL TRAIL

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α-Difluoromethylornithine (DFMO) is a specific, irreversible inhibitor of ornithine decarboxylase (ODC) and precludes intracellular accumulation of putrescine and spermidine. We have recently completed a phase IIb randomized double blind placebo-controlled study of α-DFMO vs placebo in patients with actinic keratoses. Following a 30 day placebo run-in to evaluate adherence, fifty patients were randomized to one of two treatment arms: those applying placebo to their left arm or α-DFMO cream to their right arm and vice versa. Study participants applied the cream twice daily, recording use on a calendar. Following the placebo run-in, participants underwent skin punch and shave biopsies for evaluation of polyamine content, H-ras gene mutation status, p53 gene mutation status and protein expression (i.e. p53 and PCNA) labelling indices. At the end of six months of treatment, participants underwent repeat skin biopsies and blood draws. Skin biopsies, taken after six months with treatment with α-DFMO, are also being evaluated for mutations in H-ras and p53 as well as p53 protein expression and PCNA labelling indices.

701 CLONAL p53 MUTATIONS AS TUMOR MARKERS

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Because tumors are known to develop through a series of genetic changes that accumulate during histopathologic progression, we have begun efforts to characterize these molecular changes in head and neck cancer (HNSC). We examined preinvasive lesions for the presence of allelic loss of chromosome 9p21–22, thought to harbor a critical tumor suppressor gene in many tumor types. We found 70% loss of heterozygosity in both preinvasive lesions and invasive tumors. Additionally, approximately 25% of preinvasive lesions were found to harbor p53 mutations and this increased to 45% in invasive lesions. We then used p53 mutations as specific markers to develop molecular assays to test the surrounding mucosal margins and lymph nodes from HNSC patients to detect rare cancer cells. In more than half of these patients, we found infiltrating tumor cells in the residual margins following resections. Furthermore, in approximately one-third of these patients additional lymph nodes that were negative by histology were found to contain rare cancer cells. The detection of infiltrating neoplastic cells in histologically benign tissue may aid in the surgical management of these patients by assessing the extent of tumor spread.

702 THE RODENT NON-GENOTOXIC HEPATOCARCINOGEN NAFENOPIN AND EGF ALTER THE BALANCE BETWEEN HEPATOMA MITOSIS AND APOPTOSIS

J Gill, C Molloy, R Roberts. *Zeneca Central Toxicology Laborarory, Alderley Park, Macclesfield, UK.* Sponsor: I Kimber

The responses of a series of rat hepatoma cell lines (FaO, HTC, RH1) to the rodent non-genotoxic hepatocarcinogen and peroxisome proliferator (PP) nafenopin were studied to determine if this PP acts with EGF, a naturally occurring liver growth regulator, to perturb the balance between mitosis and apoptosis. EGF enhanced the growth of FaO cells (well differentiated) and HTC cells (intermediate differentiation) but not of the poorly differentiated RH1 cell line. Nafenopin also increased FaO cell growth but, surprisingly, retarded the growth of both HTC and RH1 cells.

Since population expansion kinetics result from mitosis and death, replicative DNA synthesis (RDS) and apoptotic cell death were measured in HTC cells. EGF elevated RDS and suppressed cell death. However, nafenopin depressed HTC net population expansion via a suppression of cell death coupled to a more marked inhibition of RDS. The apparent paradox of a carcinogen suppressing population expansion was investigated using soft agar cloning to reveal sub-populations with differing growth kinetics. FaO cells formed few colonies under control conditions (30/50 000 cells) but gave a 4.5-fold increase in response to stimulation with EGF and nafenopin. HTC and RH1 cells showed many more control colonies (500 and 1000/50 000 cells, respectively), but only a 2- or 1.5-fold increase under stimulation, respectively. Thus, the PP nafenopin acts with EGF to stimulate selective clonal expansion by altering the balance between mitosis and apoptotic cell death: the role of these two parameters may vary with progressing transformation. At later stages, cells are refractory to EGF and nafenopin, suggesting that genetic changes may have superseded such factor-dependence.

703 SUPPRESSION OF APOPTOSIS BY THE NON-GENOTOXIC RODENT HEPATOCARCINOGEN AND PEROXISOME PROLIFERATOR NAFENOPIN

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The hypolipidaemic drug nafenopin is one of the peroxisome proliferator (PP) class of rodent non-genotoxic hepatocarcinogens. Suppression of apoptosis by PPs has been implicated as one of the mechanisms contributing to their hepatocarcinogenicity. We investigated the effect of nafenopin on hepatocyte apoptosis *in vitro* using primary cultures of rat hepatocytes and the Reuber hepatoma cell line FaO. Nafenopin reversibly maintained the viability of primary rat hepatocytes for at least four weeks whereas control cultures died within 8 days. The maintenance of hepatocyte viability was associated with a significant decrease in the number of cells exhibiting condensed and fragmented nuclei typical of apoptosis. Apoptosis could be induced in hepatocytes by the negative regulator of liver growth. TGFβ1. Co-addition of nafenopin significantly reduced this TGFβ-induced apoptosis by 50–60%.

TGFβ1 also induced apoptosis in the FaO hepatoma cell line. Cell death was accompanied by detachment of cells from the monolayer and detached cells exhibited the chromatin condensation and DNA fragmentation patterns typical of apoptosis. Co-addition of nafenopin to TGFβ1-treated FaO resulted in a significant increase in the number of attached, viable cells. In contrast, nafenopin did not retard the onset of apoptosis induced by the DNA-damaging agents etoposide and hydroxyurea. We conclude that the PP nafenopin can provide hepatocytes with a survival advantage although this protection is dependent on the nature of the apoptotic stimulus. It is likely that such a survival advantage contributes to multistage hepatocarcinogenesis.

704 EFFECTS OF ACRYLONITRILE AND METHACRYLONITRILE ON THE FORESTOMACH (FS) OF MALE F344 RATS: A COMPARISON OF CELL PROLIFERATION AND APOPTOSIS

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Acrylonitrile (AN) and methacrylonitrile (MAN) are two structurally related chemicals which are used in the production of plastics and acrylic fibers. Whereas the toxicity of AN is well characterized, little is known regarding MAN. One target of AN carcinogenicity is the rat FS. Present work is part of an effort designed to predict the potential toxicity/carcinogenicity of MAN. Earlier, we showed that AN and MAN induced a dose-dependent increase in mucosal cell proliferation in the FS of male F344 rats as determined by bromodeoxyuridine (BrDU) incorporation and expression of proliferating cell nuclear antigen. In contrast, AN but not MAN caused a dose-dependent increase in FS hyperplasia. Subsequently, the effect of AN and MAN on apoptosis was evaluated in rat FS using the *in situ* end labeling (ISEL) method. At doses of AN and MAN which induced a 2.3 fold increase in BrDU incorporation, apoptosis was 5 and 18 fold greater than controls, respectively. The effects of AN and MAN on apoptosis are inversely proportional to their hyperplastic response and may explain the lower efficacy of MAN in inducing FS cell proliferation. In conclusion, induction of cell proliferation and apoptosis by the two chemicals as well as the fact that they are metabolized via similar pathways suggest that they may cause qualitatively similar long-term toxicity.

705 PYRROLIZIDINE ALKALOIDS INCREASE CALCIUM LEVELS AND INDUCE DNA FRAGMENTATION IN RAT LIVER

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Acute liver injury was produced in rats 24 hr after the ip administration of the pyrrolizidine alkaloid (PA), monocrotaline (150 mg/kg) or a PA mixture (100 or 180 mg/kg) from the plant, *Senecio jacobaea*. The liver injury was indicated by marked increases in plasma alanine amino-transferase and aspartate aminotransferase and by massive hepatic centrilobular necrosis, for the 180 mg/kg of mixed PAs. Treatment with these alkaloids caused a significant increase in the concentration of calcium in mitochondria, cytosol and lysosomal fractions of rat liver. In rats given the PA mixture, 180 mg/kg, the calcium contents of the nuclear and microsomal fractions were increased 62-fold and 11-fold, respectively. Livers from these animals showed extensive DNA damage characterized by DNA fragmentation. The DNA fragments appeared in the ladder pattern characteristic of apoptosis and of Ca^{2+}-mediated endonuclease activation. However, only a few scattered cells showed evidence of apoptosis by light microscopy after 24 hr at the 180 mg/kg dose. These results demonstrate that calcium elevation and inter-nucleosomal DNA fragmentation accompany PA-induced liver necrosis and are not confined to apoptotic cell death. (Supported by NIH Grants ES00210 and USDA Cooperative State Service; 94-CRAH-0-6041).

706 APOPTOTIC CELL DEATH INDUCED BY CARCINOGENIC CHROMIUM IN NORMAL HUMAN LUNG FIBROBLASTS: MODULATION BY VITAMIN PRETREATMENT

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Hexavalent chromium compounds are documented human carcinogens. Although both the soluble and insoluble forms of hexavalent chromium are genotoxic to cultured cells, the particulate forms of chromium are more carcinogenic in experimental animals than soluble chromium compounds. This difference may be due to the ease by which soluble compounds may be cleared from the exposed tissue compared to the persistence of particulate chromium in the tissue. Previous work in our laboratory has shown that apoptosis is the mode of cell death of CHO-AA8 cells exposed to soluble sodium chromate (Blankenship, et al, 1994, *Toxicol. and Appl. Pharmicol.* 126: 75–83). Present experiments demonstrate that particulate lead chromate also induces apoptotic cell death, as revealed by nuclear condensation and fragmentation, and internucleosomal DNA fragmentation. Inhalation of chromium compounds results in an accumulation of chromium particles in the deep lung. Present experiments focus on the mode of cell death of normal human lung cells exposed to hexavalent chromium. Here we report apoptosis as a mode of cell death in normal human lung fibroblasts exposed to cytotoxic doses of sodium chromate. Intracellular reduction of Cr(VI) involves the production of DNA damaging active oxygen radicles, as well as intermediate oxidation states of chromium which may lead to cell death. Experiments were performed to determine if pre-treatment of cells with the antioxidants alpha-tocopherol and ascorbic acid have a protective effect on the cytotoxicity of sodium chromate. Protection from chromium-induced cytotoxicity was observed in cells pre-treated with both antioxidants.

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707 ACETAMINOPHEN-INDUCED HEPATOCELLULAR APOPTOSIS *IN VIVO* SHOWS SIGNS OF LOSS OF MITOCHONDRIAL VOLUME REGULATION

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Drugs and chemicals may turn on select intracellular molecular processes to induce suicidal death, apoptosis (APO), in addition to unprogrammed death, necrosis (NEC). Evidence in favor of NEC pin points diverse molecular mechanisms coupled with several concrete targets, whereas, fundamental events culminating APO still remain elusive. It is believed that: (i) ladder-like DNA fragmentation, (ii) nuclear condensation in the absence of loss of volume regulation of organelles, and (iii) preferential expression of C-myc, bcl-2, or p-53 are the sole diagnostic criteria to define APO. While DNA-laddering is no longer considered unique to APO, absence of loss of volume regulation still dominate as one of the principal features. To understand this event, male ICR mice (30–35 g) were treated with hepatotoxic doses of acetaminophen (500 mg/kg,ip) for 2–24h. Blood was collected upon sacrifice for ALT activity, and liver for histology & electron microscopy (EM). Portions of liver were also preserved in $liqN_2$ for DNA analysis. Hepatotoxicity (ALT) strongly correlated with qualitative and quantitative DNA fragrnentation ($r^2 = 0.9$) and cell death due to APO & NEC. EM sections discerned nuclear condensation typical of APO by 4h in many cells. Perplexed hepatocytes showed signs of APO & NEC both, suggesting that, smart cells upon receipt of appropriate toxic signals commit suicide via APO, whereas the cells which fail to recognize toxic signals encounter severe toxic assaults. In addition, many APO cells expressed frank nuclear condensation reactions coupled with a severe loss of mitochondrial volume (swelling) regulation during early hours of APO. In conclusion, this study proposes that absence of loss of organelle's volume regulation may not be granted as an unique criteria to differentiate APO from NEC similar to DNA-laddering.

708 ABROGATION OF ACETAMINOPHEN-INDUCED HEPATO- AND NEPHROTOXICITY BY 3-AMINOBENZAMIDE, A DNA REPAIR MODULATOR

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Whether classical hepatocellular necrogens have the potential to induce apoptosis (APO), in addition to necrosis (NEC) is an open question. Our recent studies (Ray et al., 1994) indicate that acetaminophen (APAP) turns on both APO and NEC in the liver *in vivo*, and DNA remains as the principal target of APAP-assault. This study examined whether: (i) 3-Aminobenzamide (3AB), a modulator of DNA integrity can protect mice from APAP-induced hepatotoxicity and nephrotoxicity; (ii) can protect DNA from endonuclease attack, and (iii) can also block Ca^{2+} influx into the hepatocellular nucleus? Male ICR mice (10 wk old) were treated with a hepatotoxic dose of APAP (500 mg/kg,ip) and were sacrificed 0, 2, 4, 6, 12, 18 & 24 hours later. In the cytoprotective group, mice were either pretreated with APAP (500 mg/kg, ip) alone, or APAP followed by 3-AB (400 mg/kg, ip) 1h later. Animals were sacrificed 6–24h later and blood was collected to determine serum ALT, and BUN levels. Liver and kidney were sampled for nuclear total Ca^{2+}, DNA, histology and electron microscopy (EM). Data indicate that 3AB completely protected from APAP-induced hepatotoxicity and nephrotoxicity (at 24h: ALT 14,000 U/L reduced to 40 U/L, BUN 60 mg/dl reduced to 20 mg/dl) at all the hours (6–24h). 3AB also prevented APAP-induced DNA fragmentation in the liver (>300% reduced to control by 24h) and kidney (>200% reduced to control by 24h). Similar to biochemical findings, EM sections revealed that 3AB was also effective in abolishing both APO and NEC in the liver. Tissue histology of liver and kidney mirrored the biochemical and EM data. This study suggests that: (i) DNA remains as a key target in APAP toxicity, and (ii) APAP may turn on APO and NEC both *in vivo* which may be antagonized by effective DNA repair modulators like 3AB.

709 EFFECT OF DETOXIFICATION ENZYME INDUCERS ON APOPTOSIS IN HT 29 COLON CELLS

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Agents in fruits and vegetables that are thought to protect against colon cancer include fiber and inducers of the detoxification enzymes glutathione S-transferase (GST) and NAD(P)H:quinone reductase (QR). Fiber is converted to sodium butyrate (NaB) by microflora in intestines and this butyrate promotes cellular differentiation. Because apoptosis is the final stage of differentiation of colonocytes and detoxification enzyme levels change during differentiation we have investigated the effect of enzyme inducers and NaB on cell apoptosis. Treating cells for 24 h with 25 uM benzylisothiocyanate (BIT) or 100 uM dimethylfumarate (DMF) increased cell lifting 20- and 4-fold, respectively, compared to controls. Detached cell viability was above 85% for up to 16 h and fell sharply thereafter. The lifted cells exhibited characteristic apoptotic DNA fragmentation and condensation of chromatin as visualized by propidium iodide staining. Treating HT 29 human colon cells 3 d with 5 mM NaB to differentiate the cells prior to exposure to 25 uM BIT increased cellular apoptosis 25% above the rate due to NaB alone. These findings indicate that the inducers of GST and QR may protect against colon carcinogenesis by both enzyme induction and increasing the rate of apoptosis of damaged epithelial cells. (Supported by NIH HL 39968).

710 SELECTIVE MITOMYCIN-C-INDUCED CYTOTOXICITY AND APOPTOSIS IN B-LINEAGE LYMPHOCYTES

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Many drugs and environmental chemicals are selectively toxic towards B-lineage lymphocytes *in vivo*. We studied three candidate mechanisms for mitomycin C (MMC)-induced differential B versus T lymphocyte toxicity; these were differential formation of DNA interstrand cross-links (ICL) in active genes, cell cycle checkpoint blockage, and engagement in apoptosis. The avian B-(BLC) and T-(TLC) lymphoid cell lines, DT40 and CU159, were exposed to graded dosages of MMC for one hour, and allowed to recover in drug-free medium. Similar levels of MMC-induced DNA ICL were detected in the ribosomal RNA gene cluster of the two cell lines. However, B-lineage cells were selectively blocked at the G_2 cell cycle checkpoint. T-lineage cells replicated with damage in the DNA template. In addition, MMC induced rapid and extensive apoptosis in B-lineage cells including the DT40 cells and in bursal-resident lymphocytes *in vivo*. Some 60% of bursacytes were engaged in apoptosis by 5 hours after MMC exposure. These results show the selective MMC-induction of a cell cycle block and apoptosis in B-lineage lymphocytes. Resistance in T-lineage cells involves capacity to continue replication even with DNA ICL and high threshold for induction of apoptosis.

711 BIOAVAILABILITY OF METALS IN RISK ASSESSMENT

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The bioavailability of metals is a key uncertainty in risk assessment. We propose a series of analytical tests and a protocol for measuring internal dose of metals in various organisms. Concentrations of the metal would be measured periodically in blood or other tissues of rats administered the test material in their feed or water. Curve-fitting or pharmacokinetic models could be used to estimate the equilibrium concentration in the tissue. The ratio of the estimated internal dose to the administered dose would be compared to a similar ratio for the form of the metal which was tested to establish the RfD. The latter would be based on published data or a parallel study. Similar studies of uptake by edible plants, earthworms, fish, and sediment-dwelling organisms could be used to develop food-chain transfer factors. In parallel, a series of chemical sequential extraction procedures are suggested. As data are gathered for various wastes, contaminated soils, sediments, etc., correlations can be developed between the various indicators of uptake and extractability. Eventually, it may be possible to estimate the parameters needed for human and ecological risk assessment (within a confidence interval) from a more limited battery of tests. Standardization of test methods would facilitate this information-gathering process.

712 DETERMINATION OF THE ORAL BIOAVAILABILITY OF SOLUBLE ARSENIC AND ARSENIC IN SOIL AND HOUSE DUST IN CYNOMOLGUS MONKEYS

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Recent studies have addressed risk assessment issues related to the bioavailability of arsenic from soils affected by past smelter operations. The oral bioavailability of arsenic was previously determined in rabbits to be 50% ± 5.7 for soluble arsenic and 24% ± 3.2 for arsenic in soil. In the current study, the monkey was chosen to examine an animal model with more physiological and anatomical similarity to humans. Using a cross-over design with a minimum washout period of 14 days, three female Cynomolgus monkeys were given a single oral administration via capsules of soil (0.62 mg As/kg BW) or house dust (0.26 mg As/kg BW) or dosed with sodium arsenate intravenously or by gavage (0.62 mg As/kg BW). Excreta samples (feces, urine, and cage rinse) were collected from each animal every 24 hours up to 120 hours. Total amounts or arsenic recovered in the urine and feces were determined. The absolute bioavailability for the oral routes of administration were calculated in comparison with each animal's intravenous data. Mean normalized absolute percent bioavailability values were 67.6, 19.2, and 13.8 percent for the gavage, house dust, and soil groups, respectively. The bioavailability of soil and house dust arsenic relative to dissolved arsenic was 20 and 28 percent, respectively. This finding suggests that risks associated with the ingestion of arsenic in soil or house dust will be reduced compared to ingestion of comparable quantities of arsenic in drinking water. This reduced uptake of soil and dust arsenic should be accounted for in assessing risks of arsenic exposure.

713 LEACHING OF LEAD INTO FOODS STORED AND PREPARED IN LEAD-GLAZED CERAMICS

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Lead-glazed ceramic (LGC) bowls were purchased directly from a factory in Central Mexico. The bowls were from the same batch and were similar in shape, weight and size, and all were confirmed positive for leachable lead using a quick color test. Groups of four bowls were subjected to twenty sequential overnight extractions using 4% acetic acid, to a minimum of ten sequential overnight extractions using red salsa (pH = 4.6 to 4.8), or were used to prepare cooked beans (50 g per preparation) ten times. Aliqouts of all extracts analyzed using atomic absorption spectroscopy. The results were quite variable within each group. Acetic acid leached the greatest quantity of lead, up to 2000 ppm in the first extract, and sequential extractions resulted in a rapid decline in leachable lead to as low as 15 ppm. Salsa leached lower levels of lead ranging from as low as 8 ppm to as high as 515 ppm in the first extraction. Subsequent extractions with salsa also showed a successive decline in leachable lead. Cooking beans in the LGC bowls leached the lowest amounts of lead (generally less than 10 ppm) however the decline with successive preparations was much slower. The results showed considerable variability in leachable lead, probably due to variability inherent in the manufacturing process. Because of this variability, prediction of risk of ingested leached lead from LCG bowls will be difficult. (This study was supported by a grant from the UCMEXUS Program)

714 ABSORPTION OF LEAD FROM CONTAMINATED SOIL BY WEANLING FISCHER 344 RATS

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Lead contamination of the environment is of major concern because of its toxicity and lasting effect on the neurological development of young children. Previous work has demonstrated that lead absorption, and thus toxicity, is highly dependent upon the form of lead ingested. The present study compares the absorption of lead from a soluble lead salt, lead acetate, a sparingly soluble lead salt, lead sulfide, and from lead contaminated soil. Weanling male Fischer rats were fed lead salts, contaminated soil, control soil or combinations thereof for 7, 15, or 44 days. Toxicity, as indicated by the inhibition of blood Δ-ALAD, was greatest in animals receiving lead acetate followed by lead acetate + control soil > lead sulfide > lead-contaminated soil respectively. Δ-ALAD inhibition was dose dependent in animals receiving lead acetate or lead acetate + control soil, but not in other groups. Lead absorption, as evidenced by blood lead concentrations, was significant in all groups and was near maximum for each

group within the first 7 days of exposure. Accumulations of lead in bone, however, continued to increase throughout the study. Concentrations of lead in bone were highest following administration of lead acetate, followed by lead sulfide and lead contaminated soil, respectively. Administration of control soil in the diet with lead acetate decreased lead adsorption as evidenced by both lower blood and bone levels. Results of these studies confirm previous observations that lead absorption is highly dependent on the form of lead ingested and demonstrate that lead found in contaminated soil is more poorly absorbed than other forms of lead. Lower absorption of lead from contaminated soil may be due to factors such as particle size or the presence of other components of soil in combination with the "armoring" process typical of soil lead. These factors should be considered when calculating assessments of human health risks associated with exposure to soil lead. (Supported by Contract No. N01-ES-15320).

715 BIOAVAILABILITY OF LEAD (Pb) IN WINE, BY STABLE ISOTOPE DILUTION

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Epidemiologic studies have reported dose-response relationships between alcohol consumption and blood lead concentrations (BPb), apparently as a consequence of lead exposure from wine. We conducted a non-randomized crossover study to determine the fraction of Pb absorbed from wine, using the technique of stable isotope dilution. In order to prepare an experimentally useful wine, we stored a liter of sherry wine, with an initial Pb concentration of 81 μg/L in a lead crystal decanter containing a low $^{206}Pb/^{207}Pb$ ratio (i.e. an Australian fingerprint). When the Pb concentration reached 2,950 μg/L, we removed the "spiked" wine from the decanter, by which time its $^{206}Pb/^{207}Pb$ ratio was 1.076. Four adult volunteers (mean BPb of 2.10 μg/dl, mean blood $^{206}Pb/^{207}Pb$ ratio of 1.203) were admitted to the clinical research center to drink a glass of non-spiked and spiked wine, two weeks apart. Twenty-four hours after drinking spiked wine (containing a dose of 250 μg Pb/70 kg) their mean BPb plateaued at 4.05 μg/dl while their mean blood $^{206}Pb/^{207}Pb$ ratio fell to 1.137. On average, at 24 hours, 41.4% of the administered wine Pb dose was in the blood. To calculate the fraction of Pb absorbed we made use of historical studies with iv ^{203}Pb, which indicate that at 24 hours 55% of the dose is retained in the circulation. Thus, in these four subjects we estimate the average amount of Pb absorbed from the wine was 75.5%.

716 CONCOMITANT ADMINISTRATION OF INTRAVENOUS ^{206}Pb TO ENABLE SIMULTANEOUS COLLECTION OF ORAL AND INTRAVENOUS AREA-UNDER-THE-CURVE (AUC) DATA FOR DETERMINATION OF ORAL BIOAVAILABILITY OF LEAD CONTAINING SOIL AND SLAG IN SWINE

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The oral bioavailability of lead from residential soil or slag in female, 15 kilogram swine (n = 28) was determined by simultaneous oral administration of the test material (ca 30–600 micrograms lead per kilogram body weight) and ^{206}Pb intravenously (via jugular vein; ca 2 micrograms per kilogram body weight). For individual animals, the AUC (15 minutes through 144 hours post-dosing) was calculated for each oral and intravenous dose administered. The bioavailability of lead from the various lead containing substrates was very variable (range 0–11.9%; n = 24). Intravenous ^{206}Pb gave blood level versus time curves where C_{max} occurred later than the first sampling at 15 minutes (T_{max} mean = 1.5h, sd = 0.9h; n = 28). This may be due to saturable binding in the lungs at this dose level, ca 30 micrograms. This results in higher than expected AUC values for total doses of ca 30 micrograms compared with AUC values obtained with total doses of ca 3000 micrograms. This may be due to a combination of factors such as the frequency of sampling and the delayed C_{max}. The disposition of lead at low doses warrants further investigation.

717 ABSOLUTE BIOAVAILABILITY OF LEAD CONTAMINATED SOILS

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The objective of this study was to compare two methods for determining the bioavailability of lead in soil samples. Juvenile male microswine were gavaged with a single dose of test soil. Additional groups were given a single intravenous injection of lead acetate solution containing a lead-dose equivalent to that of the gavage dose. Blood was drawn from all groups at various intervals after dosing, and analyzed for lead content by atomic absorption spectroscopy. The absolute bioavailability was measured. An in vitro method was then used in which the bioavailability of lead in the same soil samples was determined using a procedure designed to model the gastrointestinal physiology of microswine. The in vitro method was calibrated against a microswine gavage experiment where the pH, ratio of solid/liquid and emptying time of the GI tract was measured. The in vitro method provided an estimate of the maximum bioavailability of lead in soil compared to results obtained in vivo. The major determinant of bioavailability in microswine appeared to be the mineral composition of the soil samples.

718 DEVELOPMENT OF A PHYSIOLOGICALLY BASED TEST TO ESTIMATE LEAD BIOAVAILABILITY

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A physiologically based bioaccessibility test (PB/BT) that mimics gastrointestinal (GI) tract function and chemistry has been developed to estimate lead bioavailability. The test incorporates GI tract parameters representative of a human child, including stomach and small intestinal pH, soil-to-solution ratio, stomach mixing, and stomach emptying rates. Testing at multiple stomach pH values (1.3, 2.5, and 4.0) indicates that Pb bioaccessibility is strongly pH dependent. Pb bioaccessibility is also controlled by adsorption and precipitation reactions occurring in the neutral environment of the small intestinal simulation. In addition to providing mechanistic data on factors controlling Pb bioaccessibility, the PB/BT closely approximates Pb bioavailability study results for three substrates in Sprague-Dawley rats. The correlation between PB/BT results and Pb bioavailability studies in rats provides validation of the PB/BT method. The PB/BT is therefore proposed as an appropriate screening method to estimate Pb bioavailability from soil and provide site-specific evaluation of Pb exposure to children.

719 ASSESSMENT OF POTENTIAL HEALTH HAZARDS ASSOCIATED WITH BARIUM LEACHED FROM GLAZED CERAMICWARE

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Ceramic glazes contain numerous elements which have the potential to leach into food or beverages that are handled or stored in ceramicware. For instance, the propensity for lead to leach is well established as are its adverse health effects. Recently, barium salts have been investigated as one of the alternatives to lead in the frit formulations for glazes. This preliminary evaluation addressed the potential health hazards associated with barium at levels that might leach from glazed ceramicware. A set of ceramicware, consisting of 5 teacups and a pitcher, was examined for extractable barium by using the standard AOAC leach test (973.32). Exposure to barium that adults (18–44 yr) might encounter using the vessels for coffee, tea, or orange juice was estimated. The exposure estimate was derived from values for intakes of the beverages and for the barium migration from glazed ceramicware test samples. An established reference dose (RfD) for barium exposure for the critical effect of hypertension was identified (IRIS, EPA, 1990). The potential hazard associated with the leaching of barium from glazed ceramicware was found to vary with the level of use. Consuming beverages in amounts up to the 95th %tile would not result in total barium intake in amounts that exceed the RfD; consuming large quantities (>95th %tile) of beverages such as tea or coffee from glazed vessels might. This suggests that for a small portion of the population of users intake of barium may be in quantities that warrant further consideration as a potential health hazard, particularly for individuals with pre-existing hypertension. Analysis of a broad sample of ceramicware and study of barium leaching behavior under actual use conditions are needed to further assess the significance of these findings.

720 *IN VITRO* PERCUTANEOUS ABSORPTION OF MERCURY FROM WATER AND SOIL THROUGH HUMAN SKIN

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Mercury is of environmental concern as a contaminant in water and soil, and

its potential toxicity by damage to discrete areas of the brain and kidney. The objective was to determine percutaneous absorption in human skin for health risk assessment. Mercury as [^{203}Hg]-mercuric chloride in water or soil (Yolo County 65-California-57-8) was administered to human skin (0.5 μg/cm^2 containing 1 μCi) for 24 hours. Receptor fluid accumulation (n = 9) from water was 0.07 ± 0.04% for fresh skin and 0.07 ± 0.05% for frozen skin. From soil, accumulation in receptor fluid was 0.07 ± 0.03% from fresh skin and 0.07 ± 0.01% from frozen skin. Skin was washed with soap and water after the 24 hour dosing period and assayed for mercury content. From water vehicle dosed at increasing volumes (10, 100, 1000 μl) skin content was 23.0 ± 11.7%, 35.4 ± 15.2%, and 27.1 ± 24.8% respectively. From soil dosed at increasing volumes (5, 10, 40 mg) skin content was 10.4 ± 6.8%, 6.1 ± 2.0%, and 7.2 ± 10.8%, respectively. Thus human skin will accumulate large amounts of mercury from water and soil. A single short 30 minute exposure (simulate swim, bath) of mercury in water followed by soap and water wash resulted in 0.01 ± 0.00% receptor fluid accumulation, which increased to 0.09 ± 0.05% ($p < 0.001$) when perfusion was continued another 48 hours. Skin content was 5.5 ± 5.2% of dose after only the 30 minute exposure. Further study showed that receptor fluid accumulation was increased from 0.06 ± 0.03% to 0.19 ± 0.06% ($p < 0.000$) when perfusion flow rate was increased from 1.25 ml/hr to 3.0 ml/hr.

The results show that human skin has a great capacity to accumulate mercury (as mercuric chloride) from water and soil. Partition of mercury from skin to receptor fluid is dependent upon in vitro perfusion parameters. In vivo, mercury accumulated by skin probably does become absorbed systemically.

721 CYTOKINE GENE EXPRESSION AND PRODUCTION IN Hep G2 CELL LINE AS INDICATORS FOR CHEMICAL-INDUCED HEPATOTOXICITY

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Cytokine gene expression and production in the Human hepatoblastoma Hep G2 cell line was compared to that in rat hepatocytes. TNF and IL-8 production were measured by bioassay using L929 cells and by ELISA, respectively. Cytokine gene expression was determined by RT-PCR. Although no increase in IL-8 or IL-8 mRNA was found, TNF and TNF mRNA increased in Hep G2 cells cultured with 100 ng/ml LPS. Both TNF and IL-8 mRNAs elevated significantly in Hep G2 cells stimulated with 25–400 U/ml TNF. A dose-response for TNF was observed in rat hepatocytes treated with 0–200 ng/ml LPS. Dose-responses of TNF, IL-1 alpha, and IL-6 were also found in rat hepatocytes cultured with 0–200 ng/ml LPS or 0–400 U/ml TNF. In addition, TNF production was significantly elevated in Hep G2 cells cultured with glucose-glucose oxidase, a free radical generating system. Hep G2 cells, as a model to evaluate hepatotoxicity caused by agents which induce reactive oxygen species, is being examined.

722 PRODUCTION OF LEUKOCYTE CHEMOTACTIC FACTOR(S) BY ISOLATED HEPATOCYTES: EFFECT OF ALLYL ALCOHOL

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Allyl alcohol (AA) was used as a model hepatotoxicant to define interactions between parenchymal and nonparenchymal cells during inflammatory liver injury. Participation of Kupffer cells and circulating inflammatory leukocytes in AA-induced hepatotoxicity *in vivo* has been shown previously (Przybocki *et al., Toxicol. Appl. Pharmacol.,* 115: 57–63, 1992). The goal of present experiments was to determine if hepatocytes injured by AA act directly to induce migration of leukocytes into the liver. Therefore, production of chemotactic factor(s) released by hepatocytes maintained in primary culture and exposed to a non-lethal dose of AA (100 μM) was tested. Conditioned media from control and AA-treated (100 μM) hepatocytes was collected after a 24 h incubation with either vehicle or AA. Chemotactic activity of conditioned media was assessed using microchemotaxis chambers (Falk *et al., J. Immunol. Methods,* 82: 315–320). Undiluted conditioned media from control and AA-treated hepatocytes was placed in the bottom wells of the microchemotaxis chamber and a mixed population of leukocytes isolated from rat blood was added to the top wells. The apparatus was incubated for 1 h and chemotactic activity assessed by determining the amount of leukocyte migration to the opposite side of the filter as indexed by cell counts or by β-glucuronidase activity contained on the filter. Normal hepatocytes released factor(s) that stimulated leukocyte migration. Undiluted conditioned media from hepatocytes treated with AA (100 μM) significantly reduced the number of leukocytes migrating through the filter. Thus, hepatocytes directly produce chemotactic factor(s) for leukocytes which are altered in the presence of AA. (Supported, in part, by NIEHS grant ES-05052).

723 ROLE OF TUMOR NECROSIS FACTOR-α (TNF-α) AND NITRIC OXIDE (NO) IN ACETAMINOPHEN HEPATOTOXICITY

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Treatment of male LEH rats with acetaminophen (AA, 800 mg/kg) results in extensive centrilobular necrosis. This is preceded by an accumulation of activated macrophages (MP) in these regions which have been implicated in tissue injury. In the present studies, we analyzed the role of TNF-α and NO, two liver MP-derived mediators in AA-induced hepatotoxicity. Immunohistochemical studies using specific antibodies revealed a time-dependent increase in the expression TNF-α and inducible nitric oxide synthase (iNOS) protein, as well as iNOS mRNA as determined by *in situ* hybridization, in necrotic regions of the liver. Expression of these mediators was maximal between 12 and 24 hr after AA treatment. Pretreatment of rats with $GdCl_3$ (7 mg/kg), which inhibits hepatic macrophage functioning, completely prevented hepatic necrosis and macrophage infiltration induced by AA. $GdCl_3$ pretreatment also blocked the accumulation of TNF-α and iNOS protein and iNOS mRNA in the liver. Although pretreatment of rats with anti-TNF-α antibody or the NOS inhibitor, N^G-methyl-l-arginine blocked TNF-α and iNOS expression, respectively, hepatic necrosis was unaffected. These data suggest that other inflammatory mediators may play a more prominent role in AA-induced hepatotoxicity. (Supported by NIH GM34310 and the NJ Cancer Commission).

724 ACETAMINOPHEN-INDUCED CHANGES IN HEPATIC NF-κB, AP-1 AND NF-IL6 DNA BINDING ACTIVITY IN MICE

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Acetaminophen (APAP) overdose has been shown to cause hepatotoxicity. Some of this damage can be attributed to inflammatory mediators including cytokines such as TNF-α and IL-1. Transcription factors, such as NF-κB, AP-1, and NF-IL6, have been shown to control the gene expression of these cytokines. In this study we examined the changes in the DNA binding activity of these transcription factors following administration of APAP to adult mice. In saline-treated mice, the nuclear expression of NF-κB, NF-IL6 and AP-1 did not change over time. However, the nuclear expression of NF-κB and NF-IL6 decreased and the binding activity of AP-1 increased with the onset of APAP-induced liver damage. Immunostaining showed that hepatic NF-κB is located in the cytoplasm and nuclei of parenchymal and endothelial, but not Kupffer cells. Prior administration of gadolinium, an inhibitor of Kupffer Cells, prevented the decrease in NF-κB expression, but did not change the binding activity of AP-1 in APAP-treated mice. These data suggest that NF-κB, AP-1 and NF-IL6 expression is altered in response to APAP overdose and that Kupffer Cells may be partially responsible for these changes.

725 MECHANISM(S) OF ULTRAVIOLET RADIATION-INDUCED TNF-α RELEASE

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Keratinocytes are uniquely exposed to ultraviolet radiation (UV) by virtue of their anatomical location. It has been demonstrated that UV irradiation can induce the production of several cytokines, such as IL-1α and β, IL-6, IL-8, TNF-α, ect.. These cytokines are not only involved in the mediation of local inflammatory reactions within the epidermis, but may also enter the circulation and thus cause systemic effects. The purpose of the present study was to evaluate, using a human keratinocyte cell line NCTC 2544, the mechanism(s) of UV-induced TNF release. Confluent cells were exposed at different doses of UVA/UVB (0–300 mJ/cm^2), TNF release was evaluated in the conditioned media 24 hours after irradiation. TNF release can be partially prevented both by adding an anti human IL-1α antibody after irradiation, suggesting an autocrine effect of IL-1α in inducing TNF release and by adding cysteine (10 μM), a glutathione precursor, or glutathione (1 μg/ml) indicating also a role of oxidant species. One underlying mechanism for the initiation of UV-induced erythema may be the photochemical production of activated oxygen species, which working as fast-acting second messanger may directly activate transcription factors, such as NFκB, necessary for induced expression of TNF gene. Indeed, tosylphenylchloromethylketone (20 μM), an IκB;-α protease inhibitor, is able in our system to virtually abrogate UV-induced TNF release as does the combination of anti human IL-1α antibody and pyrrolidine dithiocarbamate (100 μM), a potent NF-κB; inhibitor.

726 HEPATOCYTE DIFFERENTIATION DETERMINES SENSITIVITY TO CYTOKINE TOXICITY

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The cytokines, tumor necrosis factor-α (TNF) and interferon-γ (IFN), may potentiate chemical toxicity in the liver. TNF alone (0.1 μg/ml) or in combination with IFN (100 U/ml) produce cytotoxicity in primary cultures of mouse hepatocytes cultured on rat tail collagen (Billings and Adamson, 1993). This cytokine induced toxicity, however, is highly dependent on cell culture conditions. The present study was undertaken to examine the cellular mechanisms that are involved in the observed differences in cytokine sensitivity. Primary cultures of hepatocytes plated at different cell densities exhibit different morphological characteristics as well as significantly different toxic responses to TNF and IFN in the presence of the protein synthesis inhibitor, cycloheximide (Cyclo). We hypothesized that the state of hepatocyte differentiation is involved in these differences. To investigate this theory, we determined the state of hepatocyte differentiation by examining both DNA synthesis and albumin synthesis at two different cell densities (21,300 and 63,800 cells/cm^2). At theses densities, TNF + IFN + Cyclo cytotoxicity was 70.0 ± 9% and 32.9 ± 2.8%, respectively. Cells plated at the low cell density exhibited a significantly higher rate of DNA synthesis than the higher cell density, as indexed by a 2-4 fold difference in ^{3}H-thymidine incorporation. Albumin synthesis, a measure of hepatocyte differentiation, was determined by a specific ELISA immunoassay. Albumin synthesis was 5.4 ± 0.3 vs. 0.6 ± 0.3 μg/ml/600,000 cells/24 hrs in the cells plated at the high cell density vs. the low cell density. The addition of TNF + IFN did not significantly affect ^{3}H-thymidine incorporation or albumin synthesis at either of the cell densities. These results indicate that the cells plated at 21,300 cells/cm^2 are dedifferentiating in culture and this is not due to the effects of TNF + IFN. This dedifferentiation may be responsible for the observed sensitivity to TNF + IFN. Supported by NIH grant DK 44755.

727 KUPFFER CELL INACTIVATION PROTECTS AGAINST LIPOPOLYSACCHARIDE-INDUCED HEPATIC INJURY DESPITE NEUTROPHIL ACCUMULATION AND TUMOR NECROSIS FACTOR-α (TNF-α) RELEASE

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Following exposure to lipopolysaccharide (LPS), neutrophils (PMNs) rapidly accumulate in the liver sinusoids, and midzonal hepatic necrosis develops by 6 hr. PMNs play a critical role in producing parenchymal tissue injury. The requirement for Kupffer cell (KC) activity in the development of LPS-induced hepatotoxicity was examined by employing $GdCl_3$, which selectively inactivates and eliminates KCs. Female, Sprague-Dawley rats (180–225 g, N = 4–7) were pretreated with either saline or $GdCl_3$ (7 mg/kg, iv) 24 hr prior to receiving either LPS (4 mg/kg, iv) or saline vehicle and rats were killed 6 hr post-LPS. As expected, exposure to LPS increased plasma alanine aminotransferase and isocitrate dehydrogenase activities, indicative of hepatic injury (1677 ± 795 U/L and 1711 ± 828 U/L, respectively). These increases were prevented by $GdCl_3$ pretreatment (130 ± 22 U/L and 75 ± 17 U/L, respectively). $GdCl_3$ did not prevent hepatic accumulation of PMNs following treatment with LPS or the increase in plasma TNFα that occurred 1.5 hr after administration of LPS. These results suggest that KC activity is required for the development of hepatic injury following LPS exposure, but is not required for release of TNFα or the influx of PMNs into the liver. (Supported by NIH grant ES05722).

728 TUMOR NECROSIS FACTOR DEFICIENT MICE ARE RESISTANT TO ENDOTOXIC SHOCK

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In order to investigate the roles of tumor necrosis factor (TNF) and lymphotoxin alpha (LTa) for the host resistance, the *Tnf* and *Lta* genes were simultaneously inactivated in mice by homologous recombination. TNFa/LTa–/– mice develop normally. We report an increased resistance to lipopolysaccharide-induced toxicity (LPS) in the D-galactosamine-LPS model tolerating about 1000 fold higher doses of LPS. Furthermore, the mutant mice are resistant to the lethal effect of staphylococcus enterotoxin. Therefore, we demonstrate a resistance to endotoxin and enterotoxin in TNF/LTa–/– mice as reported for TNF-RI-knockout mice. Furthermore, mutant mice have a significantly increased susceptibility to Listeria monocytogenes infection.The TNF/LTa–/– mice may be useful to investigate the role of TNF and LTa in pathogenesis of several toxic conditions, in which a contributing factor provided by endogenous TNF or LT are expected.

729 *IN SITU* DETECTION OF HEPATIC IL-6 TRANSCRIPT EXPRESSION FOLLOWING ACUTE DMN EXPOSURE

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Recent studies have demonstrated that exposure to the hepatotoxicant DMN initiates a pro-inflammatory response. The purpose of this study was to monitor molecular and histopathological changes in liver following a single acute dose of DMN *in vivo*. Mice were exposed to a single ip dose of either vehicle (PBS) or DMN (1.5, 3.0, or 5.0 mg/kg). Livers were collected at 15, 30, 60, 120, 180, and 360 min after exposure, and the tissues separately cryopreserved and paraffin embedded for *in situ* hybridization and histopathological analysis, respectively. *In situ* hybridizations were performed using an *in vitro* transcribed radiolabeled [^{33}P] riboprobe specific for IL-6. Resident peritoneal macrophages stimulated with endotoxin *in vitro* served as the positive control, and sense IL-6 riboprobes served as the negative control. At 15 min post-exposure, transcripts for IL-6 were detected in liver sections from animals that received either a 3.0 or 5.0 mg/kg dose of DMN. After 30 min post-exposure. IL-6 transcripts were still observed in the 5.0 mg/kg treated animals but were not observed in the 3.0 mg/kg treated animals. Neither vehicle nor 1.5 mg/kg DMN induced IL-6 transcripts at any of the time points measured. Furthermore, no IL-6 transcripts were detected at 60 min or thereafter in any DMN dosage group. Histopathological analysis revealed an increase in widely scattered foci of non-parenchymal cells 3 hr after DMN exposure irrespective of the dosage that was not observed in vehicle treated animals. Immunohistochemistry showed these foci to include T-cells and macrophages. These results show the rapid induction of hepatic inflammation following an acute DMN exposure.

730 THE EFFECTS OF PROPANIL ON CYTOKINE PRODUCTION DURING LISTERIA MONOCYTOGENES INFECTION

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The liver is the principal site for the metabolism of environmental toxins. It is also the primary target organ for the replication and subsequent clearance of infection by the gram positive bacteria, *Listeria monocytogenes*. Propanil, a herbicide which is hydrolyzed in the liver, has been shown to have immunotoxic effects in mice. We have found that exposure to propanil at the time of *L. monocytogenes* infection affects cytokine production in a dose dependent manner. The production of IFN-γ and IL-6 by spleen cells was suppressed after exposure to high doses of propanil, and enhanced after low dose exposure. In addition, our results show that spleen cells from mice treated with propanil seven days prior to infection with *L. monocytogenes* have increased production of IL-6 and decreased production of IFN-γ three days post-infection. Liver nonparenchymal cells (NPC) from propanil-treated animals also produced significantly less IFN-γ. IL-6 production by liver NPC, in contrast to the spleen, was decreased as well. Our studies demonstrate that propanil has a differential effect on cytokine production in the spleen and liver during *L. monocytogenes* infection. Future experiments will determine the specific cell populations affected by exposure to propanil. Supported by NIEHS grant ES04758.

731 EFFECTS OF *IN VITRO* EXPOSURE TO PROPANIL AND ITS METABOLITE ON IL-6 PRODUCTION BY MURINE MACROPHAGES

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Propanil (3,4-dichloropropionanilide) is a herbicide that is used extensively in rice and wheat production to control weeds. Approximately 70% to 80% of the total U.S. rice crop is treated with propanil, accounting for more than 95% of the 9.5 to 11.5 million pounds of active ingredient used annually. It has been found that propanil is hydrolyzed in the liver by acylamidase, and is immunotoxic to mice. The previous studies showed that *in vivo* treatment of propanil affected macrophage cytotoxic activity. Our studies focused on the immunotoxic effect of propanil and its major metabolite 3,4-dichloroaniline (DCA) on murine macrophage cytokine production. We found that *in vitro* exposure to propanil or DCA caused significant decreases in interleukin 6 (IL-6) production by LPS-stimulated C57Bl/6 peritoneal macrophages as well as

the murine macrophage cell line, IC-21. The inhibitory effects of propanil and DCA were both in a concentration-dependent manner. Exposure to propanil or DCA after an initial stimulation with LPS for ≤2 hrs resulted in reduced IL-6 production, whereas stimulation with LPS for 4 to 6 hrs prior to propanil or DCA addition did not. Based on the known timing of IL-6 transcription, this suggests that propanil or DCA may be affecting IL-6 production prior to gene transcription. Northern blot analysis will be performed to determine the effects of propanil and DCA on IL-6 message level. Therefore, we conclude that propanil affects murine macrophage IL-6 production, which may, in part, be responsible for the overall immunotoxicity of this compound. (Supported by NIEHS grant ES04758.)

732 THE IMMUNOTOXIC EFFECT OF *IN VITRO* EXPOSURE OF SPLEEN CELLS TO PROPANIL ON THE PRODUCTION OF IL-2, IL-6, AND IFN-γ

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Propanil is a post-emergent herbicide used extensively on rice and wheat crops in the United States. Previous studies in our lab showed that spleen cells from animals treated *in vivo* with propanil produce significantly lower levels of IL-2 and IL-6 but not IFN-γ after *ex vivo* stimulation with Concanavalin-A (ConA). In this study we investigated *in vitro* propanil effect on T cell cytokine production. Spleen cells of C57BL/6 mice were cultured *in vitro* and propanil added either before, during, or after stimulation with ConA. Cell culture supernatants were harvested and IL-2, IL-6, and IFN-γ were determined, by specific ELISA. The results showed that when propanil was present during the entire culture period, the production of three cytokines was inhibited. Treatment of spleen cells with propanil prior to activation with ConA inhibited IL-6 production, but not IL-2 and IFN-γ production. Propanil added after T cell activation with ConA inhibited IL-2 and IL-6 production but not IFN-γ production. Northern blot analysis of IL-2 and IL-6 messenger levels was carried out using IL-2 and IL-6 cDNA probes. The results showed a decrease of both IL-2 and IL-6 messenger levels in the group in which propanil was present during the entire stimulation process. The result demonstrates the direct inhibitory effect of propanil on cytokine production. The differential inhibition of the three cytokines indicates that propanil does not inhibit cytokine production by inhibiting general protein synthesis. Results from the Northern blot analysis imply that the decreased cytokine production of IL-2 and IL-6 was at least partially due to an effect of propanil on transcription. (Supported by NIH grant ES04758.)

733 COMPARISON OF POTENTIAL HEALTH RISKS FROM RADIOACTIVE AND NONRADIOACTIVE CONTAMINATION

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As part of an environmental/remedial investigation of a former military weapons storage area, a human health risk assessment was conducted to assess the toxicity and potential health risks associated with the site-related chemicals of potential concern. The site was used to store and process nuclear and conventional weapons. Analytical results on soil samples identified radioactive and nonradioactive compounds as site-related contaminants. The purpose of this report is to compare the methodology for characterizing the toxicity and potential health risks from radioactive and nonradioactive compounds, present the results of the risk assessment, and how they may affect risk management and remediation decisions. Compounds detected at the site included thorium, radium, uranium, and plutonium. The predominant nonradioactive compounds detected included polycyclic aromatic hydrocarbons, pesticides, and inorganic chemicals. The results of the risk characterization identified potential cancer risks exceeding the USEPA acceptable risk range. The primary contributors to the cancer risk were the radioactive nuclides. The risk assessment was used to identify site areas for possible remediation, assist in developing preliminary remediation goals, and evaluate residual risk, especially when considering natural levels of background radiation.

734 EVALUATION OF THE TOXICITY AND HEALTH RISKS OF SOIL-RELATED RADIOACTIVE CONTAMINATION AND POTENTIAL MIGRATION TO GROUNDWATER

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A human health risk assessment was conducted on a former nuclear weapons storage facility and evaluated potential health risks from exposure to contaminated soil. Although the groundwater is not currently used for domestic purposes, if chemicals in the soil migrate to groundwater additional health risks may result. The purpose of this study is to assess the toxicity and potential human health risks of soil-related, radioactive contamination migrating to groundwater. The primary chemicals of potential concern (CPCs) at the site were radioactive nuclides including thorium, radium, uranium, and plutonium. The potential migration of the CPCs from the soil to the groundwater was evaluated using the computer code RESRAD. The model output was compared to current site conditions and used to predict maximum future impacts to groundwater assuming remediation to established soil clean-up criteria.

The concentrations of the CPCs predicted in the groundwater were assumed to be the exposure point concentrations. Potential human health risks were assessed using exposure scenarios based on domestic use of the groundwater. Characterization of potential health risks was performed using USEPA methodology and by comparison to federal drinking water Maximum Contaminant Levels (MCLs). Although evaluation of the soil contamination previously identified potential health risks, this study showed that the migration to groundwater and associated health risks are not as readily assessed. This evaluation identified several limitations in the methodology for evaluating migration of radioactive soil CPCs to groundwater and in assessing the potential human health risks.

735 RADIONUCLIDES IN SEWAGE SLUDGE: A STATUS REPORT

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Recent discovery of radioactive contamination at sewage treatment plants have raised concern about release of radionuclides into sewage plants that may ultimately contaminate sewage and ash. The extent of radioactive contamination of sewage, ash, or related products is unknown. The Nuclear Regulatory Commission regulates facilities which release radioactive materials into sewage treatment plants, and EPA regulates sewage sludge, ash and related products. Sources and inventory of radioactivity in sewage sludge including inadvertent discovery of elevated concentrations of cobalt-60 in sludge and ash at the Southerly Sewage Treatment Plant and on nine cases of reported radioactive contamination at sewage systems over the last 10 years are summarized and discussed. Next steps to determine whether radionuclides in sludge warrant regulation are also discussed.

736 WILLAMETTE RIVER BASIN GEOGRAPHIC INITIATIVE: A COMPREHENSIVE APPROACH TO IDENTIFY, ASSESS, AND REDUCE ENVIRONMENTAL HEALTH RISKS TO LOCAL COMMUNITIES

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This on-going study characterizes the geographic distribution of environmental chemical releases in the Willamette River Basin using multiple databases in order to identify, rank, and reduce potential environmental health risks to local communities. The Willamette River Basin comprises an area of over 30,000 km^2 and supports approximately 70% of Oregon's population (1.7 million people). In the first phase, the Toxic Release Inventory (TRI) database was analyzed because over 80% of the reporting facilities in Oregon are located in the Willamette River Basin. Following geocoding, 30% of the submitted TRI facility coordinates were found to be incorrect and were revised. Using 1990 Census data, demographics including: race, hispanic status, housing value, and income on a block level were tabulated for neighborhoods within 0.5 and 1.0 mile of each TRI facility. A chronic toxicity index developed by Air, Radiation and Toxics Division, U. S. EPA Region III was used to rank TRI releases on the basis of toxicity alone. This media-specific index relies on oral toxicity data and a standard dose scale (carcinogenic and non-carcinogenic effects) to qualitatively rank chemical releases from TRI facilities. In the second phase, additional chemical release databases such as water discharge permits, hazardous waster sites, as well as a fish contamination database for the Willamette River will be overlaid in order to assist in identifying and prioritizing potential environmental health risks. (EPA, Region X Contract #X-000976-01-0).

737 EVALUATION OF POSSIBLE HUMAN HEALTH RISKS OF COMMON LAWN CARE PESTICIDES

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Public concerns exist on human health risks resulting from pesticide use on residential lawns, gardens, and recreation areas such as parks and golf courses.

Populations at risk from exposure to lawn care pesticides are presumably homeowners, general public, and pesticide applicators. Based on the limited data on most used pesticides in lawn care, we attempted to estimate possible environmental concentrations (EC) and threshold concentration limits (TCL) of selected pesticides. TCL, acute median Lethal Doses (LD_{50}), and Reference Doses (RfD) were compared with EC values to assess exposure and possible health impact to the general public. The results show that the calculated maximum ECs did not exceed the health-based acceptable exposure levels in most scenarios. The findings are discussed in relation to the possible exposures in both children and adults. Data do not appear to suggest that judicious use of lawn care pesticides, strictly according to label directions, increases direct exposure to lawn care pesticides or adversely impacts human health. Such expedient approaches are important in a regulatory setting to identify chemicals that may pose unacceptable risks.

738 "CLEANUP LEVELS" FOR PHOSPHORUS AND IODINE CONTAMINATION IN SOIL

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The reaction wastes, chiefly phosphorus (P) and iodine (I), were found to have contaminated the soil after clandestine methamphetamine production at several residences ceased. The objective of this analysis is to determine the acceptable concentrations ("cleanup levels") of P and I in soil.

Hydroiodic acid used in methamphetamine synthesis was produced by mixing red phosphorus, crystalline iodine, and hydrogen gas. As a common practice in this type of clandestine operation, wastes (including iodide, iodine, and phosphorus) were dumped in pits in the backyard. In this instance, the operation was discovered, shut down, and waste soil was excavated. After excavation, total P and total I for soil at the bottom of the pits were at the detection limits of 10 ppm and 50 ppm, respectively, while the excavated soil piles had an I concentration up to 1300 ppm. Thus, health-based "cleanup levels" of P and I in soil were needed to determine whether the excavated soil required remediation. These Screening Levels (SLs) were calculated following the methodology described in the "Preliminary Endangerment Assessment Guidance Manual" published by Calif. Dept. of Toxic Subs. Control (1994). Toxicologies of P and I are unique because both are essential nutrients for humans. A Reference Dose (RfD) for P was derived from available data while the Recommended Dietary Allowance (RDA) was used for iodine derivation. Using standard residential exposure assumptions, SLs for P and I in soil were calculated to be 1400 ppm and 600 ppm, respectively. These values were used as guidance in determining the cleanup strategy at this property.

739 DEVELOPMENT OF ANNUAL SOIL INGESTION DISTRIBUTIONAL ESTIMATES OF 64 CHILDREN BASED ON DAILY SOIL INGESTION VALUES

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The present analysis provides individual soil ingestion distributions of 64 subjects based on up to eight individual daily soil ingestion values. The median of the estimated daily average soil ingestion values over one year is 75 mg/day while the upper 95% value is 1,750 mg/day. This is the first distributional assessment of individual daily soil ingestion values and represents an important advance over previously published soil ingestion estimates that were dependent on averaged values and therefore were unable to incorporate daily variation. This model estimates that 10% of children will ingest on average approximately 1,200 mg of soil per day over the course of a year. The model-based estimates suggest that excessive soil ingestion may be more common than previously thought. These findings potentially have important implications for how soil ingestion rates are incorporated into site-specific risk assessment activities.

740 APPLICATION OF RISK ASSESSMENT MODELLING TO CALCULATE ACCEPTABLE SOURCE CONCENTRATIONS OF TRICHLOROETHYLENE AT A MAJOR MANUFACTURING COMPLEX

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By combining site specific risk assessment techniques and trichloroethylene (TCE) degradation analyses, we were able to demonstrate that toxicologically insignificant chemical concentrations (less than 1.0 μg/l) will be available to impact human and ecological receptors. The MYGRT™ solute transport model was used to back calculate acceptable concentrations of the primary contaminant, TCE, that could be left at the source area. Based on the environmental degradation rates of TCE and its breakdown products, conservative assumptions indicated that concentrations greater than 700 μg/l at the source area would yield insignificant concentrations at the nearest receptor interface. Key components of the model's application included a correlation of the degradation rates of TCE with the production and relative toxicity of the resultant breakdown products. Modeling results, combined with a mixing zone analysis of the predicted input at the receptor, established that the associated risks would be within acceptable limits. This process of combining risk assessment techniques and delivered dose of TCE can be used to significantly change the regulatory compliance status of many hazardous waste sites.

741 SOIL DEGRADATION OF TRICHLOROETHYLENE TO VINYL CHLORIDE: EFFECT ON CARCINOGENIC RISK ASSESSMENT

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Risk estimates for contaminants in soil can be influenced substantially by environmental degradation and loss. When loss and degradation are considered, risk estimates may be reduced as much as 2–3 fold for compounds with soil half-lives of 7–9 years and orders of magnitude for compounds with shorter half-lives. Reducing cancer risk estimates to account for loss of contaminants from soil is valid only when the soil half-life reflects a decline in carcinogenic potential. This can occur via physical movement of the contaminant out of soil (e.g. leaching; volatilization) or via production of noncarcinogenic degradation products. For contaminants that are degraded directly to noncarcinogenic products or otherwise lost from soil, the reduction in risk is calculated directly from the decline in concentration of the parent contaminant during the exposure interval and the corresponding reduction in dose over the interval. However, when soil degradation results in production of carcinogenic metabolites, estimation of cancer risks is more complex. We modeled the soil degradation of trichloroethylene to vinyl chloride and calculated the magnitude of the impact on cancer risks using a range of published soil half-lives for the parent contaminant and the metabolites. The critical considerations for incorporating soil half-lives into cancer risk estimates were the rate of decline of the parent contaminant, the rate of production of carcinogenic metabolites, the carcinogenic potency of the metabolites and their soil half-lives.

742 HAZARD ASSESSMENT OF 1,3,5-TRINITROBENZENE (TNB) CONTAMINATION OF GROUNDWATER AT PUEBLO ARMY DEPOT, COLORADO

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TNB has been detected as an environmental contaminant of water and soil near trinitrotoluene production waste sites and at certain Army installations. A recent survey at Pueblo Army Depot showed TNB contamination in groundwater (5.6 μg/L). Previously the risk based concentration of TNB for tap water was derived on the basis of 1,3-dinitrobenzene toxicity data which was about 1.8 μg/L. As part of the preliminary remediation goal (PRG), we have initiated a hazard assessment for TNB. The hazard index (HI) for TNB was calculated on the basis of RfD value (0.0039 mg/kg/day) derived from the subchronic NOAEL (3.9 mg/kg/day) of rat feeding studies. The HI values for adult and child were found to be 0.04 and 0.12, respectively. These HI values are much below the target hazard quotient = 1. The PRG level in water for TNB is 140 μg/L for adults, which is about 25 fold higher than the detected level (5.6 μg/L). Our data suggest that observed TNB levels in water will not pose a hazard for non carcinogenic effects. (This abstract does not necessarily reflect U.S. Army/ U.S. EPA policy.)

743 HUMAN HEALTH RISK ASSESSMENT OF THE SEALAND RESTORATION INC. SUPERFUND SITE

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The Sealand Restoration Inc. (SRI) site located in Lisbon, N.Y. briefly operated as a land farming disposal facility for industrial contaminated waste oils and vegetable oils. The facility also accepted solvents and PCB-contaminated industrial wastes and solvents and was closed and listed as an NPL site in 1990.

A baseline human health risk assessment was conducted as part of a remedial investigation/feasibility study. Reasonable maximum exposure and cen-

tral tendency exposure risk analyses were conducted for both current and future land-use of the site. Current on-site exposure pathways were limited to trespasser scenarios and off-site exposures were limited to groundwater pathways (ingestion, inhalation, dermal contact). Possible future land-use exposure pathways were assessed for residential adults and children.

Total carcinogenic and non-cancer risks were assessed for exposure to surface water, sediment and soil using standard USEPA exposure scenarios and assumptions. For off-site residents, ingestion of naturally-occurring arsenic in groundwater contributed 98% of the total carcinogenic risk. Groundwater also accounted for 99% of the cancer and non-cancer risk to future on-site residents. Vinyl chloride was the major contributor to the cancer risk, while acetone accounted for 93% of total non-cancer hazard.

744 ESTIMATING RISKS ASSOCIATED WITH FORMER MANUFACTURED GAS PLANT (MGP) SITES

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A synoptic public health and ecological risk analysis was developed, as part of a site restoration plan, for a former MGP site in a heavily populated area of a large eastern U.S. city. It evaluated heavy metal, petroleum product, and coal tar derivative contamination in various environmental media. The purpose of the evaluation was to integrate the risk assessment into a risk management plan. A risk management decision-making methodology was developed to protect various subpopulations and the environment. Exposure was estimated for several pathways, including inhalation, direct contact, and incidental ingestion by current subpopulations around the site and two potential future on site subpopulations, as well as potential biotic populations/communities. Exposure estimates were compared with various criteria (e.g., chronic/subchronic RfDs) and used with carcinogenic slope factors to estimate carcinogenic risk. Estimates of potential risk ranged from insignificant to carcinogenic levels above 10^{-4} for certain on site exposures resulting from carcinogenic polycyclic aromatic hydrocarbons. No potential ecological risk was predicted. This evaluation demonstrates the importance of integrated planning, data quality objectives, and future site use considerations in developing a coherent, credible picture of potential risks from such sites essential for sound risk management decision-making.

745 MAXIMUM EXPOSED INDIVIDUAL SCREENING PROCEDURE FOR MULTIPLE EMISSION SOURCES

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A major chemical company proposed to use a transportable Thermal Destruction Unit (TDU) for treating solid and non-aqueous liquid wastes from remedial activities in western New York. At the request of NYSDEC, IT developed a cumulative impact assessment protocol to evaluate the impact of all significant point sources in the vicinity of the proposed TDU on the Maximum Exposed Individual (MEI).

Instead of using the maximum ground-level air concentrations to identify the location of the MEI, the protocol utilized a "pilot risk assessment" at all 3,800 grid points to locate the point of maximum risk from all combined sources. The maximum air concentrations of five carcinogenic metals (arsenic, beryllium, cadmium, chromium and nickel) and 2,3,7,8-TCDD-eq. from five significant emission sources and fugitive facility emissions were utilized. The two exposure pathways in this pilot risk assessment were inhalation during residential usage and ingestion of home grown fruits and vegetables potentially exposed to 2,3,7,8-TCDD-eq. dry deposition. The cancer risk from each of these exposure pathways was calculated at each node of the receptor grid (100 meter intervals). The point of highest total risk was then determined, and a detailed Human Health Risk Assessment was completed for this location using all the identified Constituents of Concern and pathways as per NYSDOH Draft Guidance for Exposure Assessment of Municipal Solid Waste and Hospital Waste Incinerator Emissions.

746 REPRODUCTIVE RISK IN THE SEMICONDUCTOR INDUSTRY: IS THE CAUSE KNOWN?

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A study of the reproductive histories of semiconductor industry employees (Pastides et al, 1988) reported an increased risk (1.98) of spontaneous abortions among 34 pregnancies of female employees in manufacturing, somewhat greater in non-photolithography than in photolithography jobs. Two multiplant studies (Schenker et al., 1992 and Gray et al., 1993) attempted to confirm these results and to identify the cause. Schenker et al.'s study of 470 pregnancies of women in fabrication confirmed the finding; Gray et al.'s study of 586 pregnancies of women in clean room jobs did not. Both groups examined exposure opportunities for specific chemicals and other stressors. Photoresist chemical components had become the focus of study because of the known reproductive risk of certain ethylene glycol ethers (EGME, EGEE, EGMEA, and EGEEA) used in the photoresist processes, although the exposure levels in this industry had been demonstrated to be far below the known levels of toxicity for these chemicals. Epidemiologic analysis on the variety of chemicals used in the processes and the number of mixtures of chemicals used demonstrated that effect(s) from exposure to any of the glycol ethers could not be distinguished from those of the other constituents of the chemical mixtures. Thus, no independent risk from individual chemicals can be ascertained.

747 THE PUBLIC HEALTH IMPACT OF THE OXYGENATED FUELS PROGRAM IN NORTH CAROLINA

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Sponsor: M Genter

The Environmental Epidemiology Section (EES) evaluated and reviewed toxicological data on carbon monoxide (CO) and methyl tertiary butyl ether (MTBE) to determine whether there was a public health rationale for continuing the oxygenated fuels program in North Carolina. EES reviewed the human epidemiological data base for CO in order to determine the lowest effect of CO on humans and the no effect level and to relate this data to blood levels of carboxyhemoglobin. A review of data from the first two years of the oxygenated fuels program was requested to determine if MTBE was lowering ambient CO levels in a statistically-significant manner during the program period. EES also reviewed the toxicological data for MTBE which included a detailed review of several carcinogenicity studies. The findings of this review indicated that current CO ambient levels are well below the estimated ambient CO concentration where no observable adverse health effects would be expected and therefore, should not pose any public health risks. The study also found that MTBE failed to lower CO ambient levels in a statistically-significant manner. Finally, EES determined that MTBE was an animal carcinogen, and that increasing exposure at the gas pump to 16% by volume during the program season may pose an increased cancer risk. EES concludes that the oxygenated fuels program utilizing MTBE in North Carolina produces no significant benefit to public health at this time and may indeed be posing an increased health risk to the public.

748 DEVELOPMENT OF RISK-BASED FISH CONSUMPTION ADVISORIES

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A simple model for risk-based fish consumption advisories is proposed in which allowable daily fish intake (g/day) is calculated and then translated into a public consumption advisory stating the number of permissible meals per day, week or month. Benefits of this model are that it offers a risk-based approach that makes use of systemic toxicity and cancer risk value(s) for the measured contaminant, incorporates chemical mixtures methodology, and does not rely on assumptions about amount of fish consumed. As a result, fish consumption advisories can be determined for a wide variety of chemicals and for different sites and exposed populations. The model has been used to develop fish consumption advisories for Native Americans in the Pacific Northwest and in Florida, residents of rural areas near the Great Lakes and Asian Americans in California. For example, considering systemic toxicity, ranges of allowable fish intakes (g/day) for Lake Michigan trout (>25 inches) were calculated to be 4–23 based on chlordane, 13–51 based on DDT, 14–60 based on Dieldrin and 1–3 based on PCB using quantified contaminant concentrations. Because of the low calculated allowable intake for fish with PCB and for the mixture of these chemicals, the public fish consumption advisory for trout might be "do not eat". Fish consumption advisories based on the same chemicals in fish from the Black River allowed more frequent consumption (perhaps 1 meal/month) due to lower contaminant levels. Validation of this model was conducted by comparing the fish consumption advisories derived from this model with those developed by other methods for the above sites. The advisories, while somewhat variable, were fairly consistent among the sites.

750 HUMAN HEALTH RISK ASSOCIATED WITH THE IMPACT OF DRILLING MUD ON GROUND WATER SUPPLIES

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Property owners near exploratory drilling operations of an underground gold mine experienced turbidity and a degradation in the taste and odor of drinking water from underground sources. Drilling mud (Clear Mud Fluidril Mud System manufactured by Nalco), used during diamond drilling and coring operations, was believed to be the cause of contamination. A hydrogeological assessment of the site and a health-risk evaluation of drinking water were conducted. Data from the ore drilling operations and drinking water wells were used to characterize groundwater flows. Analyses of well and spring water using standard EPA methods did not detect individual drilling mud constituents, but the amount of drilling mud used was correlated with total organic carbon concentrations. A model of contaminant plume movement was used to predict the concentration of total carbon in well water as a function of time. Free acrylamide was identified as the principle contaminant of concern for both carcinogenic and noncarcinogenic effects. Deterministic risk-based concentrations (10^{-6}) for free acrylamide were calculated to be 0.011 mg/l for oral and 14 mg/l for dermal pathways, respectively. Comparisons of the modeled acrylamide concentrations at each residence to concentrations corresponding to a 10^{-6} lifetime cancer risk indicated that it was extremely unlikely that 10^{-6} risk levels were exceeded. Risk of systemic toxicity was evaluated by comparing the EPA health advisory levels to upper limit concentrations present at each water source. Although the aesthetic effects of contamination were undesirable, drilling mud constituents were judged to not be harmful to human health under the reported exposure conditions.

751 STREAMLINING THE SUPERFUND RISK ASSESSMENT PROCESS

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The EPA has instituted the Superfund Accelerated Cleanup Model (SACM) to expedite the cleanup of Superfund sites. Under the auspices of this program new initiatives are taking shape to streamline the Superfund process while maintaining human and environmental protection and pursuing fiscal responsibility. As part of this initiative, a streamlined approach for risk assessments has been developed for Wright-Patterson AFB which utilizes conservative, health-based, site-specific cleanup goals as screening criteria. Sites with contamination that results in a carcinogenic risk estimate less than 1×10^{-6} or a noncarcinogenic hazard index of less than 1 are eligible for no-further-action. While sites with contamination that results in a carcinogenic risk estimate greater than 1×10^{-4} or a non-carcinogenic hazard index of greater than 1 are considered for early remedial action. The sites that fall within this screening risk range are subjected to a full quantitative risk assessment to determine if remediation is warranted. This type of early screening can save 3 years in the schedule for a single operable unit while as much as 5 to 7 years can be saved in a multi-operable unit facility. Screening methodologies, derivations of screening criteria and site-specific examples are presented. (Abstract does not necessarily reflect Air Force policy.)

752 EFFECTS OF PROPYLENE OXIDE ON NASAL EPITHELIUM IN F344 RATS: IMPLICATIONS FOR WORKER HEALTH RISK

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In chronic inhalation studies, propylene oxide (PO), widely used in the chemical and food industries, induced nasal tumors in F344 rats. Previously, we demonstrated that the nonneoplastic lesions observed after 4 weeks of PO exposure were similar to those described in long-term studies, and that compensatory cell proliferation is a major component in the mechanism of PO carcinogenicity (Toxicologist 14: 151, 1994). Furthermore, the nonneoplastic lesions and cell proliferation were reversible upon cessation of PO exposure, and 50 ppm was determined to be the NOEL for PO exposure in nasal tissues of rats. These data and a method for extrapolating nasal dosimetry between species, based on pulmonary ventilation rates and nasal cavity surface area, were used to establish a safe workplace exposure limit. A range of values for the human equivalent concentration was calculated using Monte Carlo simulations and approximately 1000 iterations. The results show that a reasonable workplace exposure limit, using the lower 90th percentile on the distribution of human equivalent concentration, is 6.5 ppm. Based on these results and other relevant workplace exposure data, 5 ppm is recommended as the workplace exposure limit for PO.

753 ANALYSIS OF 1,4-DIOXANE CARCINOGENICITY DOSIMETRY

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Carcinogenicity evaluation of 1,4-dioxane (Dx) is based on positive mouse and rat bioassays (Kociba, 1974). Dx cancer activity depends on −I and +P in I/P (initiator or promoter) experiments (Pereira/Lundberg). Since Dx genotoxicity is weak, carcinogenicity is primarily by tumor promotion.Dx metabolizes → β-hydroxyethoxy-acetaldehyde → β-hydroxyethoxy-acetic acid (urine end product). The aldehyde tissue levels are likely excessive at high [Dx] doses and may contribute to the promotion. Agencies currently presume extreme low-dose activity (*elda*) down to the [0,0] which makes even minute amounts of Dx of concern. Dx dosimetry is examined here in mice and rats; both exhibit high-dose only activity in bioassays, i.e. the response has an inflection point below which zero excess cancers are produced. The *elda* default presumption is unlikely based on: (1) actual incidence data, (2) metabolic considerations, and (3) a promotion mechanism [and thus reversible] all of which suggest that the inflection point represents a Dx cancer threshold. This rationale is a simpler, and more direct, explanation of the data than the default presumption, i.e. lower-dose nonresponders merely fall below the sensitivity level of the bioassay. These considerations should factor into a modern-day risk assessment.Views expressed are those of the author and not of any agency of the U.S. government.

754 COMPARISON OF CANCER RISK VALUES FOR EPOXIDE COMPOUNDS ADMINISTERED TO RODENTS BY GAVAGE

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Styrene oxide (SO) and diglycidyl resorcinol ether (DGRE) are used primarily in the production of epoxy resins, and ethylene oxide (EO) and propylene oxide (PO) are used primarily as chemical intermediates. These epoxides are irritating to the gastric epithelium when administered to rodents by gavage. Consequently, they effectively induce proliferative lesions in the forestomach consisting of hyperplasia, hyperkeratosis, squamous cell papillomas and squamous cell carcinomas (SCC). The nonneoplastic effects suggest that cytotoxicity/cell proliferation maybe involved in the mode of action of these epoxides. The relative carcinogen potencies of these four epoxide were investigated based on their potential for inducing SCC in rodents. Dose-response analysis was conducted using EPA's linearized multistage model to derive ED_{10} values. The data show that the ED_{10} values for SO, DGRE, EO, and PO range from 0.13 mg/kg/day for DGRE in male rats to 6.4 mg/kg/day for styrene oxide in female rats (≈50-fold difference). The corresponding potency factor ($1/ED_{10}$) based on millimoles of compound showed about a 90-fold difference between the relative potencies of DGRE and SO. These data suggest that DGRE is the most potent epoxide and SO the least.

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755 DETERMINATION OF DERMAL TAR ADHERENCE AND TRANSFER RATES FOR USE IN RISK ASSESSMENTS

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A risk assessment evaluating potential exposures to tar seeps in a crude oil production field slated to be developed into residential units included the evaluation of dermal and oral exposures. The methodologies used to evaluate these exposures was the transfer-event-based method for oral and direct exposure for dermal. Both the transfer-event-based method and direct dermal exposure require the input parameter of the skin adherence rate for the evaluated material (tar in this study). In addition, the transfer-event-based method requires and additional parameter of the transfer rate of the material from the skin to a transfer vehicle (*e.g.*, food, cigarette). These parameters were not available in the literature for tar. This study determined both of these parameters for use in the quantitative risk assessment. Six human volunteers had either 50 or 5 cm^2 of skin surface area masked with tape. They then handled a sample of an active tar seep until full contact with the entire skin surface area was achieved. The tape was removed and the tar wiped off the skin using tared filter papers dipped in acetone. The filter papers were allowed to dry prior to re-weighing. The average contact rate was 2.6 mg of tar per cm^2. Dry rated filter paper was also handled by the volunteers to simulate a transfer vehicle. The paper was re-weighted to determine the mass of tar transferred to the paper during handling. The experiment determined that 23% of the tar adhering to the skin transferred to the transfer vehicle.

756 POTENCY EQUIVALENCY FACTORS FOR SOME POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) AND PAH DERIVATIVES

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Potency Equivalency Factors (PEFs) relative to benzo[a]pyrene (BaP) (PEF = 1) have been derived for 21 polycyclic aromatic hydrocarbons (PAHs) and PAH derivatives. PEFs have been derived only for PAHs with demonstrated carcinogenicity in bioassays. Data for PEF development were selected in the order: complete quantitative risk assessment, expedited risk assessment, tumor data from inhalation exposure, tumor data from intratracheal or intrapulmonary administration, tumor data from oral administration, tumor data from skin painting studies, tumor data from subcutaneous or intraperitoneal administration, genotoxicity data, and structure activity information. PEF values were rounded to the nearest factor of 10 to account for the uncertainty in the estimates. Values ranged from 0.01 for chrysene to 10 for 1,6-dinitropyrene, 6-nitrochrysene, and three dibenzpyrenes. Since the PEFs will be used in risk assessment settings, PEFs were estimated only for PAHs currently classified as carcinogens. A much larger number of PAHs and PAH derivatives are considered mutagenic or genotoxic and may have limited evidence for carcinogenicity, but these compounds are not considered in this evaluation.

757 COMPARISON OF RELATIVE POTENCY OF TISSUE DOSE VS. ADMINISTERED DOSE OF PCBs IN THE MOUSE

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Some PCBs are known to elicit dioxin-like responses in laboratory animals. Steps to estimate human health risks have been taken in the form of Toxic Equivalency Factors (TEFs). In order to estimate relative potency for the PCBs, 77, 105, 118, 126, 156, and 169, female B6C3F1 mice were exposed orally to test chemicals 5 days/wk for 13 wks. By looking at the ability to induce EROD, a marker for CYP1A1, in liver and skin, TEFs were determined for each compound. The order of potency (TCDD = 1) for these PCBs based on administered dose was 126 (0.01) > 169 (0.001) ≫ 77 (0.00001) ≥ 156 (0.000001) = 118 (0.000001) = 105 (0.000001) for each compound, in the liver and skin. In comparison, the order of potency based on tissue dose was 126 (0.01) = 77 (0.01) > 169 (0.001) ≫ 156 (0.00001) = 105 (0.00001) = 118 (0.00001) for each compound, in the liver and skin. Based on tissue dose, the monoortho PCBs(156, 105, and 118) are 10 times more potent in the liver than estimates using administered dose. PCB 77 appeared to be 100 times more potent based on tissue dose. However, nearly all of the induction of PCB 77 can be accounted for due to the presence of PCB 126 contaminate in the liver and skin samples from the 77 groups. Differences in the TEFs can be attributed both to the compound's affinity for the Ah receptor and the rate at which it is eliminated. Although PCB 126 is the only compound with significant impact on the overall TEQ, differences between administered dose and tissue dose should be taken into account when using TEFs for other polyhalogenated aromatic hydrocarbons. (This abstract does not necessarily reflect US EPA policy.)

758 PCB MIXTURE RESPONSE BASED ON INDIVIDUAL CONGENER STRUCTURE NEUROACTIVITY RELATIONSHIPS FITTED BY RIDGE REGRESSION AND UNIVERSAL KRIGGING

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As a continuation of SAR (structure activity relationship) studies on neuroactivity of PCBs (polychlorinated biphenyl congeners), two statistical methods were applied to the response of 24 of the 209 possible PCBs. The response is the ability of PCBs to affect an *in vitro* test system which consists of a primary culture of cerebellar granule cells being exposed to various doses of the PCB for 15 minutes. The resultant increase in [^{3}H]-phorbol ester binding indicates Protein Kinase C activation/translocation. The area under the dose response curve was also used as a dependent variable. The independent variables included the numbers of chlorines at the ortho, meta, and para positions, the log of the partition coefficient and measures of the Boltzman ratio. The adequacy of the prediction using ridge regression or Universal Krigging, and the predictability of the neuroactivity of mixtures of PCBs based on the additivity assumption will be discussed. (This abstract does not necessarily reflect U.S. EPA policy.)

759 COMPARISONS OF BODY BURDENS IN HUMANS AND EXPERIMENTAL ANIMALS EXPOSED TO DIOXINS

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Over the last 30 years an abundance of studies demonstrated that 2,3,7,8-tetrachlordibenzo-p-dioxin (dioxin) is a potent toxicant in experimental animals. While the toxic effects of dioxin in experimental animals are clear, the toxic effects in humans are less certain. The present study is a survey of the literature comparing the estimated body burdens of dioxins which produce effects in experimental animals to the estimated body burdens in humans which have been associated with effects. In addition, a comparison of the *in vitro* effects of dioxins in human and animal tissues is also presented. This survey indicates that dioxins produce similar effects, such as enzyme induction, proliferation and alterations in growth factors, in human and animal tissue *in vitro* and these effects occur at similar concentrations. The TEF method was used to estimate human body burdens of dioxin-like compounds in humans including dioxins, dibenzofurans and dioxin-like PCBs. Human body burdens were estimated from serum dioxin concentrations (lipid adjusted) assuming dioxins are equally distributed in body fat and that an adult has 18% body fat. In the general population, average background exposures lead to approximately 60 ppt TEQ in the serum, resulting in body burdens of 9 ng/kg. Populations accidentally exposed have body burdens ranging from 14–7,000 ng/kg. For effects clearly associated with dioxin exposure, such as chloracne and induction of CYP1A1, humans and animals respond at similar body burdens. Increased tumor incidence in animals is observable at body burdens ranging from 500–1,600 ng/kg. Non-cancer effects in animals occur at body burdens ranging from 7–10,200 ng/kg. The data indicate that average background human body burdens are 50–180 times less than body burdens that produce carcinogenic effects in animals and within an order of magnitude for some non-cancer toxic effects. (This abstract does not necessarily reflect USEPA policy).

760 THE RELATIVE CARCINOGENIC HAZARD POSED BY POLYCHLORINATED DIBENZO-p-DIOXIN AND DIBENZOFURAN, AND AROCLOR 1254 AT A TRANSFORMER MAINTENANCE AND REPAIR FACILITY

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The carcinogenic hazard of polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) relative to polychlorinated biphenyl (PCB) mixtures was evaluated to determine the need for separate sampling and analysis of PCDDs and PCDFs in soils at a transformer maintenance and repair facility. Such testing should only be considered necessary if the relative hazard posed by PCDDs/PCDFs is significant compared to that posed by PCBs. The relative carcinogenic hazard was evaluated using cancer slope factors for PCBs and 2,3,7,8-TCDD, and the International Toxic Equivalency Factor (I-TEF) scheme to evaluate toxicity of PCDD/PCDF congeners. PCDD/PCDF concentrations reported in the scientific literature measured in transformer fluids handled during routine maintenance activities were used to conservatively estimate PCDD/PCDF concentrations potentially associated with PCBs at the site. The results of the analysis indicate that the relative carcinogenic hazard posed by PCDDs and PCDFs associated with PCBs at the site was less than 1.3% of the hazards estimated for the PCBs. Considering the many other sources of uncertainty in the hazard assessment, it was concluded that quantification of PCDD/PCDF levels was not justified at this site.

761 QUANTITATIVE ASSESSMENT OF TCDD MEDIATED LOW DOSE EFFECTS ON NON-CANCER ENDPOINTS USING INCREMENTAL POTENCY FACTORS

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Application of the Power Law and Sigmoid-Emax functions to immune and biochemical endpoints that were associated with acute Tetrachlorodibenzo-p-dioxin (TCDD) administration demonstrated that high dose data can impact the assessment in the low dose range. To alleviate this potential estimation problem, low dose subsets from six published or peer reviewed data sets measuring these non-cancer endpoints were fit to the Power Law function. Estimation of benchmark doses of ED_{10}, ED_1, $ED_{0.01}$, and $ED_{0.0001}$ were made from the fits of these six studies to the Power Law function. Because TCDD effects have been purported to occur at background levels, the incremental

potency factor was calculated for each of the six studies by subtracting the $ED_{0.0001}$ (arbitrarily set as background level) from the $ED_{0.01}$ to determine the dose attributed to a 1% change over background in the reported immune suppression or enzyme induction data. Results of this analysis indicate that this 1% change occurs at doses from 2.9×10^{-8} to 3.4×10^{-6} μg TCDD/kg for enzyme induction and 4.1×10^{-7} to 1.8×10^{-5} μg TCDD/kg for immune suppression. The quantification of an incremental potency factor incorporates both a biologically-based dose-response function and low dose data. In addition, this technique allows the assessment of the impact of TCDD when added to the already contaminated environment. Therefore this technique has significant implications in regulatory activities focusing on TCDD source reduction. (Research supported by NIEHS CENTER grant ESO5022).

761a RISK REDUCTION THROUGH MECHANISM-BASED MOLECULAR DESIGN OF NON-CARCINOGENIC AROMATIC AMINE DYES

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Aromatic amine dyes form one of the largest and commercially most important group of dyes, with a wide spectrum of applications. The recognition that a number of them are human carcinogens prompted a search for safer replacement products. The aromatic amines require metabolic activation for carcinogenicity and the mechanisms of bioactivation are now known in considerable detail. Carcinogenicity data are available for many aromatic amines with different ring structures and different ring substituents, thus allowing Structure-Activity Relationships (SAR) analysis and the determination of structural requirements for carcinogenic activity. SAR analyses revealed that several molecular parameters are critical for the bioactivation and carcinogenic potential of aromatic amines; cancer risk of populations exposed to aromatic amine dyes in their manufacture, processing, or use can be reduced by mechanism-based modifications of their chemical structures. Some of these modifications include: (1) altering the position of the amino/amine-generating group(s) in the aromatic ring(s); (2) altering the position of substituent(s) with respect to the amino/amine-generating group(s); (3) introducing bulky substituent(s) *ortho* to the amino/amine-generating group(s); (4) sulfonation of the aromatic ring(s) bearing the amino/amine-generating group(s); and (5) replacing electron-conducting intercyclic linkages by electron-insulating intercyclic linkages.

(The contents of this paper do not necessarily reflect the views and policies of the USEPA).

762 DOPAC-MEDIATED PROTEIN CROSSLINKING IS POTENTIATED BY MANGANESE, COPPER, AND IRON

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Oxidation of endogenous catechols is suspected to play a role in the degeneration of neurons in patients with Parkinson's disease, and some evidence suggests that increased levels of certain transition metals increase the deleterious effects of oxidized catechols. We investigated the possibility that 3,4-dihydroxyphenylacetic acid (DOPAC) may undergo spontaneous oxidation to cause protein-protein crosslinking. We also tested the effect on the reaction of Fe(II), Fe(III), Cu(II), Mn(II), and Mn(III) ions. Reactions were incubated for 12 hours under physiological conditions (37°C, pH 7.4). The crosslinking of Nα-acetyl-L-lysine (NAL, 50 mM) to chicken egg albumin (10 mg/ml) by DOPAC was detected by ELISA, using antibody 1400/3, a rabbit polyclonal antiserum that recognizes NAL crosslinked to protein. The presence of crosslinked protein species was confirmed by polyacrylamide gel electrophoresis. DOPAC without added transition metal ions led to the appearance of crosslinked immunoreactive product in a concentration-dependent manner; the highest quantities of product were obtained at a concentration of 1.0 mM DOPAC. Increased quantities observed in reactions containing each of the metal ions tested. For Fe(II), Fe(III), Cu(II), and Mn(II), the increase in crosslinking reactivity was greatest with metal ion concentrations ranging from 0.1 mM to 1.0 mM, with decreased reactivity at higher concentrations. In contrast, Mn(III) led to proportional increases in immunoreactive product at Mn(III) concentrations up to 10 mM. At concentrations of 1.0 mM DOPAC and 0.1 mM metal, the order of crosslinking reactivity was: Cu(II) > Mn(II), Mn(III) > Fe(II), Fe(III). Appearance of melanin, assessed by uv-vis spectroscopy, and disappearance of DOPAC, measured by HPLC-EC, were observed in the reactions, in conjunction with the appearance of immunoreactive product. The results indicate that oxidation of DOPAC leads to protein-protein crosslinking, an effect potentiated by transition metal ions, particularly Cu(II) and Mn(III).

763 INDUCTION OF α- AND μ-GST AND ALTERATION IN ESTROGEN RECEPTOR EXPRESSION IN RAT ASTROCYTOMAS

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Exposure to chemical carcinogens may alter expression of glutathione S-transferases (GSTs) and estrogen receptor (ER) in the rat; these alterations may contribute to the development of preneoplastic or early neoplastic changes. To test this association, pregnant Wistar-Kyoto dams were given a single i.p. dose of N-ethyl-N-nitrosourea (ENU; 30 mg.kg^{-1}) on gestational day 15. Offspring developed intracranial tumors 8–12 months later. Tumors included ependymomas, malignant meningiomas, mixed gliomas and malignant astrocytomas. Paraffin sections (5 μm) of brain and spinal lesions were immunostained for α-, μ- and π-GSTs, glial fibrillary acidic protein (GFAP), S100 protein and galactocerebroside (galc) using antisera (GSTs) or monoclonal antibodies (GFAP, S100, galc). Astrocytomas immunopositive for GFAP show increased expression of α- and μ- but not π-GST in tumors and tissue adjacent to well-defined tumor masses. Similarly, samples of human glioblastoma multiforme showed increased expression of α- and μ-GST. Western blot analysis of tumor-bearing and control rat brain reveal elevated and atypical expression of ER only in malignant astrocytomas but not other tumors. Data from Northern blot analysis of primary cultures of astrocytomas and tumors *in situ* indicate atypical expression of ER-mRNA in malignant astrocytomas in rat. These results suggest that aberrant expression of estrogen receptor may be linked to the degree of malignancy of the tumor and that α- and μ-GSTs are induced in and around malignant astrocytomas. ENU-induced intracranial malignancies in rat are good models for studying processes involved in the induction and growth of astrocytomas. (Supported by NIH ES06103, ES05955 and ES05022).

764 INHIBITION OF INTRACELLULAR CALCIUM BUFFERING SYSTEMS IN RAT CEREBELLUM BY POLYCHLORINATED BIPHENYL MIXTURES AND CONGENERS: STRUCTURE-ACTIVITY RELATIONSHIPS

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Studies from our laboratory indicate that polychlorinated biphenyl (PCB) congeners *in vitro* perturbed signal transduction mechanisms including cellular Ca^{2+}-homeostasis and protein kinase C translocation at concentrations where no cytotoxicity was observed. We have now investigated the structure-activity relationship (SAR) of three PCB mixtures, 24 PCB congeners and one dibenzofuran for their effects on intracellular Ca^{2+}-buffering systems in rat cerebellum. Ca^{2+}-buffering was determined by measuring mitochondrial and microsomal Ca^{2+}-sequestration using radioactive $^{45}CaCl_2$. All three mixtures studied, Aroclor 1016, Aroclor 1254 and Aroclor 1260 were equally potent in inhibiting microsomal and mitochondrial Ca^{2+}sequestration with IC_{50} values of 6–8 μM. 1,2,3,7,8-Pentachlorodibenzofuran had no effect on Ca^{2+}-buffering systems. The SAR among the PCB congeners revealed: (1) congeners with *ortho-/meta-* or *ortho-, para*-chlorine substitutions were the most potent in inhibiting Ca^{2+}-buffering (IC_{50} = 2.4 to 22.3 μM); (2) congeners with only *para-*, but without *ortho*-substitutions were not effective in inhibiting Ca^{2+}-buffering systems (3) increased chlorination was not related to the effectiveness of these PCB congeners. The present SAR studies indicate that the effects of most PCB congeners *in vitro* may be related to an interaction at specific sites having preference for low lateral substitution or lateral content (*meta-* or *para-*) in the presence of *ortho*-substitution. (Supported by USEPA award #CR 818550-01-0 to PRSK).

765 NIFEDIPINE AND TETRODOTOXIN (TTX) DELAY THE ONSET OF THE METHYLMERCURY (MeHg)-INDUCED Ca_e^{2+}-DEPENDENT INCREASE IN $[Ca^{2+}]_i$, IN NG108-15 CELLS

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MeHg causes multiphasic alterations in the fluorescence of the Ca^{2+} selective probe fura-2 in NG108-15 cells, synaptosomes, and cerebellar granule cells. In NG108-15 cells the alterations involve an initial increase in $[Ca^{2+}]_i$ originating from an intracellular pool. This phase is followed by an increase in the concentration of an endogenous polyvalent cation, possibly Zn^{2+}. Finally, there is an increase in $[Ca^{2+}]_i$ resulting from an influx of Ca_e^{2+}. The objective of

this study was to define the mechanisms mediating the influx of Ca_e^{2+} during the second Ca^{2+} phase. The L-type Ca^{2+} channel antagonist nifedipine (0.1 to 10.0 μM) caused a concentration-dependent delay in the time-to-onset of the second Ca^{2+} phase. Because at these concentrations the effect of nifedipine may not be specific for L-type Ca^{2+} channels and because the L-type Ca^{2+} channel agonist Bay K-8644 and the nonspecific Ca^{2+} channel blocker Ni^{2+}, had no effect on this phase, it was postulated that the effect of nifedipine may be at sites other than the L-type Ca^{2+} channel. The Na^+ channel antagonist, TTX (1 μM) significantly delayed the onset of the second Ca^{2+} phase from 13.2 ± 2.2 to 23.8 ± 0.7 min. Moreover, similar to nifedipine, TTX also delayed the onset of the other phases. It is hypothesized that MeHg may gain access to critical intracellular sites in NG108-15 cells and that entry may involve plasma membrane voltage-dependent Na^+ channels. Supported by NIH grant ES03299 and an ASPET Undergraduate Summer Research Internship to AY.

766 EFFECTS OF AGING ON THE ANTIOXIDANT ENZYMES, SUPEROXIDE DISMUTASE, CATALASE, GLUTATHIONE PEROXIDASE AND GLUTATHIONE IN DIFFERENT REGIONS OF MOUSE BRAIN

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Recently we have generated evidence that the neurotoxicant MPTP and the drug of abuse methamphetamine induce neurotoxicity via oxidative stress in an age-dependent manner. The present study was designed to evaluate whether there are any selective changes in the activity of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and in glutathione (GSH) levels in different regions of the mouse brain. Male C57/B6N mice, 1, 6 and 24 months of age were sacrificed and brains were dissected into caudate nucleus (CN), frontal cortex (FC), hippocampus (HIP), cerebellum (CE) and brain stem (BS). SOD and GSH-Px activities (U/mg protein) were higher in CN, CE and BS than in HIP and FC. Activity of CAT was similar in all regions of the brain across these age groups. GSH levels were higher in CN and FC than in HIP, CE and BS. The activities of these enzymes were different in different regions of the brain, however, there were no significant age-related changes in their activities. These data indicate that the activity of these enzymes does not deteriorate with age in mice.

767 DISTRIBUTION OF GLUTATHIONE AND GLUTATHIONE-RELATED ANTIOXIDANT ENZYMES IN MITOCHONDRIA AND CYTOSOL OF CULTURED CEREBELLAR ASTROCYTES AND GRANULE CELLS

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The distribution of glutathione (GSH) and GSH-related antioxidant enzymes involved in cellular defense against reactive oxygen species in nervous system has been extensively studied. However, little is known about the subcellular distribution of GSH systems in cultured neural cells. The present study investigates the distribution of mitochondrial and cytosolic GSH and GSH-related enzymes in cultured cerebellar astrocytes and neurons. Cerebellar astrocytes and granule cells obtained from 2 day- and 8 day-old Sprague-Dawley rats, respectively were grown in Dulbecco's modified Eagle's medium. Mitochondrial and cytosolic compartments were separated using digitonin (0.1 mg/ml) treatment and rapid centrifugation. Mitochondrial content of GSH in cultured astrocytes and granule cells was 0.36 and 0.44 nmol/mg protein, respectively, and accounted for 2.4% and 4.6% of the respective total cellular GSH. Cytosolic GSH levels and activities of cytosolic glutathione reductase (GR), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) in astrocytes were 2–3 times higher than those found in granule cells. In contrast, granule cells contained higher mitochondrial GSH levels and greater mitochondria/cytosol GR, GPx and GST activities as compared to astrocytes. Although *in vitro* treatments with 100 μM ethacrynic acid depleted both cytosolic and mitochondrial GSH in cultured astrocytes and granule cells in a time-dependent fashion, cellular GSH in granule cells was more resistant to GSH-depleting agents than astrocytes. These results demonstrate the heterogenous distribution of GSH and GSH-related enzymes in subcellular fractions of cultured cerebellar astrocytes and granule cells and in different neural cell types which may provide a basis for selective cellular and/or subcellular expression of neurotoxicity. (Supported by ES06103, ES05955 and ES05022).

768 MODULATION OF CYANIDE-INDUCED ENHANCEMENT OF NMDA RESPONSES IN RAT CEREBELLAR GRANULE CELLS

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In neuronal cells, the enhancement of NMDA-induced elevation of $[Ca^{2+}]_i$ by cyanide has been suggested to involve an interaction of cyanide with NMDA-type glutamate receptors. The present studies were conducted to investigate this putative interaction using rat cerebellar granule cells. In $MgCl_2$-free Locke's solution that contained glycine, the addition of 50 μM NaCN showed 49.9% enhancement of the 50 μM NMDA-stimulated increase of $[Ca^{2+}]_i$. NaCN had no significant effect on basal $[Ca^{2+}]_i$ or the 0.5 and 5 μM NMDA-induced responses. In the absence of glycine, the NMDA (50 μM) stimulation was decreased 37.5%, while NaCN-induced enhancement of NMDA response was about the same magnitude as compared to that in the presence of glycine. Addition of $MgCl_2$ (1.2 mM) resulted in a 67% reduction of the NMDA response and decreased 65.9% of the NaCN-induced enhancement when compared to values obtained in the $MgCl_2$-free media. Cyanide's action was abolished by the NMDA receptor antagonists MK-801 and APV, which blocked the NMDA response by 61 and 80.5%, respectively. Modulation of the NMDA receptor redox regulatory site by 1 mM SNP or ISDN (nitric oxide donors) resulted in 64.9 and 49.9% reduction of the NMDA response. However, the NaCN-induced enhancement was not altered by NO treatment. These results suggest that the enhancement of NMDA-induced elevation of $[Ca^{2+}]_i$ by cyanide is not due to an interaction with the glutamate binding site or the receptor-associated ion channel but mediated by cyanide's action on one or more of the modulatory site(s) of NMDA receptor complex. (Supported by NIH Grant ESO4140).

769 CYANIDE INDUCED NEUROTOXICITY INVOLVES NITRIC OXIDE GENERATION LINKED TO NMDA/GLUTAMATE RECEPTOR ACTIVATION

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Cyanide-induced neuronal death resembles excitotoxicity and involves a rise of cytosolic Ca^{2+}, generation of reactive oxygen species (ROS) and lipid peroxidation (LP). The mechanism underlying the generation of ROS in cyanide toxicity remains unclear. In the present study, the generation of ROS by KCN was analyzed by microfluorescence in 2,7-dichlorofluorescin (DCF) loaded cerebellar granule cells. KCN produced a conc. (0.25–200 μM) dependent generation of ROS over a 10 min period. Pretreatment with MK-801 (1 μM) blocked the KCN induced generation of ROS. To determine the contribution of nitric oxide (NO) and ROS to the increase of DCF fluorescence observed after KCN, L-NAME (NO synthase inhibitor), hemoglobin (Hb), superoxide dismutase (SOD) and catalase were added. Hb (10 μM) or L-NAME (300 μM) blocked the formation by 60%, whereas SOD (100 U) or catalase (100 U) attenuated the KCN-induced ROS by 35–40%. Coapplication of SOD and Hb blocked the fluorescent signal. A positive correlation was observed between LP and ROS formation. MK-801 blocked LP induced by KCN whereas L-NAME partially diminished it. Addition of SOD along with L-NAME decreased LP to control levels. This correlated with complete protection by MK-801 and partially by L-NAME (1.0 mM) from KCN-induced cytotoxicity. Through NMDA receptor action cyanide can produce an excitotoxic-like response involving NO and ROS generation. (Supported by NIH grant ES04140.)

770 NEUROBEHAVIORAL CHANGES AND *IN VITRO* AND *IN VIVO* INHIBITION OF BRAIN MITOCHONDRIAL CYTOCHROME C OXIDASE BY SODIUM AZIDE

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Sodium azide (NaN_3) is known to inhibit mammalian cytochrome *c* oxidase (Cox) activity, to induce pathological lesions in the striatum, optic nerves and optic tracts, and to elicit movement disorders in macaques. NaN_3 is proposed to cause striatal damage by perturbing oxidative phosphorylation and activating a glutamate-mediated excitotoxic mechanism, presumably secondary to Cox inhibition. This study examines the inhibition of Cox activity in brain mitochondria of mice and their neurobehavior following NaN_3 administration. Brain mitochondria were treated for 15 min with NaN_3 (0.2–1.0 mM) at pH 7.0 and 30°C. A concentration-dependent inhibition of Cox activity was observed (IC_{50}, 1 mM). Mice (n = 5) were injected with NaN_3 (19.6 mg/kg, i.p.)

in saline or with saline alone. Within 10 min, the NaN_3-treated mice became withdrawn, showed palpebral closure and increased respiratory rate. Signs of lethargy, recumbency, hyperreflexia, arched back, raised hair coat, slow and occasional listeriogenic movement were also evident. At 24 h, control and NaN_3-treated mice (n = 4/group) were decapitated, brain mitochondria prepared and Cox activity determined. A significant ($P < 0.05$) mean inhibition (34%) of Cox activity was observed in NaN_3-treated mice. These results suggest that NaN_3-induced blockade of COX, a phenomenon also reported in Alzheimer's disease, elicits energy deficits that triggers the pathological process which culminates in striatal damage. Supported in part by NIH grant NS 19611.

771 COMPARISON OF THE TIME COURSE AND IONIC DEPENDENCE OF DOMOIC ACID AND GLUTAMATE EXCITOTOXICITY IN PRIMARY CULTURES OF RAT CEREBELLAR GRANULE CELLS

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Cerebellar granule neurons from 8 day old rat pups were utilized at 12 days in culture in normal physiologic medium at 22°C. Toxicity was assessed by direct counting of neurons and by measurement of lactate dehydrogenase (LDH) efflux into the medium. After a two hour incubation, glutamate caused a maximal 65% reduction of viable neurons (EC_{50} = 19.1 μM) and domoate produced a reduction of 79% (EC_{50} = 2.9 μM). Substitution of choline chloride for NaCl prevented the induction of toxicity by either toxin, whereas replacement of calcium with cobalt increased the level of toxicity. Addition of 100 mM sucrose prevented these acute responses, indicating significant osmotic effects triggered by these neurotoxicants. To further assess the degree to which toxicity could be influenced by osmotic rupture of the neurons, the experimental protocol was altered so that incubation buffer could be collected for assay of LDH efflux immediately after the acute exposure and 24hrs after replacement of untreated media. When performed in the presence or absence of sucrose, this procedure enabled separation of the excitotoxic response to each agent into two components: an acute phase and a more slowly developing neurodegenerative phase. At the longest exposure times, a greater degree of neuronal damage attributable to osmotic lysis was induced by domoate than glutamate. Domoate and glutamate toxicity could be further differentiated based upon the exposure time required to produce toxicity.

772 EAA-INDUCED NEUROTOXICITY *IN VITRO* IS ASSOCIATED WITH A SUSTAINED INCREASE IN *c-fos* mRNA LEVELS

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As part of an EC collaborative programme to develop *in vitro* neural systems for pharmacotoxicological research, we have studied the role of expression of the c-*fos* proto-oncogene in excitatory amino acid (EAA)-induced toxicity using primary neuronal cultures. L-Glutamate is the major excitatory neurotransmitter in the CNS. We have investigated the possibility that c-*fos* expression may be a marker for excitatory amino acid-induced nerve cell death. Stimulation of cerebellar granule cell cultures with low micromolar concentrations (10 μM) of L-glutamate led to a transient induction in the levels of c-*fos* mRNA which peaked at 30 min and returned to basal levels by 2 h. In parallel experiments, in which leakage of lactate dehydrogenase (LDH) activity into the extracellular medium was used as an indicator of cell death, it was shown that 10 μM glutamate was not toxic to these cells. In contrast, it was observed that exposure of cerebellar granule cells to higher, toxic concentrations (250 μM) of glutamate and two sulphur-containing excitatory amino acids of neuropathological interest, L-homocysteate and S sulpho-L-cysteine, led to increased c-*fos* mRNA levels which were sustained for at least 4 h. This sustained c-*fos* expression could be inhibited by the NMDA receptor antagonist, APV, but not by the AMPA receptor antagonist, CNQX. These preliminary data linking neurotoxicity of excitatory amino acids with sustained increased levels of c-*fos* mRNA *in vitro* are particularly interesting in the light of a recent report (Smeyne *et al.* Nature (1993) 363 166–169) that continuous c-*fos* expression precedes programmed cell death *in vivo*. This research indicates that sustained c-*fos* expression may be an important marker for neurotoxicity screening *in vitro*. This research was supported by the CEC BIOTECHNOLOGY programme – contract BIOT-CT93-0224.

773 INHIBITION OF TYROSINE HYDROXYLASE (TH) ACTIVITY BY DIALKYLDITHIOCARBAMATES IN PC12 CELLS AND FIBROBLASTS THAT EXPRESS TH

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Inhibition of TH has been proposed as a mechanism for the motor and behavioral effects of dialkyldithiocarbamate exposure. Diethyldithiocarbamate (DEDC), dimethyldithiocarbamate (DMDC), and methyldithiocarbamate (MDC) were examined for their ability to inhibit TH activity in PC12 cells and CHO fibroblasts that expressed TH (CHO/TH). Full length rat TH cDNA was subcloned into pRc/CMV and used to transfect CHO monolayers. Stable CHO/TH transfectants were selected with G418 and high expressing clones were identified by immunoblotting with a monoclonal antibody for TH. TH activity in CHO/TH cultures was tetrahydrobiopterin dependent. DEDC or DMDC did not significantly alter viability of PC12 cells or CHO/TH cells at ≤100 muM for 18 hr; the ED50 for each compound was approximately 5 mM in both cell lines. In contrast, the ED50 for MDC was 32 or 74 uM in PC12 or CHO/TH cultures, respectively. DEDC and DMDC (5 to 500 uM) produced concentration-dependent reductions in PC12 dopamine and dopac as well as in CHO/TH dopa levels as assayed by HPLC/EC. Reduction of PC12 catechols was not due to enhanced release into the medium. *In vitro* assay of PC12 TH activity was 80.2% or 82.4% of control following exposure to 100 uM DEDC or DMDC, respectively. Immunoblots of DEDC or DMDC exposed PC 12 or CHO/TH cells probed with anti-TH monoclonal antibodies did not reveal any change in immunoreactivity. DEDC and DMDC, but not MDC, are low potency cytotoxins that inhibit TH activity in tissue culture. Inhibition of TH activity in CHO/TH cultures indicates that DEDC and DMDC act directly on TH.

774 LEAD AND ZINC ALTER [^{3}H]-DCKA BINDING TO THE STRYCHNINE-INSENSITIVE GLYCINE SITE OF THE NMDA RECEPTOR COMPLEX

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We have previously shown the lead (Pb) interacts at high and low affinity zinc (Zn) binding sites on the NMDA receptor complex to inhibit receptor activation (Guilarte et al, *The Toxicologist* 14(1): 83, 1994). The exact molecular mechanism(s) for the inhibitory effects of these divalent cations, however, is not known. A previous study has suggested that Zn inhibits NMDA receptor activation via non-competitive antagonism of glycine binding (Yeh et al., *Mol. Pharm.* 38:14, 1990). In the present study, we found that Zn enhanced the binding of the glycine site antagonist [^{3}H]-DCKA via different concentration-dependent mechanisms. Scatchard analysis showed that at low concentrations (10–50 μM), Zn enhanced the binding of [^{3}H]-DCKA by increasing binding affinity (decreased Kd) with no change in the maximal number of binding sites (Bmax), while at high concentrations (250 μM), Zn decreased binding affinity (increased Kd) but increased Bmax. Pb at low concentrations (10–50 μM) did not alter binding parameters while a 100 μM Pb concentration significantly decreased binding affinity (i.e. increased Kd) with no change in Bmax, suggestive of a competitive interaction. These preliminary studies suggest that Zn and Pb may be inhibiting NMDA receptor activation by altering or maintaining the conformation of the glycine site in an antagonist-preferring state. [Supported by NIEHS grant #ES06189 to TRG].

775 EFFECT OF ALUMINUM ON IRON UPTAKE AND TRANSFERRIN RECEPTOR REGULATION IN PRIMARY OLIGODENDROCYTE CULTURE

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Transferrin (Tf) is a major transport protein for both iron (Fe) and aluminum (Al) and it can mediate cellular uptake of these elements via cell surface Tf receptors. To study the effect of Al-Tf on Tf receptor regulation in oligodendrocytes, cell suspensions were obtained from newborn Sprague-Dawley rats and cultured in MEM plus 10% FBS, antibiotics, insulin (2 ng/ml) and glucose (0.6%). For Al and Fe uptake studies, cells were transferred to Tf-free medium and incubated for 4 hrs at 37°C with apo-, Fe-, or Al-Tf (100 μg/ml). To quantitate cell surface Tf receptors, preincubation (4 hr, 37°C) was performed with apo-, Al- or Fe-Tf and homologous receptor binding studies were subsequently conducted with ^{125}I-Fe-Tf at 4°C. Incubation with AlTf led to dose-related increases in cellular Al. Incubation with either Al- or Fe-Tf led to a similar 50% decrease in ^{59}Fe uptake and a decrease in surface Tf binding. Receptor binding studies showed decreased receptor numbers and affinities (B_{max}, K_d) after Al- or Fe- Tf incubation. Receptor sites/cell were 1.05, 0.60

and 0.46 × 10^5 after incubations with equivalent amounts of apo-, Fe-, and Al-Tf respectively. We conclude that AlTf downregulates surface Tf receptors on oligodendrocytes in a manner similar to FeTf and may thus limit Fe uptake in these cells, which have a high Fe requirement for myelination. Supported by ES04190.

776 DIADENOSINE TETRAPHOSPHATE (AP4A) INCREASES THE OXIDATION OF 1-METHYL-4-(2-METHYLPHENYL)-1,2,3,6-TETRAHYDROPYRIDINE (2'CH3-MPTP) IN MOUSE BRAIN MITOCHONDRIA

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We have previously demonstrated that extracellular application of the endogenous dinucleotide Ap4A to PC12 cells increases the toxicity of 2'CH3-MPTP by what appears to be a monoamine oxidase (MAO) mediated event. We have extended this work to investigate the effect of Ap4A on the capacity of mouse brain mitochondrial preparations to oxidize 2'CH3-MPTP. The addition of 300–1200 M Ap4A significantly ($P<0.001$) increases the rate of 2'CH3-MPTP oxidation is a dose-dependent manner. With no Ap4A present, addition of 250 μM of the MAO inhibitor deprenyl (a dose selective for MAO-B) inhibits the rate of oxidation by 66–72% ($P<0.001$). 250 μM of the MAO inhibitor chlorgyline (a dose selective for MAO-A) inhibits the rate of oxidation by 18% ($P<0.001$). The addition of Ap4A had no significant effect upon the deprenyl induced inhibition of 2'CH3-MPTP oxidation but significantly ($P<0.05$) attenuated the chlorgyline induced inhibition. These results provide support for the hypothesis that Ap4A induced enhanced toxicity of 2'CH3-MPTP in PC12 cells is MAO mediated. The oxidation of 2'CH3-MPTP is carried out primarily, but not exclusively, by MAO-B. MAO may have a role in the pathogenesis of idiopathic Parkinsonism. Ap4A may modulate the activity of both MAO-A and MAO-B in the human brain and therefore may also be involved in the development of Parkinson's Disease and other clinical states associated with central dopamine dysfunction.

777 POLYCHLORINATED BIPHENYLS REDUCE DOPAMINE CONTENT IN STRIATAL SLICES FROM IMMATURE AND ADULT RATS

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Polychlorinated biphenyls (PCBs) are widespread environmental neurotoxicants associated with behavioral changes in human infants, behavioral and neurochemical changes in experimental animals and reductions in dopamine (DA) content of pheochromocytoma (PC12) cells *in vitro*. In order to study the neurochemical effects of PCBs in a more complex *in-vitro* system, we exposed 350 μm thick striatal slices from immature and adult rats to a 1:1 mixture of Aroclors 1254 and 1260. DA and DOPAC (the major metabolite of DA) concentrations in slices and media were determined by HPLC. PCBs reduced slice DA content in a time and dose-dependent manner with a 65–80% reduction observed after 6 h exposure at 200 ppm. There were no significant differences in neurochemical responses between striatal slices from adult or immature rats. DA and DOPAC concentrations in medium increased following exposure to PCBs suggesting that the decreased slice DA content may be due to enhanced release and metabolism of DA. These results suggest that, in addition to inhibition of DA synthesis, additional mechanisms may be responsible for the decreases in brain DA content observed following *in-vivo* exposure to PCBs. Supported by NIEHS Grant ESO4913.

778 REGION-SPECIFIC EFFECT OF 2,3,7,8-TETRACHLORO-DIBENZO-p-DIOXIN (TCDD) ON EXCITATORY POST-SYNAPTIC POTENTIALS IN RAT HIPPOCAMPAL SLICES

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Environmental neurotoxicants such as halogenated aromatic hydrocarbons (HAHs) and lead are known to impair cognitive function and learning. The cellular basis of impaired brain function is poorly understood. However, the brain region known as the hippocampus is a likely site of toxic action because of its well-known roles in learning and memory, as well as its propensity to accumulate neurotoxicants. One function of the hippocampus that can be measured readily is long-term potentiation (LTP), which is widely studied as a neurological substrate for learning and memory. Excitatory post-synaptic potentials (EPSPs) are often used to study the process of LTP. In this study, effects of HAHs on EPSPs were characterized in hippocampal slices from rat brain. 2,3,7,8-TCDD and 1,2,3,4-TCDD were used because these HAHs are prototypical potent and weak aryl hydrocarbon receptor agonists, respectively. Slices were obtained from the middle or ventral third of the hippocampus of male Sprague-Dawley rats. For each slice, peak amplitudes during a 15 min recording period (1 recording/min) were averaged and used as the baseline (100%). Subsequent EPSP recordings were expressed as % of baseline. After 15 min of baseline activity in artificial cerebrospinal fluid, slices were superfused for 30 min with either vehicle (0.1% DMSO) alone, vehicle + 100 nM 2,3,7,8-TCDD, or vehicle + 100 nM 1,2,3,4-TCDD. DMSO had no effect on EPSPs. 2,3,7,8-TCDD and 1,2,3,4-TCDD did not alter EPSPs in slices from the middle third of the hippocampus. However, in slices from the ventral third of the hippocampus, 2,3,7,8-TCDD significantly decreased EPSPs (59.48 ± 11.23% at 15 min), whereas 1,2,3,4-TCDD was inactive (98.37 ± 3.85%). Surprisingly, by 30 min in continued presence of 2,3,7,8-TCDD, the inhibition of EPSPs was reversed. The results of this study suggest that hippocampal target sites for 2,3,7,8-TCDD-induced neuroactivity are region-specific in male Sprague-Dawley rats. *Funded by NIH Superfund grant to Dr. Stephen Safe (P42-ES04917).*

779 LINDANE BLOCKS $GABA_A$-MEDIATED INHIBITION AND MODULATES PYRAMIDAL CELL EXCITABILITY IN AREA CA1 OF THE RAT HIPPOCAMPAL SLICE

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Electrodes were placed to record excitatory postsynaptic potentials (EPSPs) and population spikes (PSs) evoked in CA1 pyramidal cells by Schaffer collateral/commissural stimulation. Stimuli of various intensities were employed to evaluate input/output relationships before and after lindane perfusion, and a paired pulse technique was used to measure recurrent collateral-mediated inhibition. The most significant effect of perfusing slices with lindane (25 uM) was a complete reversal of recurrent collateral-evoked inhibition. With longer perfusions multiple PSs developed, particularly on responses to the second of the paired stimuli. Lindane (50 uM) also completely reversed the enhanced recurrent collateral inhibition produced by prior perfusion of slices with the injectable anesthetic agent, propofol. An analysis of input/output relationships at varying stimulus intensities showed that lindane (25 uM) shifted both EPSP and PS amplitude vs stimulus intensity curves to the left, indicative of a change in transmitter release and/or signal transduction in pyramidal cell dendrites. Similar effects are produced by other $GABA_A$ antagonists, so it is conceivable that all of these changes may result from a reduction of inhibition in this area of the hippocampus.

780 METHYLMERCURY (MeHg) ACTS AT MULTIPLE SITES TO BLOCK HIPPOCAMPAL SYNAPTIC TRANSMISSION

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To identify the sites at which MeHg blocks hippocampal synaptic transmission, we applied intracellular microelectrode recording and iontophoresis techniques to rat isolated hippocampal slices. Effects of MeHg were compared on the responses of CA1 neurons to orthodromic stimulation of Schaffer Collaterals, antidromic stimulation of the alveus, direct current injection at cell soma and iontophoresis of glutamate or N-methyl-d-aspartate (NMDA). At 20 and 100 μM, MeHg blocked action potentials evoked by presynaptic stimulation of Schaffer Collaterals and by current injection at cell soma similarly. Times to block by 100 μM MeHg were 43 ± 7 and 44 ± 7 min, respectively. Concurrent block of the two responses presumably occurred due to rapid depolarization of neuronal membranes. In contrast, MeHg blocked action potentials evoked by antidromic stimulation of the alveus more rapidly than it blocked current injection-evoked action potentials at CA1 neuronal soma. Times to block were 42 ± 4 and 49 ± 5 min, respectively ($p < 0.05$). MeHg also blocked the responses of CA1 neurons to iontophoresis of glutamate or NMDA. Times to block were 63 ± 9 and 63 ± 8 min, respectively, which was significantly slower than block of the corresponding orthodromically-evoked responses ($p < 0.05$). Compared to excitatory postsynaptic potentials (EPSPs), inhibitory postsynaptic potentials (IPSPs) seemed to be more sensitive to MeHg because block of IPSPs by MeHg occurred prior to block of EPSPs. Time to block of IPSPs was 25 ± 4 min while time to block EPSPs was 50 ± 5 min. Thus, MeHg may act at multiple sites to block central synaptic transmission. Supported by NIH grant ES03299.

781 PRENATAL AND POSTNATAL EXPOSURE TO ETHANOL DIFFERENTIALLY AFFECT THE GENERATION OF NEURONS IN THE HIPPOCAMPAL FORMATION OF THE RAT

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Neurons in the hippocampal formation (the hippocampus and the dentate gyrus) are produced over a long period, from gestational day (G) 15 into adulthood. Neurons born prenatally arise from the ventricular zone and those born postnatally derive from an auxiliary zone (the intrahilar zone). This study tests the hypothesis that prenatal ethanol exposure has a different impact on the generation of neurons in the hippocampal formation than does postnatal exposure. Rats were exposed to ethanol between G5 and G21 or between postnatal day (P) 4 and P10 and then examined on P21. Each rat was injected with [^{3}H]thymidine on G15, G18, G21, P6, P9, or P12. Prenatal exposure *decreased* the total number of hippocampal and dentate gyral neurons, whereas postnatal exposure significantly *increased* the number of dentate gyral neurons (with no effect on the hippocampus). These changes echoed ethanol-induced alterations in neuronal generation; e.g., prenatal exposure reduced the production of prenatally generated dentate gyral neurons, but postnatal exposure increased neuronal generation. Thus, the effects of ethanol are defined by the timing of the exposure relative to the schedule of cell proliferation and by the location of the proliferative population. Funded by the V.A. and N.I.H. (AA06916, AA07568, and DE07734).

782 DEVELOPMENTAL LEAD EXPOSURE ALTERS N-METHYL-D-ASPARTATE (NMDA) RECEPTORS IN THE RAT HIPPOCAMPUS: AN AUTORADIOGRAPHIC STUDY

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We have previously shown that lead (Pb) inhibits NMDA receptor function *in vitro* and *in vivo* (Guilarte and Miceli, *Neurosci. Lett.* 148: 27, 1992). In the present study, we have used quantitative autoradiography of the N-Methyl-D-Aspartate (NMDA) receptor non-competitive antagonist (+)-MK-801 in the tritiated form ([^{3}H]-MK-801) to determine the effects of developmental exposure to Pb on NMDA receptors in the rat hippocampus. Pb exposure caused significant increases (25–49%) in [^{3}H]-MK-801 binding in the hippocampus and entorhinal cortex (ECTX) of postnatal day (PN) 14 rats relative to controls. A small but significant reduction (16%) and increase (15%) in [^{3}H]-MK-801 binding were also measured in the CA1 region and subiculum of PN28 rats, respectively. No significant changes in [^{3}H]-MK-801 binding were observed among any of the groups at PN56. The increase in [^{3}H]-MK-801 binding measured in Pb-exposed developing rats may represent a compensatory response to the direct inhibitory effects of Pb on the NMDA receptor *in situ*. These findings indicate that there may be a critical developmental window during which Pb effects are most pronounced, and the magnitude and direction of these changes is highly dependent upon the age of the animal. It is suggested that NMDA receptors play an important role in the developmental toxicity of Pb. [Supported by NIEHS grant #ES06189 to TRG].

783 Pb-INDUCED REDUCTIONS IN DOPAMINERGIC ACTIVITY IN RAT NUCLEUS ACCUMBENS — A MICRODIALYSIS STUDY

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Long-Evans hooded (21-day-old, male) rats were exposed to sodium acetate (control) and 50 ppm lead acetate in deionized distilled water for 90 days and the basal and potassium (100 mM) induced release of dopamine (DA) was studied in nucleus accumbens (NA) using the microdialysis technique. After the 90 day exposure period, rats were anaesthetized with urethane (1.5 g/kg body wt) and microdialysis probes (2 mm) were placed in NA (AP 2.7 mm, L 1.5 mm, V −7.5 mm) using a stereotaxic apparatus and perfused with artificial CSF (aCSF) at a rate of 2 μl/min. After collecting 3 fractions for measuring basal levels, the perfusion fluid was switched to aCSF containing high potassium and 3 additional fractions were collected for measuring stimulus induced release of DA. The samples were analyzed for DA and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) using high performance liquid chromatography. The results indicated that both basal and potassium induced release of DA were found to be significantly reduced in Pb treated rats (blood Pb 18 μg/dl) as compared to controls (blood Pb 4 μg/dl). Similar reductions were also observed for the metabolites DOPAC and HVA. These results along with the our previous findings of reduction in tissue DA and its metabolites in NA of Pb exposed rats indicates that chronic exposure to Pb results in reduced dopaminergic activity in NA. Supported by the ATSDR/MHPF Grant No. U50/ATU 398948-02).

784 KAINIC ACID ADMINISTRATION INCREASES SEROTONIN TURNOVER RATE IN RAT BRAIN REGIONS

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Intraperitoneal kainic acid (KA) administration to rats induces severe convulsions and neurotoxicity. Seizures are initiated in limbic structures, primarily in the hippocampus. Investigations of this model revealed marked changes in neurochemical parameters as, for example, increase of noradrenaline (NA), dopamine (DA) and serotonin (5-HT) metabolites. The present study was designed to observe the effect of KA on serotonin turnover rate in brain regions. We tested 4 different groups: 1) Rats were administrated with saline solution (SS) and SS, 2) KA (10 mg/kg, i.p.) + SS, 3) pargyline (PA, 75 mg/kg, i.p.) + SS and 4) KA + PA. PA was used to estimate 5-HT turnover rate. The second injection was administrated 2 hrs after of first treatment. Animals were killed by decapitation 1h later and brain regions dissected out. The effect of KA on serotonin turnover rate was a significant increase in hippocampus (54%), frontal cortex (139%) and striatum (74%). Results indicate that KA brain accelerates regional 5-HT turnover rate.

785 NEUROCHEMICAL EFFECTS OF STYRENE EXPOSURE: A STUDY ON CATECHOLAMINE AND SEROTONIN METABOLISM AND ITS IMPLICATIONS TO SOME BEHAVIORAL EFFECTS IN RATS

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Neurological disorders involving both the central and peripheral nervous system have been reported in workers exposed to styrene monomer. Groups of male Sprague-Dawley rats (12 rats per group) received 0.25 and 0.5 g per kg b.w. per day of styrene in corn oil by gavage, 5 days a week for 13 consecutive weeks. Control group of 12 rats received an equal volume of corn oil. The regional distribution of dopamine (DA), norepinephrine (NE), 5-hydroxytryptamine (5-HT) and their metabolites was determined in striatum hippocampus, brain cortex and cerebellum, as well as in lateral olfactory tract. Neuronal turnover, estimated by determining the ratios of the metabolites to neurotransmitters, was found to be altered in certain specific regions by styrene. Thus DOPAC/DA ratios was significantly decreased in striatum in groups exposed to higher dose of styrene. HVA/DA ratio was also significantly decreased at the same high dose level. However, no statistically significant changes in striatal concentration of 5-HT or NE were noted. After termination of exposure, animals showed reduced motor activity and grip strength and lack of coordination. These data suggest that a disturbance in the endogenous levels of dopamine leading to a generalized central and peripheral disorder of their metabolism may be involved in the development of neurotoxicity due to styrene. (Supported in part by IRSST).

786 THE TOXICITY OF LOBELINE ON CENTRAL DOPAMINE (DA) NEURONS IN THE RAT

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Sponsor: R Yokel

Lobeline (α-lobeline), an alkaloid of *Lobelia inflata*, has been used as a smoking deterrent aide. It has been reported to produce pharmacologic effects similar to, but weaker than, those produced by nicotine. Due to its acute toxicity (nausea, vomiting, severe heartburn, epigastric pain and dizziness) and lack of data concerning long-term use, it's use should be limited to 6 weeks. The present study examined the toxicity of lobeline on striatal (DA) neurons *in vitro* and *in vivo*. *In vitro*, in superfusion studies, both lobeline and nicotine evoked [^{3}H]DA release from rat striatal slices in a concentration-dependent manner. Lobeline evoked 10–32 fold more [^{3}H]DA release than nicotine at high concentrations (10 – 100 μM), but only 30–50% of that evoked by nicotine at low concentrations (0.01 – 1 μM). Furthermore, lobeline (30 – 100 μM) depleted DA content about 50% and increased dihydroxyphenylacetic acid (DOPAC) content 3–4 fold, suggesting that lobeline enhances DA utilization. However, *in vivo* studies demonstrated that lobeline administration acutely (0, 1, 3, 10 and 30 mg/kg s.c.) and subacutely (0, 3 and 10 mg/kg/day for 10 days, s.c.) did not alter DA and DOPAC content in rat striatum. Potential long-term toxicity of a range of doses is currently being investigated. These findings may be significant to evaluate lobeline as a long-term smoking deterrent. (Supported by a grant from the Tobacco and Health Research Institute, Lexington, KY).

787 A SINGLE INJECTION OF IBOGAINE PRODUCES SIGNIFICANT CHANGES IN NITRIC OXIDE SYNTHASE (NOS) ACTIVITY AND MONOAMINE CONCENTRATIONS IN MOUSE BRAIN

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The alkaloid ibogaine has been proposed as a possible treatment for drug addiction, however, its mechanism of action and neurotoxic potential are still unknown. The present study was designed to determine whether a single injection of ibogaine would produce significant neurochemical alterations in the mouse brain. Adult male C57/B6N mice were dosed with 50 mg/kg ibogaine, ip, and sacrificed 0.5, 1, 2, 4 and 24 hour later. Brains were dissected for neurochemical analyses. In vitro exposure to ibogaine (0.5–250 uM) produced dose-dependent decreases in NOS activity in cerebellum as measured by a radioactive method in which the rate of conversion of ^{3}H-arginine to ^{3}H-citrulline was monitored. In vivo exposure to ibogaine also produced a significant decrease in NOS activity in the striatum, hippocampus and cerebellum. Monoamine concentrations measured by HPLC/EC showed a significant depletion of dopamine (DA) at 0.5, 1, 2 and 4 hr after drug administration, however, levels returned to control values 24 hr after dosing. Serotonin (5-HT) concentration in striatum decreased 4 and 24 hours after dosing. These data indicate that a single injection of ibogaine can produce significant alterations in NOS activity, and DA and 5-HT concentrations which suggest that ibogaine has a complex neurotoxicity profile and could interfere with several major neural functions.

788 HALOPERIDOL AFFECTS ACCUMULATION OF TRIADIMEFON IN RAT BRAIN

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The use of pharmacological challenges in toxicological studies is often a powerful approach for developing mechanistic hypotheses. Frequently, however, such mechanistic studies are confounded by changes in bioavailability resulting from co-administration of two or more test compounds. To minimize confounds in the interpretation of data, it is important to know the influence of the model drug on the brain disposition of the test drug(s). We have reported that the triazole fungicide triadimefon (TDF) potentiates the neurochemical changes (i.e., increases in DOPAC and HVA) caused by the dopamine antagonist haloperidol (HAL; see *Neurochemical characterization ...* by Walker QD et al. this meeting). In the present study, all rats were given one of three treatments: 1) HAL (1 mg/kg, i.p.); 2) TDF (30, 56, 100 or 300 mg/kg, i.p. in corn oil); 3) TDF (30, 56, 100 or 300 mg/kg) in combination with HAL (1 mg/kg, i.p.). In all cases, rats were given HAL 45 min prior to sacrifice, and TDF 30, 60, or 120 min before. Following sacrifice, the TDF content of the brain (minus the corpus striatum) was assessed using a gas chromatographic assay. Thirty minutes after treatment, brain TDF was consistently higher in rats which had been treated only with TDF (low→high TDF doses: 2.55 ± 0.0, 7.41 ± 1.68, 11.8 ± 3.8 and 23.4 ± 6.8 μg/gm) than in rats treated with TDF and HAL (1.17 ± 0.99, 3.66 ± 0.63, 6.64 ± 1.70 and 12.3 ± 0.2). Similar brain concentrations of TDF were found, however, when the rats were treated with TDF 120 min before sacrifice. These data indicate that administration of HAL prior to, or concurrent with TDF, results in a decrease in the brain concentrations of TDF, thus confounding direct toxicological comparisons. The mechanism by which this effect occurs is not known. (Supported in part by HD03110, ES01104 and ES07126).

789 NEUROCHEMICAL CHARACTERIZATION OF THE DOPAMINERGIC EFFECTS OF TRIADIMEFON

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Previous work from this laboratory has led to the hypothesis that the triazole fungicide triadimefon (TDF) causes behavioral toxicity (e.g., dopamine-mediated stereotyped behavior) principally by increasing availability of extracellular dopamine (e.g., via inhibition of the dopamine transporter). The present study was designed to compare the neurochemical effects of TDF with drugs that are known to affect dopamine metabolism. Stimulants like methylphenidate (MP), but not amphetamine (AMP), can potentiate the increase in the dopamine metabolites DOPAC and HVA caused by the blockade of D_2 dopamine autoreceptors by haloperidol (HAL). To characterize further TDF as a non-amphetamine- like stimulant, two treatment regimens were used. In the first study, rats were administered HAL (1 mg/kg, i.p.) or vehicle at 0 min, combined with a second test drug (e.g., AMP, TDF, etc.) at 15 min. At 45 min, the animals were sacrificed, after which the content of dopamine, DOPAC, and HVA in the striatum was measured. In this study, AMP (3 mg/kg, ↓26%), as expected, partially reversed the HAL-induced increases in DOPAC. Conversely, PCP (10 mg/kg, ↑32%), GBR-12909 (20 mg/kg ↑51%), nomifensine (20 mg/kg, ↑42%), or MP (10 mg/kg, ↑57%) all potentiated the increases in DOPAC caused by HAL. In a similar fashion, TDF potentiated the HAL effect on DOPAC (*100 mg/kg*): ↑16% after 30 min; *300 mg/kg*: ↑27%, ↑63%, and ↑53% after 30, 60 and 120 min, respectively) and HVA (data not shown) concentrations. Taken together, these data are consistent with the hypothesis that the behavioral toxicity of TDF involves an increase in extracellular dopamine coming from the same pools that are affected by MP or uptake inhibitors, but not AMP. (Supported in part by HD03110, ES01104 and ES07126).

790 PERIPHERAL BENZODIAZEPINE RECEPTORS IN CHEMICAL-INDUCED NEURONAL DAMAGE: A POTENTIAL BIOMARKER OF NEUROTOXICITY

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The development of a biomarker for generalized neuronal damage could be a useful tool to assess the adverse effects of chemicals on the CNS. Peripheral benzodiazepine receptors (PBR) are present in very low concentrations in the CNS, but are dramatically increased following neuronal damage. We have used quantitative autoradiography of [^{3}H]-PK11195, a selective ligand of the PBR, to assess neuronal damage induced by the known neurotoxicant trimethyltin (TMT). Adult male Long-Evans rats (250–300 g) were injected i.p with a 8 mg/kg dose of TMT and euthanized at one day to six weeks post injection. The brains were removed, and the right hemisphere used for [^{3}H]-PK11195 autoradiography while the left hemisphere was used for analysis of glial fibrillary acidic protein (GFAP) in the hippocampus. Analysis of [^{3}H]-PK11195 binding in the hippocampus revealed that the CA3 region had the largest increase in binding beginning 7 days after TMT injection and persisted beyond six weeks. Significant increases in binding were also present in other brain regions consistent with known TMT neuronal damage. The time course of the increases in [^{3}H]-PK11195 binding was highly correlated with increased GFAP levels. These findings suggest that the quantitative assessment of [^{3}H]-PK11195 binding to PBR in the brain could represent an alternative biomarker to assess chemical-induced neurotoxicity. [Supported by a pilot project from NIEHS grant No. ES03819 to TRG].

791 REGIONAL ANALYSIS OF TOTAL RNA AND DNA CONTENT IN THE RODENT BRAIN FOLLOWING Pb EXPOSURE

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Lead (Pb) is a neurotoxicant which is known to produce cognitive, morphological and biochemical alterations in the brain. We have previously observed that exposure to Pb selectively altered neuronal and glial gene expression. The objective of these studies was to examine whether Pb's effects on brain gene expression is accompanied by disturbances in macromolecular synthesis. Animals were lactationally exposed to 0.2% Pb and sacrificed on postnatal days (PND) 6, 9, 20, 30. The cerebellum, hippocampus and neocortex were isolated and their RNA and DNA content were simultaneously determined from the same preparation using the TriReagent™. The cerebellum exhibited a decreased ratio of RNA/DNA content from PND 9–20 in Pb-exposed animals resulting from changes in both macromolecules. The ratio of RNA/DNA content were transiently elevated on PND 6 in the hippocampus and on PND 9 in the cortex due to increased levels of RNA. These data suggest that Pb interferes in both proliferation and differentiation events in the cerebellum, a region known to undergo the majority of its growth in the postnatal period. However, in cortical regions with predominantly prenatal maturation, Pb may have a greater effect on RNA production. The changes in the ratio of RNA/DNA content in these regions may be due to either a transient modulation of differentiation events or a response to CNS injury.

792 ETHANOL INHIBITS MUSCARINIC RECEPTOR-INDUCED PROLIFERATION OF RAT CORTICAL ASTROCYTES

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Inhibition of muscarinic receptor-stimulated phosphoinositide metabolism has been suggested as a mechanism involved in the developmental neurotoxicity of ethanol (JPET 250, 541, 1989). Activation of muscarinic receptors coupled to phosphoinositide metabolism has been shown to cause proliferation of glial cells (Nature 340, 146, 1989). We have further characterized this mitogenic response in rat cortical astrocytes. These cells express mRNA for m_2 and m_3 muscarinic receptors, as measured by RT-PCR. Carbachol (0.1–10 mM) caused a dose-dependent increase in proliferation, measured by incorporation of ^{3}H-thymidine into DNA, with a maximal stimulation of 260% of basal. The full agonists methacholine and acetylcholine had similar effect, while the partial agonist bethanechol was less effective. The protein kinase C (PKC) antagonist H7 and down-regulation of PKC by pre-incubation for 24 h with 300 nM TPA, significantly inhibited carbachol-induced proliferation. Ethanol inhibited basal proliferation of astrocytes by a maximum of 30–40% at concentration of 100–200 mM. On the other hand, carbachol-stimulated proliferation was inhibited by ethanol at much lower concentrations: 40% inhibition by ethanol was observed at 10 mM, while at 50 mM inhibition was 90%. These results suggest that acetylcholine-stimulated astrocyte proliferation may be a relevant target for ethanol, and may play a relevant role in ethanol-induced microencephaly. (Supp. in part by AA-08154).

793 MEASUREMENT OF EXPOSURE TO ACRYLONITRILE (ACN) IN CIGARETTE SMOKERS BY ANALYSIS OF N-(2-CYANOETHYL)VALINE (CEVal) IN HEMOGLOBIN (HB)

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ACN, used to manufacture plastics and fibers, is carcinogenic in rats and is found in cigarette smoke. ACN binds to Hb, and an assay for CEVal, formed by reaction with the N-terminal valine, has been developed using a modified Edman degradation. This study investigated CEVal formation in Hb from non-smokers and smokers. *N*-(2-hydroxyethyl)valine (HEVal), an adduct from reaction of Hb with ethylene oxide, and elevated by exposure to ethylene and ethylene oxide in cigarette smoke, was measured in parallel. Blood samples, collected from 34 people who smoked either 0, 1, or 2 packs of cigarettes/day, were analyzed for HEVal and CEVal. The adducts were determined by gas chromatography with negative ion chemical ionization mass spectrometry following derivatization with pentafluorophenylthiohydantoin. Information was obtained by questionnaire on smoking history, occupation and exposure to chemicals. In non-smokers (N = 16), CEVal and HEVal levels were 13 ± 33 and 30 ± 66 (mean ± S.D.) fmol/mg globin, respectively. For 1 pack/day smokers (N = 11), CEVal and HEVal levels increased to 213 ± 69 and 237 ± 148 fmol/mg, respectively. For 2 pack/day smokers (N = 7), CEVal levels were 359 ± 146 fmol/mg, and HEVal was 308 ± 87 fmol/mg. Both groups of smokers showed increases in CEVal and HEVal compared with non-smokers. The ratio of CEVal/HEVal was 1.11 ± 0.39 for the smokers. Cigarette smoking results in Hb adducts derived from ACN. Exposure to ACN from cigarette smoke should be considered in assessment of low-level exposure in industrial settings using CEVal as a biomarker, and in assessment of risk from exposure to ACN. Measurement of HEVal and CEVal should aid in distinguishing exposure derived from cigarette smoke and low-level workplace exposure.

794 EFFECT OF THIOSULFATE, 1-BENZYLIMIDAZOLE AND N-ACETYL-L-CYSTEINE ON BIOMARKERS OF ACUTE ACRYLONITRILE INTOXICATION IN RATS

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Biomarkers of acute acrylonitrile (AN) intoxication include increased tissue cyanide (CN) levels, depletion of glutathione (GSH) and increased covalent binding (CB) of 2,3,-^{14}C-AN to tissue protein. Antidotes directed against one or more of these biomarkers of intoxication were examined, namely thiosulfate (TS) — 0.5 g/kg, 1-benzylimidazole (BI) — 1 mg/kg, and N-Acetyl-L-Cysteine (NAC) — 10 mmole/kg, all given ip immediately after the administration of an LD90 (115 mg/kg) dose of AN given sc. Biomarkers were assessed at the time of peak effect of this dose of AN (2h). All antidotes were effective in lowering cyanide levels to between 5–15% of levels found in the blood (191 nmole/ml) and brain (25.8 nmole/gm) of unprotected rats. Only NAC was able to protect or reverse the depletion of tissue thiol levels. Thiol levels, expressed as a percent of saline-treated controls, for AN- vs AN + NAC-treated rats were as follows: blood (56% vs 260%), liver (6% vs 54%), kidney (14% vs 154%), brain (61% vs 113%), glandular stomach (13% vs 144%), forestomach (16% vs 176%), skeletal muscle (62% vs 210%). Only NAC produced substantial protection against AN covalent binding. NAC decreased AN covalent binding in all tissues examined by 40–50% except blood which was reduced by 20%. These data indicate that only NAC was able to protect rats against all three biomarkers of acute AN intoxication and thus would be expected to be more efficacious than antidotes directed only toward the CN component of toxicity. (Supported by NIH grant ES-06141).

795 ISOLATION AND CHARACTERIZATION OF A 170 kDa TRIFLUOROACETYLATED PROTEIN FROM LIVERS OF HALOTHANE-TREATED RATS

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Halothane hepatitis is thought to be due to an immune reaction against liver microsomal proteins that are trifluoroacetylated (TFA-antigens) by the reactive trifluoro-acetyl chloride metabolite of halothane. In order to understand how the immune system comes in contact with TFA-antigens, we have investigated their processing in primary cultures of rat hepatocytes. While studying the turnover of 57, 58, 59, 63, 80, 82 and 100 kDa TFA-antigens, we found that a 170 kDa trifluoroacetylated protein (TFA-170) co-precipitated with the 57, 58, 59 and 82 kDa proteins during immunoprecipitation with specific antisera. The turnover rate of TFA-170 was found to be faster than other known TFA-antigens. The *N*-terminal amino acid sequence of TFA-170, isolated from rat liver microsomes, did not show any homology to known proteins. The results suggest that the 170 kDa protein may be important in the functioning of other microsomal proteins. It may also have a role in the etiology of halothane hepatitis. (JLM is partially supported by the Robert Wood Johnson Foundation.)

796 KUPFFER CELLS FROM HALOTHANE EXPOSED GUINEA PIGS CARRY TRIFLUOROACETYLATED (TFA) PROTEIN ADDUCTS

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The anesthetic, halothane, is bioactivated by the liver cytochrome P450 system to the reactive intermediate, trifluoroacetyl chloride, which can acylate liver protein. This covalent binding of TFA may result in hapten formation leading to the induction of an immune response and ultimately halothane hepatitis. Little is known about the mechanisms that could lead to the recognition of TFA-adducts by cells of the immune system. Kupffer cells are phagocytic, antigen presenting cells in the liver which are capable of processing and presenting TFA-adducts to the immune system. TFA-adducts have been detected in Kupffer cells from rats exposed to halothane; however, more recent studies using alternate isolation techniques have not been able to reproduce these results. The guinea pig was used in this study, as a sensitive animal model to investigate an immune-mediated mechanism for halothane hepatitis. Guinea pigs were exposed to 1.0% halothane, 40% oxygen for 4 hours. Kupffer cells were isolated on days one through nine post-exposure, by liver perfusion with collagenase and pronase, separation on a metrizamide gradient and purification by elutriation. Using gel electrophoresis and Western blotting techniques, it has been demonstrated that Kupffer cells obtained from halothane treated guinea pigs, do carry TFA-adducts as recognized by an anti-TFA antibody. Apparent molecular weights of polypeptides bound by TFA were 129, 93, 85, 80, 76, 67, 54, 48, 46 kDa. Bands were most prominent by day two with the majority diminishing by day seven. Distinct TFA-adducts with molecular weights of 124, 113, 106 kDa appeared on day 4. We propose that Kupffer cells acquire adducts via phagocytosis of TFA-proteins released from dying hepatocytes. This work suggests a role for Kupffer cells in the presentation of altered proteins in the liver to cells of the immune system.

797 MOCA-DNA ADDUCT FORMATION IN HUMAN UROEPITHELIAL CELLS ANALYZED BY ^{32}P POSTLABELING

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As part of our effort to investigate the carcinogenicity of 4,4'methylene-bis(2-chloroaniline) (MOCA) and develop biomarkers of effects of exposure to occupational carcinogens, N-OH-MOCA-DNA adduct formation was examined

in a human uroepithelial cell (HUC-PC) line sensitive to neoplastic transformation by 4-aminobiphenyl. HUC-PC cells were a generous gift from the Department of Human Oncology, University of Wisconsin Comprehensive Cancer Center (UWCCC). HUC-PC were incubated with 0, 2.5, 5 and 10 μM N-OH-MOCA for 24 hours. Following incubations, cells were harvested, DNA isolated, and analyzed by ^{32}P postlabeling for N-OH-MOCA-DNA adducts. At 10 M, two adducts were identified. The major adduct, with chromatographic properties similar to N-(deoxyadenosine-8-yl)-4-amino-3 chlorobenzyl alcohol, formed at a level of 430 ± 23 fmol/μg DNA ± S.E.. The same major adduct was found in DNA from exfoliated urothelial cells found in urine of a worker acutely exposed to MOCA and in liver DNA of rats dosed with MOCA. The site of MOCA-induced tumor formation in the rat is the liver and in humans MOCA is a suspected bladder carcinogen. The minor adduct was found (261 ± 158 fmol/μg DNA ± S.E.) in the 10 μM-treated HUC-PC, but was not detectable in HUC cells treated at the lower doses. However, this adduct was observed in rats dosed with MOCA. In the concentrations examined, there was no significant difference in the level of major adduct in the HUC cells. Preliminary results suggest that only the 10 μM treated HUC-PC were malignantly transformed.

798 CYTOCHROME P450 CATALYZED COVALENT BINDING OF METHOXYCHLOR (M) TO RAT HEPATIC, MICROSOMAL IODOTHYRONINE 5'-MONODEIODINASE (IDI)

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Previously we have shown that acetaminophen forms adducts with three microsomal (*ER*) proteins, calreticulin and two forms of thiol:protein disulfide oxidoreductase, *Q2* and *Q5* (Zhou *et al. Toxicologist* 14: 358, 1994). Halothane shows a similar, although more extensive binding pattern (Pohl *et al.* Adv. Exper. Med. Biol. 283: 111, 1991). The formation of these adducts is catalyzed by P450. In *in vitro* incubations *M* also forms adducts with hepatic *ER* proteins (Bulger *et al.* Tox. Appl. Pharmacol. 68: 367, 1983) but is not hepatotoxic *in vivo*. This reaction is catalyzed by CYP2B. Rat, liver microsomes were incubated with ^{14}C-*M* and NADPH and *ER* proteins purified as previously described (J. Biol. Chem. 266: 20337, 1991). Solubilized *ER* was precipitated with polyethylene glycol 6000 (*PEG*). The ^{14}C was found only in the *PEG* supernatant. FPLC of the supernatant on MonoQ and MonoP gave one ^{14}C labeled protein with Mr = 55 kDa. The N-terminal sequence was identical to *IDI*, which differs from *Q5* by a substitution of Ala and Ile for Pro 39. Sequencing of tryptic peptides separated by HPLC and gel permeation suggested that *M* binds to cysteine 27 or 30 or lysine 31. The adduct formation with *IDI* may decrease T_3 formation and decrease body thyroid activity. It is unclear why *M* forms adducts with proteins differing from those which bind acetaminophen and halothane. (Support from NIH Grant ES-00834 (DK)).

799 KINETIC STUDIES DEMONSTRATING THE FORMATION OF MULTIPLE POOLS OF N-ACETYL-P-BENZOQUINONE IMINE (NABQI), DURING CYSTEINE (CYC) CONJUGATION AND PROTEIN BINDING OF ACETAMINOPHEN (AC) BY HEPATIC MICROSOMES FROM MALE MICE

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We previously found that there are marked kinetic differences between the hepatic, microsomal catalyzed *AC* binding to protein and to *CYC* suggesting that two pools of *NABQI* are formed during the P450 catalyzed metabolism of *AC*: one on the cytosolic surface and the other within the microsomal lumen. We have extended these studies with mouse microsomes and again found that the rate of *CYC* binding was almost 1000 x's greater than protein binding. Ethanol feeding increased protein binding 97%, but *CYC* binding by only 33%. Protein binding was linear for 20 min while *CYC* binding was linear for only 5 min. CO, imidazole and metyrapone profoundly inhibited *CYC* binding, but had much less effect on protein binding. SKF-525A and KCN had similar effects on both reactions. Low [NADH] had little effect on protein binding, but at higher concentrations the rate plateaued. On the other hand, [NADPH] and [NADH] > 0.32 mM caused a precipitous decline in *CYC* binding. NADPH reduced *NABQI* binding to protein while NADH had little effect. *NABQI* reduction to *AC* was the same for NADPH and NADH. Phenobarbital induction had no effect on the LD_{50} or protein binding but increased *CYC* binding 2.24 x's. These data in mice agree with our previous data in rats indicating that two kinetically distinct pools of *NABQI* are formed during microsomal *AC* metabolism.

800 BINDING OF ^{14}C-ACETAMINOPHEN (AC) TO MOUSE, HEPATIC, MICROSOMAL (ER) PROTEINS

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In previous studies we found that in *in vitro* incubations *AC* forms hepatic, *ER* protein adducts. This binding correlates with *in vivo* toxicity. Furthermore, we have previously reported that when we incubated mouse microsomes with ^{14}C-*AC* and NADPH and purified the *ER* proteins (JBC 266: 20337, 1991) by solubilizing and precipitating them with polyethylene glycol 6000 (*PEG*), the ^{14}C was equally divided between the supernatant and pellet. Chromatography of the supernatant on MonoQ and MonoP gave two ^{14}C-labeled proteins with Mr's = 52 and 55 kDa. The N-terminal sequences of the labeled proteins were identical to those of calreticulin, an intraluminal, *ER* Ca^{2+} binding protein, and *Q5*, a thiol:protein disulfide oxidoreductase (*TPDO*) respectively. The *PEG* pellet was solubilized with Emulgen 911. Chromatography on MonoQ and octyl Sepharose gave a single ^{14}C-labeled protein with Mr = 54 kDa. The N-terminal sequence was identical to that of the *TPDO* isoform, *Q2* (*ibid*). *AC* binding was 130, 34 and 25 mol% for *Q2*, *Q5* and calreticulin respectively. In the previous study we purified the trypic digests by HPLC. We have now added a gel permeation chromatography step after the HPLC and find that the *AC* bound to lysine 288 of *Q2*, lysines 202, 208, 209, 366 or 370 of *Q5*, and lysines 225, 233 or 239 of calreticulin. We found no cysteine adducts. These data lend further support to our hypothesis that AC hepatotoxicity is initiated by ε-N-lysine adduct formation. This may be a general model for hepatotoxicity since similar adducts have been reported in halothane hepatotoxicity (Pohl *et al.* Adv. Exper. Med. Biol. 283: 111, 1991).

801 TIMECOURSE OF CARCINOGEN-DNA ADDUCT LEVELS IN TARGET AND NON-TARGET ORGANS OF FEMALE MICE CHRONICALLY EXPOSED TO 4-AMINOBIPHENYL BY TOPICAL ADMINISTRATION

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4-aminobiphenyl (4-ABP) is a potent bladder carcinogen in humans and dogs and a liver and bladder carcinogen in mice. However, little data exist regarding DNA adduct formation following topical application. We examined the kinetics of 4-ABP DNA adduct formation in target and nontarget tissues of mice chronically treated. Female ICR:BR HSD mice were topically treated twice weekly with 10 or 50 nmol of 4-ABP. Groups of animals were sacrificed after 3, 6, 12, 16, 21, or 30 weeks of treatment. Skin, lung, liver and urinary bladder DNA was obtained and analyzed for 4-ABP adducts using ^{32}P-postlabelling. The relative adduct levels increased with increasing doses to 8.4 in liver, 26.7 in urinary bladder, 4.5 in skin and 0.7 in lung. These data are consistent with what is known of the metabolism in these tissues. Skin and liver, which possess CYP1A2 activity, metabolically activate 4-ABP to form DNA adducts while the lung, a nontarget tissue, does not exhibit significant CYP1A2 activity and does not metabolically activate 4-ABP. Supported by USEPA grant #R818088.

802 COVALENT BINDING OF REACTIVE METABOLITES (RM) OF NAPHTHALENE (NA), 1-NAPHTHOL (NOL), NITRO-NAPHTHALENE (NN) AND 4-IPOMEANOL (IPO) TO PROTEINS IN MOUSE BRONCHIAL AIRWAYS

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Parenteral administration of NA, NN or IPO but not NOL to mice results in selective necrosis of pulmonary Clara cells. RM generated by P450 become bound covalently to proteins in the lung and there is an association between the extent of binding and severity of Clara cell injury. These studies examined the binding of RM from NA, NOL, NN, and IPO to proteins in dissected airways. Incubation of murine airways with 5, 50 or 500 μM ^{3}H-NA resulted in time and concentration dependent binding of RM to proteins in tissue and medium. At low NA concentrations (5 μM), the total amount bound in tissue was 30–50% of that bound in the medium. At higher substrate concentrations/longer incubation times, total binding was higher in tissue than medium. At all time points, the specific activity (nmoles/mg protein) of RM binding was higher in medium than tissue. Proteins were separated by SDS PAGE. Tissue proteins at approximately 50, 45, 25, 20, 14 and <5 kDa were labelled and the intensity of labelling corresponded to the intensity of staining with Coomassie blue. In medium, proteins of 70, 50, 45 and 25 kDa were labelled. The electrophoretic patterns of proteins in the medium were similar at low and

high substrate concentrations indicating that cytotoxic concentrations of NA do not stimulate the preferential release of proteins into the medium. Incubation of airways with ^{14}C-NOL resulted in higher levels of bound metabolites in medium than in tissue at 10, 50, 100 μM substrate. The pattern of labelling was similar to that with NA. Total RM bound from NN (250 μM) and from 4-IPO (5, 50 and 500 μM) were higher in tissue than in medium. These studies suggest that the pattern of RM binding in isolated airways is similar for both NA and NOL and that this pattern reflects the relative amounts of individual proteins present in tissue/medium. Supported by NIEHS 04311, 04699 and 06700.

803 ANTIBODY DETECTION OF COVALENT BINDING OF QUINONE METABOLITE OF NAPHTHALENE TO CLARA CELL PROTEINS

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Cytotoxicity of naphthalene is associated with the covalent binding of its reactive metabolites to proteins. 1,2-Naphthoquinone was found to covalently bind to cysteine residues of proteins of Clara cells incubated with naphthalene (Zheng et al *Toxicologist* 1994, 14, 115). To further identify the target proteins of the quinone metabolite of naphthalene, we raised polyclonal antibodies by immunizing rabbits with 1,2-naphthoquinone carrier protein adducts and used the antibodies as a probe to detect naphthoquinone cysteine moiety of the protein adducts. A high titer of polyclonal antibodies was obtained by antiserum dilution tests. Competitive ELISA showed that the antibodies specifically recognize 1,2-naphthoquinone *N*-acetyl cysteine adduct. No cross activity toward *N*-acetyl cysteine and its naphthalene oxide adduct was observed. For covalent binding studies, we incubated fleshly isolated Clara cell with naphthalene (0.5 mM). The cell homogenates were developed by SDS-PAGE, followed by Western blotting and immunostaining using the polyclonal antibodies we raised. Three protein bands with *Mr* 27, 28 and 52k dalton were detected by the antibodies. This finding indicates that the quinone derived metabolite of naphthalene selectively binds to Clara cell proteins *in vitro*.

804 TOXICITY OF N-ACETYL-p-BENZOQUINONE IMINE (NAPQI) AND DIQUAT IN CHINESE HAMSTER OVARY (CHO) CELLS

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Glutathione reductase (GR) protects tissues from oxidant injury by catalyzing the reduction of glutathione disulfide (GSSG) to glutathione (GSH). To study the role of GR under different oxidant stresses, we transfected CHO cells with an antisense cDNA for human GR by using a full length expression vector positioned antisense and downstream from the human metallothionein IIa promoter and co-transfected with a neomycin resistance gene to obtain a stable cell line. These cells (G17) have about half the GR activities of the parent CHO cells. Acetaminophen (AP), at concentrations up to 100 mM showed no toxicity in either cell line. NAPQI caused dose-dependent depletion of GSH in both CHO and G17 cells, from initial concentrations of 51.8 and 38.6 nmol/mg pro, respectively. Dose-dependent cell toxicity, as assessed by LDH leakage, also was observed, but with no difference in the two cell lines. As we have observed previously in studies on the effects of tert-butyl hydroperoxide in these cells, the GR-deficient G17 cells were more susceptible than were the parent CHO cells to the toxicity of diquat. Supported by NIH GM44263 and the Society for Pediatric Research.

805 INVESTIGATION OF HEPATOPROTECTION AFFORDED BY REPEATED EXPOSURE TO ACETAMINOPHEN

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Acetaminophen (A) toxicity is associated with its biotransformation to N-acetyl-p-benzoquinone imine, which, after glutathione is depleted, binds to protein thiols as 3-(cystein-S-yl)A-protein adduct (3-Cys-A). Previously, we reported remarkable resistance to supra-toxic doses of A (up to 65 g/day) in a physician who ingested progressively more A in codeine- and A-containing tablets as a consequence of Percocet addiction. To emulate this, mice were pretreated for eight days with increasing doses of A (A-pretreated). Controls received saline (S-pretreated). S-Pretreated mice challenged with 500 or 1000 mg/kg A on day 9 developed typical hepatotoxicity and the higher dose was lethal. In contrast, A-pretreated mice were protected as evidenced by an almost 400% increase in LD50. A-Pretreatment resulted in a >40% decrease in hepatic P450, decreased CYP2E1 activity, and >40% decreases in microsomal CYP2E1 and CYP1A2. Immunohistochemical staining for 3-Cys-A revealed an atypical distribution of A-protein adducts. Staining for PCNA revealed normal cell proliferative activity in S-pretreated animals, but increased proliferative activity as a consequence of A-pretreatment. Collectively, the data indicate that the association between covalent binding and toxicity may become uncoupled in A tolerance; that the mechanism of protection involves both inactivation of P450 and an ongoing cell proliferative response (autoprotection); and that the assessment of toxicity following repeated or chronic A exposure must consider parameters besides those used to assess acute A overdose.

806 IMMUNOCHEMICAL DETECTION OF PROTEIN ADDUCTS IN MICE TREATED WITH TRICHLOROETHYLENE

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Trichloroethylene is an industrial chemical with widespread occupational exposure and is a major environmental contaminant. It has been associated in the causation of the life threatening autoimmune disorder systemic sclerosis-like syndrome. The mechanism may involve the covalent modification of proteins that elicit an autoimmune response. To investigate whether formation of trichloroethylene-protein conjugates is involved in sclerosis-like syndrome, we synthesized an immunogen by derivitizing keyhole limpet hemocyanin (KLH) with dichloroacetic anhydride. Rabbits were immunized with dichloroacetic anhydride-KLH, and produced a high titer of antiserum that recognized dichloroacetic anhydride-treated rabbit serum albumin in an ELISA. Anti-dichloroacetic anhydride-KLH was used to detect modified proteins on Western blots of mouse liver fractions obtained 3 hours after dosing with 0, 500, or 1000 mg/kg of trichloroethylene. Several cytosolic protein adducts were detected, whereas the microsomal and 10,000xg pellet fractions did not contain detectable adducts. In a separate experiment, mice were pretreated for 8 days with 0.1% acetone to induce CYP 2E1 and then were injected *i.p.* with 0, 1000 or 2000 mg/kg of trichloroethylene. Western blots of liver and lung showed the presence of several protein adducts, the most prominent being 47–52 kDa in the microsomal fraction, suggesting binding to CYP 2E1. These results show that trichloroethylene binds to discrete protein fractions in mouse liver and lung; further analysis is required to elucidate the potential of these modified proteins to elicit autoimmunity.

807 MECHANISM OF ACETAMINOPHEN HEPATOTOXICITY: COVALENT BINDING VERSUS OXIDATIVE STRESS

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The hepatotoxicity of acetaminophen is mediated by the metabolite N-acetyl-p-benzoquinone imine. This species, which is both an electrophile and an oxidizing agent, may covalently bind to critical proteins as acetaminophen-protein adducts or may cause oxidative damage. The toxicity may be initiated by either mechanism. To determine which occurs first we administered a hepatotoxic dose of acetaminophen (400 mg/kg) to mice and assayed for acetaminophen-protein adducts at 0, 1, 2, 4, and 6 hours by a Western blot assay using an anti-acetaminophen antibody. We also assayed for increased protein aldehyde formation (a hydroxyl radical catalyzed reaction) by a Western blot assay which uses an initial derivitization with 2,4-dinitrophenylhydrazine followed by an anti-dinitrophenyl antibody. Acetaminophen increased serum ALT levels at 4 and 6 hours, but not at 1 or 2 hours. Acetaminophen plus $FeSO_4$ (100 mg/kg) significantly increased serum ALT at 2 hours and levels were higher than acetaminophen alone at 4 and 6 hours. Fe alone did not increase ALT; however, Fe alone increased the intensity of staining in the Western blot assay for protein aldehydes over control at 2, 4, and 6 hours. Acetaminophen did not cause an increase in protein aldehydes over control at any time, nor did acetaminophen plus Fe. Acetaminophen-protein adducts were detected in acetaminophen and acetaminophen plus Fe mice at 2 and 4 hours. Acetaminophen appears to quench protein oxidation and presumably the oxidative process. These data are consistent with covalent binding as the primary mechanism of toxicity and argue against a role for oxidative stress in acetaminophen hepatotoxicity.

808 EFFECT OF OXYGEN DEFICIENCY ON ACETAMINOPHEN (APAP) TOXICITY IN MICE

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O_2 deficiency results in impaired glucuronidation and sulfation of APAP in rats while increasing the bioactivation to form GSH-derived products. This combination of effects could result in O_2 deficiency increasing APAP toxicity. To study this, we have performed initial experiments with CD-1 mice exposed to air (20.9% O_2; n = 8) or 10% O_2 (n = 7) for 8 days. Animals were fasted for 16 h and administered APAP (400 mg/kg) in propylene glycol. Urine was collected for 12 h, and animals were sacrificed. Blood plasma levels of glutamic-oxaloacetic acid transaminase were lower while urinary γ-glutamyltranspeptidase values were higher in the 10% O_2 group. Pathological scoring of liver and kidney injury was consistent with the interpretation that O_2 deficiency decreases hepatotoxicity and potentiates nephrotoxicity. Urinary output of APAP metabolites was decreased in the 10% O_2 group, reflecting impaired renal function, impaired hepatic metabolism, or both. Because tissue O_2 deficiency is common in aging humans with pulmonary or cardiac insufficiency, vascular obstruction or anemia, O_2 deficiency may be an important contributor to drug toxicity. (Supported by NIH HL-39968 and DK-44510).

809 THE 58 KDA ACETAMINOPHEN (APAP) BINDING PROTEIN (58-ABP) MAY BE A COMMON TARGET IN REACTIVE INTERMEDIATE TOXICITY

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Although APAP covalent binding to the 58-ABP has been associated with toxicity, it is not known if this target is unique for APAP. To evaluate this, mice were given phenobarbital (80 mg/kg/day × 4 days) and were killed 4 hr after challenge with 800 mg bromobenzene (BrB)/kg. Liver cytosols from BrB treated or control mice were eluted through Sephadex G-25 and incubated with APAP and mouse liver microsomes. The incubates were centrifuged and the resultant cytosolic fractions were analyzed by Western blotting with anti-APAP antibody. The APAP binding patterns in cytosols from control or BrB treated mice were then compared. Binding to 58-ABP was selectively decreased in liver cytosol from BrB treated mice. However, binding to other cytosolic targets was minimally affected by BrB. Immunochemical analysis with anti-58ABP indicated that the selective decrease in APAP binding did not result from diminished 58-ABP content. Also, inclusion of cytosols pre-exposed to BrB did not alter the capacity for microsomal activation of APAP as determined by HPLC analysis of APAP-N-acetyl cysteine conjugate formation. These results suggest that the 58-ABP may be a common target for electrophiles in reactive intermediate toxicity. [NIH GM31460 and ES07163; UCCBTox; American Liver Found. (JEM)]

810 5-β SCYMNOL (5βS) DECREASES ACETAMINOPHEN (APAP) TOXICITY BUT NOT COVALENT BINDING

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APAP toxicity may involve both arylative and oxidative mechanisms. 5βS is a shark bile salt which has been demonstrated to act as an antioxidant and free radical scavenger, *in vitro*. To determine if 5βS protected against APAP-induced hepatic or renal toxicity, 3–4 month old male CD-1 mice were given APAP (500 mg/kg). 5βS (100 mg/kg) was given at 0 or 2H after APAP. Plasma SDH at 12 hr after APAP alone was 1,630 U/l and BUN was 19 mg/dl vs 20 U/l and 10 mg/dl, respectively, in controls. Either simultaneous or 2hr delayed treatment with 5βS significantly diminished the APAP-induced SDH increase while only the simultaneous pretreatment prevented the BUN elevation. 5βS alone did not increase tissue glutathione content. Immunochemical analysis of APAP covalent binding indicated that 5βS did not alter protein arylation either qualitatively or quantitatively. These results suggest that 5βS did not impair APAP activation and are consistent with 5βS protection resulting from a post-arylative, antioxidant mechanism. (NIH GM31460, ES07163, UConn Ctr. Biochem. Tox.)

811 CHARACTERISATION OF TAMOXIFEN INDUCED-DNA ADDUCTS FORMED IN RAT LIVER

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Following oral administration, tamoxifen is a potent hepatocarcinogen in the rat. Analysis of rat liver DNA by ^{32}P-postlabelling has shown that large numbers of DNA adducts are formed. These can be visualised by autoradiography and give a very distinctive pattern of up to 12 adduct spots. Adducts formed in rat liver following treatment with ^{14}C labelled tamoxifen, were eluted from TLC plates and examined by accelerator mass spectrometry. This result showed that the ^{32}P-postlabelled spots were associated with covalently bound ^{14}C tamoxifen. Binding was dose dependent, as the dose of ^{14}C labelled tamoxifen increased so did the ^{14}C detected in liver DNA. An HPLC system has also been developed using on line radioactive detection to separate and identify postlabelled tamoxifen-DNA adducts. Oligonucleotides of the four individual DNA bases were reacted *in vitro* with tamoxifen using horseradish peroxidase activation. These were postlabelled and results show that only guanine forms adducts which elute to the same place as the adducts produced *in vivo* by tamoxifen.

812 *IN VITRO* ACTIVATION AND COVALENT BINDING OF VELNACRINE MALEATE (MENTANE®) TO HUMAN AND RAT HEPATIC MICROSOMAL PROTEIN

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Mentane is an effective drug for the treatment of Alzheimer's Disease (AD) and is the hydroxylated metabolite of Tacrine. The possible formation of reactive metabolites and covalent binding to microsomal protein during its biotransformation was tested *in vitro* after incubation of radiolabelled Mentane with rat or human liver microsomes under a variety of conditions. Covalent binding was only observed in the presence of a NADPH generating system and in the presence of oxygen; little binding was detected in the absence of either cofactor. With the appropriate cofactors and in the presence of GSH little covalent binding was observed. Binding was enhanced when Mentane was incubated with microsomal protein from rats pretreated with phenobarbital, dexamethasone, 3-methylcholanthrene, or pyrazole but inhibited when incubated with microsomes from rats pretreated with piperonyl butoxide or trichloropropane. Results indicate that reactive electrophilic metabolites may be formed which are cytochrome P-450 dependent and which bind to protein. Additional metabolic studies indicate that this binding may be due to impurities associated with the test material. These results may indicate the mechanism of liver damage observed in a small number of AD patients receiving this compound.

813 COVALENT INTERACTIONS MEDIATE INACTIVATION OF RAT LIVER CLASS 2 ALDEHYDE DEHYDROGENASE BY 4-HYDROXYNONENAL AND MALONDIALDEHYDE

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The α, β-unsaturated aldehydic products of membrane lipid peroxidation 4-hydroxynonenal (HNE) an malondialdehyde (MDA) have been characterized as substrates and inhibitors of several aldehyde dehydrogenase (ALDH) isoforms. Previously in our laboratory, HNE was shown to be a competitive, or possibly mixed-type inhibitor, of Class 2 ALDH-mediated acetaldehyde oxidation, whereas MDA was classified as an irreversible inhibitor of Class 2 ALDH-mediated formaldehyde oxidation, in experiments using purified enzyme and lysed mitochondria, respectively. The possibility that electrophilic aldehydes target critical moieties within ALDH prompted us to assess the molecular interactions of HNE and MDA with affinity-purified rat liver Class 2 ALDH. The mechanisms by which HNE and MDA inhibit ALDH were complex, where inhibition was dependent on aldehyde concentration or time of exposure. Spectrophotometric assays where enzyme, in the presence of pyridine nucleotide (5 mM), was exposed to HNE (>17 μM) or MDA (>5.0 μM) for 30 seconds prior to the addition of acetaldehyde (150 μM suggested a mixed-type reversible inhibition of ALDH. In contrast, increased pre-incubation times (30 minutes) to HNE (>62 μM) or MDA (>1.5 μM) inactived the enzyme irreversibly. Covalent alkylation of Class 2 ALDH by HNE and MDA was visualized in ELISA's employing antibodies reactive toward HNE- and MDA-protein adducts, wherein immuno-absorbance increased with the occurrence

of HNE- and MDA-ALDH adducts. Enzyme inactivation corresponded with formation of HNE-derived protein hydrazones, HNE-depleted protein thiols, and HNE-induced alterations in electrophoretic mobility of Class 2 ALDH 54 kDa monomers during SDS-PAGE. These results may implicate HNE and MDA as mechanism-based inhibitors of Class 2 ALDH wherein Cys-302 may be adducted. (Supported by NIH/AA05370 and NIH/AA9300).

814 INHIBITION OF CARBAMYL PHOSPHATE SYNTHETASE I (CPS I) BY HEPATOTOXIC DOSES OF ACETAMINOPHEN (AP) IN MICE

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We administered hepatotoxic doses of AP (400 mg/kg, ip) to male ICR mice fasted for 18 h. Livers were homogenized 2 and 6h after dose and subcellular fractions were isolated and derivatized with monobromobimane (mBBr). Proteins were separated by SDS-PAGE and visualized by fluorescence. The bands in control and AP-treated fractions were visually identical at 2 and 6h except for a protein of 130 kD. Although there was no difference in the abundance of this protein, the absence of mBBr-derived fluorescence was almost complete in the mitochondrial fractions 6h after AP, indicating the disappearance of mBBr-reactive thiol groups. N-terminal amino acid sequence analysis revealed that 13 out of 14 amino acids were identical with rat and human CPS I. In the protein we isolated from mouse liver, a gln to lys substitution was observed in the 5th position. Plasma ammonia levels in AP-treated mice increased from 68 ± 12 to 338 ± 150 μM, $p > 0.01$. CPS I activities in triton-treated 10,000g supernatants were lower in AP-treated mice, 0.024 ± 0.009 than in controls 0.085 ± 0.015 U/mg pro, $p > 0.001$, even in the presence of dithiothreitol. These data suggest that AP metabolites alkylate CPS I in vivo, inhibiting this enzyme and leading to accumulation of ammonia, which may contribute to AP-induced hepatic damage. Supported by NIHGM44263.

815 EXPOSURE-RELATED DIFFERENCES IN DNA ADDUCT FORMATION AND CELLULAR PROLIFERATION BY 1,2,3-TRICHLOROPROPANE

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1,2,3-Trichloropropane (TCP) is an industrial intermediate and solvent that induces high incidences of tumors at multiple sites in rats and mice when administered chronically by gavage. The data are being used to predict human risk from potential exposure to TCP in drinking water at significantly lower concentrations. Risk assessment may be affected by the difference in route of exposure. Gavage administration, which results in high bolus concentrations compared to more protracted exposure from other routes, may increase genotoxicity and cell proliferation. We have examined the effects of exposure route on the formation of DNA adducts and the induction of cellular proliferation by TCP, comparing administration by gavage and drinking water. Male B6C3F1 mice were administered ^{14}C-TCP for one week by gavage or in drinking water at the low dose (6 mg/kg) used in the NTP carcinogenesis bioassay. Two target organs (forestomach and liver) and two nontarget organs (glandular stomach and kidney) were examined for DNA adduct formation. Adducts were hydrolyzed from DNA, isolated by HPLC, and quantitated by measuring HPLC fractions for radioactivity. In the forestomach, liver, and kidney, gavage administration of TCP resulted in 1.4 to 2.4-fold higher yields of the major DNA adduct, previously identified as S-[1-(hydroxymethyl)-2-(N7-guanyl)ethyl]glutathione. Significant differences in cell proliferation, as determined by incorporation of bromodeoxyuridine, were also observed for the two routes. In the forestomach, the principal target organ for TCP carcinogenesis, gavage administration for two weeks resulted in an approximately two-fold higher cell proliferation rate relative to administration in drinking water. Our findings of exposure-related differences in DNA adduct formation and cell proliferation suggest that risk assessment based on the existing animal tumor data from gavage administration may overestimate human risk.

816 DNA ADDUCTS AND GRANULOCYTIC DIFFERENTIATION IN HL-60 CELLS FOLLOWING TREATMENT WITH BENZENE METABOLITES

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Benzene hematotoxicity is mediated by its reactive metabolites. Although reactive metabolites of benzene covalently bind to DNA. the significance of DNA adduct formation in the mechanism of benzene toxicity is not clear. We investigated the covalent binding of the benzene metabolites hydroquinone (HQ) and 1,2,4-benzenetriol (BT) using the DNA [^{32}P]postlabeling method and explored the potential relationship between DNA adduct formation and cell differentiation in human promyelocytic (HL-60) cells, a model system for studying hematopoiesis. Maturation of HL-60 cells to granulocytes, as assessed by light and electron microscopy, was significantly inhibited in cells that were pretreated with the benzene metabolites HQ or BT prior to inducing differentiation with retinoic acid. The capacity of retinoic acid-induced cells to phagocytose sheep red blood cells and to reduce the dye nitro blue tetrazolium, two functional parameters characteristic of mature, differentiated neutrophils, was also inhibited in cells pretreated with HQ or BT. These benzene metabolite treatments induced DNA adduct formation in HQ but not in BT treated cells. These results indicate that whereas HQ and BT each block granulocytic differentiation in HL-60 cells, DNA adducts only form following HQ but not BT treatment. Thus, DNA adduct formation may be important in HQ but not in BT toxicity. Supported by NIH T32 Training Grant #ESO7148.

817 PROTEIN PHOSPHORYLATION ASSOCIATED WITH ACETAMINOPHEN-INDUCED HEPATOTOXICITY

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The major target associated with acetaminophen (APAP) hepatotoxicity is a 58 kDa protein (58ABP). Six amino acid sequences of the 58-ABP are potential sites for protein phosphorylation. *In Vitro* studies indicate that the 58-ABP is a good substrate for both protein kinase A and casein kinase II. To determine whether APAP alters the phosphorylation of targeted proteins *in vivo*, ^{32}P-incorporation into cytosolic proteins of cultured mouse hepatocytes was examined on 2D-PAGE after APAP administration for 2, 4, 8, and 12 hr. While cytotoxicity was not significant until 12 hr after APAP, altered protein phosphorylation was detected within 4 hrs with phosphorylation of 3 proteins with M.W. and (pI) of p20 (6.2); p25 (6.3); and p58 (5.8–6.0) increasing and that of p23 (5.9) decreasing. Inhibition of P-450 by metyrapone diminished the altered phosphorylation. Menadione increased the phosphorylation of p20 and p58 but not of p25, while dibutyrl cAMP increased p20 phosphorylation. Only the phosphatase inhibitor, okadaic acid, mimicked the phosphorylation seen after APAP. These data suggest that the inhibition of a protein phosphatase after APAP administration may play an important role in signaling cell damage. (NIH GM-31460 and the Univ. of CT Center for Biochemical Toxicology).

818 IS THE 58 kDa ACETAMINOPHEN BINDING PROTEIN (58-ABP) AN ELECTROPHILE SCAVENGER?

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Acetaminophen (APAP) covalent binding to the cytosolic 58-ABP has been associated with target organ toxicity. To determine if the amount of 58-ABP influences binding to other target proteins the pattern of APAP binding was examined in mice exhibiting different levels of 58-ABP. In liver, but not kidney, the amount of 58-ABP varied with individual male CD-1 mice. By contrast, male AJ mice consistently had little 58-ABP in liver cytosol, while testosterone treatment (500 mg/kg, sc) of female CD-1 mice for 2 days increased hepatic 58-ABP content 6 days later. At 4 hours after challenge of mice from the above groups with 600 mg APAP/kg, the cytosolic covalent binding was determined by Western blot with anti-APAP. The blots were then stripped and re-probed with anti-58-ABP. The level of APAP bound to the 58-ABP target corresponded with 58-ABP content. In cases where 58-ABP was poorly expressed, APAP adducts were more prominently detected on other protein targets. These data suggest that binding to the 58-ABP may spare other potential targets of APAP electrophile attack and support a role of the 58-ABP as a preferred APAP electrophile scavenger in cytosol. [NIGM31460, NIESO7163, UConn Center for Biochemical Toxicology, and Amer. Liver Foundation (JEM)].

819 LIVER PROTEIN ADDUCTS OF DICLOFENAC IN PATIENTS WITH NSAID-INDUCED LIVER INJURY

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Nonsteroidal antiinflammatory drugs (NSAIDs) including diclofenac, are associated with rare cases of idiosyncratic adverse reactions in the liver. Immunoallergic reactions or metabolic abnormalities have been suggested as

possible mechanisms of injury. We previously demonstrated that diclofenac acyl glucuronide forms covalent protein adducts in rat liver and cultured rat hepatocytes. To investigate whether such drug adducts also occur in human liver, we have used a specific polyclonal anti-diclofenac antibody raised in rabbits against diclofenac-KLH, as a probe to detect immunochemically diclofenac adducts in liver sections of patients with NSAID-associated liver injury. Confocal laser scanning microscopical analysis revealed a strong immunofluorescence signal that was present across the hepatic lobule of a patient with diclofenac-associated hepatic injury, but no antigens were recognized in the liver of a patient with ketodolac associated hepatitis. Furthermore, the antibody did not react with epitopes in normal human liver, nor with hepatic tissue derived from a hepatitis C patient. These results indicate that in human liver a reactive metabolite of diclofenac may form stable adducts with hepatic proteins, and that these adducts may play a role in the pathogenesis of NSAID-associated hepatitis.

820 COVALENT MODIFICATION OF RAT LIVER DIPEPTIDYL PEPTIDASE IV BY ACYL GLUCURONIDE METABOLITES OF DICLOFENAC

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Diclofenac is a nonsteroidal anti-inflammatory drug that causes severe hepatotoxicity in some individuals. We recently have found that acyl glucuronide metabolites of diclofenac bind covalently to proteins in plasma membrane of rat liver. The major diclofenac-protein adducts have apparent M_r of 110, 140, and 200 kDa. We now have purified the 110 kDa diclofenac-labeled protein from plasma membrane fractions of liver from diclofenac-treated rats. *N*-terminal amino acid analysis showed that the 110 kDa protein target corresponds to dipeptidyl peptidase IV (EC 3.4.14.5, DPP-IV), a serine exopeptidase that cleaves dipeptides from the *N*-terminus of polypeptides containing proline at the penultimate position. The putative role of covalently modified DPP-IV in diclofenac hepatotoxicity is currently under investigation.

821 CHARACTERIZATION AND IDENTIFICATION OF INDIGENOUS DNA-ADDUCTS (I-COMPOUNDS) IN RAT BRAIN

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Indigenous DNA-adducts (I-compounds) are considered biomarkers of aging tissues. Thus far, few studies have been conducted to investigate the accumulation patterns of I-compounds in the brain during aging. Particularly, identities of age-dependent I-compounds has largely remained unknown. In the current study, the authors have determined the amounts of I-compounds in the brains of male Fisher 344 rats at ages 1, 6, 12, 18, and 24 months using the ^{32}P-postlabeling technique. The results indicate that I-compounds increase in the brain age-dependently from 6 to 24 months of age. Total I-compound levels increased 3.5-fold from 6 to 24 months. Contrary to the results of other investigators, brains of 1 month old rats contained the highest levels of I-compounds, which may be due to their hypermetabolic status. In an effort to characterize and identify I-compounds, different deoxynucleosides were co-incubated with malondialdehyde (MDA). The results show that only dGMP-MDA adducts overlap with I-compounds of the brain DNA-adducts map. Five dGMP-MDA adducts have been identified to be responsible for the brain DNA-adducts map. The brain is known to contain high levels of lipids, and MDA is a key product of lipid peroxidation. Therefore, the accumulation of I-compounds with aging may be an index of indirect oxidative damage to DNA as evidenced by the presence of MDA-DNA adducts. [Supported by grants awarded by NIH (R01 CA60994, R01 CA61764), The American Federation For Aging Research, and The Alzheimer's Association (FSA-94-024)].

822 STRAIN COMPARISON STUDIES ON THE METABOLISM OF BENZENE AND TRANS,TRANS-MUCONALDEHYDE BY LIVER CELL FRACTIONS FROM DBA/2N AND C57BL/6 MICE

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The greater sensitivity of DBA/2N mice towards the hematotoxic effects of benzene (Bz) compared with that of C57BL/6 mice could be related to increased formation of ring-opened Bz metabolites, including trans,trans-muconaldehyde (MUC), and/or differences in the metabolism of MUC, a microsomal hematotoxic dialdehyde metabolite of Bz. Studies were performed on the metabolism of MUC and Bz by DBA/2N and C57BL/6 mouse liver cytosol supplemented with NAD^+ and mouse liver microsomes supplemented with NADPH, respectively. Incubations were carried out for various time periods and the metabolites were analyzed using RP-HPLC with detection at 250–275 nm. In both the DBA/2N and C57BL/6 cytosol experiments, MUC was metabolized to the mono- and di- oxidation and reduction products and also to the acid-alcohol analog of MUC. The production and rate of formation of the metabolites detected in MUC incubations with cytosol from either mouse strain was similar suggesting that differences in mouse strain sensitivity to Bz are unrelated to MUC metabolism. Bz was metabolized by DBA/2N and C57BL/6 liver microsomes to hydroxylated metabolites and to two ring-opened products, MUC and 6-hydroxy-trans,trans-2,4-hexadienal (CHO-M-OH). Similar amounts of MUC but increased amounts of CHO-M-OH were formed by microsomes of the C57BL/6 strain compared to that of the DBA/2N strain. At present, the role of CHO-M-OH in relation to Bz hematotoxicity is not clear and the latter results invite further study. Supported by NIH grant ES02558 and NIEHS Center grant ES05022.

823 DNA ADDUCTS OF PHENOLIC COMPOUNDS RESULTING FROM THE FORMATION OF QUINONE METHIDE INTERMEDIATES

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Alkylated phenols produce a variety of cytotoxic and genotoxic effects. A potentially important route of bioactivation involves oxidation by cytochrome P450 to quinone methides which are sufficiently electrophilic to form covalent bonds with amino and thiol side chains of proteins and with nucleophilic sites of DNA bases. Structures of the latter adducts have not been well characterized, so the present investigation was initiated to determine which bases are affected and the specific sites of bond formation. Quinone methides were synthesized by chemical oxidations of 4-methylphenols containing varying substituents in the 2- and 6-positions. These were combined with the individual deoxynucleosides and adduct formation detected by HPLC. Only purine-containing dNs reacted, with dG forming a major and a minor adduct, and dA yielding one adduct. These products were purified and analyzed by ^{1}H NMR and mass spectrometry. The data demonstrated that the major and minor dG adducts formed at the N^2 and N-1 positions, respectively, and dA binding occurred at N^6. Calf thymus DNA was treated with quinone methides, hydrolyzed, and analyzed to confirm the sites of adduct formation with intact DNA. (Supported by NIH grant ES06216).

824 OXIDATION AT C-1 CONTROLS CYTOTOXICITY OF DDD BY RABBIT AND HUMAN LUNG CELLS

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Isolated rabbit Clara cells and a transformed human bronchial epithelial cell line, BEAS-2B, were used in the present study to investigate the mechanism of cytotoxicity of 1,1-dichloro-2,2-bis(p-chlorophenyl) ethane (DDD), a persistent insecticide and stable metabolite of 1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane (DDT). Both BEAS-2B cells and rabbit Clara cells were highly susceptible to DDD toxicity and were partially protected by 1-aminobenzotriazole (ABT), a suicide substrate inhibitor of cytochrome P450 enzymes. DDD (0.05 mM) killed 47 ± 1.8% of rabbit Clara cells and 42 ± 7.9% of BEAS-2B cells after 3 hours, and 84 ± 3.0% of rabbit Clara cells and 80 ± 14% of BEAS-2B cells after 6 hours. Consequently, DDD is the most potent Clara cell toxin recognized to date. The cytotoxicity of DDD to these cells was decreased by deuterium substitution at the C-1 position. Rabbit Clara cells incubated with ^{14}C-DDD produced the fully oxidized acetic acid metabolite (DDA) but DDA was not formed when DDD was co-incubated with ABT. These observations support the hypothesis that the cytotoxicity of DDD to susceptible subpopulations of rabbit and human lung cells is due at least in part to cytochrome P450-mediated oxidation of DDD at C-1. The rate-determining step for the production of the cytotoxic intermediate is proposed to be the formation of a highly reactive acyl halide intermediate that is readily hydrolyzed to the stable, nontoxic DDA. (Supported by USPHG grant ES056289 from the National Institute of Environmental Health Sciences).

825 OXIDATION OF CLOZAPINE BY HYPOCHLORITE TO A HIGHLY DELOCALIZED NITRENIUM ION

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Clozapine is an important new antipsychotic drug but its use is limited by the induction of agranulocytosis. We have shown that other drugs associated with agranulocytosis are metabolized to reactive metabolites by activated neutrophils or hypochlorite, which is the major oxidant produced by activated neutrophils. We had previously reported that clozapine reacts with hypochlorite to form a reactive intermediate with a λmax of 460 nm which we suggested was a nitrenium ion. In the present study we present further evidence for the structure of this intermediate.

The reactive intermediate in the reaction of clozapine with HOCl was observed by use of a flow system in which the reactants were fed into a mixing chamber and the products flowed directly into a Sciex API III mass spectrometer. The major product was observed at m/z 325 which is 2 mass units less than the protonated molecular ion of the parent drug and was unstable. Several stable products were observed by LC/MS at m/z 343 which is 16 mass units greater than the protonated molecular ion of the parent drug and they were also produced by the oxidation of clozapine by the combination of myeloperoxidase, hydrogen peroxide and chloride ion. The reactive intermediate was trapped by GSH and several conjugates were formed. NMR spectra of a major conjugate indicated GSH bound to the aromatic ring of the clozapine molecule at the 9 position.

These data provide further evidence for the formation of a formal nitrenium ion in which the positive charge is highly delocalized. Most of the products appear to represent nucleophilic attack of water or GSH on the aromatic ring. Such a reactive intermediate formed by activated leukocytes in the bone marrow could be responsible for clozapine-induced agranulocytosis.

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826 N-ACETYLTRANSFERASE-CATALYZED PRODUCTION OF N-ACETOXY PROCAINAMIDE (APA) AND ITS REACTION WITH GLUTATHIONE (GSH)

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Chronic use of procainamide (PA) can cause drug-induced lupus (SLE) in humans. We have previously demonstrated that procainamide can be oxidized to hydroxylamine procainamide (HAPA) by rat and human microsomes as well as by activated leukocytes (both neutrophils and monocytes). HAPA is further converted to a nitroso derivative that appears to be responsible for covalent binding to microsomal protein, suggesting a potential mechanism of toxicity. We hypothesized that APA, produced by N-acetyltransferase isozymes (NAT1 and NAT2) from HAPA, may also contribute to the covalent binding and toxicity of procainamide. We set out to synthesize APA, to determine its reactivity with GSH as an indication of its ability to bind to protein and to determine if it is produced by the NATs.

APA was synthesized by reaction of HAPA with acetyl cyanide in the presence of triethylamine. Its identity was determined by the fragmentation pattern on mass spectrometry. NAT1 and NAT2 were obtained by heterologous expression of the cloned human gene in *E. Coli.* HAPA was incubated with each NAT in the presence of acetyl-CoA. Both NAT1 and NAT2 converted HAPA to APA and diacetylPA. Studies of the reaction between APA and GSH indicated that a reaction occurred but it was relatively slow and the major products were PA, HAPA and GSSG rather than a stable adduct. The products were identified by mass spectrometry. From these data it appears that, unlike the analogous acetoxy metabolites of carcinogenic amines, reaction of APA with GSH is slow and leads to GSSG with little adduct formation. This is presumably due to the electron withdrawing effect of the amide group which would destabilize a nitrenium ion intermediate.

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827 *IN VITRO* ALKYLATION OF OXYTOCIN BY S-(2-CHLOROETHYL)GLUTATHIONE AND CHARACTERIZATION OF ADDUCTS BY TANDEM MASS SPECTROMETRY AND EDMAN DEGRADATION

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S-(2-chloroethyl)glutathione (CEG) is an alkylating agent that is formed by glutathione conjugation with 1,2-dichloroethane. The model peptide, oxytocin, was alkylated by CEG and tandem mass spectrometry (MS) used to identify the alkylation sites of CEG on oxytocin, as a prelude to work on proteins. A Sciex triple quadrupole MS with argon as a collision gas was used to sequence the adducts. It was found that mono-, bis- and tris- adducts can result from alkylation of reduced oxytocin and tandem MS differentiated mono adducts at Cys-6 from those at Cys-1. Manual Edman degradation was performed by adding 5% PITC in pyridine to the adduct followed by cleavage with neat TFA. These results eliminated the possibility that alkylation was at Tyr-2 rather than Cys-1 in the case of the bis adduct and mono adduct Cys-1. A mono adduct-homo-dimer resulting from alkylation at Cys-6 and disulfide bridge formation through Cys-1 was identified as well. Oxidized oxytocin also formed two minor adducts, representing less than 5% of oxytocin present in the reaction mixture. These findings demonstrate that alkylation sites of CEG on oxytocin did occur, as evidenced by tandem MS, and should aid in the identification of CEG-alkylated amino acids in proteins. (Support by NIEHS grant ES-00040 and ES-00210).

828 COLOCALIZATION OF A 3-METHYLINDOLE REACTIVE INTERMEDIATE AND P450 TRANSCRIPTS IN LUNG CELLS

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3-Methylindole (3MI) selectively causes damage to pulmonary tissues. 3MI is bioactivated to toxic intermediates by cytochrome P450 enzymes and two P450's that have been shown to metabolize 3MI to toxic intermediates are CYP2F1 and CYP4B1. Covalent binding of the electrophilic 3-methyleneindolenine intermediate to proteins is a plausible mechanism of 3MI-mediated lung damage. It is likely that certain pulmonary cells that express high amounts of P450 enzymes also avidly bind the 3-methyleneindolenine. The location of the CYP450's was investigated using *in situ* hybridization with specific mRNA probes to *2F1* and *4B1* cDNAs. Tissue slices from different species were hybridized with each of the probes. An antibody was developed to adducts of 3MI by dehydration of indole-3-carbinol to form 3-methyleneindolenine in the presence of metallothionein to produce the thioether conjugates of 3-methyleneindolenine and the conjugates were injected into sheep and serum was collected. The immunoreactivity of the serum was analyzed by ELISA for reactivity with several compounds, such as 3MI, 3-methyloxindole, 3-hydroxy-3-methyloxindole, cysteine, N-acetylcysteine, glutathione, N-acetylcysteine and glutathione conjugates of 3MI, and metallothionein. Only the thioether adducts of 3-methyleneindolenine cross-reacted with the antibody. The lung tissues from rabbits that had been exposed to 3MI were highly immunoreactive, while liver and kidney tissues possessed less than 24% of the reactivity of the lung. *In situ* hybridization of lung tissues showed CYP2F1 mRNA in pulmonary alveolar epithelial cells. Immunochemical and *in situ* analysis demonstrated the colocalization of P450 transcripts and 3MI adducts in pulmonary epithelial cells. (Supported by USPHS grant HL13645 from the National Heart, Lung, and Blood Institute).

829 PHENOXYL RADICAL-CATALYZED OXIDATION OF THIOREDOXIN: SELECTIVE FORMATION OF DISULFIDES

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Thioredoxin is the most important cellular redox buffer besides glutathione. This small oxidoreductase, M(r) 11700, exists in most tissues in μM concentrations and contains a pair of cysteines that participates in thiol/disulfide exchange reactions with numerous proteins. We have found that the protein is also an excellent one electron reductant of phenoxyl radicals generated by horseradish peroxidase-catalyzed oxidation of phenol, p-cresol, and etoposide. Redox-cycling of phenols was accompanied by selective oxidation of thioredoxin sulfhydryls to disulfide. HPLC/UV-VIS measurements showed that the ratio of SH/phenol oxidation was 15:1 under the conditions used. At the end of the reaction, oxidized thioredoxin was quantitatively recovered in the reduced form with dithiothreitol. Oxidation of the sulfhydryls to sulfoxy derivatives, oxidation of other amino acid residues, and formation of covalent adducts with phenolic metabolites (quinones) was not detectable by LC-MS. While the thiyl radical of glutathione is readily detected with the spin trap 5,5-dimethyl-1-pyrroline N-oxide (DMPO), no ESR detectable DMPO-thiyl adducts were found during the oxidation of thioredoxin. Similarly, oxidation of vicinal sulfhydryls of dihydrolipoic acid did not produce DMPO-thiyl spin-adducts indicating fast intramolecular cyclization to disulfide. Measurements of the superoxide dismutase-sensitive chemiluminescence response of lucigenin demonstrated that thioredoxin oxidation was accompanied by release of superoxide, most likely via disulfide radical-mediated one electron reduction of oxygen. We propose that formation of disulfides and superoxide release is characteristic of the phenoxyl radical-catalyzed oxidation of vicinal sulfhydryls

in both small thiols and disulfide forming oxidoreductases. The results indicate that the cytotoxicity of phenolic compounds can be partly explained by phenoxyl-radical-mediated oxidative stress and by direct modification of sensitive enzymes, channels, and receptors.

830 IMMUNOHISTOCHEMICAL DETECTION OF RENAL ADDUCTS IN RATS TREATED WITH THE SEVOFLURANE DEGRADATION PRODUCT, COMPOUND A

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The anesthetic sevoflurane, $FH_2C\text{-}O\text{-}CH(CF_3)_2$ is unstable in CO_2 absorbents and breaks down into ([fluoromethyl-2,2-difluoro (trifluoromethyl) vinyl ether], (cpd A). Inhalation of cpd A causes toxicity in the corticomedullary zone (CMZ) of the rat kidney. Cpd A may be biotransformed to a cysteine S-conjugate, which could be converted by cysteine conjugate β-lyase into the reactive metabolite, $CF_3CH(OCH_2F)CSF$. This species may acylate proteins and cause toxicity, analogous to that seen with cysteine conjugates of other halogenated ethenes. To test this idea, rats were exposed for 3 hrs to increasing doses of cpd A and killed 24 hrs later. Immunohistochemical staining of kidney sections with trifluoroacetyl antisera showed that adducts were formed in the region of CMZ necrosis. These results indicate that toxicity produced by cpd A may be due to tissue adduct formation. (Drs. El Eger II and MJ Laster of UCSF supplied the tissues used in this study. Support was provided by Ohmeda and the Robert Wood Johnson Foundation.)

831 METABOLISM AND TOXICITY OF 4-HYDROXY-PHENYLACETONE IN RAT LIVER SLICES: COMPARISON WITH ACETAMINOPHEN

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Acetaminophen (APAP) is oxidized by cytochrome P450 to a reactive quinoneimine which is thought to be responsible for its hepatotoxic effects. 4-Hydroxyphenylacetone (4-HPA) is a structural analog of APAP in which the amine group is replaced by a methylene group. Following a similar metabolic pathway 4-HPA would be oxidized to form a reactive quinone methide intermediate. We compared the metabolism and toxicity of 4-HPA and APAP in rat liver microsomes and precision-cut liver slices. Metabolites from microsomal incubations with [^{3}H] glutathione were quantified by reverse phase HPLC with a radioactivity detector, while toxicity in liver slices was assessed by measuring loss of intracellular potassium levels. Both 4-HPA and APAP formed glutathione conjugates in microsomal incubations. The rate of conjugate formation with 4-HPA was 3-fold greater than that of APAP. In rat liver slices a concentration of 5 mM 4-HPA killed approximately 50% of hepatocytes after 6 h incubation, while APAP was not toxic at concentrations up to 50 mM. N-Acetylcysteine protected slices from 4-HPA-induced toxicity, while phenobarbital enhanced metabolism and toxicity. In summary, 4-HPA is more hepatotoxic than APAP and this may be due to differences in metabolic rate and/or type of reactive intermediate formed. (ES06016).

832 THE ROLE OF OXYGEN-DERIVED FREE RADICALS AND LIPID PEROXIDATION IN CIGUATERA FISH POISONING

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The toxic dinoflagellate *Ostreopsis lenticularis* grows in coral reefs which exhibit substantial daily variability in oxygen tension (pO_2). The initial objective of this study was to determine whether pO_2 regulated the toxicity of *O. lenticularis*. A 2.9-fold increase in toxicity (LD_{50}) was found in cells cultured at 63% pO_2 relative to normoxic (21% pO_2) controls. These data indicated that the increased toxicity was regulated by enhanced oxidative stress resulting from exposure to elevated ambient pO_2. *O. lenticularis* cells grown under hyperoxic conditions also showed a mean increase of 42% in lipid peroxidation [measured as malonaldehyde (MDA)-TBARS] relative to normoxic controls.

Mice were injected (ip) with one sublethal dose of pure preparations of either ciguatoxin 1, maitotoxin 2, brevetoxin 2, or saxitoxin and sacrificed at 12, 24, 48 and 72 h after injection. A high variability (0.003–24.10) in the MDA ratio (experimental/control) was found in the livers (cytosolic fraction) of these animals which depended on the type of toxin and the time in which samples were obtained. Cytosolic preparations of livers (n = 3) of large (5.9–6.8 kg) highly toxic barracuda showed a mean increase of 85% in MDA when compared with livers (n = 2) from small (1 kg) and presumably non-toxic barracuda.

Although hyperoxia is a factor regulating the toxicity of *O. lenticularis*, the precise mechanism(s) of action has not been yet elucidated. Hyperoxia may cause an increase in peroxidation of polyether toxins present in *O. lenticularis* which may also result in an increased peroxidation of membrane phospholipids. Similarly, fish and humans exposed to these toxins may experience elevated levels of liver lipid peroxidation.

833 THE EFFECT OF 4-ALKYL SUBSTITUTION ON THE RATE OF ISOMERIZATION OF *o*-QUINONES TO *p*-QUINONE METHIDES: POTENTIAL BIOACTIVATION MECHANISM FOR CATECHOLS

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Previously we have shown that an additional bioactivation pathway for the hepatocarcinogen safrole (1-allyl-3,4-methylenedioxybenzene) exists which may contribute to its toxic effects: initial O-dealkylation of the methylenedioxy ring forming the catechol, hydroxychavicol (HC, 1-allyl-3,4-dihydroxybenzene), 2-electron oxidation to the *o*-quinone (4-allyl-3,5-cyclohexadien-1,2-dione), and isomerization forming the more electrophilic *p*-quinone methide (2-hydroxy-4-allylidene-2,5-cyclohexadien-1-one) [Bolton, J. L.; Acay, N. M.; Vukomanovic, V. *Chem. Res. Toxicol.* 1994, 7, 453–450]. In the present investigation, we explored the effects of changing π-conjugation at the 4-position on the rate of isomerization and reactivity of the quinoids formed from 4-propylcatechol (**1**), 2,3-dihydroxy-5,6,7,8-tetrahydronaphthalene (**2**), and 4-cinnamylcatechol (**3**). We selectively oxidized the catechols to the corresponding *o*-quinones or *p*-quinone methides and trapped these reactive electrophiles with glutathione (GSH). The results indicate that extended π-conjugation at the *para* position enhances the rate of isomerization of the *o*-quinone to the quinone methide. These data provide further evidence that formation of these electrophilic quinone methides from *o*-quinones may be a general bioactivation pathway for 4-alkylcatechols and their methylenedioxy precursors.

834 KINETIC DEUTERIUM ISOTOPE EFFECTS ON THE ISOMERIZATION OF 4-PROPYL-*o*-QUINONE TO ITS TAUTOMERIC QUINONE METHIDE

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We have recently shown that *o*-quinones can isomerize to quinone methides at rates which depend on the type of substituent at the 4-position. In the present investigation, we explored the mechanism of this isomerization reaction using 4-propyl-*o*-quinone and its deuterated analog. The results show that the isomerization reaction is general base-catalyzed which suggests that amino acids on proteins with basic side chains could catalyze the reaction in vivo. The Bronsted β value was determined to be 0.23 ± 0.02 consistent with the transfer of a proton in the rate-determining step. The rate/pH profile generated from the buffer dilution plots showed first-order dependence on hydroxide ion concentration from pH 7.8 – 10. From pH 6 – 7.8, the reaction was independent of pH suggesting that other processes compete at low buffer concentration in this pH region. Substitution of the benzyl CH_2 group with CD_2 dramatically slows the isomerization reaction. The kinetic deuterium isotope effect on quinone methide formation was determined by measuring the amount of quinone methide trapped as GSH conjugates from the 4-propyl-*o*-quinone and its deuterated analog. The isotope effect on product formation was 5.6. These data provide further evidence that formation of these electrophilic quinone methides from *o*-quinones could be catalyzed by basic residues in vivo and that the reaction could be inhibited by deuterium substitution at the benzyl methylene group. (Supported by NIH grant ES06216).

835 DIFFERENTIALLY EXPRESSED GENES ASSOCIATED WITH 5-AZA-2′-DEOXYCYTIDINE (d-AZA)-INDUCED HINDLIMB DEFECTS IN THE SWISS WEBSTER MOUSE

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d-AZA is an S-phase cytotoxic agent. It inhibits methylation of DNA, a process which serves as an epigenetic regulator of gene expression. We have shown that d-AZA causes temporally-related defects. Gestational day (GD) 10 treatment induced severe long bone defects of the hindlimb but not forelimb. This

suggests that alterations in gene function contribute to the observed pattern of defects. We conducted subtraction hybridization studies to identify differentially expressed genes. d-AZA was administered by a single i.p. injection (1.0 mg/kg) in the morning on GD 10. Hindlimb buds were collected from both treated and untreated embryos 4, 12, and 24 hours post-treatment. The tissues were frozen in liquid nitrogen then stored at −70°C. The mRNA was isolated and a portion of each sample representing both treated and untreated tissues was converted to cDNA (accounting for up- or down-regulation of genes). The treated cDNA was hybridized with untreated photobiotin labeled mRNA (and vice versa). Nonhybridized cDNA was recovered. This cDNA was amplified via polymerase chain reaction. Products were recovered and ligated to SK-pBluescript plasmid. *E. coli* competent cells were transformed with the ligated-plasmid and cells plated. Inserts ≈400bp in size were isolated in the 4 hr untreated sample and the 12 hr treated samples and sequenced. One clone was identified as a member of the murine B1 family of repetitive sequences. These sequences have been associated with posttranscriptional control of gene expression. mRNAs containing certain repetitive sequences are expressed as a function of cell growth, transformation, and embyonic development. This suggests that the clone isolated may also have a role in embryonic development.

836 THE EFFECT OF AROCLOR 1242, 1254 AND POLYCHLORINATED BIPHENYL ISOMERS ON GROWTH IN CHICK EMBRYOS

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Polychlorinated biphenyls (PCB) are ubiquitous in the environment and biomagnify in fish-eating birds. Domestic and wild avian species exposed to PCBs have demonstrated increased embryo mortality and decreased growth. Studies were carried out (two experiments, 20 embryos per treatment) to investigate the embryonic profile of growth related hormones as well as growth parameters in 17 day embryos after *in ovo* PCB treatment. Aroclor 1242, 1254 (6.67, 0.67, 0.067 ppm), 3,3′,4,4′-tetrachlorobiphenyl (TCB; 0.02, 0.002, 0.0002 ppm), 2,2′,6,6′-TCB or 3,3′,5,5′-TCB (both at 0.2, 0.02, 0.002 ppm) or vehicle were injected into day 0 fertile eggs. On day 17 of incubation, femur length, body, liver, heart and bursal weights were measured. Plasma was analyzed for triiodothyronine (T_3), thyroxine (T_4), growth hormone (GH) and insulin-like growth factor-I (IGF-I). Pituitary GH concentrations were also determined. Aroclor 1254 (6.67 ppm) and 3,3′,4,4′-TCB (0.02 ppm) treatment increased embryo mortality. Aroclor 1254 and 1242 (6.67 ppm) and 3,3′,4,4′-TCB (0.02 ppm) treatment decreased body weight, femur length, liver to body weight ratio and increased the heart to body weight ratio ($p < 0.05$). Aroclor 1254 (6.67 ppm), 3,3′,4,4′-TCB (0.02 ppm), 2,2′,6,6′-TCB (0.02 ppm) treatment increased plasma T_3 ($p < 0.05$). Aroclor 1242 (6.67 ppm) increased plasma T_4 ($p < 0.05$). Treatment with 3,3′,5,5′-TCB (0.02 ppm) increased femur length and pituitary GH whereas 0.002 ppm 3,3′,5,5′-TCB decreased plasma GH ($p < 0.05$). The other PCBs had no effect on pituitary or plasma GH. The PCB treatments did not alter plasma IGF-I concentrations. From these results, it appears that chick embryo growth related hormones are sensitive to PCBs. Each PCB isomer resulted in their own toxic effects, the toxic or dioxin-like 3,3′,4,4′-TCB had detrimental effects, similar to the Aroclors, on 17 day chick embryos. The non-dioxin like PCB isomers (2,2′,6,6′-TCB and 3,3′,5,5′-TCB) should not be discounted because they also are toxic but affect hormones in a different manner than the dioxin-like PCB isomers. (Supported by NIEHS Training Grant ES07148).

837 DEVELOPMENTAL EFFECTS OF PURE FUMONISIN B_1 IN CD1 MICE

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Developmental toxic effects of aqueous culture extract from *Fusarium moniliforme* were recently reported in mice and included maternal deaths, reduced maternal body weight gain, increased embryonic resorptions, reduced pup weights and fetal malformations including cleft palate, hydrocephalus and ossification deficits. Role of fumonisin B_1 (FB_1) in the induction of these developmental effects was evaluated in this study by administering 0 to 100 mg/kg of pure FB_1 on gestational days (GD) 7 thru 15 to pregnant CD1 mice and assessing maternal health and fetal development at the end of gestation. Doses of 25 mg/kg or higher produced an increase plasma ALT and increased hepatocyte nuclear diameter. FB_1 doses of 50 mg/kg or higher also resulted in significantly reduced material body weight gains and the number of live pups. Surviving pups exhibited hydrocephalus of the lateral and third ventricles at doses of 12.5 mg/kg or higher. Alterations in sphingosine:sphinganine ratios occurred in maternal but not fetal livers. These results suggest that the developmental effects of *F. moniliforme* culture extract are largely due to FB_1 and that the effects of FB_1 in the fetus may be mediated through it's maternal hepatotoxic effects.

838 2,2′,4,4′,5,5′-HEXACHLOROBIPHENYL AS AN ANTAGONIST OF THE TERATOGENICITY OF 3,3′,4,4′,5-PENTACHLOROBIPHENYL IN C57BL/6 MICE

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Polychlorinated biphenyls (PCBs) are widespread environmental contaminants. Among the PCBs, 3,3′,4,4′,5-pentachlorobiphenyl (PeCB) is the most toxic coplanar PCB congener. Recent studies have demonstrated the teratogenicity of PeCB in mice. This study was designed to investigate the potential of 2,2′,4,4′,5,5′-hexachlorobiphenyl (HeCB) as an antagonist of the teratogenicity of PeCB in C57BL/6 mice. Pregnant mice were administered orally a single dose of PeCB (dissolved in corn oil) at concentrations ranging from 522 to 2088 μg/kg body weight, either alone or in combination with a dose of HeCB (271 mg/kg) on day 10 of gestation. Fetuses were examined on day 17 for the presence of cleft palate and hydronephrosis. The results indicated a concentration-dependent increase in the incidences of cleft palate (ranging from 21 to 100%) in PeCB treatment groups. HeCB alone did not induce cleft palate. Cotreatment of dams with PeCB plus HeCB significantly reduced the number of fetuses with cleft palate in all treatment groups, except the group treated with the highest dose of PeCB (i.e., 2088 μg/kg). PeCB (1044, 783 or 522 μg/kg) produced 64, 45 and 21% of fetuses with cleft palate, respectively; whereas, the same doses of PeCB plus HeCB resulted in only 13, 4 and 0% of fetal cleft palate. Interestingly, HeCB did not antagonize PeCB-induced hydronephrosis in any of the treatment groups. The results from this study demonstrate that HeCB is a partial antagonist of PeCB-induced teratogenicity in C57BL/6 mice. (Supported by NIH P42-ES04917).

839 DEVELOPMENTAL EXPOSURE TO AROCLOR 1254 CAUSES LOW-FREQUENCY HEARING LOSS IN RATS

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The developing auditory system has been shown to be sensitive to the disruptive effects of hypothyroidism. Therefore, we assessed the effects of developmental exposure to Aroclor 1254, a thyrotoxic PCB mixture, on auditory function. Long-Evans rats (10/dose) received A1254, p.o., at 0, 1, 4, or 8 mg/kg from GD 6-PND 21. We assessed offspring at a number of ages for: plasma thyroid hormone concentrations body weight, survivorship, motor activity, auditory startle amplitudes and auditory thresholds. Circulating T4 concentrations were sharply reduced in a dose-dependent fashion in all PCB-exposed groups at PND 1, 7, 14 and 21. Mild reductions in T3 concentrations were apparent in the 4 and 8 mg/kg groups after PND 7. Persistent body weight deficits (up to 30% below controls) were apparent in treated pups by PND 7, and pup mortality ranged from 20–30% at the two highest doses. Auditory startle amplitudes were reduced in young (PND 24), but not adult, offspring. No effects on motor activity were detected at any age. Importantly, A1254 caused permanent hearing deficits (20–30 dB threshold shift) at the lowest frequency tested (1 kHz), whereas auditory thresholds were not significantly affected at higher frequencies (4, 16, 32 or 40 kHz). These data suggest a link between the dosage- and/or age-dependent effects of PCB-induced disruption of thyroid function and the ontogeny of the cochlea, i.e. later developing regions of the cochlea (lower frequency regions) may be more affected than earlier developing regions. Current investigations combining PCB treatment with thyroxine supplementation may further a possible link between PCB-induced developmental thyrotoxicity and auditory dysfunction.

840 EXPOSURE TO NOISE AND ADVERSE PREGNANCY OUTCOMES

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Noise is a common environmental pollutant. Although epidemiological studies on the effect of other environmental pollutants upon reproductive outcomes have been rapidly expanding at the present time, little attention has been focused on noise exposure. Recently more work has been done toward understanding the hazardous effects of noise exposure on the biological systems. Noise influences the gestation and makes the growth and development

of the organisms obscure. In this study, the prenatal effects of noise on the pregnant MFI mice were carried out. The 90 dBL_{Aeq} (8 hr) dose was used once a day for 5 days a week during gestation period. The prenatal effects: gestation periods, little size, still birth, neonatal mortality of life births, and birth weight were observed. The prenatal noise exposure caused a significant reduction in gestation period ($P < 0.01$), birth body weight ($P < 0.05$), liver weight ($P < 0.05$) and blood triglyceride level ($P < 0.1$) of the progeny. It also caused a significant elevation in blood GPT ($P < 0.1$) and GOT levels ($P < 0.01$) in addition to the reduction of the hot plate resistance time (analgesia) ($P < 0.05$) in these animals.

841 COMPARISON OF THE *IN VIVO* AND *IN VITRO* EFFECTS OF A TRIAZOLE ANTIFUNGAL ON DEVELOPMENTAL TOXICITY IN THE RAT

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ICIA0282 is a triazole antifungal that is teratogenic in the rat. The purpose of the current studies was to evaluate the effects of a single oral dose of ICIA0282 given at different days of gestation and compare these changes to those observed in rat whole embryo culture (WEC). ICIA0282 (600 mg/kg) was administered to pregnant Wistar rats (11/ group) as a single gavage dose on each of gestation days 7–16 (mating = gd1). Foetuses were evaluated after C-section on gd 22. Embryos (gd = 9.5) were explanted from dams and cultured in the presence of ICIA0282 (1 – 25 μg/ml) for 48 hours before evaluation of growth and differentiation. Additionally, pregnant females were also given a single dose of 300 mg [^{14}C] ICIA0282/kg on gd 15 and at various times after dosing (1–48 hours) radiolabel and parent compound were measured in placenta, foetus and maternal plasma. Examination of pups indicated a very high incidence of specific abnormalities. The foetal incidence of major abnormalities approached 100% on a number of treatment days. Selected defects exhibiting marked time dependency included: open eye (60% foetal incidence; d11), abnormal tympanic annulus (83%; d10), abnormal zygomatic arch (93%; d11), cleft palate and shortened ribs (98%; d15). In WEC the most pronounced effect was the fusion of the branchial arches (>5 μg/ml). Peak maternal and foetal concentrations occurred after 12 h and in maternal plasma <50% of radiolabel was present as parent compound after 1 h. In the foetus at 24 h there was negligible metabolism. Thus there was a good concordance between the craniofacial abnormalities observed *in vivo* and fusion of the branchial arches (precursors of such structures) *in vitro*. Kinetic data indicated a relative accumulation of ICIA0282 in the foetus which was directly responsible for the induction of developmental toxicity.

842 POTENTIAL ROLE FOR ALTERATIONS IN EPIDERMAL GROWTH FACTOR AND *c-myc* IN SECALONIC ACID D-INDUCED CLEFT PALATE IN MICE

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The in-vivo role of epidermal growth factor (EGF) and the oncogene *c-myc* in normal and abnormal palate development was evaluated by using secalonic acid D (SAD) induced cleft palate in CD 1 mice as a model. Pregnant mice receiving 0 or 35 mg/kg of SAD were sacrificed at 12 hr intervals between 13.5 and 15.0 of gestation and epidermal growth factor (EGF) levels and expression of the oncogene *c-myc* were measured in secondary palate using RIA, blotting and in-situ hybridization techniques. Results of our study indicated the presence of EGF and the adsence of *c-myc* expression in normal palate throughout the period of study. Peak EGF levels occured on gestational day (GD) 14.5, the critical period of palate developmental. Exposure to the cleft palate teratogen SAD resulted in a significant reduction in EGF levels between GD 13.5 thru 14.5 and an induction of *c-myc* expression in both epithelial & mesenchymal regions throughout the period of study. These results suggest important roles for alterations in EGF and *c-myc* in abnormal palate development.

843 MATERNAL EXPOSURE TO POLYCHLORINATED DIPHENYL ETHERS: HEPATO-, FETO-, AND THYRO-TOXICITY IN RATS

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Polychlorinated diphenyl ethers (PCDEs) are not produced for commercial use, but are present in the environment at detectable levels. Their structural similarity to PCBs suggests that they may have similar effects on the environment.

We evaluated the developmental toxicity of 3 PCDEs: 2,2′,4,4′,5,5′-hexaCDE, 2,2′,4′,5′,6-pentaCDE and 2,3′,4′,6-tetraCDE (PCDE 32, 35, 37, respectively), and examined their effects on maternal P450 and on maternal and juvenile T_4 & T_3. Sprague-Dawley rats were gavaged with PCDEs (25, 50, 75, or 100 mg/kg/d), or corn oil vehicle, on gestation days (GD) 6–15.

PCDE 35 decreased the number of pups born and also decreased weight at birth. PCDEs 32 & 37 did not decrease the number of pups born or the number surviving to postnatal day (PD) 5, but PCDE 32 decreased pup weights at birth and PCDE 37 decreased pup weight through day PD 5.

On GD 16, cytochrome P450 and thyroid hormone levels (T_3 and T_4) were measured in dams. PCDE 32 induced total cyt. P450 and alkoxyresorufin-*O*-dealkylases (EROD, PROD), while PCDEs 35 and 37 had no effect. T_4 was significantly depressed by PCDE 35 and 37; PCDE 32 had no effect. No PCDE affected T_3 levels. On PD 16, juvenile thyroid hormone levels were measured. PCDE 32 & 35, but not PCDE 37, depressed T_4 levels. PCDE 32 and 35, but not PCDE 37, affected T_3 levels.

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844 SEQUENTIAL ALTERATIONS IN PALATAL MESENCHYME OF RETINOIC ACID-EXPOSED MURINE EMBRYOS

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Retinoic acid (RA) is a potent inducer of cleft palate. In mice, exposure on gestation day (GD) 12 results in a 24 hour delay in shelf elevation. The objective of this study was to identify morphologic and functional changes causing this delay. On GD 12, gravid CD-1 mice were given an oral dose of RA or vehicle. Embryos were collected at 8 hour intervals beginning on GD 13–15. Serial sections of embryos were stained with H&E and with a battery of lectins. By mid-GD 13, the oral palatal mesenchyme of control and treated embryos was aligned perpendicular to the basement membrane with a slightly increased cell density. The central mesenchyme began to orient along the palatal axis. In controls, prior to shelf elevation (early GD 14), oral and central mesenchyme had decreased cell density (increased matrix hydration) with a loss of lectin (PNA) staining, while the nasal mesenchyme remained condensed and stained positive for lectin (PNA). In contrast, after RA-treatment, central and oral mesenchyme remain oriented similar to mid-GD 13. On mid-GD 14, the palatal shelves of control embryos were fused and their mesenchyme consisted of hydrated extracellular matrix. The shelves of treated embryos were still vertical with little mesenchymal hydration. By GD 15, some RA-treated embryos had horizontal palatal shelves with signs of hydration. Between GD 13–14, palatal epithelium was similar in control and treated embryos. The peridermal layer of the medial edge epithelium sloughed on GD 14. In non-fused palates of treated embryos, peridermal cells regenerated to a 2 cell epithelial lining. These data indicate that exogenous RA alters mesenchymal hydration (causes delayed elevation) without significantly affecting epithelial maturation.

845 DIAZEPAM AFFECTS ALL-TRANS-RETINOIC ACID- AND RESTRAINT-STRESS-INDUCED DEVELOPMENTAL DEFECTS IN CD-1 MICE

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Maternal restraint stress significantly increases all-*trans*-retinoic acid (tRA) teratogenicity in mice. We administered diazepam (Dz) prior to and/or during maternal restraint to determine if an anxiolytic agent could mitigate this effect of stress. Mated female CD-1 mice were allotted to 10 groups: (1) vehicle control (carboxymethyl cellulose) [C], (2) restraint only [R], (3) diazepam only [Dz], (4) tRA only [tRA], (5) restraint and tRA [R + tRA], (6) restraint and diazepam [R + Dz], (7) tRA and diazepam [tRA + Dz] (8–10) restraint, tRA, and diazepam, with diazepam given at 0 [R + tRA + Dz(0)], 4 [R + tRA + Dz(4)], or both 0 and 4 [R + tRA + Dz(0,4)] hr into the restraint period. Diazepam, tRA, and vehicle (control) were given p.o. on gestation day (gd) 9 (plug = day 1). Dams in groups 3 and 6–8 were given 10 mg/kg diazepam 15 min. before restraint; group 9 was dosed 4 hr into the restraint period; group 10 was dosed 15 min. before restraint and 4 hr after. Mice were kept supine with Elastakon® surgical tape for 12 hr, 9:00 a.m. to 9:00 p.m. Dams were killed on gd 18 and given a teratological examination. Dz alone had no effect, and the incidence of supernumerary ribs was less in the R+Dz group than in the R group, indicating a protective effect of Dz. Fetal defects were increased in the R + tRA group in comparison with the tRA group. However, the tRA + Dz group had more exencephalies and a fewer fused ribs than the tRA group. The R + tRA + Dz(0) group (group 8) had more exencephalies than groups 1–7; the R + tRA + Dz(4) group had less exencephalies than group 8 but appeared to have more short tails and rib or vertebral fusion than groups 1–7. The R +

tRA + Dz(0,4) group had the lowest fetal weights and highest incidences of exencephaly, short tail, and fused vertebrae of all groups. All three of these groups given restraint, tRA, and Dz had lower fetal weights than any others. These results suggest that induction of supernumerary ribs in mouse embryos by maternal restraint is mediated through maternal psychogenic stress, as the effect was attenuated by an anxiolytic agent. The triple combination treatment (R + tRA + Dz) results are more difficult to interpret, as Dz appeared to enhance the teratogenicity of tRA in the presence of restraint.

846 MULTIPLE FACTORS CONTRIBUTE TO THE TOXICITY OF THE AROMATIC RETINOID, TTNPB (RO 13-7410)

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The aromatic retinoid, (E)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthylenyl)-1-propenyl] benzoic acid (TTNPB) is 1000-fold more teratogenic than all *trans*-retinoic acid (tRA) in several species and in a cell-based model of teratogenesis, the mouse limb bud culture. Factors responsible for the remarkable potency of TTNPB were investigated including: binding to retinoid nuclear receptors (RARs and RXRs) and cytosolic binding proteins (CRABPs) and the metabolic disposition of TTNPB. Competitive binding affinities and dissociation constants were determined for TTNPB and compared to those of tRA. For binding assays, nucleosol or cytosolic fractions were obtained from COS-1 cells transfected with cDNAs encoding the appropriate nuclear receptor or binding protein. TTNPB binds to RAR α, β and γ with K_ds of 2.5, 2.7 and 1.8 nM, respectively. tRA binds RAR subtypes with slightly higher affinities, having K_ds of 0.3, 0.2 and 0.5 nM, respectively. The ability of TTNPB to compete with [^{3}H]tRA for CRABPI and CRABPII resulted in IC_{50}s of 1800 and 255 nM, respectively. In contrast, tRA yielded IC_{50}s of 67 and 70 nM, respectively. 10 μM TTNPB did not compete with [^{3}H]9-cis RA for binding to RXR α, β or γ. Mouse limb bud cell cultures, a well characterized model for retinoid teratogenesis, were used to compare the metabolic disposition of TTNPB and tRA. Disappearance of TTNPB was significantly slower than that of tRA over 72 hours. In cell uptake experiments, both retinoids reached approximately equal concentrations in the cell; however, TTNPB reached peak concentration and disappeared from the limb bud cell at a significantly slower rate than that of tRA. Collectively, these results indicate that multiple factors including high affinity binding to RARs, lower affinity binding to CRABPs and metabolic resistance contribute to the remarkable potency of TTNPB.

847 PRETREATMENT WITH Zn OR LOW DOSES OF LIPOPOLYSACCHARIDE (LPS) PROTECTS AGAINST LPS DEVELOPMENTAL TOXICITY

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The developmental toxicant LPS (from *Salmonella*), is known to induce the Acute Phase Inflammatory Response (APR), a systemic response to tissue injury. In the pregnant CD-1 mouse, the LPS-induced APR includes induction of hepatic metallothionein (MT) synthesis. MT is a metal binding protein which can be induced by metals, hormones, and various chemical agents. Induction of maternal hepatic MT alters Zn distribution, including increased liver Zn, decreased plasma Zn and decreased Zn distribution to the litter. Zn supplementation administered as a pretreatment to chemical insult ameliorates the developmental toxicity of LPS. The mediator of this protective effect is uncertain. We hypothesized that although Zn may protect against LPS possibly via improving embryonal Zn status, other MT inducing agents may also have protective properties and that the mediator may be MT. We pre-administered (PTX) Zn acetate (10 mg/kg) or a low dose of LPS (0.01 mg/kg) i.p. to pregnant mice 24hr prior to GD 9 administration of LPS (0, 0.05 mg/kg). We co-administered (Co-Tx) Zn acetate (10 mg/kg, 5 mg/kg) i.p. 0hr prior to GD9 LPS (0, 0.05 mg/kg). Maternal liver and plasma were taken for trace element and/or MT analysis. Embryos were examined on GD 10 for viability and dysmorphogenesis. LPS produced maternal weight loss and embryolethality. The Zn PTX and the low LPS (0.01 mg/kg) PTX protected against embryolethality whereas the Zn Co-Tx appeared to exacerbate the effect. Protection against LPS developmental toxicity by 0.01 mg/kg LPS PTX suggests protection may be mediated by MT. This abstract does not necessarily reflect EPA policy. TML: Training Agreement T901915.

848 ENHANCEMENT OF METHYLMERCURY-INDUCED EMBRYOTOXICITY BY MATERNAL STRESS IN THE MOUSE

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Both methylmercury and maternal restraint (R) stress during gestation produce embryotoxic and teratogenic effects in mammals. The purpose of this study was to determine whether R may enhance the developmental toxicity of methylmercury chloride (MMC) in mice at a dose which is embryotoxic and teratogenic by itself. On day 10 of gestation, three groups of plug-positive females were treated with MMC alone (25 mg/kg, p.o.), R alone (14 hr), or MMC (25 mg/kg, p.o.) with R (14 hr). An additional group of untreated females received water p.o. (control group). There was no maternal mortality due to R alone. However, MMC alone produced a 14.3% of mortality, whereas combined MMC and R treatment caused a 35.3% of deaths. Body weight gain was also significantly reduced in this group, compared with the other groups. On day 18 of gestation, the mice were killed and gravid uteri examined. There were no completely resorbed litters in the control group or in the R group. In contrast, a percentage of 50% of totally resorbed litters due to MMC alone was observed. This percentage was increased to 100% in the group treated with MMC and R. Maternal stress in mice, which by itself was no embryotoxic and teratogenic, significantly increased maternal mortality as well as the rate of resorptions.

849 Mi-DMSA AND PREVENTION OF METHYL MERCURY TERATOGENESIS IN MICE

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Methyl mercury is embryotoxic and teratogenic in mammals. In this study, the protective activity of monoisoamyl *meso*-2,3-dimercaptosuccinate (Mi-DMSA), a new dimercaptosuccinic acid (DMSA) analog with enhanced lipophilic properties, on methyl mercury-induced developmental toxicity was assessed in mice. A series of four Mi-DMSA injections was given s.c. 0.25, 6, 24, and 48 hr after p.o. administration of 25 mg/kg of methyl mercury chloride (MMC) given on day 10 of gestation. Mi-DMSA effectiveness was tested at 0, 23.8, 47.5, and 95 mg/kg. Cesarean sections were performed on gestation day 18. All live fetuses were examined for external, internal, and skeletal abnormalities. Oral MMC administration resulted in an increase in the number of resorptions and a decrease in fetal body weight. Moreover, the incidence of cleft palate (57.6%) and micrognathia (15.3%), as well as the number of skeletal variations were found to be increased in the fetuses of the positive control group. Amelioration by Mi-DMSA of MMC-induced embryolethality was not noted at any dose. However, MMC-induced teratogenicity was reduced at 23.8, 47.5, and 95 mg/kg. The decreases in the incidence of cleft palate (21.9%) and micrognathia (0%) were especially remarkable at 95 mg/kg. The above results show that Mi-DMSA offers only modest encouragement for its possible therapeutic potential in pregnant women exposed to MMC.

850 URETHANE, AN INDUCER OF EMBRYONIC Zn DEFICIENCY, CAUSES EXCESS CELL DEATH IN RAT EMBRYOS

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Programmed or physiological cell death (PCD) occurs in a predetermined spatial and temporal pattern during normal embryogenesis. Increases in the extent of PCD can lead to abnormal development. Many physiological/toxic insults increase PCD, including dietary Zn deficiency. In this study, the vital dye neutral red was used to identify the pattern and extent of PCD in embryos after maternal treatment with urethane. Urethane is developmentally toxic, and has been shown to induce metallothionein in the dam, eliciting a systemic redistribution of Zn including a secondary embryonic Zn deficiency. Urethane (1 g/kg, n = 16) was given ip to Sprague Dawley rats on gestation day (GD) 10. Controls received vehicle and were pair-fed to treated rats (n = 11), or no injection and *ad lib* fed (n = 13). Embryos were excised on GD 11, just subsequent to the peak maternal metallothionein induction, and incubated in a dilute solution of neutral red for 30 min at 37°C. This visualizes cell death as red stippled areas. The normal pattern of PCD on GD 11 includes the otic pit, eye, and limited amounts in forebrain, somites and tail bud. Urethane treatment induced more marked cell death in these regions as well as in branchial arches, and staining was more intense. There was no increased cell death in

pair-fed controls vs. ad lib fed. Increased cell death was no longer evident 48 hr after treatment. The timing of cell death induced by urethane is coincidental with altered maternal and embryonic Zn distribution. Furthermore, the pattern is similar to that reported for dietary Zn deficiency. These results support the hypothesis that altered Zn status plays an important role in adverse developmental outcome after urethane treatment.

851 TERATOGENICITY IN THE RAT AFTER REPEATED INTRAVENOUS INJECTION OF MANGANESE, EITHER AS A COMPLEX (MANGAFODIPIR TRISODIUM, MNDPDP), OR AS THE INORGANIC CHLORIDE

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Although the general toxicity of manganese is well known, its teratogenicity has never been clearly demonstrated until recently (D Grant, E Holtz & D Shaw. Acta Radiol. 1994, 35 (Suppl 394):48). To investigate this further, pregnant female rats (Crl: CD (SD) BR VAF/Plus) were injected via the caudal vein with physiological saline (control), inorganic manganese ($MnCl_2$), complexed manganese used as an hepatobiliary MRI contrast agent (mangafodipir trisodium, or MnDPDP), or with the DPDP ligand (N,N'-dipyridoxylethylenediamine-N,N'-diacetic acid 5,5'-bis-(phosphate)). An additional group of rats was also given 400 μmol $MnCl_2$/kg by oral gavage. Treatment was daily from days 6–17 inclusive of gestation. All rats (24/group) were killed on day 20 of pregnancy. The incidences of skeletal abnormalities of foetuses in the various groups were as follows: control, 7.3%; $MnCl_2$, 30 μmol/kg and 6 μmol/kg, 85.0% and 13.6% respectively; MnDPDP, 40 μmol/kg, 89.9%; DPDP, 40 μmol/kg, 6.8%. The major abnormalities were rib irregularities, and distorted/misshapen long bones, scapulae and clavicles. There were also reductions in the degree of ossification. In the 30 μmol/kg $MnCl_2$ and 40 μmol/kg MnDPDP groups, foetal weights were significantly reduced. Embryolethality and maternal toxicity were not increased in any of the groups. Oral dosing with $MnCl_2$, and intravenous administration of DPDP, produced no overt treatment related adverse effects on either dams or foetuses. It is concluded that Mn is clearly teratogenic to the rat when given repeatedly by intravenous injection during organogenesis, either as the inorganic chloride or when complexed to an organic chelator, but not when given orally at much greater dosages, probably due to the limited intestinal absorption of manganese and the highly efficient first pass uptake by the liver, thereby reducing bioavailability to the foetus.

852 EMBRYOTOXICITY AND FETOTOXICITY OF MANGANESE IN MICE: VARIABILITY WITH THE DAY OF EXPOSURE

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Exposure of pregnant mice to manganese (Mn) induces embryotoxic and fetotoxic effects. The aim of this study was to determine a critical gestational day of developmental toxicity caused by Mn (II) in mice. Single s.c. injections of 50 mg/kg of Mn(II) chloride were given to Swiss mice on one of gestational days (gd) 9–12. No maternal deaths, abortions, or early deliveries were observed. The dams were killed on day 18 of pregnancy and their uterine contents examined. Live fetuses were observed for external, internal and skeletal malformations and variations. Although embryolethality, evidenced by an increase in the number of late resorptions and in the percentage of postimplantation loss, was noted in all the Mn-treated groups, it was especially notorious when Mn was injected on days 9 or 10 of gestation. Significant reduced fetal body weight was also found on days 9 and 10, but not in gestational days 11 and 12. Although there were no external, internal or skeletal malformations, the incidence of skeletal variations was increased in all the Mn-treated groups. According to these results, gd 9 and 10 would be the most sensitive time for Mn-induced developmental toxicity in mice.

853 PRE- AND POSTNATAL DEVELOPMENT OF RATS EXPOSED TO BORIC ACID (BA) ON GESTATIONAL DAYS (GD) 6 TO 15

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Exposure of CD® rats to BA (CAS 10043-35-3) during gestation resulted in developmental toxicity including reduced fetal body wt (≥ 0.1% BA in feed), enlarged brain ventricles (≥ 0.4% BA) and craniofacial malformations (0.8% BA; Heindel *et al.*, FAAT 18, 266–277, 1992). In order to determine the repeatability, dose-response characteristics and postnatal sequelae of these findings, BA (0, 0.4, 0.5, 0.6 or 0.8% in feed) was provided to timed-mated Sprague-Dawley-derived (CD®) rats (42–76/group) from gd 6 to 15. Dams terminated on gd 20 or postnatal day (pnd) 21 ingested 0(0), 299(283), 361(368), 432(434) or 549(562) mg BA/kg/day. Maternal clinical signs, body wt and food/water intake were measured at regular intervals; liver and right kidney were weighed at termination. Fetuses (gd 20) and pups (pnd 0–21) were evaluated for growth, viability, and gross morphology of the head (external and internal). Maternal wt gain during (gd 6–15) and after (gd 15–20) treatment was decreased at all doses, but gestational wt gain corrected for uterine wt was not affected. Maternal relative liver wt increased at 0.8% BA (gd 20) and decreased at ≥ 0.6% BA (pnd 21); relative kidney wt increased at ≥ 0.5% BA (gd 20). Postimplantation mortality was increased at all doses by pnd 21 (9, 17, 30, 64 and 97%). Fetal body wt was decreased at all doses (79, 70, 62 and 51% of controls on gd 20). By pnd 21, body wt deficits persisted only at 0.8% BA (76% of controls). Craniofacial malformations (primarily anophthalmia or microphthalmia) occurred at 0.8% BA. After adjusting for fetal body wt by covariate analysis, minimal ventricular enlargement was not dose-related, but hydrocephaly was increased at ≥0.4% BA. These results confirm and extend the previous study. Supported by NTP/NIEHS Contract No. NO1-ES-95255.

854 DEVELOPMENTAL TOXICITY OF THIOPHENOL (THIO) IN RATS AND RABBITS

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THIO, used in the production of pesticides, polymers, and pharmaceuticals, and as a food additive, was tested for developmental toxicity in Sprague-Dawley rats (25/group) and New Zealand White rabbits (15–26/group). THIO was given po in corn oil to rats [0, 20, 35, or 50 mg/kg/day; gestational days (gd) 6–15] or rabbits (0, 10, 30, or 40 mg/kg/day; gd 6–19). Maternal body wt., food and water consumption (rats) were recorded. On gd 20 (rats) or 30 (rabbits), maternal organs and fetuses were weighed, and fetuses were examined for malformations (external, visceral, and skeletal). In rats, maternal body wt. change and food consumption were depressed in all THIO-treated groups on gd 6–9. Gravid uterine wt. was decreased, and relative maternal liver wt. was increased at 50 mg/kg/day; kidney wt. was unaffected. Increased postimplantation loss and incidence of external malformations, and decreased live litter size were observed at 50 mg/kg/day. Female fetal body wt. was decreased at 35 mg/kg/day. In rabbits, maternal food intake tended to be lower, and body wt. change was decreased at 30 and 40 mg/kg/day on gd 12–15. Gravid uterine wt., liver and kidney wt. were unaffected. Postimplantation loss, litter size, average fetal body wt. and morphological development were unaffected. In summary, for rats, 20 mg/kg/day THIO was the low observed adverse effect level (LOAEL) for maternal toxicity, based on transient decreases in maternal wt. gain and food intake. A maternal no observed adverse effect level (NOAEL) was not determined. The developmental NOAEL in rats was 20 mg/kg/day; clear evidence of developmental toxicity was seen at ≥ 35 mg/kg/day. In rabbits, the maternal NOAEL was 10 mg/kg/day. Maternal toxic effects at ≥ 30 mg/kg/day were minor. The developmental NOAEL was ≥40 mg/kg/day. Supported by NTP/NIEHS Contract NO1-ES-95255.

855 DEVELOPMENTAL TOXICITY OF WIN 59010-2 IN SPRAGUE DAWLEY RATS

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Mangafodipir trisodium (MFTS) is a manganese chelate (Mn to chelate ratio, 1:1) developed as a contrast agent for magnetic resonance imaging of the hepatobiliary system. The intended clinical dose is 5 μmol/kg. The potential for MFTS to induce embryotoxicity and/or teratogenicity in Sprague Dawley rats was investigated. Twenty five inseminated rats/group were intravenously administered 0, 2, 5, or 20 μmol/kg MFTS from days 6–17 of gestation. Maternal toxicity was not seen at any dose of MFTS. There were no treatment effects on the number of fetuses, fetal viability, number of resorptions, implantations and corpora lutea, or the percent of pre- and post-implantation loss. However, at 20 μmol/kg fetal body weights were significantly decreased (3.3 g vs 3.9 g for control), abnormal limb flexures were increased (38/270 vs 0/285 for control), and an increase in malformations was observed (141/270 vs 0/285 in control). The malformations were of a specific type which included angulated or irregularly shaped clavicle, femur, fibula, humerus, ilium, radius, scapula, tibia and/or ulna. To better define the developmental stage which is most susceptible to the effects of MFTS, a segmented developmental study was conducted. Twelve inseminated rats were administered 0, 20, 40 or 80 μmol/kg MFTS on

days 6–8, 9–11, 12–14 or 15–17 of gestation. Skeletal malformations, identical to those seen in the previous study, were increased in a dose-dependent manner with the highest incidence occurring in females dosed from days 15–17 of gestation. Results from a third study where 15 rats/group were intravenously dosed with 0, 5, 20 or 40 μmol/kg $MnCl_2$ indicated that Mn is the active moiety responsible for the specific malformations caused by MFTS.

856 TERATOGENIC EFFECT OF ALCOHOL OR/AND COCAINE ON CF-1 MICE

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The prevalence of simultaneous ingestion of ethanol by cocaine users was increased recently. Therefore, pregnant mice were used to study the teratogenic effect of this combination. In the alcohol group, pregnant females were given 0.6 gm/kg twice daily, 5 hrs apart by gavage. Cocaine hydrochloride 20 mg/kg was injected intravenously. In combined group, pregnant females received both treatments. The treatment was started from day 6 to day 15 of gestation and dams were sacrificed at day 18. The number of dead and live fetuses was determined as well as the number of resorptions. Half of each litter was used for visceral examination, the other half was eviscerated, and examined for skeletal anomaly. The results revealed a significant increase in skeletal anomalies in the 3 treated groups. Although the skull and the paw anomalies increased in cocaine and alcohol groups, the significant increase in combined group was observed. A synergistic effect of cocaine/alcohol group on sternum anomalies such as drop wrist and clubbing of the foot were found. Abdominal anomalies such as hydronephrosis and ectopic kidney were significantly increased in combined group. This study revealed that the combination of alcohol and cocaine is more deleterious than each drug alone.

857 DEVELOPMENTAL TOXICITY STUDIES IN MICE, RATS AND RABBITS ADMINISTERED NICOTINE VIA SUBCUTANEOUS OSMOTIC PUMPS

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Mated female CD-1 mice, CD rats and inseminated NZW rabbits were assigned to one control and three or four experimental groups of 25 animals each. Nominal dosage levels of 2.5, 10, 25 and 50 mg/kg/d (mice) or 1.0, 2.5 and 6 mg/kg/d (rats and rabbits) were administered subcutaneously via osmotic pumps. The pumps were implanted at gd 6 (mice and rats) or 7 (rabbits) and the dosages were administered for the life of the pumps ($\approx$ 2 weeks). The control group received sterile 0.9% NaCl on a comparable regimen. Uterotomies were performed on gd 17 (mice), 20 (rats), or 29 (rabbits). Maternal toxicity, as evidenced by decreases in body weight gain and food consumption, was present at 25 mg/kg/d and higher for mice, at 2.5 mg/kg/d and higher for rats and at 6 mg/kg/d for rabbits. Additionally, whole litter resorptions were present at 25 mg/kg/d and higher (mice), and the clinical observations of unkempt appearance (rats) and decreased defecation (rabbits) at 6 mg/kg/d. Developmental toxicity was demonstrated only in mice as evidenced by decreases in mean fetal and uterine wts. at 50 mg/kg/d. Increased incidences of unossified hyoid (mice) and sternebra (mice and rats) were noted at the highest dose levels. The maternal NOEL of nicotine in these studies was 10 (mice), 1 (rats) and 2.5 (rabbits) mg/kg/d and 25 (mice), and 6 (rats and rabbits) mg/kg/d with respect to developmental toxicity.

858 COMPARATIVE TERATOLOGICAL STUDIES ON TCDD, ENDRIN AND LINDANE IN C57BL/6J AND DBA/2J MICE

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The teratogenic effects of endrin and lindane have been determined and compared to those induced by TCDD in the fetuses of C57Bl/6J and DBA/2J mice, after the administration of single oral doses to pregnant mice on day 12 of gestation. TCDD produced dose-dependent decreases in fetal weight, fetal thymic weight and placental weight, and dose-dependent increases in fetolethality, cleft palate formation and hydronephrosis at doses of 10–30 and 30–60 μg/kg body weight in C57BL/6J and DBA/2J mice, respectively. No maternal death was observed at the given doses in both strains of mice. Endrin (4.5 and 6 mg/kg body weight) and lindane (30 and 45 mg/kg body weight) produced significant decreases in fetal weight, fetal thymic weight and placental weight, in C57BL/6J and DBA/2J mice. However, endrin and lindane caused 0–30 and 10–30% maternal deaths, respectively, at the above mentioned doses. Neither cleft palate nor hydronephrosis were induced by endrin or lindane in both strains of mice. The results suggest that TCDD-induced cleft palate and hydronephrosis may involve mechanisms that are Ah-receptor mediated. However, other fetotoxic effects induced by TCDD as well as the fetotoxic effects of endrin and lindane may involve other mechanisms that are not related to the Ah-receptor.

859 PRELIMINARY EVALUATION OF PHENYTOIN TERATOGENICITY IN TRANSGENIC MICE DEFICIENT IN THE p53 TUMOR SUPPRESSOR GENE

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While widely known as a tumor suppressor gene, p53 also serves as a teratological suppressor gene in transgenic p53-deficient mice treated with the DNA-arylating teratogen benzo[a]pyrene. To determine the embryoprotective role of p53 for the anticonvulsant drug phenytoin, which enhances the formation of reactive oxygen species and oxidises DNA, homozygous p53-normal (+/+) and heterozygous p53-deficient (p/+) dams were mated with heterozygous p53-deficient (p/+) males. Pregnant dams were treated on days 12 and 13 of gestation (plug = day 1) with a teratogenic dose of phenytoin (65 mg/kg ip) and sacrificed on day 19. The incidence of fetal resorptions (in utero death) and postpartum lethality were respectively 3-fold higher and 6-fold higher in p/+ p53-deficient dams than in +/+ p53-normal dams ($p < 0.05$). In the p/+ p53-deficient dams, 11 of the 13 observed resorptions could be genotyped using the polymerase chain reaction. Only one resorption was +/+ p53-normal, and p/p p53-deficient resorptions occurred with greater frequency than p/+ p53-deficient resorptions. In p/+ deficient dams, over half of the postpartum lethality was in p/p p53-deficient fetuses. These results indicate that DNA damage constitutes an important molecular mechanism of phentyoin teratogenicity, and suggest that p53 may protect the embryo from a broad spectrum of DNA-damaging teratogens. (Support: Medical Research Council of Canada).

860 p53 — A TERATOLOGIC SUPPRESSOR GENE? BENZO[a]PYRENE (B[a]P) TERATOGENICITY IN TRANSGENIC P53-DEFICIENT MICE

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If DNA damage mediates chemical teratogenesis, then p53, a so-called tumor suppressor gene, may be critically embryoprotective. This study evaluated whether pregnant heterozygous p53-deficient dams were more susceptible than homozygous p53-normal controls to B[a]P teratogenicity. Pregnant dams were treated with the CYP1A1 inducer 2,3,7,8-tetrachlorodibenzo-p-dioxin 1 μg/kg ip on gestational day (GD) 7 (plug = GD 1) and B[a]P, 200 mg/kg ip, on GD 10. Dams were sacrificed on GD 19 and the implantations (fetuses and resorptions) were removed. DNA from fetal resorptions was amplified by the polymerase chain reaction and genotyped for p53. Compared to p53-normal dams, p53-deficient dams had respective 2-fold, 2.5 fold, and 4-fold increases in B[a]P-initiated fetal resorptions, a teratologic index and postpartum lethality ($p < 0.001$), with no difference in fetal body weight. Fetal resorptions reflecting *in utero* death occur almost exclusively with p53-deficient embryos. This is the first direct evidence that p53 may be an important teratological suppressor gene that protects the embryo from DNA-damaging chemicals and developmental oxidative stress. (Support: Medical Research Council of Canada).

861 THE *IN VITRO* EVALUATION OF CROSS-SPECIES STEREOSELECTIVE DYSMORPHOLOGY OF THE ENANTIOMERS OF THE VALPROIC ACID ANALOGUE 2-N-PROPYL-4-PENTYNOIC ACID (4-yn-VPA)

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We have previously reported the in vitro differential stereoselective dysmorphogenesis of the R(+) and S(−) enantiomers of 4-yn-VPA in mice. In order to determine if the stereoselectivity was species specific, we evaluated the dysmorphogenic potential of these isomers as well as valproic acid (VPA) to

gestational day 9 rat embryos in whole embryo culture (WEC). Aqueous solutions of the sodium salts of R-4-yn-VPA, S-4-yn-VPA, 50%R-50%S-4-yn-VPA or VPA were added to the culture medium to give 0, 0.075, 0.15, 0.3, 0.6 or 1.2 mmol/l and embryos were evaluated 48 hours later. The S-4-yn-VPA enantiomer gave clear concentration dependent dysmorphology as well as effects on developmental score, somite number and head length. The primary anomalies were neural tube defects, rotational defects, as well as somite and heart effects. Embryolethality was observed only at the 1.2 mmol/l concentration. The R-4-yn-VPA enantiomer was neither embryotoxic nor dysmorphogenic at any concentration. The 50/50 mixture of R and S isomers appeared to be more embryolethal and dysmorphogenic than VPA. VPA significantly reduced all parameters and was dysmorphogenic at the highest concentration but was not embryolethal. The potency rank order for the four chemicals was S(−) > S(−)/R(+) > VPA ≫ R(+) and is comparable to that observed in mice by both in vivo and in vitro exposure. These data demonstrate that the stereoselective dyemorphology for these enantiomers as observed across species is not related to maternal metabolism.

862 FORMALDEHYDE EMBRYOTOXICITY IN MOUSE CONCEPTUSES GROWN IN WHOLE EMBRYO CULTURE

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Recent evidence shows methanol to be both embryotoxic and teratogenic. The metabolism of methanol to formaldehyde and formate has been well characterized in mature organ systems but has not been studied in the developing conceptus. As a reactive and short-lived intermediate, the potential of formaldehyde to be the cause of embryotoxicity has been discounted. Active biotransformation of methanol in the visceral yolk sac (VYS), however, may produce significant quantities of formaldehyde to result in embryotoxicity. Direct addition of formaldehyde to the culture medium containing gestational day 9 (GD 9) CD-1 mouse conceptuses on at doses between 0–10 μg/ml produced a dose-dependant loss of viability, an increase in abnormal axial rotation and decreased crown-rump length when assessed on GD 10. At formaldehyde concentrations of 1.0 and 5.0 μg/ml, however, embryonic (348.5 ± 74.3 and 314.2 ± 64.14 μg) and VYS (259.3 ± 55.3 and 220.7 ± 45.1) protein levels were actually increased significantly above control values by 20% (embryo = 288.2 ± 6.0 μg and VYS = 215.8 ± 4.0 μg). DNA levels in the embryo and VYS were also increased to a similar degree at these doses. Significant decreases in these parameters were seen only as a dose-dependant reduction of DNA levels in the VYS which declined by 47% and 55% at formaldehyde concentrations of 6.0 and 8.0 μg/ml, respectively. In comparison with the effects of direct addition of methanol, formic acid and sodium formate to the culture medium containing similar staged mouse embryos growing in whole embryo culture, formaldehyde appears to be capable of producing lethality and malformations at a thousand-fold lower concentration than observed for methanol, formic acid or sodium formate. It is reasonable to suppose that formaldehyde produced through embryonic biotransformation, even in small amounts, may contribute to the overall embryotoxicity of methanol in mice. This work was supported by EPA grant CR821964.

863 DYSMORPHOGENIC AND METABOLIC EFFECTS OF HYPOGLYCEMIA ON THE EMBRYONIC HEART

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Normal heart development requires glucose (glu) as a substrate for energy production and cellular proliferation. Hypoglycemia is a common clinical condition, particularly as a consequence of diabetic therapy, but its effect on cardiogenesis has not been well studied. The *in vitro* method of whole-embryo culture (WEC) was used to expose mouse embryos for 6 hr to 150(Cntl), 80, 40, or 20 mg/dl glu at 20-somite and 30-somite stages. Hypoglycemic medium was prepared with serum from rats injected IP with 50 IU insulin prior to blood collection and adjusted, as needed, with concentrated glu. Heart rate (HR) and vascular perfusion (VP) were measured after 2, 4, and 6 hr in WEC, and somite numbers and morphology of heart and pericardium were recorded after 6 hr. Hearts were then excised and incubated in 2-deoxy-D-(^{3}H)glucose for 30 min in WEC to determine glu uptake in response to hypoglycemia. In 20-somite embryos, HR was decreased at 2 and 4 hr by ≤40 mg/dl glu and at 6 hr by ≤80 mg/dl glu, whereas VP was decreased at 2, 4, and 6 hr by ≤80 mg/dl glu, compared to Cntls. Somite numbers and morphology of heart and pericardium were significantly affected at the 20-somite stage by glu levels of ≤80 mg/dl for 6 hr. In 30-somite embryos, HR was decreased at 2 and 4 hr by 20 mg/dl glu and at 6 hr by ≤40 mg/dl glu, whereas VP was decreased at 2 hr by 20 mg/dl glu and at 4 and 6 hr by ≤40 mg/dl glu. Somite numbers were decreased by 80 and 20 mg/dl glu, and morphology of heart and pericardium were affected by glu levels of ≤40 mg/dl for 6 hr. In 20-somite embryos, glu uptake was increased by exposure to 40 and 20 mg/dl glu compared to Cntls. In 30-somite embryos, glu uptake was increased by exposure to 80, 40 and 20 mg/dl glu compared to Cntls. Significance of these results is not currently understood but may represent a compensatory change in glu uptake by the embryonic heart in response to hypoglycemia. (Supported by American Heart Assoc. #NC-93-GS-37).

864 ADDITION OF GLUCOSE TO DEFINED CULTURE MEDIA FOR THE DEVELOPMENT OF *IN VITRO* PRODUCED BOVINE EMBRYOS AND ITS EFFECTS ON EMBRYO SURVIVAL

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Three variations of a defined medium (DCM) were investigated in an effort to improve blastocyst yield over that of a TCM-199 control medium. Oocytes obtained from ovaries at slaughter were cultured in maturation medium (TCM-199 + estrus cow serum (ECS), pyruvate, estradiol, FSH and LH) for 20h, fertilized with 1 × 10^6 swim-up selected spermatazoa per ml for 8h, and randomly allocated to the following development media: (1) DCM (CR1aa (Rosenkrans et al 1990) + 0.11 mM EDTA + 10 ng/ml EGF, n = 100); (2) DCM + 5.6 mM glucose added at 72 h post-insemination (hpi) (DCM72); n = 107); (3) DCM + 5.6 mM glucose added at 120hpi (DCM120), n = 102); or (4) control (TCM-199 + 10% ECS (CC), n = 73). Embryos were staged at 24h intervals throughout the 168h development period. At termination of culture on d7, blastocysts were assigned a quality grade (1 = good, 2 = fair, 3 = poor). Four replicates were performed. Kaplan-Meier and Cox Proportional Hazards Regression methodology was employed to compare dynamics of embryonic mortality throughout the culture period. Percent oocytes cleaved, percent blastocysts developed, and percent grade 1 blastocysts were analyzed using Freidman 2-Way ANOVA and Multiple Comparisons Procedure. Percent oocytes cleaved did not differ between treatments (overall = 83%, p = 0.55). Percent blastocysts developed was highest for DCM72 (23.4%, p = 0.013). Percent blastocysts in DCM72 differed significantly from DCM and DCM120 ($p < 0.05$), but not from CC ($p > 0.15$). Percent grade 1 blastocysts did not differ between treatments (p = 0.22). Survival probability and hazard ratios did not differ between treatments (p = 0.735 and 0.705, respectively). Greatest losses of embryos occurred between 48–96hpi. From these data, it can be concluded that the defined medium, DCM72, is an effective alternative to the undefined medium, CC, for the production of bovine blastocysts. (Supported by NCARS).

865 1,3-BIS(2-CHLOROETHYL)1-NITROSOUREA (BCNU) INHIBITION OF GLUTATHIONE DISULFIDE REDUCTASE POTENTIATES THE *IN VITRO* EMBRYOTOXICITY OF 2-NITROSOFLUORENE

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Intracellular glutathione (GSH) is hypothesized to protect against 2-nitrosofluorene (NOF) induced flexure defects in gestational day 10 (GD10) rat conceptuses *in vitro* via formation of GSH adducts and 2-aminofluorene (AF). A reaction scheme proposed by Mülder et al (Chem.-Biol. Interact., 39, 111–127, 1982) suggests that two GSH-dependent oxidation steps are necessary to convert NOF to the less toxic metabolite, AF. In this study, the GSSG-reductase inhibitor BCNU was utilized in a whole embryo culture system to inhibit reduction of oxidized GSH. Following a 2 hour pre-treatment with 25 μM BCNU, GD10 rat conceptuses were treated with 25 μM or 50 μM NOF and cultured for 24 hr. Morphological assessment and biochemical analysis of vehicle, BCNU, NOF, and NOF + BCNU treated groups was then completed. Total GSH levels were measured in embryo and visceral yolk sac in all groups during the first 5 hr of NOF treatment following BCNU. Inhibition of GSSG-Rd increased the incidence of flexure defects in the 50 μM NOF treatment group from 46% to 74% and decreased crown-rump length from 3.2 ± 0.1 to 3.0 ± 0.1. BCNU + 25 μM NOF exposure did not affect the incidence of flexure defects or crown-rump length. The BCNU pre-treatment increased visceral yolk sac protein and decreased embryonic DNA in both 25 and 50 μm NOF treatment groups relative to NOF alone. Embryonic GSH levels decreased in the BCNU + 50 μM NOF treatment group beginning at 3 hr after addition of NOF to the culture media and continued to decrease through the next 2 hr of the 5 hr sampling period. Embryonic cysteine levels ware lower in the BCNU + 50 μM NOF group when compared to controls. Embryonic

GSH concentrations decreased sharply in the BCNU + 25 μM NOF group at 3 hr but returned rapidly to control levels by 4 hr. These results indicate that BCNU potentiates the embryotoxic effects of 50 μM NOF, due to irreversible GSH oxidation, efflux, and a consequent decrease in AF formation. The transient decrease in embryonic GSH followed by rapid recovery in the BCNU + 25 μM NOF group may indicate that this dose was not sufficient to overwhelm GSH reduction capacity. Supported by NIH grants ES07062 and ES05235.

866 IDENTIFICATION OF PROXIMATE TOXICANT FOR ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY USING RAT WHOLE EMBRYO CULTURE

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Previous studies have shown that the onset of ethylene glycol (EG)-induced developmental toxicity corresponds with a dose-dependent shift in EG metabolism, leading to accumulation of a weak acid metabolite, glycolic acid (GA), and a resultant metabolic acidosis. To determine if GA accumulation is responsible for developmental toxicity, the effects of GA vs. parent EG were compared in whole embryo culture. Day 10.5 rat embryos were cultured for 46 h in media containing 0.5, 2.5, 12.5, 25 or 50 mM EG (all pH 7.5) or with GA at these same concentrations (pH 7.5–4.3). These concentrations reflected peak maternal plasma levels observed at a NOEL dose (11 mM EG, < 2 mM GA), LOEL dose (21 mM EG, 2 mM GA) or dose causing malformations (43 mM EG, 13 mM GA). EG up to 50 mM and GA at $\leq$ 2.5 mM were essentially without effect, while 12.5 mM GA significantly inhibited embryo growth, protein content and morphology score. Higher GA levels were lethal. Dysmorphogenesis, mainly of the craniofacial region, was found in 70% of the 12.5 mM GA embryos vs. 0% in controls. To determine the role of reduced pH in vitro, a second experiment was conducted in which embryos were cultured in 12.5 mM GA (pH 6.7), 12.5 mM sodium glycolate (pH 7.4) or in control medium (pH 7.4 or 6.7). Glycolate at pH 7.4 and control medium at pH 6.7 affected growth and development, although to a lesser extent than GA at pH 6.7. These results support the hypothesis that EG developmental toxicity requires a shift in metabolism leading to high levels of GA, which then acts both intrinsically and via induction of metabolic acidosis. Sponsored by the EG Panel of the Chemical Manufacturer's Association.

867 EMBRYOTOXICITY OF METHANOL, FORMALDEHYDE, AND SODIUM FORMATE IN THE RAT CONCEPTUS *IN VITRO*: EXPOSURE BY DIRECT INTRAAMNIOTIC MICROINJECTION

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Microinjection of chemical agents directly into the amniotic fluid (AF) has been shown to be an effective means of experimentally bypassing the visceral yolk sac (VYS) and facilitating direct initial exposure of the embryo proper. In order to evaluate the role of VYS biotransformation in methanol embryotoxicity we have microinjected methanol and two major metabolites, formaldehyde and formate, into the AF of gestational day 10 rat conceptuses and made assessments for altered growth and malformations. Formaldehyde was found to be the most toxic of all compounds tested, producing decreases in viability to 42% and 8% of controls following microinjections of 1.5 and 3.0 μg of formaldehyde, respectively. Sodium formate elicited a similar decrease in viability beginning at a dose of 5.0 μg. Low doses of formaldehyde (0.2–0.5 μg) resulted in an apparent increase in embryonic protein (358.6 $\pm$ 13.3 μg for formaldehyde and 286.4 $\pm$ 14.3 μg for controls) and crown rump length (3.5 $\pm$ 0.2 mm for formaldehyde and 3.37 $\pm$ 0.2 mm for controls), but these parameters decreased significantly below 50% of control values at higher doses. In comparison, microinjection of methanol at doses in excess of 175 μg failed to reduce viability below 80% or cause embryonic protein to be reduced by more than 20%, although significant increases in abnormal axial rotation and decreases in crown rump length were observed. Higher doses of methanol were not possible due to severe distension of the amnion. Calculated toxic concentrations of methanol, formaldehyde and sodium formate, based on an estimated volume of intraamniotic fluid of 500 nl, produced final concentrations in the AF of 350, 4, and 10 mg/ml, respectively. These results indicate that the metabolites of methanol produce embryotoxicity at concentrations similar to those reported to elicit similar effects when added directly to the culture medium. The 100-fold excess of methanol required to elicit any similar effects suggests that this compound requires significant VYS metabolism in order to produce embryotoxicity. This work was supported by EPA grant CR821964.

868 EFFECTS OF ALKYLATING AGENTS ON GROWTH AND DIFFERENTIATION OF P19 EMBRYONAL CARCINOMA CELLS: MODULATION OF TOXICITY BY O^6-BENZYLGUANINE

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Effects of alkylating agents (AA) on growth and differentiation of P19 embryonal carcinoma cells were determined. P19 cells are pluripotent murine embryonal carcinoma cells which can be induced by RA to differentiate into cells which are biochemically and morphologically similar to CNS cells. The AAs tested, methylnitrosourea (MNU), ethylnitrosourea (ENU), methylmethanesulfonate (MMS), and ethylmethanesulfonate (EMS), have produced developmental toxicity following either *in vivo* or *in vitro* exposure. Relative potency previously determined in primary cultures of differentiating rat embryo midbrain (MB) cells was MMS > MNU > ENU > EMS. In P19 cells, effects on growth were assessed by a neutral red dye assay, and differentiation was assessed following uptake of ^{3}H-γ-amino butyric acid. Concentration dependent effects on viability and differentiation were observed, with inhibition of differentiation occurring at significantly lower concentrations than effects on viability. With the exception of ENU, concentrations which caused effects on growth and differentiation in P19 cells were comparable to those causing similar effects in micromass MB cells, suggesting that this established cell line may be useful for studying mechanisms of developmental toxicity. Inhibition of O^6-alkylguanine-DNA-alkyltransferase (AT) by O^6-benzylguanine (O^6-Bg), a potent and specific inhibitor of AT, had a much greater effect on the toxicity of methylating as compared with ethylating agents. Since AA induced effects on viability and differentiation were both impacted by O^6-Bg exposure, these observations suggest that O^6-alkGua adducts can contribute to cytotoxicity and inhibition of differentiation. Our results also suggest that O^6-alkGua lesions may be more deleterious for processes involved in cell differentiation than for those involved in cell viability. NIH ES-03157 and ES-07032.

869 PRELIMINARY CHARACTERIZATION OF TOTAL PEROXIDASE (Px) AND GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD) ACTIVITIES IN HUMAN AMNIOCYTES

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Amniocytes are embryonically-derived cells that may reflect the genetic and enzymatic determinants of individual fetal susceptibility to teratogenic drugs and/or environmental chemicals. This study evaluated human amniocytes from different fetuses to determine the activities and intraindividual variability in the respective potential xenobiotic bioactivating and cytoprotective enzymes Px and G6PD. Amniocytes were homogenized in 0.1 M sucrose buffer. Px activity was determined based on the enzyme-catalysed reduction of 5-phenyl-4-pentenyl-hydroperoxide to its alcohol product 5-phenyl-4-pentenyl alcohol with the reducing cosubstrate phenol. G6PD activity was determined spectrophotometrically using a standard assay kit. Baseline activity of both enzymes was evident in amniocytes from all fetuses, with respective variations of 2-fold and over 10-fold in Px and G6PD activities. To our knowledge this is the first direct evidence for baseline enzymatic activity and variability for Px and G6PD activities in human amniocytes, which may reflect fetal activities. Such variability may contribute to individual teratological susceptibility following *in utero* exposure to xenobiotics. (Support: Medical Research Council of Canada).

870 CONTINUOUS INTRAVENOUS INFUSION OF RATS FOR FERTILITY STUDIES TO INTERNATIONAL CONFERENCE ON HARMONISATION (ICH) GUIDELINES

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Sponsor: B Broxup

Continuous intravenous infusion in animals via an indwelling catheter is often used in preclinical safety evaluations studies to mimic the proposed clinical route and treatment regimen. The feasibility of using this route for fertility and early embryo development studies to meet the ICH guidelines was established in both males and females and subsequently definitive studies were performed.

Medical grade silastic catheters were surgically implanted under general anesthesia in the vena cava via the femoral vein 1 week prior to the initiation of treatment. Animals were continuously infused, using a jacket and tether

system, with saline at a rate of 0.4 ml/hr prior to treatment and at rates of 1.5 to 2.5 ml/kg/hr during treatment. Males were treated for 28 days prior to mating, during mating and until necropsy. Females were treated for 14 days prior to mating, during mating and up to day 7 of gestation, after which the catheters were tied off and inserted subcutaneously and the jacket and tether system removed. The treated animals were placed with untreated partners for mating. The females (treated and untreated) were given a cesarean section on day 20 of gestation and the ovaries and uterine contents examined. Male reproductive assessments (epididymal evaluations and testicular pathology) were performed at the necropsy examination. Comparison of the reproductive parameters of the continuously infused males or females to concurrent noncatheterized animals and/or historical control data showed no differences for mean day to mating, mating and fertility indices or conception rates. Similarly, male reproductive assessments were unaffected.

It was concluded that the presence of an indwelling catheter, jacket and tether system did not adversely effect fertility in male and female rats.

871 VALIDATION OF THE ALTERNATIVE REPRODUCTIVE TEST PROTOCOL (ART) TO ASSESS TOXICITY OF METHOXYCHLOR IN RATS

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The study was designed to assess reproductive effects of methoxychlor(M), an estrogenic pesticide, in male and female F0 and F1 Long-Evans rats using the ART protocol. M was given daily at 0, 50, or 200 mg/kg/day to F0 male and female rats, starting at 3 weeks of age (WA) and continuing for 14 weeks in males and through gestation and lactation in females (18 weeks). Other males, treated at 0 and 200 mg/kg/day, were fitted with intra-atrial cannulae and challenged with GnRH or hCG at 14 WA. In F0 males, preputial separation was delayed, occurring at postnatal day (PND) 41, 45 and 49 for 0, 50, and 200 mg/kg dose groups. At 16 WA, F0 males were paired with estrogen-primed, virgin females. While mounting behavior was normal, ejaculated sperm counts were decreased. Epididymides, ventral prostate, seminal vesicle weights, and caudal epididymal sperm counts were reduced ($p < 0.05$) at both doses. In challenged males, M suppressed both GnRH and hCG-stimulated testosterone; LH was significantly higher ($p < 0.05$) at 15 min. after GnRH. In F0 females, vaginal opening was accelerated occurring at PND 29, 26, and 25 at 0, 50, and 200 mg/kg/day, respectively. When females at 200 mg/kg/day were mated to non-treated males, no conceptions occurred. At necropsy (21 WA), uterine (200 mg/kg) and ovarian (50 and 200 mg/kg) weights were reduced ($p < 0.05$). At 10 WA, untreated F1 offspring were bred within dose group for 12 weeks. F1 females from 50 mg/kg/day F0 dams produced fewer litters and had reduced litter sizes ($p < 0.05$). Thus, M altered male and female reproductive parameters. These results are similar to Gray et al. (FAAT 12: 92–108, 1989) using the ART protocol. (CR820335-01-0; conclusions do not reflect EPA policy).

872 EXPOSURE CONCENTRATION AND TIME (CxT) RELATIONSHIPS IN THE DEVELOPMENTAL TOXICITY OF METHANOL IN MICE

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Inhaled methanol is developmentally toxic in pregnant mice exposed for ten days to 2,000 ppm or above (7 hr/d on gestation days [GD] 6–15). Effects in fetuses include increased incidence of cervical ribs (2,000 ppm), cleft palate and exencephaly (5,000 ppm), and decreased fetal weight and resorptions (10,000 ppm). Single-day exposures (7 hr @ 10,000 ppm) indicate that GD 7 is the most sensitive day for these effects. In this study, pregnant CD-1 mice on GD 7 were exposed to methanol by inhalation at different concentrations (C; 0, 2, 3, 5, 10 or 15 × 10^3 ppm) and durations (T; 1, 2, 3, 5, 7 hr). Maternal blood was collected from some animals immediately after exposure and remaining animals were killed on GD 17 to assess fetal outcome. Maternal blood methanol, assayed by GC, increased with the product of CxT to a mean of 4966 μg/ml for a 7 hr exposure @ 15,000 ppm (CxT = 105,000 ppm · hr). For exposures with like CxT, blood methanol levels were higher with shorter duration, higher concentration exposures. Embryo/fetal death, cleft palate and multiple skeletal defects were significantly increased at CxT $\geq$ 70,000 ppm · hr. The most sensitive endpoint was induction of cervical rib, which occurred in groups with CxT $\geq$ 15,000 ppm · hr (3 hr @ 5,000 ppm or 1 hr @ 15,000 ppm). Developmental toxicity correlated well with peak maternal blood methanol concentration, but further work is needed to compare correlation with area under the blood concentration × time curve.

This abstract does not represent EPA policy.

873 COMPARISON OF NICOTINIC RECEPTOR BINDING AND BIOTRANSFORMATION OF CONIINE IN THE RAT AND CHICK

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Coniine, an alkaloid from *Conium maculatum* (poison hemlock), is a known teratogen in many domestic species with maternal ingestion resulting in arthrogryposis of the offspring. We have previously shown that rats are not susceptible and rabbits only weakly susceptible to coniine-induced arthrogryposis. However, the chick embryo does provide a reproducible laboratory animal model of coniine-induced teratogenesis. The reason for this cross-species variation is unknown. The purpose of this study was to evaluate coniine binding to nicotinic receptors and to measure coniine metabolism in vitro between susceptible and nonsusceptible species. Using the chick model, neither the peripheral nicotinic receptor antagonist d-tubocurarine chloride nor the central nicotinic receptor antagonist trimethaphan camsylate blocked the teratogenesis or lethality of 1.5% coniine (50 μl/egg). Trimethaphan camsylate enhanced coniine-induced lethality in a dose dependent manner. Neither nicotinic receptor blocker prevented nicotine sulfate-induced malformations but d-tubocurarine chloride did block lethality in a dose-dependent manner. Coniine binding to nicotinic receptors isolated from rat diaphragm and brain, fetal rat brain, and chick leg muscle and brain was also assessed. The IC_{50} for coniine binding in rat diaphragm was 314 μM while that for chick leg muscle was 70 μM. Also, [^{125}I]-α-bungarotoxin binding alone was low in rat fetal thigh tissue. For central nicotinic receptors, the IC_{50}'s for maternal rat brain, fetal rat brain, and chick brain were 1.1 mM, 0.82 mM, and 0.27 mM, respectively. There were no differences in coniine biotransformation in vitro by microsomes from rat or chick livers. Differences in receptor affinity and/or quantity between the rat and chick may explain, in part, the differences in susceptibility of coniine-induced teratogenesis between these two species.

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874 TOXICOKINETICS OF 2-METHOXYACETIC ACID IN PREGNANT MICE FOLLOWING 2-METHOXYETHANOL INHALATION EXPOSURE

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A 1993 proposal by the Occupational Safety and Health Administration (OSHA) would drastically reduce the permissible exposure levels to certain ethylene glycol ethers known to be reproductive and developmental toxicants in laboratory animals. 2-Methoxyethanol (2-ME) is among the targeted chemicals, and inhalation is the suspected route of human occupational exposure. No inhalation pharmacokinetic data from animals exist to support the new regulatory standard. In designing the present studies, an inhalation concentration of 2-ME that was expected to produce teratogenic maternal plasma (MP) and embryo (E) levels of 2-methoxyacetic acid (2-MAA) was extrapolated from the PBPK gestation model developed at CIIT. 2-MAA is the oxidation product of 2-ME and the putative proximate toxicant. On gestation day (GD) 11, mice inhaled the TLV (5 ppm) for up to 8 hr or 500 ppm for up to 6 hr. In another exposure design, mice first inhaled the TLV for 4 hr, followed by 30 min at 500 ppm. Levels of 2-MAA in MP, E and extraembryonic fluids (EAF) were determined at various times during and after 2-ME inhalation. Developmental toxicity and digit malformations were assessed on GD 18 in fetuses from other 2-ME exposed dams. At 5 ppm, the 8 hr 2-MAA levels were between 30 and 70 μM, while 6 hr at 500 ppm produced 2-MAA concentrations of >8 mM in each of the three compartments that were analyzed. The latter 2-MAA values exceeded the PBPK model predictions following 2-ME inhalation. In the combination exposure, 2-MAA levels at the end of the 30 min burst were 325–525 μM, with a peak of 680 μM in EAF 3 hr later. In the 5-ppm-only study, 2-MAA concentrations reached 30–70 μM. On GD 18, developmental toxicity, including malformations, occurred exclusively in mice that had inhaled 500 ppm 2-ME for 6 hr. All litters (n = 10) and all fetuses were affected. In contrast, neither the 5 ppm only nor the 30 min exposure to 500 ppm of 2-ME on top of a continuous inhalation of 5 ppm had any detectable adverse developmental effects in near-term fetuses.

875 RHESUS MONKEYS NEONATAL MODEL

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Development of a neonatal non-human primate model allows for testing the

toxic activity of compounds, or the transmission of infectious agents (test articles) via intra-uterine, para-parturient or lactational means. While delivery of the neonate via cesarian section provides a controlled experimental model, it also requires special care to facilitate successful acceptance of the neonate by the dam post delivery, and experienced human intervention to increase survivability if the neonate is rejected. Mason Laboratories has established a neonate model in rhesus monkeys which is used routinely to assess the transmission of infectious agents, developed several techniques to increase the chance of maternal acceptance, and established a rigorous hand-rearing program for rejected infants. A cesarian section is performed at 155 to 160 days of gestation. The techniques used to increase the chance of acceptance include scenting the neonate with the dam's blood and peritoneal fluids obtained from surgery prior to introduction; alleviating the dam's post-operative discomfort with analgesics; ensuring a quiet and familiar environment; and allowing the bonding process to occur with little interruption. In the event that the neonate is not accepted an extensive hand-rearing feeding schedule begins, which includes, monitoring clinical condition by body weight, fecal and urine output, activity level, and general well-being. To date these techniques have resulted in 60% acceptance rate of neonates. Out of the 40% rejected, we have successfully hand-reared 60% of the infants. We have concluded that a variety of methods help ensure a high rate of maternal acceptance providing for a successful model to study the role of various routes of transmission of infectious agents or compounds with potential toxic effects in neonates.

876 REPRODUCTIVE SAFETY EVALUATION OF PACLITAXEL IN RATS

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Paclitaxel, a new anticancer agent used primarily in the treatment of ovarian cancer, was evaluated in a series of three reproductive safety studies using intravenous administration in rats. In a Segment I fertility and general reproduction study, paclitaxel was given to male rats for 63 days and to female rats for 14 days before mating, at doses of 0, 0.1, 0.3, or 1.0 mg/kg/day. Daily dosing continued until scheduled sacrifice after completion of the mating period in males or gestation day 7 in females; females were sacrificed and cesarean-sectioned on gestation day 20. Although the mating index was unaffected at all doses tested, decreased fertility occurred at 1.0 mg/kg. In male rats, body-weight gains and food consumption were decreased at 1.0 mg/kg, resulting in a no-observable-effect level (NOEL) of 0.3 mg/kg. In female rats, 1.0 mg/kg decreased body-weight gains and food consumption, as well as adrenal, uterine, and ovarian weights. Additional effects at 1.0 mg/kg included decreased corpora lutea, implantations, and live litter sizes, as well as increased resorptions, resulting in maternal and developmental NOELs of 0.3 mg/kg. In a Segment II teratology study with a postnatal phase, pregnant rats were given doses of 0, 0.15, 0.3, or 0.6 mg/kg on gestation days 7 through 17. There were no effects on any maternal parameters, and no changes in the offspring, other than delayed hair growth at 0.6 mg/kg (maternal and developmental NOELs of 0.6 and 0.3 mg/kg, respectively). In a Segment III perinatal and postnatal study, doses of 0, 0.1, 0.3, or 1.0 mg/kg were given to pregnant/lactating rats on gestation day 17 through lactation day 21. Maternal body weights, food consumption, and thymus, heart, and uterus weights were decreased at 1.0 mg/kg. Effects in the offspring also occurred at 1.0 mg/kg and included delayed hair growth, incisor eruption, and testes descent, as well as prolonged latencies in an olfactory-choice task, decreased body weights, and decreased spleen and liver weights (maternal and developmental NOELs of 0.3 mg/kg).

877 TWO-GENERATION REPRODUCTION STUDY WITH GLUTARALDEHYDE (GA) IN THE DRINKING WATER OF CD® RATS

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Male and female CD® rats (F0) were given GA (CAS #111-30-8) in the drinking water at concentrations of 0, 50, 250, or 1000 ppm for a 10-week prebreed period and through mating, gestation and lactation (females only). Resulting offspring (F1) continued on the same treatment regimen as their parents (from prebreed through lactation). Twenty-eight animals/sex/generation were evaluated in each dose group. Evaluations for parental (F0 and F1) animals included measurements of body weight, food and water consumption and clinical observations. Offspring evaluations included survival indices and body weight measurements from birth through weaning. All F0 and F1 animals were necropsied and select tissues were examined microscopically. Ten offspring/sex/ group/generation were necropsied at weaning. Treatment with GA resulted in aversion to the drinking water as evidenced by consistent reductions in water consumption in both generations throughout the prebreed (both sexes) and gestational periods at 250 and 1000 ppm. Slightly reduced water consumption was seen in the 50 ppm group during gestation only. Food consumption was slightly reduced at 1000 ppm in F0 and F1 parents during the prebreed periods and during the last week of gestation for F0 and F1 females. There were no effects on parental fertility and mating performance or on pup viability and litter size in any generation. At 1000 ppm, average litter weight was reduced from Days 21–28 postpartum in the F1 offspring and from Days 14–28 in the second generation of offspring (F2). No treatment-related lesions were noted at necropsy of parental or weanling animals. The NOEL for adult toxicity in this study was 50 ppm; the NOEL for offspring was 250 ppm. The NOEL for reproductive effects was >1000 ppm.

878 REPRODUCTIVE EFFECTS OF ETHOXYQUIN IN S-D RATS ASSESSED BY A CONTINUOUS BREEDING PROTOCOL

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The potential reproductive toxicity of ethoxyquin in Sprague-Dawley rats was evaluated using the Reproductive Assessment by Continuous Breeding Protocol (NTP, 1989). Ethoxyquin was nominated for testing due to the relative lack of reproductive toxicity information; it is used in both agriculture and industry. Ethoxyquin in corn oil was administered by oral gavage at doses of 0, 75, 150, and 300 mg/kg/day to 20 animals/sex/group. Rats were individually housed for one week and then cohabitated in breeding pairs for 16 weeks. During cohabitation, litters were euthanized after evaluation on Postnatal Day 1 (PND). Litters born (F_1) after Week 17 were reared by the dam until weaning (PND 21); selected weanlings were reared in same sex groups until PND 81 ± 10. At PND 81 ± 10, F_1 animals were cohabitated within groups for up to seven days and necropsied after delivery of litters. Fertility of F_0 rats was reduced at 300 mg/kg, manifested as decreased litters per pair (22%), live pups per litter (22%), and increased cumulative days to litter (8% to 17%). At the crossover mating to determine the affected sex, pups born to treated females weighed approximately 20% less than controls; no effects were observed in litters from ethoxyquin-treated males. Body weights of high-dose F_0 and F_1 males were 7–20% less than controls throughout the study. In F_0 and F_1 animals of all treated groups liver and kidney weights were increased (13% to 56%) with increasing dose. No effects of ethoxyquin exposure were observed in F_1 breeding pairs. In conclusion, these data indicate that ethoxyquin had renal and hepatic effects at ≥75 mg/kg/day and was a female reproductive toxicant in Sprague-Dawley rats at 300 mg/kg/day, based on increased cumulative days to litter and decreased live pup weight during the crossover mating.

879 WHAT CAN BE LEARNED FROM THE MOUSE REPRODUCTIVE ASSESSMENT BY CONTINUOUS BREEDING DATABASE?

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The National Toxicology Program uses the RACB design as a definitive 2-generation test of reproductive toxicity. Data from 82 RACB studies using Swiss CD-1 mice were evaluated for relationships between endpoints. Some of the pertinent findings were: (1) Using data from treated and control mice, a significant association was found between epididymal sperm count and # live pups: greater sperm counts correlate with more pups/litter up through 9×10^5 sperm/mg cauda, well into the control range. This means that, for a *population*, even small decreases in sperm count can lead to decreased fertility. This differs from the *individual*, wherein a ≅90% reduction in sperm number must occur to decrease an individual's fertility. (2) Sperm motility was a strong predictor of # pups/litter only when motility dropped to <50% of control. There was a correlation between sperm motility and count. (3) More compounds were reproductive toxicants in females (58 of 60 studies) than in males (37/60). A significant number (23/60) were reproductive toxicants *only* in females; just 2 were toxic only to males. (4) In treated F_0 mice, a longer estrous cycle correlated with fewer pups/litter; this correlation did not hold for F_1. However, (5) 30–40% of the tested compounds produced significant changes in the F_1 reproductive organ weights or indices *without* similar changes in the F_0's; these compounds appeared more toxic if given during gestation/maturation. (6) In F_0 controls, dam weight correlated to # live pups. This association was strongest for the first litter and weakest for the last. These examples highlight some of the associations that can be found in this large and accessible database.

880 REPRODUCTIVE EFFECTS OF DICYCLOPENTADIENE IN S-D RATS ASSESSED BY A CONTINUOUS BREEDING PROTOCOL

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Dicyclopentadiene (DCP), was evaluated for reproductive toxicity in Sprague-Dawley rats using the Reproductive Assessment by Continuous Breeding Protocol (NTP, 1989). DCP is used in pesticides, fuel additives and adhesives and was selected for study because of its presence in the environment and lack of reproductive toxicity data. DCP was administered by gavage in corn oil at dose levels of 10, 30, and 100 mg/kg to animals that were housed individually for one week and then cohabitated for 16 weeks (20 animals/sex/group). Newborn litters were euthanized after evaluation on postnatal day (PND) 1. Litters born after Week 17 were reared until PND 21 and selected weanlings were administered the same dose levels as their respective parents. On PND 81 ± 10, F_1 animals were cohabitated within groups for one week and necropsied following delivery of the litters. Reproductive toxicity was observed in the 100 mg/kg group females: 28% fewer F_1 pups born live, 8% lower adjusted live F_1 pup weights, higher F_1 pup mortality, increased cumulative days to litter, and decreased F_1 pup survival in the final litter. At 30 mg/kg there was a 4% decrease in female pup weight. At the crossover mating, pup weight was reduced (9%), in the DCP-treated females, while no effects were observed in litters from DCP-treated males. At necropsy, DCP caused a 2%, 7%, and 17% increase in liver weights and a 16%, 15%, and 16% increase in kidney weights in males from the 10, 30 and 100 mg/kg groups, respectively. Microscopically, an increase in the incidence of clear cell foci was observed in the livers of 30 and 100 mg/kg rats. In the second generation, DCP at 100 mg/kg caused a 12% reduction in F_2 pup weight in the presence of increased F_1 liver and kidney weights. The reproductive effects of DCP were not greater than those observed in the first generation. Thus, DCP is a reproductive toxicant, but not selectively so, as there were systemic toxicities at and below reproductively toxic dose levels.

881 INHIBITION OF STEROID GLUCOSYLTRANSFERASE ACTIVITY BY PENTACHLOROPHENOL AT CONCENTRATIONS THAT REDUCE SURVIVAL AND FECUNDITY OF DAPHNIA MAGNA

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Alterations in steroid metabolism by environmental endocrine disrupters can significantly affect steroid hormone-dependent processes such as growth and reproduction. Exposure to the fungicide pentachlorophenol (PCP) has been shown to elicit a variety of endocrine-related adverse effects. The present study was undertaken to establish whether concentrations of PCP that adversely affect survival, growth, or reproduction of *Daphnia magna,* during chronic exposure, also elicit changes in steroid hormone metabolism. Survival or reproduction of daphnids was significantly reduced from exposure to 1.0, 0.5 and 0.25 mg/L PCP. Following chronic exposure to PCP, daphnids were incubated with [^{14}C]testosterone and the testosterone metabolic profile was assessed. The rate of phase I hydroxy metabolites formed was not significantly different from controls. However, phase II steroid glucosyltransferase activity decreased in a PCP concentration-dependent manner for three of the metabolites produced. This effect occurred at PCP concentrations that reduced survival or reproduction. These results demonstrate that PCP alters steroid biotransformation activities at concentrations that affect survival and reproduction. Thus, this biochemical parameter may serve as a biomarker of chronic toxicity associated with PCP.

882 COMPARATIVE DEVELOPMENTAL TOXICITY OF 2-METHOXYETHANOL (2ME), METHOXYACETIC ACID (MAA) AND METHOXYACETALDEHYDE (MALD) IN *DROSOPHILA*

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To assess the developmental toxicity of selected glycol ethers and their metabolites, *Drosophila melanogaster* larvae were reared on media containing equimolar concentrations of 2ME or its acid metabolite MAA (both documented mammalian developmental toxicants), or its acetaldehyde metabolite MALD. *Drosophila* were exposed throughout development (egg through third instar larvae) to 0.085 mM, 0.17 mM or 0.34 mM of each chemical in culture vials, with each vial containing 1 g of powdered medium and 5 ml of distilled water or a solution of test chemical in water. The highest concentration corresponded to 26 mg/vial of 2ME, a concentration previously shown in our laboratory to increase the incidence of bent humeral bristles in developing flies. A mated, untreated, Oregon-R wild-type female (Mid-American *Drosophila* Stock Center) was added to each culture vial and allowed to oviposit for 20 hours, then removed. Emerging adults, collected over 10 days, were examined microscopically (25x) for bent humeral bristles or wing blade notches — morphological endpoints shown to occur with an increased incidence in flies exposed to developmental toxicants. All chemical treatments statistically increased the incidence of bent bristles compared to concurrent controls (chi-square). The percentage of flies with bent humeral bristles observed with each chemical and at each concentration were: 2ME, 4.1, 9.0 and 11.1%; MAA, 7.7, 7.0 and 12.5%; MALD, 8.8, 6.9 and 15.3%. No bristle defects were seen in controls (n = 242). These results indicate that exposure to 2ME or its aldehyde or acid metabolites can induce defects in developing flies and support further utilization of this *Drosophila*-based assay as a pre-screen in developmental toxicology studies.

883 EFFECTS OF HYPERVITAMINOSIS A ON GAMETOGENESIS IN THE NEMATODE *CAENORHABDITIS ELEGANS*

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Vitamin A (retinol) is required for normal growth, development and reproduction, thus, it is an essential nutrient in the diet of most organisms. Being fat-soluble, retinol is primarily stored in the liver. Although required for survival, excess amounts of retinol result in the pathological condition of hypervitaminosis A, which is characterized in *somatic* tissues by edema, weight and hair loss, fatigue, skeletal abnormalities, headaches and death. Much less is known about the effects of hypervitaminosis on gametogenesis, primarily during pachytene of meiosis prophase I, when homologous chromosomes are in the process of pairing. Results of the present study identified decreases in viability and fecundity, and changes in ultrastructural morphology of the gonad and nuclei through the F_3 generation. Such changes correlated with increased concentrations of retinol in the growth medium. Synaptonemal complexes were not present in nematodes treated with high retinol concentrations (81, 243 and 729 μg/ml) although had normal ultrastructure at lower concentrations.

884 DEVELOPMENTAL EFFECTS OF LOW-FREQUENCY MAGNETIC FIELDS IN MICE

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Sponsor: K Savolainen

The possible adverse effects on pregnancy of low-frequency magnetic fields caused by alternating current have been under concern for some time. In most studies, no or only slight effects on fetal development have been observed in mammals exposed to low frequency magnetic fields. In CBA/S mice increased incidence of resorptions have been observed. The present study attempted to replicate the earlier results in CBA/S mice and study further the effects of magnetic field on pregnancy and embryonic development in mice. The pregnant CBA/S mice were exposed continuously to a 20-kHz sawtooth magnetic field from day 0 to day 18 of pregnancy for 24 h/day until necropsied on day 18. Control animals were under similar conditions without magnetic field. The magnetic field strength was 12 A/m peak to peak (flux density 15 μT). On gestation day 18 the uterine contents, ovaries and very early resorptions were examined. Maternal blood samples were collected for determination of hematological parameters. In other experiments the development of fertilized mouse eggs exposed to 50-Hz sinusoidal magnetic field (field strength 10 A/m r.m.s) were studied. Slight increase of resorptions were observed, otherwise results were negative. Supported by the Swedish Work Environment Fund.

885 EVALUATION OF THE SUBCHRONIC, DEVELOPMENTAL, AND REPRODUCTIVE TOXICITY OF 60 Hz MAGNETIC FIELDS

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A series of four *in vivo* studies was performed to evaluate the possible effects of exposure to power frequency magnetic fields (EMF) on overall health status, fetal development, and reproductive function in rodents. In all studies, animals were exposed continuously for 18.5 hrs/day to pure sinusoidal 60 Hz magnetic fields of 10 G, 2 G, 20 mG, or 0 G (control), or intermittently (1 hr on, 1 hr off) to 10 G fields. In the subchronic toxicity study, F344 rats and B6C3F1 mice (10/sex/group) were exposed to EMF for 8 weeks; survival, body

weight, clinical signs, hematology, serum chemistry, gross pathology, organ weights, and histopathology (50 tissues/animal) were evaluated. In the teratology study, timed-pregnant Sprague-Dawley rats (55/group) were exposed to EMF from gestation days 6 through 19. After fetal delivery by caesarian section on day 20, litter size, litter weight, % live births, and number of resorption sites were determined. All fetuses (>3,000) underwent gross external, visceral, and detailed skeletal examinations. The multi-generation reproductive toxicity study employed the Reproductive Assessment by Continuous Breeding protocol; this study included 40 breeding pairs of Sprague-Dawley rats per group. Magnetic field exposures induced no mortality or clinical evidence of toxicity in any group in any study. Positive findings in the subchronic toxicity studies were limited to mild reticulocytosis in male rats exposed intermittently to 10 G fields; all other clinical parameters were normal, as were histopathology findings in both species. EMF exposure had no effect on fetal development or reproductive function in rats. These data indicate that continuous or intermittent (1 hour on, 1 hour off) exposure to pure (transient-free) 60 Hz magnetic field strengths of up to 10 G induces no significant subchronic, developmental, or reproductive toxicity in standard animal model systems. The potential carcinogenicity of 60 Hz EMF in rats and mice, and any possible biological effects of magnetic field transients or harmonics remain to be evaluated. (NO1-ES-25351 from NIEHS)

886 AN ASSESSMENT OF THE DOMINANT LETHAL EFFECTS OF LIQUID PROPELLENT XM46 IN THE RAT

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Liquid propellent XM46 is a developmental propellent for use in the Advanced Field Artillery System. The present study was undertaken as part of a battery of tests to determine the potential health risks associated with exposure to this compound. The dominant lethal effects of XM46 were studied in the Crl:CD-1 mouse. Male mice were administered XM46 (0, 100, 200 OR 400 mg/kg/day) by gavage for 5 days. A group of positive control animals received a single i.p. injection of cyclophosphamide (60 mg/kg). Two days following treatment, male mice were cohabited with 2 untreated females for a period of 7 days. This weekly mating sequence was continued for 7 weeks. Female mice were euthanized 14 days after the mid-week of the cohabitation period and their uteri examined for implantation sites, resorption sites, live fetuses and dead fetuses. Male mice receiving the 400 mg/kg/day dose of XM46 weighed significantly less at the conclusion of the dosing period than did control males. Analysis of the female data failed to reveal evidence of a dominant lethal effect.

887 ACRYLAMIDE INDUCES DNA-ADDUCTS IN MALE GERM CELLS AND CAUSES PREIMPLANTATION ABNORMALITIES AND DOMINANT LETHAL MUTATIONS IN MICE

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Acrylamide is a widely-used industral chemical. It has been shown to induce dominant-lethal mutations and heritable translocations. We used two new approaches to assess the male-mediated genotoxicity of acrylamide. Male C57Bl/6J mice were treated with acrylamide (40 and 50 mg/kg b/w) for 5 days and mated with female C3H/J mice for ten subsequent weeks. The frequency of embryos with preimplantation abnormalities in the mated females was determined 4 day p.c. and the level of DNA-adducts determined in the sperm of exposed males by accelerator mass spectrometry. Acrylamide induced the highest frequency of preimplantation abnormalities after one week, when almost all eggs (86.2%) in females at 4 days p.c. were either unfertilized, arrested in cleavage after 1–3 divisions or had lysis and degeneration of blastomeres. At the end of the 10 week period the frequency of abnormalities returned to background level. Data on preimplantation loss collected at 15–16 days of pregnancy by the classical dominant lethal assay were in good agreement with the estimations obtained on the 4th day of pregnancy. Further, the dynamics of DNA adducts in sperm collected during the first two weeks after treatment correlated well with preimplantation abnormalities in embryos, fathered by treated males. This suggests that formation of DNA-adducts is one of the molecular mechanisms of male-mediated genotoxicity of acrylamide in mice. We conclude that acrylamide is a potent inducer of mutations in male germ cells and abnormalities at the preimplantation stages of embryogenesis in progeny. These studies augment the known reproductive effects of acrylamide in experimental mammals and indicate a potential risk for humans. (Supported by NIEHS grant P30 ESO1896. Work performed under the auspices of the U.S. DOE by LLNL under contract #W-7405-EN6-48; supported by IAG NIEHS Y01-ES-10203-00).

888 OFLOXACIN-INDUCED JOINT CARTILAGE LESIONS IN IMMATURE RATS ARE IDENTICAL WITH LESIONS INDUCED BY MAGNESIUM-DEFICIENT DIET

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Five week old rats (4 male, 3 female) with diet-induced magnesium deficiency showed cartilage lesions in knee joints (e.g. swollen matrix, clefts). Diet was fed for 9 days. Magnesium concentrations (mean ± sd) in serum were 0.18 ± 0.05 mmol/l (control: 0.82 ± 0.10 mmol/l) and magnesium concentrations in cartilage and bone samples corresponded to approximately 50% of the concentrations measured in controls. Single oral doses of 600 mg ofloxacin/kg b wt induced histologically identical lesions in 5 of 7 animals studied. Ofloxacin plasma levels after this dose were approximately 10-fold higher than therapeutic plasma levels. Incidence of lesions in rats with fluoroquinolone treatment *plus* magnesium deficiency was higher than in rats with regular magnesium concentrations (n = 18 each group).

We conclude that quinolone-induced arthropathy is probably caused by a deficit of available magnesium in joint cartilage due to the formation of quinolone-magnesium-chelate complexes.

889 A STUDY OF THE ESTROGENIC ACTIVITY AND TOXICITY OF TRYCOL®6961 (NONOXYNOL-4) IN RATS

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The purpose of this study was to determine in rats, the potential estrogenic activity and toxicity of Trycol®6961 (Nonoxynol-4). This study consisted of four dose groups (10 rats/group) of ovariectionized female Sprague Dawley rats; a negative control group which received 1000 mg/kg distilled water (Group 1) orally, an estrogen activity positive control group which received subcutaneously 1.0 μg β-estradiol/rat (Group 2) and treatment dose groups receiving either 250 mg/kg or 1000 mg/kg Trycol®6961 (Groups 3 and 4, respectively). The animals received their treatments for four consecutive days and were sacrificed on the day after the last treatment. Comparison was made between the groups of uterine weights, physical observations, body weights and macroscopic necropsy data. The positive control group uterine weights were statistically significantly increased ($p < 0.001$) compared to each of the other groups, an expected and desired pharmacological effect. In the 1000 mg/kg Trycol®6961 dose group, four out of ten animals died. The animals of this dose groups exhibited a number of toxic signs which tended to be more pronounced and appeared earlier in those animals that died. Not toxic signs were seen in any of the animals in any of the other groups. Thus, to the rats in this study, Trycol®6961 administered orally did not induce an increase in uterine weights and by this parameter. Additionally, after four consecutive doses, this material is toxic at 1000 mg/kg but not at 250 mg/kg.

890 A STUDY OF THE ESTROGENIC ACTIVITY AND TOXICITY OF TRYCOL®6961 (NONOXYNOL-4) IN MICE

J H Berke [1], J M Mitchell [2]. [1] *W.R. Grace & Co., — Conn. Lexington, MA;* [2] *MB Research laboratories, Spinnerstown, PA*

The purpose of this study was to determine in mice, the potential estrogenic activity and toxicity of Trycol®6961 (Nonoxynol-4). The study consisted of four dose groups (10 mice/group) of ovavectomized female Swiss Webster mice; a negative control group which received 1000 mg/kg of distilled water (Group 1) orally, an estrogen activity positive control group which received subcutaneously 0.1 μg β-estradiol/mouse (Group 2) and treatment dose groups receiving either 250 mg/kg or 1000 mg/kg Trycol®6961 (Groups 3 and 4, respectively). The animals received their treatments for four consecutive days and were sacrificed on the day following the last treatment. The uteri from each animal were excised and weighed. Comparison of uterine weights, physical observations, body weights and macroscopic necropsy data was made between groups. The positive control group uterine weights were statistically significantly greater ($p < 0.001$) than those of each of the other groups, an expected and desired pharmacological effect. The uterine weights of the animals in the treatment groups were similar to those of the negative control group. No toxic

signs were seen in any of the animals of this study. Thus, in the mice of this study, oral dosing of Trycol®6961 at either 250 mg/kg or 1000 mg/kg did not cause an increase of uterine weights compared to the negative control group and is not considered to have estrogenic activity as measured by this parameter. In addition, neither dose level of Trycol® caused signs of toxicity as determined by the parameters evaluated.

891 ASSESSING CONTAMINANT BIOAVAILABILITY AND HEALTH EFFECTS TO EUROPEAN STARLINGS INHABITING A HAZARDOUS WASTE SITE

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The Rocky Mountain Arsenal (RMA) is a future wildlife refuge with concerns for detrimental effects to wildlife from chemical contamination. This site has been extensively characterized for chemical residue, but little research to date has investigated bioavailability and potential health effects to wildlife. In our study, we investigated the reproductive success, P450 induction, histopathological changes, immunological alterations, and chemical residue analyses of selected tissues and food items of European starling (*Sturnus vulgaris*) nestlings reared on RMA. Nestboxes were erected on 16 sites with known contaminant soil concentrations. Nestboxes were monitored regularly to determine egg survival, hatching success, nestling survival and overall nesting success. Nine day old nestlings were injected with 1×10^9 sheep red blood cells and were collected on day 15 post-hatch for assessment of chemical bioaccumulation and biomarker responses. These results show evidence of reproductive dysfunction on contaminated sites; while reproduction on the reference sites appeared normal, nestboxes on contaminated sites had decreased egg and nestling survival and nestboxes on heavily contaminated sites had no egg survival. More detailed chemical and biomarker analyses are currently in progress. This research provides important data for the ecological risk assessment of the RMA before and after remediation and provides a diagnostic tool for monitoring the health of wildlife inhabiting hazardous waste sites. Supported by U.S. Fish and Wildlife Service and NIEHS ES04696.

892 DIFFERENTIAL DIAGNOSIS OF ORGANOCHLORINE AND HEAVY METAL EXPOSURE USING PORPHYRIN PROFILES IN NESTLING EUROPEAN STARLINGS

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Tissue and excreta porphyrin concentrations (porphyrin profiles, PP's) can be a qualitative or quantitative biomarker of environmental toxicants such as PHAH's and heavy metals. We evaluated the differential diagnosis of PP's by measuring hepatic PP's of nestling European starlings treated with Aroclor 1254, hexachlorobenzene, dieldrin, mercuric chloride, lead acetate, and sodium arsenate. Nestlings were treated with the above toxicants on post-hatch days 5, 8, 11, and 14, euthanized on post-hatch day 15. Nestlings treated with a total of 42 and 130 mg/kg Aroclor 1254 exhibited an increase of 4-carboxylporphyrin and 7- and 4-carboxylporphyrin, respectively, in dose related manner. Mercuric chloride caused an increase in only 2-carboxylporphyrin at 8 mg/kg. The PP evaluations of nestlings treated with hexachlorobenzene (800 mg/kg), dieldrin (20, 40, and 80 mg/kg), lead acetate (40, 200, and 400 mg/kg), and sodium arsenate (12 mg/kg) are currently in progress. These findings extend the view that nestling starling PP's may serve as a diagnostic tool to monitor toxicant bioavailability to wildlife. Supported by NIEHS ES04696.

893 BIOMARKER ASSESSMENT OF CONTAMINANT EXPOSURE AND EFFECTS IN KESTRELS AT ROCKY MOUNTAIN ARSENAL

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Rocky Mountain Arsenal (RMA), formerly the site of chemical weapons and pesticide production, has been managed as a National Wildlife Refuge since October 1992. A biomonitoring program was initiated in 1993 to evaluate current effects of site contaminants in wildlife species and to determine any changes that may occur as a result of remediation activities. The American kestrel is being evaluated as a sentinel species for this program. Kestrel pairs nesting at RMA were studied to determine relationships between contaminant exposure, tissue burdens, biomarker responses and reproduction. Adult foraging areas were determined using telemetry and nestling exposure was estimated by collection of prey items for analysis. Nests were monitored to determine reproductive success, nestling growth and survival. One chick was collected from each nest 21 days after hatching for tissue residue and analysis of biochemical markers (hepatic alkoxyresorufin oxidation and glutathione conjugation activities, total renal porphyrin content, brain biogenic amine content and lymphocyte proliferation *in vitro*).

894 PROPHYLACTIC ADMINISTRATION OF H-7, AN INHIBITOR OF PROTEIN KINASE C, IMPROVES THE SURVIVABILITY OF CYANIDE INTOXICATION

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We recently presented evidence suggesting that the protein kinase C (PKC) inhibitor H-7 [1-(5-isoquinolinesulfonyl)-2-methylpiperazine] protects micropigs against cyanide toxicity *(Maduh et al. FASEB J. 8: A647, 1994)*. We now report the protective effect of H-7 against cyanide in an additional species, the White Carneau pigeon, *Columba livia* (WCP). Animals, weighing 450–600 gm, were placed under isoflurane (2%) anesthesia and administered (i.v.) NaCN (1.86, 2.2, or 3.2 mg/kg), n = 5. Sixty min following NaCN administration, all the animals in the 1.86 mg/kg dose group, survived as did 3 of the 5 in the 2.2 mg/kg dose group. None of the 5 animals in the 3.2 mg/kg treatment group survived. The LD_{50} was calculated to be 2.25 mg/kg, which was used to test for the H-7 effects. A group of 5 WCPs was administered 2.25 mg/kg NaCN alone while another group of 6 received 1 mg/kg dose of H-7, i.v., 30 min before the same NaCN dose was injected. Through the experimental period of 60 min, 3 out of the 5 animals treated with 2.25 mg/kg NaCN alone survived whereas 5 of the 6 pretreated with H-7 survived. The present observations complement the earlier findings of protection by H-7 pretreatment in the micropig over an identical 60 min time course following NaCN, and are consistent with the hypothesis that H-7 ameliorates cyanide toxicity in the intact organism. It is proposed that H-7, a potent PKC inhibitor, may be of value in the search for novel pharmacologic cyanide antagonists.

895 INTERACTION OF WATER QUALITY AND NUTRITIONAL STATUS ON PERFORMANCE AND IMMUNE FUNCTION IN BROILER CHICKS

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Adverse health effects of drinking water contaminants have been observed in plants, animals, and humans living near hazardous waste sites. In this study a randomized block with 3 × 3 factorial design was used to determine if there is an interaction between effects of drinking water contaminants and suboptimal nutritional status on performance and immune function in male broiler chicks after 21 and 49 days of exposure. Experimental drinking water contained arsenic, benzene, cadmium, lead and trichloroethylene at low levels (0.9, 1.25, 5.1, 7.0 and 6.5 ppm) and high levels (9, 12.5, 51, 70 and 65 ppm); while experimental diets included diet containing 50% added vitamins and minerals (V&M), and diet without added V&M. Increasing levels of contaminants in drinking water and decreasing levels of V&M in diet resulted in decreased feed and water intake, decreased weight gain, and suppression of natural, humoral, and cell mediated immune response. These effects were more pronounced in chicks simultaneously exposed to contaminants in drinking water and reduced levels of V&M. These findings suggest this agricultural species (chicken) is more sensitive than the laboratory animal (mice) that was used in earlier studies involving contaminants in drinking water.

896 GENERATION OF PHOSPHINE IN WHITE PHOSPHORUS TOXICITY

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White Phosphorus, P_4, has been implicated in thousands of waterfowl deaths in Eagle River Flats, Anchorage, Alaska. Lethality, however, could not be correlated with the level of P_4 found in the gizzard, nor in the fatty tissues of these ducks. It has been hypothesized that the toxicity of P_4 may be due to its metabolite(s) rather than the parent compound. In vitro studies were undertaken to determine if phosphine (PH_3), a toxic gas, was generated from P_4

by various rat tissues. Tissue samples, such as blood, brain, liver, stomach and small intestine, as well as the gastrointestinal contents of the F344 rat were used. Tissue homogenates were made with 0.025 M Tris buffered saline (pH 7.0) and placed in 40 ml vials. One set of samples was boiled for 30 minutes to denature enzymes and microorganisms. Samples were incubated in 37°C water bath prior to and subsequent to the addition of P_4 (1 mg). Phosphine gas in the head space was determined and quantified by GC at times up to 2 hrs after P_4 exposure. By 2 hours, all samples produced phosphine. The production of PH_3 was significantly greater in the biologically viable samples compared to the heat inactivated samples ($p < 0.05$) for the blood and the brain samples. However, with the liver samples, the heat inactivated samples produced more PH_3 than the biologically viable samples. These results imply that the conversion of P_4 to PH_3 is both biologically and chemically mediated and the level of PH_3 produced is highly tissue specific. The results further imply that the toxicity of P_4-exposed waterfowl may in part be due to PH_3. Experiments are under way to determine if PH_3 can be generated in vivo from animals that have been exposed to P_4.

897 MEDICAL STUDIES OF THE POISONOUS LAND AND SEA SNAKES FOUND IN AND AROUND SOMALIA

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Studies were carried out to determine the comparative toxicity and pathophysiology of 20 of the more poisonous snakes indigenous to Northern Africa. Included were 2 snakes from the family *Colubridae,* 8 snakes from the *Viperidae* family, 8 from the *Elapidae* family and 2 representative sea snakes from the family *Hydrophidae*. Anesthetized dogs were used in the study. Vital physiological functions were continuously recorded as were changes in the blood coagulation system and any tissue damage produced by the venom at the site of envenomation. *Colubridae* venom killed at an average of 10 hours at an average dose of 5.0 mg/kg. *Viperidae* venom produced death in 3 hours with an average lethal dose of 3.8 mg/kg. Depression of 1st and 2nd stage clotting factors and a decrease in firinogen and platelets, was seen. Intravascular coagulation were also observed with moderate hemolysis and local hemorrhage. *Elapidae* venom produced death in 1.7 hours. The average lethal dose was 1.85 mg/kg. Death appeared to be primarily due to respiratory paralysis. Moderate hemolysis was seen with no intravascular coagulation or tissue damage. *Hydrophidae* venom consistently produced death in less than 30 minutes. No tissue damage or changes were seen in the hematological parameters monitored. Death was due to respiratory paralysis. It would appear from these studies that many snakes found in Northern Africa and adjacent areas can indeed be dangerous to man.

898 INTERSPECIES SENSITIVITY AND SPECIFICITY OF PLASMA SEX STEROIDS AS BIOMARKERS IN CIRCUMPOLAR ARCTIC FAUNA

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Plasma sex steroids show promise as sensitive biomarkers for certain organic contaminants and heavy metals. We investigated the relationship between 17β-estradiol and testosterone and tissue burdens of a large suite of heavy metals and organochlorines (As, Cd, Cu, Hg, Ni, Pb, Se, Zn, PCBs, DDTs, HCHs, and chlordanes) in five freshwater fish species and two small mammals from the U.S. and Russian Arctic. Correlation analysis indicated chemical specificity. Plasma 17β-estradiol and testosterone were negatively correlated only with Pb in liver in both a freshwater fish (arctic grayling, *Thymallus arcticus*) and a small mammal (arctic ground squirrel, *Spermophilus parryi*). The strongest relationships were for testosterone in male fish ($R^2 = 0.82$), and for 17β-estradiol in female squirrels ($R^2 = 0.57$). Our results also indicate prominent differences in species sensitivity. For example, grayling exhibited a stronger biomarker response at lower tissue Pb concentrations than lake trout (*Salvelinus namaycush*) collected from the same sites. Liver concentrations of ΣPCBs, DDTs, HCHs and chlordanes were typically higher in lake trout and ground squirrels than grayling (1–50 ng/g wet wt. versus 0.2–10 ng/g). Combinations of life history and physiological species differences probably underlie sensitivity of plasma sex steroids to contaminants. (Supported by USEPA grant CR818187).

899 ANATOXIN-A: RISK ASSESSMENT OF A TOXIN DERIVED FROM BLUE-GREEN ALGAE

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Blooms of blue-green algae occur in drinking water reservoirs. These algae contain potent toxins, of which the alkaloid anatoxin-a is among the most common. This study was designed to generate data to permit a risk assessment of exposure to pure anatoxin-a in drinking water.

Pharmacological studies confirmed that anatoxin-a is a potent nicotinic agonist inducing neuromuscular blockade, which is reversible at sub-lethal doses.

When administered to mice by gavage for 28 days, the No Observable Effect Level was 0.1 mg/kg/day. No evidence of maternal or developmental toxicity was seen when anatoxin-a was administered to mice from Day 6 to 15 of pregnancy at doses of 2.5 mg/kg/day.

The margin of safety for exposure to anatoxin-a, utilising these data considering a pregnant woman and a 10 kg child and assuming a concentration of 1 mg/ml in drinking water, is about 3 orders of magnitude.

It is concluded that blooms of blue-green algae in domestic reservoirs, although in many respects undesirable, are unlikely to present a significant environmental health risk from anatoxin-a.

900 MICROCYSTIN-LR: TOXICOLOGICAL ASSESSMENT OF A TOXIN DERIVED FROM BLUE-GREEN ALGAE

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Blooms of blue-green algae have been known for centuries and have caused poisoning in domestic and farm animals. Some produce potent hepatotoxic peptides known as microcystins. The objective of this study was to determine the risk of microcystin-LR exposure through drinking water.

Three series of experiments were undertaken, a comparison of acute effects following administration by the intraperitoneal and oral routes in mice and the oral route in rats, an investigation of the effects on the developing foetus in the mouse and a 13 week repeat dosing study in the mouse.

In the acute studies, the mouse was the most sensitive species; microcystin-LR was some 30 to 100 times less toxic to the mouse by the oral route than intraperitoneally. Administered to the mouse from Day 6 to 15 of pregnancy at doses causing maternal toxicity, there were no increases in foetal deaths or abnormalities. The No Observable Effect Level was 600 mcg/kg/day orally. In the 13 week study, hepatotoxicity was seen at 200 and 1000 mcg/kg/day accompanied by changes in blood chemistry. The No Observable Adverse Effect Level was 40 mcg/kg/day orally.

A preliminary risk analysis suggests that contamination of drinking water by microcystin-LR is unlikely to present a significant environmental health risk.

901 EXTOXNET — A UNIQUE APPROACH TO ENVIRONMENTAL TOXICOLOGY OUTREACH

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EXTOXNET is an *ad hoc* consortium of university toxicologists, environmental chemists and scientists from other disciplines who are interested in promoting public education and university outreach programs related to toxicants in the environment. This consortium was formed in 1983 and now has been institutionalized with funding from the the USDA National Pesticide Impact Assessment Program. EXTOXNET now consists of four partners: the National Coordination Center at University of California-Davis, the Western Regional Access Center at Oregon State University, the Eastern Regional Access Center at Cornell University and the Midwest Regional Access Center at Michigan State University. EXTOXNET has developed a variety of written materials including almost 150 pesticide profiles, 25 toxicology information briefs, a number of issue documents and other written resources and databases. These were originally available only in hard copy form but can now also be accessed by computer via ALMANAC, GOPHER and MOSAIC systems administered at the Western Regional Center. In addition to providing written information, EXTOXNET members also respond to phone, mail and computer inquiries. EXTOXNET is set up to provide a coherent, rapid, national response to media reports, such as recent television programs about pesticides in foods. Expansion of EXTOXNET to include regional centers in other sections of the country is planned.

902 EFFECTS OF REDUCED LEADED PETROL CONSUMPTION ON LEAD CONCENTRATIONS IN EDIBLE VEGETABLES

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The reduction in the use of lead (Pb) in gasolines has induced a decrease of the atmospheric Pb concentrations. Vegetables absorb Pb from the soil and from surface deposits on parts of vegetables exposed to polluted air. In a previous survey, the levels of Pb in edible vegetables from Tarragona Province (Catalonia, NE Spain) were determined for 1988–1989 when the consumption of leaded gasolines in that area surpassed 99% of the total consumption. The purpose of the present study was to evaluate the impact of the reduction of leaded petrol on the concentrations of Pb in edible vegetables grown in several locations from Tarragona Province. Lead content was determined in 492 samples belonging to 20 species. The samples were subjected to Pb analyses by atomic absorption spectrophotometry. An average decrease for Pb concentrations of 53.2% could be observed (71.4 μg/kg in the present study vs. a mean value of 152.7 μg/kg found in the previous survey). Spinachs showed the lowest reduction in Pb content (6%), whereas the highest reduction was observed for onions (87%). Only a few species: chard, spinach, parsley, and leek, which were the species with the highest Pb levels, showed higher Pb concentrations (8–81%) in the industrial area than in the agricultural area of Tarragona Province. These results corroborate the expected decrease of atmospheric Pb as a consequence of antipollution policies on gasolines.

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903 CHARACTERIZATION OF CONTEMPORARY SOURCES OF ENVIRONMENTAL LEAD CONTAMINATION IN THE SAN FRANCISCO BAY AREA BY THEIR STABLE ISOTOPIC COMPOSITIONS

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The systematic elimination of lead alkyl additives in gasolines in the United States has reduced atmospheric lead contamination in the San Francisco Bay area by orders of magnitude, and increased the relative contributions of other sources of environmental lead contamination. Particulate lead aerosols derived from both contemporary emissions and the resuspension of previously discharged lead with dust, still constitute a source of environmental lead contamination in humans, as do urban soil and drinking water. This is illustrated by stable lead isotopic compositions of lead in environmental samples and human blood. In contrast, the primary source of contemporary lead in the waters of San Francisco Bay appears to be derived from historic emissions of industrial lead aerosols that have been sequestered in bay sediments. The amount of lead accumulated in those sediments is so large that the relatively negligible amounts of lead remobilized from the sediments by diagenic processes are now equal to or greater than or all other contemporary inputs of lead to the bay from natural and anthropogenic sources. This corroborates the proposal that elevated concentrations of environmental lead will be a persistent problem in spite of order of magnitude reductions in industrial lead emissions.

904 ESTROGEN AND Ah RECEPTOR LIGANDS IN PULP AND PAPER MILL BLACK LIQUOR

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Pulp and paper mill effluent contains a complex mixture of compounds which adversely affect aquatic wildlife at both the physiological and population level. These effects include compromised reproductive capacities and the induction of mixed-function oxygenase activities. Laboratory and caged fish studies suggest that the effects are due to estrogen- and dioxin-like compounds present in mill effluent. Using a recombinant receptor/reporter gene assay, black liquor, a component of mill effluent, was examined for the presence of estrogen receptor (ER)- and Ah receptor (AhR) ligands. Based on the dose-response induction of a luciferase reporter gene, black liquor contains 4 ± 2 ppb 17β-estradiol (E2) equivalents and 10 ± 4 ppb TCDD equivalents. The ER-mediated induction was confirmed by antagonism of the response using the pure antiestrogen ICI 164,384. Gel retardation assays demonstrated that black liquor could also transform the AhR into a DNA binding complex. Moreover, the ability of black liquor to inhibited the E2 induction of pS2Luc suggests that it contains ligands with antiestrogenic properties. Therefore, the adverse effects observed in fish exposed to mill effluent may be due to the endocrine disruptive activities of ER and AhR ligands present within black liquor. (Supported by funds from MRC of Canada, the Pharmaceutical Manufacturers Association of Canada/Health Research Foundation and the Canadian Network of Toxicology Centres.)

905 MICRODIALYSIS OF A CACTUS PLANT TO STUDY UPTAKE OF CADMIUM FROM CONTAMINATED SOIL

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Microdialysis is a relatively new research tool for sampling the extracellular fluids of plants. The technique is virtually non-invasive and non-injurious to the plant. A CMA/12 microdialysis probe with 4 mm membrane length was employed to study relative recovery (RR) of Cd from a standard solution containing 5 ppm, at flow rates of 1, 2 and 5 μl/min. Measurements were taken at 25°C. Good RR was observed under these conditions. Uptake of Cd was then determined on a prickly pear cactus (Opuntia sp.) suspended in soil saturated with a 5 ppm Cd solution and allowed to stand for 5 days. The CMA/12 probe was inserted at a strategic point in the cactus and microdialysis samples were taken at a flow rate of 1 μl/min. The analyte was measured by GFAAS and the uptake of Cd was significant. Microdialysis was concluded to be a viable method of studying uptake and distribution of Cd in Opuntia sp. at various stages of plant development.

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906 THE CONTRIBUTION OF SOIL HALF-LIFE TO RISK ASSESSMENT CONSERVATISM

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Risk estimates for contaminants in soil are typically calculated by assuming that chemical concentrations remain unchanged over time. In reality, microbial and physicochemical processes can substantially diminish contaminant concentrations in soil. To address the effect of chemical half-lives on estimates of chemical exposures from soil and consequent human health risks, a Monte Carlo analysis was performed comparing "point estimates" of risk (i.e., single risk values determined by assuming a constant value for each of the variables in the risk equation) to risk distributions (i.e., risk ranges determined through the use of probability density functions for the variables in the risk equation) for the chemicals benzo(a)pyrene (BaP) and pentachlorophenol (PCP) at hypothetical soil concentrations of 100 ppm. Variables in the risk equation for which probability density functions are available in the published literature include soil ingestion rate (SIR), exposure frequency (EF), exposure duration (ED), and body weight (BW). A sensitivity analysis was performed by comparing point estimates of risk for BaP and PCP to the risks corresponding to the 95th percentile of the risk distribution for these chemicals determined by using a probability density function for a single equation variable (e.g., SIR) while assuming constants for the remaining variables (i.e., EF, ED, BW). This allowed for an assessment of the degree of conservatism that a particular variable contributes to the point estimate of risk. Results of these analyses indicated that soil half-life is by far the greatest single contributor to conservatism in the risk equation for soil ingestion, even for a persistent soil chemical such as BaP ($t_{1/2}$ = 1.5 years). Thus, accounting for chemical half-life will produce the greatest reduction in unnecesary conservatism when determining risks from chemical exposure in soil.

907 DECHLORINATION OF WATER-SOLVATED PENTACHLOROPHENOL BY CHEMICALLY MODIFIED MONTMORILLONITE CLAYS

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Rapid dechlorination of water-solvated pentachlorophenol (C_5P) by two proprietary montmorillonites was investigated. Nevalite montmorillonite (M_1) and standard calcium montmorillonite (M_2) were chemically modified. Aqueous 100 ppm C_5P was reacted with M_1 and M_2 for 10 hr. Disappearance of C_5P and appearance of dechlorination products were detected by ultraviolet spectrophotometry and capillary gas chromatography/quadrupole mass spectrometry. Treatment with M_1 (2.5% w/v) resulted in sorption of 42% of the initial concentration of C_5P from solution. Following desorption, 57.9% of bound C_5P was dechlorinated to products of lesser toxicity. M_2 (2.5% w/v) sorbed 53% of the solvated C_5P and dechlorinated 33.8% of bound C_5P to products of lesser toxicity. Chemical and mechanistic studies of these dechlorination reactions, in conjunction with toxicological screening, suggest that these clays may facilitate the chemical remediation of C_5P-contaminated groundwater. (Supported by NIEHS P42ES04913-04 and TAES H6215).

908 BIOREMEDIATION APPLICATIONS OF ORGANO-PHOSPHATE HYDROLASE: INTEGRATING GENETIC ENGINEERING WITH REACTOR ENGINEERING

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Organophosphate (OP) neurotoxins have been successfully hydrolyzed by both genetically engineered recombinant *E. coli* strains and a native *Flavobacterium* strain. These strains were cryoimmobilized in polyvinyl alcohol to form stable and environmentally safe biocatalyst beads. External mass transfer and diffusion limitation effects in the biocatalysts are currently being investigated to improve the rate of hydrolysis. The biocatalyst effectiveness is limited by nonoptimal bead size and low solubilities and diffusivities of OP substrates in an aqueous environ. These parameters have a significant effect on the observed reaction rate. Biocatalyst beads with diameters of 2, 1.2 and 0.8 mm hydrolyzed 5 mM of pesticide paraoxon at rates of 0.236, 0.368 and 1.06 mol/sec/m^3 catalyst, respectively. Biocatalyst beads with a 2 mm diameter hydrolyzed 10, 5 and 1 mM of paraoxon at rates of 0.328, 0.236 and 0.102 mol/sec/m^3 catalyst. Engineering studies to establish an optimal biocatalyst system will allow for an efficient, cost-effective bioremediation technology for OP compounds and their neutralized products.

909 MICROBIAL DEGRADATION OF TRINITROTOLUENE UNDER AEROBIC CONDITIONS

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Nitroaromatics are not only used as a variety of herbicides, pesticides, and precursors of plastics and dyes, but also as explosives, such as 2,4,6-trinitrotoluene (TNT). Even though TNT is extremely toxic to microbial systems in general, several bacterial isolates that degrade these compounds have been identified. We report here on 3 bacterial strains that are effective as bioremediation agents for solubilized TNT under aerobic conditions. One of these strains has been identified as a *Bacillus sp.* The other two tentatively have been identified as *Pseudomonas sp.* and *Staphylococcus sp.* These strains differ in their toxic sensitivity to TNT, rate of TNT degradation, and growth rates when grown in the presence of TNT as a co-metabolite. While growth of the *Pseudomonas sp.* remains unaffected in the presence of 100 ppm TNT, growth of the other two strains is adversely affected by greater than 50 ppm TNT. TNT-dependent NADH/NADPH oxidation by the crude cell extract was assayed, and the *Bacillus sp.* appears to have the highest level of activity. This enzyme activity is constitutively expressed and its expression is not dependent on the growth medium. Initial attempts at purification of this enzymatic activity by combinations of salting out methods and various chromatographic procedures have shown promise. Both biochemical and molecular genetic approaches are being utilized in the search for the specific enzymes/genes involved in the initial steps of TNT modification/biodegradation under aerobic conditions.

910 ASSESSING THE ENVIRONMENTAL IMPACTS OF EMISSIONS FROM A CHEMICAL FACILITY

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Exhibit F of Alaska's Air Quality Control Permit to Operate requires facilities in Alaska to complete an "18 AAC 50.400(c)(2) Compliance Showing for Potential Environmental Impacts." The permit stipulates that "No person may permit any emission which is injurious to human health or welfare, animal or plant life, or property, or which would unreasonably interfere with the enjoyment of life or property." Risk to environmental receptors (i.e., plants, animals, property, and unreasonable interference with the enjoyment of life or property) from emissions from the facility were assessed through a comprehensive literature search. Ecological assessment criteria, including the plant and animal species indigenous to the area; and ecotoxicity and bioaccumulation information, were used in determining potential impacts. Nuisance properties of chemical substances, such as odor, were considered in evaluating interference with the enjoyment of life or property. Three data bases were searched for environmentally-relevant articles specific to 46 emitted chemicals. Over 5,000 articles were identified for review. The abstracts from all of the articles were reviewed and screened for relevant environmental receptors. The selected 237 articles were summarized, critiqued, and evaluated for concentration-effects relationships. Concentrations provided in the literature were compared with modeled concentrations at receptor points. An assessment of relevance to the area surrounding the facility, a qualitative evaluation of impact, and summaries of the impacts of each of the chemicals as documented in the articles were presented in the final report. This methodology provides a means by which to evaluate environmental impacts from a large number of emitted chemicals.

911 A COMPARISON OF AMES ASSAY AND MUTATOX™ IN ASSESSING THE MUTAGENIC POTENTIAL OF CONTAMINATED SEDIMENT

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The Ames assay and Mutatox™ were compared to evaluate the ability of Mutatox™ to identify genotoxic potential in dredged sediments. The Ames assay has been used extensively in the testing of environmental contaminants in air, water, and sediment. Mutatox™, a bacterial bioluminescence test was developed for use as a genotoxicity bioassay.

Ten sediments with varying degrees of contamination were soxhlet-extracted and were divided into a crude sample and a clean sample using silica-gel column chromatography. Both the Ames plate incorporation tests and Mutatox™ tests were conducted using metabolic activation. The Ames assay indicated a positive mutagenic response in 16 of the 20 sediment extracts while Mutatox™ indicated a positive mutagenic response in 12 of the 20 extracts. In a side by side comparison of the Ames assay and Mutatox™, 17 out of 20 sediment extracts had similar responses, both positive and negative.

Mutatox™ compared favorably with the Ames assay and shows promise as a screening tool to assess sediment genotoxicity when used with Ames assay as a final confirmation.

912 INDUCTION OF HSP-60 IN ISOCHRYSIS GALBANA EXPOSED TO DISPERSANT AND PRUDHOE BAY CRUDE OIL PREPARATIONS

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Oil spills represent a serious threat to a variety of marine organisms, from phytoplankton to marine mammals. Surface inhabitants may benefit from the application of dispersing agents, yet little is understood of the disposition of the dispersed components of a spill. The objective of the current research was to determine the influence of dispersants on crude oil components at concentrations at the no observable effect level (NOEL). HSP-60 proteins, members of the chaperonin family of stress proteins, are induced in response to a wide variety of environmental agents, including UV light, heavy metals, and xenobiotics. These proteins are found in mitochondria and chloroplasts of eukaryotes and facilitate folding and assembly of proteins. To aid in establishing the NOEL for *I. galbana,* induction of HSP-60 was selected as a sublethal endpoint for algal cultures exposed to water-accommodated fractions (WAF) of Prudhoe Bay Crude Oil and Corexit® 9527 dispersant. Western blotting techniques, using HSP-60 protein specific antibodies, were used to isolate and identify induced HSP-60 in *I. galbana.* Studies indicated that WAF produced a dose-related response in *I. galbana,* which increased as a function of time. Dispersant alone showed the greatest induction, producing a double band, while combined WAF-dispersant showed less induction, suggesting a possible competition between crude oil and algae for dispersant interaction. Results showed that HSP-60 protein synthesis was induced in *I. galbana* in response to exposure to WAF, dispersant, and WAF-dispersant preparations.

913 TOXICITY AND ACCUMULATION OF THE WATER-SOLUBLE FRACTION (WSF) OF CRUDE OIL (CO) AND PARTIALLY COMBUSTED CRUDE OIL (PCO) FROM KUWAITI OIL-FIRES ON THE MARINE FISH, MENIDIA BERYLLINA

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As a consequence of the Iraqi invasion in Kuwait, a substantial amount of CO and PCO were emitted into the marine environment. The aim of this study was to examine to toxicity of the WSF of CO and PCO on a marine fish, *Menidia beryllina*, in 16-day flow-through tests. Specific growth rate (SGR) was studied as a function of total petroleum hydrocarbon (TPHC) concentration in water. Water and tissue levels of polycyclic aromatic hydrocarbons (PAH) were analyzed by gas chromatography (GC) and GC-mass spectrometry (GC-MS). Cytochrome P4501A (CYP1A) was measured using western blotting techniques. SGR reductions were observed in fish exposed to PCO and CO, with TPHC

water-concentrations being 10 fold higher in CO exposures (67 – 145 ppb) than in PCO exposures (4 – 12 ppb). GC and GC-MS water sample analysis indicated that PAHs contributed more to the TPHC content of the PCO WSF than did CO. No PAHs were detected in whole fish homogenates. Preliminary analysis of whole fish tissue cytochrome CYP1A concentrations indicated that exposure to the PCO-WSF resulted in CYP1A induction.

914 INTERACTIVE EFFECTS OF PENTACHLOROPHENOL (PCP) AND WATER SALINITY IN THE RED ABALONE AS MEASURED BY *IN VIVO* ^{31}P NMR

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Application of in vivo ^{31}P NMR for elucidating the sublethal biochemical actions of toxicants in intact aquatic organisms, and the influence of natural stress factors, has previously been demonstrated by our research group. Recently, using a flow-through chamber, abalones (*Haliotis rufescens*) were exposed to PCP in flowing seawater (14°C) at a sublethal water level (1.2 mg L^{-1}) and at a water salinity of either 25, 35, or 45‰, representing the coastal variation that might result during natural precipitation or municipal desalination activities. After exposure, they were maintained in clean flowing seawater (again at either 25, 35, or 45‰) for 16 h to check recovery, and phosphagen changes in foot muscle were monitored by surface probe-localized ^{31}P NMR. Upon PCP exposure, phosphoarginine levels declined, inorganic monophosphate (P_i) levels concurrently increased, and the spectral P_i resonance shifted, indicating intracellular acidification; during recovery all biochemical endpoints returned to normal. However, both the onset and intensity of effects were potentiated by increased water salinity. While PCP alone is a potent uncoupler of mitochondrial oxidative phosphorylation, use of different water salinities simulated an additional stress imposed on intertidal animals by transcient or seasonal environmental changes. Interactions between pollutants and natural stresses can alter effects, often explaining unanticipated environmental actions.

915 MEASUREMENT OF ABALONE ARGININE KINASE (AK) KINETICS BY *IN VIVO* ^{31}P NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY

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Previous studies from this laboratory developed ^{31}P NMR spectroscopy techniques to noninvasively assess sublethal effects of pentachlorophenol (PCP) on cellular bioenergetics in the red abalone (*Haliotis rufescens*). PCP exposure produced changes in ^{31}p NMR visible metabolites, including concurrent decreases in phosphoarginine (PA) and increases in inorganic phosphate (P_i). Interestingly, adenosine triphosphate (ATP) significantly decreased prior to complete PA loss, suggesting that in addition to uncoupling oxidative phosphorylation PCP may inhibit AK. To further elucidate molecular mechanisms of PCP toxicity, we have initiated in vivo NMR saturation transfer experiments in red abalone to noninvasively measure the pseudo-first-order rate constant for AK catalyzed phosphate exchange between PA and ATP. Studies were conducted at 7.05 T using a custom-built 1-cm dia., 1-turn surface-coil probe tuned to 121.1 MHz. Abalone (1 yr old) were mounted in a perfusion chamber oriented coaxial to the coil. Under control conditions, selective saturation of the γ-ATP resonance (10 s, 200 mW) caused a 16% reduction in the PA resonance. Signal attenuation resulted from chemical exchange between PA and ATP prior to full longitudinal relaxation and corresponded to a rate constant of 0.11 s^{-1} for AK catalyzed ATP formation. Direct, in vivo measurement of this reaction provides a novel and valuable addition to NMR techniques for noninvasive assessment of molecular mechanisms of environmental toxicity and is being used to determine the effects of PCP exposure on AK.

916 SUBLETHAL EFFECTS OF TRIBUTYLTIN AND HYPOXIA IN ADULT AND LARVAL ABALONE AS MEASURED BY *IN VIVO* ^{31}P NMR

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Current methods of in vivo toxicity testing rely heavily upon observed behavioral or morphological changes to determine toxic endpoints. However, organisms may experience detrimental effects at toxicant levels well below those which produce obvious changes. Consequently, the goal of this study was to use ^{31}P NMR to directly compare the sublethal effects of tributyltin (an inhibitor of oxidative phosphorylation) and hypoxia in adult and larval red abalone (*Haliotis rufescens*). Utilizing a teflon and glass flow-through exposure system, levels of phosphoarginine, ATP, and inorganic phosphate were monitored while the organisms were exposed to the compound, or to functional hypoxia by the cessation of water flow, and then allowed to recover. Relative toxicity was determined by the decrease in levels of phosphoarginine and/or ATP, the subsequent increase in inorganic phosphate, and the change in intracellular pH (measured by the shift in inorganic phosphorous resonance). Organisms were exposed for 2 h to hypoxia or 2 ppm of tributyltin, followed by clean seawater recovery periods of 2 h for larvae and 5 h for adult animals. Adult abalone are intertidal organisms and moderately resistant to oxidative stress, while larvae do not normally encounter low-oxygen environments, which may account for the observed increased resistance of the adult animals to both stressors. This comparative study may lead to a greater understanding of sublethal effects, toxic mechanisms, and the development of resistance to xenobiotics in these organisms.

917 TROUT CYP1A1: GENE STRUCTURE, SEQUENCE, AND COMPARISON WITH THE MAMMALIAN ORTHOLOGOUS GENE

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Previous studies in this laboratory have demonstrated the presence of the Ah receptor and 3-methylcholanthrene-induced CYP1A1 mRNA in trout liver. We have now sequenced the trout (*Oncorhyncus mykiss*) *CYP1A1* gene, and 1,864 bp of the 5′ flanking region. The gene spans less than 4.1 kb, comprising seven exons and six introns. The transcriptional start site was determined by primer extension. Including the first noncoding exon of 119 bp, the entire mRNA is 2,740 bp in length, and the open reading frame, starting with nucleotide 32 of exon 2, encodes a protein of 522 amino acids (M_r = 59,241). All exon/intron junctions obey the GT/AG rule. Structurally, the trout gene is roughly similar to mammalian *CYP1A1* genes, regarding the size of exons and introns—with the exception of the trout intron 1 (only 523 bp, compared with 2–3 kb in mammals). The trout *CYP1A1* 5′ flanking region contains five putative aromatic hydrocarbon response elements (AhREs), which are found upstream of all mammalian *CYP1A1* genes and many other dioxin-inducible genes and are involved in controlling transcription via binding of Ah receptor-inducer complexes. (Supported by NIH Grants R01 AG09235, R01 ES06321, R01 ES06811, and P30 ES06096.)

918 APPLICATION OF THE GAS-UPTAKE CHAMBER TO DETERMINE UPTAKE AND ELIMINATION KINETICS OF VOLATILE CHEMICALS IN SMALL FISH

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A proper understanding of the toxicity of a compound often starts with detailed knowledge of its uptake and elimination kinetics. There are several theoretical models to describe this behavior of chemicals in fish, however, these models are only validated for chemicals with high octanol-water partition coefficients (Kow) (log Kow > 3.4). The purpose of this study was to validate these models for compounds with log Kow as low as 1.25. Static exposure of the fish is via a gas-uptake chamber containing water and air. The mathematical description of this system uses the following assumptions, a) instantaneous distribution of chemical between water and air, and b) a first order one-compartment model describes the kinetics of the chemical in fish. Uptake and elimination rate constants (k1 and k2, respectively) were determined separately for dichloromethane (log Kow = 1.25), 1,1,1-trichloroethane (log Kow = 2.49), and 1,3-dichlorobenzene (log Kow = 3.60). The kinetic constants were determined in two species of fish, rainbow trout (Oncorhynchus mykiss) and fathead minnow (Pimephales promelas), using animals of similar size. Differences in the rate constants were not observed between species. Uptake rate constants increased with increasing octanol-water partition coefficient, whereas the elimination rate constants were inversely related to Kow. The data indicate that a linear model adequately describes the latter relationship.

919 EFFECT OF METHYL tert-BUTYL ETHER AND NAPHTHALENE ON THE DEVELOPING EGGS OF FUNDULUS HETEROCLITUS, AN ESTUARINE FISH

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Methyl tert-butyl ether (MTBE) is used to increase the oxygen content of fuels. Levels of MTBE in gasoline in some areas of the United States can be as high as 15%. Studies were carried out to examine the toxic effects of MTBE, naphthalene (a toxic, water soluble component of gasoline), and mixtures of MTBE and naphthalene on *F. heteroclitus* embryos. Early in development, the eggs of *F. heteroclitus* were exposed to naphthalene at 6.0 mg/L, 0.6 mg/L, and 0.06 mg/L; MTBE at 300 mg/L and 30 mg/L; and concomitantly to naphthalene and MTBE. Each treatment group had 25 eggs which were exposed and monitored individually. No deaths occurred before day 14 (late fetal stage). The highest mortality (24%) occurred in the group exposed to 6.0 mg/L naphthalene. MTBE was shown in a previous experiment to cause 100% death at 3 g/L. The primary lesions seen in the embryos exposed to naphthalene alone were hemorrhage, edema, and hypopigmenation of red blood cells. These lesions were also observed upon exposure to lethal concentrations of MTBE. When the eggs were exposed to combinations of MTBE and naphthalene, the toxic effects from the naphthalene were not increased. (Supported by NIEHS ESO7148 and Rutgers/NOAA CMER Program).

920 ACUTE AND CHRONIC TOXICITY OF ALKYLAMINES TO CERIODAPHNIA DUBIA AND RAINBOW TROUT

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The acute and chronic toxicity of ethylamine, diethylamine, triethylamine and butylamine to *Ceriodaphnia*, the acute toxicity of dibutylamine to *Ceriodaphnia*, and the acute toxicity of methylamine and triethylamine to rainbow trout (*Oncoryhynchus mykiss*) were evaluated in a series of static renewal (*Ceriodaphnia*) or flow-through (rainbow trout) studies. Exposure concentrations were verified by HPLC analysis after derivatization of primary and secondary alkylamines with 7-chloro-4-nitro-2,1,3-benzoxadiazole (NBD-Cl), and direct injection using cation exchange and conductivity detection for triethylamine. In the static renewal studies, test solutions were renewed every 24 hours and were analyzed at 0, 24 and 48 hours (acute studies), and additionally at 7 days in the chronic studies. Rainbow trout flow-through studies were analyzed at 0 and 96 hours. The *Ceriodaphnia* 48-hour LC_{50} and 7-day maximum acceptable toxicant concentration (MATC) were, respectively; ethylamine (7.9 and 4.4 ppm); diethylamine (4.6 and 2.4 ppm); triethylamine (17 and 10 ppm); and butylamine (8.2 and 4.3 ppm). The *Ceriodaphnia* 48-hour LC_{50} for dibutylamine was 8.4 ppm, and the rainbow trout 96-hour LC_{50}'s for triethylamine and methylamine were 38 and 9.6 ppm, respectively. These results showed that low molecular weight alkylamines were only slightly to moderately toxic to aquatic species, and importantly, no marked cumulative toxicity was observed as demonstrated by the *Ceriodaphnia* acute to chronic toxicity ratios (48 hour LC_{50}/7-day MATC) of less than 2.

921 HEPATOCARCINOGENESIS IN MOSQUITOFISH (*GAMBUSIA AFFINIS*): ASSESSING CELL PROLIFERATION, p53, AND DNA ADDUCTS INDUCED BY ALKYLATING AGENTS

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A need exists for fish sentinel species for monitoring environmental changes. These studies were performed to examine the suitability of western mosquitofish (*Gambusia affinis*) as a sentinel species. Neoplastic lesions were reduced in laboratory-reared mosquitofish fry and adults using single pulse exposures to 1 or 10 ppm methylazoxy-methanol acetate, a model alkylating agent. Histopathology, EM, and immunohistochemistry were performed on groups of fish killed at 8 intervals from 24 h to 41 wk post-exposure. Neoplastic changes were found in the liver in approximately 20% of the exposed fish by 8 wk post-exposure and increased to approximately 70% by 41 wk; these included altered foci, hepatocellular carcinomas and cholangiocarcinomas. Other changes included widespread apoptosis, overexpression of p53 in approximately 38% of the tumors, and significantly increased cell proliferation index as measured by PCNA immunohistochemistry. Tumors were found in less than 1% of controls. Levels of the DNA adducts O^6- and N^7-methylguanine were measured in the liver in additional alkylating agent exposures using reverse isotope dilution-GC/mass spectrometry. Adapted for small fish species, this method gave adduct detection limits at the femtomole level. O^6-methylguanine adducts at 10 pg/μg of DNA were found at dose levels that induced tumors. The carcinogen sensitivity, biology, and wide range of this fish appear to indicate excellent potential for its use as a warmwater sentinel species in biomarker studies. (Supported in part by a U.S. Department of Energy grant.)

922 TOXICITY OF PESTICIDES TO THE PLANARIAN *DUGESIA DOROTOCEPHALA*

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Planaria are a valuable aquatic invertebrate model for investigation of chemical toxicity and are potentially useful for environmental biomonitoring studies. These studies provide toxicity data for a variety of pesticides in planaria. Mature asexual *Dugesia dorotocephala* were exposed to the agents in reconstituted water for 14 days. Exposure solutions were prepared and changed daily. Minimum concentrations causing 100% mortality were estimated for DDT (2 ppm), DDE (10 ppm), kelthane (2 ppm), dieldrin (1 ppm) and endosulfan (2 ppm). For the fungicides chlorthalonil, dichloran, and benomyl, the values were 2 ppm, 50 ppm, and 250 ppb, respectively. The toxicity of a series of triazine herbicides tested appeared to be directly related to the water solubility of the agents. In another study, the toxicity of a commercial formulation of carbaryl was compared with that of the pure compound. Exposure of planaria to 2 ppm carbaryl diluted from the formulation caused 50% mortality whereas none resulted after treatment with pure carbaryl at 10 ppm. These results illustrate the important influence that "inert ingredients" included in pesticide formulations can have on toxicity to aquatic species.

923 MULTIVARIATE ANALYSIS OF AQUATIC TEST CONDITIONS AFFECTING THE TOXICITIES OF FOUR METALS TO THE NEMATODE *CAENORHABDITIS ELEGANS*

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The *Caenorhabditis elegans* aquatic toxicity test shows promise for use as part of a standard bioassessment protocol, yet no widely accepted standardized test procedures exist. This allows for possible inconsistent results due to varied test conditions. A multivariate approach was undertaken to determine possible sources of variability in acute and chronic responses to four metals (Cd, Pb, Cu, and Hg). Specifically, the presence or absence of a bacterial food source, the presence or absence of background ions in the test solution, and the developmental stage or history of the nematodes were all examined for their effects on mortality, effects on development, and reproduction in the presence of each of the four metals at various concentrations. It was found that a lack of background ions universally increased sensitivity to the toxicants, while other factors were less important in tests. Differential responses both within and among the experimental blocks were noted for all three endpoints. Recommendations for future standardization of protocols were determined.

924 TOXICOLOGICAL IMPLICATIONS OF MICROSOMAL ENZYME INDUCTION IN THE DEVELOPMENT OF NOVEL COMPOUNDS: INTRODUCTION

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Induction of the cytochromes P450 is a common finding during pre-market evaluation of novel compounds. Over the past several years there has been an explosion of information on the biochemistry of P450's at both the gene and protein level. There is, however, a paucity of data on the significance of microsomal induction in experimental models as it relates to potential human exposure. All too frequently conclusions about the significance of induction are made on inadequate or erroneous information leading to decisions that may inappropriately result in discontinuation of a promising compound or visa-versa. Currently, within the pharmaceutical industry, there is no systematic approach to the development of inducers. Some companies refuse to develop inducers while others don't even routinely screen for the effect. The primary focus of the workshop will be on the development of drugs, but the nature of the questions being addressed will give the workshop a broader scope. The emphasis will be on providing practical information about the toxicologic implications of microsomal induction and its relevance to humans. Topics covered will include induction and carcinogenesis, the significance of isozyme profiles and the utility of *in vitro* and transgenic models.

925 INTERSPECIES TOXICOLOGY: MOLECULAR BASIS OF DIFFERENTIAL RESPONSE

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Recently, attention has been focused on the differences that exist in interspecies responses to toxicant exposures. The objective of this symposium is to present timely examples wherein a molecular approach has been used to evaluate toxicant response in animal models and how data from these models can be extrapolated to other species, including humans. Information has begun to emerge that key enzyme systems involved in activation and detoxification of chemicals and their regulation differ at the molecular level in different species, leading to difficulties in the interpretation of toxicity data when extrapolating across species. The issue of how the metabolism of compounds can act as a determinant of toxicity in various species will be addressed by the first speaker. Many animal models utilizing recoverable transgenes have been used to study genetic damage induced by toxic agents. In the second talk, the relationship between genotoxicity data obtained in transgenic animals of different species and the disadvantages and advantages of the various transgenic model systems available will be addressed. The third speaker will cover specific examples of carcinogen specificity in the spectrum of ras family oncogene mutations in various species and tumor types. The last of the four speakers will discuss issues relating to the use of molecular techniques to establish the relevance of animal models to the human disease, using breast cancer as a paradigm for extrapolating carcinogenicity data across various species.

926 XENOBIOTIC METABOLIZING ENZYMES: SPECIES DIFFERENCES IN GENE STRUCTURE AND REGULATION

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Investigators interested in drug development and toxicology are hampered by ethical issues limiting their ability to conduct studies in their ultimate target subject, i.e. human. As such, any one of several animal models are generally used. Given that most xenobiotics are metabolized to a significant degree, the desire to be able to choose the best animal model has served as an impetus for the study of species-specific xenobiotic metabolism. With the elucidation of over 200 cytochrome P450 cDNA and/or gene sequences, it is clear that some species-specific metabolism is due to the divergence of a particular gene family from a single ancestral gene post-speciation. This is particularly true for the cytochrome P4502 gene family and one or more examples will be discussed. However, species differences in the regulation of orthologous genes also appears to be involved. For example, flavin-containing monooxygenase (FMO) 1 is the predominant enzyme expressed in rabbit liver whereas FMO3 is the predominant hepatic enzyme in the human. The underlying mechanism for this difference has yet to be determined. Our laboratory has carried out an extensive study on the negative regulation of *CYP1A1*, which also appears to be species specific. Although the *trans*-acting factor mediating the negative regulation of this gene appears conserved between the rodent and human, its *cis*-responsive element is present on the human *CYP1A1* gene, but not the orthologous rodent genes. This observation may explain some of the species specific expression patterns observed for this particular enzyme. Such studies have led to a much greater understanding of species differences in metabolism. This knowledge should be useful in the selection of optimal animal models and in the extrapolation of animal data to the human.

927 MOUSE VERSUS RAT: CROSSING THE SPECIES BARRIER IN TRANSGENIC MUTATION ASSAYS

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The relationship between the *in vivo* response to chemical exposures in one species is not necessarily reflective of exposures in a related species. Carcinogenesis testing has revealed differential responses in such similar species as mice and rats. The inclusion of multiple species in the rodent bioassay has increased detection of carcinogens that may have gone undetected using only one species. As with carcinogenesis, mutagenesis can be affected by many species specific factors, including metabolic activities, cell turnover rates, DNA repair rates, tissue sensitivity, etc. The application of multi-species mutagenesis testing will provide information about similar as well as differential genotoxic responses to mutagenic compounds and perhaps offer insight into species specific mutational initiation of carcinogenesis. Comparative *in vivo* genotoxicity testing in multiple species is possible using the lambda/*lacI* transgenic mutation assay. Transgenic mice (C57BL/6 and B6C3F$_1$) and rats (Fischer 344) carrying the recoverable *LacI* marker gene have been used to measure differential spontaneous and induced mutant frequencies in multiple tissues. This comparative approach to assessing genetic toxicity in identical transgenic target genes may enhance the accuracy of interspecies extrapolations and more closely estimate the risk of human exposure.

928 SPECIES VARIABILITY AND CHEMICAL SPECIFICITY OF *ras* ONCOGENE ACTIVATION

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Among the most common molecular events in spontaneous and chemically-induced rodent tumors, and in human tumors, is mutational activation of the *ras* gene. The mutation frequency in *ras* can vary markedly from one species to another. For example, the mutation frequency in the K-*ras* oncogene of mouse, human and rat lung adenocarcinomas varies from >90%, >30% to <5% of tumors, respectively. The molecular basis for these differences in mutation frequency is not understood. Investigations in strain A/J mice have shown that carcinogens induce specific mutations in codons 12, 13 or 61 of the K-*ras* oncogene in lung tumors that coincide with the profile of adducts they produce in lung DNA. The known variation in lung tumor susceptibility in different mouse strains appears to be due to a heritable 37 base pair deletion in the second intron of the K-*ras* gene. Thus, in mice, two molecular events in the K-*ras* gene; *i.e.*, an inherited deletion and a point mutation in codons 12, 13 or 61, appear to be important for lung tumor development.

929 COMPARATIVE MAMMARY CARCINOGENESIS IN THE RAT AND MOUSE

S Sukumar, K McKenzie, Y Chen. *Oncology Center, The Johns Hopkins University school of Medicine, Baltimore, MD.* Sponsor: C Walker

Rodent mammary tumors induced by chemical carcinogens have proven to be very useful in the genetic analysis of initiation, promotion and progression of mammary carcinogenesis. We are studying rat and mouse mammary carcinomas induced by the chemical carcinogens, nitroso-methyl urea and 7, 12-dimethylbenz(a)anthracene.The rat mammary gland is susceptible to chemical carcinogens as early as the first week after birth. The mouse mammary gland remains refractory to carcinogens until induced to branch out by using exogenous prolactin. Also, the mouse mammary gland progresses to carcinoma through an easily identifiable preneoplastic stage of either hyperplastic alveolar nodules or ductal hyperplasia. The earliest genetic event observed in the mammary gland of carcinogen treated rodents is the activation of Ha-*ras* oncogenes. The *ras* oncogene containing initiated mammary gland cells remain quiescent until they are subjected to the promotional influence of estrogenic hormones during puberty. The influence of hormones, and diet during the time of initiation and promotion on the incidence and the type of tumors induced is profound. Progression events in mammary cancer include loss of hormone dependence and metastasis to local and distant sties. Genetic analysis of these tumors reveal the involvement of H-*ras* oncogenes, IGF2, PRAD-1, and MK genes during tumor progression. Thus, comparative studies in two rodent species helps us to gain insight on the influence of physiological factors on the genetics of multistep mammary carcinogenesis.

930 MECHANISMS OF INFLAMMATORY LIVER INJURY: ADHESION MOLECULES AND CYTOTOXICITY OF NEUTROPHILS: INTRODUCTION

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During recent years, increasing experimental evidence suggested that hepatic nonparenchymal cells, in particular Kupffer cells and neutrophils, can contribute significantly to the pathogenesis of liver injury in various chemical toxicities. Indeed, neutrophils are central to the mechanism of injury after hepatic ischemia-reperfusion and endotoxemia. In this symposium, an overview of critical aspects of neutrophil-dependent liver injury will be presented. Molecular mechanisms of neutrophil arrest, adhesion and stimulation in the microvasculature will be discussed. Cytokines such as TNF-α and IL-1 as well as lipid peroxidation products play important roles in neutrophil recruitment and activation. Mechanisms by which activated neutrophils injure rat hepatocytes will be considered. Interactions between neutrophils and contractile, perisinusoidal, fat-storing cells and their role in hepatic microvascular failure will be explored. Results of these and other investigations are leading to increased understanding of the complex interactions between neutrophils and tissues that result in injury.

931 MECHANISMS OF NEUTROPHIL ROLLING AND TRANSENDOTHELIAL MIGRATION

C W Smith. *Baylor College of Medicine, Houston, TX.* Sponsor: H Jaeschke

Neutrophils must resist the shear forces of flowing blood in order to localize at sites of inflammation. In post-capillary venules, they accomplish this through a sequence of adhesive and activation steps involving at least 3 gene families of adhesion molecules. On cytokine-stimulated endothelium in flow chambers, the initial tethering and rolling events under physiologic shear involve all three members of the selectin family each in a unique spatial and temporal context. The arrest of rolling neutrophils apparently requires chemotactic activation (e.g., IL-8), rapidly signaling (within seconds) both confirmational changes in leukocyte integrins (CD11a/CD18; CD11b/CD18) that promote binding to endothelial ligands (e.g., CD54), and downregulation of the selectin-dependent adhesion. Following arrest, adherent neutrophils are markedly shear resistant and migrate through the endothelial monolayer within ≈5 min, requiring chemokinesis, leukocyte integrins and CD54 (ICAM-1) on the endothelium. Transmigrated neutrophils exhibit markedly increased production of reactive oxygen, and adhesion is an important co-signal. CD11b/CD18 interactions with CD54-expressing parenchymal cells are particularly effective and may be cytotoxic. Thus, the cascade of events that allow neutrophil localization and cytotoxic activity provide several potential points for therapeutic interventions such as blocking key adhesion molecules or activating stimuli.

932 MECHANISMS OF NEUTROPHIL-INDUCED LIVER INJURY *IN VIVO*

H Jaeschke. *The Upjohn Company, Kalamazoo, MI*

Neutrophils contribute to liver injury during ischemia-reperfusion, endotoxin shock and certain drug toxicities *in vivo*. Inflammatory mediators such as TNF-α, complement factors (C_{5a}) and injury products cause the initial accumulation of neutrophils in the hepatic microvasculature (sinusoids), however, extravasation and binding to hepatocytes is necessary for significant hepatocellular injury to occur. Monoclonal antibodies against the β_2 integrin Mac-1 (CD11b/CD18) on neutrophils and its counterreceptor intercellular adhesion molecule-1 (ICAM-1) do not inhibit neutrophil accumulation in the vasculature but reduce extravasation and adherence-dependent reactive oxygen formation and thus protect against neutrophil-induced liver injury. Upregulation of Mac-1 *in vivo*, i.e. increased expression of preformed receptors on the surface of neutrophils, can be induced by complement and TNF-α within minutes after mediator formation. Enhanced expression of ICAM-1 on endothelial cells and the new expression on hepatocytes involves de novo protein synthesis and thus requires several hours. ICAM-1 mRNA is regulated *in vivo* by TNF-α and IL-1. After the initial activation by cytokines and complement, injury products, e.g. lipid peroxidation products, become mainly responsible for further neutrophil recruitment and activation. The emerging complex network of inflammatory mediators and adhesion molecules involved in the pathogenesis of neutrophil-induced liver injury offers multiple possibilities for therapeutic interventions.

933 MICROCIRCULATORY FAILURE IN THE LIVER: NEUTROPHIL PLUGGING AND SINUSOID CONTRACTION

M G Clemens. *The Johns Hopkins School of Medicine, Baltimore, MD.* Sponsor: H Jaeschke

Neutrophils (PMN) accumulate in the liver during inflammatory states and inhibition of this accumulation attenuates injury; however, intravital microscopy studies show that the areas of microvascular shutdown do not correlate spatially with the sites of neutrophil infiltration and PMNs are rarely observed plugging sinusoids. Very recent evidence shows that active contraction of the sinusoids mediated by the perisinusoidal fat storing cells (FSC) results in sinusoidal narrowing and disruption of normal flow patterns. Contraction of the FSCs can be stimulated by endothelin or constrictor eicosanoids which are upregulated under conditions in which PMNs accumulate and may be released from the marginated PMNs suggesting that PMN effects on the liver microcirculation may be mediated by release on vasoactive substances. This effect may be exacerbated during chronic inflammation since inflammatory cytokines stimulate phenotypic transformation of the FSCs to myofibroblasts resulting in increased contractile capacity. FSC contraction is inhibited by nitric oxide which is induced in the liver under some inflammatory conditions but its distribution is patchy and may be further decreased by neutrophil-derived superoxide giving rise to focal sinusoidal shutdown and patchy necrosis. The sinusoidal narrowing further enhances trapping of PMNs in the sinusoids. Thus the contribution of PMNs to microvascular injury in the liver are exerted via direct toxicity plus stimulation of the release of vasoactive substances that act directly on the sinusoids to cause constriction and, ultimately microvascular failure.

934 MECHANISMS OF NEUTROPHIL-MEDIATED HEPATOCELLULAR INJURY *IN VITRO*

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Neutrophils (PMNs) arriving at a site of inflammation are armed with multiple mechanisms by which they can contribute to tissue injury directly or indirectly. These include release of reactive oxygen species, proteolytic enzymes, cytokines, and arachidonic acid metabolites. To investigate mechanisms important in PMN-mediated injury to liver, studies were performed *in vitro* using cocultures of isolated, rat hepatocytes (HCs) and PMNs. Initial studies revealed that PMNs activated with either f-met-leu-phe (fMLP) or phorbol ester (PMA) damaged HCs and that toxicity caused by PMA but not fMLP required cell contact. The mechanism does not involve reactive oxygen species or nitric oxide, but it is mediated in part by proteases, the physical and chemical properties of which have been characterized. The roles of tumor necrosis factor and glutathione status have also been investigated. The consequence of PMN activation is influenced by hepatocellular defense mechanisms, and HCs inhibit superoxide anion production by PMNs. The mechanism of this effect as well as potential contributions of PMN activation to chemically induced liver injury will be discussed. (Supported by ES05722 and ES04911.)

935 LEAD INHIBITS NITRIC OXIDE PRODUCTION BY MURINE MACROPHAGES

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The heavy metal lead is an environmental toxicant that can compromise host defense systems and induce pathophysiological changes in many organ systems. We investigated whether Pb can affect nitric oxide (NO) production by macrophages (Mϕs), because NO has been suggested to play important roles in Mϕ-mediated defense against various pathogenic microorganisms and tumor cells. Low levels of $PbCl_2$ (Pb) were observed to effectively suppress NO production by murine splenic Mϕs. Since soluble and surface membrane factors from activated $CD4^+$ T helper cells are the primary regulators for NO production by Mϕs, we further assessed the susceptibilities of the pathways triggered by some of the T cell factors. NO production induced by soluble T cell cytokines, interferon-γ (IFN-γ) and tumor necrosis factor-α was readily blocked by low levels of Pb; whereas, NO production enhanced by surface molecules of activated T helper cells was less sensitive to the inhibition by Pb. Moreover, the status of Mϕs influences the cell's susceptibility to Pb, in that RAW cells, a murine Mϕ cell line, which are in a more advanced stage of differentiation than normal splenic Mϕs based on their phenotypic and functional characteristics, are differentially modulated by cytokines and Pb. IFN-γ or bacterial lipopolysaccharide (LPS) alone was able to induce substantial NO production by RAW cells. NO production by RAW cells was less sensitive to Pb inhibition than splenic Mϕs, and the RAW cell response to IFN-γ was less sensitive to Pb than its response to LPS. Unlike splenic Mϕs, the inhibition of RAW cells by Pb was overcome by high doses of IFN-γ or the combination of IFN-γ and LPS. These phenomena suggest that NO production by Mϕs in particular stages of differentiation have different sensitivities to Pb. Together, our results provide an explanation for some of the Pb-induced abnormalities in host defense. (Supported by ES03179).

936 *IN VITRO* EFFECTS OF ORGANOPHOSPHATES ON REACTIVE OXYGEN AND NITROGEN SPECIES PRODUCTION OF MACROPHAGES

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Organophosphates (OP) are used extensively in agriculture and in industry. Although the neurotoxicity of OP is well established, their immunotoxicity is not well known. We have previously shown that various species of OP (tri-o-tolyl phosphate, triphenyl phosphate, triphenyl phosphite, triphenylphophine oxide, and diphenyl phosphate) decrease the ability of murine peritoneal elicited macrophages to kill the opportunistic pathogen *Candida albicans*, while showing minimal effects on the phagocytic ability *in vitro*. In this study we have investigated the *in vitro* effects of OP on the production of superoxide, hydrogen peroxide, and nitric oxide by elicited and resident murine peritoneal

macrophages activated with IFN-γ and/or LPS. Elicited and elicited, IFN-γ activated macrophages, but not LPS activated macrophages exposed to OP exhibited decreased levels of all these oxygen species. Oxygen radical production by resident macrophages was slightly reduced in OP-treated unactivated cells. The levels of oxygen species produced correlated with the dose-response curves for candidacidal activity for both elicited and resident macrophages. This may suggest that OP adversely affects the production of reactive oxygen species that are required for killing *C. albicans* efficiently.

937 NITRITE PRODUCTION OF PERITONEAL MACROPHAGES AFTER *IN VIVO* AND *IN VITRO* EXPOSURE TO DEXAMETHASONE

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The anti-inflammatory action of dexamethasone *in vivo* has been attributed to the suppressive action of glucocorticoids on the macrophage production of reactive nitrogen intermediates (RNI) *in vitro*. However, the effects of dexamethasone administered *in vivo* and the resulting production of RNI from peritoneal macrophages *in vitro* has not been investigated. In this study, peritoneal macrophages from B6C3F1 female mice were isolated and examined for RNI production *in vitro* after exposure to dexamethasone *in vivo*(0.1–30.0 mg/kg/day) for 3, 6, or 16 days. In addition, normal peritoneal macrophages were exposed to dexamethasone *in vitro* at comparable concentrations expected after an *in vivo* exposure. For each exposure regimen, nitrite production was measured in response to interferon-γ (IFN-γ), and/or bacterial lipopolysaccharide (LPS). After *in vivo* exposure to dexamethasone, macrophages produced an equal or greater concentration of nitrite than control macrophages after stimulation with IFN-γ, LPS or IFN-γ and LPS. In contrast, continuous exposure of macrophages to dexamethasone during stimulation *in vitro* caused dose dependent inhibition of nitrite production. However, the inhibition was much less pronounced when LPS and IFN-γ together were used to stimulate the macrophages than when either was used separately. Similar results were observed after *in vitro* exposure to dexamethasone for 24 or 72 hours followed by a 0–24 hour recovery period. Consequently, immunosuppressive doses of dexamethasone *in vivo* did not decrease the production of nitrite 24 hours after the last dose, whereas nitrite production was suppressed 24 hours after the last exposure to dexamethasone *in vitro*. There is no evidence that a "glucocorticoid resistant" population of macrophages are selected for *in vivo*. However, there may be other cytokines and/or other stimulants that alone or in combination may attribute to the maintenance of nitrite production of peritoneal macrophages *in vivo*. These observations have implications with regard to the mechanism of glucocorticoid-mediated anti-inflammatory and immunosuppressive action *in vivo*.

938 ULTRAVIOLET LIGHT INHIBITS NITRIC OXIDE PRODUCTION BY PAM 212 MURINE KERATINOCYTES

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The biological effects of ultraviolet light in the UVB range (290–320 nm) on the skin are of concern because exposure is thought to be a causative factor in the development of cancer. UVB irradiation has been shown to induce systemic immunosuppression and inhibit tumor rejection. The mechanisms underlying immunomodulation initiated by UVB light are not well understood. However recent studies indicate that keratinocyte responses to UVB are important in the generation of immunosuppression. Cytokine induction of reactive nitrogen species is critical in immunological responses to tumor cells, as well as during inflammation. We found that PAM 212 keratinocytes respond to the inflammatory cytokine γ-interferon by producing nitric oxide. Nitric oxide production these cells was dependent on the presence of 1-arginine in the cell culture medium and was inhibitable by the nitric oxide synthase inhibitor n-monomethyl l-arginine. Exposure of keratinocytes to UVB light in the presence of γ-interferon inhibited the production of nitric oxide. This effect was dose related in a range of 1–100 mJ/cm^2 UVB. Inhibition of nitric oxide production by UVB light may be one mechanism by which keratinocyte immunological responses are altered by this xenobiotic. (Supported by NIH grant ES 03647.)

939 MECHANISMS OF ULTRAVIOLET LIGHT-INDUCED SUPPRESSION OF KERATINOCYTE NITRIC OXIDE PRODUCTION

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Irradiation of the skin with ultraviolet light from the UVB region of the solar spectrum (290–320 nm) is thought to be a causative factor in the development of cancer as well as a mediator of systemic immunosuppression. Recent studies by our laboratory have demonstrated that keratinocyte production of nitric oxide, a reactive intermediate critical to immune functions, is inhibited by UVB exposure. In mammalian cells nitric oxide is produced by nitric oxide synthases. This family of enzymes is comprised of cytokine inducible as well as constitutively expressed isoforms. Interestingly, in keratinocytes nitric oxide production is stimulated by inflammatory cytokines, whereas nitric oxide synthase is constitutively expressed, suggesting the enzyme is regulated post translationally. To determine if UVB acts to suppress nitric oxide production by direct action on nitric oxide synthase or through alterations to its regulatory pathway, we purified keratinocyte nitric oxide synthase using ion exchange and gel filtration chromatography followed by chromatofocusing. The enzyme readily converted arginine to citrulline in an oxygen dependent manner, displayed a sharp pH maximum of 7.5, and was heat labile. The Km for l-arginine was 4 μM. Inhibition of enzyme activity by carbon monoxide confirmed a heme containing center. Enzyme activity was inhibited by structural analogs of l-arginine including n-monomethyl l-arginine and n-nitro l-arginine, as well as by several imidazole analogs. Although nitric oxide production by intact keratinocytes was inhibited by UVB irradiation (30 mJ/cm^2), treatment of lysates had no effect on enzyme activity. Taken together our data suggests that UVB regulation of the isoform of nitric oxide synthase expressed by keratinocytes is post-translational.

940 SERINE, BUT NOT GLYCINE OR CALCIUM CHANNEL BLOCKERS, ATTENUATES 2-METHOXYETHANOL INDUCED THYMIC APOPTOSIS

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2-Methoxyethanol(2-ME) is a water-miscible organic solvent that is toxic to the testes, the embryo, the lymphoid and hematopoietic tissues. Many questions persist about the mechanism of 2-ME toxicity to these target sites. However, simple physiological compounds (eg, serine and glycine) attenuate the embryo and testicular toxicity of 2-ME while calcium channel blockers provide protection against 2-ME induced testicular toxicity. We have recently detected rapid induction of thymic apoptosis in rats treated with 2-ME. Our objective was to determine whether the simple physiological compounds D-serine and glycine or the calcium channel blockers verapamil and diltiazem would attenuate 2-ME induced thymic apoptosis. Adult male Sprague-Dawley rats (370–410 g) were pretreated with saline, D-serine, glycine, verapamil or diltiazem thirty minutes prior to 2-ME (500 mg/kg, p.o) and sacrificed at three hours. Thymus sections were stained with methylene blue. Morphological changes characteristic of apoptosis were detected in thymus sections from animals given 2-ME or calcium channel blockers. Only pretreatment with D-serine diminished the extent of 2-ME induced apoptosis. *Conclusion:* 2-ME induced thymic apoptosis can be attenuated by D-serine but not by calcium channel blockers.

941 SUSCEPTIBILITY OF HUMAN T CELLS TO MERCURY INDUCED APOPTOSIS IS DEPENDENT ON GLUTATHIONE CONTENT

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The goal of this investigation was to examine the cytotoxic properties of low levels of $HgCl_2$ and MeHgCl on human T cell viability. Cells were incubated with varying concentrations of mercurial compounds and viability was assessed by propidium exclusion. Minimal reduction in T cell viability was noted 1–4 hours after treatment. However, after exposure to mercurials for 24 hours, cell death was apparent. Electron microscopy and other assessments of cells revealed early nuclear alterations characterized by hyperchomaticity, nuclear fragmentation and condensation of nucleoplasm. There was a concomitant increase in intracellular levels of calcium. To further explore the mechanism of cell death, we measured the glutathione-glutathione disulfide ratio and the energy charge ratio. We found that mercury induced depletion of glutathione without changing the energy charge ratio. Moreover susceptibility to the cytotoxic effects of mercury was directly related to intracellular glutathione levels.

We conclude that these and other observed alterations are consistent with the notion that mercury initiates cytotoxic changes consistent with programmed cell death.

942 METHYLMERCURY POTENTIALIZES APOPTOSIS INDUCED BY DEXAMETHAZONE IN MOUSE BONE MARROW CELLS

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Mouse strains with H-2^s, but not H-2^b haplotype, are susceptible to autoantibody development following mercury treatment. We have recently observed an abnormal stimulation of bone marrow cells and B lineage cells in SJL (H-2^s) and C57BL/6 (H-2^b) mice treated with various doses of methylmercury (MeHg). To determine the mechanisms involved in such stimulation, we studied the *in vitro* effect of low doses of MeHg on total, adherent and nonadherent bone marrow cells. We observed a slight stimulation of metabolic activity, as measured by the tetrazolium salts uptake, of bone marrow marrow adherent cells with the lower dose of MeHg. At higher doses, according to mouse strain, inhibitory effects were observed in adherent and nonadherent bone marrow cells. Cytofluorometric analysis of incorporation of propidium iodide in total bone marrow cells treated with various doses of MeHg revealed the presence of DNA fragments with hypodiploid suggesting induction of programmed cell death (apoptosis) by MeHg. However, lower levels of apoptotic cells occurred in MeHg treated bone marrow cells. In contrast, levels of apoptotic cells were potentialized by MeHg in dexamethazone-treated bone marrow cells. Levels of apoptotic bone marrow cells differed in SJL and C57BL/6 mice. Such MeHg toxic effects were also evidenced in bone marrow B lineage cells. These results suggest that abnormal stimulation of B lineage cells may be related to disorders in the normal apoptotic process during B cell maturation. (Supported by CRSNG grant).

943 INTERLEUKIN-2 PROTECTS MURINE T-LYMPHOCYTES FROM GLUCOCORTICOID-INDUCED APOPTOSIS BY ALTERING TRANSCRIPTIONAL EVENTS

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In this work, we have investigated the ability of interleukin-2 (IL-2) to rescue CTLL-2 cells, an IL-2 dependent murine T-cell clone, from either dexamethasone (DEX; methyl in position 16α), betamethasone (BM; methyl in position 16β) or triamcinolone (TRIAM; hydroxyl in position 16)-induced programmed cell death. Using a quantitative DNA fragmentation assay, we showed that higher concentration of IL-2 were required to protect CTLL-2 cells against DEX-induced apoptosis as compared to BM or TRIAM. Then, we demonstrated, using transient transfection of MMTV-CAT plasmid (containing glucocorticoid-responsive elements; GRE), that the transcriptional activity of glucocorticoid-receptor (GC-GR) complexed to DEX was higher than the one induced by BM. Indeed, IL-2 was able to repress this transcriptional activity. We are currently investigating the role of AP-1 (Fos-Jun) in its ability to interact with GR-GC, since it has been shown that IL-2 was able to induce AP-1.

944 IDENTIFYING THE NOAEL IN CATEGORICAL TOXICITY DATA: A STATISTICAL APPROACH

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A No-Observed-Adverse-Effect-Level (NOAEL) for toxicity of an agent is determined from both biological and statistical considerations. The statistical part of the problem requires estimating the maximal dose without increased severity or frequency of toxic response. A method based on the Akaike Information Criterion (AIC) has been previously proposed by the current authors for the case with only two response levels (presence or absence of a specific endpoint) (*EHP, 102, 95–101, 1994*). It is shown here that repeated applications of that method to suitably defined subsets of data provide the maximum likelihood estimate of the NOAEL for each severity level in the more general case of categorical data, where the model assumed for ordinal categorical data is a slight modification of the continuation-ratio logit model. Several examples of data from controlled exposure of rodents illustrate the method using both conditional and unconditional maximum likelihood.

945 APPLICATION OF THE BENCHMARK DOSE TO NONCANCER EFFECTS OF TRICHLOROETHYLENE EXPOSURE

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Reevaluation of the risk assessment of trichloroethylene (TCE) to better account for the evolving understanding of its activity as a rodent carcinogen, will also require revisiting other toxicities with which this chemical is associated. The toxicity that would limit exposure depends upon the methods used to derive dose-response values. TCE has been tested for a wide range of effects over a wide range of exposure conditions. Effects studied have included anaesthetic and other neurological changes, reproductive and developmental toxicity, and toxicity to liver and other organs. Some of these effects are highly species dependent (i.e., kidney toxicity) while others occur across species. Relatively few studies included multiple dose levels. Those that did were evaluated using statistical models to determine a best fit. From this a benchmark dose (BMD) corresponding to a selected increase in response was chosen. The statistical lower bound was determined that could be used to replace the traditionally used no-observed-adverse-effect-level (NOAEL). These results are compared with the traditional NOAEL/Uncertainty Factor approach. Limitations of data quality is one of the most difficult problems even for this extensively studied chemical.

946 COMPARISON OF SOME METHODS FOR ESTIMATING ORAL DOSES FOR BORON (B) REGULATORY PURPOSES

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The US EPA must set regulatory levels for chemicals that will protect public health. For noncarcinogens, these levels are usually based on an estimate of a "safe" or nontoxic exposure dose. The current approach to this problem is to derive an oral reference dose (RfD) by selecting a no-observed-adverse-effect level (NOAEL) and dividing by one or more uncertainty factors (UFs). Based on this approach the current RfD for B is 0.09 mg/kg-day. A suggested alternative to the current approach is to estimate a Benchmark dose (BMD) and divide by one or more UFs to derive the RfD. A BMD for B, of 9.3 mg/kg-day was estimated using a polynomial model and was defined as the lower 95% confidence limit on the dose that causes a 5% decrease in fetal body weight. Dividing this BMD by a UF of 100 gives an RfD of 0.09 mg/kg-day. Using a categorical regression (CR) approach it is possible to calculate the probability of an adverse effect above the RfD. For B a CR of reproductive and developmental data suggests an estimate of 0.25 for the probability that a dose group would exhibit an adverse effect at 10-fold above the RfD. The use and limitations of each approach will be discussed. This does not necessarily reflect EPA policy.

947 USE OF STRATIFIED ORDINAL REGRESSION FOR COMBINATION OF TOXICOLOGICAL DATA AND MODEL SELECTION

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Categorical regression analysis of toxicological data for exposure-response assessment allows studies to be combined by analyzing different quantitative measurements categorized on the basis of severity. A stratified regression model addresses identifiable systematic differences between studies (such as different species, strains, or sexes) by allowing some model parameters to vary between strata. Evaluation of a hierarchy of models with different levels of stratification provides a means for testing the significance of differences between strata in potency or duration-dependence. The analysis is used to identify sensitive endpoints or species, to determine the appropriateness of combining data, and to select the most appropriate model. This analytic approach may also be used to evaluate the sensitivity of the analysis to inclusion of specific studies, application of interspecies dosimetric adjustments, or the designations of effect severity. For example, acute inhalation toxicity data for phosgene showed little difference in duration-dependence between species, but greater potency in mice suggesting a model stratified on species, with a common duration parameter. Methyl isocyanate showed no differences between species or sexes, suggesting valid combination of data in an unstratified model.

948 DOSE-RESPONSE CURVES AND REGULATORY POINT ESTIMATES: IMPLICATIONS FOR NON-CARCINOGENIC RISK

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Regulatory risk assessments often unnecessarily limit the use of existing toxicology data. For estimating risks of non-carcinogenic effects, EPA's hazard index for mixtures and toxic equivalence (TEQs) procedures depend on the accuracy of point estimates of toxicity, e.g., RfDs. TEQs and the hazard index disregard the actual shape of the dose-response curve and implicitly assume linearity and no threshold, even though thresholds and non-linear curves are common for non-carcinogens. Several non-linear dose-response curves were examined to determine the potential magnitude of the effect of such assumptions on the accuracy of the estimated toxic response. A linear extrapolation of a quadratic dose-response curve, for example, can result in a 10-fold inaccuracy in estimated toxic response for each 100-fold change in dose. Probit dose-response curves, e.g., resulting from a normal tolerance distribution of response, demonstrate larger discrepancies under specified conditions. Such effects are magnified when numerous exposures that are small fractions of the regulatory point estimates are summed using TEQs or the hazard index.

949 BOOTSTRAP ESTIMATION OF PARAMETER VARIANCE FOR A SIMULATION MODEL OF CHEMICAL CARCINOGENESIS

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Estimates of parameter variance for mechanism-based, quantitative models are needed for testing hypotheses concerning differences in division or apoptosis rates resulting from dissimilar experimental regimens. We have adapted bootstrap methodology to obtain variance estimates for numerical simulation models, and describe here the application of this methodology to a numerical model for simulation of data on growth of preneoplastic lesions in carcinogenesis experiments.

A simulation model was developed for the transition of cells from one stage to the next and for clonal growth within stages [Conolly & Kimbell, *TAP* 124: 284–295 (1994)]. In the current research, estimation of the simulation model parameters was formalized by developing a grid-search bisection algorithm to automate the process of finding optimal fit of the model's output to observed data on the number and size of clones from a study on peroxisome proliferator-induced hepatocarcinogenesis. This code was then embedded within a larger algorithm for implementation of bootstrap resampling of the data. The bootstrap samples were used as input to the simulation model, leading to new point estimates of the parameters. As per bootstrap theory, the sample standard deviation of these point estimates leads to estimates of the parameter standard errors.

This methodology maintains consistency among various users, allows for hypothesis testing, and permits direct comparison between parameter estimates derived from analytic models and simulation models.

950 BAYESIAN COMBINATION OF CANCER RISK ESTIMATES

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In current practice for cancer dose-response assessment, calculation of the geometric mean of the unit risks for a number of studies is sometimes performed to combine data. However, neither slope factors nor unit risk estimates are parameter estimates in the classic statistical sense, but rather are lower confidence limits of parameter estimates. As such, they have no statistical properties which would allow for their combination, and calculation of the geometric mean represents an ad hoc attempt at data combination. Differences in data sets may not be apparent when described by slope factors alone. A Bayesian approach is proposed that provides graphical display of the data that aids the decision of when it is appropriate to combine data and that statistically provides for data combination which results in greater accuracy (narrower limits on the estimate) when data are combined. These properties of the Bayesian approach are illustrated by its application to data on aldrin and acrylonitrile, two chemicals that have undergone quantitative dose-response assessment and data combination based on the geometric mean as described. The value associated with a 10% cancer risk for aldrin was 0.0049 versus 0.0068 mg/kg-day for the ad hoc versus Bayesian approaches, respectively, while the Bayesian approach for acyrlonitrile suggests that the data should not be combined.

951 A PARALLELOGRAM APPROACH FOR SAFETY EVALUATION OF INGESTED ACETALDEHYDE

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Acetaldehyde (AA) is a natural constituent of many foods. The median oral intake of this aldehyde is estimated to be 4.3 mg/day (90th percentile = 15.5 mg/day). Data on the chronic toxicity and carcinogenicity of ingested AA are unavailable. However, the inhalation carcinogenicity and toxicity of AA are well studied. In addition, chronic toxicity data on the closely-related aldehyde, formaldehyde (FA), are available via inhalation and oral routes of administration. Comparison of these data provides three corners of a parallelogram (AA-inhalation; FA-inhalation; FA-oral) and allows estimation of the last corner — oral AA. Via inhalation and ingestion, FA and AA cause injury only at the contact sites (nasal or gastric tissues); no systemic toxicity is found. Three measures of FA or AA toxicity are available for comparison to each other and between oral and inhalation routes of administration: cellular damage, DNA-protein crosslink formation, and tumorigenesis. On a dosimetric basis, inhaled AA is 15–30 fold less potent than FA in producing each of these effects in the nose. Comparison of the oral and inhalation data reveal the stomach is less sensitive than the nose to toxic effects of both AA and FA. On a dosimetric basis, both FA and AA are several-fold less potent in inducing gastric than nasal DNA-protein crosslinks and cytotoxicity. Finally, although carcinogenic via the inhalation route, orally administered FA does not produce a carcinogenic response. The decreased potency of AA compared to FA, coupled with the failure of FA to produce a tumorigenic response when administered orally, strongly suggests that ingested AA is not likely to be carcinogenic.

952 RESTRICTED VERSUS UNRESTRICTED MODELS: WHY IS ONE THE MAGIC NUMBER?

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The Weibull model has been proposed for calculation of a benchmark dose (BMD) for dose-response assessment of noncancer toxicity. As defined by Crump (1984), the model is

$$P\,(\text{response}) = \gamma + (1-\gamma)[1 - \exp(-\beta x^{\alpha})],$$

where P is probability, x is the exposure concentration, and α, β, and γ are parameters to be estimated (γ is the non-zero background rate of response). The parameter α has been restricted to be ≥ 1 by some authors, as has the slope of the log-logistic regression model. The restriction of $\alpha \geq 1$ guarantees that the slope is not infinite at the origin, and eliminates the possibility of fitting a supralinear model to the data. However, the slope is finite even in the unrestricted case when plotted on a logarithmic scale. Restriction of $\alpha \geq 1$ has some historical precedent in approaches to cancer risk assessment but is basically an arbitrary constraint and can result in significantly poor estimation of the BMD. Supralinear models (with $\alpha < 1$) may better fit data that reflect saturation phenomena or the responses of a sensitive subpopulation. Restricted and unrestricted models (Weibull and log-logistic) are applied to an epidemiologic data set to illustrate the impact on resultant BMD estimates and goodness-of-fit.

954 INTELLIGENT TOXICOLOGY PREDICTION SYSTEM

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This project was undertaken to demonstrate the feasibility of integrating two Artificial Intelligence technologies, intelligent data discovery and neural networks, to predict a chemical's potential toxicity and carcinogenicity through biological data from short term tests. The approach used data discovery software and a large database of sub-chronic and chronic test results to find predictors of carcinogenic outcomes. A neural network using the discovered predictors as input nodes was developed, trained and tested. Examples of predictors are: liver and kidney organ to body weight ratios, hematology and blood chemistry effects, genetic toxicity, chemical toxicity, chemical structural activity, and 90-day body weight changes. A variety of neural network paradigms such as backward propagation and learning vector quantization were investigated to determine the neural network design. The results of this project indicate that it is feasible to use intelligent data discovery and neural networks to predict a chemical's potential carcinogenicity from the results of short-term tests and structural alert information. Data discovery software was able to identify a set of subchronic biological factors that were used to design a neural network and

the neural network trained to an acceptable 87% classification rate. Testing of the neural network also proved very promising. Only two false negative results and six false positive results were produced by the neural network with the remaining differences falling in the equivocal call categories. Another network making a prediction solely as a positive or negative call correctly identified 143 out of 175 exact calls, in this situation, performing at an accuracy rate of 82%. Work continues to refine the neural network training data set and to increase the number of observations in the database.

955 GROWTH, DEVELOPMENT, AND BEHAVIOR IN NEONATAL RATS GIVEN RECOMBINANT HUMAN EPIDERMAL GROWTH FACTOR$_{1-48}$

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To assess effects of supraphysiologic doses of human recombinant epidermal growth factor$_{1-48}$ (rhEGF$_{1-48}$) on neonates, 10 litters of Wistar rats/treatment group were given 0 (formulated vehicle), 10, 100, or 1000 μg/kg daily by subcutaneous injection on Postnatal Days (PN) 1 through 6. Clinical signs, body weight, acquisition of developmental landmarks and reflexes, and behavior were monitored during treatment and for 5 weeks posttreatment. Body weight gain at 1000 μg/kg during treatment was significantly less than control, but was comparable to control posttreatment. Precocious eye opening, vaginal opening, and preputial separation occurred at 100 and/or 1000 μg/kg. No treatment-related effects were seen on initial stages of locomotor acquisition; however, progression to the final stage of adult locomotion was delayed at 1000 μg/kg. Acquisition of negative geotaxis, wire maneuver, acoustic startle reflex, and visual placing also were delayed at 1000 μg/kg. Evaluation of locomotor activity on PN 28 and 42 and acoustic startle (including habituation and prepulse inhibition) on PN 42 revealed no treatment-related effects. These results indicate that rhEGF$_{1-48}$ accelerated some aspects of physical development, but transiently suppressed reflex and behavioral development and somatic growth.

956 MORPHOLOGIC CHANGES IN RATS TREATED POSTNATALLY WITH RECOMBINANT HUMAN EGF$_{1-48}$

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To assess the effects of fragment of human recombinant epidermal growth factor (rhEGF$_{1-48}$) on neonates, one-day-old rats were administered 10, 100, or 1000 μg/kg/day subcutaneously for 6 days postnatally and examined on Day 7, 14, 21, and 28 thereafter. Epidermal exfoliation at all doses and pale livers at 1000 μg/kg were noted at Day 7; stunted growth and delayed pelage development occurred at 1000 μg/kg on Days 7 and 14. Low incidence corneal opacities occurred on Days 14 and 21 at 1000 μg/kg. Microscopic skin changes were transient and dose-related including epidermal hyperplasia/hyperkeratosis and delayed adnexa development at all doses. Retinal hypocellularity with increased mitoses in the outer nuclear area occurred at 1000 μg/kg on Day 7 only. Retained foci of photoreceptor cells were found in the layer of rods and cones in a 100 μg/kg pup at Day 28 and in 1000 μg/kg pups on Days 14, 21, and 28. Increased frequency of retinal rosettes occurred at 1000 μg/kg on Days 14, 21, and 28. Kidneys of 1000 μg/kg pups had hypercellular epithelial foci in cortex at Days 14, 21, and 28 and foci of tubular degeneration were increased above controls at 1000 μg/kg on Day 21. Hepatocyte vacuolation occurred in 1000 μg/kg pups on Day 7 only. Thus, high doses of rhEGF$_{1-48}$ induced only subtle and generally transient structural changes in tissues of neonatal rats.

957 CARDIOVASCULAR EFFECTS OF RECOMBINANT HUMAN EGF$_{1-48}$ IN CYNOMOLGUS MONKEYS AFTER INTRAVENOUS ADMINISTRATION FOR TWO WEEKS

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Epidermal growth factor (EGF) is known to cause vasodilation in canine vascular beds following intraarterial administration. The cardiovascular effects of EGF or its active fragments have not been characterized in nonhuman primates. In the present study, a bioactive 48 amino acid fragment of human EGF (EGF$_{1-48}$) was administered daily by bolus injection to 2 monkeys per sex at 0, 10, 100, 500 (females only), and 1000 (males only) μg/kg for up to 2 weeks. Electrocardiography (ECG), blood pressure, and heart rate (HR) were evaluated pre- and 30 minutes postdose on Days 1 and 14. On Day 1, systolic pressure was decreased from predose by 39, 33, and 37% in males and by 32, 45, and 55% in females; and diastolic pressure was decreased by 27, 35, and 26% in males and by 24, 46, and 49% in females at 10, 100, 500 (females only) and 1000 (males only) μg/kg, respectively. Transient postdose pallor was observed with dose-related incidence and severity. Males at 1000 μg/kg had persistent pallor, weak pulse, hypotension, and did not tolerate dosing for more than 5 days. On Day 14, hypotensive changes similar to those observed on Day 1 occurred in the remaining males, and in females at 100 and 500 μg/kg. ECG and HR parameters in the EGF$_{1-48}$ treated groups were comparable to controls. These data demonstrate the hypotensive properties of EGF$_{1-48}$ in cynomolgus monkeys and suggest that EGF$_{1-48}$ causes vasodilation with reduced peripheral resistance in nonhuman primates, but without compensatory changes in HR.

958 EVALUATION OF THE SUBCHRONIC TOXICITY OF AN ORAL PRODRUG OF THE ANTI-HIV NUCLEOTIDE ANALOG (9-2-PHOSPHONYLMETHOXYETHYL)ADENINE (PMEA)

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The subchronic toxicities of the anti-HIV nucleotide analog PMEA and its bis-pivaloyloxymethyl oral prodrug (bis-POM PMEA) were characterized in 13-week repeated dose studies in cynomolgus monkeys. Monkeys (4/sex/group) received either PMEA iv at doses of 3, 8 or 20 mg/kg/d or bis-POM PMEA po at doses of 1, 5 or 25 mg/kg/d for 13 weeks. Bis-POM PMEA is approximately 30% orally bioavailable (as PMEA). Nephropathy was the dose-limiting toxicity present in the PMEA high dose (mild-moderate) and bis-POM PMEA mid and high dose groups (minimal-mild). PMEA treatment also produced a dose-dependent exfoliative dermatitis (characterized by increased melanization, acanthosis and chronic inflammation of the upper thorax, face and extremities) and a minor high dose erythroid depletion. Bis-POM PMEA administration produced no skin or erythroid effects, but did result in minimal-moderate increases in ALT, AST and CK (skeletal muscle isozyme) that were dose-dependent, readily reversible, and not associated with any correlative histopathological changes. Thus, PMEA and bis-POM PMEA have a common target organ toxicity, but possess unique secondary systemic toxicities presumably reflecting differences in pharmacokinetic and/or distribution profiles.

959 THE IMMUNOGENICITY OF A HUMAN PROTEIN DRUG (TUMOR NECROSIS FACTOR BINDING PROTEIN; TNFBP) IN ANIMAL TOXICOLOGY STUDIES

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Tumor necrosis factor binding protein (TNFbp) was modified by the addition of a polyethylene glycol (PEG) moiety to increase its half-life. Studies were conducted in animals to assess the preclinical safety of this modified TNFbp. Rats treated subcutaneously every other day for 14 days developed a serum antibody response to TNFbp. Microscopic evaluation of the kidneys showed slight mesangioproliferative glomerulopathy. Since this lesion could be the result of immune complex formation, immunohistochemistry was performed. The kidney glomeruli were found to be positive for C3 and IgG. In theory, the addition of the PEG moiety to TNFbp may result in altered characteristics of the molecule that lead to increased immunogenicity in humans, with subsequent toxicity similar to that seen in the animal studies. We have shown in rat studies that the addition of the PEG moiety did not significantly increase the intrinsic immunogenicity of the molecule. Animal studies may be helpful to some degree in predicting the evolution and consequences of an immune response to human proteins administered to humans. However, the definitive information on the immunogenicity and clinical impact of a recombinant human protein in humans will need to be obtained from clinical trials.

960 PRECLINICAL SAFETY EVALUATION OF rhuMAb OZ: A HUMANIZED F(ab')$_2$ TO THE CD18 RECEPTOR

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rhuMAb OZ is a humanized F(ab')$_2$ which binds to the CD18 receptor on the surface of neutrophils, preventing adherence to the endothelium, and subsequent extravasation into the tissue. rhuMAb OZ is specific for human and chimpanzee leukocytes. To support administration to humans by intravenous (IV) infusion, safety studies were conducted in chimpanzees and studies to as-

sess non-specific toxicity were conducted in mice. Tissue cross-reactivity studies were conducted on a panel of human and rhesus monkey tissues and acute local tolerance and hemolytic compatibility studies were performed. Mice receiving rhuMAb OZ at IV doses of up to 100 mg/kg/injection, every 12 hours (q12h) for 14 days, exhibited no adverse clinical or pathological findings. Consistent with the pharmacologic activity of rhuMAb OZ in chimpanzees, increases in WBC counts up to 4-fold over pretreatment values, due to an increase in absolute neutrophils, were evident as early as 1 hour post dose, following multiple doses of rhuMAb OZ (q12h × 3, 5 or 25 mg/kg). Although histopathological examination is precluded in chimpanzees, no adverse effects were observed on other parameters evaluated in this study. The results of human tissue cross-reactivity studies indicated that rhuMAb OZ binds to a receptor expressed on macrophages, neutrophils and monocytes; staining was also observed in interstitial and perivascular stromal cells of various organs. Rhesus monkey tissues were less reactive. rhuMAb OZ produced no signs of local irritation in rabbits following a single IV injection of a 5 mg/mL solution, did not cause hemolysis of human or monkey erythrocytes and was compatible with human and monkey serum and plasma.

961 PHARMACOLOGICAL AND TOXICOLOGICAL EVALUATION OF rhuMAb-E25, A HUMANIZED MONOCLONAL ANTIBODY TO IgE, IN RAGWEED SENSITIZED CYNOMOLGUS MONKEYS

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The purposes of this study were to assess serum IgE concentrations, safety, pharmacokinetics and skin reactivity following administration of rhuMAb-E25 in cynomolgus monkeys after exposure to an allergen. Six female monkeys were assigned to 3 groups (2/group). All animals were administered ragweed extract (40 μg/kg at 0.2 mL/kg) every 2–4 weeks beginning on Day 1. rhuMAb-E25 was administered to animals after ragweed injections on Days 71, 85, 99, 127, 169 and 190. Initially Groups 1 and 2 received 5 and 10 mg/kg of rhuMAb-E25 subcutaneously, respectively, and Group 3 received 10 mg/kg of rhuMAb-E25 intravenously. Later doses (Days 169 and 190) were increased to ensure that serum levels of rhuMAb-E25 were greater than the total serum IgE concentrations for each animal. Dose levels for 4 of the 6 animals were increased to 50 mg/kg. All animals survived to scheduled sacrifice, and no test material-related overt clinical or pathological signs of toxicity were observed. There was no evidence of antibody formation to rhuMAb-E25. rhuMAb-E25 had a terminal half-life of ≈1 week. Total serum IgE increased 6-fold due to rhuMAb-E25:IgE complex formation and the decrease in clearance of the complex relative to free IgE. Following sufficient doses of rhuMAb-E25, free IgE levels decreased to undetectable concentrations. Intradermal skin sensitivity to ragweed was elicited in all animals and diminished after treatment with rhuMAb-E25. Reactivity to ragweed returned ≈55 days after rhuMAb-E25 treatment was terminated. These data suggest that rhuMAb-E25 may be able to ameliorate allergic responses. This test system proved to be an acceptable model for eliciting an allergic response and demonstrating suppression of this response with rhuMAb-E25.

962 TOXICOLOGY OF M-CSF IN CYNOMOLGUS MONKEYS BY IV INFUSION FOR 28-DAYS

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Human Recombinant Macrophage-Colony Stimulating Factor (M-CSF) was administered by intravenous infusion to Cynomolgus monkeys (2/sex) at 0.1, 0.3, 0.7 and 1.0 mg/kg/day, 1 × daily for 2 hr/day for 28 consecutive days. Control animals (2/sex) received placebo. Animals in the 0.7 mg/kg/day group were held for an additional 4 week treatment-free period and then sacrificed. Criteria evaluated for treatment effect included physical observations, ophthalmoscopy, electrocardiography, body weight, food consumption, clinical pathology, necropsy, organ weights and histopathology. In addition, antibody and pharmacokinetic evaluations were performed throughout study. There were no M-CSF related signs of toxicity during the course of the study. One female in the 0.1 mg/kg/day group was sacrificed on day 24 with septicemia. Animals in the 0.7 and 1.0 mg/kg/day group exhibited a slight decrease in platelets between days 4 to 12; recovery occurred during the second half of the study. None of the values reached levels considered to be clinically relevant. No effects related to M-CSF administration were evident in macroscopic or microscopic evaluations. There was no detectable pattern of increasing anti-M-CSF antibody production over time. M-CSF at all dose levels was completely eliminated within 24 hours with no accumulation evident. This dosing regimen was shown to be remarkably free of toxicities noted in previous IV monkey studies where M-CSF was given by bolus or continuous infusion.

963 DETERMINATION OF TISSUE DISTRIBUTION OF A PLASMID VACCINE USING POLYMERASE CHAIN REACTION (PCR) AND *IN SITU* DNA HYBRIDIZATION

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The increasing use of nucleic acid-based therapeutics has created a need for methods for determination of tissue distribution and levels. Radiolabel methods may not always be appropriate since degraded nucleic acids can be reincorporated into endogenous nucleic acids. We discuss the results of a tissue distribution study for APL400-001, a plasmid that expresses genes derived from the HIV-1 virus and could be used as a vaccine for the treatment and/or prevention of AIDS. Rabbits were sacrificed at 4 hours, 24 hours, or 7 days after i.m. treatment with 400 μg APL400-001. Tissues were screened for presence of the plasmid by PCR. *In situ* DNA hybridization was used to detect distribution of plasmid at the histologic level in injected muscle. In positive tissues, the number of copies of plasmid per mg of tissue was determined by competitive, quantitative PCR. The test article remained principally within the skin and muscle of the injection site and within blood plasma. 10^{10} copies per mg of tissue were present in injected muscle at 4 hours after injection and 1.7×10^5 copies remained in injected skin 7 days after injection. At 4 hours after injection plasmid was present in the perimysium surrounding bundles of muscle fibers and to a lesser degree in the endomysium surrounding individual fibers. These studies demonstrate that PCR and *in situ* hybridization are suitable for sensitive detection of polynucleotides in tissues and body fluids. (Supported by NIAID Contract NIAID-DAIDS-N01-A1-15111 and Apollon, Inc.)

964 TOXICITY AND IMMUNOTOXICITY STUDIES OF A THERAPEUTIC ANTI-HIV PLASMID VACCINE IN RABBITS AND MICE

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APL400-001, a plasmid that expresses genes derived from the HIV-1 virus, is a candidate for use as a nucleic acid-based vaccine for the treatment and/or prevention of AIDS. Single- and repeat-dose (once weekly for 4 weeks) toxicity studies in male and female New Zealand White rabbits, and an immunotoxicity study designed to evaluate cellular immune and humoral responses and natural killer (NK) cell activity in female BALB/c and CBA/Ca (NK assay only) mice dosed once weekly for 4 weeks, were carried out. The plasmid [100 μg, 200 μg (mice), or 400 μg (rabbits)] was administered in a vehicle containing the facilitating agent bupivacaine HCl by injection to the posterolateral muscle of the hindleg. Endpoints in the toxicity studies included clinical signs, body weight and temperature, clinical pathology (hematology, clinical chemistry, and urinalysis), feed consumption, ophthalmology, gross pathology and organ weights at necropsy (24 hr and 2 weeks after the last injection), and histopathology. No evidence of adverse effects were observed following single or repeated i.m. injections with the plasmid, APL400-001. Treatment with the plasmid did not result in changes in mitogen-induced T and B cell proliferation or in alloantigen-induced T cell proliferation, NK activity, or anti-KLH-specific IgM or IgG antibody titers. No alterations in splenic T, B, or macrophage numbers were observed in any treatment groups. (Supported by NIAID Contract NIAID-DAIDS-N01-A1-15111).

965 PANCREATIC EFFECTS OF FPL 14294KF, A CCK-8 ANALOG, IN RATS AND DOGS

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Cholecystokinin octapeptide (CCK-8) has been shown to cause effects on the exocrine pancreas. Therefore, FPL 14294KF [Hpa(SO_3H)-Met-Gly-Trp-Met-Asp-MePhe-NH_2], a CCK-8 analog, was evaluated to determine its potential to induce pancreatic toxicity. Rats received intravenously single doses or repeated daily doses for 30 days, while dogs received intranasal doses for 30 days. Treatment-related effects were similar for all studies and included dose-related increases in serum amylase, lipase and trypsin-like immunoreactivity (measured in dogs) and microscopic pancreatic acinar cell changes (zymogen granule depletion, vacuolation and necrosis). In rats, these effects were shown

to occur within 2 hours after a single dose. In repeated dose studies, the pancreatic effects appeared to be more severe during the first week of dosing than on day 30. Pancreatic findings observed with FPL 14294KF are consistent with effects previously described for CCK-8 and related compounds in rats and dogs; however, pancreatic injury has not been reported in humans administered CCK. Thus, the relevance of these animal models for assessing the potential human pancreotoxicity of CCK-8 analogs requires further study.

966 PRECLINICAL TOXICOLOGY AND PHARMACOKINETICS OF AMYLIN ANTAGONIST PEPTIDE

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Peptide GI150747 is an antagonist of amylin, a naturally occurring peptide which may play an important role in the pathology of diabetes. The preclinical toxicology and pharmacokinetics of GI150747 were explored in studies in which GI150747 was administered to the rat and dog at dose levels of 0, 6, 12, and 24 mg GI150747 (free peptide)/kg/day using a 12-hr/day infusion period for 14 days. Group sizes were 10 rats per sex for toxicology and 5 rats per sex for pharmacokinetics. Four dogs of each sex were used for both the toxicology and GI150747 pharmacokinetic analyses. For both dog and rat, no compound-related effects were found for clinical signs, body weight gain, food consumption, hematology, clinical chemistry, urinalysis, relative and absolute organ weights, ophthalmology, or gross or microscopic histopathology. In addition, no changes in blood pressure, heart rate, or ECG were seen in dogs.

Exposure of GI150747 to all animals was confirmed on Days 1 and 14. GI150747 did not accumulate in plasma after 14 days of dosing. The mean plasma concentrations at steady state (C_{ss}) generally increased in proportion to the increase in dose on both days. Plasma C_{ss} on Day 14 in the high dose groups were about 200 and 400 nM in rats and dogs, respectively. The C_{ss} values were slightly decreased from those on Day 1 in the rat, but were not different from Day 1 values in the dog. Systemic clearance (CL_s) was about 40 mL/min/kg in both species, and generally did not change after repeated administration of the compound. Half-life was about 5 minutes in each species. Overall, there were no major sex- or dose-related differences in C_{ss}, CL_s, or half-life of GI150747 on either day.

967 ACUTE INTRAVENOUS PHARMACOLOGY AND TOXICITY OF VASOACTIVE INTESTINAL POLYPEPTIDE IN THE RAT, MOUSE AND DOG

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Vasoactive intestinal polypeptide (VAIP) is a naturally occurring rapidly metabolized peptide present in most major organ systems. Its primary effects are as a potent vasodilator and as a neurotransmitter. It may also act as a bronchodilator, ameliorate bronchoconstriction and stimulate release of prolaction and growth hormone.

Porcine VAIP was administered intravenously at dose levels of 15 and 150 ug/kg to Sprague Dawley rats, at 75 and 150 ug/kg to CD-1 mice, and at 1.5 and 15 ug/kg to dogs. Each dose group consisted of 5 males and 5 females (rodents) or 2 of each sex (dogs). In dogs, blood pressure, mean arterial pressure, and pulse rate were recorded pre-dosing and at intervals thereafter. After dosing, dogs were observed continuously until no effects were apparent, and twice a day thereafter. Rodents were observed continuously for 60 minutes after injection, at 4 hr post-dose, and then daily. On day 7, animals were necropsied.

In rats immediate peripheral vasodilation, increased respiration, and lateral head movement were observed at both dose levels; the effects disappeared within 15 minutes. Mice showed immediate dose-dependent clinical signs: mild vasodilation of rear paws at 75 ug/kg and peripheral vasodilation and decreased respiration at 150 ug/kg. These effects disappeared within 3 minutes after dosing. Dogs showed immediate, transient tachycardia and hypotension which was dose-related in intensity and duration. All 3 species showed no deaths, no effect on body weight gain, and no VAIP-related findings at necropsy. All effects were related to expected pharmacology. No evidence of untoward toxicity after single high dose administration was seen.

968 EFFECTS OF RESTRAINT ON PREGNANT RATS PLACED IN NOSE-ONLY INHALATION EXPOSURE SYSTEM

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This study was designed both to validate a nose-only exposure system and to evaluate the effects of confinement on timed presumed pregnant CD rats. Female rats were assigned to the study on the basis of confirmed mating (day 0 of gestation [GD0]). Attempts were made to balance body weights between exposure groups. Rats were placed in the exposure tubes and positioned in the exposure chamber randomly once each day for six hours on GD6 through GD15 (Phase 1) or for one hour each day on DG16 through day 20 of lactation [LD20] (Phase 2). Preconditioning the rats to the exposure restraints was not incorporated into Phase 1. Phase 2 animals were placed into the exposure tubes daily for ½ h for three days prior to GD16. Flow rate (30 liters per minute), temperature (20±2°C) and relative humidity (40–60%) were monitored continuously. Clinical observations were recorded daily. Body weights were recorded on (Phase 1) GD0, GD6-GD20 and (Phase 2) GD0, GD6, GD10, GD15 through GD21, LD1, LD4, LD7, LD14 and LD21. Rats from Phase 1 were sacrificed on GD20 and were evaluated for corpora lutea, gravid uterine weight, and status of implantation sites. Fetuses were counted, weighed, sexed and examined for external alterations. Rats from Phase 2 were sacrificed on LD21 and necropsied; F1 generation rats were weighed and evaluated for viability and growth on Days 1, 4, 7, 14 and 21, and were sacrificed on day 21 postpartum and necropsied. The developmental parameters evaluated fell within the range of our historical controls and were essentially comparable with control groups from gavage, dermal and intravenous studies conducted at this facility. These data indicate that restraint of pregnant rats in exposure tubes and apparent confinement-related stress do not induce developmental toxicity in this nose-only inhalation exposure system.

969 *IN VITRO* ENZYMATIC ANALYSIS OF POLYURETHANE SUBSTRATES

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The aim of this research is to determine the lability of TDI-(2,4-toluene diisocyanate) and MDI-(diphenylmethane-4,4'-diisocyanate) based polyurethane substrates to enzymatic attack and to identify the responsible enzyme(s) and cell types. A 6-aminoquinoline-based fluorogenic substrate was synthesized containing a urethane bond through the free amine which shifted fluorophore λem. Multiple enzymes detailed in the polyurethane degradation literature were assayed with this substrate. The rate of hydrolysis was followed by free fluorophore release. None of the enzymes tested showed activity with this substrate. This may be due to structural differences between the synthetic substrate and native polyurethane. Therefore, we shifted to assaying polyurethane degradation via o-phthalaldehyde (OPA) detection of free amine release. Standard enzymes used in the literature, e.g. papain and urease, have little extrapolative value to the human physiological environment. Literature reports have noticed macrophage-characteristic scarring of explanted polyurethane prosthetics. This led us to propose experiments examining macrophage, especially aveolar, enzymatic processing upon the polyurethane structure. We are interested in the elucidation of polyurethane processing by enzymes and determination of processing products. We believe the hydrophobic pocket of an enzyme's active site would favor stable isocyanate intermediate formation and lead to protein functional group modification over water-induced hydrolysis to the corresponding aromatic amine.

970 EFFECTS OF 4-WEEK VAPOR INHALATION OF DECAMETHYLCYCLOPENTASILOXANE (D_5) ON FISCHER 344 RATS

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Decamethylcyclopentasiloxane (D_5), a volatile cyclomethicone, is a component of many deodorants and other personal care products. To evaluate the vapor inhalation toxicity of D_5, a 4-week nose-only exposure study was conducted in which five groups of Fischer 344 rats (10 male/10 female per group) were exposed to 0, 0.44, 0.65, 1.50, or 2.27 & 3.06 mg D_5/l of air, 6 hours/day, five days/week. Animals exposed to D_5 exhibited no statistically significant differences in mortality, body weight, food intake, or other clinical observations when compared with controls. The two highest exposure groups had significant increases in mean corpuscular volume and triglyceride concentration. Females in the highest exposure group had significant differences in both relative and absolute liver weights, with hepatocellular hypertrophy found in six of these animals. Other morphological changes observed in the nasal cavity and lungs of the highest exposure group were regarded as an effect of irritation. Further evaluation regarding the biological relevance of these results is ongoing.

971 1-MONTH REPEATED DOSE INHALATION TOXICITY STUDY WITH OCTAMETHYLTETRASILOXANE IN RATS

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A 1-month inhalation study was conducted with Octamethyltetrasiloxane (D_4) which is a volatile siloxane having a number of industrial and commercial applications. Groups of 10 male and 10 female Fischer 344 rats were exposed to O, 2.5, 5.0, 9.0, or 12.0 mg D_4/L, 6 hrs/day, 5 days/weeks for four weeks. Body weights, food consumption, and clinical signs were recorded. Twenty-four hours following the last exposure, animals were sacrificed and blood and urine collected for hematology, clinical chemistry, and urinalysis. A complete necropsy was performed. Selected organ weights were obtained from tissues taken for histopathology. D_4 exposure caused no significant adverse effects on body weight, food consumption, hematology, clinical chemistry, or urinalysis parameters evaluated. Clinical signs were confined to the 9.0 and 12.0 mg/L dose groups and consisted of abnormal posture, gait and hunched posture. A significant dose related increase in liver weights occured in males and females at all doses. Females were more sensitive. A decrease in thymus weights and increase in adrenal weights were observed in males (12.0 mg/L) and females (9.0 & 12.0 mg/L). A nonsignificant, dose related decrease in ovarian weight was seen in females. Histopathological lesions consisted of hepatocellular hypertrophy, thymic atrophy, ovarian atrophy and vaginal mucification. All lesions were confined to the two highest doses. Electron microscopy of the livers showed a dose-related decrease in the size of the mitochondria. an increased proliferation of the smooth endoplasmic reticulum, and cellular hypertrophy. Additonal studies are in progress in Fischer 344 rats to further characterize these changes.

972 SUBCHRONIC INHALATION TOXICITY OF 4-VINYLCYCLOHEXENE IN RATS AND MICE

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4-Vinylcyclohexene (4-VCH), a dimer of 1,3-butadiene (BD), is formed during BD production and is used in polymer manufacturing. In previous oral studies, 4-VCH produced hyaline droplets in male rat kidneys and ovarian atrophy and/or tumors in female mice. In a 2-wk inhalation study, 9 of 10 mice exposed to 1500 ppm 4-VCH died after 4 days of exposure. Subsequently, groups of CD rats and $B6C3F_1$ mice were exposed 6 hrs/day, 5 days/wk for 90 days to 0, 50 (mice only), 250, 1000, or 1500 (rats only) ppm 4-VCH. Other groups were concurrently exposed to 1000 ppm BD. Non-adverse effects in rats included elevated mean liver weights in the 1000 and 1500 ppm 4-VCH groups and the BD group and an increased accumulation of hyaline droplets (likely due to α_{2u}-globulin) in kidneys of all exposed male rat groups. The only adverse effect in rats was a low body weight gain in the 1500 ppm group. In mice, 15 of 20 in the 1000 ppm 4-VCH groups died by test day 12. Microscopically, there was ovarian atrophy in mice exposed to 1000 ppm 4-VCH (moderate) and 1000 ppm BD (mild to moderate). Male mice exposed to BD had testicular degeneration and atrophy; males and females had mild hemolytic effects and low platelet counts. The NOAEL for 4-VCH in rats was 1000 ppm, and for mice was 250 ppm. (Supported by the Butadiene Panel of the Chemical Manufacturers Association).

973 A 90-DAY SUBCHRONIC TOXICITY STUDY OF 1,1,1,2,3,3,3 HEPTAFLUOROPROPANE (FM-200™) IN RATS

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The potential subchronic toxic effects of FM-200™, a non-ozone-depleting substitute for existing halon fire extinguishants, were studied via whole-body inhalation exposure to rats. A preliminary 14-day repeated-exposure study, which utilized concentrations of 1, 2, 5 and 10%, caused no toxicity. Exposure concentrations of 2, 5 and 10.5% (in air) were selected for the subsequent 90-day study. The high exposure concentration was selected at 1.5X the expected effective fire extinguishing concentration of 7%. The study was comprised of three groups of 12 male and 12 female SD Crl:CD® BR rats that were exposed for six hours per day, five days per week, for a minimum of 65 exposures under a standard 90-day subchronic EPA TSCA protocol. A concurrent control group of identical design was exposed to filtered air on a similar regimen. Analytically confirmed test atmospheres were generated in 500-liter, NYU-type exposure chambers, operated under dynamic conditions, ensuring a minimum oxygen content of 19% and homogeneous distribution of FM-200™. Oxygen was supplemented in the 10.5% group. Daily clinical observation, and weekly body weight and food consumption measurement revealed no signs of toxicity at the tested concentrations of FM-200™. Hematology and serum chemistry parameters, evaluated during the fourth and thirteenth weeks of exposure, were unaffected by FM-200™. Exposure to FM-200™ induced no oculopathic changes at the concentrations administered. FM-200™ had no effect on gross anatomy, organ weights or selected tissues examined microscopically. The NOEL for systemic toxicity of FM-200™ was 10.5% ppm when administered as a daily six-hour exposure, five days per week, for 13 consecutive weeks. This NOEL provides an approximate 35,000-fold margin of safety over the FM-200™ exposure which humans may receive under proposed conditions of use.

974 *IN VIVO* ESTERIFICATION OF FATTY ACIDS BY METHANOL

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Human exposure to methanol (MeOH) is likely to increase in view of its proposed future use as an alternative automotive fuel. Since alcohols are known to esterify the fatty acids in the body and some of those esterified fatty acids are toxic, we studied the formation of fatty acid esters of methanol in male Long-Evan rats given a single oral dose of 3.5 ml/kg MeOH in saline. Three rats were sacrificed at each time point, 0 (controls), 1, 3, 6, 12 and 24 hr following the treatment. Lipids, after being extracted from whole blood, liver, pancreas and brown fat, were separated by TLC and the fatty acid ester fractions were analyzed by GC using DB225 capillary column. Significantly high levels of C-16:0, C-18:0, C-18:1, C-18:2, C-18:3 and C-20:4 fatty acid methyl esters were found in the pancreas (mean values 5.29, 1.11, 9.66, 7.73, 0.56 and 2.79 mg/g wet weight, respectively) as compared to those in brown fat and liver. In whole blood, only low levels of C-16:0, C-18:0 and C-20:4 fatty acid methyl esters could be detected at all time points. The levels of fatty acid methyl esters were found to be highest at 1, 3 and 24 hr in pancreas, liver and brown fat, respectively. Pancreas appears to be the major organ involved in the biosynthesis of fatty acid methyl esters. A low concentration of the fatty acid methyl esters was also detected in control rats indicating their endogenous formation. Toxicity and biological significance of these fatty acid methyl esters are not presently known.

975 PULMONARY RETENTION AND TISSUE DISTRIBUTION OF ^{239}Pu NITRATE IN F334 RATS AND SYRIAN HAMSTERS INHALING CARBON TETRACHLORIDE

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Carbon tetrachloride (CCl_4) has been used extensively in the nuclear weapons industry for cleaning and plutonium extraction. Therefore, workers in this industry may have been exposed to this combination of toxicants. Potential for future worker exposure to this combination of materials exists during "cleanup" operations at weapons production sites. The purpose of this study was to compare the inhalation toxicity of CCl_4 in F344 rats and Syrian hamsters and to determine the combined effects of inhaled CCl_4 and ^{239}Pu nitrate ($^{239}PuNO_3)_4$. F344/Crl rats (36 M/36 F) and Syrian hamsters (36 M/36 F) were exposed to 0, 5, 20, or 100 ppm CCl_4 6 hr/day, 5 days/wk for 16 wk. After 4 wk of CCl_4 exposure, subgroups of 30 animals/species/sex/exposure level were exposed once, nose-only to aerosols of $^{239}Pu(NO_3)_4$. The remaining animals were exposed once, nose-only to ^{85}Sr-labeled fused aluminosilicate particles (^{85}Sr-FAP) in order to evaluate the effects of CCl_4 on lung particle clearance. Subchronic inhalation of CCl_4 combined with ^{239}Pu had no effect on weight gain of male or female rats, but depressed growth in male and female hamsters in a concentration-dependent manner. Six wk after ^{239}Pu inhalation, liver lesions were present in rats and hamsters exposed to 20 or 100 ppm CCl_4 plus ^{239}Pu. CCl_4 inhalation had no effect on the pulmonary deposition or clearance patterns of ^{85}Sr-FAP in rats or hamsters. Results to date suggest that hamsters are more sensitive than rats to inhaled $CCl_4/^{239}$Pu and that subchronic inhalation of CCl_4 does not affect alveolar macrophage function. Evaluation of the pulmonary retention and tissue distribution of the ^{239}Pu is underway. (Research sponsored by the Assistant Secretary for Defense Programs and the U.S. DOE/OHER under Contract No. DE-AC04-76EV01013.)

976 INHALATION ACUTE TOXICITY OF METHACRYLIC ACID AND BUTYL, ETHYL, AND METHYL METHACRYLATES IN RATS

D P Kelly. *Du Pont, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE.* Sponsor: N D Krivanek

Methacrylic acid (MAA) and the tested methacrylates are used as intermediates or monomers in the production of widely used polymers. Groups of five male and five female rats were exposed, nose only, to test atmospheres containing essentially all vapor in the ethyl methacrylate (EMA) and methyl methacrylate (MMA) experiments, and to mixed aerosol/vapor in the MAA and butyl methacrylate (BMA) experiments. Exposure times were four hours except for methyl methacrylate where one hour exposures were conducted. The four-hour median lethal concentrations (LC50s) for MAA and EMA were 7.1 and 55 mg/l. The four-hour approximate lethal concentration for BMA was 29 mg/l. No deaths occurred in rats exposed for one hour to MMA vapor at concentrations approximating the lower explosive limit (up to 74 mg/l). On an acute inhalation basis EMA, MMA, and BMA are considered to have very low toxicity; MAA is considered to have slight toxicity. The order of four-hour inhalation toxicity was MAA > BMA > EMA.

977 VINYLNORBORNENE (VNB): NINETY-DAY VAPOR INHALATION STUDY IN RATS

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VNB, used as a chemical intermediate, was investigated for toxicity after repeated subchronic vapor exposure. Sprague Dawley® rats were exposed to VNB concentrations of 0, 5, 25, and 150 ppm. Each exposure group had 10 rats/sex, while the 0 and 150 ppm groups had an additional 5 rats/sex as a 4-week recovery group. Females in the 150 ppm group had a decrease in body weights with an occasional decrease in food consumption. At the Week 14 sacrifice, the kidney (absolute and relative body and brain weights) and liver (absolute and relative body weights) weights were increased in the males from the 150 ppm group, and at the Week 18 sacrifice (recovery), liver (absolute and relative brain weights) weights were also increased. However, no clinical pathology or histopathological findings were observed, indicating no liver dysfunction. At the Week 14 sacrifice, hyaline droplet inclusions in the renal proximal tubules of the males were present (minimal to mild) in all VNB exposure groups (nonstatistically significant in the 5 ppm group). No associated damage to the proximal tubules was observed. At the Week 18 sacrifice, none of the male kidneys in either of the recovery groups were observed to have hyaline droplets. Immunohistochemical evaluation for $alpha_{2u}$-globulin of the male kidneys revealed no VNB-related findings at either sacrifice, contradictory to a previous 9-day study. Based on the nonstatistically significant increased incidence of hyaline droplets in the male kidneys from the 5 ppm group, a no-observed-adverse-effect level (NOAEL) was determined to be 5 ppm under the conditions of this study in rats.

978 AN INHALATION ONCOGENICITY STUDY IN RATS OF METHYLETHYLKETOXIME

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Fischer 344 rats (80/sex/group) were exposed 6 hrs/day, 5 days/week for 26 months via whole-body inhalation to MEKO (methylethylketoxime) vapor concentrations of 0, 15 ± 1 (X ± SD), 75 ± 2 and 374 ± 10 ppm. Survivorship at 26 months (34% in the males; 60% in the females) was unaffected by the MEKO exposures. Microscopically, MEKO related findings included: congestion of the spleen with pigment in reticuloendothelial cells and extramedullary hematopoiesis, hepatocellular carcinoma in the male 374 ppm rats, a dose related increase in hepatocellular adenomas in the males at 75 and 374 ppm, an increase incidence of basophilic foci in males and females at 374 ppm (with increased severity in the males in the 15, 75 and 374 ppm group males and 374 ppm group females). In the nasal turbinates, primarily the dorsal meatus, degeneration of the olfactory epithelium was seen in both males and females at 374 ppm with less severe findings at 75 and 15 ppm. These changes consisted of thinning of the olfactory epithelium with some loss of apical cytoplasm and fewer layers present.

In conclusion, under the conditions of this study, MEKO produced changes in the olfactory epithelium in all MEKO exposed groups and was a liver oncogen in male rats at 75 ppm.

979 ISOPROPANOL (IPA) VAPOR INHALATION ONCOGENICITY STUDY IN FISCHER 344 RATS

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An inhalation study was performed to assess the oncogenic potential of IPA in rats. Groups of 75 rats were exposed to concentrations of 0, 500, 2500, or 5000 ppm for 6 hours/day, 5 days/week, for at least 104 weeks. Ten rats/sex/group were assigned to a Week 73 interim sacrifice group. Evaluations included clinical observations, body and organ weights, opthalmology, clinical pathology, and anatomic pathology. Signs of narcosis were observed in animals during exposures to 2500 and 5000 ppm. Increased mortality and a decreased mean survival time were noted for male rats from the 5000 ppm group. Body weight and body weight gain were increased for both sexes from the 2500 and 5000 ppm groups throughout the study. No exposure-related ophthalmic lesions or changes in hematologic parameters were observed for either male or female exposed rats. Urine chemistry changes indicative of impaired kidney function (i.e., decreased osmolality and increased total protein, volume, and glucose) were noted for rats from the 2500 (males) and 5000 (males and females) ppm groups. Increased liver and kidney weights were observed for males and/or females from the 2500 and 5000 ppm groups. Concentration-related increases in testes weight were observed at the interim sacrifice. A number of nonneoplastic lesions were observed for male and female rats from the 2500 and 5000 ppm groups, with the most significant lesions being observed in the kidney and associated with chronic renal disease. The only neoplastic lesion noted was an increase in interstitial cell adenomas of the testes for male rats which was considered to represent marked hyperplasia and not autonomous growth. Thus, the no-observed-effect level (NOEL) for toxic effects was 500 ppm for both sexes of rats. The NOEL for oncogenicity effects for male and female rats was determined to be greater than 5000 ppm.

980 CHRONIC TOXICITY AND ONCOGENICITY OF DIMETHYLACETAMIDE (DMAC) BY INHALATION EXPOSURE TO RATS

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The potential chronic toxicity and oncogenicity of DMAC was evaluated by exposing male and female Crl:CD®BR rats to either 0, 25, 100 or 350 ppm DMAC for 6 hr/d, 5 d/wk for 2 years. Body weights were measured every other week. Clinical pathology evaluations occurred at 3, 6, 12, 18, and 24 months. Sacrifices were conducted at 12 and 24 months. Hepatic cell proliferation was measured at 2 wks, 3 and 12 months. Rats exposed to 350 ppm had lower body weight and increased sorbital dehydrogenase activity. Serum cholesterol and glucose concentrations were higher in 100 and 350 ppm females. There were no compound-related effects on survival, hematology, or urinalysis parameters. Exposure to 100 or 350 ppm produced increased absolute and/or relative liver weights, hepatic focal cystic degeneration, hepatic peliosis, biliary hyperplasia (350 ppm only), and lipofuscin/hemosiderin accumulation in hepatic Kupffer cells. Males exposed to 350 ppm also had higher absolute and relative kidney weights which correlated with an increase in severity of chronic progressive nephropathy. No increase in hepatic cell proliferation was observed. DMAC was not oncogenic under the conditions of the study. The NOAEL was 25 ppm.

981 CHRONIC TOXICITY AND ONCOGENICITY OF DIMETHYLACETAMIDE (DMAC) BY INHALATION EXPOSURE TO MICE

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The potential chronic toxicity and oncogenicity of DMAC was evaluated by exposing groups of 78 male and female Crl:CD-1®BR mice to either 0, 25, 100 or 350 ppm DMAC for 6 hr/day, 5 days/wk for 18 months. Body weights were measured every other week. Clinical pathology evaluations occurred at 3, 6, 12, and 18 months. The terminal sacrifice occurred at 18 months. Hepatic cell proliferation was measured at 4 wks, 3 and 12 months. There were no compound-related effects on body weight, incidence of clinical signs, survival, or hematology parameters. Exposure to 100 or 350 ppm produced increased

absolute liver weight, and relative liver weight was increased in 350 ppm females. Accumulation of lipofuscin/hemosiderin in hepatic Kupffer cells and centrilobular single cell necrosis occurred at concentrations of 100 and 350 ppm. Females exposed to 350 ppm also had a significantly higher incidence of bilateral, diffuse retinal atrophy. No increase in hepatic cell proliferation was observed. DMAC was not oncogenic under the conditions of the study. The NOAEL was 25 ppm.

982 IDENTIFICATION AND QUANTITATION OF S-(N-METHYLCARBAMOYL)-N-ACETYLCYSTEINE (AMCC) IN THE URINE OF METHYL ISOCYANATE (MIC) INHALATION-DOSED RATS USING LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY (LC-MS/MS)

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Six male Sprague-Dawley (CD) rats were exposed to 20.1 ppm MIC (±1.2 ppm SD) using a Battelle-designed, nose only exposure unit. Exposure concentrations were established by sampling with adsorbent tubes containing 1-(2-pyridyl)piperazine followed by HPLC analysis of the MIC derivative. Urine was collected over dry ice for 21 hours postexposure. Direct analysis of urine specimens by ion spray LC-MS/MS led to the detection of AMCC, which was quantified by selected reaction monitoring using a stable isotope labeled internal standard. An average of 691 μg (±163 μg SD) AMCC per animal was excreted.

This is the first demonstration that inhalation-exposed rats excrete AMCC in their urine. These results reinforce the hypothesis that MIC, despite its high reactivity, crosses the alveolar-blood barrier, possibly as the corresponding glutathione adduct. The latter conjugate has been postulated to act as a systemically available, latent toxin; which by reverting spontaneously to free MIC, causes damage to a variety of internal organs.

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983 PULMONARY TOXICITY ASSESSMENTS OF INHALED ETHYLENE OXIDE/PROPYLENE OXIDE COPOLYMER LUBRICANTS IN RATS

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The pulmonary toxicities of 5 different ethylene oxide/propylene oxide (EO/PO) copolymer commercial lubricant candidates were assessed by exposing groups of rats for 3 consecutive days (6 hr/day) to aerosols and evaluating pulmonary parameters at selected postexposure time periods. Groups of rats were exposed either to 22 mg/m^3 of UCON® 50-HB-5100, 42 mg/m^3 of UCON® 50-HB-2000 (one rat died in each of these 2 groups), to 110 mg/m^3 of Pluronic® L31, to 99.4 mg/m^3 of Pluronic® L64, or to 111 mg/m3 of UCON® 75 H-1400. The lungs of rats were lavaged (BAL) at 0 hrs (i.e., immediately after), 2 days, 1 wk, 1 and 3 months postexposure. Cellular and biochemical data demonstrated a substantial pulmonary inflammatory response concomitant with increases in BAL fluid levels of LDH, protein, alkaline phosphatase, and N-acetyl glucosaminidase as well as edema in the lungs of rats exposed to UCON® 50-HB-5100 or 50-HB-2000 (less severe). In contrast, the lungs of rats exposed to Pluronic® L31 and L64, and UCON 75 H-1400 demonstrated only slight and reversible pulmonary inflammatory effects. The results from this study validate this inhalation bioassay technique for predicting the pulmonary toxicity of inhaled aerosols by confirming the effects measured in a previous 2-week inhalation toxicity study with these same compounds. (Supported by the American Fibers Manufacturers Association).

984 THE ASSESSMENT OF TOXICITY AFTER EXPOSURE TO A PYROTECHNICALLY-GENERATED AEROSOL

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Fisher 344 rats (250 to 300 g) were exposed to the by-products of SFE, a pyrotechnically-generated aerosol fire extinguisher. Exposures were at nominal concentrations of 50 or 80 g SFE/m^3 for 15 or 60 minute exposure periods. A 700 L whole body inhalation chamber was used for the exposures and consisted of a supply/exhaust system, aerosol generator and exhaust scrubber. Exposures were conducted under static chamber conditions. The chamber atmosphere was analyzed for aerosol concentration, size (mass median aerodynamic diameter) and distribution (geometric standard deviation). Clinical observations were taken throughout the exposure. Animals were euthanized at 1 hr, 6 hrs, 24 hrs, 7 d or 14 d post-exposure and underwent wet/dry lung weight determination, histopathological examination and enzyme analyses. *Results:* No deaths occurred during the study. Animals exhibited signs of irregular breathing cycles, coughing, incoordination and lethargy during each exposure. Often, animals were lateral-recumbent by 15 minutes and remained as such throughout the duration of the exposure. Head pulling or straining was observed frequently. As loads and exposure length increased, these clinical signs became more pronounced. No lesions were noted in the trachea, lung, heart and abdominal organs upon gross examination. A reversible edema developed only in animals exposed to a nominal concentration of 80 g/m^3 for a 60-minute exposure period. The presence of edema was supported by weight/dry lung weight determination. No inflammation was noted. No alterations were noted in enzyme analyses of bronchoalveolar lavage fluid.

985 ACUTE AND REPEATED DOSE INHALATION TOXICITY EFFECTS OF PYROTECHNICALLY DISSEMINATED TEREPHTHALIC ACID SMOKE (XM83 GRENADE)

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Acute and repeated dose inhalation studies using Fisher 344 rats were conducted to assess the health hazard potential of pyrotechnically generated TPA and its combustion products. Acute exposure levels ranged from 150–1,900 mg/m^3 for 30 minutes and repeated dose exposures ranged from 128–1,965 mg/m^3 for 30 min/day for 5 days. Exposed and control rats were evaluated for toxic signs, bronchoalveolar lavage (BAL), cellular chemiluminescence (CL), and histopathologic changes. During exposure, rats showed slight to moderate lacrimation, rhinorrhea, lethargy and dyspnea which reversed within 1-hr post-exposure. No deaths occurred, even at the highest smoke concentrations. Other than a temporary increase in white blood cell count, all BAL and CL parameters were within normal limits. Histopathological changes were confined to dose-related nasal necrosis and inflammation in both acute and repeated dose exposures at levels above 900 mg/m^3. Major pyrotechnic combustion products formed were formaldehyde, benzene and carbon monoxide. These by-products were above their respective threshold limit values at various dose levels.

986 PULMONARY TOXICITY OF COPPER INDIUM DISELENIDE (CIS), COPPER GALLIUM DISELENIDE (CGS), AND CADMIUM TELLURIDE (CdTe) IN RATS

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CIS, CGS, and CdTe are compounds that may see widespread usage in the photovoltaic and semiconductor industries. Toxicity tests were conducted on these compounds because of their increased use, the potential for worker exposure, and the lack of toxicity data. Because inhalation is the most likely route of worker exposure, initial studies examined the pulmonary toxicity of these compounds after intratracheal instillation. Female Sprague-Dawley rats were administered 0, 12, 25, 50, or 100 mg/kg body weight (6 rats/dose). At 72 hr after treatment, body weight gain was significantly decreased in the 100 mg/kg CIS group, and in all CdTe dose groups. Lung weights were increased in most treated rats, with CdTe causing the greatest increase. Total numbers of cells in bronchoalveolar lavage fluid (BALF) were significantly increased in treated rats, and were greatest in the 100 mg/kg CIS group. Decreases in the %AMs and increases in the %PMNs and lymphocytes were observed in BALF of all treated rats. Slight to moderate increases in lactate dehydrogenase (LDH) activity were observed in BALF from CIS- and CGS-treated rats; marked increases were observed in CdTe-treated rats. BALF protein was significantly increased in rats treated with CIS and CdTe. Based on changes in lung weight, and BALF indices, CdTe was the most toxic for the lung; CIS had intermediate toxicity and CGS was the least toxic.

987 AN INHALATION EXPOSURE SYSTEM USING A NOSE-ONLY EXPOSURE TOWER FOR TOXICOKINETIC STUDIES OF CHEMICAL TERATOGENS IN MICE

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An inhalation system with 32 individual holding chambers was developed to expose unrestrained pregnant mice and allow removal of animals from the test chemical atmosphere at intermediate time points without exposure inter-

ruption. Maternal toxicity resulting from the stress of restraint during nose-only exposures is undesirable and makes a whole-body type of exposure system preferable. However, such apparatus does not lend itself to easy removal of individual animals during the exposure. We adapted a nose-only exposure tower (RCC-type, CR Equipment SA, Gland, Switzerland) and modified it to serve as a whole-body exposure system for mice. An individual mouse is able to move freely in a tube designed to hold a rat. The inlet at each exposure port is extended to the back of the animal-holding tube to promote mixing. Each holding tube can be removed while the inhalation exposure is ongoing, without disturbing conditions in other tubes or the entire system. This facilitates removal of animals to develop time series data on the tissue distribution of the toxicant. An atmosphere of 10,000 ppm methanol or of 500 ppm 2-methoxyethanol was generated and passed through the 32-port nose-only system at a flowrate of 12 to 15 l/min. Chemical concentration was monitored using a MIRAN 1A infrared spectrometer to sample at the inlet and outlet of the system. Gas samples were taken from individual tubes with 100 μl syringes for GC analysis. The variability of concentration of the two chemicals in individual tubes, when empty, was within ±5%, or, containing mice, was within ±7% of the inlet concentration.

988 PHARMACOKINETICS OF PENTACHLOROBENZENE IN F344 RATS

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As part of our effort to carry out a research project on physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) modeling of a number of cholorobenzene isomers, certain basic pharmacokinetic information on pentachlorobenzene (PCBz) in F344 rats was obtained. PCBz is used primarily as a precursor in the synthesis of pentachloronitrobenzene, a fungicide, and as a flame retardant. PCBz is usually biotransformed to pentachlorophenol (PCP), tetrachlorohydroquin (TCHQ), and tetrachlorobenzoquinone (TCBQ), metabolites often more toxic than the parent compounds. Since the kinetics of *in vivo* biotransformation of PCBz is not clear, we evaluated the tissue level and distribution of PCBz and its major metabolite, PCP, in F344 male rats up to 72 hours after a gavage treatment at a dose of 4 mmol/kg body weight. Tissue levels of compounds in blood, liver, kidney, and fat were measured by HPLC. For HPLC analysis, a short analytical column (C18, 3μ, 10 cm × 4.5 mm) and gradient eluent system with methanol and water were used for optimum resolution. The retention time, detection limit, and recovery with solvent extraction from tissues were 15 min., 3 ppm, and 70% for PCBz and 13 min., 0.1 ppm and 80% for PCP, respectively. Peak blood levels of PCBz were reached 6 hours after treatment, while PCP peaked after 72 hours. Fat tissues, a major sink for PCBz, contained 20–30 times more PCBz than the liver. The concentrations of PCP were usually less than 10% of those found for PCBz.

989 A PHARMACOKINETIC MODEL OF ANAEROBIC *IN VITRO* CCl_4 METABOLISM

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Carbon tetrachloride (CCl_4) is a potent hepatotoxic agent whose toxicity is mediated through cytochrome P-450-dependent metabolism. Previous results from anaerobic *in vitro* experiments with hepatic microsomes isolated from male F-344 rats indicate that chloroform ($CHCl_3$) formation from CCl_4 may be non-linear with dose. In that work, the dose was expressed as the amount of CCl_4 added to the vial. In the present study, a pharmacokinetic model was developed to calculate the concentration of CCl_4 in the microsomal suspension. Hepatic microsomes were treated with CCl_4 under anaerobic conditions. Initial rates of $CHCl_3$ formation over a 5 min incubation period were monitored by headspace GC/ECD. Dose-response curves, based on total amount of CCl_4 added to the microsomes, revealed a nonlinear, biphasic metabolism. There appears to be a high affinity saturable phase at lower CCl_4 concentrations followed by a linear phase at higher CCl_4 concentrations. The best fit kinetic constants for the saturable phase were 0.0014 μmoles/min (Vmax) and 15 μM (Km). The linear phase rate constant (kf) was 0.00077/min. In conclusion, a pharmacokinetic model has been developed for *in vitro* metabolism of CCl_4 that estimates metabolic rates in terms of CCl_4 in the microsomal suspensions. (This abstract does not necessarily reflect EPA policy.)

990 DISPOSITION AND PHARMACOKINETICS OF 3,3′,4,4′-TETRACHLOROAZOBENZENE (TCAB) IN MALE FISCHER 344 RATS

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3,3′,4,4′-Tetrachloroazobenzene (TCAB) is a contaminant generated during the synthesis of 3,4-dichloroaniline (DCA) and DCA-derived pesticides. TCAB is isosteric to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and has been shown to bind Ah receptors. Following oral administration of [^{14}C]-TCAB (3.2 and 32 mg/kg), 39–45% of the dosed radioactivity was excreted in the urine and 53–56% was recovered in the feces within 48 hr. Less than 6% of the dosed radioactivity remained in the tissues examined at 96 hr. Biliary excretion of the radiolabel was also investigated following a single iv dose of [^{14}C]-TCAB (3.2 mg/kg). Approximately 33% of the administered radiolabel was excreted in the bile during 6 hr. TCAB metabolites in urine and bile were tentatively identified using LC/MS. An O-glucuronide of TCAB was detected in the bile. In contrast, conjugated derivatives of hydroxylated mono or dichloroaniline derivatives were detected in the urine. Some of these metabolites were also acetylated. Following iv administration, the disappearance of [^{14}C]-TCAB from blood was monitored. The pharmacokinetic profile was consistent with a multicompartment model. Pharmacokinetic parameters calculated from the experiment reveal that the compound is readily cleared from the blood with a $t_{1/2}$ of 3.9 hr, clearance of 0.6 L/kg/hr, and an apparent volume of distribution of 3.3 L/kg. The rapid elimination of TCAB as compared to TCDD ($t_{1/2}$ = 12–34 days) and metabolism to more polar compounds should reduce its interaction with the Ah receptor and decrease Ah receptor-mediated toxicity. Efforts are under way to further characterize the urinary and biliary metabolites. (Supported by NIEHS, NO1-ES 35367 and Southwest Environmental Health Sciences Center Grant, P30 ES06694).

991 TOXICOKINETIC STUDIES OF RIFAMPIN IN BEIGE MICE

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Rifampin is a commonly used antimycobacterial drug. The beige mouse is proposed as an animal model for evaluation of drugs indicated for treatment of *Myocbacterium avium* complex (MAC) in humans. This study compared oral and IV plasma pharmacokinetics of single doses of rifampin ranging from therapeutic to toxic in uninfected and MAC-infected beige mice. Mice were infected with suspensions of MAC administered IV. Rifampin doses were 30, 100 and 150 mg/kg for both routes of administration. The 100 and 150 mg/kg IV doses were toxic to infected mice, causing 60 and 100% mortality, respectively. No mortality occurred in the orally-dosed groups or uninfected IV-dosed groups. Pharmacokinetic parameters were comparable in infected and uninfected mice. Rifampin was rapidly and virtually completely absorbed at the two lower dose levels, with peak plasma concentrations occurring from 0.5 to 2 hr. Clearance in 30 mg/kg mice compared well with values reported for humans given therapeutic doses. Clearance and AUC demonstrated dose-dependency in mice, paralleling observations in humans given varying doses of rifampin. Findings suggest that the beige mouse is an appropriate model to establish relationships between plasma levels of rifampin and toxicity for extrapolation to human MAC patients.

992 KINETIC STUDIES OF CARBON DISULFIDE IN MALE AND FEMALE FISCHER 344 RATS

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Carbon disulfide (CS2) is an important industrial chemical widely used in the production of rayon, cellophane, fungicides, and biocides. A study was conducted to characterize the uptake and elimination kinetics of CS2 and to look for variations in CS2 blood levels resulting from repeated exposure of male and female Fischer 344 rats by inhalation for 13 weeks. Multiple blood samples were obtained from individual rats via jugular cannula during and after a single nose-only inhalation exposure to 50, 500, or 800 ppm CS2. Uptake and elimination kinetics and steady state concentration for free CS2 were characterized using the blood CS2 concentrations measured in these samples. The apparent volume of distribution and the elimination half time, 5L/kg and 23 minutes, were estimated from similar samples obtained following a single 50 mg/kg iv dose. Blood samples were taken after 5 hours of exposure to CS2 in rats previously exposed to 50, 500, or 800 ppm CS2, 6 hours per day for 1.5,

3.5, 8.5, or 12.5 weeks to determine the effect of previous exposure on the steady state blood concentration. Urine collected from the same animals was analyzed for 2-thiothiazolidine-4-carboxylic acid (TTCA) as a biomarker of exposure. The values for both the steady state CS2 blood concentration and total urinary TTCA corrected for differences in estimated ventilation rate, were relatively consistent over the 13 weeks of exposure and between sexes.

993 TOXICOKINETICS OF THE FLAME RETARDANT 2,2-BIS (BROMOMETHYL)-1,3-PROPANEDIOL IN RATS AND MICE

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The toxicokinetics of the flame retardant 2,2-bis(bromomethyl)-1,3-propanediol (BMP), recently demonstrated by the National Toxicology Program to be a multisite carcinogen in rats and mice, have been investigated to further characterize BMP-induced toxicity. These studies indicated that BMP was readily absorbed from the gastrointestinal tract of either male F344 rats or B6C3F1 mice and rapidly excreted, primarily in urine. Greater than 90% of a total dose of 150 mg [^{14}C(U)] BMP/kg was excreted in urine within 72 hr following po administration to rats. The rate of excretion of BMP-derived ^{14}C in urine was not affected by dose up to 600 mg/kg. Excretion of BMP-derived ^{14}C in mice was similar to rats following po administration of the low dose. However, the high dose was acutely toxic to mice. BMP-derived ^{14}C in urine of both rats and mice was excreted primarily as a glucuronide conjugate of the parent compound. Following iv injection of 15 mg/kg to rats, BMP disappeared rapidly from blood, with the excretion rate of BMP-derived ^{14}C in urine similar to that following gavage. Within 6 hr, over 50% of an iv administered dose was excreted in bile of cannulated rats. Greater than 99% of the BMP-derived ^{14}C excreted in bile consisted of the same glucuronide conjugate found in urine. These results indicate that BMP undergoes rapid conjugation and excretion following absorption from the gut. There is little or no evidence that BMP is an alkylating agent or is metabolized to an alkylating agent.

994 THE ACUTE DERMAL TOXICITY, ABSORPTION AND ELIMINATION RATES OF CrO_3 (Cr^{6+}), $CrCl_3$ (Cr^{3+}), Na_2SeO_3 AND SeO_2 IN *EISENIA FOETIDA*

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Earthworms represent an important entry point of soil contaminants, such as Se and Cr into the food web. Of biological significance are Cr^{3+}, a putative micronutrient, Cr^{6+}, a respiratory irritant and human carcinogen and the diet supplement, Na_2SeO_3. Se and Cr compounds are present in the environment because of industrial processes, erosion of natural geological formations and run-off from agricultural fields. Data describing the absorption and elimination of these compounds in earthworms are useful in environmental risk assessment. The *objective* of this study was to determine acute dermal toxicity, absorption efficiency and elimination half-life of CrO_3, $CrCl_3$, Na_2SeO_3, SeO_2 in *E. foetida*. All exposures were for 48 hr using individual worms housed in glass vials lined with filter paper (65 cm^2 surface area) on which 1.0 ml of each test chemical was applied. For the elimination study, worms were exposed to 1 mg/cm^2 for 48 hr and sampled after 0.5–42 days. All worms were rinsed, weighed, euthanized, digested with HNO_3/1% H_2O_2 and prepared for analysis by atomic absorption spectroscopy. The order of acute toxicity and LC_{50} values (mg/cm^2) were CrO_3 (6.2) = SeO_2 (7.0) > $CrCl_3$ (18.1) = Na_2SeO_3 (24.4). Few changes in body burden (mg/g dry body wt) occurred for exposures less than 2.5 mg/cm^2. The slopes of the body burden-exposure concentration relationship were determined. There were concentration dependent increases in the (slope) for $CrCl_3$ (12.4) > SeO_2 (7.3) > Na_2SeO_3 (1.5). CrO_3 body burden remained at or below 14 mg/g dry wt. The order of the half-life of elimination (days) was $CrCl_3$ (6.4) < Na_2SeO_3 (9.8) < CrO_3 (16.3) < SeO_2 (42.7). *Conclusion:* All compounds exhibited zero order elimination kinetics. Except for SeO_2, elimination decreased markedly once minimum levels of metal were reached. Exposed earthworms may retain these metals indefinitely thus increasing the risk of introduction of Se and Cr into higher trophic levels of the food web.

995 MATERNAL-FETAL TOXICOKINETICS OF METHANOL

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Methanol (MeOH) has been shown to be teratogenic in rodents, and its proposed use as a motor fuel has raised concern regarding the potential for MeOH-induced developmental toxicity in humans. To construct a toxicokinetic model to predict fetal risk following maternal exposure, the disposition of MeOH in the fetal environment must be determined. In the present study, the disposition of MeOH in the rat amniotic fluid was examined through use of *in vivo* intrauterine microdialysis (MD) to estimate fetal MeOH concentrations. MD probes were inserted into the uteri of anesthetized (urethane, 1 g/kg i.p.) pregnant Sprague-Dawley rats (gestational day 20); MeOH was administered as either an i.v bolus (100 or 500 mg/kg) or an i.v. infusion (100 or 1000 mg/kg/hr). Samples of both maternal blood and uterine microdialysate were collected for MeOH analysis by gas chromatography. The resulting concentration-time data were consistent with saturable maternal MeOH elimination and apparent first-order transfer of MeOH between maternal and fetal compartments. At distribution equilibrium, the concentrations of MeOH in the fetus exceeded those in the dam by approximately 25%. The initial rate of fetal permeation decreased linearly with increasing maternal blood MeOH concentration (r^2 = 0.910). These data indicate that MeOH may decrease blood flow to the fetal compartment in a concentration-dependent manner, thereby decreasing the rate of MeOH presentation to the conceptus. (Supported by HEI Agreement 90-10 and NIEHS training grant T32 ES07126).

996 PHARMACOKINETIC PARAMETERS FOLLOWING INTRAPERITONEAL AND SUBCUTANEOUS ROUTES OF EXPOSURE TO CHLORPYRIFOS IN FEMALE RATS

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Chlorpyrifos (CPF) is an organophosphate insecticide which exhibits its toxicity by inhibiting acetylcholinesterase. We previously noted very persistent neurochemical changes following acute subcutaneous exposure to chlorpyrifos. In the present study, female rats were dosed with CPF either by subcutaneous (sc, 200 mg/kg/2 ml in peanut oil) or intraperitoneal (ip, 60 mg/kg/ml in peanut oil) routes. Blood and brain samples were collected at different time intervals. CPF and its major metabolites, chlorpyrifos-oxon (CPF-O) and 3,5,6-trichloro-2-pyridinol (TCP), were extracted from tissues and analyzed using an HPLC method. CPF could not be detected in blood at any time point following sc administration. 3,5,6-TCP was detected in blood and brain at 30 minutes after sc injection and was present in the blood for as long as 30 days. CPF concentrations peaked in the brain at 8 hrs after sc treatment. Blood concentration of CPF was much higher in the intraperitoneally exposed rats. Pharmacokinetic analysis of the ip data indicates first order absorption and elimination of CPF and 3,5,6-TCP. The half lives of CPF and 3,5,6-TCP were 10 and 56 hr, respectively, following ip administration. Peak concentration of CPF (0.74 μg/ml blood) was reached at 7.1 hrs and volume of distribution was 1.65 liters following ip administration. Overall data indicates that subcutaneous administration forms a depot at the site of injection and CPF is rapidly absorbed but slowly released from the brain tissue. A previously developed physiologically based pharmacokinetic model for organophosphates was used to further analyze the pharmacokinetic data and to tie the kinetics to the pharmacodynamics of enzyme inhibition. (Supported by cooperative agreement CR820229-01 with USEPA).

997 THE DISPOSITION AND PHARMACOKINETIC PARAMETERS FOR 3,3′,4,4′-TETRACHLOROAZOBENZENE (TCAB) AND 3,3′,4,4′-TETRACHLOROAZOXYBENZENE (TCAOB) IN THE FISCHER-344 MALE RAT

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3,3′,4,4′-tetrachloroazobenzene (TCAB) and 3,3′,4,4′-tetrachloroazoxybenzene (TCAOB) are analogs of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). They are formed as byproducts during the synthesis of industrial products. TCAB and TCAOB exhibit similar toxicity and Ah receptor binding characteristics to those defined for TCDD. In this study, the disposition and pharmacokinetic parameters of TCAB and TCAOB were examined in male F-344 rats. The animals received ^{14}C-TCAB (3.2 or 32 mg/kg oral, 3.2 mg/kg i.v.) or ^{14}C-TCAOB (3.4 or 34 mg/kg oral, 3.4 mg/kg i.v.) and the excretion of [^{14}C] was monitored over 96 hr. For oral TCAB, 39% and 45% of the [^{14}C] was eliminated in the urine and 56% and 53% eliminated in the feces for the low and high doses, respectively. Following the low and high oral doses of ^{14}C-TCAOB, 35% and 30% of the [^{14}C] was eliminated in the urine and 55% and 54% eliminated in the feces. At 96 hr after dosing the adipose tissue:blood ratios for TCAB were 2.4 and 11.4 and for TCAOB were 8.0 and 25.9, for the

low and high doses of each compound, respectively. Pharmacokinetic parameters indicate that the compounds are readily cleared from the blood (TCAB: $t_{1/2}$ = 3.9 h, CLs = 0.97 L/kg(h); TCAOB $t_{1/2}$ = 8.2 h, CLs = 0.78 L/kg(h)). Thus, TCAB is cleared from the blood at a faster rate than TCAOB. Both compounds are eliminated faster than TCDD. Therefore, TCAB and TCAOB should express lower levels of Ah receptor associated toxicities than those defined for equal molar doses of TCDD. (Supported by ES35367 and Center Grant P30 ES06694).

998 PHARMACOKINETICS AND TOXICITY OF RECOMBINANT-METHIONYL HUMAN INTERLEUKIN-2[ALANINE-125] (r-metHuIL-2[ala-125]) FOLLOWING INTRAVENOUS AND SUBCUTANEOUS ADMINISTRATION IN RATS

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Comparative toxicity studies in rats were performed to evaluate the response to r-metHuIL-2[ala-125] following two or four weeks of daily intravenous (IV) or subcutaneous (SC) administration, as well as pharmacokinetic (PK) and pharmacodynamic (PD) analyses. PK analysis indicated that r-metHuIL-2[ala-125] showed excellent bioavailability and classical nonlinear concentration profiles. PD responses to IV or SC dosing with r-metHuIL-2[ala-125], as measured by WBC, were comparable. Toxicity studies (6, 30, and 150 μg/kg/day) indicated that r-metHuIL-2[ala-125], either IV or SC, was associated with increased circulating and infiltrating levels of lymphocytes and eosinophils. Bone marrow lymphoid hyperplasia and splenic extramedullary hematopoiesis were similarly observed with each route. This pattern of effects was considered an exaggerated PD response to r-metHuIL-2[ala-125]. Hepatocyte single cell necrosis was observed following both IV and SC administration and was considered to be a toxic response to high doses of r-metHuIL-2[ala-125]. The no observable adverse effect level for r-metHuIL-2[ala-125] via IV administration was 6 μg/kg/day, while that for SC administration was 30 μg/kg/day. Data suggest that SC administration may be a preferred clinical route of administration.

999 PHARMACOKINETIC PROFILE AND PLACENTAL TRANSFER OF A SINGLE INTRAVENOUS DOSE OF [^{14}C]CHLORPYRIFOS IN SPRAGUE-DAWLEY RATS

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The pharmacokinetic profile and placental transfer of [2,6-pyridinyl-^{14}C]chlorpyrifos (*O, O*-diethyl *O*-3,5,6-trichloro-2-pyridinyl phosphorothioate) were determined following a single intravenous injection of 5 mg (10 μCi)/kg in pregnant Sprague-Dawley rats, 14–18 days of gestation. The intravenous level data of chlorpyrifos (CPF) and its metabolites, chlorpyrifos oxon (CPFO) and 3,5,6-trichloropyridinol (TCP), were fitted with a three-compartment, open-model system for plasma, brain, liver, and placenta. On the other hand, the pharmacokinetic profile in the fetus followed a two-compartment, open-model system. The plasma relative residence (R_R) values for CPFO and TCP compared to CPF were 13.71 and 17.03, respectively. The apparent half-life of elimination of CPF, CPFO, and TCP were 17.01, 11.17, and 16.5 h, respectively. The AUC_{plasma} for CPF, CPFO, and TCP were 3,506, 48,074, and 59,718 ng·hr/ml, respectively. The total plasma clearance (CL) and volume of distribution (V_d) for CPF were 0.49 liter/h/kg and 0.21 liter/kg, respectively. The ratio k_{21}/k_{12} of 0.56 and the ratio k_{31}/k_{13} of 0.04 suggest slower equilibrium and slower return of CPF from the deep peripheral compartment to the central compartment when compared to the shallow compartment. The ratio β/k_{10} of 0.017 indicates that only 1.7% of the CPF dose in the body localized in the central compartment and was available for elimination at any time. Overall, the pharmacokinetic profile of an intravenous injection of CPF indicates that k_{13} and k_{31}, the ratio of entry into and return of [^{14}C]CPF from the deep peripheral compartment, respectively, and not k_{10}, the true elimination rate constant, are the rate-limiting factors in the elimination of the insecticide from the body.

1000 PHARMACOKINETICS AND BIOAVAILABILITY OF RWJ-26127 IN THE RAT

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The objectives of this study were to characterize the single dose pharmacokinetics and determine the absolute bioavailability of the experimental osteoporosis drug, RWJ-26127 (3-trifluoromethyl-benzylphosphonic acid), in male and female Sprague-Dawley rats (202–315 g). RWJ-26127 was quantified by a novel HPLC assay in the plasma fractions obtained from serial blood samples collected from each rat over 24 h following administration of the drug. Fasted male and female rats displayed rapid absorption following single oral 10 mg/kg gavage doses in 0.9% saline with the Cmax occurring at about one hour. The mean (±SD) AUC value (N = 6) was significantly lower in the male (18.6 ± 4.0 μg·h/mL) than in the female (29.3 ± 5.1 μg·h/mL), and the Cmax values showed the same trend (5.1 ± 2.0 vs. 10.6 ± 2.2 μg/mL) although it did not reach statistical significance. The terminal half-lives were similar (0.7 ± 0.4 vs. 1.1 ± 0.2 h for males and females, respectively). The mean (±SD, N = 6) terminal half-lives (1.0 ± 0.6 and 0.7 ± 0.4 h for males and females, respectively) after intravenous administration of single bolus 10 mg/kg doses of RWJ-26127 were equivalent to the values obtained following oral administration. AUC values of 26.8 ± 5.6 and 32.5 ± 9.0 μg·h/mL were obtained which resulted in estimates for clearance of 6.42 ± 1.18 and 5.48 ± 1.53 mL/min/kg and for Vdarea of 542 ± 317 and 265 ± 51 mL/kg, respectively, for males and females. The absolute bioavailability of the 10 mg/kg oral gavage dose was 69.4 ± 15.0 and 90.3 ± 15.6% for male and female rats, respectively. In conclusion, RWJ-26127 is rapidly and extensively absorbed and rapidly eliminated in rats.

1001 TOXICOKINETICS OF TAZAROTENE AND ITS METABOLITE (AGN 190299) IN MICE FOLLOWING SINGLE INTRAPERITONEAL ADMINISTRATION OF TAZAROTENE

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Tazarotene, a potent acetylenic retinoid, is intended for the treatment of psoriasis and acne in man. Mice were intraperitoneally dosed with tazarotene at 0.1–100 mg/kg for evaluating the potential of tazarotene to induce micronuclei in the mouse bone marrow erythropoietic cells. Blood samples were collected at 0, 2, 4, 24, 48 and 72 hours post-dose and blood concentrations of both tazarotene and its major active metabolite, AGN 190299, were determined by a GC-MS analysis. Tazarotene was detected in the blood of most animals and the concentrations increased with an increase in tazarotene dosage. However, the blood tazarotene concentrations were scattered, probably due to variations in drug absorption or metabolism. AGN 190299 immediately appeared in the blood with its Cmax at less than 2 hours post-dose. At the highest dosage level, the Cmax of AGN 190299 in males and females were 7.85 and 10.2 μg/ml, indicating extensive systemic drug exposure. Cmax and AUCs of AGN 190299 was linearly correlated to the dosage of tazarotene with $r^2 > 0.998$, demonstrating linear pharmacokinetics of AGN 190299 at tazarotene dosage levels of 0.1–100 mg/kg. Drug bioavailability, as measured by AUCs of AGN 190299, was 20–30% except for that at the lowest dosage level (≈100%). Elimination half-life of AGN 190299 after intraperitoneal administration was about 9–17 hours, which is much longer than the half-life after intravenous dosing (90 min). The longer half-life of AGN 190299 in the present study, therefore, seemed to be absorption rate-limited. The difference in Cmax of AGN 190299 between the sexes was not statistically significant, although higher blood concentrations of AGN 190299 were seen in female animals.

1002 TOXICOKINETICS OF THE METABOLITE (AGN 190299) OF TAZAROTENE IN MICE, RATS, RABBITS, PIGS AND MONKEYS FOLLOWING MULTIPLE ADMINISTRATION OF TAZAROTENE

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Tazarotene, an acetylenic retinoid, is a novel agent for topical treatment of psoriasis and acne. *In vivo,* tazarotene is subjected to rapid and extensive hydrolysis to its major active metabolite, AGN 190299, in most species. The toxicokinetic characteristics of AGN 190299 were studied in various animal species following daily oral (dietary or gavage) or topical skin administration of tazarotene at dosage levels of 0.025–1 mg/kg/day. Systemic drug exposure based on AGN 190299 blood concentrations varied among the animal species. Following oral gavage administration at 0.05 mg/kg/day, the Cmax of AGN 190299 in pregnant rabbits, pregnant rats and monkeys were 60.4, 17.2 and 6.2 ng/ml, respectively. Dietary administration showed lower systemic bioavailability than that after gavage administration. After an 18-month dietary dosing at 0.05 mg/kg/day, the maximal blood AGN 190299 concentrations in rats were 0.77 ng/ml. Following topical administration at 0.1–0.125 mg/kg/day, mice

showed the highest blood AGN 190299 concentrations (20 ng/ml), rats intermediate (5.0 ng/ml), and pigs the lowest (2.24 ng/ml). The Cmax and AUCs of AGN 190299 in the blood of animal species tested were linearly correlated to the increase in tazarotene dosage. Drug accumulation was not observed following long-term administration of tazarotene. Overall, drug exposure in the animal species at all dosage levels was generally much greater than that in man after multiple topical administration at 0.1 mg/kg/day (average Cmax = 0.7 ng/ml). Since significant toxicological effects were not observed at low dose levels in animals, the toxicokinetic data from animal species established a good safety margin for tazarotene in clinical use.

1003 THE PHARMACOKINETICS (PK) OF 3'-AZIDO-3'-DEOXYTHYMIDINE (AZT) AND ITS METABOLITES IN B6C3F$_1$ MICE

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Metabolism of AZT to 3'-amino-3'-deoxythymidine (AMT) may be a primary cause of AZT-associated myelotoxicity in AIDS patients. Characterization of the *in vivo* metabolism of AZT could therefore improve the understanding of AZT toxicity. The present study was conducted to characterize the plasma concentration × time (cxt) profile of AZT and its major metabolites, AMT and AZT-glucuronide (GAZT), in mice. Male and female mice (11 weeks old) were given 100 mg AZT/kg body weight as a single iv or oral dose. At 2.5, 5, 10, 15, 20, 30, 40, 60, 90, 120, 150, 180 and 240 min postdose (iv) and 2.5, 5, 10, 15, 20, 30, 40, 60, 90, 120, 150, 180, and 300 min postdose (oral), blood was collected by cardiac puncture under CO_2 anesthesia (n = 3/timepoint). For each sample, AZT, AMT, and GAZT were measured. The cxt profiles were similar for both sexes. Model-independent analysis of the iv and oral AZT data yielded the following PK parameters, respectively (mean for both sexes): half-life, 21 and 40 min; clearance, 26 and 26 mL/min; and mean residence time, 24 and 39 min. The volume of distribution and absolute bioavailability were 0.79 L and 70%, respectively. AZT was rapidly metabolized as evidenced by the appearance of AMT and GAZT in plasma within 2.5 and 5 min, respectively, following iv and oral administration. AMT levels persisted for up to 5 hr postdose, while GAZT levels were usually non-detectable by 1 hr. The estimated area under the cxt curve was ≈100 fold greater for AZT than AMT and GAZT. In summary, these baseline data will be used to evaluate potential alterations in the PK and metabolism of AZT in the presence of other drugs commonly used in the treatment of AIDS patients. Supported by NIH Contract N01-ES-15307.

1004 TOXICOKINETICS (TK)-BASED DOSE SELECTION FOR CARCINOGENESIS STUDIES OF PHARMACEUTICS: AN ALTERNATE APPROACH TO MAXIMUM TOLERATED DOSE (MTD)

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The use of MTD as the top dose in rodent carcinogenesis studies of pharmaceuticals with low toxicities has often resulted in administration of very large doses. Examples are provided of TK-based dose selection as an alternate to MTD for conducting the carcinogenesis studies of two non-genotoxic pharmaceuticals, compounds A and B. These compounds were well tolerated up to the oral doses of 250 and 800 mg/kg/day, respectively, in 3-month toxicity studies in rats. The systemic exposure [plasma drug area under the curve (AUC)] values for compound A at the doses of 125 and 250 mg/kg/day were 50.3 and 115.7 μg·hr/ml, respectively. These AUC values exceeded the AUC of compound A in humans (0.05 μg·hr/ml) at the maximal clinical dose (0.05 mg/kg/day) by approximately 1000 and 2000-fold, respectively. The dose of 125 mg/kg/day was selected as the top dose for compound A because it provided a very large safety margin of approximately 1000-fold. The AUC value for compound B and its major metabolites plateaued at the dose of 200 mg/kg/day, and this dose was selected as the top dose for compound B. These results suggest that TK-based end-points provide a rational basis of top dose selection with relevance to human safety.

1005 THE TOXICITY AND TOXICOKINETIC PROFILE OF LG 1-05 FOLLOWING INTRAVENOUS INFUSION IN THE CYNOMOLGUS MONKEY

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Sponsor: A B Wilson

LG 1-05 (acetyl starch) is a derivative of natural starch with a molecular weight of approximately 200,000 daltons and a molar substitution of 0.5. LG 1-05 is intended for use as either a plasma expander or haemodilution agent. LG 1-05 was given *via* daily intravenous infusion (4 h.day^{-1}) to 36 laboratory-bred cynomolgus monkeys (*Macaca fascicularis*) at dose levels of 1.2, 2.4 and 4.8 mg.kg^{-1}.day^{-1} for 28 days after which 12 animals were retained for either a 4 or 8 week post-treatment observation period. There were no clinical reactions to treatment. Biochemical data showed a marked and dose-related increase in AST; in the absence of any histological correlates, this finding is equivocal. Histology revealed foamy macrophage accumulations in several organs and tubular vacuolation/hydropic degeneration in the kidneys of animals that received 2.4 and 4.8 mg.kg^{-1}.day^{-1}. These changes, however, were shown to be partially reversible at the end of the recovery period. LG 1-05 was estimated in monkey serum using an Anthrone condensation method and results were expressed in mmol of glucose (breakdown product of LG 1-05). Toxicokinetic data showed peak serum levels of glucose were attained 1 h after the end of the 4 h infusion, after which the serum glucose levels showed a slow decline.

1006 AROCLOR 1242 ACTIVATES RAT NEUTROPHILS BY A MECHANISM INDEPENDENT OF G PROTEINS AND PROTEIN TYROSINE KINASES

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Polychlorinated biphenyls (PCBs) activate isolated neutrophils by a mechanism which likely involves phospholipase C-dependent hydrolysis of membrane phosphoinositides (PI). Other neutrophil activators such as fMLP stimulate PI turnover by binding to a membrane receptor which is coupled to phospholipase C by a pertussis toxin-sensitive G protein. Tyrosine phosphorylation is also involved in neutrophil activation by fMLP since inhibitors of protein tyrosine kinases (PTKs) attenuate responses to this agent. To determine whether similar signal transduction pathways are operant in PCB-induced activation of neutrophils, superoxide (O_2^-) production was measured in glycogen-elicited, rat neutrophils exposed to the PCB mixture, Aroclor 1242, in the presence of pertussis toxin (50 ng/ml), the PTK inhibitor, genistein (10^{-4}M) or vehicle. Neutrophils produced significant O_2^- when stimulated for 30 minutes with either 10 μg/ml Aroclor 1242 or 10^{-6}M fMLP. Pretreatment with pertussis toxin for 90 minutes or genistein for 15 minutes abolished the response to fMLP; however O_2^- release in response to Aroclor 1242 was unaffected by these inhibitors. To role out an involvement of pertussis toxin-insensitive G proteins in neutrophil activation by PCBs. O_2^- was measured in digitonin-permeabilized cells in the presence and absence of the nonhydrolyzable GDP analogue, GDPβS (10^{-3}M). Permeabilized neutrophils produced significant O_2^- in response to Aroclor 1242 or fMLP. GDPβS, at a concentration which abolished fMLP-induced O_2^- production, did not alter the response to Aroclor 1242. These results suggest that, although fMLP activates rat neutrophils to produce O_2^- by a mechanism which involves G proteins and PTKs, these signal transduction pathways are unlikely to be involved in the mechanism of PCB-induced neutrophil activation. (Supported by NIH grant ESO4911).

1007 AROCLOR 1242 ACTIVATES NEUTROPHILS BY A MECHANISM WHICH INVOLVES PHOSPHOLIPASE A$_2$

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Isolated, rat neutrophils produce superoxide anion (O_2^-) when exposed *in vitro* to the mixture of polychlorinated biphenyls, Aroclor 1242. The mechanism of neutrophil activation involves, as an early event, phospholipase C-dependent hydrolysis of membrane phosphoinositides. Little is known, however, about subsequent signal transduction mechanisms in this response. Other agents which activate neutrophils through phospholipase C stimulate O_2^- production by a mechanism which involves phospholipase A$_2$ (PLA$_2$)-dependent release of arachidonic acid. Therefore, the current study was undertaken to deter-

mine whether similar mechanisms are important in PCB-induced O_2^- production. Superoxide anion production was measured in glycogen-elicited, rat neutrophils stimulated with Aroclor 1242 (10 μg/ml) or fMLP (10^{-6}M) in the presence of inhibitors of the secretory PLA_2 ($sPLA_2$), manoalide (10 μM), quinacrine (100 μM) and bromophenacyl bromide (BPB; 10 μM). Manoalide, quinacrine and BPB abolished O_2^- production in response to Aroclor 1242 and fMLP. In neutrophils prelabeled with 2 μCi/ml ^{3}H-arachidonic acid, Aroclor 1242 caused release of ^{3}H-arachidonic acid which was inhibited by manoalide (10 μM). These results suggest that $sPLA_2$-induced mobilization of arachidonic acid is important in the mechanism of O_2^- production by rat neutrophils exposed to Aroclor 1242. (Supported by NIH grant ESO4911).

1009 IMMUNOSUPPRESSIVE ACTIVITY OF POLYCHLORINATED BIPHENYL MIXTURES AND CONGENERS: NON-ADDITIVE (ANTAGONISTIC) INTERACTIONS

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The dose-response inhibition of the splenic plaque-forming cell (PFC) response and serum IgM units to the antigen, trinitrophenyl-lipopolysaccharide, was determined for several polychlorinated biphenyls (PCBs) mixtures and congeners in female B3C3F1 mice. The ED_{50} values for Aroclor 1260, 1254, 1248 and 1242-induced immunotoxicity varied by less than two-fold from 355 to 699 mg/kg. The range of ED_{50} values for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), 3,3′,4,4′-tetra-chlorobiphenyl (CB), 3,3′,4,4′,5-pentaCB, 3,3′,4,4′,5,5′-hexaCB, 2,3,3′,4,4′-pentaCB, 2,3′,4,4′,5-pentaCB, 2,3,3′,4,4′,5-hexaCB, 2,3,3′,4,4′,5,5′-heptaCB, 2,2′,3,3′,4,4′,5-heptaCB and 2,2′,3,4,4′,5,5′-heptaCB were 4.6 to 4.9, 134 to 245, 4.7 to 7.0, 6.9 to 11.1, 88,000 to 121,000, 122,000 to 132,000, 99,000 to 157,000, 89,000 to 129,000, 117,000 to 240,000, and 132,000 to 238,000 μg/kg, respectively. The immunotoxicity-derived toxic equivalency factors (TEFs) for these congeners were calculated from the ED_{50} (TCDD) / ED_{50} (congener) ratios and the TEF values were within the range of those previously reported. The values were used to calculate toxic equivalent (TEQ) ED_{50} values for the PCB mixtures. The ED_{50} (observed) / ED_{50} (calculated) ratios gave values of 0.9 to 1.6, 4.5 to 6.8, 15.3 to 26.9, and 13.4 to 23.0 for Aroclors 1260, 1254, 1248 and 1242, respectively, indicating that for Aroclors 1254, 1248 and 1242 (but not Aroclor 1260), the TEF approach overestimates toxicity due to non-additive (antagonistic) interactions of the PCBs.

1010 DECREASED MOTOR ACTIVITY PRODUCED BY ACUTE AND REPEATED EXPOSURE TO AROCLOR 1254 IN ADULT RATS

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While considerable research has focused on the neurotoxicity of developmental exposure to polychlorinated biphenyls, including Aroclor 1254, relatively little is known about exposures in adult animals. This experiment compared the effects of acute and repeated exposure to Aroclor 1254 on the motor activity of adult rats. Male LE rats (60d) were tested for motor activity in a photocell device before and after acute exposure to Aroclor 1254 (0, 100, 300 and 1000 mg/kg, p.o., N = 9/group). Motor activity was decreased in a dose-related manner 45-min following exposure. ED50s were 1000 mg/kg for horizontal activity and 300 mg/kg for vertical activity. Recovery of activity occurred over 4 weeks after 300 mg/kg but was incomplete after 1000 mg/kg. The highest dose also produced severe weight decreases, and death in 4 of 9 rats. Additional rats received 0, 10, 30 and 100 mg/kg Aroclor 1254, 5 days/week, for 4 weeks (N = 9/group). Testing occurred before dosing and at weekly intervals throughout dosing. No effects were obtained after the first exposure. Thereafter, activity levels decreased in a dose-related manner. These results demonstrate a cumulative and persistent effect of Aroclor 1254 on the motor activity of adult-exposed rats.

1011 SEX DIFFERENCES IN THE TISSUE ACCUMULATION AND METABOLIC PROFILES OF AROCLORS 1016, 1242, 1254, AND 1260 IN SPRAGUE-DAWLEY RATS

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Adipose, liver, and brain tissue taken from Sprague-Dawley rats fed Aroclors 1016, 1242, 1254, or 1260 at levels ranging from 25–200 ppm for 3–12 months as part of an ongoing 2 yr chronic toxicity/carcinogenicity study was analyzed for 118 PCB congeners. All rats showed a dose-dependent accumulation of total PCB in tissue lipids. By 12 months, male *vs.* female lipid PCB accumulation for Aroclors 1016, 1242, 1254, and 1260 were 162 *vs.* 297, 96 *vs.* 237, 1074 *vs.* 3222, and 1779 *vs.* 3229 ppm, respectively, indicating 2–3 times as much accumulation in females as in males. Furthermore, females accumulated Aroclors 1254 and 1260 to an extent tenfold that of either Aroclor 1016 or 1242. The congener-specific analysis of the residual PCBs indicated 95–99% losses of all congeners with adjacent unsubstituted *meta, para*-positions from all test groups as a result of their susceptibility to oxidative metabolism. However, the losses of PCB congeners that were *para*-substituted, *meta*-unsubstituted were found to be sex-, dose-, and Aroclor-dependent. The clearance patterns for these congeners suggested that induction of P4501A-like PCB-metabolizing activity was similar in both sexes, and nearly independent of dose, whereas that of P4502B-like activity was greater in males and dose-dependent.

1012 EFFECT OF AROCLOR 1254 EXPOSURE ON BULLFROG (*RANA CATESBEIANA*) HELMINTH PARASITE BURDEN AND CYP1A1 ACTIVITY

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Frog populations are declining worldwide and it has been suggested that environmental contaminants are a major factor in this decline. Polychlorinated biphenyls (PCBs) are widely distributed in sediments and produce a number of toxic and biochemical effects in most animal species. It has been suggested that one effect may be decreased resistance to parasite infestation. A series of experiments was initiated to determine if Aroclor 1254 (a PCB mixture) exposure affected parasite susceptibility in the bullfrog. Adult bullfrogs were dosed with 0, 10 or 300 mg/kg Aroclor 1254 dissolved in corn oil by gavage. The bullfrogs were euthanized after 7 days and the number and species of helminth parasites ascertained. Neither the number of nematode species nor the number of trematode species were affected by the treatment in either frogs that survived until the end of the treatment or those that succumbed during the exposure period. There was a dose-dependent decrease in parasite burden in those frogs that survived the exposure period whereas there were no differences in parasite intensity in those frogs that failed to survive the exposure period. Moreover, there was a concomitant increase in CYP1A1 activity in those frogs surviving until the end of the exposure period. The results of these experiments suggest that parasite burden and sensitivity to Aroclor 1254 exposure may be interrelated. Supported by NIEHS 04696.

1013 ORTHO-SUBSTITUTED POLYCHLORINATED BIPHENYLS (PCBS) ACTIVATE RYANODINE RECEPTOR TYPE I IN CEREBELLUM AND SKELETAL MUSCLE

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Recent epidemiological studies revealed that children and animals prenatally exposed to low level of polychlorinated biphenyls (PCBs) showed neurodevelopmental deficiencies. However, the molecular mechanism of the neurotoxicity caused by these environmental contaminants is still unclear. Radioligand binding analysis with [^{3}H]ryanodine, a conformationally sensitive probe for ryanodine receptor type 1/Ca^{2+} channel complex (RyR1) of skeletal muscle and cerebellum sarcoplasmic and endoplasmic reticulum (SR/ER), demonstrated that a commercial PCB mixture, Aroclor 1254, significantly enhanced the activity of RyR1 in both skeletal muscle (EC_{50} = 1.3 μM) and cerebellum (EC_{50} = 35 μM) SR/ER preparations. Aroclor 1254 released the inhibitory effect of mM of Ca^{2+} and Mg^{2+}, which are known physiological antagonists. Structure-function studies with select PCB congeners indicated that ortho-substituted PCBs, but not the planar PCBs, possess activity towards RyR1. The 22′35′6 pentachloro-PCB congener was found to activate [^{3}H]ryanodine occupancy 6 to 10-fold with an EC_{50} of 0.3 μM and 17 μM in skeletal muscle and cerebellar preparations, respectively. In contrast, the co-planar congener 33′44′5 PCB showed no detectable activity on the RyR1/Ca^{2+} channel complex. Spectrometric determination of active uptake and release of Ca^{2+} using the metallochromic dye antipyrylazo III revealed that non-planar, but not co-planar, PCB congeners induce the release of vesicular Ca^{2+}, an effect completely blocked by known RyR1 antagonists, ryanodine (μM) or ruthenium red (nM). Ca^{2+} ATPase and SERCA pump activity were shown not to be affected by channel active PCB congeners, indicating specificity and a stringent structure-function relationship at RyR1. Binding of ortho-substituted PCBs to RyR1 on SR/ER membranes, appears to account for their ability to mobilize

intracellular Ca^{2+} and contribute significantly to altered cellular Ca^{2+} signaling and function. This newly identified mechanism may contribute significantly to the neurotoxicity of ortho-substituted PCBs.

1014 CHLORINATED DIHYDROXYBIPHENYLS AND QUINONES: SYNTHESIS, REACTIVITY, AND CYTOTOXICITY STUDIES

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We are evaluating the metabolic activation of chlorinated biphenyls to hydroxylated metabolites and quinones and the reactivity of these metabolites. As a part of this effort, a series of chlorinated dihydroxybiphenyls were synthesized using a combined metalation/cross-coupling strategy followed by demethylation of the precursor dimethoxybiphenyls. The dihydroxybiphenyls were oxidized to the corresponding quinones using DDQ. These compounds have been used to estimate the reactivity towards nucleophiles such as glutathione and N-acetyl-L-cysteine as well as establishing the redox potentials using cyclic voltammetry. The dihydroxybiphenyls and quinones were employed in toxicity studies using yeast (*Saccharomyces cerevisiae*). The toxicity was influenced both by the number and position of the chlorine substituents as well as by the position of the hydroxyl groups. The toxicity increased with 1–2 chlorines in position 2 < 3 < 2,5 < 4 < 3,5 < 3,4 for 2′,5′ as well as 2,′3′ and 3′,4′-dihydroxybiphenyls. Similarly the toxicity increased with the dihydroxy groups in the 2′,5′ < 2′,3′ < 3′4′ position. Quinones were always 2–10x more toxic then their corresponding dihydroxybiphenyls. (Supported by NIH RO1 CA 57423).

1015 REDUCTION OF ESTROGEN RECEPTOR CONCENTRATION IN MINK CONSUMING GREAT LAKES FISH

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Ranch-bred mink were fed diets supplemented with PCB-containing carp from Saginaw Bay, MI, to provide 0.25, 0.5, and 1.0 ppm total fish during three generations. Non-PCB-containing fish were substituted for the Saginaw Bay carp to provide a control diet. Half of the P_1 and F_1 animals were transferred to the control diet after whelping of the subsequent generation. PCBs have been demonstrated to have anti-estrogenic effects in animal species other than the mink, and to decrease estrogen receptor concentrations both in experimental animals and in cultured cell lines. Mink have been shown to be very sensitive to reproductive disruption by the PCBs contained in Great Lakes fish. Estrogen receptor concentration was measured by binding of ^{3}H-estradiol at saturating conditions in cytosol from uterine and hepatic tissues from each animal. Estrogen receptor concentrations decreased with increasing Great Lakes fish consumption.

1016 KINETIC OF THE DISTURBANCES ELICITED BY HEXACHLOROBENZENE IN DIFFERENT ZONES OF KIDNEY. METABOLIC STUDIES

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Studies on haem and lipid metabolisms were performed in cortical, medullary and papillary zones of rat kidney as function of hexachlorobenzene (HCB) intoxication time. They included the level and synthesis of phospholipids (PL) (^{32}P incorporation), porphyrin content, uroporphyrinogen decarboxylase (URO-D) activity and (URO-D) inhibitor generation, as well as parameters of oxidative stress. Cortex is the only kidney zone that accumulates porphyrins (4th) week) due to HCB effect. In this zone URO-D activity diminishes from 2nd week while the inhibitor is present from the 3rd week of treatment. Oxidative stress is evident from the early times. PL of renal cortex respond to xenobiotic action, lowering their turnover during all treatment time. This fact results in an increase in phosphatidilcholine content that falls at chronic intoxication times. However, papilla shows an increase in PL turnover to maintain steady the amount of phosphatidilcholine in membranes along the treatment.

On the whole, the three kidney zones respond in different way to HCB effect in both haem and lipid metabolism.

1017 ASSESSMENT OF HEXACHLOROBENZENE (HCB) CYTOTOXICITY IN CULTURED RAT HEPATOCYTES

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HCB is a persistant and widely distributed contaminant in the environment. It is known to induce liver porphyria and tumors in female rats. An *in vivo* study demonstrated that liver cell proliferation is increased in porphyric rats. In order to establish whether or not these effects are related to a sex-related toxicity in the liver, hepatocytes isolated from rats of both sexes were incubated with HCB and cytotoxicity was determined. Three other toxins, namely carbon tetrachloride (CCl_4), mercury chloride ($HgCl_2$) and 3-methylcholanthrene (3-MC) were used to evaluate the relative cytotoxicity of HCB. Hepatocytes were isolated from male or female Sprague-Dawley rats (45–50 days old) and incubated in Williams' medium-E supplemented with 100 nM insulin, 100 nM dexamethasone, 2 mM L-glutamine and 2% FCS. After 3 h, the medium was replaced by serum-free medium. The following day, the hepatocytes were treated for 24 h with various concentrations of the four toxins (0.5 μM–25 mM). Cytotoxicity was measured by determining the secretion of albumin, LDH leakage and protein contents. The results showed a relative low cytotoxicity for CCl_4, intermediate for both HCB and 3-MC, and a high cytotoxicity for $HgCl_2$. No sex differences in cytotoxicity of the tested substances could be detected. Therefore, the sexual dimorphism in hepatic carcinogenicity seems not to be related to a sex-specific cytotoxicity *in vitro*. (Supported by MRCC).

1018 ACCUTE TOXICITY, GENOTOXICITY AND CARDIAC SENSITIZATION POTENTIAL OF CF_3I (TRIFLUOROIODOMETHANE)

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CF_3I is being considered by the USAF as a candidate for replacement of Halon 1301 (CF_3Br), the fire extinguishant currently used as a total flooding agent in both occupied and unoccupied spaces. As part of the preliminary screening of CF_3I an initial evaluation of its toxicity was undertaken. A series of 4-h nose-only inhalation exposures were performed on Fischer 344 rats at 1.0 and 0.5% CF_3I. Groups of rats were sacrificed 0, 3 and 14 days postexposure. No signs of toxic stress were noted during or following exposure. Inhalation of CF_3I at these concentrations did not result in any observable toxicological effects. CF_3I was positive in the *Salmonella typhimurium* histidine (*his*) reversion mutagenesis assay. The L5178Y/*tk* mouse lymphoma cell mutagenesis assay showed that CF_3I did not induce gene or chromosomal mutations in mammalian cells *in vitro*. A positive evaluation in the mouse bone marrow micronucleus test indicated CF_3I was clastogenic *in vivo*. Cardiac sensitization testing of CF_3I using beagle dogs showed a no observable effect level at 0.2% and a lowest observable adverse effect level at 0.4%. Since the proposed use concentration for fire extinguishing is 7.0%, the cardiac sensitization results eliminate neat CF_3I for use as a flooding agent in occupied spaces but it is still a viable option for use in unoccupied spaces. (Supported by Dept. of the Air Force Contract No. F33615-90-C-0532).

1019 A COMPARATIVE STUDY OF TCDD EFFECT IN INTACT CELLS AND CELL-FREE CONDITIONS USING NIH 3T3 CELLS

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TCDD induced protein phosphorylation in ^{32}P-H_3PO_4 labeled NIH 3T3 cells after 15 min of treatment and this effect continued throughout the time course of treatment (up to 180 min). The same stimulatory effect of TCDD was observed when phosphorylation was performed *in vitro*. In that case cells were treated with TCDD for 5 and 10 min followed by isolation of the 7000 xg supernatant fraction and phosphorylation with γ-^{32}P-ATP for 60 sec. The phosphoproteins were then analyzed by SDS-PAGE. In another set of experiments using substrate peptides for tyrosine kinase, MAP 2 kinase, PKA and PKC with optimal conditions for each kinase, it was found that TCDD stimulated tyrosine kinase activity by 18% after 5 min of treatment of intact cells. MAP 2 kinase activity was highly increased (150% of control values) after 10 min of TCDD treatment. Under cell-free conditions (i.e. *in vitro* addition of TCDD to the 7000 xg supernatant or 100,000 xg cytosol), the results were similar to those obtained after treatment of intact cells. In some cases, TCDD induced more kinase activity under cell-free conditions than in intact cells. For example, MAP 2 kinase activity was increased by up to 50% after 10 min treatment of intact cells while an increase of 66% was observed after 10 min of TCDD treatment under cell-free conditions. Using the cell-free system, in the

presence of tyrosine kinase substrate peptide (RR-SRC) in combination with heparin, TCDD reduced the incorporation of ^{32}P into the substrate while in combination with genistein, TCDD did not produce further effect than that obtained with genistein alone. It can be concluded that TCDD produced changes in protein phosphorylation of NIH 3T3 cells as early as 5–15 min in intact cells and under cell-free conditions as well. Also, among the four kinases we have tested, MAP 2 kinase was the most responsive after 10 min of treatment with TCDD followed by tyrosine kinase.

1020 HALOACETATE INDUCED OXIDATIVE DNA DAMAGE IN THE LIVERS OF MALE B6C3F1 MICE

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Brominated and chlorinated haloacetates (HAs) are water chlorination byproducts. Dichloroacetate (DCA) and trichloroacetate (TCA) are carcinogenic in rodents, but little is known about their brominated analogs. A series of experiments involving acute administration and inclusion of HAs in drinking water were undertaken to determine whether oxidative stress could be playing a role in their toxicology. Acute oral doses of 300 mg/kg induced an oxidative stress characterized by increases in hepatic concentrations of thiobarbituric acid reactive substances (TBARS) that was paralleled by increases in 8-hydroxy-2′-deoxyguanosine (8-OH-dG) concentrations in nuclear DNA of the liver of male B6C3F1 mice. The relative potencies of the HAs in this experiment were dibromoacetate (DBA) $\geq$ bromochloro-acetate (BCA) $\gg$ bromodichloroacetate (BDCA) > TCA > DCA. Administration of the HAs in drinking water for periods of up to 10 weeks resulted in dose-related increases in nuclear 8-OH-dG with DBA and BCA, but not with TCA or DCA. These data suggest that oxidative damage to DNA may play a role in the chronic toxicology of brominated haloacetates, but will contribute little to the effects of TCA and DCA. (Supported by NIEHS RO1-ESO4648 & AWWARF 702-91).

1022 COMPARATIVE TOXICOLOGY OF THE CFCS AND HFC ALTERNATIVES

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The Montreal Protocol requires the phase out of chlorofluoro-carbons (CFC) by 1996. International toxicology programs have been established by producers to examine the safety of CFC alternatives. The HFC compounds include HFC-152a, HFC-23, HFC-143a, HFC-134a, HFC-125 and HFC-32. The CFC to be eliminated are CFC-12, CFC-11, CFC-13, CFC-113, CFC-114, CFC-115 and CFC-13B1. The HFCs and CFCs exhibit very low acute inhalation toxicity as shown by 4-hr LC50 values in rats. The principal acute effect with most of these compounds is an anesthetic effect observed during exposure at high concentrations but this is rapidly reversible on exposure termination. In screening studies with dogs, the HFCs sensitized the heart to an intravenous epinephrine challenge. Similar results were obtained with the CFCs. In subchronic inhalation studies, rats were exposed 6 hr/day, 5 days/wk for 10–90 days. The HFCs and CFCs exhibited little to no toxicity, with weak anesthesia being the principal effect during exposure. In addition, these compounds were not cumulatively toxic. None of these compounds were mutagenic in either *in vitro* or *in vivo* tests. In summary, the HFC alternatives are similar or lower in toxicity compared to the CFCs that they are intended to replace. Many of the toxicity studies conducted with some HFCs were sponsored by the Program for Alternative Fluorocarbon Toxicity Testing (PAFT).

1023 NONLINEAR KINETICS OF TRICHLOROETHYLENE (TCE) IN RATS: SOLVENT-INDUCED CYTOTOXICITY FOLLOWING PORTAL VEIN ADMINISTRATION

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In a previous study in rats (Lee *et al.*, 1994), it was observed that hepatic clearance of TCE, injected via the portal vein (PV), decreased as the dose of TCE increased. The objective of the present study was to assess the ability of hepatocellular damage to reduce the capacity of the liver to metabolize TCE. A cannula was implanted into either the jugular vein (JV) or PV of male S-D rats (300–380 g). TCE (16 and 64 mg/kg) was incorporated into a 5% aqueous Emulphor® emulsion and injected into the JV or PV. Some animals received 0.5 ml of p-nitrophenol (PNP) (50 mM) through the PV cannula 3 min before TCE dosing. Serial serum and hepatic microsomal samples were prepared and liver samples examined microscopically. There was a marked reduction of P450 and elevation of serum enzymes 1 hr after PV injection of TCE. Selective reduction of P450IIE1, the primary isozyme responsible for TCE metabolism, was not observed. PNP-pretreatment failed to protect from TCE hepatotoxicity. Periportal rather than centrilobular necrosis was seen, implying that the cytotoxicity resulted from solvent effects of high TCE concentrations. Thus, hepatic extraction of TCE in rats given PV doses $\geq$ 16 mg/kg appears to be reduced primarily by nonspecific hepatocellular membrane damage, rather than by TCE metabolite-mediated P450 inhibition. (This abstract does not necessarily reflect EPA policy. Supported by EPA Cooperative Agreement CR-820447).

1024 KINETICS OF MOBILIZATION OF PCB CONGENERS AND DDE BY LIPID MICROEMULSION INFUSIONS; IMPLICATIONS FOR BIOMARKERS OF EXPOSURE ASSESSMENT

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Serum levels of persistent chlorinated organics such as PCBs and DDE are being used as biomarkers of cumulative exposure and body burden in epidemiological studies. The validity of serum levels as a biomarker is dependent upon the mobility of these lipophilic xenobiotics between serum and body lipid compartments. The purpose of this study was to assess the rate of equilibration of several endogenous PCB congeners and DDE from body stores with a xenobiotic free lipid lead introduced by iv infusion. ASAI elective surgery patients receiving propofol anesthesia by continuous iv infusion in a lipid microemulsion served as the study population. Serum from timed blood samples taken before and during infusion were analyzed for total lipid content (gravimetric) and amount of PCB congeners (IUPAC #) 138, 153, and 180, and DDE (GC-ECD). There was a tendency toward increased serum xenobiotic levels with increased serum lipid lead, however, there were considerable inter-individual and congener specific variation in both the magnitude and kinetics of mobilization. The most dramatic increase was seen in a patient whose levels of PCB congeners 138, 153, and 180 were elevated 316, 342 and 480% respectively after a 38% increase in serum lipid. The DDE increase in that patient was only 26%. Further studies are required to assess the mechanism responsible for the inter-individual and congener specific differences in lipid induced xenobiotic mobilization, and their implications for use of serum levels as a biomarker for exposure or cumulative body burden of these pollutants.

1025 MECHANISMS OF TOXICITY OF CARBON TETRACHLORIDE AND CHLOROFORM IN PRIMARY CULTURE OF RAINBOW TROUT HEPATOCYTES

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Sponsor: M A Davis

The main obstacle in establishing long-term primary cultures of rainbow trout (*Oncorhynchus mykiss*) hepatocytes has been the poor attachment to the culture dish. We have established primary cultures of trout hepatocytes in which differentiated liver function is maintained in cells grown on polylysine coated culture dishes. Induction of TAT (tyrosine amino transferase) by dexamethasone was observed up to 22 days. Two model substances metabolized by cytochrome P450, carbon tetrachloride (CCl_4) and chloroform, were used to study the suitability of the model system in toxicological research. The LD_{50} was 5 mM for CCl_4 and 24 mM for $CHCl_3$, determined as LDH release. Reduced glutathione (GSH) depletion did not precede toxicity indicating that initial events in the toxicity was not related to depletion of GSH. The relationship between lipid peroxidation and the toxicity of the solvents was determined by measuring the LDH release in the presence of the antioxidant DPPD (N,N,-diphenyl-p-phenylenediamine). DPPD was able to protect the cells only at concentrations around LC_{50}, i.e. low level toxicity. In this study we further looked at the time course of induction of the cellular oncogenes c-fos, c-myc and c-jun in response to CCl_4 by using Northern blotting techniques.

1026 EFFECT OF AROMATIC HYDROCARBONS ON THE CARBOXYHEMOGLOBIN FORMATION AND METABOLISM OF DICHLOROMETHANE IN RATS

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The effect of a single exposure to several aromatic hydrocarbons on the metabolic activity responsible for the metabolic conversion of dichloromethane (DCM) to carbon monoxide (CO) was investigated using adult female rats. In rats treated orally with either benzene (1.5 ml/kg), toluene (2.0

ml/kg), or *m*-xylene (2.0 ml/kg) 16 to 24 hr prior to DCM (3 mmol/kg, ip), the carboxyhemoglobin (COHb) levels plateaued at 21%, 16%, or 23%, respectively, 120 min following the DCM treatment whereas the maximum COHb level reached approximately 10% in rats treated with DCM only. Disulfiram (3.4 mmol/kg, ip) blocked the COHb elevation completely suggesting that the metabolic conversion of DCM to CO is mediated by cytochrome P-450 (CYP) 2E1. Also the hepatotoxicity induced by carbon tetrachloride (2 mmol/kg, ip), in which the CYP 2E1 activity has been known to play an important role, was potentiated significantly by pretreatment of rats with each aromatic hydrocarbon as determined by increases in serum sorbital dehydrogenase, glutamic pyruvic transaminase, and glutamic oxaloacetic transaminase activity. *In vitro* studies showed that the hepatic microsomal metabolism of nitrosodimethylamine, *p*-nitrophenol, *p*-nitroanisole, and cytochrome P-450 contents were all increased by a single treatment of rats with each aromatic hydrocarbon. The present study indicates that the mechanism responsible for the increased COHb formation from DCM is the induction of CYP 2E1 by a single exposure of rats to either benzene, toluene, or *m*-xylene. (Supported by KOSEF 921-1600-023-2).

1027 THE EFFECT OF TRICHLOROACETATE (TCA) AND DICHLOROACETATE (DCA) PRETREATMENT ON THE METABOLISM OF BROMOTRICHLOROACETATE (BDCA) IN THE B6C3F1 MOUSE

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Previous research demonstrated that pretreatment with TCA and DCA causes a modification in thiobarbaturic acid reactive substances (TBARS) following acute doses of TCA and DCA. TCA pretreatment inhibited TBARS formation from acute doses of TCA and DCA whereas DCA pretreatment potentiated TBARS formation from acute doses of DCA. It is hypothesized that this effect is due to changes in haloacetate metabolism caused by pretreatment. Therefore, mice were pretreated for 14 days with 1 g/L, TCA or DCA and on the 15th day were given an acute dose of ^{14}C-BDCA via gavage and placed in metabolism cages for 24 hours. TCA pretreatment resulted in a significant increase in the amount of ^{14}C eliminated in urine. DCA pretreatment caused a significant increase in the amount of $^{14}CO_2$ eliminated with a corresponding decrease in the amount of ^{14}C, eliminated in urine. HPLC analysis of acid extractable urinary metabolites showed that TCA pretreatment resulted in significant increases in the amount of BDCA that was eliminated unchanged. DCA pretreatment caused a significant decrease in the amount of oxalate that was eliminated in urine, whereas monochloroacetate and glycolate levels were increased. We conclude that TCA inhibits BDCA metabolism whereas DCA substantially alters BDCA metabolism. Therefore, there are changes in BDCA metabolism that may be related to altered TBARS responses. (Supported by NIEHS RO1-504648).

1028 DISPOSITION AND SEX-DEPENDENT EXCRETION OF S-PENTACHLOROPHENYL-N-ACETYLCYSTEINE IN THE RAT

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Previous studies have shown that the urinary excretion of several N-acetylcysteine (NAC) derivatives, some of which are the common final metabolites of chlorinated pesticides such as hexachlorobenzene and pentachloronitrobenzene (PCNB), show notable sex-related differences in rats. The structural requirements for the appearance of these differences seem to be highly specific since they are shown when rats are given S-pentachlorophenyl-NAC (PCP-NAC) but not when given a closely related derivative such as S-pentafluorobenzyl-NAC. In order to asses if this differential excretion may affect the disposition and the toxicokinetics of PCP-NAC and/or its precursors, a single i.p. dose of [^{14}C]PCNB was given to male and female Wistar rats and the total radioactivity was followed up in blood, urine and faeces. The concentration of the parent compound and the main metabolites in liver and kidney were determined specifically by GLC or HPLC. Three days after i.p. administration female rats had excreted 69% of the radioactivity by urine and 25% by faeces while male rats had excreted 11% by urine and 64% by faeces. The large urinary difference was due, as expected, to a very much higher concentration of PCP-NAC in female urine while in faeces the different radioactivity content could not be ascribed either to the mercapturate or to pentachloroaniline (PCA). In spite of the differences, maximum peak and disappearance of total radioactivity from the blood was similar in both sexes. Half life of PCP-NAC in organs was also similar in males and females. These results suggest that the hepatic hydrolysis of the mercapturate and the biliary excretion of metabolites in males can match the very efficient PCP-NAC renal active secretion of the females.

1029 DISTRIBUTION OF XENOBIOTICS INTO HAIR AS A BIOMARKER OF EXPOSURE

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Exposure to a xenobiotic can result in its eventual incorporation into a growing hair shaft and may serve as a biomarker of exposure. To understand the distribution of xenobiotics into hair we are developing animal models to serve as a guide for future human studies. Sprague-Dawley rats were administered codeine at 10, 20, 40, or 60 mg/kg/day, i.p., daily for 5 days, or 40 mg/kg/day by constant i.v. infusion for 5 days. Hair was collected 14 days later and analyzed for codeine and morphine by positive-ion chemical ionization GC/MS on a Magnum™ ion trap mass spectrometer. The concentrations of codeine in rat hair were 0.29 ± 0.05, 0.57 ± 0.05, 0.96 ± 0.10, 1.93 ± 0.18 ng/mg hair for the 10, 20, 40 and 60 mg/kg/day doses, respectively (mean ± SEM; n = 10). The concentration of codeine in rats that received a constant infusion was 2.89 ± 0.70 ng/mg hair (n = 6). In another study, athymic (nude) mice had human scalp hair transplanted onto their backs. Following codeine administration at 20 mg/kg/day, i.p., daily for 5 days, the concentration in hair was 2.84 ± 1.03 ng/mg hair (n = 5). The incorporation of xenobiotics into rat hair is dose-dependent; greater concentrations of xenobiotics are incorporated into hair with constant i.v. infusion than with daily i.p. injections. These data also demonstrate that the rat and mouse-human-hair transplant models are suitable for the study of the incorporation of xenobiotics into hair. Supported by NIDA grant DA 07809.

1030 THE INCORPORATION OF XENOBIOTICS AND POLAR METABOLITES INTO DARK AND LIGHT COLORED HAIR

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The incorporation of xenobiotics and their conjugated metabolites into light and dark colored rat hair was investigated in Sprague-Dawley (SD) and Dark-Agouti (DA) rats. Codeine was administered at 40 mg/kg/day i.p. for 5 days. Fourteen days later, hair was collected and analyzed for codeine and morphine by positive-ion chemical ionization GC/MS on a Magnum™ ion trap mass spectrometer. Conjugates of morphine in rat hair were estimated by the difference between acid hydrolyzed specimens (total) and unhydrolyzed (free) specimens. HPLC was used to confirm the presence of glucuronide conjugates. Concentrations of free codeine in the hair of SD and DA rats were 0.98 ± 0.10 and 6.02 ± 1.23 ng/mg hair, respectively (mean ± SEM; n = 10). Concentrations of free morphine in SD and DA rat hair were 0.34 ± 0.04 and 0.51 ± 0.11 ng/mg hair, respectively. Morphine conjugates in SD and DA rat hair were 0.42 ± 0.05 and 0.65 ± 0.23 ng/mg hair, respectively. Dark-colored hair appears to incorporate codeine and morphine to a greater extent than light-colored hair, although differences in the metabolism between SD and DA rats must be considered. This study demonstrates that xenobiotics and their polar conjugates are incorporated into rat hair and hair color plays an important role in the degree of incorporation. Supported by NIDA grant DA 07809.

1031 DISPOSITION OF 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D) IN MATERNAL AND FETAL RABBITS

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Exposure to the organic acid herbicide 2,4-D has been associated with neurotoxicity including mental retardation in humans after prenatal exposure. In order to develop a risk assessment model for 2,4-D toxicity, the distribution of 2,4-D was examined in maternal and fetal rabbits. Pregnant New Zealand rabbits (28–30 days GA) were anesthetized with Ketamine/Xylazine and the femoral vein and artery were catheterized for drug administration and sampling. Dams received iv ^{14}C-2,4-D (12.5 μCi/kg) with cold sodium 2,4-D (1, 10, or 40 mg/kg) in saline. Blood and tissue were collected from 10 to 120 min after dosing. The relative maternal exposure as determined by the tissue AUC:plasma AUC ratio (40, 10, and 1 mg/kg) from 0 to 2 hr was: plasma

(1, 1, 1), kidney (0.67, 1.20, 1.09), uterus (0.35, 0.31, 0.36), heart (0.25, 0.21, 0.32), ovary (0.24, 0.20, 0.23), liver (0.22, 0.16, 0.19), mammary gland (0.20, 0.14, no data), fat (0.04, 0.06, 0.07), forebrain (0.02, 0.02, 0.02). Fetal to maternal plasma AUC ratios were 0.16, 0.07, and 0.09 after the 40, 10 and 1 mg/kg dose, respectively. Although the fetal brain tissue to media ratio was unity in vitro, (1.03 ± 0.1, mean ± SD), fetal brain AUC's were 1% or less of the fetal plasma AUC's indicating the brain barrier system to 2,4-D is active in the late gestation fetal rabbit. (JAS supported in part by an ORISE/FDA Postdoctoral Fellowship).

1032 INTERSPECIES COMPARISONS OF THE REGIONAL BRAIN DISTRIBUTIONS OF INHALED 1,1,1-TRICHLOROETHANE IN MICE AND RATS

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The disposition of 1,1,1-Trichloroethane (TRI) in three and seven different brain regions of mice and rats, respectively, was compared over the time course of TRI inhalation. Both species were exposed to TRI by inhalation at dose levels of 3500 and 5000 ppm for 10, 30, 60, and 120 min. Rat brains were dissected into medulla oblongata(MO), cerebellum(CB), hypothalamus(HY), hippocampus(HC), striatum(ST), midbrain(MB), and cortex(CX), and mouse brains into MO, CB and CX. TRI contents in blood and brain samples were determined by headspace GC analysis. In both species, uptake of inhaled TRI in blood and each brain region was very rapid, and the highest concentrations were found in MO (182 and 216 μg/g for rats at 3500 and 5000 ppm, respectively, and 179 and 199 μg/g for mice at 3500 and 5000 ppm, respectively). TRI concentrations were always higher in the brain tissues than in blood, ranging from a 1.17 ratio for mouse CX:blood at 3500 ppm to a 3.03 ratio for rat MO:blood at 5000 ppm. CX had the lowest TRI concentrations among the three regions studied in mice, ranging from 55 to 75% of TRI concentration found in MO. Relatively lower TRI concentrations in rat were found in ST, CB, and HC (as 64, 69, and 70%, respectively, of TRI concentration found in MO of the same dose level). Blood:brain partition coefficients were derived from the AUC data. Varied lipid contents in the brain regions appear to be a primary factor for the differential TRI distribution in the brain of mice and rats during inhalation exposure. (Supported by AFOSR 910356).

1033 CUTANEOUS DISPOSITION OF ^{14}C-HYDRAMETHYLNON (HMN) IN THE MALE RAT

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A modified procedure of the EPA Dermal Absorption Guidelines (Subdivision F, §85-3) was conducted in which young adult male Sprague-Dawley rats (8 weeks old) were dosed with a 2.15% w/w gel preparation of ^{14}C-HMN (CAS No. 67485-29-4) to obtain skin absorption estimates for HMN from a routinely-used gel formulation. Two dose groups of 4 rats were dosed with ca. 1.3–2.2 g gel/10 cm^2 application area (109.6–166.9 mg/kg), then terminated at 10- or 24-hr collection points. A maximum of 1.1% of the applied ^{14}C was recovered in excreta, dose site skin, and carcass at 10 hr. Remaining unabsorbed dose recovery (89.2 ± 4.0%) encompassed gel removed from the dose site (48%), from containment materials (16%), and from dose site skin decontamination (26%). Overall, 90.3 ± 4.2% of the applied ^{14}C was recovered after 10 hr. For 24-hr rats, 1.5% of the applied ^{14}C was recovered in excreta, dose site skin, and in carcass for animals analyzed at this later interval. Remaining unabsorbed dose recovery (90.5 ± 5.3%) again included ^{14}C recovered in dose site gel residues (43%), containment materials (22%), and dose site skin decontamination rinses (26%). The overall recovery at 24 hr was 92.0 ± 6.0%. The protocol used required that additional rats be evaluated for chemical persistence if more than 1% of the applied ^{14}C penetrated skin in the previous studies. Therefore, since this 1% protocol limit for absorbed dose was exceeded in both test groups, an additional 2 groups of 4 animals were dosed as before, with dose washed off at 10 hr. Urine and feces were collected at 24-hr intervals over a 1- or 2-week duration to examine test substance persistence. An overall average of 0.6% of the applied ^{14}C dose was absorbed in either the 1- or 2-week groups. Thus, only marginal levels of HMN-derived ^{14}C were detected by 2 weeks after exposure to this dose range, suggesting that accumulation of HMN residues is not likely to occur during any routine use of this chemical in a gel formulation.

1034 CONCENTRATIONS OF BUTADIENE EPOXIDES ARE HIGHER IN LUNGS AND LIVERS OF MICE THAN RATS AFTER INHALATION OF 1,3-BUTADIENE (BD)

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Butadiene monoxide (BMO) and butadiene diepoxide (BDE) are the putative DNA-reactive metabolites of BD, a carcinogen in mice and rats. Mice are more sensitive than rats to the carcinogenic effects of BD, and lungs and livers, among other tissues, are target organs for BD-induced tumors in mice, but not rats. This study measured the concentrations of BMO and BDE and glutathione (GSH) in lungs and livers of male B6C3F1 mice and Sprague-Dawley rats during and after 6 hr exposures to 625, 1250 or 8000 (rats only) ppm BD. Lung and liver samples (n = 3–6) were collected at 3 and 6 hr of exposure and at 6 and 12 min after exposure. BMO and BDE were extracted into methylene chloride and quantified by gas chromatography-mass spectrometry. Since BD epoxides can be conjugated with GSH, the non-protein sulfhydryl content of lung and liver were quantified colorimetrically. Exposures greater than or equal to 625 ppm resulted in higher BMO concentrations in lungs and livers of mice compared to rats. In mice exposed to 625 and 1250 ppm, peak BMO concentrations in lungs (2.6 and 3.7 nmol/g) at 3 or 6 hr of exposure were higher than in livers (0.58 and 0.93 nmol/g). Rats exposed to 625 and 1250 ppm had lower BMO concentrations in lungs (0.16 and 0.31 nmol/g) and livers (0.06 and 0.16 nmol/g) than mice. Rats exposed to 8000 ppm had peak concentrations of BMO in lungs (1.3 nmol/g) and livers (1.2 nmol/g) that were nearly identical. BDE detected in lungs, but not livers, of mice; peak lung concentrations of BDE in mice were 0.71 and 1.5 nmol/g after exposure to 625 and 1250 ppm, respectively. BDE was not detected in rat tissues. GSH depletion was dependent on exposure concentration and duration of exposure. Mouse lung sustained the greatest magnitude of depletion (26% of control at 6 hr of exposure to 1250 ppm). The data suggest that GSH plays an important role in the kinetic behavior of BD, and that the greater sensitivity of mice to BD-induced carcinogenicity may be explained, in part, by the higher levels of both BMO and BDE in lungs and BMO in livers of mice compared to rats. (Supported in part by the Chemical Manufacturers Association and a NIEHS grant ES0558702.)

1035 DISPOSITION AND METABOLISM OF ^{14}C-LENACIL

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Lenacil is a selective herbicide used for sugar beet cultivation. A single oral dose of 10 or 1000 mg/kg was administered to male and female rats to study the disposition and metabolism of ^{14}C-lenacil. Recovery of radioactivity ranged from 92–100%. Nearly all the dose was excreted in the feces and urine with approximately 90% eliminated within 48 hours. Excretion of radioactivity in the feces was 82–85% of the high dose compared to 12–34% of the low dose. Retention of radioactivity in the tissues seven days after dosing was very low. Approximately 35% of the dose was identified in the urine. The major biotransformational pathway in both sexes was hydroxylation of the pyrimidine ring. Plasma levels of radioactivity were analyzed through 72 hours after dosing. The mean elimination half-life ranged between 22 and 58 hours postdose. The time to reach maximum concentration (Tmax) was dose dependent, and the areas under the plasma radioactivity curve (AUCs) were dose proportional. In conclusion, these results indicate that once lenacil is absorbed, it is metabolized and excreted with negligible tissue residues.

1036 URINARY CHROMIUM CONCENTRATIONS IN HUMANS FOLLOWING INGESTION OF SAFE DOSES OF HEXAVALENT AND TRIVALENT CHROMIUM: IMPLICATIONS FOR BIOMONITORING

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The New Jersey Department of Health (NJDOH) recently completed a urinary chromium (Cr) biomonitoring survey of over 2,000 residents of Hudson County. New Jersey suspected to live on or near sites containing chromite-ore processing residue (COPR). The mean urinary Cr levels were reported to be significantly higher in the Hudson County residents (0.22 μg/L) than in a "control" group of unexposed persons (0.17 μg/L) (Fagliano, 1994). However, such findings are uninterpretable from a health perspective until the relationship between increased urinary Cr and potential health impacts is understood. In this investigation, human exposure studies were conducted to assess urinary Cr excretion following ingestion of safe Cr doses much higher than typical dietary levels, and to therefore provide a basis of comparison to the

NJDOH data. The objective of the study was to measure the urinary excretion of Cr following the ingestion of reference doses (RfDs) of Cr(VI) as potassium chromate (K_2CrO_4) and Cr(III) as chromic oxide (Cr_2O_3) each over a 3-day period with a "no dose" period between the two substances. Loading doses of Cr(III) dietary supplements (200 μg Cr/day chromium picolinate) were ingested for 7 days prior to the ingestion of the Cr(VI) and Cr(III) RfDs to prevent "chromium deficiency". All urine voids were collected on 11 designated days of the study and analyzed for Cr, creatinine, specific gravity, and volume. Study results indicate that urinary Cr concentrations were orders of magnitude *above* the NJDOH results for: (1) the picolinate loading phase, and (2) the Cr(VI) phase. These results indicate that exceedance of background concentrations is not necessarily indicative of an unacceptable health risk, and that the levels measured by NJDOH are indicative of a safe level of exposure.

1037 ABSORPTION, DISTRIBUTION AND EXCRETION OF ^{14}C-OCTAMETHYLCYCLOTETRASILOXANE (D_4) FOLLOWING NOSE-ONLY VAPOR INHALATION EXPOSURE IN THE RAT

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Octamethylcyclotetrasiloxane (D_4) is a common ingredient found in personal care products. Due to its volatility there is potential for human exposure to D_4 via inhalation. Adsorption, distribution and excretion of ^{14}C was studied in F344 rats following a single 6 hr exposure to a target concentration of 700 ppm ^{14}C-D_4. Immediately following exposure, one group of animals was solubilized and total ^{14}C body burden was determined. Excreta animals were housed in individual metabolism cages for 168 hr post-exposure for collection of urine, feces and expired air. Additional animals were euthanized at predetermined time points for blood and tissue collection. The radioactivity content was measured in all samples. Approximately 5% of the nominal concentration was retained in the body burden animals at the end of exposure. Of the retained radioactivity, 43 and 32% was excreted in the urine, 27 and 34% was found in expired volatiles, 13 and 14% was eliminated in feces, and 11 and 12% remained in the carcass at 168 hr post-exposure in the male and female rats respectively. In both male and females <3% was eliminated as CO_2. In a pilot study with males, the tissues with the highest concentration of radioactivity immediately following exposure were small intestine, large intestine, adrenals, kidney, liver and fat. Plasma half life (terminal) was similar in males and females. Clearance of radioactivity from the tissues followed the clearance from the plasma for most tissues except fat, which had a slower clearance.

1038 EFFECT OF ENTEROHEPATIC CIRCULATION ON THE METABOLISM OF CHLORAL HYDRATE IN THE MALE F334 RAT

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Trichloroethylene (TRI), a common environmental pollutant, is oxidatively metabolized to trichloroacetate (TCA), a known rodent carcinogen, and trichloroethanol (TCE) via a chloral hydrate (CH) intermediate. In order to determine the effect of enterohepatic circulation of these TRI-derived metabolites, rats were given a venous infusion of 200 mg/kg CH over a period of 3 hr. In paired experiments, blood and urine was collected from rats which were either bile duct cannulated, or which had intact biliary systems. The peak blood concentration and halflife of total TCE (free + glucuronide) were both approx half that of the intact rats. For TCA, the time to peak blood concentration was reduced from approx 5 hr to less than 1 hr, following termination of infusion. Again, the halflife and the AUC were reduced by approx 2 by bile duct cannulation. Analysis of bile from the cannulated animals shows large amounts of total TCE, peaking at about 1 hr. TCA was also detected in bile, but at about 1/10 the amount of TCE. In the urine, there is about twice as much total TCE from non-cannulated rats, whereas the TCA levels were actually about 4X higher in the urine from cannulated animals. This study shows that enterohepatic circulation is a significant pathway for metabolites derived from TRI by the oxidative pathway. These data are to be used to help explain the kinetics of these metabolites in humans exposed to TRI. (EPA CR #822577).

1039 TRANSPLACENTAL TRANSFER OF LEAD IN NON-HUMAN PRIMATES (*MACACA FASCICULARIS*): USE OF SERIALLY ADMINISTERED STABLE ISOTOPE TRACERS OF LEAD TO ELICIT CONTRIBUTION OF MATERNAL BONE LEAD TO BLOOD LEAD AND THE FETUS

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It is unclear whether the contribution of bone-lead [Pb-BONE] to blood lead [PbB] remains stable during pregnancy given the considerable mineral demands of the developing fetal skeleton and the associated physiological changes in the mother. To measure the contribution of lead previously accumulated in maternal bone to the fetus, 5 cynomolgus monkeys ([PbB] range 30–80 μg/dl) with lifetime exposure to 'common' lead, were serially administered lead of different isotopic fingerprints i.e., enriched in ^{204}Pb, ^{206}Pb and ^{207}Pb. Serial [PbB] measurements were carried out pre-, during and post-pregnancy. Bone biopsies were taken at intervals and fetal bone harvested. Analysis for isotope ratios and lead concentration was by thermal ionization mass spectrometry. Using an end-member 'un-mixing' procedure (transformation), early data indicate that about 20% of lead in fetal bone is derived from historic maternal [Pb-BONE] deposits. Because of the complexity of the biokinetics of lead (and of bone-seeking elements in general) in bone tissue and blood, a physiologically-based model was developed to enable interpretation and elucidation of changes in [PbB] during pregnancy. Its application to the experimental data is presented. *Supported by NIEHS Contract No.: N01-ES-05285.*

1040 CYP1A1 ACTIVITY, ELIMINATION & LIVER DISPOSITION IN RATS EXPOSED TO 3,3′4,4′-TETRACHLORO BIPHENYL (PCB#77)

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PCB#77, an environmental pollutant induces CYP1A1 activity in rats which is a sensitive biomarker of exposure to halogenated aromatic hydrocarbons. To investigate the time course of enzyme induction, fecal excretion and liver disposition, groups of four male Fisher rats were dosed intraperitoneally with UL-^{14}C-PCB#77 at two dose levels (0.5 & 5 mg/kg bw). Rats were held for 16 days at the low and for 44 days at the high dose levels. At the low dose the initial and maximum enzyme inductions were observed at 8 hrs and Day 1, respectively, while at the high dose the initial induction was detected at 2 hr and by 8 hr it reached the maximum level which lasted till Day 7. It took 16 days for the CYP1A1 activity reach the basal level at the low dose and for 44 days at the high dose. About 95% of PCB#77 was excreted through bile at the end of both exposures. Parent and metabolites were detected in liver which was analyzed by HPLC. At the low dose, PCB#77 and metabolites were detected till Day 16 and Day 4, respectively. At the high dose, parent compound was detected till the end of the exposure and metabolites till half way through the experiment.

1041 DEVELOPMENT OF A BIOLOGICALLY-BASED KINETIC MODEL FOR AQUEOUS SOLUBLE, DIFFUSION LIMITED CHEMICALS

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Much of the recent effort in biologically-based kinetic (BBK) modeling has focused on the description of perfusion limited chemicals, i.e., chemicals whose permeability with respect to plasma membranes is sufficiently high that it can be assumed that the chemical reaches equilibrium with tissues during transit through the tissue. For chemicals with low permeability this assumption does not hold and the chemical is diffusion limited, i.e., membrane/cellular barriers are rate limiting in the uptake of the chemical into a tissue. Modeling of this condition requires the specific inclusion of permeability parameters in the differential equations that describe the transport of chemicals into tissue. This study describes the theoretical consequences of the diffusion limited condition on the short term (<1 h) kinetics of chemicals in rats following an i.v. dose and uses experimental data from kinetic studies with trichloroacetic acid to illustrate these effects. For chemicals with low permeability/high aqueous solubility, the major effect is that plasma concentration remains elevated at early times relative to chemicals with high permeability.

1042 DISPOSITION OF FORMAMIDE IN F-344 RATS AND B6C3F1 MICE

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Formamide is a chemical solvent used in the manufacture of a wide variety of pharmaceutical, chemical, and agrochemical products. Formamide is of toxicologic interest based on the potential for human exposure and its structural similarity to acetamide, a rat hepatocarcinogen. The present studies were designed to characterize the dispositional fate of formamide in rodents. When administered by intravenous injection, [^{14}C]formamide was rapidly metabolized and excreted by both rats and mice. Clearance was somewhat more rapid in mice in which greater than 80% of the dose was excreted in 24 hours compared with approximately 70% for rats. Mice metabolized greater than 50% of the dose to $^{14}CO_2$ vs. approximately 30% for rats. A greater portion of the dose was eliminated in the urine by rats (40%) compared with mice (30%). Excretion via the feces and volatile breath was minor (≈1–3%). Urine and CO_2 were also primary routes of excretion in rats exposed to formamide by inhalation, however elimination was somewhat slower than that observed after intravenous injection. Except for low concentrations in adipose tissue, tissue distribution in rats was fairly uniform at 24 hours. In summary, formamide is rapidly cleared via the urine and CO_2, and tissue distribution in both species following both routes of administration shows little potential for bioaccumulation.

1043 INVESTIGATION OF THE FACTORS INFLUENCING DICHLOROACETIC ACID METABOLISM *IN VITRO*

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Dichloroacetic acid (DCA) is major metabolite of trichloroethylene (TRI), a common groundwater contaminant. DCA has been reported to cause hepatocarcinogens in rodents. We have shown that DCA metabolism is dependent on a cytosolic protein, and therefore not P-450 dependent. However, the products of DCA metabolism have not been clearly identified. Initial experiments performed with nuclease and protease ruled out binding of DCA as an explanation for the disappearance of DCA from cytosolic incubations. Experiments were then conducted to determine if a specific cofactor dependence for DCA metabolism existed. Mouse liver cytosol was incubated with either nicotinamide or flavin cofactors at a concentration of 0.9 mM or 0.25–5 mM glutathione (GSH) and with 20–50 μg/ml DCA for 3 to 20 minutes at 37°C. The incubations were derivatized and analyzed to assess DCA removed from solution. DCA metabolism increased with increasing concentration of GSH. Mouse liver cytosol was then incubated with 0.1–5 mM diethyl maleate (DEM), TCA or monochloroacetic acid (MCA) and 20–50 μg/ml DCA for 5–30 minutes at 37°C. The same analysis was done to calculate μg DCA removed. While TCA appeared to have no effect on DCA metabolism, MCA and DEM had varying effects on DCA metabolism. Results from this research can be used to support further investigation of the products of DCA metabolism. (Supported by DoD Contract No. F33615-90-C-0532 and funded by Strategic Environmental Restoration Defense Program (SERDP))

1044 SECONDARY TOXICOLOGICAL EVALUATION OF CE-1037: SUMMARY OF 28 DAY CONTINUOUS INFUSION STUDY IN THE BEAGLE DOG, INCLUDING PLASMA AND EXCRETORY PROFILE

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CE-1037 (Bis (4-(2′-carboxy-2′-methylpropyl-sulfonyl)phenyl)2,2′-(1,4-Phenylene)diisobutyrate) is a potent a selective inhibitor [IC_{50}, 0.45 nM] of human neutrophil elastase (HNE) developed for the treatment of acute respiratory distress syndrome. Preclinical toxicological evaluation has involved the continuous infusion of this compound in beagle dogs at three dose levels (100, 300 & 1000 mg/kg/day) for 28 days. All doses were well tolerated over the 28 day time course. Incidences of drug related stress were largely limited to the high dose group, e.g., a reduction in hematocrit and plasma protein with an increase in total bilirubin. All high dose animals showed red blood cell crenation which is indicative of slight hemolysis. Histopathological examination revealed a thickening of the gall bladder wall and slight inflammation of the gall bladder, stomach, kidneys and liver in this group. Therefore, the 300 mg/kg/day dose was considered to be the no toxic effect level after 28 days of infusion. Analysis of the plasma indicated that there is a dose dependent increase in the systemic levels of parent compound. In addition, it was observed that at steady state concentrations there was a 3 to 4:1 ratio of CE-1155 to CE-1037. This hydrolytic product, CE-1155, is also a selective, but less potent [IC_{50}, 10 nM] inhibitor of HNE. Preliminary studies have indicated the presence of both CE-1037 and CE-1155 in dog bile, whereas only CE-1155 has been detected in dog urine.

1045 PRIMARY TOXICOLOGICAL EVALUATION OF CE-1037, COMPARATIVE PLASMA METABOLISM, ACUTE RAT TOXICOLOGY AND RADIOLABELED DISPOSITION STUDIES

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CE-1037 (Bis (4-(2′-carboxy-2′-methylpropyl-sulfonyl)phenyl)2,2′-(1,4-Phenylene)diisobutyrate) is a potent inhibitor [IC_{50}, 0.45 nm] of human neutrophil elastase (HNE) which is being developed for the treatment of Acute Respiratory Distress Syndrome (ARDS). Incubation of CE-1037 with rat, guinea pig and human plasma has indicated in vitro half lifes of approximately 60, 75 and 100 minutes, respectively. This work also indicated the formation of an esterase hydrolytic product, CE-1155, which also has HNE inhibitory activity [IC_{50}, ca. 10 nM]. Acute rat toxicology studies were performed by administering the compound by continuous infusion over a 24 hour period. The upper limit for this work was set by the solubility limits of the compound in vehicle (ca 40 mg/ml in 5% dextrose) and resulted in a maximal dose of 2400 mg/kg/day. This infusion resulted in no mortalities or significant effect on clinical chemistry. Distribution and excretion studies using ^{14}C-labeled CE-1037 were performed over 2 hour administration periods at doses equivalent to 1000 mg/kg/day. The results of this work indicated that the primary route of elimination is fecal, e.g. 88% of radioactive dose was found in the feces at 24 hours with only 6% in the urine. HPLC analysis of the urine shows no CE-1037 or CE-1155. In conclusion, these data would indicate rapid hepatic uptake of CE-1037 with a resultant short plasma half life, i.e. less than 1 minute *in vivo*.

1046 PRIMARY TOXICOLOGICAL EVALUATION OF CI-0694: ACUTE TOXICOLOGY IN THE MOUSE AND RADIOLABELED DISPOSITION STUDIES IN THE RAT

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CI-0694 (5-(sulfamethoxazolyl)-glutaryl-dexamine) has been developed to specifically suppress the immune response to sulfamethoxazole (SMX) observed in HIV infected individuals. CI-0694 is a 10K-dextran based entity with a defined number of arrayed SMX epitopes (25–50). Immunological research has indicated that this new molecular entity may be successfully administered by the sub-cutaneous (s.c.) route. CI-0694 at approximate doses of 67.667 and 1,333 mg/kg (s.c.) had no observable effects on the clinical chemistry of naive mice over a 48 hour time course. A similar result was obtained in mice where an IgG response to SMX has been induced by previous exposures to KLH-SMX (keyhole limpet hemocyanin protein with SMX arrayed). Administration (s.c.) of ^{14}C-labeled CI-0694 to rats at a dose of approximately 8 mg/kg and 5 μCi per animal allowed for the disposition of total radioactivity to be characterized. This work has indicated that the compound is primarily excreted in the urine as approximately 40% of the total administered radioactivity is found in the urine at 10 to 12 days post administration. Less than 4% of the radioactivity is found in the feces over the same interval. HPLC analysis of the urine would indicate, i.e. by co-elutions, that over the first 48 hours the radioactivity in the urine is associated with a "CI-0694-like" material. The tissue distribution of total detectable radioactivity was localized to liver, spleen, and specific lymph nodes. Together, the data suggests that CI-0694 is well absorbed from the S.C. injection site and distributed to immunologically relevant sites.

1047 ROLE OF THE MUCOPROTEIN SURFACE COAT, DIETARY BULK, AND MUCOSAL CELL TURNOVER IN THE INTESTINAL DISPOSITION OF BENZO(a)PYRENE(BaP)

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The disposition of ^{3}H-BaP equivalents from the diet was examined at the lumen-mucosal cell interface for the proximal, medial and distal regions of the catfish intestine. ^{3}H-BaP (500 μCi, 20 μg/kg), was administered via gavage in maintenance diet to catfish. One group of fish was subsequently fasted while the other fed until collection of intestinal tissues (24, 48, 72, 96, 144 and 192h). ^{3}H-BaP concentrations in the distal intestine of fasted animals were significantly higher than proximal regions at 24, 48, 72 and 96h. Similar differences were evident for medial regions at 48 and 96h. Depuration of ^{3}H-BaP

was much slower for the distal intestine than the proximal or medial regions. Microautoradiography demonstrated a persistent and intense regional binding of ^{3}H-BaP to the mucoprotein surface coat on the lumen surface of the distal intestine in fasted animals. Computer enhanced image analysis of ^{3}H BaP signal within the villi demonstrated a comparatively higher and more persistent signal for the distal intestine. Feeding subsequent to dosing in the second treatment abalated ^{3}H-BaP whole tissue levels, the ^{3}H-BaP containing surface coat and ^{3}H-BaP derived signal in the villi.

In a similar but separate experiment ^{3}H thymidine (600 μCi) was administered via ip injection to examine mucosal cell turnover in relationship to BaP turnover. ^{3}H-Thymidine incorporation was greatest in the distal regions and the number of labelled cells reduced with feeding. These results suggest that ^{3}H-BaP is regionally more available to sensitive colonic tissues as a result of the mucoprotein surface coat. (NIEHS ES 05781)

1048 ABSORPTION OF ORGANIC TOXICANTS BY THE LUNGS: MODELING PORTAL-OF-ENTRY DOSIMETRY

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To assess target dose when inhalants induce toxicity to the airway epithelium requires knowledge of the mechanism of absorption. Whereas absorption of less lipophilic solutes is limited mainly by the local rate of blood perfusion in the lungs, absorption of highly lipophilic toxicants is limited by the diffusion rate through the airway epithelium (*TAP 121*:328, 1993). To relate both modes of clearance, a new model was developed simulating absorption of organic solutes with lipid/aqueous partition coefficients ($PC_{1/a}$) larger than one. The model describes the dosimetry to the airway mucosa by simulating concentration gradients perpendicular to the air interface. Critical input data were morphometry, blood perfusion, metabolism, and distribution of membrane lipids within the cross-section of the mucosa. Transport of toxicants was assumed to occur through molecular diffusion, partitioning into lipid membranes, membrane trafficking, and perfusion of the subepithelium. Results showed that absorption of toxicants was perfusion-limited when the $PC_{1/a}$ was <100, giving clearance half-times in the range of 1.4–7 min. For $PC_{1/a}$s > 100, there was a gradual shift toward clearance being limited by the rate of diffusion through the epithelium. Slower absorption during diffusion-limited clearance led to prolonged exposures of the airway epithelium. Local metabolism in the epithelium may enhance diffusion to the capillary bed by reducing the lipophilicity of a toxic substrate, albeit at the risk of generating reactive and potentially toxic metabolites. (Research supported by NIH Grant RO1-4422 from NEIHS, and the Swedish Work Environment Fund Grant 91-0359 in facilities provided by the U.S. DOE/OHER under Contract No. DE-AC04-76EV01013. 1) Also at: National Inst. Occup. Health, Sweden)

1049 CONCENTRATION DEPENDENCE OF SEVOFLURANE METABOLISM IN RATS

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Biotransformation of the anesthetic, sevoflurane [CF_3-CH(CF_3)-O-CH_2F] produces F^- and hexafluoroisopropanol (HFIP). Groups of male wistar rats (N = 5) were anesthetized with sevoflurane at one inspired concentration (0.5–3.75%) during spontaneous ventilation in a 180 l chamber. Body temperature was monitored and maintained at 37°C. At 30 and 60 min into the exposure, blood was collected for measurement of sevoflurane concentration, plasma fluoride, and plasma hexafluoroisopropanol. After 60 min of anesthesia, urine was collected for determination of F^- and HFIP excretion. Blood sevoflurane concentration increased linearly, over the range 0.125–3.75%, with inspired anesthetic concentration. End anesthetic plasma concentration of F^- were 14 μM at 30 min and 22 μM after 60 min. End anesthetic plasma concentration of HFIP were 8 μM after 30 min and 11 μM after 60 min. Plasma concentration of fluoride ion, plasma HFIP, and urinary excretion increased as sevoflurane concentration increased up to 1.25%. Above 1.25% sevoflurane, plasma concentration and urinary excretion of metabolites were dose independent. Thus sevoflurane metabolism is not dose-related in rats at anesthetic concentrations above 1.25%, possibly because sevoflurane is metabolized by cytochrome P450 2E1 a saturable enzyme system. These data indicate that production of potentially toxic sevoflurane metabolites may be dose-independent at clinically-used concentrations. (Supported by Abbott Laboratories).

1050 CHLOROFORM BIOTRANSFORMATION IN RATS IS INHIBITED BY COEXPOSURE TO CARBON TETRACHLORIDE

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Chloroform (CHCl3) and carbon tetrachloride (CCl4) are common contaminants at hazardous waste sites. In the present study, gas uptake techniques and physiologically based pharmacokinetic (PBPK) modeling have been applied to examine the metabolic interaction of this particular binary combination. Male F344 rats (176 ± 6 g) were exposed (N = 3) to CHCl3 alone in gas uptake chambers using the following initial concentrations: 500, 1000, 3000 ppm. A second set of animals was coexposed under similar conditions to the same CHCl3 concentrations and 1000 ppm CCl4. For all three initial concentrations used, CHCl3 chamber concentrations for the CHCl3&CCl4 mixture were higher than concentrations for CHCl3 alone. The ability to detect changes in declining chamber concentration due to the metabolic interaction was a function of the initial chamber CHCl3 concentration. The largest difference between coexposure and CHCl3 alone was at 500 ppm and decreased as initial CHCl3 concentration increased. Sensitivity analysis corroborated that changes in metabolic rate are most evident in the mid-range concentrations. These gas uptake results are consistent with inhibition of CHCl3 metabolism by the coexposure. PBPK modeling simulations will be used to evaluate the mechanism of inhibition. Serum markers of hepatotoxicity were measured 48 hours after gas uptake exposure, while urinary markers of nephrotoxicity were measured at 0, 24, and 48 hours. Preliminary analysis at 48 hours showed that neither markers were markedly altered by coexposure. In summary, the understanding of the metabolic interaction between these two compounds may lead to an improved assessment of the toxicological response for this mixture. (This abstract does not necessarily reflect EPA policy).

1051 DRUG ACCUMULATION AND INDUCTION OF ALVEOLAR MACROPHAGE PHOSPHOLIPIDOSIS AFTER AMIODARONE (AD) TREATMENT IN TWO STRAINS OF RATS

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AD and its primary metabolite, desethylAD (dAD), can cause a phospholipidosis in humans and animals. We have reported previously that Fischer-344 (F-344) rats are more susceptible than Sprague-Dawley (SD) rats to the induction of alveolar macrophage (AM) phospholipidosis and tissue accumulation of drugs following 1 week of AD treatment. The purpose of this study was to examine the comparative susceptibilities of these strains after a longer treatment period. AD was given to male F-344 and SD rats at a dose of 100 mg/kg, 5 days/wk for 4 wks. A biochemical and ultrastructural phospholipidosis was induced in AMs of F-344s but not SDs. Plasma concentrations and tissue levels of AD and dAD were still significantly higher in F-344s than SDs with the respective values being similar to those at 1 week. Therefore, even after 4 wks of AD, SDs were nonresponsive to the induction of AM phospholipidosis, and general tissue accumulation of drugs was much less than for F-344s. The differences between strains appears related to dispositional factors of the drug. (Supported by a grant from Procter & Gamble Pharmaceuticals)

1052 2,4,5,2′,4′,5′-HEXACHLOROBIPHENYL (2HCB) AND 3,4,5,3′,4′,5′-HEXACHLOROBIPHENYL (3HCB) INCREASE BILIARY EXCRETION OF 7,12-DIMETHYLBENZ[a]-ANTHRACENE (DMBA) IN RAINBOW TROUT

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Di-ortho polychlorinated biphenyls (PCBs) are prominent environmental contaminants and their interaction with the Ah-receptor may be more significant than previously thought. Four weeks after ip injection with 50 to 250 mg 2HCB/kg or 5 or 25 mg 3HCB/kg, rainbow trout were either ip injected with 10 μmol [3H]DMBA/kg and liver and gallbladder removed 16 hr later, or liver microsomes were prepared. Biliary excretion of [3H]DMBA increased in 2HCB but not 3HCB treated fish. Ethoxyresorufin-O-deethylase activity (EROD) is catalyzed by cytochrome P450 1A. Liver microsomal EROD activity increased with 2HCB dose. EROD increased after the low but not the high 3HCB dose. In another experiment, rainbow trout were fed 0.06 or 0.22 μg 2HCB/g fish/day or 0.0019 or 0.0078 μg 3HCB/g fish/day for 4, 8, or 12 weeks. At each time fish were ip injected with 10 μmol [3H]DMBA/kg. Sixteen hours later liver, bile, mesenteric fat, stomach, muscle, kidney, and plasma were sampled. [3H]DMBA concentra-tion in bile increased with dose for 2HCB and

3HCB treated fish. Ah receptor-mediated cytochrome P450 1A induction may partially explain di-ortho and non-ortho PCB-altered [3H]DMBA biliary excretion. (Supported by USPHS grant ESO5543).

1053 COMPARATIVE HEPATIC DISPOSITION OF NAPHTHYLISOTHIOCYANATES IN THE RAT

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1-Naphthylisothiocyanate (ANIT) produces cholangiolitic hepatitis in the rat, whereas 2-naphthylisothiocyanate (BNIT) is not hepatotoxic. The mechanisms responsible for the disparate toxic potentials of these isothiocyanates are not known but may be related to differences in hepatic disposition. Therefore, we compared BNIT disposition in bile and plasma with that of ANIT after oral administration of 100 mg isothiocyanate/kg rat. The effect of glutathione depletion on the disposition of each compound was also evaluated. ANIT and BNIT concentrations in bile and plasma were determined by HPLC analysis. Bile concentrations of BNIT ranged between 140 μM at 1 hr and 5 μM at 24 hr. BNIT concentrations in bile at 1 and 4 hr were 5-fold greater than those of ANIT. BNIT administration increased bile glutathione concentration (4-fold) at 1 and 4 hr. ANIT and BNIT had similar effects on bile glutathione. Accumulation of both isothiocyanates in bile was markedly inhibited by pretreating rats with a dose of BSO that decreased hepatic glutathione by 75%. These results indicate that both ANIT and BNIT accumulate in bile and suggest that glutathione is involved in the transport of both isothiocyanates into the biliary tree. The difference in hepatotoxicity between ANIT and BNIT cannot be explained by differences in hepatobiliary disposition. (Supported by NIH grant ES04139.)

1054 2-METHOXYETHANOL METABOLISM, FETAL DISTRIBUTION, AND MACROMOLECULAR ADDUCT FORMATION IN THE RAT: THE EFFECT OF RADIO-FREQUENCY RADIATION

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Previous work demonstrated that exposure to 2-methoxyethanol (2ME), a teratogenic agent, and Radiofrequency (RF) radiation resulted in greater than additive fetal malformations (Nelson *et al.* 1991). The current study evaluated the metabolism of ^{14}C-labeled 2ME and the distribution of methoxyacetic acid (MA) in maternal and fetal tissues of pregnant Sprague-Dawley rats either exposed to RF radiation at 10 mHz or sham conditions. Additionally, adduct formation for both plasma and fetal protein was tested as a possible biomarker for the observed 2ME/RF teratogenicity. Rats were administered [ethanol-1,2-^{14}C]-2ME (150 mg/kg, 161 μCi/rat average) by gavage on day 13 of gestation immediately before RF radiation body temperature elevation to 42°C/30 min. Concurrent sham and RF rats were sacrificed at 3, 6, 24 or 48 hr for harvest of maternal blood, urine, fetuses and embryonic fluid. Tissues were either digested for determination of radioactivity or deproteinized with TCA and analyzed by HPLC for quantification of 2ME metabolites. Results show the presence of 2ME and seven metabolites, with the major metabolite, MA, peaking at 6 hr in the tissues tested. MA, the proximal teratogen, was detectable in maternal serum, urine, fetus and embryonic fluid 48 hr after dosing. Clearance of total body ^{14}C was significantly reduced for the RF animals ($p > 0.05$) for the 24–48 hr period, but MA values for serum, fetus and embryonic fluid were similar for both sham and RF rats. Additionally, no difference was noted for 2ME metabolite profiles in urine or tissue for sham or RF rats thus eliminating an effect of RF radiation on MA production as a possible explanation for the reported RF/2ME synergism. Subsequently, serum and fetus protein-bound adducts were evaluated by analysis of covalently bound radioactivity. Serum protein binding was significantly higher for sham than RF rats at 3- and 6-hr — highest for sham rats at 6 hr (519 ± 95 μg as parent 2ME/g of protein) whereas RF serum values were highest at 24 hr (266 ± 79 μg/g protein). Fetal protein binding was significantly higher for sham rats at 6 hr, but binding was highest for both groups at 24 hr (sham = 229 ± 71 μg/g, RF = 185 ± 48 μg/g). Formation of protein adducts after 2ME is thought to be related to levels of methoxyacetaldehyde, a reactive intermediate in the formation of MA. These results suggest that no positive correlation exists for covalent binding in the fetus which would explain RF/2ME synergistic malformations. However, Nelson *et al.* (1991) suggested that the malformations may be RF related and are exacerbated by 2ME. In comparison with urinary metabolites, the relatively slow elimination of adducted serum 2ME indicates that analysis of protein-bound concentrations may be a valuable tool for long-term biomonitoring of workers.

1055 COMPARISON OF TISSUE CONCENTRATIONS OF BUTADIENE EPOXIDES IN SPRAGUE-DAWLEY RATS AND B6C3F$_1$ MICE EXPOSED TO LOW LEVELS OF 1,3-BUTADIENE (BD) BY INHALATION

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Butadiene monoepoxide (BDO) and butadiene diepoxide (BDO$_2$ are mutagenic metabolites of BD. Tumors occur in mice at chronic BD exposure concentrations as low as 6.25 ppm, while a carcinogenic response occurs in rats only after exposures above 1000 ppm. In the present study, BDO and BDO$_2$ were measured in several tissues of Sprague-Dawley rats and B6C3F$_1$ mice during and following a 4 h inhalation exposure to 62.5 ppm BD. The metabolites were isolated by cryogenic vacuum line distillation, then quantified using multidimensional gas chromatography/mass spectrometry. After 2 h, BDO levels in blood, heart and fat of mice were 3- to 8-fold greater than those in rats. In mouse lungs, a target organ for BD carcinogenesis, BDO levels reached 48 ± 8 pmol/g tissue at 4 h. In rat lungs, BDO was not detected above control levels at any point during the exposure. BDO$_2$, the more genotoxic of the two epoxides, was detected in blood, heart, lung, fat, spleen, and thymus of mice at 2 and 4 h at levels that ranged from 50 ± 8 pmol/g (2 h spleen) to 205 ± 15 pmol/g (4 h blood). In rats, BDO$_2$ concentration in blood at 4 h was 2.1 ± 0.8 pmol/g, while levels of this metabolite were below the detection limit in the other tissues examined. In most tissues, the epoxides had decreased to control levels by 30–60 min following the end of the exposure. The increased levels of the epoxide metabolites, particularly BDO$_2$, observed in mouse tissues suggest that differences in BD metabolism may account for species differences in susceptibility to BD carcinogenesis. (Research sponsored by CMA under Funds-in-Agreement No. DE-FIOY-91AL66351 with the U.S. DOE/OHER under Contract No. DE-AC04-76EV01013.)

1056 KINETICS OF HEXACHLOROBENZENE IN THE LACTATING RAT

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The objective of this study was to examine the kinetic behavior of stored hexachlorobenzene (HCB) as it is mobilized during lactation in the rat. HCB is transferred to the mammary tissue and milk via blood flow from storage depots such as fat tissue and is then ingested by nursing pups. Sprague-Dawley rats were given bolus oral doses of either 10 or 100 mg HCB dissolved in corn oil/kg body wt two weeks prior to breeding. Milk, blood and tissue concentrations of HCB were measured periodically throughout a 3-week period of lactation. Another group of rats was given an intravenous (IV) dose of 1.0 mg HCB dissolved in DMSO/kg body wt. on Day 9 of gestation. Milk and blood concentrations were measured during a 3-week lactation period. Achieved maternal blood and milk HCB concentrations were 0.3–0.6 ppb and 4.0–5.0 ppb, respectively, for the 10 mg/kg dose group and 3.0–4.0 ppb and 40.0–53.0 ppb, respectively, in the 100 mg/kg dose group. Achieved maternal blood and milk concentrations from IV dosing were 0.08–0.1 ppb and 0.9–1.0 ppb, respectively. Tissue analyses of dams and pups from the 10 mg/kg dose group showed that fat contained the highest concentration of HCB and that achieved pup tissue and blood concentrations of HCB surpassed corresponding maternal blood and tissue concentrations. Preliminary analysis of the kinetics of HCB during lactation using a PB-PK model indicates that lactational transfer of HCB is a significant exposure route for nursing pups.

1057 DIFFERENTIAL SYSTEMIC EFFECTS OF STEROIDAL AND NON-STEROIDAL ANTI-ANDROGENS (AA) AFTER REPEATED TOPICAL ADMINISTRATION

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Non-steroidal (i.e., Flutamide — FL) AA lack the partial agonist, antigonadotropic effects induced by steroidal (i.e., Cyproterone acetate — CA) AA. The present experiments were done to better define the systemic toxicological effects of these different classes of AA after repeated percutaneous administration. Young, male, Sprague Dawley rats received CA or FL intravenously (i.v.) at doses of 1.0, 10.0 or 50.0 mg/kg/day for 7-days or, percutaneously at doses of 10, 50 or 125 mg/kg/day for 14-days. As corrected for body weight differences, 7-days of i.v. dosing with CA and FL induced comparable decreases in prostate and seminal vesicle weights and did not affect body and adrenal gland weights. However, after 14 days of topical administration: (a) the dose-response curve for prostate and seminal vesicle effects shifted leftward for CA

compared to FL; (b) CA but not FL depressed body weight gain, and; (c) CA decreased and FL increased adrenal gland weights. These data suggest that the 14-day topical drug administration protocol used in the experiments, can distinguish skin penetration and pharmacodynamic differences in systemic toxicological effects of percutaneously administered steroidal and non-steroidal AA.

1058 ASSESSMENT OF THE *IN VIVO* METABOLIC INTERACTION OF CHLOROFORM ($CHCl_3$) AND TRICHLOROETHYLENE (TCE) IN RATS BY CLOSED CHAMBER METHODS

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Concurrent oral exposure of rats to $CHCl_3$ and TCE results in reduced hepatic and renal toxicity relative to $CHCl_3$ alone (Lilly *et al.*, 1992). The present study investigated the *in vivo* metabolic interaction of $CHCl_3$ and TCE by closed chamber methods. Male F344 rats (≅70 days old) were exposed individually (n = 3) to four initial concentrations of $CHCl_3$ or TCE of approximately 100, 500, 1000, or 3000 ppm. The uptake curves were examined with a physiologically-based pharmacokinetic (PB-PK) inhalation model. Both TCE and $CHCl_3$ metabolism were modeled successfully by a single saturable pathway. The allometrically scaled maximum velocities (V_{MAXC}) for $CHCl_3$ and TCE were estimated as 4.4 and 4.5 mg/hr/kg, respectively. Sensitivity analysis indicated that 500 ppm of either TCE or $CHCl_3$ was an optimal concentration for estimation of V_{MAXC}. Rats were then exposed to the following initial concentrations of the binary combinations: 500 ppm $CHCl_3$ with 10, 500, or 2000 ppm TCE; and 500 ppm TCE with 10, 500 or 2000 ppm $CHCl_3$. The uptake curves of TCE and $CHCl_3$ during combined exposures were examined by a PB-PK model that included an equation to accommodate three different mechanisms of inhibition: competitive, noncompetitive, or uncompetitive. The metabolic interactions of TCE and $CHCl_3$ were best described as competitive. TCE competitively inhibited $CHCl_3$ metabolism; similarly, $CHCl_3$ competitively inhibited TCE metabolism. Inhibition of $CHCl_3$ metabolism by TCE in rats may contribute to the antagonism of $CHCl_3$ toxicity by TCE. (Funded by EPA/UNC-CH TA-T901915. This abstract does not necessarily reflect EPA policy.)

1059 TISSUE SOLUBILITY AND KINETICS FOR METABOLITES OF TRICHLOROETHYLENE-CHLORAL AND TRICHLOROETHANOL

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Trichloroethylene (TCE) is a common groundwater contaminant and is used as a degreaser. The metabolism of TCE plays a key role in its pharmacokinetics and pharmacodynamics. Thus, the determination of accurate tissue solubility and kinetics are important in the development of a PBPK model. This study focuses on 2 main metabolites: chloral (CH) and trichloroethanol (TCOH). Studies were conducted in $B_6C_3F_1$ mice since this species was used in the NCI/NTP carcinogenicity studies on TCE. Partition coefficients for CH and TCOH were determined. using the nonvolatile method of Jepson et al. (Fund. Appl. Tox. 1994). Tissues used in CH studies were pre-treated with 20% lead acetate in insure no metabolism occurred during the incubation period. IV studies provide the time course for tissue and blood levels, clearance rates as well as the role of enterohepatic recirculation in the metabolism of TCE. Accurate kinetic measurements *in vivo* can lead to a PBPK model in mice with potential for use to calculate the risk in humans. (Supported by USAF #F33615-90C-052 and SERDP).

1060 THE EFFECTS OF DIET, *AD LIBITUM* (AL) OVER-FEEDING, AND MODERATE DIETARY (CALORIC) RESTRICTION (DR) ON SURVIVAL AND SPONTANEOUS NEOPLASMS IN SPRAGUE DAWLEY (SD) RATS

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Our studies compared the effects of AL overfeeding and moderate DR of three diets on SD rat survival, degenerative disease and neoplasia. SD rats were fed diets varying in protein, fiber and energy content by AL or 25% DR or 35% DR. At two years SD rats fed all diets by DR (but within the range of reported AL food consumption) had improved survival. The tumor incidence in all sites by two years was similar between AL and DR groups, however, a decreased age-adjusted incidence of pituitary adenoma was seen in both sexes, of pancreatic islet cell carcinoma in males and of mammary gland tumors in females. DR females also had a decrease in mammary gland tumor multiplicity. These tumors had a growth time and tumor volume that was not statistically significant between AL and DR groups. Moderate DR appears to only delay the time of onset but not the progression of these tumors whether measured by age-adjusted incidence, growth time, tumor doubling time, or the time between initial detection and death. Moderate DR appears to produce its effect by delaying the onset of spontaneous tumors and degenerative disease, but not by preventing their spontaneous development in a two-year period.

1061 EFFECTS OF MODERATE DIETARY RESTRICTION (MDR) ON TOXICITY AND TOXICOKINETIC (TK) PROFILES OF NON-GENOTOXIC PHARMACEUTICALS IN RATS

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Our previous studies have shown that MDR increases the life span and delays or prevents the age-related mortality, diseases and spontaneous tumors seen in Sprague-Dawley (SD) rats. Three-month toxicity/toxicokinetic studies were conducted to determine if MDR alters the maximum tolerated dose (MTD), the no-observed effect level (NOEL), and the toxicokinetic profile (systemic exposure) of four different non-genotoxic pharmaceuticals in SD rats [compared to ad libitum (AL) over-feeding]. The MDR males and females received 24 and 17 g of food/day, respectively (the majority of the food was consumed). In AL animals, the average food consumptions were 32 and 24 g/day in males, and females, respectively. The estimated MTDs for compounds A, B, C, and D in AL animals were approximately 250, 400, 125 and 125 mg/kg/day, respectively. In MDR animals, these compounds were found to have higher NOEL and the estimated MTDs for compounds A, B, C and D were 500–1000, >500, >250 and >500 mg/kg/day, respectively. Toxicokinetic data indicted that the steady-state systemic drug exposures were either equal or higher in MDR rats when compared to AL rats. The results suggest that the MDR rats are healthier than the AL rats, and therefore, can tolerate higher doses at equal or greater systemic drug exposure.

1062 EFFECT OF FOOD RESTRICTION ON PLASMA LIPID PEROXIDATION, PROTEIN OXIDATION AND MITOGEN-INDUCED LYMPHOCYTE PROLIFERATION IN AGED RATS

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Decreased immune function associated with aging has been demonstrated in humans and animals. The reactive oxygen species (ROS) hypothesis of aging may contribute to this decline in the immune response. In this study the authors compared the levels of lipid peroxidation and oxidatively modified proteins in plasma, and mitogen-induced lymphocyte proliferation in spleen cells from 5, 18, and 31 month-old ad libitum fed (AL) versus food-restricted (FR, 50% of the mean caloric intake of the AL) Fischer 344 male rats. The results reveal that AL rats show an age-related decrease in proliferative response of splenic lymphocytes to phytohemagglutinin (PHA) and concanavalin (Con A). This functional decline in T-lymphocytes is inversely correlated to both increased molondialdehyde (MDA) levels and protein carbonyl content in the plasma during aging. Food restriction, however, can prevent the decrease in proliferative response of T-cells to mitogens in aged rats. Also, FR rats showed significantly lower lipid peroxidation and protein carbonyl contents in plasma than those of AL-fed rats. The above observations suggest that the age-associated declines in immune function might be related to the oxidative modification of macromolecules, which may lead to enzyme inactivation and cell senescence. One of the mechanisms by which food restriction improves decreased immune function in aged rats is hypothesized to be via the reduction of ROS production and subsequent oxidative damage to macromolecules. (Supported by grant awards by NIH, R01 CA60994, R01 CA61764; American Federation for Aging Research, and Alzheimer's Association, FSA-94-024).

1063 EFFECTS OF FENBUCONAZOLE ON THYROID FUNCTION AND HEPATIC CLEARANCE OF L-THYROXINE IN MALE RATS

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A slight increase in the incidence of thyroid tumors was observed in male rats after 2 years of dietary exposure to fenbuconazole. Since fenbuconazole is not genotoxic, a nongenotoxic mechanism was investigated. Groups of CD male

rats were fed 0, 8, 800, 1600 and 3200 ppm (a.i.) fenbuconazole for 4 and 13 weeks. Compound-related effects on the structure and function of the thyroid were observed at doses of 800 ppm and greater and included: increases in thyroid weight, altered thyroid histopathology (diffuse follicular cell hypertrophy/hyperplasia), decreases in serum T4 conc. (34–53%), and increases in serum TSH conc. (58–106%). The thyroid changes were reversible and considered secondary to compound-related effects on the hepatic metabolism and biliary excretion of L-thyroxine. Liver weights were increased 21–53% at doses of 800 ppm and greater. The biliary excretion of L-thyroxine, predominantly as the glucuronide, was increased 2-fold at 4 and 13 weeks in treated rats (3200 ppm). The *in vitro* activity of hepatic microsomal UDP-glucuronosyltransferase, with L-thyroxine as substrate, was increased in fenbuconazole fed rats (3200 ppm) at 4 and 13 weeks (25–54% per g microsomal protein and 300–337% per whole liver basis). These findings support a nongenotoxic mode of action for thyroid tumor development, and have clear implications for dietary risk assessment of fenbuconazole.

1064 SERUM CONCENTRATIONS OF THE CYCLIN DEPENDENT KINASES SERVE AS BIOMARKERS FOR SPONTANEOUS TUMORS IN DOGS, CATS AND WOODCHUCKS

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Cyclin-dependent kinases (CDK), which serve as fundamental regulators of cell division in all eukaryotic cells, have been shown to be present at elevated concentration in more than ninety percent of cancerous tissues and transformed cells examined. The objective of this study was to quantify CDK concentrations in the serum of animals with a diagnosed cancer. Sera of 9 dogs and 2 cats with a variety of cancers, and 10 woodchucks with hepatocellular carcinoma (WHC), were assayed by immunoblotting of SDS-PAGE gels and ELISA using anti-CDK2 antibodies. The immunoblots and ELISA values of sera CDK2 from the animals with tumors were compared to immunoblots and CDK2 values of sera from 140 healthy dogs, 60 healthy cats and 9 healthy woodchucks. Immunoblots revealed an increase in serum CDK2 as a result of tumor burden in dogs, cats and woodchucks compared to normal animals. ELISA results indicated that serum CDK2 was increased an average of 3.8-fold in dogs and cats with tumors and an average of 4.3-fold with WHC ($p < 0.05$) relative to animals without tumors. This study indicates the potential of using serum CDK2 as a biomarker for the identification of tumor bearing animals.

1065 SIX MONTH EXPOSURE OF STRAIN A/J MICE TO CIGARETTE SIDESTREAM SMOKE

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Male strain A/J mice were exposed to sidestream smoke (SS) generated from Kentucky 1R4F reference cigarettes. Chamber concentrations were 4 mg/m^3 of total suspended respirable particulate matter (TSP). Animals were exposed for 24 weeks for 6 hours a day, 5 days a week. One-week cumulative labeling indices were significantly increased in the large intrapulmonary airways during the first week, and in the respiratory epithelium of the nasal and maxillar turbinates during the first two weeks of exposure, then returned to control values. Signs of increased cell proliferation were again found in the nasal turbinates during the 9th and 16th exposure weeks. After 24 weeks, lung tumor incidence and multiplicity were the same in smoke-exposed as in filtered air-exposed animals. Analysis for K-ras mutations in tumors obtained from ETS and control mice suggested that exon 2 might be a specific target for K-ras mutations. The mutations resulted in a base change in the middle adenine in codon 61 resulting in the substitution of either arginine (AT-GC transition) or leucine (AT-TA transversion) for the wild type glutamine in ETS treated animals. It was concluded that duration of exposure was too short or concentration of TSP was too low to increase tumor incidence or multiplicity, but that ETS favored the development of tumors bearing K-ras mutations in exon 2 (codon) 61.

1066 A LIFE-TIME IMPLANT STUDY OF SILICONE MAMMARY GEL IN FEMALE SPRAGUE DAWLEY RATS

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Previous life-time studies on silicone mammary gel provided no evidence of chemical carcinogenicity but demonstrated an implant associated sarcomatous mass known as solid state tumorigenesis (SST). To confirm the lack of chemical carcinogenic potential of the silicone gel, a life-time study was conducted in female Sprague Dawley rats to identify distant site neoplasia when SST is present as a confounding factor. Test animals were implanted s.c. with three volumes (0.8 ml, 3.6 ml, 16.0 ml) of silicone gel distributed over four dorsal implant sites and then monitored for 104 weeks. Age matched sham-operated and polyethylene (PE) disk (0.5 cm, 1.0 cm, 2.0 cm diameters) implanted rats served as primary and secondary noncarcinogenic controls respectively. To allow for censorship of the SST animals and thereby best evaluate the chemical carcinogenic potential, each treatment group was comprised of 100 animals. Parameters evaluated included body weights, food consumption, clinical and urine chemistries, and histopathology of over 50 tissues and organs. Subsequent analyses were conducted comparing 1) all animals, 2) animals without SST, and 3) animals with SST to sham controls. The only evidence of increased tumor rates was a dose-response incidence of SST at the silicone gel (2%, 2%, 43%) and PE (1%, 19%, 52%) implant sites. There was no evidence indicating silicone gel or PE has any chemical carcinogenic potential and little, if any evidence to support an adverse treatment related effect with regards to the other previously mentioned end points.

1067 NEOPLASTIC INITIATION AND DNA MODIFICATION OF TAMOXIFEN, TOREMIFENE AND DIETHYLSTILBESTROL IN FISCHER 344 AND SPRAGUE-DAWLEY RATS

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Tamoxifen (TAM) and Toremifene (TOR) are triphenylethylene antiestrogenic drugs related to diethylstilbestrol (DES). TAM and DES were found to be hepatocarcinogenic in rats causing DNA adduct formation in rat livers, whereas TOR produced neither effect. In the present study, the process of initiation of hepatocarcinogenesis was monitored using the placental form of glutathione S transferase (GST-P) to quantify liver altered loci per cm^2 of liver and bromodeoxyuridine (BrdU) special stains. Liver DNA modification was monitored through ^{32}P-post-labeling. Female Sprague-Dawley (SD) and Fischer 344 (F344) rats were given TAM doses of 5, 10, 20, 40 mg/kg bw, TOR doses of 21.2, 42.4 mg/kg bw and DES doses of 20, 40 mg/kg bw given by gavage daily for 12 weeks. Groups were killed at 2 and 12 weeks. There was no mortality in any of the groups but a significant dose related decrease in all TAM, TOR, DES-exposed groups, greatest with DES followed by TAM and TOR. The decrease was more pronounced in the SD strain. The hepatocellular morphological changes exhibited a time and dose related increase in the number of foci of cellular alteration and increased proliferation indices with the highest values in high dose TAM followed by high dose DES in both strains. The SD values were significantly higher than the F344 values. TAM, but not DES or TOR produced DNA adducts in the livers of both strains.

1068 INHIBITION OF UTERINE SMOOTH MUSCLE TUMOR GROWTH BY TAMOXIFEN

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Modulation of gynecological tumor growth by hormones and antihormones is in the mainstream of contemporary issues surrounding estrogen-responsive tumor development. The antiestrogen tamoxifen (TAM), for example, has received much recent attention because of its tissue-specific actions and the possible increased risk of endometrial cancer associated with its use. This is the first study to report on the effects of TAM on uterine smooth muscle tumor development, which is of great interest due to the high incidence of uterine leiomyoma in the population. In a novel rat hereditary cancer model, myometrial tumors develop spontaneously with a high frequency, and seven cell lines have been derived from these tumors. The cell lines express receptors for estrogen (Kd = 0.7 nM) as determined by Northern and specific binding analysis. The lines proliferate in response to nanomolar concentrations of estradiol. We now show that TAM will inhibit the growth of these tumor cells in a dose-dependent fashion (2.5–10 μM TAM) *in vitro*. We also extend our *in vitro* findings into an *in vivo* system by demonstrating that TAM treatment will inhibit nude mouse tumor formation by the cell lines. These studies show that the Eker rat model is a unique system in which to evaluate tumor response to endogenous or exogenous hormones or antihormones.

1069 EFFECTS ON CELL CYCLE KINETICS AS A POSSIBLE MECHANISM OF ESTRADIOL PROMOTION OF HEPATOCARCINOGENESIS

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Low- (9.5 μg/kg/day) or high-dose (95 μg/kg/day) 17β-estradiol (E2), administered as a promoter in a two-stage model of hepatocarcinogenesis, fails to increase the cumulative BRDU labeling index when compared with DEN (150 mg/kg) controls, suggesting that regenerative hyperplasia does not completely explain the increase in number and/or size of EAF observed. When a pulse marker for cell proliferation (PCNA) is used, promotion with high-dose E2 increases the labeling index at study termination (Days 39/40) two-fold compared with the DEN-control group. Additionally, compared with the DEN- and SAL-control groups, promotion with either low- or high-dose E2 increases the percentage of cells in S phase at study termination. Promotion with E2 does not affect the early synchronization of the cell cycle however, because 7 days after initiation the majority of proliferating hepatocytes are in G_1. This suggests effects on cell cycle kinetics as a possible mechanism for the promotion of experimental hepatocarcinogenesis by E2 in the rat. (Supported in part by NIEHS Center grant #ES05022 and Berlex Laboratories).

1070 EFFECTS OF TRANSFORMING GROWTH FACTOR-α ON DIMETHYLNITROSAMINE-INDUCED HEPATIC INJURY IN MICE

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Alkylating hepatotoxins cause early DNA fragmentation that correlates well with toxic cell death (*J Pharmacol Exp Ther* 263: 387). A suspected cause-effect relationship between DNA damage and hepatotoxicity resulted in our examination of DNA fragmentation and cell entry into the division cycle during hepatic injury. Our working hypothesis states that an initial wave of cell death caused by alkylation results in release of mitogens, localized proliferation of DNA-damaged cells, and abortive transit of recruited cell through the division cycle culminating in their toxic death. The present studies examined the endogenous protein mitogen, transforming growth factor-alpha (TGF-α), for potentiation of dimethylnitrosamine (DMN) injury. ICR mice received 25 mg/kg DMN ip alone or with human recombinant TGF-α (100 μg/kg ip, at +2 or −2 & +6 hr). TGF-α alone increased liver DNA 23–29% at 24 hr. DMN toxicity was mitogenic, increasing liver DNA by 49%. Surprisingly, TGF-α antagonized DMN mitogenesis (DNA increased 31–29%). DMN toxicity elevated ALT in serum 73-fold (4460 vs 61 U/L in control) and DNA fragmentation 2.4 fold (4.04% vs 1.66%). TGF-α suppressed DMN elevation of ALT in a dose-dependent manner (3440 U/L with one and 1920 U/L with two doses). These findings indicate that TGF-α protected against DMN liver injury by suppressing toxicity-associated proliferation (NIH ES 06345).

1071 COMPARATIVE EFFECTS OF DIELDRIN AND PHENOBARBITAL UPON HEPATIC PRENEOPLASTIC AND NEOPLASTIC LESION DNA SYNTHESIS AND TUMOR PROMOTION IN B6C3F1 MICE AND F344 RATS

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Dieldrin, a chlorinated pesticide, is a hepatocarcinogen selectively in the mouse but not in other species (including rats). It appears that dieldrin is functioning through nongenotoxic mechanisms during the tumor promotion stage of the cancer process. Since mice have a high spontaneous rate of hepatic tumors, the present study was undertaken to help elucidate whether the observed mouse specific hepatocarcinogenic effect of dieldrin was related to the promotion of already initiated hepatocytes in the mouse. Preneoplastic hepatic foci were formed in both rat (F344) and mouse (B6C3F1) with diethylnitrosamine. Following foci formation, mice and rats were treated with either dieldrin (0.0, 0.1, 1.0, and 10.0 mg/kg diet) or Phenobarbital (PB) (a tumor promoter in both mouse and rat liver) (0, 10, 100, and 500 mg/kg diet) for 7, 30, or 60 days. At each sampling time, DNA synthesis in both lesions and surrounding liver was determined as well as lesion number, size, and type. Dieldrin and PB increased foci DNA synthesis and size in the mouse, while only PB increased DNA synthesis and size in rat foci. In summary, only mouse preneoplastic liver foci responded to the tumor promotional effects of dieldrin suggesting a possible mechanism for the observed species specificity of dieldrin-induced hepatic cancer.

1072 SELECTIVE INDUCTION OF DNA SYNTHESIS BY DIELDRIN IN MOUSE LIVER

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Dieldrin is an organochlorine hepatocarcinogen that has been shown to induce hepatic cancer only in mice. Previous studies have established dieldrin as a nongenotoxic carcinogen. It has been suggested that nongenotoxic carcinogens may produce cancer through the induction of DNA synthesis in the target organ which may in turn either induce newly initiated cells through altered DNA repair, promote previously initiated cells, or both. In the present study, the dose responsive effect of dieldrin (a mouse liver carcinogen) and Phenobarbital (PB) (a mouse and rat liver carcinogen) on the induction of DNA synthesis in rodent liver was examined. F344 rats and B6C3F1 mice were treated with either 0.0, 0.1, 1.0, 3.0, 10.0 ppm dieldrin or 0, 10, 50, 100, 500 ppm PB for 7, 14, 21, 28 or 90 days. ^{3}H-thymidine was administered to each animal via osmotic pump 7 days prior to sacrifice. DNA synthesis was evaluated in histologic sections by autoradiography. PB induced DNA synthesis in both rat and mouse liver in a dose responsive manner. In the rat and mouse, hepatic DNA synthesis peaked at the 7 day sampling period. Only mouse liver exhibited increased DNA synthesis following treatment with dieldrin with the peak occurring after 14 days. For both PB and dieldrin, a centrilobular distribution of labeling was seen. These results show that dieldrin and PB produced a species specific increase in DNA synthesis that correlated with observed tumorigenicity.

1073 ROLE OF OXIDATIVE STRESS IN DIELDRIN INDUCED MOUSE HEPATOCARCINOGENESIS

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The production of reactive oxygen species by toxic chemicals has been implemented in acute and chronic disease states including cancer. Dieldrin, an organochlorinated insecticide, selectively induces hepatic tumors in mice but not rats. The mechanism for the induction of hepatic cancer by dieldrin and the observed species selectivity of this effect is not known. We hypothesized that induction of oxidative stress by dieldrin may contribute to its observed hepatic toxicity and carcinogenicity. In the present study we examined the induction of oxidative stress (using 8-hydroxy-2-deoxyguanosine (oh8dG) malonaldehyde (MDA) as markers) by dieldrin, both in vitro (cultured hepatocytes) and in vivo (in rat and mouse liver). Dieldrin produce a dose responsive and species specific (mouse only) increase in oh8dG and MDA in vitro. Both subchronic and chronic in vivo treatment with dieldrin produced an increase in liver and urinary oh8dG in the mouse. Oh8dG formation correlated with hepatic DNA synthesis and liver tumor induction in the mouse when compared to control. Subsequent evaluation showed that dieldrin induced oxidative stress correlated with a decrease in hepatic vitamin E levels in the mouse. Dieldrin had no effect on hepatic vitamin E levels in the rat. These studies suggest that the induction of oxidative stress may be a mechanism by which dieldrin and other chlorinated compounds may selectively induce hepatic cancer in the mouse.

1074 CORRELATION BETWEEN THE INDUCTION OF OXIDATIVE STRESS AND RODENT SUSCEPTIBILITY TO HEPATOCARCINOGENESIS

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A number of rodent liver carcinogens have been proposed to function through the induction of oxidative stress. In the present study we examined the relationship between species specific hepatocarcinogenesis and oxidative stress. Both background levels of oxidative stress in normal rodent liver and the production of oxidative stress and reactive oxygen species (ROS) by liver tumor promoters were examined. Spontaneous hepatic tumor incidence in rodents follows a strain and species dependent pattern with mice strains (C3H>B6C3F1>C57/BL) having higher rates than rats. In the present study we found a higher level of 8-hydroxy-2′-de-oxyquanosine (oh8dG) and lower antioxidant capacity in mice than in rats. These results correlated with the observed spontaneous liver tumor pattern suggesting a greater oxidative environment and lower protective capability to oxidative stress in mice than rats may contribute to the observed greater hepatic cancer incidence. Using spin trap or native DNA co-incubated with liver microsomes, Phenobarbital (PB)

(a mouse and rat liver tumor promoter) and dieldrin (a mouse only liver promoter) increased ROS and oh8dG in a dose and species specific manner. The P450 activity of the microsomes was key to the ROS and oh8dG formation. These results showed a direct correlation between the induction of oxidative stress and the species specific tumorigenic effects of these compounds.

1075 AFLATOXIN B_1-INDUCED OXIDATIVE DAMAGES IN THE LIVER OF F344 RATS

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Aflatoxin B_1 (AFB_1) has been shown to be a potent hepatocarcinogen in laboratory animals. The mechanism of AFB_1 genotoxicity and hepatocarcinogenesis, however, is not fully understood. The present study examined the induction of lipid peroxidation as well as DNA oxidative damages by AFB_1 in the liver of F344 rats. Two lipid peroxidation products, malonaldehyde (MDA) and conjugated dienes, were measured in liver homogenate and sub-cellular fractions. 8-Hydroxydeoxyguanosine (8-OHdG), a form of DNA oxidative lesions, were determined in isolated hepatic DNA. The result showed time- and dose-dependent increases of all three indices following a single *i.p.* injection of AFB_1 (100 μg/100 g body wt.), indicating that AFB_1 was capable of inducing oxidative damages in the liver. Lipid peroxidation was localized in certain cellular organelles such as microsomes, nuclei and mitochondria, but not in the cytosol. Hepatic DNA was also extensively modified by AFB_1-induced oxidative damages (as high as 45 8-OHdG adducts per 10^6 dG). Pretreatment of rats with the antioxidant selenium and the ion-chelator deferoxamine significantly inhibited lipid peroxidation as well as 8-OHdG formation, suggesting that hydroxyl free radicals might be involved as the reactive oxygen species. These results provide evidence that AFB_1 causes oxidative DNA damages in rat liver, which may constitute an important pathway in AFB_1 hepatocarcinogenesis.

1076 HEPATITIS VIRUS SURFACE ANTIGEN INDUCES NITRIC OXIDE SYNTHESIS IN HEPATOCYTES

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Nitric oxide ($NO^{\bullet}$) can react with DNA to exert untoward effects including deamination and/or indirect alkylation leading to mutagenesis. Endogenous production of $NO^{\bullet}$ and hepatocarcinogenic N-nitroso compound are increased in woodchucks with chronic woodchuck hepatitis virus (WHV) infection. We now report WHV surface antigen (WHsAg) stimulates hepatocytes to express $NO^{\bullet}$ synthase to produce $NO^{\bullet}$ in culture. $NO^{\bullet}$ synthesis by hepatocytes was positively correlated to WHsAg concentration, and was inhibited by N^G-monomethyl-L-arginine and anti-WHsAg antibody. These data, when considered in light of the known genotoxicity of $NO^{\bullet}$, raise the possibility that viral hepatitis increases the risk of liver cancer by increasing the production of $NO^{\bullet}$. This provides a new mechanism by which hepatitis B virus infection increases the risk of liver cancer.

1077 THE TIME/DOSE RESPONSE OF OVARIAN ATROPHY AND TUBULAR ADENOMAS IN MICE FOLLOWING ORAL ADMINISTRATION OF MGK® REPELLENT R.11

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Carcinogenicity studies in CD_1 mice resulted in an increase of tubular adenomas of the ovary. Tubular adenomas are secondary to ovarian atrophy and pituitary stimulation. The time course and dose response have been studied to demonstrate the correlation of atrophy and adenomas and establish a no effect level. Mice received 5, 30, 60, 90 and 500 mg/kg/day and groups were killed at 30, 60, 90 days and 26, 55 and 80 weeks. Mice receiving 500 mg/kg/day at 26 weeks showed ovarian atrophy, 6/10 and at 55 weeks all had ovarian atrophy and 7/10 mice tubular. At 80 weeks atrophy was present in 31/37 mice from the 500 mg/kg/day group and 8/40 from the 90 mg/kg/day group. Tubular hyperplasia was only observed in the high dose group. Tubular adenomas, 9/37, granuloma cell tumour, 3/37, and luteoma, 4/37, were observed in the high dose group. No tubular adenomas were seen in the 90 mg/kg/day group. The findings show a clear dose and time related occurrence of atrophy, hyperplasia and adenomas with a no observable effect level of 60 mg/kg/day.

1078 A COMPARISON OF URINARY CHEMISTRY CHANGES IN MALE AND FEMALE RATS AND MICE TREATED WITH SODIUM SACCHARIN

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Male rats fed high doses ($\geq$3%) of sodium saccharin (NaSac) develop urothelial cell proliferation and increased bladder tumor formation. Calcium phosphate precipitate forms in the urine of these rats and may be part of the mechanism by which the bladder changes occur. These changes occur to a lesser extent in the female rat and not at all in mice. We treated F344 male and female rats and Swiss male and female mice with 7.5% NaSac for twelve weeks. Urinary chemistry parameters evaluated showed similarities between the males and females in each species in pH level, protein and NaSac concentration, and various minerals. Calcium levels were higher in control rats compared to control mice. Treatment further increased levels in male rats but not in the mice or female rats. This increased level of calcium may play a role in the mechanism by which NaSac selectively causes bladder changes in the male rat. Supported by NIH grant #CA32513 and a grant from Intl. Life Sci. Inst.

1079 CELL KINETIC STUDY OF SQUAMOUS CARCINOMA DEVELOPMENT DURING BLADDER TRANSITIONAL CELL CARCINOGENESIS IN RATS TREATED WITH BUTYL-(4-HYDROXYBUTYL)-NITROSAMINE

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To clarify the origin and development of the squamous cell carcinoma (SCC) of rat bladder, cell kinetics of the squamous and transitional neoplastic lesions induced by butyl-(4-hydroxybutyl)-nitrosamine (BBN) was investigated. A total of 220 male F344 rats, aged 6 wks, were given 0.05% BBN in the drinking water fort 12 wks and then given normal tap water. Rats were sequentially sacrificed at 8, 12, 18, 30 and 60 wk. Bromodeoxyuridine (BrdU) was applied as a single injection or by contineous labeling using osmotic mini pumps for 4 or 7 days before sacrifice. The bladder were fixed and processed for routine staining with hematoxylin-eosin, and for immunohistochemical demonstraion of BrdU. Small neoplastic squamous lesions (NSL) were firstly observed in transitional cell papillomas and carcinomas (TCP/TCC) at 18 wk. And then the number and area of NSL in the TCP/TCC was increased during the course of the study. Flash and continuous BrdU labeling revealed NSL to consist of cells showing twofold high mitotic activity compared with surrounding TCP/TCC cells. These results suggest that NSL cells might derive from TCP/TCC cells, and this highly labeled NSL replace the TCP/TCC and develop to bladder SCC.

1080 *IN VITRO* EXPOSURE OF HUMAN UROEPITHELIAL CELLS TO OCCUPATIONAL CHEMICAL RESULTS IN ALTERATIONS IN PROTEIN EXPRESSION AS DETERMINED BY HIGH RESOLUTION TWO DIMENSIONAL POLYACRYLAMIDE GEL ELECTROPHORESIS

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A study has been initiated to investigate the carcinogenicity of 4,4'-methylene bis(2-chloroaniline) (MOCA) and 2-phenyl-1,4-benzoquinone (PBQ) and to identify biomarkers of effects of exposure to these workplace-associated suspect carcinogens. High resolution two dimensional polyacrylamide gel electrophoresis (2D PAGE) was used to examine changes in protein expression in chemically-treated human uroepithelial cells (HUC). Previously, HUC has been shown to be sensitive to malignant transformation by 4-aminobiphenyl (4-ABP) and its metabolites (Bookland, et. al., *Cancer Research*, 52, 1606–1614, 1992). HUC was incubated for 24 hr with the controls, dimethyl sulfoxide (DMSO) or N-OH-AABP, as well as N-OH-MOCA (2.5 and 10 uM), or PBQ (1.5 and 3.5 uM) which resulted in 25% and 75% cell kill, respectively. A 2D PAGE protein map of treated HUC was developed using the ISODALT method of Anderson. Electrophoretic pattern comparison across treatment groups by scanning densitometer and computer analysis revealed quantitative changes in a specific subset of proteins in the 10 uM MOCA-treated HUC and N-OH-AABP-treated HUC but not the DMSO-treated HUC. Preliminary studies of tumor incidence in athymic mice suggest that neo-plastic

transformation occurred only in the 10 uM MOCA-treated HUC. These results show that occupational chemical exposure of HUC results in 2D PAGE protein alterations which may be useful for the identification of biomarkers associated with bladder tumorigenesis.

1081 TWO-YEAR DERMAL STUDY OF TRIETHANOLAMINE IN F344 RATS AND B6C3F$_1$ MICE

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Human dermal exposure to triethanolamine (TEA, CAS No. 102-71-6) occurs during the normal use of many cosmetic preparations. F344 rats received TEA in acetone by topical application at doses of 0, 32 (males only), 63, 125, and 250 (females) mg/kg. Male B6C3F$_1$ mice received TEA at doses of 0, 200, 630 and 2000 mg/kg and female mice received doses of 0, 100, 300, and 1000 mg/kg. Animals were treated for 5 days/week for up to 103 weeks. An interim evaluation conducted during Week 66 included 10 animals/dose group of each sex. After two years, dosing was suspended and survivors terminated two weeks later, followed by complete necropsy and histopathology. Treatment was not associated with an increased incidence of spontaneous deaths or moribund terminations. The growth rate of treated animals was similar to that of their respective control groups. Irritation at the site of application increased with dose level (rats and male mice). Microscopic examination of interim and two year rats (particularly females) revealed dose-related acanthosis, chronic-active inflammation, and epidermal ulcerations. There were no treatment-related lesions at the application site in mice, although acanthosis apppeared to be a treatment-related change at the 66-week interim termination. In mice, there was a treatment-related increase in the number of liver tumors at necropsy which were classified predominately as hepatocellular adenomas upon microscopic examination. (Supported by NIEHS Contract No. NO1-ES-85226).

1082 NINETY-DAY TOXICITY STUDIES OF 2-METHYL-IMIDAZOLE IN FISCHER RATS AND B6C3F1 MICE

P C Chan, M L Wenk. *NIEHS, RTP, NC and Microbiological Associates, Inc., Bethesda, MD.* Sponsor: J R Bucher

2-Methylimidazole (2MI) is used as chemical intermediate in the manufacture of pharmaceuticals, photographic and photothermographic chemicals, dyes and pigments, agricultural chemicals and rubber. 2MI is also used as polymerization crosslinking accelerator and hardener for epoxy resin systems for semiconductor potting compounds and soldering masks. 2MI has been identified in ammoniated hay forage for livestocks and in caramel coloring, soy sauce, Worchestershire sauce, wine, ammoniated molasses, caramel-colored syrups, and cigarette smoke. Toxicity studies of 2 MI was conducted because of its widespread use, potential for widespread exposure in food to humans, lack of toxicity data, and suspicion of carcinogenicity. Groups of 10 male and 10 female Fischer rats and B6C3F1 mice were dosed fed 2MI at 0, 625, 1250, 2500, 5000 or 10000 ppm for 90 days. All animals survived. No signs of neurotoxicity were observed. Mean body weights were significantly reduced in male rats at ≥2500 ppm and in female rats at 10000 ppm. Food consumption was reduced in male and female rats at ≥5000 ppm. Relative liver and kidney weights were increased in male and female rats. Serum T3 and T4 levels were decreased and TSH levels increased significantly in male and female rats at ≥2500 ppm. Dose-related thyroid follicular cell hyperplasia occurred in male and female rats; in 2 of 10 male rats at 10000 ppm the lesions were large. Uterine atrophy and adrenal cortical necrosis were also observed in the 2 highest dose groups of female rats. Mean body weights were significantly reduced in male and female mice at ≥5000 ppm. Food consumptions were similar among the male and female mouse groups. Relative kidney and liver weights were increased in dosed male and female mice. Thyroid follicular cell hyperplasia and splenic hematopoietic cell proliferation were found in male and female mice at ≥2500 ppm. Hemosiderin was observed in kidney tubules in male mice at ≥1250 ppm and in female mice at ≥2500 ppm. Serum TSH levels were increased and T3 and T4 levels were decreased in male and female mice. Induction of thyroid follicular cell hyperplasia in rats and mice by 2MI may be due to enhanced metabolism of T3 and T4 by increased hepatic UDPglucuronosyltransferase activities. This indirectly stimulated TSH secretion and in turn thyroid gland hyperplasia. Prolonged exposure to 2MI in rats and mice would probably lead to thyroid follicular cell adenoma formation.

1083 MODIFYING EFFECTS OF ADI DOSE MIXTURES OF 20 OR 40 PESTICIDES ON THE MEDIUM-TERM CARCINOGENESIS MODELS IN RATS

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The modifying effects of acceptable daily intake(ADI) dose mixtures of 20 or 40 pesticides of interest because of large quantity manufactured or suspectable carcinogenicity in rats, were investigated using our medium-term liver and multi-organ bioassays for rapid detection of carcinogens. No adverse effects in terms of body weight, organ weights, and tumor incidences were observed in rats exposed to the mixtures of 40 or 20 pesticides. In contrast, elevated quantitative values for immuno-histochemically demonstrated GST-P positive hepatocyte foci, as well as increased incidences of preneoplastic renal lesions, were found in rats given captofol used as a positive control. The safety factor used for ADI by the Japanese Ministry of Health and Welfare and the FAO/WHO is usually 100. There are a number of potential problems associated with the safety factor approach, including the fact that the observation of no treatment-related effects may depend on the number of animals exposed and dose levels used, and biological justification for general use may therefore be lacking. However, the present results indicate that the regulation of pesticides based on ADI levels is appropriate with regard to carcinogenicity.

1084 CARCINOGENICITY OF TRIFLURALIN — NOT TRIGGERED BY ITS IMPURITY N-NITROSODI-N-PROPYLAMINE (NDPA)

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Trifluralin is a selective pre-emergence herbicide used worldwide on more than 28 crops. Several years ago, the US EPA raised concerns about trifluralin regarding its carcinogenic potential. There was concern because trifluralin products contained significant levels of a carcinogenic contaminant — NDPA. Trifluralin, with or without NDPA contamination, was studied in a medium-term bioassay based on the induction of preneoplastic lesions in the liver. Rats were administered diethylnitrosamine (DEN) i.p. at a dose of 200 mg/kg bw and two weeks later were treated with different samples of trifluralin at a dose of 5000 ppm in the diet for six weeks and then killed; all rats had a partial hepatectomy at week 3. Hepatocarcinogenic potential was assessed by comparing the number and area of glutathione-S-transferase (placental form)-positive foci in the liver with those of controls given DEN alone.

Positive results were noted with all samples of trifluralin with or without NDPA contamination. The present results would suggest that trifluralin itself (without contaminant) was hepatocarcinogenic in this bioassay.

1085 SUBCHRONIC TOXICITY EVALUATION OF TETRYL IN FISCHER 344 RATS

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Subchronic toxic effects of the explosive chemical N-Methyl-N-2,4,6-tetranitroaniline (tetryl) in male and female Fischer 344 rats were evaluated by feeding Purina® rodent chow supplemented with varied concentrations of tetryl (0, 200, 1000 and 3000 mg/kg diet) for ninety days. Food intake was reduced in both sexes at all dose levels throughout the study and resulted in a significant decrease in terminal body weights in the 3000 and 1000 mg tetryl dose groups. The calculated average tetryl intake was 14, 69, and 199 mg/kg BW/day for females and 13, 62 and 180 mg/kg BW/day for males. An increase in the relative liver and kidney weights in both sexes in the 3000 and 1000 mg tetryl dose groups and an increase in the relative spleen weight in the 3000 mg dose group were noted. Hemoglobin content was decreased and methemoglobin levels were increased in both sexes in the 3000 and 1000 mg tetryl dose groups. Decreased red cell count and increased reticulocyte count from both sexes in the 3000 mg tetryl dose group were apparent. Histopathological examinations revealed pigment deposition and erythroid cell hyperplasia in spleen, and tubular degeneration and cytoplasmic droplets in kidney, from both high dose groups. From this study the no observed adverse effect level (NOAEL) of 13 mg/kg/day was established. (This abstract does not necessarily reflect U.S. EPA/U.S. Army policy.)

1086 CHRONIC TOXICITY OF AROCLOR MIXTURES ADMINISTERED IN THE DIET TO SPRAGUE-DAWLEY RATS FOR TWELVE MONTHS

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A side-by-side evaluation of the chronic toxicity/oncogenicity of the polychlorinated biphenyls (PCBs) Aroclors 1016, 1242, 1254 and 1260, at dose levels ranging from 25–200 ppm in diet, is ongoing in male (M) and female (F) Sprague-Dawley rats. The highly chlorinated PCB mixtures Aroclor 1260 and Clophen A 60 (60% Cl) have been demonstrated in previous chronic studies to induce hepatic tumors in rodents, while the less chlorinated PCB mixtures, Aroclor 1254 (54% Cl) and Clophen A 30 (42% Cl), have not produced tumorigenic responses. Interim evaluations have been conducted after 13 and 39 weeks (F only), and 26 and 52 weeks (M & F) for this 2-yr study. Parameters measured include feed consumption, body weight, serum chemistry, hematology, urinalysis and organ weights, as well as evaluations of appearance and behavior and macroscopic and histomorphologic lesions. Dose-related decreases in body weight gain were recorded for M receiving Aroclor 1254 and for F receiving Aroclors 1242, 1254 and 1260. Dose-related increases in aspartate aminotransferase were measured for F receiving Aroclors 1242 and 1254, and slight treatment-related increases in serum cholesterol were measured for M receiving Aroclors 1016, 1254 and 1260. Treatment-related increased relative (to brain) liver weight was measured for M (but not F) in all Aroclors, with dose-related increases evident for Aroclors 1254 and 1260. Histomorphologic evaluation of livers revealed treatment- and dose-related centrilobular hepatocellular hypertrophy in both M & F.

1087 COMBINED CHRONIC TOXICITY/ONCOGENICITY STUDY WITH GLUTARALDEHYDE (GA) IN THE DRINKING WATER OF RATS

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Fischer 344 rats (100/sex/group) were dosed with GA at 0 (control), 50, 250, or 1000 ppm in drinking water 7 days/week for at least 104 weeks. The mean GA intake over the entire study was 4, 17, and 64 mg/kg/day for the males and 6, 25, and 86 mg/kg/day for the females. Interim sacrifices (10/group/sex) were performed after 52 and 78 weeks of treatment. There were no treatment-related effects on mortality or incidence of palpable masses. Body weights and gains and food and water consumption in the mid and high dose groups of both sexes were generally decreased throughout the study. Water consumption for the low dose group of both sexes tended to be decreased throughout the study. In the mid and high dose groups, compensatory changes to decreased water consumption included decreased urine volume and pH with increased osmolality, protein and bilirubin along with increased kidney weights. Upon gross and/or microscopic examination, evidence of gastric irritation, without evidence of a localized oncogenic effect, was present in some mid and high dose group animals. Large granular lymphocyte (LGL) leukemia, a common and spontaneously occurring neoplasm in Fischer 344 rats, was found at a high incidence in animals from all dose groups and was significantly increased in all GA-treated groups of female rats. The significance of the increased incidence of LGL leukemia in female rats was unclear but there was no evidence of a direct carcinogenic effect of GA. However, a clear NOEL was not established for this study due to the decreased water consumption in all dose groups of male and female rats and increased incidence of LGL leukemia in all dose groups of female rats.

1088 EVALUATION OF CHEMICALLY-RELATED DECREASED TUMOR INCIDENCES IN NTP RODENT CARCINOGENICITY STUDIES

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The frequency of chemically-related decreases in tumor incidence was examined in 218 two-year carcinogenicity studies carried out by the National Toxicology Program (NTP). For certain site-specific tumors, the frequency of chemically-related tumor decreases exceeded chance expectation. Certain of these tumors, most notably pituitary and mammary gland neoplasms in F344 rats and liver tumors in B6C3F1 mice, are known to be associated with reduced body weight, and the decreased body weights that frequently occurred in dosed animals were primarily responsible for the decreases observed for these neoplasms. Decreases in leukemia incidence in F344 rats occurred primarily for chemicals that produced splenic alterations in 90 day studies. Reduced survival in dosed groups also influenced the frequency of tumor decreases. We concluded that most chemically-related tumor decreases observed in NTP studies can be explained by random variability, reduced body weights and/or survival in dosed groups and (in the case of leukemia) splenic alterations.

1089 EFFECTS OF ORGANOPHOSPHATE INSECTICIDES (OPI) ON THE BLOOD-BRAIN BARRIER (BBB)

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OPI are inhibitors of acetylcholinesterase and are potent toxins in the central nervous system (CNS). Although OPI are known to increase (BBB) permeability, the mechanism is not understood. In these experiments on rats, we studied the ability of different OPI to inhibit cholinesterase (CE) *in vitro* and *in vivo* and to increase BBB permeability *in vivo*. Several OPI that do not require metabolism for activity, including diisopropyl fluorophosphate and paraoxon, inhibited CE activity in preparations of isolated microvessels when exposed to the drugs *in vitro*. However, two OPI that require activation, parathion and malathion, were ineffective. CE activity was also measured in brain microvessels isolated from rats 10 min after an i.m. injection of paraoxon. The lowest concentration to inhibit CE was observed at 60 μg/kg. Finally, changes in permeability of the BBB was examined using horseradish peroxidase in animals injected with paraoxon. Again, the lowest concentration where a leaky BBB was found was 60 μg/kg. These data demonstrate a similar dose-dependence for inhibition of CE in microvessels and increase in BBB permeability. We suggest that the leak in the barrier may be due to inhibition of CE on the capillary.

1090 *IN VIVO* ACUTE EFFECTS OF CARBOFURAN AND METHYL PARATHION ON BIOMARKERS IN DISCRETE RAT BRAIN REGIONS

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The objective of this investigation was to determine the distribution of AChE and creatine kinase (CK) and CK isoenzymes in brain regions of rats treated with DMSO (controls), carbofuran (1.5 mg/kg, sc) or methyl parathion (MPTH, 5 mg/kg, ip). In controls, AChE activity was highest in striatum and least in cerebellum, while CK activity was found highest in cerebellum and least in hippocampus. Patterns of CK isoenzymes in stem, striatum, and hippocampus were very similar (86.2–88.5% CK-BB; 2.8–5.1% CK-MB; and 6.7–9.4% CK-MM). Cortex, however, had lowest CK-BB, but highest CK-MM compared to other brain structures. CK-MM isoenzyme in all brain structures had only the CK-MM3 subform. Maximal inhibition of AChE occurred in cortex with carbofuran and in cerebellum with MPTH. Striatal AChE was least inhibited. Both insecticides caused characteristic alterations in CK-isoenzymes patterns, and consequently their leakage into circulation by increased membrane permeability due to depletion of ATP and phosphocreatine. (Partially supported by Beckman Instruments, Inc.).

1091 IMMUNOHISTOCHEMICAL LOCALIZATION OF THE α AND β SUBUNITS OF CaM II KINASE IN THE SPINAL CORD OF THE HENS TREATED WITH DIISOPROPRYL PHOSPHOFLUORIDATE (DFP)

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Alterations in CaM II kinase activity have been demonstrated in several neuropathological conditions, including organophosphorus ester-induced delay neurotoxicity (OPIDN). We have previously reported that in hens exhibiting OPIDN, axons in early stages of degeneration exhibit anomalous aggregations of phosphorylated neurofilaments and that such aggregations can exhibit CaM II kinase-like immunoreactivity, as determined with a polyclonal antisera for the holoenzyme. To further characterize the axonal CaM II kinase we have now used monoclonal antisera specific for either the α or β subunit to stain sections of the spinal cord of hens killed 7 days after administration of 1.7 mg/kg DFP. In control hens, immunostaining of most axons for the β subunit was more intense than for the α subunit, except for a restricted region within the dorsal columns. In addition, non-neuronal cells, presumably oligodendroglia, astrocytes, and microglia were also immunostained. In hens treated with DFP, axons in early stages of degeneration exhibited accumulations immunoreactive for both antisera recognizing alpha and beta subunits. These observations are consistent with a role for CaM II kinase in the neurodegeneration associated with OPIDN. (Supported in part by NIEHS grant ESO5154).

1092 cRNA CLONING AND SEQUENCING OF Ca^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE II AND ITS GENETIC EXPRESSION IN DFP-TREATED HEN CENTRAL NERVOUS SYSTEM

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Diisopropyl phosphorofluoridate (DFP) is one of the organophosphorus esters that produce delayed neurotoxicity (OPIDN) in the hen and other sensitive species. A single dose of DFP (1.7 mg/kg, sc.) produces mild ataxia followed by paralysis in 7–14 days in hens. Organophosphorus esters producing delayed neurotoxicity also show increased autophosphorylation of calcium/calmodulin-dependent protein kinase II (CaM-kinase II) and increased phosphorylation of various cytoskeletal proteins. We purified and sequenced a cDNA clone for CaM-kinase II from normal adult chicken brain. Twelve positive clones were obtained by screening 250,000 plaques of λgt10 cDNA library with the ^{32}P labeled probe prepared from cloned rat CaM-kinase II α subunit. One of these plaques which had an insert of 1.8 kbp was used for sequencing. CaM-kinase II cDNA sequence showed that its α subunit contains 478 amino acids and it differs in 7 amino acids from rat CaM-kinase II α subunit. ^{32}P labeled probe corresponding to the carboxyl terminal portion of enzyme hybridized to a single genomic DNA fragment on restriction digestion with BamHI, EcoRi, or HindIII. Northern blots, however, showed mRNA species of 6.3, 3.38, 2.6, and 1.65 kb in the cerebral cortex. Studies on expression showed an increase in CaM-kinase II α subunit mRNA in the cerebellum of DFP-treated chicken. The increase was maximum after 1 day, although it remainded elevated through out the period of observation upto 20 days post-treatment. This increased expression of CaM-kinase II mRNA in the hen cerebellum after DFP treatment suggested a role of this enzyme in OPIDN. (Supported in part by NIEHS grant ESO 5154).

1093 CHANGES IN AFFINITY TO ACETYLCHOLINE (ACh) AND AFFINITY AND PHOSPHORYLATION CONSTANTS REGULATING INHIBITION OF ACETYLCHOLINESTERASE (AChE) IN DIISOPROPYL FLUOROPHOSPHATE (DFP) TOLERANT RATS

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Daily dosing of DFP in a concentration (0.5 mg/kg, sc) that as a single dose did not cause signs of cholinergic hyperactivity, produced fasciculations and central tremors between day 3 and 5. During further administration of DFP (10–20 days) no signs of cholinergic hyperactivity were seen, despite critically reduced AChE activity. The hypothesis was tested that prolonged treatment with DFP induced alterations in the kinetics of reactivity of AChE with its substrate ACh and with the inhibitor DFP. The kinetic constant Vmax and Km for ACh were determined by Lineweaver-Burke transformations. The affinity constant for DFP (Kd) and the phosphorylation constant (Kp) were determined. Slopes of inhibition curves were calculated by linear regression and converted to bimolecular rate constants. Following 5, 10, and 20 days of treatment, Km of brain was reduced from 0.069 to 0.0476 mM and Vmax from 999.5 to 133.3 μM/g/hr. The corresponding values for diaphragm were reduced to 72% and 52%. In 20 day treated rats the Kd decreased from 0.062 mM to 0.024 mM, while the Kp decreased from 2.227 to 1.594 min^{-1}. The decrease in catalytic activity (Vmax) was compensated for by an increase in affinity (Km) of AChE to its substrate ACh. The slower rate of phosphorylation combined with the increased affinity of AChE for ACh affords protection against further inhibition. These changes may allow the animals to regain normal function despite critically reduced AChE activity. Supported by NIH grant ESO5193.

1094 DIISOPROPYL FLUOROPHOSPHATE (DFP) INDUCED CHOLINERGIC HYPERACTIVITY AND LIPID PEROXIDATION (LP)

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Acute exposure to insecticides such as organophosphates and carbamates induces functional changes at cholinergic synapses that ultimately causes neuronal and muscle fiber necrosis. There is recent evidence that oxygen free radical formation may be a factor in the toxicity of these insecticides. One of the targets of free radical induced injury is peroxidation of lipids. The role of LP in DFP induced muscle and brain necrosis was investigated by quantifying the malondialdehyde-thiobarbituric acid complex using HPLC. Male Sprague-Dawley rats treated with DFP (1.0–2.5 mg/kg) were sacrificed 30–120 mins after the DFP injection. Significant increases of LP over control were observed in diaphragm when rats were treated with 1.5, 2.0, or 2.5 mg/kg of DFP. The highest increase of 96% was obtained with rats treated with 2.0 mg/kg DFP after 60 mins' exposure. A 55% increase of LP in brain was observed under the same conditions. At this time seizures and fasciculations were pronounced. When 1.0 mg/kg DFP was used, neither a significant difference of LP level from control nor seizures or fasciculations were seen. The rate of LP and acetylcholinesterase inhibition was directly proportional. In rats pretreated with cholinergic antagonists such as atropine, d-tubocurarine chloride, or with the lazaroid U 78517G(F), an antioxidant, before DFP (2.0 mg/kg), no increases in LP were observed, and necrosis of muscle fibers was significantly attenuated. It is suggested that DFP induced cholinergic hyperactivity stimulates LP leading to free radical injury in brain and muscle. Supported by NIH grant ESO4597.

1095 MOTOR NERVE CONDUCTION VELOCITY IN MIPAFOX-TREATED WISTAR RATS

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Hens with organophosphate-induced delayed neurotoxicity (OPIDN) exhibit ataxia and paresis with distal degeneration of long sensory and motor axons in spinal cord and peripheral nerve. Rats do not readily show clinical signs of OPIDN despite the presence of axonal lesions. Previous work has shown that sciatic nerve motor nerve conduction velocity (mNCV) decreases after onset of diabetes in BB-Wistar rats. As part of a study of neurotoxic interactions with diabetic neuropathy, we measured the time-course of mNCV in a nondiabetic subpopulation of BB (8–18 mo old) and normal Wistar rats (8 mo old) after single or repeated doses of the neuropathic OP, mipafox (MIP, 45 mg/kg ip in 1.0 ml/kg isotonic saline). One dose of MIP in BB rats (6/group) resulted in a maximum significant decrease in mNCV (24% of same-day control) on Day 8, and return to control by Day 17. After a 1-month recovery, a dose of MIP was given on Day 0 to the former control (group 1) and single-dosed (group 2) BB rats, followed by another dose to group 2 on Day 8, resulting in a maximum significant decrease in mNCV on Day 12 (compared to Day 0 of the same group) of 25% (group 1) and 39% (group 2). In group 2, mNCV was still depressed (37%) on Day 16, and this group had clinical signs of ataxia and paresis by Day 12. Similar results on mNCV were obtained in normal Wistar rats (5/group) given MIP or saline on Day 0 followed by either saline, MIP, or the OPIDN potentiator, phenylmethanesulfonyl fluoride (PMSF, 120 mg/kg ip in 1.0 ml/kg glycerol formal) on Day 8. Significant decreases in mNCV compared to same-day controls were found with MIP + PMSF on Days 4, 8, and 12; with MIP + MIP on Days 8 and 12; and with MIP + saline on Day 12. Surprisingly, significant decreases in mNCV were also found on Days 4–12 when PMSF was given alone on Day 0. All groups had normal mNCVs by Day 16, and in contrast to the BB rats, no clinical signs of neuropathy were seen. The results suggest that mNCV may be used to monitor development of OPIDN in rats. Future work will assess the unexpected finding with PMSF alone and the relative roles of strain and age in the clinical response. (Supported in part by Dow, Mobil, and NIH).

1096 NEUROTOXIC EVALUATION OF THE ORGANOPHOSPHATE INSECTICIDE PROFENOFOS (CURACRON®8E) IN THE RAT

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Groups of Hsd:Sprague Dawley® SD™ rats were administered, po, a single dose of 0, 95, 190 or 380 mg/kg profenofos in corn oil (acute study) or 0, 30, 135 or 600 ppm profenofos in diet for 90 days (subchronic study). Neurological tests consisting of a functional observational battery (FOB) and figure-8 maze motor activity were performed pretest, 4 to 6 hours after dosing (peak effect) and days 8 and 15 for the acute study; and pretest, weeks 4, 8 and 13 for the subchronic study. Specimens were obtained from identically treated rats at the same intervals for determination of serum and RBC cholinesterase (ChE). Brain ChE was determined at the last intervals. For the pathology evaluation, rats were perfusion fixed, necropsied, and the nervous tissues examined microscopically. In the acute study, motor activity was markedly reduced and FOB findings consistent with cholinergic, poisoning were observed at the peak effect assessments at 380 mg/kg. These effects were completely abated by day 8. Significant inhibition of serum and RBC ChE was observed at all dose levels at the peak effect interval. By day 15, the serum inhibition was completely reversed and RBC inhibition was partially reversed. No neurobehavioral effects were observed at 95 and 190 mg/kg even though RBC and serum ChE were inhibited by up to 94%. In the subchronic study, no effects on the FOB and motor activity tests were observed even though there was up to 84, 94 and 20

percent inhibition of RBC, serum and brain ChE, respectively. No pathological evidence of neurotoxicity was observed in either study.

1097 BEHAVIORAL EFFECTS AND CHOLINESTERASE (ChE) INHIBITION FROM ACUTE ORAL EXPOSURE TO THE ORGANOPHOSPHATE INSECTICIDE MONOCROTOPHOS IN RATS

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Monocrotophos was administered to Crl:CD®(SD)BR rats to determine the no-observable-effect level (NOEL) for clinical/behavioral effects; the peak ChE inhibition time(s); and NOEL for serum, red blood cell (RBC) and brain ChE activity levels. Groups of five rats/sex (8–9 weeks old) were administered, po, monocrotophos in distilled water at 0, 0.01, 0.03, 0.1, 0.3, 1, 3 or 5 mg/kg. Clinical signs were recorded at 1, 2, 4 or 6 hours after dosing and once daily up to 7 days. Serum, RBC and brain ChE activity levels were determined at 2, 4, 6 or 24 hours after dosing. Clinical signs at ≥ 1 mg/kg included flattened posture, muscle fasciculations, staining of the eyes, mouth and nose, partially formed feces or diarrhea, miosis, lacrimation, and salivation, compatible with cholinergic stimulation. The severity of these signs peaked at 1–2 hours and complete recovery was apparent by 24 hours. Depression of serum, RBC and brain ChE activity levels at 3 mg/kg appeared to peak at 2 hours (serum 80%, RBC 72% and brain 87% reduction from control) and partial recovery was demonstrable at 24 hours (30% reduction). Serum ChE activity was significantly lower for males at ≥ 0.1 mg/kg and for females at ≥ 0.3 mg/kg. Both sexes showed a lower RBC ChE activity at ≥ 0.3 mg/kg. Brain ChE activity was significantly lower for males and females dosed at ≥ 0.1 mg/kg. In summary, a single oral dose of monocrotophos resulted in a NOEL for clinical signs at ≥ 0.3 mg/kg and peak ChE activity inhibition in serum, RBC or brain samples at 2 hours. The NOEL for inhibition of ChE activity at 2 hours post-dose was 0.03 mg/kg.

1098 COMPARISONS OF THE BEHAVIORAL EFFECTS AND CHOLINESTERASE INHIBITION IN CHLORPYRIFOS-TREATED RATS

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This study examined the relationships between behavioral changes and cholinesterase (ChE) inhibition produced in rats treated with chlorpyrifos (CPF). Adult Long-Evans male rats were dosed orally with either vehicle (corn oil), or 10, 30, 60, or 100 mg/kg CPF. Rats (N = 20/dose group) were evaluated using a neurobehavioral test battery (functional observational battery and automated motor activity) the day before dosing (baseline). All rats were tested at 3.5 hr after dosing, and half of these (N = 10/dose) were sacrificed immediately after testing for collection of blood, brain and muscle tissues. The remaining rats were tested again at 24 hr, followed by sacrifice. Tissues were then assayed for ChE activity and muscarinic receptor binding (see Nostrandt *et al.*). The lowest dose, 10 mg/kg, produced no behavioral effects in rats sacrificed at 3.5 hr, however, this dose produced significant ChE inhibition in most tissues. Effects of higher doses showed maximal ChE inhibition as well as the expected cholinergic syndrome. Recovery of behavioral effects at 24 hr was evident; however, ChE inhibition in most tissues showed little recovery. The dose-response curves for the behavioral effects were not parallel to those for the effects on ChE inhibition. Correlations for behavioral and biochemical effects were generally poor since the low-dose effects on ChE were not reflected in behavioral signs at 3.5 hr, and because behavioral signs showed recovery at 24 hr unlike ChE activity. Examination of individual rat data indicated that brain ChE inhibition of >80% was obtained in rats showing lacrimation, salivation, tremors, miosis, and inhibited pupil response; >70% with mouth smacking and >60% for gait alterations, hypoactivity, and hypothermia in CPF-treated animals.

1099 DETAILED EVALUATION OF CHOLINESTERASE INHIBITION AND MUSCARINIC RECEPTOR DENSITY IN RATS TREATED WITH CHLORPYRIFOS

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Adult male rats were treated by gavage with chlorpyrifos at doses of 0, 10, 30, 60, and 100 mg/kg. Animals were evaluated behaviorally (see Moser *et al.*) at 3.5 (time of peak effect) and 24 hours after dosing and were sacrificed at their respective time point. The following tissues were collected from each animal: half brain (sectioned longitudinally so that all anatomical region were represented), frontal cortex, hippocampus, striatum, hypothalamus, cerebellum, pons/medulla, retina, liver, heart, diaphragm, and *quadriceps femoris* muscle. Trunk blood was collected and, after removing an aliquot of whole blood, plasma and erythrocytes were separated by centrifugation. Cholinesterase (ChE) activity was determined in all tissues. Inhibition of ChE was highest in blood components and liver at 3.5 hours (71–99% inhibition), with little difference in response as a function of dose and some recovery at 24 hours in the lower dose groups. In brain, a clearer dose response relationship was observed (11–92% inhibition), again with slight recovery of ChE activity at 24 hours. Generally, ChE inhibition in whole brain appeared to be representative of that in individual brain parts and retinal ChE inhibition followed a pattern similar to that of the brain. ChE inhibition in the muscular tissues examined also exhibited a clear dose-response relationship, but with inhibition in heart > diaphragm > *quadriceps*. At the higher doses, ChE inhibition at 24 hours was actually greater than that at 3.5 hours in heart and plasma, while no significant recovery was seen in the remaining muscular tissues and blood components, striatum, hypothalamus, and liver. Muscarinic receptor density was assessed in all brain regions, as well as in heart and retina. Apparent downregulation of muscarinic receptor density was noted in striatum, pons/medulla, and retina at a dose of 100 mg/kg.

1100 EFFECTS OF CHLORPYRIFOS ON HIGH AFFINITY CHOLINE UPTAKE IN RAT BRAIN

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We previously found that the organophosphorus insecticide chlorpyrifos (CPF, 280 mg/kg, sc) caused extensive and persistent (>90% for up to 14 days) inhibition of cholinesterase (ChE) activity both centrally and peripherally in rats but few signs of overt toxicity. As a reduction in acetylcholine synthesis could participate in tolerance to acetylcholinesterase inhibition, we examined the possible effects of CPF on high affinity choline uptake (HAChU), the rate-limiting step. Adult Sprague-Dawley female rats were exposed to CPF (280 mg/kg, sc) and sacrificed at 1, 2 or 7 days after treatment for measurement of HAChU, ChE activity, and quinuclidinyl benzilate (QNB) and cis-methyldioxolane (CD) binding in the cortex and/or striatum. The effect of the active metabolite of CPF (i.e. CPF-oxon) on forebrain HAChU *in vitro* was also examined. ChE activity was inhibited >90% and QNB binding was decreased (10–34%) while CD binding was increased (15–23%) 1, 2 and 7 days after treatment. HAChU was depressed (16–20%) in cortex and striatum 1 day after CPF, remained lower in striatum at 2 days, but was not different in either brain region by 7 days after treatment. Relative to the known inhibitor hemicholinium-3. CPF-oxon did not appear to be a potent inhibitor of HAChU *in vitro*. These data suggest that a compensatory reduction in HAChU, along with down-regulation of postsynaptic muscarinic receptors and up-regulation of putative presynaptic autoreceptors, may contribute to the development of tolerance to ChE inhibition following CPF exposure.

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1101 THE JOINT ACTION OF CHLORPYRIFOS AND SAFROTIN ON DEVELOPMENT OF DELAYED NEUROTOXICITY IN THE HEN

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Chlorpyrifos[*O,O*-diethyl-*O*-(3,5,6-trichloro-2-pyridinyl) phosphorothioate] is an organophosphorus (OP) ester which has been reported to produce delayed neurotoxicity in humans and chickens following high level, acute exposures. Organophosphorus compound-induced delayed neurotoxicity (OPIDN) is characterized by a delay period of 6–14 days following exposure before onset of ataxia and paralysis in severe cases. In this study, adult hens were given a single 100 mg/kg oral dose of chlorpyrifos or safrotin, propetamphos[(E)-1-methylethyl 3-[[(ethylamino)methoxyphosphinothioyl] oxy]-2-butenoate alone, or in combination. The oral LD_{50} for chlorpyrifos and safrotin were determined to be 35 and 50 mg/kg, respectively. Hens were sacrificed 24 and 96h following treatment and brain neurotoxicity target esterase (NTE) and plasma and brain cholinesterase activities were determined. Others from each treatment were allowed to survive 16 days, evaluated for clinical signs of delayed neurotoxicity and sacrificed for histopathological examination. Results showed that exposure to high levels of safrotin (≥2x the LD_{50}) did not significantly inhibit NTE activity compared to controls or produce signs

of delayed neurotoxicity. Chlorpyrifos treatment resulted in <50% inhibition of NTE and signs of mild ataxia and disturbance of gait were present at 16 days. Combined treatment with 100 mg/kg oral dose of each compound produced 73% inhibition of NTE activity at 96h. This treatment also resulted in signs of severe ataxia and hindlimb paralysis. Spinal cord tissue taken from these hens showed degeneration and enlarged axons in the lateral and ventral tracts that are typical of OPIDN. These results showed that coexposure to chlorpyrifos and safrotin resulted in greater than additive inhibition of brain NTE and development of delayed neurotoxicity at dose levels which do not produce these effects when each compound was given alone.

1102 AGE-RELATED SENSITIVITY TO CHOLINESTERASE-INHIBITING PESTICIDES: IS BRAIN ACETYLCHOLINESTERASE (ACHE) FROM YOUNG ANIMALS INTRINSICALLY MORE SENSITIVE TO INHIBITION?

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The literature indicates that young animals exhibit an increased susceptibility to the lethal effects of cholinesterase (ChE)-inhibiting pesticides. Our laboratory is engaged in defining factors which may explain that age-related sensitivity. The following are results from experiments designed to compare the kinetic parameters and intrinsic (i.e., *in vitro*) sensitivity of young (postnatal day 4; PND4) and adult (90 days) rat brain AChE activity (n = 4 per age). Both the V_{max} and K_m of AChE of PND4 brain was less than that of the adult, indicating that although there is about 30% less AChE activity in PND4 brain, the AChE has a higher affinity for the substrate, acetylthiocholine (ACtCh). Optimal ACtCh concentration for both ages was 1 mM and both showed substantial substrate inhibition at 2.5 mM. Total cholinester-ase activity in both PND4 and adult brain was composed of about 90% acetyl- and 10% butyrylcholinesterase activity. I_{50}s (30 min at 26°C), defined concomitantly for both PND4 and adult brain AChE using either chlorpyrifos oxon (CPFo), carbaryl (C), maloxon (M), or aldicarb (A), were virtually identical at both ages with the calculated I_{50}s being: CPFo = 2.8 nM, C = 500 nM, M = 120 nM, A = 1.2 μM. In summary, the AChE of immature (PND4) and adult brain are quite similar, especially regarding their *in vitro* sensitivity to inhibitors, but the *in vitro* sensitivity to inhibitors must be determined on a case-by-case basis.

1103 *IN VIVO* AND *IN VITRO* EFFECTS OF ORGANO-PHOSPHORUS (OP) INSECTICIDES ON K^+-EVOKED ACETYLCHOLINE (ACh) RELEASE FROM RAT STRIATAL SLICES

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Previous studies from our laboratory have demonstrated marked differences in toxicity following acute exposure to the OP anticholinesterases parathion (PS) and chlorpyrifos (CPF) with doses causing similar levels of acetylcholinesterase (AChE) inhibition. We hypothesized that changes in autoreceptor-mediated ACh release might differentially modify the expression of toxicity associated with exposure to different anticholinesterases. The present study examined ACh release as affected by *in vivo* or *in vitro* exposures to PS or CPF or their active metabolites, paraoxon (PO) or chlorpyrifos-oxon (CPO). ACh release was studied in a superfusion apparatus by stimulating [^{3}H]choline pre-loaded slices with 20 mM K^+ either in the presence or absence of physostigmine (PHY, 20 uM) and presence of hemicholinium-3 (10 uM). Physostigmine, as reported by others, caused a net decrease in ACh release (12–35% at 0.1–10 uM final concentration) in an atropine sensitive manner, presumably *via* indirect activation of autoreceptors. CPF (270 mg/kg, sc) and PS (18 mg/kg, sc) treatment caused similar degrees of maximal AChE inhibition but OP-dependent and time-dependent (2–14 day) changes in the PHY-mediated reduction in ACh release, suggesting differential alteration in autoreceptor function. In the presence of PHY, CPO and PO had little effect on ACh release *in vitro*. In the absence of PHY, however, ACh release was reduced by 6%, 19%, and 24% by CPO and 4%, 23%, and 31% by PO at 0.1, 1.0, and 10 uM final concentrations. *In vitro* concentrations of oxons required to modify ACh release were high relative to their AChE inhibitory potencies (IC_{50}: CPO = 6.7 nM; PO = 8.2 nM), suggesting that a direct interaction between OP and autoreceptors occurs only at high oxon concentrations. Selective compensatory changes in autoreceptor function may participate in the differential development of tolerance to acetylcholinesterase inhibition seen with different OP inhibitors.

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1104 KINETIC ANALYSIS OF THE INHIBITION OF CATFISH AND RAT BRAIN ACETYLCHOLINESTERASE BY PARAOXON OR CHLORPYRIFOS-OXON AND THE SUBSEQUENT REACTIVATION OR AGING

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In channel catfish, chlorpyrifos is much more toxic than parathion, while in rats the toxicity is reversed. The *in vitro* inhibition of brain acetylcholinesterase (AChE) from both species by paraoxon (PO) and chlorpyrifos-oxon (CO) in the interspecies toxicity difference was investigated, along with the rates at which the inhibitor-enzyme complex of each species undergoes reactivation or aging. The bimolecular rate constants (K_i) were greater in rats than in catfish resulting from much greater association constants (K_A). In both species, CO yielded higher K_i's that PO. The K_A of CO was much larger than that of PO in both species suggesting that association of the inhibitor with AChE is the principal factor in the different potencies between these two inhibitors. Only minor differences in the phosphorylation constants (k_p) were present in both species. However, in catfish, the K_i of CO was 22 fold greater than that of PO. In rats, CO was only 9 fold greater. This suggests that target site sensitivity is an important factor in the higher toxicity of chlorpyrifos to catfish but not in the higher toxicity of parathion to rats. Catfish AChE did not undergo spontaneous reactivation and there were no differences in the first order aging constants (k_a) between compounds. No differences in the first order reactivation constants (k_r) between compounds for inhibited rat AChE were found. However, the k_a for rat AChE inhibited by CO was significantly greater than for AChE inhibited by PO. This suggests that the steric positioning of the diethyl phosphate in the esteratic site is different between the two compounds, hence leading to differences in aging.

1105 TIMED INHIBITION PATTERNS OF CHLOR-PYRIFOS-OXON, PARAOXON, AND METHYL PARAOXON TO BRAIN AND MUSCLE ACETYLCHOLINESTERASE IN MOSQUITOFISH

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Previous studies have suggested that both central and peripheral acetylcholinesterases (AChE) play an important role in mediating acute toxicity of organophosphorus (OP) insecticides in fish. Three common OP insecticides are chlorpyrifos, parathion, and methyl parathion. Various concentrations of their active metabolites (the oxons) were used to identify AChE inhibition trends over 5, 10, 15, 30 and 60 min in both brain and muscle AChE assays. The muscle AChE assays suggested the presence of two separate isozymes; one was inhibited very quickly at low concentrations, whereas the other required longer periods of time and higher concentrations. The inhibition patterns of the two putative isozymes were separated by a plateau (i.e., little increase in inhibition over a one order of magnitude range of oxon concentration) at about 50% inhibition of the total activity. The brain AChE assays suggested the presence of one isozyme which was inhibited without a concentration/time plateau, and its reactivity was similar to that of the less sensitive of the muscle activities (compared within a chemical). Both tissues were most sensitive to chlorpyrifos-oxon and least sensitive to methyl paraoxon. (Supported by NIH K04ES00190 and S07RR07215).

1106 PARAOXONASE PROTECTS AGAINST POISONING BY CHLORPYRIFOS (CPS)

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Purified rabbit paraoxonase injected into mice protected the animals against the acute toxicity of the phosphorothioate, chlorpyrifos (CPS). Mice which received paraoxonase 30 min before CPS (dermal exposure, 100 mg/kg) were more resistant to cholinesterase inhibition than control animals. When paraoxonase was given 30 min after CPS, significant protection was found in all tissues, while the protective effect was reduced if paraoxonase was injected 3 hr postexposure. The fact that exogenous paraoxonase offered protection against CPS poisoning when administered both before and after the compound suggests that it may be used as an additional treatment of organophosphate poisoning. Mouse paraoxonase cDNA was amplified by RT-PCR using human cDNA sequence as primers. According to the preliminary data, mouse paraoxonase cDNA contains an open reading frame which would encode 355 amino acids, demonstrating an 80% homology to human or rabbit protein sequence. The 5'-UTR of mouse paraoxonase cDNA is being characterized, and chromosomal mapping of this gene is also in progress. The profile of postnatal development of serum paraoxonase activity was determined

in both rats and mice. Serum paraoxonase activity in rats increases with age after birth and reaches the constitutive levels at Day 25. Mice show a somewhat different age-related profile of the enzyme activity than rats. These results together with earlier data on human newborns and others' work with neonatal rats suggest that newborns should be very sensitive to organophosphate toxicity. (Supported by NIH Grant ES-05194).

1107 NEURAL β-GALACTOSIDASE (GAL), β-GLUCURONIDASE (GLU) AND ORNITHINE DECARBOXILASE (ODC) IN PROMOTED AND UNPROMOTED ORGANOPHOSPHATE INDUCED DELAYED POLYNEUROPATHY (OPIDP)

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Certain esterase inhibitors, such as phenylmethanesulfonyl fluoride (PMSF), promote the clinical neuropathy caused by certain chemicals (including OPIDP) and by nerve crush. Promotion is not related with the development of the initial lesion. The hypothesis was made that promotion might interfere with repair. Six-month old male Wistar rats were given DBDCVP (5 mg/kg s.c.) or DBDCVP (2 mg/kg s.c.) followed, 24 h later, by PMSF (120 mg/kg s.c. × 2, 24 h apart) to obtain comparable median clinical scores: 2 (range = 2–4, n = 5) and 3 (range = 1–4, n = 6), respectively (assessed on a 0–3 point scale for both walking and the rod test). Activities of GAL and GLU in tibial nerves (indicies of nerve degeneration), and ODC in dorsal root ganglia (index of nerve regeneration) were measured up to 29 days after treatment. ODC increased on days 3 and 7 to the same extent in both groups. GAL and GLU increased DBDCVP/PMSF rats on day 29, only (197 ± 49 (n = 6) and 268 ± 98% (n = 6) of controls, respectively). Control values were: 940 ± 210 and 290 ± 120 ng of methylumbelliferone liberated/h/mg of tissue, respectively, mean ± SD, n = 4. The increase correlated with clinical scores of individual animals. This supports the hypothesis that promotion has a different mechanism than that of development of OPIDP.

1108 $GABA_A$ RECEPTOR AS A TARGET FOR PARAOXON-INDUCED TOXICITY IN MAMMALIAN NEURONS

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A number of studies indicate that the neurotoxic effects induced by paraoxon are not solely due to the irreversible inhibition of acetylcholinesterase. In the present work, we used cultured rat hippocampal neurons to investigate the effect of paraoxon (3 nM–1 mM) on spontaneous postsynaptic currents. Spontaneous miniature postsynaptic currents were recorded from pyramidal-shaped neurons under the whole-cell clamp configuration. The extracellular saline solution contained: TTX, 0.3 μM (to block TTX-sensitive Na^+ channels); methyllycaconitine, 2 nM and dihydro-β-erythroidine, 200 nM (to block nicotinic receptors); atropine, 1 μM (to block muscarinic receptors); and Mg^{2+}, 3 mM (to block NMDA receptors). Paraoxon was applied at several concentrations (each for 5 min) through six parallel barrels placed 100 μm away from the soma. The frequency, peak amplitude, and decay-time constant of the miniature postsynaptic currents were determined. Paraoxon at concentrations up to 1 mM did not show any effect on glutamate-induced (CNQX-sensitive) miniature postsynaptic currents. In contrast, a few seconds of exposure of the neurons to paraoxon (3 μM–1 mM) was enough to cause a marked reduction of both the peak amplitude and decay-time constant of GABA-induced (picrotoxin-sensitive) miniature postsynaptic currents. The effects of 1–3-min exposure of the neurons to paraoxon (3 μM–1 mM) could be promptly reversed by washing. In comparison, washing only partially reversed the effects of prolonged exposure to paraoxon. These data suggest that the blockade of $GABA_A$ receptors may contribute to the toxicity of paraoxon in the mammalian central nervous system. Support: NINDS Grant NS 25296; NIEHS Grant ES05730 and T32-ES07263.

1109 EFFECTS OF CALCIUM ON PURIFIED HUMAN AND RABBIT SERUM PARAOXONASES

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Paraoxonase (PON) activity is several times higher in rabbit serum than in human serum, or serum of other mammalian species. We studied the basis of this difference. Comparison of purified rabbit and human PON has shown that the two are very similar as far as substrate specificity patterns, disulfide bonds, sugar chains, stimulation by phospholipids, and catalytic turnover numbers. Rabbit PON is more stable than human PON to heat inactivation, to electrophoresis on SDS-PAGE gels, and to treatment with EDTA or Chelex 100. The two esterases have considerable structural homology—85% identical amino acid residues, but significant differences in that rabbit PON binds Ca^{2+} more tightly than human PON. Ca^{2+} is required for both catalytic activity and for stability. Certain other metals, like Zn^{2+} and Cd^{2+}, can substitute for Ca^{2+} to maintain these enzymes in their active form, but only calcium confers catalytic activity. Recent atomic absorption studies have focused on the calcium binding properties. Equilibrium dialysis and Scatchard plot analyses have shown that human and rabbit PONs both possess two Ca-binding sites. Both independent-site and stepwise-equilibrium constant analyses show human PON to have a dissociation constant (Kd_1) for the high affinity site of $6.7 \pm 0.5 \times 10^{-7}$ M, and a Kd_2 for the low affinity site of $1.22 \pm 0.4 \times 10^{-4}$ M. For rabbit PON, the Kd_1 is $4.5 \pm 0.7 \times 10^{-8}$ M, and Kd_2 is $1.9 \pm 1.0 \times 10^{-5}$ M. Hill coefficients are 0.7 for human PON, and 0.4 for the rabbit PON. The higher Ca-binding affinities for rabbit PON are very significant. The high affinity site is essential for stability of the enzyme; removal of Ca^{2+} leads to irreversible inactivation. The low affinity site, affecting catalytic activity, is reversible. We conclude that the active centers of the two paraoxonases are very similar, but rabbit PON, binds calcium more tightly, is more stable, and is maintained at a higher steady-state concentration in the serum than human PON. (Supported by GM 46979).

1110 THE ROLE OF CALCIUM IN HYDROLYSIS OF PARAOXON BY HUMAN SERUM A-ESTERASE

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Human serum A-esterase is a calcium-dependent enzyme that hydrolyzes the organophosphate paraoxon by an Ordered Uni Bi kinetic mechanism. Incubation of various concentrations of calcium chloride with human serum A-esterase resulted in corresponding changes in $appk_3$ and *appE* for the reaction, while $appk_2$ was unaffected. Carboxyglutamic acid (CAG) prevented calcium chloride from altering $appk_3$, but not *appE*. Similarly CAG reduced the calcium-stimulated nonenzymatic hydrolysis of paraoxon, as well as the calcium-stimulated de-phosphorylation of chymotrypsin phosphorylated by paraoxon. These results suggest that calcium plays two roles in the hydrolysis of paraoxon by A-esterase. Firstly, calcium is required in order to maintain an active site. In this capacity calcium might participate directly in the catalytic reaction, or it might be required in order to maintain the appropriate confirmation of the active site. And secondly, free calcium (or calcium weakly associated with A-esterase) facilitates the removal of diethyl phosphate from A-esterase, probably by polarizing the P = O bond of the diethyl phosphate-A-esterase intermediate, thereby rendering phosphorus more susceptible to nucleophilic attack by hydroxide ions. (Supported by Grant ES04335 from NIEHS).

1111 REACTIVATION OF PARAOXON-INHIBITED ACETYLCHOLINESTERASE BY THE COMBINATION OF 2-PAM AND ORGANOPHOSPHATE HYDROLASE

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In the therapeutic treatment of organophosphorus intoxication, Acetylcholinesterase (AChE) has been shown to be reactivated by Pyridine-2-Aldoxime methyl compound (2-PAM). However the reactivation of the inhibited AChE by 2-PAM is incomplete and 2-PAM induces its own inhibition of AChE. Thus this treatment does not provide ideal therapeutic regimen. In this study, the ability to reactivate paraoxon-inhibited AChE by Organophosphate Hydrolase (OPH) in combination with 2-PAM was evaluated. It has been proved in the past studies that the enzyme is able to degrade a variety of organophosphate compounds. Initial experiments showed that OPH was able to reactivate paraoxon-inhibited AChE too, although the reactivation efficiency of OPH was rather low. When OPH and 2-PAM were used in combination to reactivate the inhibited AChE, the efficiency was much higher than that of the same concentration of 2-PAM alone and restored >90% of the AChE activity. No 2-PAM-induced inhibition was observed after the reactivation. A kinetic analysis of reactivation reaction (Kcat/Km) showed that the combination of 2-PAM/OPH was more efficient than the reactivation reaction with 2-PAM alone.

1112 INFLUENCE OF STRESS ON THE TOXICITY AND METABOLISM OF ORGANOPHOSPHORUS INSECTICIDES

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The role of stress on the oxidative metabolism of parathion (PS) and methyl parathion (MPS) was studied in male CD-1 mice. Stress was induced by five daily i.p. injections of hypertonic (1.5 M) saline whereas control animals received isotonic (0.15 M) saline (n = 5). Two hours after the last saline injection on day 5, i.p. injection of four doses of each insecticide for each pretreated group were used, and at least six mice were employed for each combination of pretreatment and insecticide dose. Animals were observed continuously for two hours and at frequent intervals thereafter to determine the time of onset of clinical signs and death. After 24 hrs, the LD50's were calculated. Intraperitoneal injection of 1.5 M saline produced a 2-fold potentiation of PS toxicity whereas at least a 40-fold protection against the toxicity of MPS was seen. The effect of 1.5 M saline on the oxidative metabolism of PS and MPS by mouse liver homogenate was then characterized. Two, 24, and 48 hrs after the last saline injection on day 5, groups of mice were sacrificed, and serum corticosterone (CORT) and liver oxidative cleavage to p-nitrophenol and oxidative desulfuration to oxon formation of PS and MPS were determined. Injection of 1.5 M saline increased serum CORT concentrations at 2 and 24 but not at 48 hours. Oxidative cleavage of both PS and MPS by liver homogenates was inhibited at 2 and 24 hours after 1.5 M saline treatment. However, this enzymatic cleavage was increased at 48 hours in both compounds. Desulfuration of MPS was decreased at 2 and 24 but not at 48 hours. Oxidative desulfuration of PS was inhibited only after 2 hours. We conclude that hormones released during stress interfere with the hepatic oxidative metabolism of organophosphorus insecticides, which in turn significantly alters the acute toxicity of these compounds.

1113 INDIRECT INHIBITION OF THE OXIDATIVE DESULFURATION OF THE ORGANOPHOSPHORUS INSECTICIDE PARATHION BY ETHANOL

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Treatment of male Swiss Webster mice with 15% ethanol in the drinking water for 6 days antagonized the acute toxicity of a challenge dose of parathion (PS) (*O,O*-diethyl *O-p*-nitrophenyl phosphorothioate) (20 mg/kg, ip), but not a challenge dose of paraoxon (PO) (*O,O*-diethyl *O-p*-nitrophenyl phosphate) (3.25 mg/kg, ip). Administration of PS 24 hours after ethanol cessation (when no ethanol could be detected in the liver) resulted in no antagonism, indicating that the presence of ethanol is necessary for the protective effect. Incubation of PS with either mouse hepatic microsomes or transfected human P450s (expressed in human β-lymphoblastoid cells) indicated that the antagonism of PS toxicity by ethanol *in vivo* is not a result of direct inhibition of P450 activity. Calculation of cytosolic redox ratios to detect possible alterations in cytosolic NADPH level, resulted in no differences between control and 6 day ethanol treated animals. These data suggest that the protection offered by ethanol exposure against PS toxicity results through an indirect mechanism not involving decreased cofactor supply. (Supported by NIEHS Grant ES04335.)

1114 PARTIAL PURIFICATION OF RAT HEPATIC A-ESTERASE

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A-esterases detoxify the organophosphate active metabolites of phosphorothionate pesticides via hydrolysis. Two hepatic enzymes with A-esterase activity were identified in native PAGE gels by activity staining with β-naphthyl acetate and Fast Garnet GBC following preparative scale isoelectric focusing. Partial purification of these two enzymes is reported here. Whole rat liver was homogenized in 0.05 M Tris buffer containing 1 mM $CaCl_2$. The sample was centrifuged at 1000 g (4°C, 15 min) followed by solubilization of supernatant with Triton X-100 and a second centrifugation at 100,000 g (4°C, 1 hr). An ammonium sulfate precipitation of this supernatant was then followed by preparative scale isoelectric focusing. Native PAGE gels verified that two proteins existed in the final preparation which hydrolyzed β-naphthyl acetate in the presence of 10^{-5} M paraoxon. Spectrophotometric assay of this preparation detected A-esterase activity toward high concentrations of paraoxon, chlorpyrifos-oxon, and toward low, physiologically relevant concentrations of chlorpyrifos-oxon. Apparent pI range for the two enzymes was 5.5–6.5. Based upon protein standards, the molecular weights of the hepatic A-esterases were greater than 100 kDa as determined by activity stained PAGE gels. (Supported by NIH ESO4394.)

1115 THE EFFECTS OF CYTOCHROMES P450 (P450) INHIBITORS ON THE TOXICITY OF THE ORGANOPHOSPHORUS INSECTICIDE PARATHION

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The organophosphorus insecticide parathion (*O, O*-diethyl-*O-p*-nitrophenyl phosphorothioate, PS) undergoes P450-dependent activation to form the cholinesterase inhibitor paraoxon (*O, O*-diethyl-*O-p*-nitrophenyl phosphate, PO). Consequently, inhibition of P450s might be expected to antagonize the toxicity of parathion. However, in the present study, pretreatment of mice with cimetidine (300 mg/kg, ip) metyrapone (100 mg/kg, ip) and methoxsalen (100 mg/kg, ip) significantly potentiated the lethality of parathion in mice. While the same pretreatment with metyrapone and methoxsalen potentiated the acute toxicity of paraoxon, pretreatment with cimetidine did not. These data suggest that metyrapone and methoxsalen exert their effect on the disposition of paraoxon, while cimetidine might alter the oxidative desulfuration of parathion. (Supported by Grant ES04335 from NIEHS).

1116 CLINICAL EXPRESSION OF 3,3′-IMINODIPROPRIONITRILE (IDPN) NEUROTOXICITY IS NOT EXACERBATED BY PHENYLMETHANESULFONYL FLUORIDE (PMSF) IN RATS

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Several distal toxic axonopathies are exacerbated by treating animals with certain esterase inhibitors such as PMSF. No reports are available on the exacerbation of proximal axonopathies such as IDPN neuropathy. Adult male Wistar rats (n = 4 per group) were given IDPN in drinking water (0.05% for 32 days or 0.2% for 14 days) followed by either PMSF (120 mg/kg sc × 2, 24 h apart) or vehicle. Treated rats consumed less water than controls and calculated IDPN intakes were 60 and 152 mg/kg bw/day in the 0.05% and 0.2% groups, respectively. The loss (0.2% IDPN) or impairment (0.05% IDPN) of the righting reflex were not exacerbated (up to 21 days from the end of dosing) by PMSF. Median (range) scores in the rod test (0 to 3 point scale) were 2(2–3), 3(2–3); 3(2–3), and 2(2–3) in the 0.05% IDPN, 0.05% IDPN/PMSF, 0.2% IDPN, and 0.2% IDPN/PMSF group, respectively. Foot-splay was not significantly affected in all groups. In conclusion, by using these clinical end-points, exacerbation of IDPN neuropathy by PMSF was not observed.

1117 CENTRAL NERVOUS SYSTEM TOXICITY OF IDPN IN THE RAT

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3′,3′-Iminodipropionitrile (IDPN) is a neurotoxic agent producing irreparable damage within the central nervous systems (CNS). As a part of study, using known neurotoxicants, eight male Sprague-Dawley rats (CD® (SD)BR/VAF Plus™) were given a single intraperitoneal injection of 2000 mg/kg of IDPN. CNS symptoms included increased incidences of ataxia, splayed gait, hypotonic gait, and soft and flabby muscle tone. In addition, the righting reflex and visual placing reflex were impaired. Characteristic retropulsion, head flick, and circling responses (waltzing) previously reported following IDPN exposure were also observed. The IDPN-induced microscopic changes included balloon-shaped axonal swellings in the ventral horns of the cervical and lumbar spinal cords and in the midbrain. Additional microscopic changes included loss of ependymal cells lining the central canal of the spinal cord, foci of necrosis and foci of gliosis in the midbrain, thrombosis of the posterior cerebral arteries between the midbrain and the cerebral peduncles and a basilar artery at the base of the medulla oblongata, and hemorrhages in the spinal cord and brain. Although these additional microscopic changes have not been previously reported, they were not observed in any of the control rats, and therefore, they were considered treatment-related effects.

1118 NEUROTOXICITY OF METHYLATED 3,3′-IMINODIPROPIONITRILE (IDPN) ANALOGUES

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Animals intoxicated with IDPN develop an irreversible behavioral abnormality characterized by excitation, chorea, and circling: the ECC syndrome. Additionally, neurofilament-filled proximal axonal swellings are observed throughout the nervous system with concurrent axonal atrophy. IDPN intoxication en-

tails cytochrome P450 oxidation, a free-radical reaction, to an as yet undetermined metabolite. To test the role of free-radical oxidation in IDPN-induced neuro-toxicity, two methylated IDPN analogues were synthesized: 2-methyl IDPN (2MeI), and 3-methyl IDPN (3MeI). Because tertiary radicals are more stable than secondary radicals, it was expected that free-radical formation would be promoted at the tertiary carbons in the methyl IDPN analogues. Thus, 2MeI would receive preferential oxidation at the C-2 carbon, and 3MeI would be likewise oxidized at C-3. In a double-blind study, 27 Sprague-Dawley rats received 3 daily ip injections of PBS, IDPN, 2MeI, or 3MeI at doses from 1.5 to 3.0 mmol/kg/day (n = 3). The animals were scored between days 4 and 14 for excitation, chorea, locomotion, and tail hanging. L3–L5 dorsal root ganglia, collected on day 14, were analyzed for evidence of axonal swellings. Full expression of the ECC syndrome was observed with IDPN-treated rats at 3.0 and 2.25 mmol/kg/day, with 2MeI only at 3.0 mmol/kg/day, whereas the complete ECC syndrome was not observed with 3MeI. This study corroborates other works showing that oxidative metabolism at the C-3 carbons of IDPN is a detoxifying reaction, but the role of C-2 oxidation in the activation of IDPN remains unclear. Supported by ES 07031 and ES 02611.

1119 INITIATION AND POTENTIATION OF ORGANOPHOSPHORUS INDUCED DELAYED NEUROTOXICITY (OPIDN) FOLLOWING REPEATED PHENYL SALIGEN PHOSPHATE (PSP) EXPOSURES

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Inhibition of neurotoxic esterase (NTE) has been the suspected mechanism for initiation of OPIDN for many years even though the specific events involved in progression of the neuropathy are still unclear. Even less understood is the mechanism through which non-neuropathic NTE inhibitors potentiate the effects of neuropathic NTE inhibitors when administered after the neuropathic agent. In the present study, we examined the ability of phenylmethanesulfonyl fluoride (PMSF, 90 mg/kg sc), a non-neuropathic NTE inhibitor, to alter development of OPIDN in 2, 5, and 8 week old White Leghorn chickens treated either 1, 2, or 3 times (doses separated by 24 hrs) with the neuropathic NTE inhibitor PSP (5 mg/kg sc). NTE activity was measured in cervical cords collected 4 hrs after the last PSP treatment. Development of neuropathy, as indicated by ataxia, was measured over a 16 day post-exposure period. All PSP-treated groups exhibited >96% NTE inhibition regardless of age or number of treatments. Two week old birds did not develop clinical signs of neurotoxicity in response to any of the treatment regimens. Five week old birds exhibited a dose-related increase in ataxia, with PMSF potentiating the effects of all three doses of PSP. The findings in 8 week old birds paralleled those in the 5 week old birds differing only by increased severity of ataxia. We have demonstrated the ability of PMSF to potentiate PSP-induced delayed neuropathy in the presence of 0–4% residual NTE activity. These findings indicate that subsequent doses of neuropathic and non-neuropathic NTE inhibitors, following virtually complete inhibition by a neuropathic agent, can modify the severity of clinical signs and they further support the idea that PMSF potentiates OPIDN through a mechanism unrelated to NTE.

1120 PHOSPHORYLATED NEUROFILAMENTS IN AXONS IN EARLY STAGES OF DEGENERATION IN THE SPINAL CORD OF HENS TREATED WITH DIISOPROPRYL PHOSPHOFLUORIDATE (DFP)

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Anomalous phosphorylation of cytoskeletal proteins is associated with pathological alterations in a variety of neurological diseases, including those associated with exposure to toxicants. Compounds such as tri-*o*-cresyl phosphate (TOCP) and DFP can produce organophosphorus ester-induced delay neurotoxicity (OPIDN), characterized by ataxia progressing to paralysis concurrent with a central-peripheral distal axonopathy. One biochemical feature of OPIDN is an increase in calcium-calmodulin dependent kinase-mediated phosphorylation of cytoskeletal proteins. We previously demonstrated that in TOCP treated hens anomalous aggregations of phosphorylated neurofilaments occur in axons of the spinal cord in early stages of OPIDN. To further characterize this phenomenon we have investigated whether similar aggregations of phosphorylated neurofilaments can be induced by DFP. Hens were killed 7 days after administration of 1.7 mg/kg DFP. Consistent with our previous observations with TOCP, anomalous aggregations of phosphorylated neurofilaments could be detected in axons in early stages of degeneration. This observation further supports the hypothesis that increases in kinase-mediated phosphorylation of cytoskeletal proteins may be involved in axonal degeneration in OPIDN. (Supported in part by NIEHS grant ESO5154).

1121 MORPHOLOGICAL AND IMMUNOCYTOCHEMICAL STUDY OF PHOSPHORYLATED NEUROFILAMENTS DURING THE EVOLUTION OF ORGANOPHOSPHORUS ESTER-INDUCED DELAYED NEUROPATHY (OPIDN)

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OPIDN is manifest by delayed degeneration of distal levels of long myelinated fibers following an appropriate neurotoxic exposure. We investigated the dynamics of cytoskeletal changes during nerve fiber degeneration in this condition, focusing on phosphorylated neurofilaments. OPIDN was produced in 5-month-old White Leghorn hens using a single 2.5 mg/kg im dose of phenyl saligenin phosphate, and manifest by progressive ataxia and weakness beginning by post-dosing day 8. Dosed hens were sacrificed (with controls) on days 4, 7, and 21. Distal (susceptible) levels of selected long myelinated spinal cord tracts and of peripheral nerves were prepared for morphological and immunocytochemical light microscopic and ultrastructural study. For immunocytochemistry, tissues were embedded in LR White resin, and a monoclonal primary antibody to phosphorylated neurofilaments (SMI-31, Sternberger Monoclonals Inc.) was used. At post-dosing day 21, various stages of OPIDN lesions were prominent including axonal swelling and myelinated nerve fiber degeneration. The latter was associated with cytoskeletal lysis, including loss of intermediate filaments immunolabelled for phosphorylated neurofilament proteins, a process consistent with proteolysis. Early transient abnormal phosphorylated neurofilament intra-axonal aggregates (Jensen et al., J. Neurosci. Res. 33: 455) were not prominent in our material. This study provides light and immunocytochemical support for a role of phosphorylated neurofilament lysis in the nerve fiber degeneration of OPIDN.

1122 IDENTIFICATION OF A 155 KDA FRACTION THAT POSSESSES NEUROPATHY TARGET ESTERASE ACTIVITY

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Microsomes isolated from 18-day old chicken embryos were treated with phospholipase A_2 to solublize the neuropathy target esterase (NTE) activity. The extract was then combined with polyoxyethylene W1 detergent and resolved by gel filtration chromatography to yield an active fraction with an approximate mass of 200 kd. This fraction was further purified by preparative isoelectric focusing and native electrophoresis to yield two separate bands possessing NTE activity. The slower migrating band was highly enriched in a 155 kd protein that was identified to be the source of the NTE activity by affinity chromatography using 3-(9′-mercaptononylthio)-1, 1, 1-trifluoropropan-2-one (MNTFP) bound to Sepharose. This represents the first report of the isolation of NTE in its active form and strengthens the identification of the 155 kd protein as the most likely candidate for NTE.

1123 AXONAL ATROPHY IS A PRIMARY NEUROTOXIC RESPONSE TO 2,5-HEXANEDIONE (HD)

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To assess the relevance of previously identified axonal atrophy to hexacarbon neuropathy (LoPachin et al., *J. Neurochem.* 63: in press, 1994), the present study quantitated fiber size in rats intoxicated with HD by two routes of exposure: oral (0.4% in drinking water) or ip (0.4 g/kg/d). Morphometric techniques were used and data were evaluated by percentile analyses. Lower, median and upper quartiles for myelinated axon areas (μm^2, mean ± SEM) were depressed significantly ($p < 0.05$) in tibial nerve of oral-treated rats prior to onset of behavioral deficits (77d of exposure) and remained depressed as rats progressed to moderate (86d) and severe (103d) toxicity (e.g., for median quartiles: control = 17.8 ± 0.2, HD at 86d = 9.4 ± 0.2). These variables were also reduced in proximal sciatic axons, but to a lesser extent. Concomitant reductions in axon circularity and g ratios support the interpretation of atrophy. Giant axonal swellings were present in both proximal sciatic and tibial nerve axons from oral-exposed rats at all time points. Intraperitoneal intoxicated rats were sampled at behavioral endpoints similar to those of oral HD-treated rats. In sciatic and tibial nerves from ip-treated rats, reductions in axon area were similar to oral exposure. However, ip treatment produced few giant axonal swellings. Results indicate that axonal atrophy is a consistent, route-independent neurotoxic effect of HD. In contrast, swellings occur

mainly as a function of oral administration which suggests ancillary mechanistic relevance. The proximal location of axonal swellings and atrophy suggest that classification of HD peripheral neuropathy as a distal axonopathy is inappropriate. (Supported by NIH grant 5-RO1 ES03830).

1124 COMPARISON OF INTRAPERITONEAL (IP) AND ORAL ADMINISTRATION OF 2,5-HEXANEDIONE (2,5-HD) TO RATS: NEUROPATHOLOGIC OBSERVATIONS

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Morphometric and clinical studies of 2,5-HD neurotoxicity in rats have demonstrated progressive neurological deficits and a dosage route-independent axonal atrophy (Lehning et al., this volume). The present study provides complementary morphological data. Male Sprague-Dawley rats were given 2,5-HD ip (0.4 g/kg, 5 daily dosages/week) or orally (0.4% [w/v] in drinking water). Neurological evaluation of gait and treadmill performance was made. Rats from the ip and oral dosage groups (with appropriate controls) were sacrificed when equivalent clinical deficits were noted, and light microscopic study of 1 μm thick sections of epoxy resin-embedded sciatic and tibial nerves, and teased fibers of the latter was conducted. These morphological evaluations focused on myelinated nerve fiber degeneration. No neurological abnormalities were noted in the 17 day ip or 77 day oral rats, and lesions in these were negative to moderate (oral > ip). By day 32 of ip or 88 of oral administration, both clinical and neuropathologic alterations were more advanced. Severe clinical and morphological neuropathic changes were noted in the 103 day oral group. A striking feature of oral dosing was the prominence of axonal swelling. As noted previously (Spencer and Schaumburg, 1977), such swellings appeared to originate paranodally. In later stages, Wallerian-like degeneration of some fibers was seen. This study demonstrates a morphological substrate for the progressively impaired neurological function in these two models of 2,5-HD toxicity, and provides support for an oral route-dependency of axonal swelling in the neuropathy (supported by NIH 5-RO1ES03830).

1125 ROLE OF BIOLOGICAL THIOLS AND AMINES IN 2,5-HEXANEDIONE-INDUCED PROTEIN CROSSLINKING

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2,5-Hexanedione (2,5-HD), the reactive γ-diketone metabolite of *n*-hexane, covalently binds to neurofilament protein to form pyrrole adducts, followed by autoxidative protein crosslinking. Recently, we characterized a pyrrole-to-pyrrole crosslink in 2,5-HD-treated peptides and protein. The present study examined the role of nucleophilic thiols and amines in the pyrrole-mediated crosslinking reaction. When pyrrolylated ribonuclease was incubated with N-acetyllysine (NAL), N-acetylcysteine (NAC), or glutathione (GSH) under air, pyrrole-to-pyrrole crosslinking was inhibited by the thiol-containing compounds, but not by NAL. Stable NAC- and GSH-pyrrole conjugates, containing a bridge from the pyrrole ring at C-3 to sulfur, were characterized by thermospray LC/MS and ^{1}H-NMR spectroscopy. In contrast, SDS-PAGE studies indicated that protein-bound thiols did not undergo reaction with pyrrole adducts to form crosslinks. Further experiments using a model pyrrole revealed that GSH was also able to directly suppress pyrrole dimerization without conjugate formation, probably via inhibition of a free radical-dependent mechanism. The results suggest that: (1) 2,5-HD-induced protein crosslinking is mediated solely by pyrrole-to-pyrrole bridging, and (2) GSH and other small thiols may inhibit the pyrrole dimerization reaction by two distinct pathways. These findings have significant implications for the mechanism of γ-diketone neuropathy. (Supported by NIH ES-05172).

1126 REGIOSELECTIVE BINDING OF 2,5-HEXANEDIONE TO HIGH-MOLECULAR-WEIGHT RAT NEUROFILAMENT PROTEINS *IN VITRO*

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We have previously shown selective binding of the neuro-toxicant 2,5-hexanedione (2,5-HD) to carboxyl-terminal domains of rat neurofilament (NF) M and H proteins *in vitro*. The present study was designed to further localize this binding in native rat NF preparations exposed to [^{14}C]-2,5-HD. Purified M and H proteins from treated NFs were subjected to CNBr cleavage, and the resultant peptides separated by Tris/tricine SDS-PAGE and electroblotted to PVDF membranes. Peptides were identified by direct sequencing of stained bands and the relative radiolabeling of each peptide was determined by densitometric scanning of fluorographed blots. For NF-M, the highest label was found in CNBr 10, a peptide corresponding to residues 678–846 at the extreme carboxyl terminus. This region of the protein includes three highly conserved lysine-containing sequences believed to be critical to its function. For NF-H, the greatest binding was localized in CNBr 7 + 8, representing an incomplete CNBr cleavage product of residues 390–810. This peptide contains essentially all of the KSP phosphorylation sites in the carboxyl terminus of H, a domain believed to control NF interactions in the axon. Only minor radiolabeling was observed in other M or H peptides. Extensive dephosphorylation of NFs prior to 2,5-HD exposure had no effect on relative adduct levels in each protein. These results provide additional support for limited and specific binding of 2,5-HD with neurofilaments. (Supported by NIH ES-05172).

1127 NGF RECEPTOR ACTIVATION IS NOT REQUIRED FOR ACRYLAMIDE-REGULATED NEUROFILAMENT SYNTHESIS IN PC12 CELLS

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The industrial neurotoxicant acrylamide has recently been found to produce nerve growth factor (NGF)-like responses in a rat pheochromocytoma (PC12) cell line. One typical response upon NGF-stimulation is about 3–6 fold increase in the synthesis of neurofilament proteins, which occurs concurrently with the neurite outgrowth. We first tested if acrylamide can mimic this NGF action. When PC12 cells were treated with acrylamide for 4 days, the synthesis of neurofilament mid-sized subunit (NFM) was increased 2–3 fold, as measured by ^{35}S-methionine labeling followed by immunoprecipitation. This is about half of the increase stimulated by NGF alone. When cells were treated with both NGF and acrylamide, the effects seemed to be additive. The NGF-stimulated NFM synthesis was blocked by 100 nM K252a, a potent and selective inhibitor of the tyrosine kinase activity of the NGF receptor gp140trk, the product of the trk protooncogene. K252a has also been shown to selectively inhibit every NGF action tested so far, indicating that the receptor activation is the initial step of the NGF signaling pathway. Therefore, we tested if this step is also required for acrylamide-induced NFM synthesis. The presence of K252a did not inhibit the effects produced by either acrylamide itself or in combination with NGF. In some cases, the effects were even enhanced. These results clearly show that NGF receptor activation is non-essential for acrylamide-regulated neurofilament synthesis in PC12 cells. We suggest that acrylamide may act through certain downstream signaling steps that lead to the transcription of neurofilament proteins.

1128 INCREASED LEVELS OF GAP-43 mRNA IN THE DORSAL ROOT GANGLIA OF RATS FOLLOWING CLINICAL RECOVERY FROM ACRYLAMIDE-INDUCED NEUROPATHY

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Acrylamide is a potent neurotoxicant in humans and laboratory animals, producing a central-peripheral distal axonopathy. Studies in our laboratory have demonstrated increased levels of GAP-43 following acrylamide treatment. Expression of GAP-43, a growth associated protein, increases at the onset of regeneration from sciatic nerve crush, continues until reinnervation is complete, and declines as clinical recovery is completed. In this study male Sprague-Dawley rats were administered 0.05% acrylamide in drinking water. On day 15 treatment was stopped and animals were evaluated for clinical signs over a 36 day period. Signs progressed from severe hindlimb splay and ataxia on day 15 to mild hindlimb splay on day 28 and no clinical signs of neurotoxicity by day 50. Frozen sections were prepared from the dorsal root ganglia (DRG) of control and treated animals and probed by *in situ* hybridization for GAP-43 mRNA. A significant increase in the number of DRG neurons expressing GAP-43 was apparent in sections from acrylamide-treated rats (64%) as compared to controls (23%) ($p < 0.05$). Control levels of GAP-43 signal are consistent with published reports. GAP-43 expression is maintained above control levels for 5–7 weeks following sciatic nerve crush, comparable to the time of exposure and recovery in this study. These results suggest that acute exposure to acrylamide produces a temporal pattern of GAP-43 expression in the peripheral nerve that may be similar to that observed following mechanical lesion.

1129 ROLE OF Na-Ca EXCHANGER IN ACRYLAMIDE-INDUCED PERIPHERAL DISTAL AXONOPATHY

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We have hypothesized that acrylamide (ACR)-induced peripheral distal axon degeneration is mediated by excess entry of Ca, possibly via reverse operation of the Na-Ca exchanger (LoPachin and Lehning, *NeuroToxicology* 15: 247–260, 1994). The purpose of this investigation was to characterize exchanger function in peripheral axons. Since exchanger activity is reversed by increased axoplasmic Na, two *in vitro* injury models associated with elevated Na, ouabain and anoxia, were investigated. Tibial nerves were stripped of epineurium and incubated for up to 3 hr in a perfusion chamber with 2 mM ouabain or under anoxic conditions. X-ray microanalysis was used to measure % water and Na, K and Ca concentrations (mmol/kg dry weight ± SEM). After two hours of incubation, both ouabain and anoxia significantly ($p < 0.05$) raised Na (control = 203 ± 12, ouabain = 1553 ± 123, anoxia = 1569 ± 132) and depleted K (control = 1691 ± 73, ouabain = 237 ± 32, anoxia = 463 ± 56) contents of myelinated axons. These elemental changes remained constant during the next hour of incubation. However, Ca concentrations were elevated significantly only after 3 hours and only in myelinated axons from anoxic nerves (control = 8 ± 2, ouabain = 11 ± 3, anoxia = 30 ± 5). For both ouabain and anoxia, mitochondria exhibited elemental changes similar to their respective axons. Loss of axolemmal Ca and Na-K ATPase activities during anoxia could account for the ability of anoxia but not ouabain to increase axoplasmic and mitochondrial Ca. These data provide baseline characterization of Na-Ca exchanger activity in peripheral axons and support its possible involvement in ACR axonopathy. Future studies will use pharmacological probes to further define and confirm exchanger activity in axons. (Supported by NIH grant 5-RO1 ES03830).

1130 ACRYLAMIDE EFFECTS ON KINESIN-BASED MICROTUBULE MOTILITY

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Transient reductions in fast anterograde axonal transport by acrylamide (ACR) have been correlated with axonal degeneration. Toxicant-induced alteration of kinesin, an anterograde motor protein, as a molecular site of action was examined. The movement of taxol-stabilized microtubules (MT) over an adsorbed bed of kinesin was observed with AVEC-DIC microscopy. Dilution of kinesin or preincubation of kinesin with N-ethylmaleimide resulted in unsteady MT motility, MT lifting off the kinesin bed, and/or total absence of attached MT. Preincubation (37°C) of kinesin with ACR (0.1 mM) produced a 5–12 fold increase in MT liftoff while preincubation with MT produced a doubling in MT liftoff. This effect was not observed at 24°C but was temperature-dependent at 30–37°C. Non-neurotoxic propionamide did not produce a significant change up to 1.0 mM. Covalent modification of kinesin, supplemented by a MT effect, is suggested as contributing to alteration of anterograde axonal transport by ACR.

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1131 MORPHOLOGICAL AND FUNCTIONAL EVALUATION OF CARBON DISULFIDE (CS_2) INDUCED PERIPHERAL NEUROPATHY IN F344 RATS

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The study objectives were to examine the progression of CS_2 distal axonopathy in the spinal cord and muscular branch of the posterior tibial nerve, and to compare the morphology with neuromuscular alterations as determined by a functional observational battery. Male and female F344 rats were exposed to 0, 50, 500 or 800 ppm CS_2 by inhalation, 6 hours/day, 5 days/week, for 2, 4, 8 or 13 wks. At 8-wks, spinal cord white matter changes consisted of prominent multifocal axonal swelling in the fasciculus gracilis (C1, C2), and dorsal corticospinal tract and ventral funiculus (L1, L2), by 13-wks these changes were more diffuse. At 8-wks, tibial nerve changes involved single fascicles containing individual swollen axons, by 13-wks, multiple fascicles had swollen axons with thin myelin sheaths and occasional degenerated axons. Functional alterations associated with the morphology included decreases in hindlimb and forelimb grip strength, and postural and gait abnormalities; these changes were evident as early as 4 wks.

1132 2,4-DITHIOBIURET DEPRESSES EVOKED RELEASE OF [^{3}H]-ACETYLCHOLINE ([^{3}H]ACh) FROM RAT PHEOCHROMOCYTOMA (PC12) CELLS

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2,4-Dithiobiuret (DTB) is a thiourea derivative which causes a delayed onset skeletal muscle weakness in rats after chronic exposure. Electrophysiological and biochemical studies indicate that DTB-induced neuromuscular weakness is caused by disruption of synaptic transmission at motor nerve terminals. The site of action is predominantly presynaptic although the precise mechanism of action of DTB is unknown. The purpose of this work was to study the presynaptic mechanisms involved in DTB-induced inhibition of ACh release. Differentiated PC12 cells were used as a model, and [^{3}H]choline uptake and [^{3}H]ACh storage and release were measured using neurochemical techniques. At concentrations of 50 μM to 2000 μM, DTB had little or no effect on [^{3}H]choline uptake or on the spontaneous release of [^{3}H]ACh but caused a concentration-dependent decrease in release of [^{3}H]ACh elicited by elevations in extracellular [K^+]. These effects were not observed at exposure durations of less than 2 hours. These alterations occurred without prominent alterations in [Ca^{2+}]$_i$ as measured by fluorescence microscopy of individual PC12 cells loaded with fura-2. DTB-induced suppression of depolarization-evoked release of [^{3}H]ACh was associated with a decreased level of [^{3}H]ACh in the vesicular pool while the cytosolic pool was unaffected. These results suggest that DTB alters the release of [^{3}H]ACh by interrupting the synthesis, translocation and/or release of [^{3}H]ACh in the vesicular pool. Supported by NIH grant NS20683 and an ASPET Undergraduate Summer Research Internship to LMN.

1133 ALTERED EXPRESSION OF THE NEURAL CELL ADHESION MOLECULE (NCAM) IN DENERVATED RAT MUSCLE WITH LOW LEVEL LEAD TOXICITY

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Growth and repair of peripheral nerve is facilitated by the transient binding of neural cell adhesion molecules (CAMS) on the neuronal surface with cell adhesion molecules on apposing neurons glia, myotubes and the extracellular matrix. Following denervation, the pattern of NCAM reverts to that seen during development, with expression of a sialylic acid-rich embryonic (E) NCAM exceeding that of the sialylic acid-poor adult (A) form believed to anchor mature neurons. Lead (Pb) exposures have been linked to peripheral neuropathy involving both the nerve and neuromuscular junction (NMJ). Since Pb has been shown to interact with a variety of cell adhesion molecules, alterations in the expression of NCAM during re-establishment of the NMJ were examined following nerve crush with or without exposure to Pb. Female Sprague-Dawley rats (150–180 g) were given citrate-buffered 3.9 mM lead acetate (PbAc) in drinking water for 90 days. Both gastrocnemius muscles were removed for Western blot analysis, immunocytochemistry or histological examination of NMJs 7–21 days after crushing the right sciatic nerve. A rabbit polyclonal antibody (AB1505) which recognizes all NCAM types and mouse monoclonal antibodies (12E3) which recognize E-NCAM were used. Studies indicate that exposure to PbAc: 1) alters morphology of the NMJ, 2) delays reinnervation post-crush, 3) induces modest increases in NCAM expression, 4) induces drastic expression of NCAM with crush and 5) does not increase sialylation of NCAM. It is hypothesized that pre-exposure to PbAc may represent a conditioning lesion resulting in a more robust remodelling response to subsequent crush injury. (Supported by NIH ES05955 & ES06103).

1134 ASSOCIATION OF SYNAPTOPHYSIN WITH DEGENERATING NERVE FIBERS IN p-BROMOPHENYL-ACETYLUREA (BPAU)-INDUCED NEUROPATHY

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Synaptophysin is an integral membrane protein of synaptic vesicles, synthesized in neuronal soma and carried by axonal transport to nerve terminals. In head injury, abnormal immunocytochemically demonstrated masses of this protein were seen in swollen axons, presumably related to traumatic alteration of anterograde transport in these neurites (Schweitzer et al., Brain Pathol. 4: 489, 1994). To evaluate whether a similar mechanism exists in toxic axonal injury, the BPAU model of dying-back neuropathy was used. This toxicant elicits its neuropathic effects by altering fast axonal transport (Oka and Brimijoin,

Mayo Clin. Proc. 67: 341, 1992). Clinical neuropathy developed within 7 days of a single ip 300 mg/kg dose of BPAU in adult female Sprague-Dawley rats. Pathological study at 14–16 days revealed bilateral distal axonopathy progressing to Wallerian-like degeneration. This study focused on microscopic examination of cervical-medullary (distal) levels of spinocerebellar tracts and fasciculus gracilis, where myelinated nerve fibers in various stages of degeneration were seen. Sections were immunocytochemically reacted with a polyclonal human synthetic anti-synaptophysin antibody (Dako). Synaptophysin-positive deposits were seen in many degenerating fibers, being particularly prominent in swollen neurites. These often corresponded to degenerating axons seen by histopathology. The presence of prominent deposits of anterogradely transported synaptophysin in axonopathic regions provides a clear immunocytochemical marker for degenerating fibers. It also indicates an obstruction of axonal transport in BPAU-induced neuropathy.

1135 CHRONIC SUBCUTANEOUS TOXICITY STUDIES WITH rhNGF IN MICE AND MONKEYS

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Nerve Growth Factor (NGF), is known to influence the development and differentiation of neurons of the sympathetic and the sensory nervous system. In order to support chronic treatment in humans, recombinant human NGF (rhNGF) was administered 3 days/week for at least 13 or 26 weeks as subcutaneous (SC) bolus injections followed by a 4-week recovery period to mice and cynomolgous monkeys. In mice administered rhNGF, transient hypoactivity following injection (1000 μg/kg) and swelling at the injection site (doses $\geq$ 100 μg/kg) were observed beginning Week 2 following the initiation of treatment. There were no other rhNGF-related clinical observations. An increase in the incidence of positive antibody titers to rhNGF was also observed in Weeks 14, 27 and 31 sera of rhNGF treated mice. In monkeys, rhNGF (75 and 250 μg/kg) produced a slight reduction in body weight gain. Upon cessation of treatment, body weights in the 250 μg/kg treatment group returned to levels which were similar to vehicle controls. An increase in the incidence of antibody titers to rhNGF was also observed Weeks 5, 13, 18, 22, 27 and 31 in some monkeys treated with 75 and 250 μg/kg (including recovery animals). In both species, there were no rhNGF-related clinical pathological findings or gross anatomical findings. In conclusion, there were no findings in the current studies that would preclude the chronic administration of rhNGF in clinical trials in humans.

1136 *IN VIVO* MECHANISTIC STUDIES OF RECOMBINANT HUMAN NERVE GROWTH FACTOR-INDUCED EFFECTS IN MICE AND RATS

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In previous studies, the subcutaneous (SC) administration of recombinant human Nerve Growth Factor (rhNGF) produced clinical observations in rats and mice which included skin discoloration, irregular respiration, edema, plasma extravasation, and/or death. Since NGF may play a role in both nervous and immune system function, the present studies examined the role(s) of these systems in producing these effects. In rats, extensive SC pretreatment with the mast cell degranulator 48/80 (2 mg/kg; 19 daily doses) completely blocked, while pretreatment with cyclophosphamide (100 mg/kg; intraperitoneal) partially reduced, the effects of SC rhNGF (0.5 mg/kg); pretreatment with the sensory fiber neurotoxin capsaicin had no effect. In mice, although a correlation between antibody formation and toxicity could not be established (due possibly to interference in the assay by rhNGF-antibody complexes), toxicity in this species could be mimicked by the co-administration of SC rhNGF (5 or 50 mg/kg) with rhNGF antibodies (intravenous doses $\geq$ 5 mg/kg) in both normal (CD-1 and Wv/+) and mast cell-deficient (W/Wv) mice. Similar co-treatments in CD-1 mice with rhNGF and antibodies to other proteins, or other protein/antibody combinations were without effect. These results suggest that while mast cells are essential in rats, they may not be critical for the development of toxicity in mice. Furthermore, the formation of antibodies may be essential for toxicity in mice.

1137 RECOMBINANT-METHIONYL HUMAN BRAIN-DERIVED NEUROTROPHIC FACTOR (r-metHuBDNF) FOR THE TREATMENT OF AMYOTROPHIC LATERAL SCLEROSIS (ALS): TOXICITY AND PHARMACOKINETICS IN RATS

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BDNF is a member of the nerve growth factor (NGF) family. r-metHuBDNF has been shown to afford protection in various models of motor neuron degeneration, findings which support the use r-metHuBDNF as a potential clinical treatment for ALS. Therefore the pharmacokinetics and toxicity associated with daily subcutaneous dosing of r-metHuBDNF to rats for up to six months was evaluated. Pharmacokinetic data indicate that r-metHuBDNF showed 10% bioavailability and a biphasic clearance pattern with maximal serum concentrations at 0.5hr post-dose. In a one-month toxicity study treatment with r-metHuBDNF (10–75 mg/kg/d) was primarily associated with reductions in body weight, body weight gain, and food consumption. Significant findings from a 3/6-month toxicity study of r-metHuBDNF treatment (2–20 mg/kg/d) were also reductions in body weight, body weight gain, and food consumption. In none of the above studies were any biologically significant effects noted on clinical pathology, nor were any target organ toxicities observed. Findings from these studies are therefore suggestive of a central nervous system-mediated pathway. Rats were subsequently administered intracerebroventricular infusions of r-metHuBDNF (2–8 μg/kg/d, 14d) resulting in food intake suppression and increased hypothalamic serotonin turnover. As hypothalamic serotonin status has been implicated in certain forms of appetite suppression, these data suggest that high doses of r-metHuBDNF may cause food intake/body weight reductions by a centrally-mediated appetite suppression mechanism.

1138 LEAD TOXICITY AS AN URBAN PUBLIC HEALTH ISSUE; BALTIMORE AS A MODEL FOR TREATMENT AND PREVENTION

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Among the most significant toxicological problems encountered in urban environments is exposure to lead. Lead exposure can commence as early as prenatally and has been associated with serious alterations in brain development in infants and toddlers. Such lead induced alterations include deficits in neurocognitive function as exemplified by significantly lower IQ as well as attentional and learning disorders. The deleterious effects of lead toxicity may indeed persist throughout the lifetime of exposed individuals. A number of unique projects have been undertaken in the city of Baltimore that are focused on the treatment and prevention of diseases attributable to lead exposure. Among these is a clinical trial to investigate the efficacy of the lead chelating drug succimer. In addition, the Environmental Protection Agency is sponsoring a randomized field study in Baltimore on Repair and Maintenance interventions for reducing lead in settled house dust and children's blood and a comprehensive lead-paint abatement program. This workshop will focus on the issue of lead toxicity as an urban public health issue and showcase some of the research, treatment and prevention programs that are ongoing in Baltimore. In addition, a representative of EPA will provide a government perspective on these programs and how they can be adapted to other urban environments. A legal representative will provide information about legislation passed in the State of Maryland that allocates funds to cover relocation of affected individuals to better housing as well as medical expenses.

1139 COMPARTMENTATION OF GLUTATHIONE: IMPLICATIONS FOR THE STUDY OF TOXICITY AND DISEASE

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That glutathione plays many roles in biological protective mechanisms has been recognized for decades. Conjugates, disulfides, and other glutathione-derived products also have been studied as biomarkers of the chemical natures or specific identities of key metabolites of various agents and such studies have been crucial in the delineation of the nature of the interactions of proximal toxins with target biomolecules. Despite the extensive evidence implicating the depletion and/or oxidation of glutathione in a wide variety of human and experimental toxicities, critical examination of such studies frequently indicates that injury is not related simply to glutathione status. Glutathione is compartmentalized at several levels and this compartmentalization appears to exert considerable influence on the relationships between glutathione depletion or oxidation and onset of injury. Although compartmentation is usually viewed

from the perspective of different intracellular pools, the significance of extracellular glutathione in functionally important pools is gaining appreciation. As the factors affecting the interactions of intracellular pools with extracellular pools are delineated, studies in humans can be designed and interpreted with greater precision and utility.

1140 TOXICOLOGICAL IMPLICATIONS OF A DISTINCT NUCLEAR GLUTATHIONE POOL

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The existence of a distinct nuclear GSH pool has been demonstrated in several laboratories, by both direct and indirect evidence. Studies from our laboratory suggest that this pool is relatively small, comprising approximately 1–2% of the total cellular GSH content. The rate of turnover of the nuclear GSH pool is much slower than that of the cytosolic GSH pool, which implies that nuclear GSH levels are protected form the normal fluctuations that occur in cytosolic GSH levels. We have shown that the cell nucleus contains enzymes that allow it to synthesize its own GSH, at a rate that can account for the maintenance of the levels present. We have not been able to demonstrate in isolated nuclei preparations any ATP-dependent uptake process for externally synthesized GSH, such as is seen in mitochondria. We have also shown that depletion of the nuclear GSH pool is possible through redox cycling, and that this depletion sensitizes cells to the genotoxicity of DNA alkylating agents. We suggest that part of this sensitization is due to lowered efficiency of DNA repair processes consequent to nuclear GSH depletion, and that protection of such processes represents a significant cytoprotective role of the nuclear GSH pool.

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1141 MITOCHONDRIAL UPTAKE OF GLUTATHIONE AND NEPHROTOXICITY

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Renal mitochondria are important targets of chemically induced cell injury because of their critical role in energy production. Glutathione (GSH) is an important intracellular defense against many chemical toxicants. Mitochondria either do not synthesize GSH *de novo* or do so at slow rates, yet they contain GSH concentrations comparable to those in cytoplasm. This suggests that transport of cytosolic GSH across mitochondrial inner membrane is the source of most of the mitochondrial GSH pool. GSH is a charged molecule inside cells; it has a net charge of -1 in cytoplasm (where pH = 7.0) and a net charge of -1 or -2 in mitochondrial matrix (where pH = 7.8). GSH was transported as the intact tripeptide into rat renal cortical mitochondria in a concentration- and time-dependent manner. Transport appeared to be electroneutral and was inhibited by γ-glutamyl amino acids, *S*-alkyl GSH analogs, and dicarboxylates (e.g., succinate, malate, glutamate), but not monocarboxylates (e.g., pyruvate, lactate). Succinate and malate also *trans*-stimulated GSH uptake. This suggests that the transport mechanism involves electroneutral exchange of GS^- (net charge = -2) with dicarboxylates and that the γ-glutamyl moiety is a recognition site on the transporter. Interaction of the transporter with intermediary metabolites suggests that metabolic or nutritional state may influence intramitochondrial GSH status. Increase of mitochondrial GSH by action of the transporter prevented inhibition of state 3 respiration induced by the oxidant *tert*-butyl hydroperoxide, demonstrating a toxicological role for mitochondrial uptake of GSH.

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1142 EXTRACELLULAR GLUTATHIONE: A CRITICAL POOL FOR CELLULAR PROTECTION

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Cells contain receptors, transport systems and enzymes in their plasma membranes that are susceptible to oxidative damage. Extracellular glutathione (GSH) may be a critical component in protection against oxidative and chemically induced damage to these systems. However, extracellular GSH concentrations are typically much lower than intracellular concentrations, and relatively few studies have addressed the regulation and functions of extracellular GSH. We have used a variety of cellular, perfused organ and in vivo systems to examine the mechanisms involved in the maintenance and utilization of extracellular GSH. Results with primary cultures of human retinal pigment epithelium show that micromolar concentrations can protect against oxidative injury without transport into the cells and without degradation to yield cysteine. In the absence of added GSH, the cells release GSH to maintain this extracellular pool. Blood plasma GSH is maintained by release of GSH from liver and other cells. Epidemiologic studies in humans show that variations in blood plasma GSH are associated with age, gender, dietary habits and health-related factors. However, the control of extracellular GSH concentration and redox status remain poorly defined. Systems exist in many cells that allow efflux of GSH and GSSG on both basal and apical surfaces. Cysteine release, especially in the small intestine, provides a mechanism to reduce GSSG via thiol-disulfide exchange. There is also some evidence that cell turnover and sloughing can contribute to extracellular GSH. Maintenance of extracellular GSH may provide an important and previously overlooked means to protect against cell toxicity caused by exogenous oxidant- and reactive electrophile-induced damage to critical plasma membrane biomolecules.

1143 GLUTATHIONE SYNTHESIS AND CLEARANCE IN HUMANS AND THE EFFECTS OF INFECTION WITH HUMAN IMMUNODEFICIENCY VIRUS

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Sponsor: C V Smith

Low glutathione (GSH) concentrations in patients with HIV infections could contribute to the immune deficiency since GSH plays important roles in the functions of lymphocytes and sulfhydryls modulate the expression of HIV *in vitro*. To investigate the mechanisms responsible for the low GSH in plasma and lymphocytes of patients with HIV, the release of GSH into the circulation, an estimate of the systemic production, was determined in patients with HIV infection using a pharmacokinetic approach. The basal plasma concentrations of total GSH (7.6 ± 3.1 vs. 11.6 ± 3.2 μM), free GSH (3.3 ± 1.3 vs. 5.3 ± 1.0) and cysteine (7.7 ± 2.6 vs. 13.4 ± 4.9) were lower in 8 infected patients than in 8 controls. Upon infusion of GSH at 1 $\mu mol \cdot min^{-1} \cdot kg^{-1}$ the plasma GSH plateaued after 20–35 min. The increments in total plasma GSH were greater in patients (43 ± 13 vs. 30 ± 5 μM). The input of GSH (12.9 ± 5.7 vs. 30 ± 11.7 $\mu mol \cdot min^{-1}$) was lower in patients ($P < 0.01$). The rates of clearance of GSH were 25 ± 7 and 35 ± 7 $ml \cdot min^{-1} \cdot kg^{-1}$ in patients and controls. During infusion of GSH the cysteine in peripheral blood mononuclear cells of the HIV-infected patients increased, but the intracellular concentrations of GSH did not increase. The present data indicate that low GSH concentrations in HIV-infected patients is due to decreased systemic synthesis of GSH rather than increased consumption.

1144 GLUTATHIONE STATUS AND METABOLISM IN HUMAN INFANTS

C V Smith, T N Hansen. *Department of Pediatrics, Baylor College of Medicine, Houston, TX*

Premature birth and associated toxicities and diseases are major sources of morbidity and mortality in the US. Many of the problems associated with prematurity arise from oxidative exposures in excess of the capacities of incompletely developed defense mechanisms. Hyperoxic ventilation and parenteral nutrition, although frequently essential to clinical management, each may contribute to the adverse effects. Premature infants have lower plasma concentrations of glutathione and higher concentrations of glutathione disulfide than are observed in healthy adults, suggesting a deficit in defense capacities and elevated oxidant stresses in these infants. Low plasma concentrations of GSH are also seen in children infected with the human immunodeficiency virus (HIV), although GSSG concentrations are not elevated. The mechanisms responsible for the low GSH associated with HIV infection are not known, but the lower plasma concentrations of cysteinylglycine we observe in HIV-infected (2.25 ± 0.31 μM) than in uninfected (3.48 ± 0.37 μM) children, are not consistent with elevated γ-glutamyltranspeptidase activities, which are often associated with preneoplastic states. Appropriate support of glutathione status may offer specific benefits in improving the health and welfare of these infants and children, as well as adults afflicted with related maladies.

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1145 CHLORINATION: ESSENTIAL PROCESS OR CANCER HAZARD? INTRODUCTION

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Chlorine has been successfully used for the control of waterborne infectious disease. In the 1970s it was found that chlorine reacted with natural organic matter present in surface waters to produce disinfection by-products (DBP). Concern focussed initially on the trihalomethanes (THM), but a wide variety of DBPs are now known to result from chlorination.

This symposium will examine the toxicological and epidemiological data on chemical hazards associated with chlorination and attempt to measure this hazard against competing microbial risks. The first presentation will discuss the available analytical epidemiological studies. A second presentation will deal with the importance of chlorination to the prevention of waterborne infectious disease. Pharmacokinetic, mechanistic and modeling information on the prototypical DBP, chloroform, are to be discussed and contrasted with data on brominated THMs to determine if it is scientifically appropriate to regulate THMs as a single toxicological class. The fifth presentation will deal with the carcinogenic properties of a potent mutagen that is produced by chlorination. The final presentation will discuss the haloacetates, carcinogenic DBPs whose concentrations approach and occasionally exceed those of the THMs. The impact that these results should have on public policy will form the basis of the closing discussion.

1146 EPIDEMIOLOGIC ASSOCIATIONS OF CHLORINATION OF DRINKING WATER AND CANCER

K P Cantor. *National Cancer Institute, Bethesda, MD.* Sponsor: R J Bull

Many epidemiologic studies of chlorination byproducts and cancer have been conducted. The first studies, conducted soon after byproducts were identified, compared geographic differences in cancer rates with the types and treatments of community drinking water used in different places. These investigations were qualitatively useful in identifying bladder, colon, and rectal cancers as those likely to be linked with chlorination byproducts. More recent studies, that obtained lifetime residential histories and other information directly from individual patients and matched controls, are useful for quantitative risk assessment. Among the challenges in conducting and interpreting epidemiologic studies of environmental contaminants are: assessment of findings where relative risks are small, as with these exposures; estimating past exposures; and separating the contribution to risk of the various components of the byproduct mix. The recent studies support an association of chlorination byproducts with rectal cancer, but decrease our confidence in earlier observations of links with bladder cancer and colon cancer. Among highly exposed groups, relative risks for rectal cancer are approximately 1.8–2.0. If confirmed, this finding will translate in the U.S. to 6,000 new cases annually among persons with long-term exposure to chlorinated surface water with historical levels of chlorinated byproducts.

1147 REDUCTION OF MICROBIAL RISKS BY CHLORINATION

J B Rose. *Department of Marine Sciences, University of South Florida, St. Petersburg, FL.* Sponsor: R J Bull

Abel Wolman has been credited with the quote "Chlorine has saved more lives than any other single chemical". Waterborne disease continues to be an important health threat throughout the world as evidenced by cholera epidemics in Africa and South America. In the U.S., there are an average of 31 outbreaks per year and with few exceptions (i.e. *Cryptosporidium* control) chlorination of water has provided the major barrier to waterborne disease. Quantification of cases and deaths avoided can be made using risk assessment and dose-response models. Based on water quality data from developing and developed countries, a risk assessment will be presented. This will consider cases of disease and deaths in the general population and in sensitive populations. Some of the health benefits achieved through chlorination will be estimated, based on the limited data that is available. For example, waters containing rotavirus, *Salmonella*, and cholera may be reduced from 90% to 99.9999% using chlorine. Annual disease risks are likewise reduced from 10^{-1} to 10^{-6} and mortality from 10^{-3} to 10^{-9}. Even at these low levels, lifetime risks may remain significant.

1148 RELEVANT RISK ASSESSMENTS FOR CHLOROFORM

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Chloroform administered by gavage induces male and female $B6C3F_1$ mouse liver tumors and male Osborne-Mendel rat kidney tumors. The mouse liver tumor response disappears if the same daily gavage dose of chloroform is instead given in the drinking water. Cytotoxicity and regenerative cell proliferation are seen in the mouse liver when chloroform is given by gavage, but not in the drinking water, suggesting that tumor formation is secondary to these effects. Dosimetry studies suggest that toxicity is not observed when chloroform is given in the drinking water because the compound is not delivered or converted to toxic metabolites at a rate sufficient to kill hepatocytes. An Osborne-Mendel male rat kidney tumor response was seen when chloroform was given in the drinking water, but only at the highest dose that also resulted in a 60% decrease in water consumption and a 25% loss in body weight. The nutritional status of this high dose group was uncertain and the validity of extrapolation of the tumor response to lower doses is questionable. Chloroform administered by inhalation for up to 90-days to $B6C3F_1$ mice and F-344 rats produced concentration-dependent responses for toxicity and regenerative cell proliferation in the male and female mouse liver, the male mouse kidney, the male and female rat kidney and nasal turbinates. Primary hepatocyte cultures allow comparisons between rodent and human metabolic rates and toxic responses. Integration of the above information yields risk assessments that are appropriate to each route of administration and that are consistent with the mode of action of this nongenotoxic/cytotoxic carcinogen. These risk assessments predict no increased risk of cancer from the levels of chloroform generated by the chlorination of drinking water.

1149 BROMINATED TRIHALOMETHANES: CARCINOGENIC RISKS AND MECHANISMS

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The brominated trihalomethanes (THMs), $CHBrCl_2$, $CHBr_2Cl$, and $CHBr_3$), are common by-products of surface water chlorination which may occur at greater concentrations than chloroform ($CHCl_3$ when source waters with high bromide levels are disinfected. In corn oil gavage studies (NTP), the brominated THMs were carcinogenic in rodents, producing tumors of the liver ($CHBrCl_2$ and $CHBr_2Cl$), kidneys ($CHBrCl_2$) and large intestine ($CHBrCl_2$ and $CHBr_3$). $CHBrCl_2$, generally the second most prevalent THM in drinking water, appears to be more carcinogenic than $CHCl_3$. A rodent tumorigenesis study of $CHBrCl_2$ in drinking water is in progress. Research has also been undertaken to examine the pharmacokinetics and biological activities of $CHBrCl_2$ in rats, and when possible, comparisons to $CHCl_3$ and $CHBr_3$ have been incorporated into the experiments. Gas uptake experiments and physiologically-based pharmacokinetic modeling suggest that $CHBrCl_2$ is absorbed into tissues and metabolized more rapidly than $CHCl_3$ and that metabolism other than oxidation may be important for $CHBrCl_2$, but not $CHCl_3$. Additional in vivo results demonstrate that $CHBrCl_2$ is a more potent acute renal toxicant than $CHCl_3$ after gavage in an aqueous vehicle. Target tissue covalent DNA binding by $CHBrCl_2$ after oral dosing was also greater than has been observed with $CHCl_3$. DNA binding by $CHBrCl_2$ was especially noteworthy in the intestinal tract and liver. In vitro microsomal protein and lipid binding by metabolites of $CHBrCl_2$ and $CHBr_3$ also exceeded that of $CHCl_3$-derived intermediates. These findings support other evidence suggesting that risk estimates for THMs in water should not rely primarily on the $CHCl_3$ data base. The potential risks posed by brominated THMs deserve serious consideration. (This abstract does not necessarily reflect EPA policy.)

1150 CANCER RISK OF 3-CHLORO-4-(DICHLOROMETHYL)-5-HYDROXY-2(5H)-FURANONE (MX) AND RELATED NON-VOLATILE CHLORINATION PRODUCTS

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Highly mutagenic, acidic compounds have been found in drinking water after chlorination of humus-containing surface waters. MX is the most potent mutagen identified. MX is rapidly degraded in the organism (Pharmacol. & Toxicol. 70:424, 1992), but it is protected to some extent by protein binding (Toxicol. Lett. 59: 187, 1993). Its acute/subchronic toxicity is moderate (Arch. Toxicol. 68:398, 1994), but it has been shown to induce sister chromatid exchange *in vivo* (Mutat. Res. 299:25, 1993). The in-life portion of a two-year bioassay in rats has been completed and the histopathology is under study. MX concentrations in Finland correlate well with the mutagenicity of drinking water. MX is responsible for 30–50% of the activity found. Mutagenic activity can be predicted by water quality parameters. Levels of mutagenic activity in Finnish drinking waters have been modeled back to the 1950s. A correlation between the modeled mutagenicity and lymphoma and cancers of the kidney, bladder, and pancreas in ecological studies. (Am. J. Public Health 84(8), 1994; unpublished data). Cohort and case control studies are ongoing.

1151 HALOACETATES: CARCINOGENIC BY-PRODUCTS OF CHLORINATION

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Brominated and chlorinated acetates are produced in the chlorination of drinking water. Dichloroacetate (DCA) and trichloroacetate (TCA) produce hepatic tumors in rodents, but brominated and mixed bromochloro analogs have received little study. Diverse biochemical mechanisms are associated with the different haloacetates which may contribute to their carcinogenic activity. The carcinogenic activity of TCA appears associated with its activity as a weak peroxisome proliferator. This mechanism is not prominent with any other haloacetate. Evidence suggests that neither DCA and TCA are genotoxic, but the haloacetates have not been adequately tested. DCA and bromochloroacetate (BCA) produce profound changes in the control of glycogen metabolism. Evidence of oxidative stress including lipid peroxidation and elevated levels 8-hydroxy-2-deoxyguanosine (8-OH-dG) in hepatic nuclear DNA *in vivo* are observed with increasing bromine substitution. Reparative hyperplasia does not appear important with any of the haloacetates, but extended treatment with DCA inhibits cell division in normal cells, but appears to increase division of the intermediate cell population. Thus, DCA may interact with cell signaling pathways to act primarily as a promoter. These data suggest that there may be little to fear from chlorinated haloacetates at low doses, but such effects cannot be ruled out with brominated analogs. (Supported by NIEHS RO1-ES04648)

1152 OXIDATIVE DEGRADATION OF AFLATOXINS USING A NOVEL METHOD OF OZONE PRODUCTION

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Development of practical methods to degrade aflatoxins using ozone (O_3) have been limited by low O_3 production capacities of gas phase-based methods and their associated costs. The objective of this study was to employ a new method of O_3 production to study the mechanisms and kinetics of degradation of aflatoxins B_1 (AfB_1), B_2 (AfB_2), G_1 (AfG_1) and G_2 (AfG_2). The O_3 gas was electrochemically generated using water and a solid polymer electrolyte (Nafion®) as a proton exchange membrane to facilitate an O_3 generating hydrolysis reaction. Aqueous solutions of AfB_1, AfB_2, AfG_1 and AfG_2 (10 μg/ml each) were ozonated with 2 and 20% ozone for 0, 15, 30, 45 s, 1.0, 2.0, 2.5, and 5.0 min. The reaction was terminated with chloroform and the extracts were analyzed by normal and reversed-phase HPLC. AfB_1 and AfG_1 were rapidly degraded with rate constants equal to 0.0603/sec and 0.0583/sec using 2% O_3. Total degradation was observed after 15 s with 20% O_3. AfB_2 and AfG_2 required 20% O_3 for degradation with rate constants equal to 0.1014/sec and 0.0831/sec, suggesting a rapid reaction of O_3 at the 8.9 double bond in AfB_1 and AfG_1. Ozonation of ^{14}C-AfB_1 resulted in a loss of 57% of radioactivity; only 7% of the remaining radioactivity could be extracted into chloroform. The hydroxylation products AfB_{2a} and AfG_{2a} were not detected by HPLC following ozonation. Degradation of aflatoxins in corn and rice was not as rapid as aqueous solutions. (Supported by TAES H6215 and ATP 999902-004).

1153 BIOAVAILABILITY OF FB_1, HYDROLYZED FB_1, AND THE FB_1-FRUCTOSE ADDUCT

E C Hopmans, S Hendrich, P A Murphy. *Department of Food Science and Human Nutrition, Iowa State University, Ames.* Sponsor: W R Bidlack

Fumonisin B_1 (FB_1) is a cancer causing toxicant commonly found in corn. Hydrolyzed FB_1 (HFB_1) has been reported in some processed corn foods and to be equally toxic as FB_1. Reaction of FB_1 with glucose or fructose resulted in loss of o-phthaldialdehyde (opa) reactivity, probably due to the formation of an FB_1-fructose adduct A preliminary study suggested this adduct formation may detoxify FB_1. FB_1 was purified from *Fusarium proliferatum* 5991 liquid culture using XAD-16-, C_8-, and semipreparative C_{18}-columns. Resulting FB_1 was 70% pure. HFB_1 was produced by refluxing FB_1 in 2 N KOH. The FB_1-fructose adducts was formed by heating FB_1 in 0.1 M fructose, pH 7, at 80 °C for 48 hr. Male Fisher 344/NHsd rats were dosed by gavage with 0.13, 1.3, or 13 μmole/kg body weight with either FB_1, HFB_1 or FB_1-fructose. Urine and feces were collected for 4 days. Urine was filtered and cleaned up using C_{18} solid phase extraction. Feces were repeatedly extracted with 0.1 M EDTA pH 5.2, and cleaned up similar to urine. FB_1 and HFB_1 were detected as opa-derivatives following reversed phase HPLC. FB_1-fructose was detected after hydrolysis as HFB_1. Rate and route of excretion and recoveries will be presented. Bioavailability was estimated as 100% (1-dose recovered/total dose).

1154 DOSE-RESPONSE CHARACTERISTICS OF SPHINGOID BASE ELEVATIONS INDUCED IN LIVER AND KIDNEY SLICES BY FUMONISIN B1

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The mycotoxin, fumonisin B1 (FB1), is a specific inhibitor of sphinganine (Sa) and sphingosine (So) N-acyltransferases. Monitoring Sa/So ratio in serum, urine or tissues can serve as a biomarker of FB1 exposure. We have reported that Sa/So ratio is elevated in liver slices dosed with FB1. We now report dose response effects, and compare the responses of liver and kidney. Slices (250 μm) were incubated with FB1, and after 20 hr Sa and So were extracted and quantitated by HPLC. So was not significantly elevated, but Sa content increased with FB1 dose from 0.5 to 10 nmol/g of tissue. Liver was slightly more responsive than kidney to these effects. In both tissues, Sa content tended to plateau at concentrations greater than 1 μM, suggesting precursor (serine?) depletion in the incubation medium. These studies will provide the basis for future studies to determine levels of fumonisins required to alter sphingoid bases *in vivo*.

1155 REPRODUCTIVE TOXICITY STUDY OF FUSARIUM MONILIFORME CULTURE MATERIAL IN RATS

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Fumonisins (FB) are mycotoxins produced by *F. moniliforme* and *F. proliferatum*. FB commonly occur in corn and corn-based foodstuffs and are potent inhibitors of sphingolipid (SL) biosynthesis. Because (a) SL are regulators of cell growth and differentiation and (b) the reproductive effects of FB are unknown, diets containing *F. moniliforme* culture material (CM) to provide 0, 1, 10, or 55 ppm fumonisin B1 (FB1) were fed to male and female rats beginning 9 and 2 weeks, respectively, before mating. CM caused nephrosis, typical of FB1, in males fed $\geq$ 10 and females fed 55 ppm FB1. No significant reproductive effects were found in males (n = 12/group), dams and fetuses examined on gestation day 15 (n $\geq$ 8/group), or dams and litters examined on day 21 *post partum* (n $\geq$ 9/group). Litter weight gain of groups fed 10 or 55 ppm FB1 was slightly decreased; however, gross litter weight and physical development were unaffected. Increased sphinganine/sphingosine ratios, a biomarker of FB exposure, were found in high-dose dams, but not fetuses, on gestation day 15. This suggests that *in utero* FB1 exposure was negligible. (Sponsored by Agricultural Research Service, USDA and National Toxicology Program, NIEHS, Interagency Research Agreement 58-6612-M-016).

1156 ROLE OF NUTRITION IN THE SURVIVAL AFTER HEPATOTOXIC INJURY

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An important factor in facilitating compensatory liver regeneration after hepatic injury is the availability of adequate and appropriate nutritional support. There is regiospecificity in the primary sources of energy utilized by the hepatocytes in the periportal versus centrilobular regions. The centrilobular region derives its energy primarily by glucose oxidation while the periportal region gets its energy by fatty acid metabolism. Recent studies in our laboratory have shown that 15% glucose in drinking water for 7 days increases lethality of four structurally and mechanistically different model centrilobular hepatotoxicants (acetaminophen, thioacetamide, chloroform and carbon tetrachloride) in male Sprague-Dawley rats (n = 10/group). One single injection of an ordinarily nonlethal dose of thioacetamide was lethal in glucose loaded rats and therefore was chosen for further studies. On the other hand, dietary palmitic acid (PA, 8% w/w) and L-carnitine (LC, 2 mg/ml) in drinking water protected the rats (n = 10/group) from an ordinarily lethal dose of thioacetamide. Serum enzyme elevations and liver histopathology revealed that actual infliction of liver injury from thioacetamide peaked between 36 to 48 hr after thioacetamide injection. Liver damage progressed thereafter in rats receiving glucose supplement, while it regressed in rats on PA-LC diet. Glucose loading did not increase the hepatic microsomal cytochromes P-450, while PA-LC dietary regimen increased P-450, suggesting lack of concordance with increased/decreased bioactivation of thioacetamide. ^3H-Thymidine incorporation studies along with proliferating cell nuclear antigen (PCNA) immunohistochemical analysis of liver sections revealed inhibition of S-phase stimulation

and decelerated cell cycle progression in glucose loaded rats as opposed to increased S-phase stimulation and accelerated cell cycle progression in rats on PA-LC dietary regimen. The proto-oncogenes *c-myc* and *v-Ha-ras* were expressed within 6 hr of thioacetamide administration while *v-fos* and *p53* were expressed at later time points in the rats on PA-LC dietary regimen. These findings underscore the decisive impact of nutritional supplements on the hepatobiology on one hand and the importance of tissue repair response in overcoming even massive hepatic injury on the other. (Supported by the Burroughs Wellcome Fund).

1157 INVESTIGATION OF THE ADSORPTION OF SECALONIC ACID D TO PHYLLOSILICATE CLAY

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Secalonic Acid D (SAD) is a mycotoxin that produces toxic and teratogenic manifestations in experimental animals. The *Hydra attenuata* bioassay has been used to screen various clay and zeolitic minerals for their ability to bind and detoxify SAD, resulting in several sorbents that were able to protect adult hydra from SAD toxicity. The present studies were undertaken to evaluate and prioritize sorbents for testing *in vivo*. Initial binding studies indicated that 5 phyllosilicate clays were capable of strongly adsorbing ^{14}C-SAD (chemisorption index, or $C\alpha > 0.70$); an acid activated phyllosilicate clay exhibited the strongest binding. The zeolite and a neutral phyllosilicate clay (P_1) had virtually no capacity for SAD (<1 nmol/mg), in contrast to the capacities of low pH phyllosilicates (20 to 250 nmol/mg). Chemisorption indices for P_1 were equal to 0.86 (at pH 2), 0.78 (at pH 7), and 0.62 (at pH 10) indicating a greater affinity of P_1 for SAD under acidic conditions. An acidic phyllosilicate clay (P_2) was shown to tightly bind ligands possessing a β-dicarbonyl or enol system ($C\alpha > 0.80$). $C\alpha$s for compounds without these groups ranged from 0.05 to 0.23. The efficacy of P_2 in reducing the bioavailability of SAD was assessed in the Sprague-Dawley rat. In preliminary experiments, SAD output in the urine of rats treated with P_2 was decreased by 18%. These findings suggest that P_2, or a similar clay, may be used to facilitate the detoxification of SAD. (Supported by NIEHS Toxicology Training Grant ES 07273-01 and ATP 999902-004).

1158 THE IMPACT OF FOOD AND COLOR ADDITIVES AND GENERALLY RECOGNIZED AS SAFE (GRAS) INGREDIENTS ON DIETARY LEAD INTAKE AND POPULATION BLOOD LEAD LEVELS

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The FDA has proposed regulations to lower allowable lead concentrations in 38 food additives and GRAS food substances. We evaluate the impact of these ingredients on dietary lead intake and population blood lead levels for four American population age groups: adult males, adult females, toddlers (approximately 2 years of age), and infants (approximately 6 to 11 months of age). We establish a high-end estimate of the contribution of these ingredients to daily dietary lead intake. Using computer simulation models, we then calculate the contribution of this dietary lead intake to the distribution of population blood lead levels, reporting the impact on both the population median and the proportion of individuals with levels exceeding health-based thresholds. We conclude that the ingredients considered by FDA's proposed regulations have, at most, a minimal impact on population blood lead levels, accounting for no more than 2% to 4% of total lead exposure. Further efforts to reduce lead exposure in the United States should focus on more substantial sources of contamination.

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1159 EVALUATION OF HAMBURGERS AND HOT DOGS FOR THE PRESENCE OF MUTAGENS

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Food products derived from meat, during heat treatment, (frying, broiling), may develop traces of mutagenic contaminants, mainly hetrocyclic amines (HA). In an effort to obtain an estimate for the average human intake of these types of mutagens, a number of commercially prepared hamburgers and hot dogs were analyzed for their mutagenic activity. The samples were extracted using liquid-liquid extraction technique at different pH values and mutagenicity of the extracts was tested in the Salmonella/microsome assay using strain TA98 with metabolic activation. Sixteen samples of hamburgers and thirteen samples of hot dogs, randomly obtained from 'fast food' commercial establishtnents, were used in this survey. The mutagenic activity of these samples varied and ranged for the hamburgers from 20 to 1042 revertants/g, and for the hot dogs from non detectable to 4875 revertants/g of the original samples. The average values were 199 and 590 revertants/g for the hamburgers and hot dogs, respectively. A wide range in mutagenicity was found even for the same type of product obtained from the same outlet at different times. This indicates an inconsistency in the heating procedures during the preparation of these foods. The results also illustrate the difficulties in accurately assessing the intake of mutagenic HAs from hamburgers and hot dogs prepared in 'fast food' outlets.

1160 PREVENTIVE AND THERAPEUTIC POTENTIAL OF SQUALENE-CONTAINING ROIDEX ON TUMOUR PROMOTION AND REGRESSION

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Recent scientific evidence has shown reactive oxygen species (ROS) play an important role in carcinogenesis. Many free radical scavengers have also been found to reduce oxidative damage to macromolecules by ROS. Roidex, an unconventional therapeutic agent used to treat skin cancer and hemorrhoids, is a mixture of antioxidants including squalene, vitamin A, vitamin E, and aloe vera in mineral oil. Our goal was to investigate the ability of Roidex to prevent the development of chemically-induced cancer and to cause regression of any tumors already formed. In the chemoprevention study, female CD-1 mice were initiated with 7,12-dimethylbenz[a]anthracene (DMBA) and promoted with 12-O-tetradecanoylphorbol-13-acetate (TPA). The mice were treated 30 min before TPA treatment with either mineral oil (vehicle control), 5% squalene, or Roidex. At the end of the experiment, there was a 33.3% prevalence of tumors (multiplicity of 1.40) in the positive control group and 26.6% (multiplicity of 1.46) and 20.0% (multiplicity of 0.46) in the 5% squalene and Roidex groups, respectively. The tumor regression study involved the selection of mice with tumors and possible regression of these tumors with Roidex treatment alone. There was a regression of 33.3% of the tumors in the Roidex-treated group (39 to 26) compared to the non-treated group whose tumors regressed only 3.4% (29 to 28). We conclude that Roidex may have both antipromotional and tumor regressive properties. Supported by Chasmuta Inc.

1161 EFFECT OF VITAMIN E ON DIELDRIN INDUCED HEPATIC DNA SYNTHESIS AND NEOPLASIA IN MICE

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The organochlorine insecticide, dieldrin, has been found to be a selective hepatocarcinogen in mice but not in other species tested. Dieldrin appears to function through nongenotoxic mechanisms. Recent evidence from our laboratory has shown that dieldrin selectively induces oxidative stress and lowers hepatic antioxidant levels (in particular vitamin E (vit E)) in mouse hepatocytes. The present study was undertaken to examine the effect of vit E supplementation on the toxic effects of dieldrin in the mouse. B6C3F1 mice were treated both subchronically (up to 90 days) and chronically (up to 540 days) with either dieldrin (10 ppm), dieldrin + vit E (200 mg/kg), vit E only, or control diet. After 7, 14, 28, 90, 300 or 540 days of treatment mice were sampled. Livers were examined for vit E levels, DNA synthesis rate, and other histopathologic changes. Dieldrin produced an increase in hepatic DNA synthesis that peaked at 14 days but remained above control levels at all sampling times. Vit E added to the dieldrin diet reduced this effect. Chronic treatment (300 and 540 days) resulted in the production of hepatic tumors in the dieldrin treated mice. Dieldrin + Vit E diet reduced the tumor incidence and multiplicity at 300 days and decreased multiplicity and tumor size at 540 days. These results suggest that dieldrin may be producing its toxic effects through oxidative mechanisms in the mouse liver and these effects can be modulated by antioxidants.

1162 EFFECT OF SODIUM SELENITE ON ANTIOXIDANT ENZYMES

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Selenium is reported to exert protective effects against carcinogenesis and alteration of antioxidant enzymes is one of the proposed mechanisms. To examine this potential mechanism, a study was conducted in partially hepatec-

tomized rats that were exposed to diethylnitrosamine to induce hepatocarcinogenesis. Animals had been pretreated with different concentrations of selenium (0, 0.5 1.0, and 2.0 ppm). Selenium treatment was continued for 30 days. Liver microsomes and cytosolic fractions were analyzed for catalase (CAT), glutathione peroxidase (GSHpx), and superoxide dismutase (SOD), and selenium content. Our data indicates that the activities of CAT, GSHpx, and SOD increase as selenium levels in liver increase, to more than 50%. Animals pretreated with 2.0 ppm Se displayed a similar pattern of results as positive controls which suggests that this concentration may be near toxic levels. Rate of lipid peroxidation in cytosolic fractions increased as selenium concentration decreases. Animals treated with 0.0 ppm, and 2.0 ppm show similar values strengthening the initial observation of toxicity at high selenium levels. Preliminary results tends to support the proposed mechanism.

1163 INDUCTION OF DETOXIFICATION ENZYMES BY SODIUM BUTYRATE AND BENZYL ISOTHIOCYANATE IN HUMAN COLON CELLS

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Dietary fiber has been suggested as a cancer preventative agent based on the positive association of fruit and vegetable intake with lower incidences of colon cancer. One mechanism proposed for this protection is the production of butyric acid from fiber fermentation by intestinal microflora. Sodium butyrate (NaB) acts as a differentiating agent in several human cell lines, including HT-29 colon cancer cells. To determine whether cell differentiation influences the effects of isothiocyanates, found in cruciferous vegetables, on the induction of detoxification enzymes, HT-29 cells were pre-incubated with or without NaB and then treated with benzyl isothiocyanate (BIT). NAD(P)H: quinone reductase (N:QR) and glutathione S-transferase (GST) activities were dose-dependently enhanced up to 250 and 500%, respectively, by NaB alone (0–5 mM). This elevation of N:QR and GST enzyme activity paralleled alkaline phosphatase activity, an indicator of cell differentiation. In cells not treated with NaB, BIT (5–25 μM) dose-dependently increased the activity of N:QR and GST up to 50%; however, the combination of 5 mM NaB and 25 μM BIT gave elevations of 300 and 500%, respectively. These findings suggests a new mechanism for cancer prevention by fiber, wherein the fiber acts synergistically with compounds in fruits and vegetables to induce enzyme activities capable of conjugating carcinogenic electrophiles. (Supported by NIH HL39968)

1164 CHEMOPREVENTION OF MAMMARY ADENOCARCINOMAS BY PREPUBERTAL EXPOSURE TO GENISTEIN

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For the past fourteen years breast cancer incidence rates have continued to rise in the United States. While genetic predisposition to cancer cannot be controlled at this time, it is believed that lifestyle factors can be modified to lessen the likelihood of neoplastic growth. One factor that can be manipulated easily is diet. A pertinent example of this is the lower rate of breast cancer observed in orientals that consume a high soy diet. We have hypothesized that genistein is the active component of soy which is responsible for this protective effect and that early exposure to genistein will be a significant factor in programming for protection. Using female Sprague Dawley rats we have demonstrated that prepubertal (days 16, 18, 20 postpartum) injections of genistein (500 μg genistein/g BW, s.c.) decrease the incidence and multiplicity (by almost 2 to 1), and increase the latency of dimethylbenz(a)anthracene (8 mg/100 g BW, injected s.c. on day 50 postpartum) induced mammary adenocarcinomas. To investigate mechanisms of action we prepared mammary whole mounts and found that prepubertal genistein treatment alters the ontogeny of mammary gland maturation and cell proliferation.

1165 EFFECT OF GENISTEIN ON CELL PROLIFERATION, DIFFERENTIATION AND APOPTOSIS IN NEUROBLASTOMA CELLS

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Neuroblastoma is a common childhood malignant tumor of the autonomic nervous system. Genistein is an isoflavone known to inhibit protein tyrosine kinases and DNA topoisomerase II. However, few studies on the effects of genistein on neuroblastoma cells have been conducted. We studied the effects of genistien on cell proliferation differentiation and apoptosis in neuroblastoma cell lines N2A, SKNSH and JC. Cell lines were treated with genistein concentrations of 0–20 μg/ml. Genistein was found to inhibit the growth of the three tumor cell lines in a dose dependent manner with IC50 values of 1–3 μM. N2A cells treated with genistein for 6 days exhibited morphological features of differentiation such as axon and dendrite extension. Mechanistic studies in all 3 cell lines showed apopotic chromatin condensation and cell shrinkage at genistein concentrations >10 μg/ml. These findings suggest that genistein may be a good candidate for a chemopreventive or therapeutic agent against neuroblastoma. (Supported by NIH Grant RO1 CA60994 and RO1 CA 61764).

1166 CHEMOPREVENTIVE EFFICACY OF DEHYDROEPIANDROSTERONE (DHEA) AND INDOMETHACIN (IM) ON TUMOR DEVELOPMENT IN A MULTI-ORGAN CARCINOGENESIS MODEL

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Although DHEA is hepatocarcinogenic, it has been experimentally demonstrated to exert anti-carcinogenic effects in various other organs. IM has also been reported to have inhibitory potential for chemically-induced carcinogenesis. Therefore effects of DHEA and IM alone and in combination were investigated in a rat multi-organ carcinogenesis model. Male F344 rats were sequentially treated with 5 carcinogens with different target organ specifities (DEN, MNU, BBN, DMH, DHPN) for 4 weeks. One week after this initiation protocol, the rats were given DHEA and IM as follows; 0.3% DHEA in the diet, 20 ppm IM in the drinking water or both chemicals together until experimental week 28. DHEA treatment enhanced hepatocarcinogenesis, but the combined treatment with IM suppressed the liver tumor incidence as compared to the DHEA alone group, while DHEA inhibited the development of thyroid tumors. In addition, this hormone also decreased the development of preneoplasias in urinary bladder and seminal vesicles. IM clearly suppressed development of preneoplasias or neoplasias in lung, and small and large intestines. The present results indicate that although DHEA and IM have significant chemopreventive efficacy against the multi-organ carcinogenesis with the exception of the DHEA-treated liver case, treatment with combination did not result in amplification of their chemopreventive effects.

1167 EFFECTS OF DEHYDROEPIANDROSTERONE AND ITS STRUCTURAL ANALOG ON AFLATOXIN B_1 CARCINOGENESIS IN TROUT

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The adrenal steroid dehydroepiandrosterone (DHEA) has chemoprotective properties towards a wide variety of conditions including cancer, obesity, diabetes, and cardiovascular disease. However, DHEA is also a carcinogen in laboratory animals, possibly due to metabolism to other steroids or through peroxisome proliferation. A structural analog of DHEA, 16α-fluoro-5-androsten-17-one (8354) has been reported to have enhanced chemopreventive activity without the steroid precursor and peroxisome proliferating effects. This study was designed to compare DHEA and 8354 in rainbow trout, a species previously shown to be highly susceptible to the carcinogenic and tumor enhancing properties of DHEA. Trout were exposed as fry to aflatoxin B1 (AFB1) or given a sham exposure, then were fed diets containing 444 ppm DHEA or 8354 for six months. Post-initiation treatment with DHEA significantly increased liver tumor incidence, multiplicity, and size compared to initiated controls. 8354 slightly increased tumor incidence ($p < 0.06$) but had no effect on multiplicity or size. Six percent of trout treated with DHEA alone developed tumors, while no tumors occurred in noninitiated trout fed control diets or 8354. Serum androstenedione and estradiol were elevated in DHEA-, but not 8354-fed trout. Peroxisomal βoxidation was not increased by either compound. This study suggests that the carcinogenicity of DHEA may be due to its steroid precursor rather than peroxisome proliferating effects. Our research strengthens other studies which advocate 8354 as an alternative to DHEA for chemoprevention. (Supported by ES04766 and ES07060).

1168 β-NAPHTHOFLAVONE, AN AFLATOXIN B_1 CARCINOGENESIS INHIBITOR, ENHANCES AND INHIBITS MICROSOMAL ACTIVATION OF AFLATOXIN B_1 IN THE RAT

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β-Naphthoflavone (BNF) is a synthetic flavonoid and model *Ah* receptor agonist that has been previously shown to inhibit aflatoxin B_1 (AFB_1)-induced hepatocarcinogenesis in both trout and rats. The mechanism of anti-carcinogenesis has been ascribed to the induction of CYP1A subfamily enzymes that enhance AFB_1 metabolism toward the less carcinogenic 9a-hydroxylated metabolite of AFB_1, aflatoxin M_1. Recent evidence from our laboratory indicate that BNF is a potent inhibitor of AFB_1 activation by trout liver microsomes as determined *in vitro* by inhibition of [^{3}H]AFB_1-DNA adduction. In this study, we examined the effect of BNF on AFB_1 activation by rat liver microsomes. We used the AFB_1-DNA binding assay and an HPLC assay whereby the primary DNA adducting species, AFB_1 8,9-epoxide, can be quantified as a stable glutathione conjugate. By this method, quantification of microsomal AFB_1 detoxication products aflatoxin M_1, aflatoxin Q_1 and aflatoxin P_1, is also possible. Similar to results obtained in the trout, we found that BNF inhibits activation, but only at less the 25 μM BNF. In contrast to the trout, when BNF is present *in vitro* at 25–200 μM, activation is enhanced up to 2-fold. At all concentrations of BNF, we found inhibition of formation of the phase I detoxication metabolites, except that AFP_1 formation was stimulated by 25, 50 and 100 μM BNF. These results suggest that, during BNF modulation of AFB_1 carcinogenesis in the rat, conditions may exist where enhancement of carcinogenesis at the initiation step may occur. However, the possibility is also suggested that inhibition of CYP bioactivation, at least at low exposures to BNF, may constitute a mechanism of inhibition of AFB_1 carcinogenesis in the rat. (This work was supported by grants CA 34732, ES 03850, ES 00210 and T32 ES 07060).

1169 INHIBITION OF AFLATOXIN B1-DNA ADDUCT FORMATION BY ELLAGIC ACID IN CULTURED HUMAN KERATINOCYTES

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The potential chemoprotective activity of ellagic acid (ELA) against the genotoxicity of aflatoxin B1 (AFB1) was investigated in a human line of spontaneously immortalized keratinocytes. The cells were preincubated with ELA (13 mM) for 24 h before incubation with both AFB1 (39 mM) and ELA (13 mM) for additional 48 h. The cellular DNA was extracted and isolated for determination of the presence of AFB1-DNA adducts (ADA). ADA were calculated from the amount of AFB1-dihydrodiol determined in a known amount of DNA using an acid-hydrolysis-HPLC procedure. Approximately 3 ADA per million nucleotides were detected in the DNA without the ELA treatment. With the ELA treatment, the ADA formation was reduced by 65%–90%. The chemoprotective activity of ELA against AFB1 appeared to be associated with the induction of AFB1 metabolic enzymes. The enzymes involved will be elaborated. (Supported by CONACYT, Mexico and by NIEHS Center Grant ES05707).

1170 EFFECTS OF PHENETHYL ISOTHIOCYANATE (PEITC), ELLAGIC ACID (EA), SULINDAC AND SUPPLEMENTAL DIETARY CALCIUM (SDC) ON THE INDUCTION AND PROGRESSION OF ESOPHAGEAL CARCINOGENESIS IN RATS INDUCED BY SHORT-TERM ADMINISTRATION OF N-NITROSOMETHYLBENZYLAMINE (NMBA)

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The potential inhibitory effects of PEITC, EA, Sulindac and SDC on NMBA-induced esophageal carcinogenesis were evaluated in rats utilizing an abbreviated NMBA treatment protocol which allowed administration of the putative inhibitors throughout the experiment, or following completion of NMBA dosing only. PEITC at 500 ppm significantly inhibited tumor incidence and multiplicity when given during, but not following NMBA treatment, demonstrating the PEITC selectively inhibits the induction, but not the subsequent progression of NMBA-induced esophageal tumors. This observation is consistent with the proposed mechanism by which PEITC inhibits nitrosamine-induced carcinogenesis (i.e., inhibition of carcinogen activation). Neither Sulindac at 125 ppm, nor SDC (2% vs 0.5% in control diet) inhibited tumor development when given during or following NMBA treatment. EA, which was administered only following NMBA treatment, significantly reduced the incidence (66.7% vs 93.3% in NMBA controls) but not the multiplicity of esophageal tumors at the high-dose (4000 ppm) level, suggesting that EA may repress tumor development when administered following NMBA treatment.

1171 EFFECTS OF EXERCISE-TRAINING ON THE HEPATOTOXICITY OF 17 α-ALKYLATED ANABOLIC-ANDROGENIC STEROIDS IN PRIMARY RAT HEPATIC CELL CULTURES

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Hepatic complications in athletes and bodybuilders after abusing anabolic-androgenic steroids (AAS) have recently been reported. Hepatic injury including cholestasis, peliosis hepatis, hyperplasia and tumors have been attributed to abuse of the 17 α-alkylated steroids. Some of these conditions have been reversed when an individual's therapy was changed to non-alkylated steroids. The purpose of this study was to evaluate the direct toxic effects of 2 commonly abused 17 α-alkylated AAS in primary liver cell cultures obtained from exercise-trained and sedentary rats. Cultures were established from 99–128 day old sedentary and swim-trained Sprague-Dawley rats and exposed to doses of 1×10^{-5}, 4×10^{-5}, and 1×10^{-4} M oxymetholone (Oxy) and methyltestosterone (MT). Swim-training consisted of two 75-min swim sessions a day, 5 days a wk, for 8–13 wks. Indices of toxicity (LDH release) were determined at 0.25, 0.5, 1, 2, 3, 4, and 6 hr for Oxy and at 2, 4, 6, and 8 hr for MT. In cultures of swim-trained rats, exposure to 1×10^{-4} M Oxy produced significant LDH release at 1, 2, 3, 4, and 6 hr, whereas in cultures of sedentary rats LDH release was significant only at 2, 3, 4, and 6 hr. Swim-trained cultures exposed to 1×10^{-4} M MT demonstrated significant LDH release at 4, 6, and 8 hr, whereas cultures of sedentary rats had significant LDH release at only 6 and 8 hr. These two 17 α-alkylated steroids (MT, Oxy) commonly abused by athletes caused injury to the plasma membrane, as seen by increased LDH release, at earlier time periods and to a greater degree in exercise-trained rats than in the sedentary group These results indicate exercise-training may enhance the hepatotoxicity of MT and Oxy.

1172 LATE PROTECTIVE EFFECT OF DIMETHYL SULFOXIDE IN THE GUINEA PIG MODEL OF HALOTHANE HEPATOTOXICITY

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Dimethyl sulfoxide (DMSO), which can inhibit P450 2E1 based metabolism, has been shown to protect the liver against acetaminophen induced necrosis if coadministered with the drug. DMSO was evaluated as a late hepatoprotective agent in the guinea pig model of halothane ($CF_3CBrClH$; **H**) associated hepatotoxicity. Male outbred Hartley guinea pigs (600–700 g) were exposed to 1.0% (v/v) **H**, 40% O_2, for 4 hr, and then administered DMSO (2 ml/kg, ip) 10 hr after the end of exposure. DMSO proved to be hepatoprotective. Plasma ICDH activity was decreased 24 hr after **H** + DMSO compared to **H** + saline(S) animals (**H** + S = 1,538 ± 646, **H** + DMSO = 357 ± 151 U/L, $p < 0.05$, $\bar{x} \pm$ SEM, N = 4, untreated control = 31 ± 2). Centrilobular necrosis that developed in 2/4 of the **H** + S group was not present in **H** + DMSO animals. Biotransformation of **H** by P450 2E1 produces the metabolite, trifluoroacetic acid (TFA) which has a plasma half-life of ≈48 hr. Following 1% **H** exposure, plasma TFA concentrations are not observed to decrease until >48 hr after due to ongoing biotransformation of residual quantities of **H**. DMSO treatment produced decreased plasma TFA concentrations 48 hr after **H** exposure (**H** + S = 1900 ± 130, **H** + DMSO = 1440 ± μM, $p < 0.03$, N = 4). Further studies will determine whether H biotransformation that occurs long (>10 hr) after acute exposure is critical to lesion development or if DMSO has other protective actions than inhibiting P450 2E1 metabolism.

1173 MINIMIZATION OF THIOACETAMIDE-INDUCED LIVER TOXICITY BY GADOLINIUM CHLORIDE

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Activated Kupffer cells release profibrogenic cytokines such as TGFβ there-

fore, it is likely Kupffer cells play a role in fibrogenesis after chronic alcohol. Because of difficulties involved with evaluating this hypothesis in chronic alcohol models, we used a model in which fibrosis is induced chemically with thioacetamide (TAA). TAA or vehicle was administered three times per week via i.p. injection. A subgroup of animals was also treated twice weekly with $GdCl_3$, a particle which selectively destroys Kupffer cells. At 2 and 4 weeks, livers were perfused and oxygen uptake and carbohydrate production were measured. Activation of Kupffer cells by carbon particles increased oxygen uptake over basal values by 5 μmoles/g/h in controls. In contrast, oxygen uptake was increased in livers from TAA-treated rats by 34 μmoles/g/h an increase largely blocked by $GdCl_3$ (7 μmoles/g/h). The basal rate of carbohydrate production (control = 60 μmoles/g/h) was reduced by TAA treatment (35 μmoles/g/h); $GdCl_3$ protected against this effect (48 μmoles/g/h). Activation of Kupffer cells with carbon stimulated carbohydrate production in controls, but not in TAA or $GdCl_3$ + TAA animals. Alkaline phosphatase (AP) in plasma was elevated about 4-fold by TAA treatment, an effect which was also largely blocked by $GdCl_3$. Plasma aspartate aminotransferase (AST) levels were unaltered in all groups. These results show that $GdCl_3$ attenuates the stimulation of carbon-induced oxygen uptake and the release of AP into the plasma caused by TAA treatment. This is consistent with the hypothesis that Kupffer cells are involved in the mechanism of hepatic fibrogenesis. (AA-03624 and ES-04325).

1174 GADOLINIUM CHLORIDE ATTENUATES ACETAMINOPHEN HEPATOTOXICITY IN MICE

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Acetaminophen (APAP) administration causes accumulation and activation of macrophages in the livers of rats, a species insensitive to the hepatotoxic effects of APAP. To explore whether hepatic macrophages participate in APAP hepatotoxicity in a sensitive species, mice were pretreated with $GdCl_3$ (two injections of 7 mg/kg,iv, 24 hrs apart) or its vehicle, then given APAP (300 mg/kg, i.p.) or its vehicle 24 hr later. This dosing regimen of $GdCl_3$ reduced the clearance of colloidal carbon in vivo, suggesting that it impaired macrophage function. APAP treatment caused liver injury as indicated by elevated plasma alanine aminotransferase (ALT) activity (3591 ± 573 IU/ml), and this was markedly attenuated by $GdCl_3$ pretreatment (1261 ± 448 IU/ml). To verify that $GdCl_3$ did not provide protection via inhibition of APAP bioactivation, liver glutathione was evaluated 1 hr after APAP administration. $GdCl_3$ treatment did not influence the decrease in liver glutathione caused by APAP. These results suggest that inhibition of hepatic macrophage function protects against APAP hepatotoxicity and that macrophages contribute to this hepatotoxic response. (Supported by NIH grant ES04139).

1175 RETINOL PRETREATMENT POTENTIATES ACETAMINOPHEN-INDUCED HEPATOTOXICITY IN THE MALE SWISS WEBSTER MOUSE

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Previous results have shown that retinol pretreatment potentiates acetaminophen-induced (APAP) hepatotoxicity in male Swiss Webster mice. The objective of this study was to further characterize the role of retinol in the poteniation of APAP-induced liver injury in the mouse. One, 2 and 3 days of retinol pretreatment (75 mg/kg/d, po) did not potentiate the hepatotoxicity of APAP. However, after 4 days of retinol pretreatment liver injury was potentiated (ALT activity of 1269 ± 280 vs. 4017 ± 811 for vehicle and retinol treatments, respectively, $p < 0.01$). When the activity of cytochrome P450 was inhibited by administration of 1-aminobenzotriazole (ABT, 50 mg/kg) there was no evidence of liver injury in the treatment groups. Therefore, APAP must be metabolically activated in order for retinol to potentiated its hepatic injury. Reactive oxygen species are involved in the potentiation of CCl4-induced liver injury in rats. To determine their role in this model PEGSOD (10,000 U/kg, iv) was administered 2 h post APAP treatment. This treatment significantly blocked the retinol-induced potentiation of APAP-induced liver injury (3834 ± 958 vs. 895 ± 441 for retinol + APAP and retinol + APAP + PEGSOD respectively). These results demonstrated that an increase in active oxygen species and iron species was present in the retinol potentiated mice. Since glutathione (GSH) is the major detoxification route for APAP, the effect of retinol on GSH was also measured. The results showed that retinol did not alter GSH significantly. From the data obtained it appears as though reactive oxygen species play a role in retinol's potentiation of APAP-induced liver injury. Further experimentation will more closely examine the role of these active oxygen species. (This work is supported by NIEHS ES05583 and NIEHS ES06095).

1176 RETINOL ALTERATION OF 1-NITRONAPHTHALENE-INDUCED INJURY BY MODULATION OF ASSOCIATED INFLAMMATORY RESPONSES

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Pretreatment of male Sprague-Dawley rats with retinol protects against 1-nitronaphthalene (1-NN) induced pulmonary toxicity, but potentiates 1-NN hepatotoxicity. The objective of this study was to further characterize the effect of retinol on the pulmonary and hepatic toxicity of 1-NN in the rat. *all-trans*-Retinol (75 mg/kg/d) or vehicle was administered orally for 7 days. On day 8, animals were given 1-NN (100 mg/kg, *ip*). At 24 hr, retinol vehicle rats showed pulmonary morphological changes included necrosis and exfoliation of the nonciliated bronchiolar epithelium, as well as infiltration of inflammatory cells into the interstitial areas around affected bronchioles. Following pretreatment with retinol, evaluation of the lungs from these animals revealed bronchiolar cell necrosis restricted to the nonciliated bronchiolar epithelial cells with no associated inflammation. In the liver, retinol pretreatment potentiated hepatocyte and bile duct epithelial cell (BEC) necrosis, as well as the associated infiltration of inflammatory cells into the areas around bile ducts. Retinol potentiation of 1-NN-induced hepatocyte necrosis was significantly reduced following pretreatment with $GdCl_3$, an inhibitor of Kupffer cell functions. However, $GdCl_3$ did not limit the amount of BEC necrosis caused by 1-NN. These results demonstrate that in the lung, pretreatment with retinol decreases the severity of 1-NN-induced toxicity apparently by inhibiting the associated inflammatory response and not by altering the initial cellular injury. In the liver, retinol potentiates 1-NN-induced liver injury apparently by increasing the infiltration of inflammatory cells, thereby causing increased hepatotoxicity. (Supported in part by NIEHS center grant, P30 ES 06694 and DuPont Graduate Fellowship).

1177 EFFECTS OF INDUCER AND INHIBITOR ON CAFFEINE-INDUCED POTENTIATION OF ALLYL ALCOHOL (AA) HEPATOTOXICITY IN ISOLATED RAT HEPATOCYTES

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Our previous studies have shown the potentiating effects of caffeine (CF) on AA-induced hepatotoxicity in isolated rat hepatocytes (Toxicologist 14: 370, 1994). The present study was designed to investigate the mechanism of such potentiating effects using isolated hepatocytes from adult male Spague-Dawley rats. AA (2.5 mM)-induced loss of hepatocyte viability after 60 min incubation was significantly reduced and returned to nearly control values when pretreated with 4-methylpyrazole (4-MP) (1 mM). Similarly, reduced hepatocyte viability due to combined AA and CF (5 mM) was prevented in the presence of 1 mM 4-MP. Pretreatment with 4-MP (an inhibitor of alcohol dehydrogenase) (0.5 mmol/kg, i.p.) for 30 min reduced significantly the increase of serum lactate dehydrogenase (LDH) induced by AA (1.8 mg/kg, i.p.) at 24 h. Similar *in vivo* pretreatment also reduced the increase of serum LDH at 24 h after combined *in vivo* treatment of AA and CF (75 or 150 mg/kg, oral). Reduced hepatocyte viability due to AA plus CF was further reduced by phenobarbital (PB) (80 mg/kg, 3 days) pretreatment. Increase of serum LDH due to AA plus CF *in vivo* was further increased at 24 h following PB pretreatment. These data suggest that caffeine potentiation of AA hepatotoxicity depends on (1) conversion of AA to acrolein and (2) metabolic activation of CF by PB-inducible cytochromes P450. (Supported by World University Services of Canada).

1178 LIPOPOLYSACCHARIDE ENHANCES THE HEPATOTOXICITY OF ALLYL ALCOHOL IN SPRAGUE-DAWLEY RATS

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Lipopolysaccharide (LPS), which derives from gram-negative bacteria, activates phagocytic cells. Kupffer cells (KCs), the resident macrophages of the liver, have been implicated in hepatotoxicity due to allyl alcohol (AA). Accordingly, the effect of LPS on AA-induced liver toxicity was examined. LPS (0.1

mg/kg iv) or saline was injected into male, Sprague-Dawley rats (200–300 gm) two hours prior to injection of AA (30 mg/kg ip) or saline. The rats were sacrificed 18 hrs after administration of AA. Activity of plasma alanine aminotransferase (ALT), and gross and microscopic pathology were used to evaluate liver damage. In a separate study, gadolinium chloride ($GdCl_3$) was used to block KC function. $GdCl_3$ (10 mg/kg iv) was administered 22 hrs prior to injection of LPS or saline. The rest of the experiment proceeded as described above. Liver injury due to either LPS or AA alone was minimal Animals treated with both LPS and AA had significant increases in ALT compared to animals treated with either LPS or AA alone. Both gross and microscopic pathology correlated with plasma ALT activity. KC inhibition reduced plasma ALT activity in AA-treated rats cotreated with LPS, suggesting LPS potentiates the toxicity of AA through a KC-dependent mechanism. These data suggest that LPS may have a synergistic role in xenobiotic-induced hepatotoxicity. (Supported by NIH Grant ES05772.)

1179 POTENTIATION OF LIPOPOLYSACCHARIDE-MEDIATED HEPATIC INJURY BY POLYCHLORINATED BIPHENYLS (PCBs)

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Neutrophils (PMNs) are required for the development of hepatic injury upon exposure to lipopolysaccharide (LPS). Following iv administration of LPS, PMNs rapidly accumulate in the liver, and midzonal hepatic necrosis is prominent by 6 hr. PCBs stimulate PMNs to release cytolytic enzymes and produce superoxide anion *in vitro*. This study examined whether PCB exposure potentiates PMN-dependent injury in a model of LPS-induced hepatotoxicity. Female, Sprague-Dawley rats (175–220 g, N = 5–11) were treated with a minimally toxic dose of LPS in saline (2 mg/kg, iv) and 1.5 hr later were exposed to the PCB mixture, Aroclor 1248 by ip injection. The animals were killed 6 hr post-LPS exposure and hepatic injury was assessed. Cotreatment with 50 mg/kg Aroclor 1248 resulted in potentiation of injury compared to LPS alone, as demonstrated from increased activities in plasma of alanine aminotransferase (200 ± 45 vs 1418 ± 513 U/L, respectively) and isocitrate dehydrogenase (37 ± 11 vs 1005 ± 540 U/L, respectively). Histopathological evaluation indicated increases in severity of hepatic necrosis in rats receiving both LPS and Aroclor 1248. Aroclor 1248 did not cause hepatotoxicity. Hepatic accumulation of PMNs was not altered by PCB exposure. PCBs may act in a fashion analogous to inflammatory stimuli in activating PMNs to become cytotoxic. (Supported by NIH grant ESO4911).

1180 MORPHINE POTENTIATION OF CARBON TETRACHLORIDE HEPATOTOXICITY IN MICE

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Previous studies have shown that cotreatment of mice with morphine through pellet implantation potentiates the mortality produced by carbon tetrachloride (CCl4). The objective of the present study was to examine more closely the exacerbation of CCl4 toxicity by morphine. ICR male mice were treated concurrently by the i.p. route with morphine and CCl4 in varying dosages. A 100 mg/kg morphine dose increased significantly the extent of hepatic injury, as indicated by serum alanine aminotransferase (ALT) activities, resulting from 0.01, 0.02, or 0.04 ml/kg CCl4. A morphine dose ≥75 mg/kg was required to produce a significant potentiation of CCl4 toxicity. Intracerebroventricular (icv) injection of morphine in doses of 0.1 μg or more also produced significant increases in serum ALT activity following CCl4 (0.02 ml/kg) administration, when compared with mice cotreated with saline icv, suggesting that morphine effects on CCl4 hepatotoxicity may originate within the CNS. Attempts to block morphine effects on CCl4 toxicity with an opioid receptor antagonist, naltrexone, were unsuccessful. However, it appears that this may be due to an effect of naltrexone itself on CCl4 toxicity. Subsequent experiments found that naltrexone increased CCl4 toxicity in a dose-dependent manner. The ability of opioid agonist [morphine] and antagonist [naltrexone] drugs to increase CCl4 hepatotoxicity has interesting mechanistic implications and merits further investigation. (Supported by ES-05216).

1181 THE ROLE OF KUPFFER CELLS IN THE POTENTIATION OF CCl_4-INDUCED LIVER INJURY BY A SINGLE DOSE OF RETINOL

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Seven days of pretreatment with retinol potentiates the hepatotoxicity of a number of chemicals in rats. The mechanism of this potentiation involves priming of Kupffer cells (KC). These primed KC release increased levels of superoxide anion and other reactive species of oxygen (ROS) to promote the progression of injury. The purpose of this study was to determine if a shorter retinol treatment regimen potentiates chemical-induced hepatotoxicity. Male S/D rats were pretreated with retinol (75 mg/kg, *po*) 24h prior to CCl_4 treatment (0.2 ml/kg, *ip*). Plasma ALT activities 24h following CCl_4 were 2687 ± 366 U/L in retinol pretreated animals, 220 ± 51 U/L in control animals receiving CCl_4 and 29 ± 1 U/L in retinol controls. Histological examination of liver sections and immunohistochemistry staining for macrophages (ED2) and neutrophils (HIS48) were performed. Severe centrilobular to mid-zonal necrosis with an increase in macrophage and neutrophil infiltration was observed in retinol pretreated animals given CCl_4. In subsequent experiments, rats were pretreated with gadolinium chloride ($GdCl_3$) to selectively destroy Kupffer cells. While this treatment caused no significant reduction in control rats receiving CCl_4, $GdCl_3$ reduced ALT activity by 55% in retinol/CCl_4 treated animals. Histological evaluation confirmed that $GdCl_3$ treatment decreased the amount of potentiated liver injury. Preliminary studies have also shown that while total P450 is not increased, CYP 2E1 activity is elevated after a single dose of retinol. These findings suggest that a single pretreatment with retinol may potentiate CCl_4 hepatotoxicity by more than one mechanism. (Supported by NIEHS ES 06095 and Center Grant #P30 ES06694).

1182 INVOLVEMENT OF KUPFFER CELL IN CCl_4-INDUCED LIVER INJURY AND THE ROLE OF CALCIUM

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The hypothesis that calcium provoke O_2^- formation by Kupffer cells and may contribute to carbon tetrachloride(CCl_4)-induced liver injury was studied in SD rats. In CCl_4-treated animals, hepatic malonaldehyde (nmole/gm liver) and plasma ALT (IU/ml) levels elevated significantly from 10.66 ± 1.07 to 54.97 ± 1.27 and from 22.2 ± 1.5 to 2229.2 ± 205.9, respectively, compared to those in controls. Activation of Kupffer cells with high dose of retinol (250,000 IU/kg/day, po, for 7 day) significantly enhanced ALT levels, while inactivation of Kupffer cells with gadolinium chloride (7.5 mg/kg/day, ip, for 2 day) attenuated the increase of plasma ALT level following CCl_4 treatment. Diltiazem (10 mg/kg/day, ip for 2 day) given in combination with retinol led to a marked decrease in ALT levels compare to the level in rats treated only with retinol against CCl_4 treatment. In order to determine any alterations in cytochrome P450 activities, the P450 content and the CYP2E1 activity were measured and all CCl_4-treated rats showed significantly lower levels compared to those in controls and vehicle-treated animals. There were significant increases in glutathione peroxidase in all CCl_4-treated rats except diltiazem treated animals. No difference was found among untreated and vehicle-treated rats. It is concluded that Kupffer cells contribute to CCl_4-induced liver injury and that calcium antagonist attenuated the increased CCl_4-induced liver injury due to activation of kupffer cells.

1183 COLCHICINE ANTIMITOSIS ALTERS THE OUTCOME OF ISOPROPANOL-POTENTIATED CARBON TETRACHLORIDE HEPATOTOXICITY

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The objective of this study was to investigate the role of cell division and tissue repair in animal survival despite significantly enhanced CCl_4 liver injury by isopropanol. Male Sprague Dawley rats (175–225 g) were treated with isopropanol (2.5 ml/kg, po) 24 hr prior to CCl_4 (1 ml/kg, ip) administration. Injury was measured as serum enzyme (ALT and SDH) elevations and ^{3}H-thymidine (^{3}H-T) incorporation into hepatonuclear DNA was measured as an index of cell division and tissue repair during a time course (0–96 hr) after the administration of CCl_4. Maximum injury was observed at 36 hr in both groups as evidenced by elevated serum enzyme levels which was concordant with the maximum glycogen depletion and minimal tissue repair. Maximum ^{3}H-T incorporation occurred at 48 hr in both the groups (ISOP + CCl_4, H_2O + CCl_4) indicating maximum stimulation of cell division. The wave of tissue regeneration continued upto 72 hr in the ISOP + CCl_4 group but returned to quiescent

control level in the H_2O + CCl_4 group by that time. Antimitotic intervention of tissue repair processes with colchicine (1 mg/kg, ip) 42 hr after CCl_4 administration led to increased mortality due to diminished tissue repair leading to unrestrained progression of liver injury, and liver failure. These findings suggest that while isopropanol-enhanced infliction of liver injury might be explained by enhanced bioactivation of CCl_4, the animals are nonetheless rescued by augmented and sustained stimulation of tissue repair. These results further underscore the importance of hepatocellular regeneration and tissue repair in the final outcome of toxicity inflicted by hepatotoxicants. (Supported by The Burroughs Wellcome Fund).

1184 *IN VITRO* EFFECTS OF ESTRADIOL (E_2) AND INDOMETHACIN (IN) ON RABBIT ERYTHROCYTE FRAGILITY (EF) CHARACTERISTICS

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An in vitro study was conducted to determine whether IN and E_2-induced decreases in rabbit hematocrit may be related to their effect on erythrocyte fragility. Two ml aliquots of treated rabbit whole blood were assayed as; Control, IN (9.6 μg/ml), E_2 (500 pg/ml) and IN + E_2, for changes in EF. Osmotic (OF) and mechanical (MF) fragility were evaluated under approximate physiological conditions by measurement of hemoglobin release at 545 nm. Blood samples at 39.5°C were assayed immediately after drug addition (*i*) and again 4 hours after incubation (*f*). Eight replicates of each experiment were run. OF results showed a significant increase ($p < 0.05$) in 50% hemolysis point between IN*i* and IN + E_2*f* when compared to their initial values and to Controls. OF hemolysis dispersion was increased over time by IN and IN + E_2. MF increased with IN, E_2 and IN + E_2 versus their initial values and the Controls. While the increase in MF from IN was greater than that from E_2, the MF from the combination (IN + E_2) was not greater than from IN alone. The IN-induced increases in both OF and MF indicate a difference in degree of interaction with the erythrocyte from that of E_2, which only affected MF and whose effect was not additive or synergistic with that of IN.

1185 TOXICITY AND POSSIBLE POTENTIATION OF COMPONENTS IN COPPER-CONTAINING WOOD PRESERVATIVE

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This study was performed to assess the subchronic toxicity of POL-NU CuRAP, a wood preservative, following oral administration in rats. The second objective was to determine whether the two active components cause potentiation of any systemic effects produced by either component when administered alone. The formulation was administered by oral gavage at doses of 250, 500 and 1000 mg/kg/day to groups of 10 rats/sex for at least 30 days. The active components of the formulation, Cunapsol-5 and Borax 10 MOL, were each given to groups of 10 rats/sex at concentrations equivalent to those found in the highest concentration of the formulation. Toxicological effects of the formulation were noted in the hematopoietic system and appeared to be associated with erythrocyte production. Lower erythrocyte count and hemoglobin and hematocrit values were observed as well as lower cholesterol and total protein levels. Changes in erythrocyte morphology were also evident. The effects obtained in animals administered the formulation could be directly attributed to either Cunapsol or Borax. Statistical comparison of affected parameters from the Cunapsol, Borax and POL-NU CuRAP groups did not reveal any evidence of potentiation after combination of the two components in the formulation. The NOEL for the formulation was 250 mg/kg/day.

1186 INHIBITION OF HUMAN TOPOISOMERASE II BY BENZENE METABOLITES

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In recent years, a number of human leukemia-inducing agents have been shown to exert their effects through the inhibition of topoisomerase enzymes. Benzene, a human carcinogen, may exert its clastogenic and carcinogenic effects through a similar mechanism. To test this hypothesis, the inhibitory effects of various phenolic and quinone metabolites of benzene (phenol, hydroquinone, 1,4-benzoquinone, catechol, 1,2,4-benzenetriol, 2,2′-biphenol and 4,4′-biphenol) on the activity of human topoisomerases I and II were studied *in vitro*. No inhibition of topoisomerase I was seen with any of the above compounds. Inhibition of topoisomerase II was not observed for hydroquinone, phenol, 2,2′-biphenol, 4,4′-biphenol and catechol at concentrations as high as 500 μM. 1,4-Benzoquinone and 1,2,4-benzenetriol inhibited topoisomerase II at 500 and 250 μM concentrations, respectively. Interestingly, following peroxidase-mediated bioactivation, partial inhibitory effects were observed at concentrations as low as 50 μM for both phenol and 2,2′-biphenol. For 4,4′-biphenol in the presence of bioactivation, a complete inhibition was observed at 50 μM and a partial inhibition at 10 μM. The addition of reduced glutathione (GSH) to the 4,4′-biphenol and horseradish peroxidase reaction system protected topoisomerase II from inhibition suggesting that diphenoquinone or another unknown metabolite formed from 4,4′-biphenol might be the reactive species. These results indicate that inhibition of topoisomerase II may be an important event in the clastogenic and carcinogenic effects of benzene.

1187 LEAD INDUCED ALTERATIONS IN HEMATOPOIETIC PROGENITOR CELLS MAY BE MEDIATED BY REACTIVE OXYGEN INTERMEDIATES

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Inorganic lead (Pb) is a hematotoxic environmental contaminant. We have previously demonstrated that 10^{-12} M Pb enhances colony forming response to granulocyte/macrophage colony stimulating factor (GM-CSF), mimicking synergism observed with c-kit ligand (CKL). We now report that reactive oxygen intermediates (ROI) may be involved in Pb induced hematopoietic alterations. H_2O_2 (HP) is a documented generator of ROIs. Pretreatment of murine bone marrow cells with 10^{-8} M HP results in a 25–50% increase in colony formation in response to GM-CSF. This data supports the newly emerging role of ROIs in intracellular signaling and modulation of cellular proliferation. CKL and HP act interchangeably to enhance GM-CSF stimulated colony formation suggesting that HP and CKL act on the same population of HPCs. HP-induced enhancement is suppressed by pretreatment with 10^{-7} M epoxybutene that has been shown to selectively suppress CKL synergism with GM-CSF. Because Pb produced a pattern of results identical to HP, we hypothesized that Pb and HP are acting on the same CKL responsive population of HPCs. Doses of Pb and HP that result in enhancement of clonogenic response do not alter intracellular [GSH] and are not believed to result in oxidative stress. Subsequently, we looked at the effects of ROI scavengers on Pb and HP induced GM-CSF enhancement. 4-Hydroxy-tempo and N-*tert*-butyl-α-phenylnitrone, nitroxide spin traps that are ROI scavengers with little or no affinity for metal chelation, completely suppressed Pb- and HP-induced enhancement of GM-CSF. Therefore we conclude that Pb- and HP-induced enhancement of GM-CSF response is mediated via a pathway involving the production of ROIs. Whether the ROIs are second messengers or modify some component in a signal transduction pathway has yet to be determined.

1188 EVALUATION OF THE ORAL 2-FLUOROANILINE DOSE-RESPONSE FOR BLOOD METHEMOGLOBIN FORMATION IN DOGS

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The production of methemoglobinemia is the most frequently reported adverse effect in humans resulting from exposure to aniline and derivatives. Blood methemoglobin (MetHb) is a sensitive *in vivo* biomarker of exposure to anilines. Risk characterization for exposure to the halogenated derivative 2-fluoroaniline (2-FA) has been complicated by the absence of adequate toxicity data. This study evaluated the oral 2-FA dose/MetHb response in dogs, a sensitive species to the MetHb-forming activity of anilines. Beagle dogs (3/sex) were administered single escalating doses of 2-FA (2 to 50 mg/kg) by gavage and, in addition to routine chemistry and hematology tests, MetHb was measured in serial blood samples (jugular vein). A superproportionate increase in maximum MetHb concentration with dose was observed, with the time to maximum MetHb response and for eventual decay to background levels also increasing with dose. The apparent no-effect (NOAEL) level was 2 mg/kg and the low-effect (LOAEL) level was 5 mg/kg (Mean 0.9 ± 0.6% SD of total Hb pigment, ≈35 min post dose). Increased heart rate (3/6 dogs) and elevated hepatic enzymes (1/6) were observed at the 50 mg/kg dose. The mean maximum MetHb concentration at the high dose was 19.6 ± 6.6% at ≈2.6 hr. Following this high dose, re-challenge at the NOAEL/LOAEL demonstrated no apparent carryover response. No other chemistry or hematologic changes were observed at all doses. These data indicate that blood MetHb is a sensitive *in vivo* biomarker of 2-FA exposure in dogs which could be used to predict the potential for other aniline-associated responses.

1189 THE EFFECTS OF INTERLEUKIN-1β AND TUMOR NECROSIS FACTOR-α ON *IN VITRO* COLONY FORMATION BY HUMAN HEMATOPOIETIC PROGENITOR CELLS EXPOSED TO DOXORUBICIN OR HYDROQUINONE

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Treatment of bone marrow (BM) cells with IL-1β or TNFα can protect hematopoietic progenitor cells (HPC) from the toxic effects of 4-hydroperoxycyclophosphamide (4HC) or γ-irradiation. Since doxorubicin (DX) and hydroquinone (HQ) appear to inhibit hematopoiesis through mechanisms similar to 4HC and γ-irradiation, it was of interest to determine whether IL-1β or TNFα also could protect from DX and HQ. BM mononuclear cells (BMMNC) or purified HPC (pHPC) were exposed to 50 ng/mL IL-1β or 25 ng/mL TNFα alone or in combination with DX or HQ for 22hr at physiological O_2 partial pressure and temperature. The cells were washed and plated in GM-CSF-, IL-3- and SCF-containing semisolid medium. Under these concurrent cytokine ± toxicant treatment conditions, neither IL-1β nor TNFα alone significantly affected CFU-C frequencies or lineage commitment compared with the medium-treated controls. However, treatment with either 100 nM DX or 30 μM HQ alone reduced CFU-C frequencies by approximately 70%. When BMMNC were used, neither IL-1β nor TNFα consistently protected CFU-C from either DX or HQ. In contrast, IL-1β or TNFα protected pHPC from DX resulting in a 100% increase in CFU-C compared to pHPC treated with DX alone. TNFα protected pHPC from HQ from all but one donor while IL-1β did not protect CFU-C from HQ. Using pHPC, maximum protection from DX was reached at IL-1β or TNFα concentrations above 10 ng/mL or 1 ng/mL, respectively. Treatment of pHPC with TNFα for at least 8hr was necessary before significant protection from DX could be detected. Therefore, we conclude that IL-1β and TNFα can act directly upon human HPC to protect them from the inhibitory effects of DX and, to a lesser extent, TNFα can directly protect HPC from HQ. (Supported by NIH grants ES05538 and ES05020)

1190 MATHEMATICAL MODELING OF THE OSMOTIC FRAGILITY OF RABBIT RED BLOOD CELLS

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The osmotic fragility (OF) test is used to determine the extent of red blood cell hemolysis (RBCH) produced by osmotic stress. RBCH is dependent upon cell volume, surface area and functional integrity of cell membranes. The variation of cell lysis with stress reflects underlying cell subpopulations and their membranes' functionality. OF was determined on citrate/phosphate/dextrose blood from New Zealand White rabbits by the method of Dacie (1954). The degree of RBCH as a function of NaCl concentration [NaCl] was determined spectrophotometrically by measuring absorbance (Abs) from hemoglobin release at 545 nm. Abs data were fitted to the equation $Abs = p3 \cdot erfc(([NaCl] - p1)/p2)$ where p3 reflects maximum RBCH, p1 measures the [NaCl] at 50% RBCH and p2 shows the dispersion in [NaCl] producing RBCH in the specimen. Parameter values for control blood were p1 = 0.4124; p2 = 0.0503; and p3 = 0.4408 with STD_{fit} = 0.0183. Addition of indomethacin (9.6 μg/ml) produced an increased fragility in the RBC's which was characterized by p1 = 0.4779; p2 = 0.0792; and p3 = 0.4205 with STD_{fit} = 0.0118. Normalization of the data to the value of p3 did not change the values of p1 or p2. These parameters can be used to quantitatively characterize Abs/[NaCl] data and compare pathological, pharmacological or toxicological effects in the OF of RBC's.

1191 ANALYSIS OF BENZENE METABOLITES AND GLUTATHIONE IN THE BONE MARROW OF B6C3F$_1$ MICE

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Our laboratory is studying the effect of inhalation of low benzene concentrations on benzene metabolites and glutathione (GSH) levels in the bone marrow of B6C3F$_1$ mice to determine if there are low-dose nonlinearities. An analytical method was developed for the analysis of metabolites (muconic acid, hydroquinone, and catechol) in blood and bone marrow samples following low concentration exposures. The method was based on extraction of the metabolites from the samples with methanol containing ascorbate, derivatization with pentafluorobenzyl bromide, followed by analysis using isotope dilution, multidimensional gas chromatography/mass spectroscopy. The method was validated by addition and analysis of 2.77, 4.4, and 2.57 ng of catechol, hydroquinone, and muconic acid, respectively, to mouse bone marrow and 10-fold higher amounts of the same metabolites to blood. The amounts were based on expected relative levels of metabolites in blood and bone marrow. Mean recoveries ± SD of seven spiked samples in the bone marrow were 103 ± 2% for catechol, 101 ± 2% for hydroquinone, and 114 ± 4% for muconic acid. Mean recoveries ± SD for the spiked blood samples were 95 ± 8% for catechol, 93 ± 32% for hydroquinone, and 101 ± 7% for muconic acid. GSH was analyzed by the method of Reed *et al.* (*Anal. Chem.* 106:55–62, 1980). Validation of the method by analysis of spiked samples indicated mean recoveries ± SD of 80 ± 7%. Initial studies in mice exposed to 60 ppm benzene for 6 hr indicated significant depletion of bone marrow GSH at the end of exposure. (Research supported by the API through an FIA DE-FI04-94AL97353 and the U.S. DOE/OHER under Contract No. DE-AC04-76EV01013.)

1192 AN EXTERNAL THORACIC DUCT-VENOUS SHUNT TO ALLOW FOR LONG TERM COLLECTION OF LYMPH AND BLOOD IN THE CONSCIOUS PIG

V Mendenhall. *TSI Mason Laboratories, Worcester, MA.* Sponsor: F Mecler

Studies involving the pharmacokinetics of quinolones or cytokines require an animal model which allows for continuous lymph and/or blood sampling. Commonly used procedures to achieve this end result in continuous drainage of the lymph. These procedures are prone to infection, catheter occlusion, and developing physiologic abnormalities. To address these problems, a method of creating an external thoracic duct-venous shunt has been developed to allow for convenient collection of thoracic duct lymph and/or blood in the conscious pig for extended periods of time, while maintaining normal lymph flow. Under strict aseptic procedures, a silastic catheter is placed in the right external jugular vein and exteriorized in the interscapular region. A similar catheter is then placed in the thoracic duct through a right thoracotomy and exteriorized in the midline posterior thoracic region. The two catheters are connected to two three-way stopcocks in series. Flow of lymph from the thoracic duct back to the external jugular vein is thus re-established. A specially prepared aluminum jacket is placed over the pig to prevent disruption of the catheters and stopcocks. The catheters are flushed twice daily with heparin. Lymph and/or blood samples can then be collected from the animal while conscious at any convenient or study-required timepoints. This shunt system in pigs has remained open and functional for over 14 days.

1193 THE HEMATOTOXIC EFFECTS OF 6-HYDROXY-TRANS,TRANS-2,4-HEXADIENAL, A REACTIVE METABOLITE OF TRANS,TRANS-MUCONALDEHYDE, IN CD-1 MICE

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6-Hydroxy-trans,trans-2,4-hexadienal (CHO-M-OH) is a reactive metabolite of trans,trans-muconaldehyde, a microsomal hematotoxic ring-opened metabolite of benzene. In order to assess potential toxic effects of CHO-M-OH on the maturation of erythroid cells in the bone marrow, ten week old male CD-1 mice were administered CHO-M-OH intraperitoneally and [^{59}Fe] incorporation into erythrocytes was measured. The uptake of [^{59}Fe] was significantly inhibited at doses of 20, 25 and 30 mg/kg. There was no inhibition of [^{59}Fe] incorporation at a dose of 15 mg/kg. In other hematotoxicity studies, bone marrow cellularity, peripheral blood cells and sulfhydryl content in bone marrow cells were examined in mice administered CHO-M-OH intraperitoneally. Administration of 10 mg/kg/day for 16 consecutive days caused a significant decrease in sulfhydryl levels of bone marrow cells but no changes in bone marrow cellularity or peripheral blood parameters as compared with controls. At a dose of 25 mg/kg/day for 4 days, there was a significant decrease in nucleated bone marrow cells. The white blood cell count, mainly lymphocytes, also significantly decreased. Our results indicate that CHO-M-OH is a potent hematotoxin in mice and could play a role in benzene toxicity. Supported by NIH Grant ES02558 and NIEHS Center Grant ES05022.

1194 BENZENE-INDUCED HEMATOTOXICITY IN MICE EVALUATED BY FLOW CYTOMETRY

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Evaluation of hematotoxicity in mice at low exposure concentrations of benzene is needed to determine if the response is the same pattern as seen

at high exposures. We developed and tested sensitive assays for evaluating hematopoiesis in the bone marrow of male B6C3F1 mice. Mice were exposed to 300 ppm benzene for 6hrs/day, 5 days/week by inhalation for 5, 12, 26, or 54 days. At each sacrifice timepoint, hematopoiesis was examined by determining the population of erythropoietic cells, granulopoietic cells and B lymphocytes in the bone marrow by specific monoclonal antibody labeling and flow cytometric analysis. The cell replication of each specific cell population was evaluated by BrdU incorporation. At all four timepoints, the total bone marrow cell count in treated mice was significantly decreased. The absolute number of B lymphocytes was 43%, 17%, and 6.3% of controls at 5, 12, and 26 days, respectively. At 54 days of exposure, the B cell population was too depleted to analyze accurately. Replication of the B lymphocytes in treated mice was significantly and progressively increased at all timepoints, reaching twice the control values by day 26. The absolute number of immature and progenitor erythropoietic cells was moderately increased at day 5 and then decreased to 74%, 51% and 51% of control values by days 12, 26 and 54, respectively. The percentage of erythropoietic cells replicating in treated mice was significantly different from controls only at the day 5 sacrifice at which time it was moderately decreased. The absolute number of granulocytic cells decreased in treated mice with only 42% and 30% of control values present at 26 and 54 days of exposure. The evaluation of specific cell populations within the bone marrow of mice demonstrated that at a high concentration of benzene, the reduction in bone marrow cells was caused primarily by cell loss and was not initiated by the blockage of DNA synthesis. This method will be used to characterize benzene-induced alterations in hematopoiesis at lower concentrations.

1195 HOST RESISTANCE TO LISTERIA MONOCYTOGENES FOLLOWING EXPOSURE TO SILICONE H.P. ELASTOMER AND MEDICAL GRADE POLYDIMETHYLSILOXANE FLUID, 1000 CS

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To examine whether silicone can elicit an immunotoxic response, host resistance to *Listeria monocytogenes* was evaluated in female B6C3F1 mice. The test materials, consisting of silicone H.P. Elastomer (1 cm × 1 mm rods) and medical grade polydimethylsiloxane fluid, 1000 cs. (1 ml) were implanted s.c. in the dorsal region. Controls included a sham, vehicle (saline), immunosuppressive (cyclophosphamide, 200 mg/kg, i.p.) and immunopotentiating (*Corynebacterium parvum*, 35 mg/kg, i.v.) treatment group. Secondary control animals were implanted s.c. with 1 cm × 1 mm rods of polyethylene or cobalt chromium. At 14, 45 or 90 days post implantation, 5 concentrations of *Listeria monocytogenes* approximating the LD 10, 30, 50, 70 and LD90 were injected i.v. to allow for expression of immunoenhancement as well as immunosuppression. Test animal mortality was then monitored daily for 14 days after *Listeria monocytogenes* challenge. No differences were noted in mortality with either of the two silicone test groups relative to the sham or saline vehicle control groups. It is concluded that the silicones examined in this test system failed to elicit an immunotoxic response.

1196 ABSENCE OF AN IMMUNOLOGICAL REACTION TO DOW CORNING® SILICONE ELASTOMER AND SILICONE FLUID

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The appearance and histological consistency of tissue capsules which form around silicone implants have raised questions regarding the immunogenicity of silicone materials. Potential systemic consequences of a specific immune reaction toward an implanted material are varied, ranging from mild, fleeting symptoms to severe disruption of the immunoregulatory functions and the development of autoimmune disease. To investigate the potential of silicone breast implant materials (H.P. Elastomer, medical grade polydimethylsiloxane fluid, 1000 cs) to elicit a specific anti-silicone immune response, evidence of immunological sensitization was examined in T cell deficient nude mice (nu/nu) and their immunologically normal heterozygote littermates (nu/+). Animals were sensitized s.c. in the dorsal area with a 1:1 mixture of the silicone test material with Freund's complete adjuvant. Four weeks later animals were challenged s.c. in the abdominal region with the silicone test material only. A heightened and/or accelerated tissue capsule response in the animals presensitized to the appropriate silicone test material would be indicative of a specific immune reaction. The resultant data profiles failed to provide any evidence of immunological sensitization. For each of the two test materials, all eleven histological parameters evaluated were equivalent between the presensitized and nonsensitized test groups at each time point examined (2, 6, and 13 weeks after challenge with a secondary implant). Accordingly, it is concluded that the formation of tissue capsules around H.P. Elastomer and medical grade polydimethyl-siloxane fluid, 1000 cs, do not represent immunologically mediated events, but merely normal wound healing responses to alloplastic materials.

1197 A COMPARATIVE HUMORAL ADJUVANCY STUDY OF LOW MOLECULAR WEIGHT CYCLOSILOXANES (D_4, D_5, D_6, & D_7) IN RATS

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D_4 has been shown to act as an immunological adjuvant by amplifying an IgG antibody response to a co-administered antigen, bovine serum albumin (BSA). To gain further insight regarding the adjuvancy potential of D_4, this present study was designed to: 1) confirm or refute the humoral adjuvancy activity of D_4, 2) compare the relative adjuvant potency of D_4 to other cyclosiloxanes (D_5–D_7), and 3) determine whether any observed adjuvancy activity is unique to silicones by testing non-silicone, personal care product ingredients. Test materials were triturated with BSA through emulsifying needles. Female Sprague-Dawley rats were injected i.m. with 0.3 ml of test preparation which contained 50 ug of BSA. Serum samples were collected at 2, 4, 6, and 8 weeks post injection and anti-BSA IgG titers were quantified by ELISA methodology. Similar to prior results, the D_4 preparation demonstrated marked adjuvant activity at 4, 6, and 8 weeks. The D_5 preparation elicited an antibody response which was similar in time course but decreased in magnitude in comparison to the D_4 response. The D_6 and D_7 preparations demonstrated marginal adjuvant activity which was equal to one another but considerably less than that of D_4 or D_5. The non-silicone preparations (mineral oil, isopropyl palmitate, glycerin, and lanolin) all elicited some degree of adjuvant activity with the exception of glycerin which was totally inactive under these experimental conditions.

1198 NATURAL KILLER CELL ACTIVITY IS ENHANCED FOLLOWING EXPOSURE TO OCTAMETHYLCYCLOTETRASILOXANE (D4)

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Octamethylcyclotetrasiloxane (D4), a volatile low molecular weight cyclic polydimethylsiloxane, is present in numerous products including personal care products (shampoos, deodorants). Furthermore, products containing predominantly high molecular weight polydimethylsiloxanes such as tablet and liquid antacids, contain small amounts of D4. The potential for human exposure by either oral, dermal, inhalation or parenteral routes is significant. To evaluate the response of natural killer (NK) cell activity to exposure to D4, B6C3F1 female mice were administered 1000 mg/kg daily by gavage for 1, 7, 14 or 35 days. After a single day exposure, minimal suppression of NK activity was apparent. However, by day 7 there was significant enhancement of the lytic capabilities of NK cells (>100% increase in LU_{10} per 10^7 spleen cells). Lytic activity was still enhanced at day 14 (approximately 50% above baseline) By day 35, despite continued daily gavage, levels of NK activity in the D4 treated animals had returned to baseline. Spleen and liver weights were increased by day 7 when compared to controls and remained above controls throughout the exposure period. The return of NK activity to control values in the D4 treated mice, along with the increase in liver size, may be caused by induction of D4 metabolism. The enhancement in NK activity observed after exposure to D4 may be due to either a direct effect on NK cells or may result from indirect mechanisms via release of cytokines. Supported by NIEHS T32-ES07087.

1199 THE EFFECT OF OCTAMETHYLCYCLOTETRASILOXANE (D_4) ON THE HUMORAL IMMUNE RESPONSE OF B6C3F1 MICE

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This study sought to determine the effect of oral D_4 on the humoral immune response to the T-dependent antigen, sheep red blood cells (sRBC), in B6C3F1 mice. Mice were gavaged with 100, 500 or 1000 mg/kg of D_4 for one, three or five days and the humoral immune response was measured *in vitro* using a Mishell-Dutton assay. An exposure and dose dependent suppression of the antibody response to sRBC was observed. Separation-reconstitution experiments of the adherent (AD;antigen presenting) and non-adherent cell (NAD;T-cells, B-cells) populations revealed a deficit in the ability of both pop-

ulations to mount an antibody response to sRBC. D_4 treated NAD cells combined with vehicle AD cells were suppressed to the same degree as the reconstituted D_4 cells. Vehicle NAD cells combined with D_4 treated AD cells were suppressed but to a lesser degree than D_4 reconstituted cells. Increasing the number of D_4 treated AD cells reconstituted with vehicle NAD cells did not return the AFC response back to control levels. Further experiments separating T-cells and B-cells revealed a deficit in the ability of D_4 treated B-cells to develop a humoral immune response. D_4 treated T-cells, however, retained the ability to elicit a humoral immune response to sRBC. Using cell surface markers, neither B-cell nor T-cell numbers were significantly decreased by D_4 treatment. The proliferative response of B cells to the mitogen, LPS, was decreased by 30% following exposure to D_4. The proliferative response of T cells to Con A was unaltered by D_4 exposure. These results suggest that gastric exposure to D_4 leads to a deficit in both APC and B-cell function, while T-cell function appears to remain intact. Supported by NIEHS T32-ES07087.

1200 EVALUATION OF THE INHALATION AND IMMUNOTOXICITY OF OCTAMETHYLCYCLOTETRASILOXANE (D_4) IN FISCHER 344 RATS FOLLOWING A 28 DAY EXPOSURE

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D_4 has been shown to act as a humoral adjuvant in rats. To assess the immunomodulating potential of D_4, the splenic antibody forming cell (AFC) response to sheep erythrocytes (sRBC) was studied in a 28 day subchronic inhalation study. Groups of 25 male and 25 female F344 rats were exposed to either 0, 0.08, 0.24, 0.73, 2.18, or 6.55 mg/l D_4 6hrs/day, 5 days/wk for 28 days. 4 males and 4 females served as cyclophosphamide (CP) (25 mg/kg) positive controls. Clinical signs, body weight and food consumption were recorded. On day 28, 10 rats/group/sex and 14 days following exposure 5 rats/sex/group (recovery animals) were sacrificed, blood and urine obtained for clinical chemistry, hematology, and urinalysis. A complete necropsy was performed. Liver, kidney, adrenal, ovary, thymus and body weights obtained and a complete set of tissues taken for histopathology. All AFC animals (10/sex/group) were immunized by i.v. injection of $\approx 2 \times 10^8$ sRBC 96 hours prior to sacrifice. CP was administered i.p. to the positive immunosuppressive controls on days 25–28. Anti-sRBC IgM antibody forming cells were evaluated in a plaque assay. D_4 exposure caused no adverse effects on body weight, food consumption, hematology, clinical chemistry or urinalysis parameters. A significant dose related increase in liver weights was observed in males (2.18 & 6.55 mg/l) and females (0.24–6.55 mg/l). Liver weights were the same as controls in the recovery animals. There were no other significant organ weight changes. Other than a few male rats with possible alpha 2-μ globulin inclusions in the kidney there were no adverse gross or histopathologic finding. D_4 exposure had no effect on spleen antibody-forming cell response to T-dependent antigen sRBC.

1201 THYMOCYTE ABNORMALITIES IN C57BL6 MICE ADMINISTERED ALL-TRANS RETINOIC ACID ON GESTATIONAL DAY 9

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Retinoids have been shown to have important developmental functions. Retinoic acid (RA) embryopathy is characterized by craniofacial, cardiac, and brain abnormalities and by thymic aplasia or hypoplasia. We characterized the nature of the RA-induced thymus abnormalities to better understand the role of RA in thymic embryogenesis. Pregnant C57Bl6J mice were given varying doses (100, 75, 50 and 25 mg/kg) of all-trans retinoic acid or sesame oil by oral gavage at gestational day 9 (plug is day 0) when thymic stroma is forming and prior to seeding with T-lymphoid stem cells. Fetuses were retrieved at gd 18; evaluations included body weight, craniofacial defects, thymus cellularity and thymocyte subsets by flow cytometric analysis. Thymocyte development was assessed by surface expression of CD4, CD8, HSA, CD44, CD25, CD3 and T-cell receptor-$\alpha\beta$ (TCR-$\alpha\beta$). Fetuses had mild to moderate craniofacial abnormalities and showed a spectrum of thymus defects within litters ranging from aplasia, decreased cellularity, abnormal thymocyte size patterns and alterations in developmental thymocyte antigens. Fetuses exposed to the lowest dose of RA (25 mg/kg) had thymi with normal cellularity but subtle changes in CD44 and TCR-$\alpha\beta$ expression. These studies may give insights into the role of RA in thymic stroma development and subsequent thymocyte and T-cell maturation.

1202 IMMUNOTOXICITY STUDIES OF BENZOPORPHYRIN DERIVATIVE IN MICE

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Benzoporphyrin derivative (BPD-MA; BPD verteporfin) is a potent photosensitizing agent which has been developed for the potential photodynamic treatment (PDT) of cancer. Preliminary studies have also shown selective immunomodulatory properties under appropriate drug and light conditions. We investigated the potential of BPD-MA to alter general immunologic parameters specifically by measuring the *in vivo* response to SRBC in the splenic plaque assay, as well as NK, CTL, and MLR activities *in vitro* 4 days after treatment with 0.1–1.0 mg/kg BPD-MA and 162 J/cm^2 of broad band red light. No adverse effects were observed. Furthermore, after PDT was administered during the peak of a primary immune response to ovalbumin, the secondary antibody response was unaffected. These data suggest that PDT using BPD-MA does not suppress (i) naive B cells, T-helper cells, antigen presenting cells or memory cells, (ii) NK cells, or (iii) the generation of cytotoxic T cells.

1203 SELECTIVE MODULATION OF IMMUNE FUNCTION PRODUCED BY METHYLENEDIOXYMETHAMPHETAMINE

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Abuse of illicit methamphetamine analogs represents a growing problem. The current study was performed to assess the potential direct immunomodulatory effect of exposure to (dl)3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy"). Splenocytes or elicited peritoneal macrophages from B6C3F1 mice were cultured in vitro at MDMA concentrations between 0.0001–100 μM. Macrophages were stimulated with bacterial LPS, and production of TNF was assessed. T-cell function was assessed by culturing splenocytes with MDMA in the presence of anti-CD3, and subsequently quantitating cytokine production. B-cell function was assessed by quantitating cellular proliferation in response to an antigen analog. Natural immunity was assessed by quantitating natural killer (NK) cell activity. T-cell effector function was evaluated by culturing lymphocytes with MDMA during a 5-day induction culture, followed by assessment of cytotoxic T-lymphocyte (CTL) activity. In vitro MDMA exposure had no effect on B-cell proliferation at any concentration tested. In comparison, production of IL-2 was significantly enhanced at concentrations as low as 0.0001 μM. IL-4 production was not affected by exposure to any concentration of MDMA, suggesting a differential alteration in T-helper cell function. NK cell function was elevated at MDMA concentrations between 0.0001 and 1.0 μM. CTL induction was significantly suppressed at 100 μM MDMA. Production of TNF was not significantly affected by any concentration of MDMA. (Supported by contract No. 271-91-9201 and Research Scientist Development Award DA-0130 from the National Institute on Drug Abuse).

1204 ASSESSMENT OF ENDOGENOUS OPIOID PEPTIDES FOR IMMUNOMODULATORY ACTIVITY

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This study assessed the direct effect of several endogenous opioid peptides (met-enkephalin, leu-enkephalin, Dynorphin A, β-endorphin) and a peptide derivative (metenkephalinamide) on *in vitro* immune functions. Murine B6C3F1 splenocytes and elicited macrophages were cultured *in vitro* with peptides at concentrations between 0.00001–10 μM. B-cell function was assessed by quantitating cellular proliferation, and T-cell regulatory function was assessed by culturing splenocytes with peptides and anti-CD3 antibody and then quantitating cytokine production. Natural immunity was assessed by culturing lymphocytes with peptides for 24 h, then quantitating natural killer (NK) cell activity. Macrophage function was assessed by quantitating IL-6 production. Peptide exposure had no significant effect on either B-cell proliferation or the production of IL-2. IL-4 production was unaffected by exposure to the enkephalins. In comparison, both dynorphin A and β-endorphin significantly suppressed IL-4 production at concentrations between 0.00001 and 0.001 μM. NK cell function was suppressed by all three enkephalins, with the greatest degree of suppression observed between 0.00001 and 0.01 μM; exposure to dynorphin or β-endorphin had no effect on NK cell function. IL-6 production was significantly enhanced by met-enkephalin and leu-enkephalin at 0.00001 and 0.0001 μM. In summary, *in vitro* exposure to endogenous neuropeptides

results in selective immunomodulation. (Supported by contract No. 271-91-9201 and Research Scientist Development Award DA-0130 from the National Institute on Drug Abuse).

1205 ALTERATIONS IN IMMUNE FUNCTION PRODUCED BY DELTA OPIOID RECEPTOR-SELECTIVE PEPTIDES

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The present study assessed the direct effect of a series of synthetic peptides selective for δ-opioid receptors on various in vitro immune functions. Murine B6C3F1 splenocytes and elicited macrophages were cultured in vitro with peptides at concentrations between 0.00001–10 μM. B-cell function was assessed by quantitating cell proliferation in response to an antigen analog, T-cell function was assessed by culturing splenocytes with peptides and anti-CD3 antibody, and subsequently quantitating cytokine production. Natural immunity was assessed by culturing lymphocytes with peptides for 24 h, then quantitating natural killer (NK) cell activity. Macrophage function was assessed by production of IL-6. Peptide exposure had limited effects on B-cell proliferation, whereas the production of IL-2 was significantly enhanced by exposure to DPDPE at concentrations between 0.00001 and 0.1 μM. Likewise, IL-4 production was significantly enhanced by DPDPE at these same concentrations. Conversely, the salt DPDPE-trifluoroacetate was inactive in both of these assays. NK cell function was significantly enhanced by in vitro exposure to a number of the peptides, with enhancement generally noted at concentrations between 0.00001 and 0.01 μM. Likewise, IL-6 production was generally augmented by δ-opioid receptor-selective peptides. These data suggest that δ-opioid agonists are broadly immunomodulatory at physiological concentrations. (Supported by contract No. 271-91-9201 and Research Scientist Development Award DA-0130 from the National Institute on Drug Abuse).

1206 EVALUATION OF TRANSPORTED LYMPHOID TISSUE FROM FISCHER 344 RATS AND B6C3F1 MICE FOR USE IN IMMUNOLOGICAL ASSESSMENT

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Studies were conducted to demonstrate the feasibility of integrating immunotoxicological and standard toxicological studies by transporting lymphoid tissue to a second laboratory site. Studies were carried out in female B6C3F1 mice and Fischer 344 rats. The T-dependent antibody forming cell (AFC) response to s-RBC and the natural killer cell response (NK) were the endpoints assessed. Spleens were removed from immunized animals and placed in Earle's Balanced Salt Solution (EBSS) on ice, for 0, 18 or 24 hours as whole spleens, sectioned spleens or spleen cell suspensions. No significant differences were seen with the spleen cell suspensions or the whole spleens at 24 hours as compared with the 0 time control. A rat NK response was evaluated at 0 and 24 hour time points. Both time points were within historical control values. The AFC assay was included in a rat inhalation study exposed at the Dow Corning Corp (DCC) facility. Whole rat spleens from DCC were transported to MCV Immunotoxicology Laboratory for assay 18 hours after sacrifice. Data is shown for the control groups: male (2056 ± 188) and female (1941 ± 290) mean AFC/10^6 spleen cells, evaluated on two separate days with no significant differences seen between the two days. These studies demonstrate that with proper shipping and temperature control, spleens can be successfully transported from one laboratory to another within a 24 hour period for evaluation.

1207 CONTACT HYPERSENSITIVITY RESPONSE TO ACRYLATE ESTERS

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Butyl Acrylate (BAC), Ethyl Acrylate (EAC) and 2-Ethyl-2-(Hydroxymethyl)-1,3-Propanediol Triacrylate (2-ETAC) were selected by the NTP for study as representatives of the acrylate class of agents. Chemicals of this class are widely used in manufacturing of numerous industrial and consumer products. The purpose of these studies was to assess the contact sensitizing potential of these acrylates using B6C3F1 mice. Irritancy studies were conducted to determine concentrations to be used for the hypersensitivity assays and to assure that the animals could tolerate 3 consecutive days of dosing. Each chemical was evaluated for its ability to elicit a contact hypersensitivity response using the Mouse Ear Swelling Test (MEST) and the Local Lymph Node Assay (LLNA). There were no treatment related effects on survival or body weights with any of the compounds tested. Testing concentrations up to 30%, there was no irritating concentrations detected with either EAC or BAC. The minimal irritating concentration for 2-ETAC was 1.0%. A positive contact hypersensitivity response to 2-ETAC was elicited with sensitizing concentrations as low as 0.03% when measured by the MEST and of 0.15% when measured by the LLNA. A positive contact hypersensitivity response to BAC was detected with sensitizing concentrations as low as 20% when measured by the LLNA, however this response was not detected using the MEST at concentrations as high as 30%. These studies did not indicate EAC as a sensitizer using either the MEST or the LLNA. These studies show that of the three acrylates tested 2-ETAC is the most potent contact sensitizer. Supported by NIEHS Contract ES 05288.

1208 IMMUNOSUPPRESSION IN RATS FOLLOWING ORAL EXPOSURE TO BIS(2-METHOXYETHYL)ETHER

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Bis(2-methoxyethyl)ether (BMEE) is one of a group of glycol ethers which is used as an industrial solvent and reaction medium for chemical synthesis. In this study, BMEE was administered daily by gavage to male F344 rats for one week at dosages ranging from 100 to 800 mg/kg/d and immune function determined. Rats exposed to BMEE at 400 and 800 mg/kg/d had reduced thymus weights in the absence of body weight changes compared to water controls. Antibody responses, measured by enumeration of plaque-forming cells (PFC), to the T cell-dependent antigen sheep red blood cells (SRBC) and the T cell-independent antigen trinitro-phenyl lipopolysaccharide (TNP-LPS) were suppressed in rats dosed at 800 mg/kg/d BMEE. Exposure of rats to 2-(2-methoxyethoxy)ethanol (MEE), the primary O-demethylation metabolite of BMEE in rats, failed to alter immune function. These data suggest that BMEE-induced immunosuppression resulted from the metabolism of BMEE via a previously described secondary metabolic pathway in the rat which involves hydrolysis of the central ether bond of BMEE to form 2-methoxyethanol (ME) followed by oxidation of ME to 2-methoxyacetic acid (MAA), which we have previously demonstrated to be immunosuppressive in the rat. (This abstract does not reflect EPA policy.)

1209 IMMUNOPHENOTYPING OF NORMAL HUMAN WORKER VOLUNTEERS: DISTRIBUTION AND RANGE STUDIES

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As part of a multi-year study of the effects of xenobiotics on the immune system of workers, blood (N = 100) was obtained from normal control volunteers from two worksites. The subjects (89 male and 11 female) were 31.9 ± 8.5 (±SD) years old and 37% were current smokers. Statistical analyses indicated asymmetric distributions for percentages (%) of CD56 (natural killer) and CD19 (B-cell), while CD3+/CD4+ (T-helper/inducer), CD3+/CD8+ (T-suppressor/cytotoxic) and CD3 (T-cell) had gaussian distributions. Percentages of total LY (median with 10th and 90th percentiles) were: CD3, 74.20 (61.66 to 84.36), CD3+/CD4+, 44.70 (34.56 to 57.98), CD3+/CD8+, 23.90 (14.16 to 31.59), CD56, 3.80 (1.11 to 6.79) and CD19, 14.00 (8.11 to 24.44). The distributions for the absolute number (#) of cells were asymmetric ($P > 0.05$) for all surface markers studied. Median numbers of LY (with 10th and 90th percentiles) were: CD3, 1642 (1170 to 2464), CD3+/CD4+, 987 (674 to 1548), CD3+/CD8+, 504 (338 to 832), CD56, 85 (20 to 180) and CD19, 320 (162 to 628). The median CD3+/CD4+:CD3+/CD8+ value was 1.87 (1.27 to 3.50) and also showed non-normal distribution characteristics. Significant age, sex and current smoking status associated differences were observed for %s and #s of some subsets. These data indicate that the distributions for #s and %s of some lymphocyte subsets are not symmetrically distributed, and that sex, smoking status, and age can have significant effects on some subsets. These results indicate the need to carefully control for these factors in the design of exposure studies of the human immune system.

1210 USEFULNESS OF ASSOCIATED INVESTIGATIONS IN THE IMMUNOTOXICITY ASSESSMENT OF DRUGS

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Functional tests are pivotal for assessing the toxicological significance of drug

effects on the immune system. However, additional parameters such as cell viability or lymphocyte subsets distribution are needed to interpret the results of functional tests as shown in a 2-week rat study.

Fischer 344 rats received an oral dose of 0, 1, 5 or 10 mg/kg cyclosporine daily for 14 days. Lymphocyte subset counts and cell viability were measured on spleen cells at the end of the treatment period. In addition, spleen cell proliferative response to the mitogens conA and STM, natural killer cell activity and plaque forming cell response were measured. A treatment-related decrease was noted for the PFC response (−63%) and the number of T cells (−28%) but no statistically significant changes in cell viability, NK cell activity and lymphocyte proliferation were found. However, the decrease in proliferative response (−24%) is highly significant (−45%) when the actual number of T cells is taken into account.

These results further support the need for a multistep strategy in the immunotoxicity assessment of drugs and chemicals, and reinforce the value of lymphocyte surface marker analysis in this context.

1211 GUINEA PIG SENSITIZATION/ANAPHYLAXIS STUDY OF FLUORESCEIN IN COMBINATION WITH RMP-7, A BLOOD-OCULAR-BARRIER PERMEABILIZER

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RMP-7, a peptide bradykinin agonist, is being developed to increase the blood-ocular permeability of diagnostic and therapeutic agents. Vitreous fluorophotometry with IV fluorescein will be used in RMP-7 dose ranging studies of permeability in man. However, fluorescein has been associated with adverse reactions in man (i.e. rare anaphylaxis in severe cases). Since bradykinin and histamine both exert pharmacological effects on the vasculature, a sensitization/anaphylaxis study was conducted in guinea pigs to investigate potential interaction or allergic response of RMP-7 with fluorescein. Animals were intravenously (via a dorsal metatarsal vein) sensitized with fluorescein on Days 1 and 5 and challenged with the following doses on Day 15: 100 mg/kg fluorescein alone, 20 μg/kg RMP-7 alone, or fluorescein (100 mg/kg) in combination with RMP-7 (20 μg/kg). Animals were monitored for acute clinical signs following the challenge dose. Positive control (horse serum) animals, also sensitized according to the same schedule, exhibited severe anaphylaxis following the challenge dose. A total of 4/11 (36%) animals receiving 100 mg/kg fluorescein showed mild generalized anaphylactic response consisting of head shaking, ear scratching, and/or hypoactivity. Only 1/11 (9%) animals treated with RMP-7 in combination with fluorescein exhibited signs of mild anaphylaxis (i.e. retching). Therefore, it was concluded that RMP-7 does not exacerbate either the incidence or severity of anaphylactic response produced by intravenous fluorescein challenge in guinea pigs previously exposed to fluorescein.

1212 SUPPRESSIVE EFFECTS OF CARBON TETRACHLORIDE ON IMMUNE PARAMETERS IN B6C3F1 MICE

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Investigations by Kaminski et al (Toxicol Appl Pharmacol 1994 May; 126(1): 98–107) showed clearly that CCL4 is immunotoxic and actions are mediated by the release of TNFβ by the liver. These studies were performed to further characterize the range of the immunotoxicity. B6C3F1 female mice were exposed to CCL4 in corn oil by gavage daily for 14 days with doses of 50, 100 and 500 mg/kg. There were no significant changes in body, brain, spleen, thymus or kidney weight. Hepatotoxicity was demonstrated by significant dose dependent increases in liver weight and serum chemistries (SGPT). CCL4 exposure produced no changes in absolute numbers of B cells, $CD3^+$ or $CD8^+$ T cells. A 15% decrease in $CD4^+$ and 39% decrease in $CD4^+CD8^+$ T cells was seen at the 500 mg/kg dose level. A dose dependent decrease in the IgM response to sRBC with a 54% maximum effect was seen. Reticuloendothelial system function was decreased dose dependently with the liver Kupffer cell targeted. No significant effects were observed on the mixed leukocyte, cytotoxic t cell and natural killer cell assays., *S. pneumoniae* (SP) and *L. monocytogenes* (LM) host resistance models were evaluated. There was a decrease in resistance to SP at the 500 mg/kg dose level. As shown by Kaminiski et al (Toxicol Appl Pharmacol 1990 Jan; 102(1): 9–20) there was a decrease in susceptibility to *L. monocytogenes.* The no effect level for the lower challenges was between 25 and 100 mg/kg. Supported by NIEHS Contract ES 05288.

1213 HALOGENATED HYDROCARBONS INHIBIT NATURAL KILLER ACTIVITY IN SPLEEN CELLS FROM EXPOSED MICE

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The common groundwater contaminants trichloroethylene (TCE) and dichloroethylene (1,1-DCE) have been reported to have a variety of effects on the murine immune system. C3B6F1 mice were injected subcutaneously with 0.02–40 μl TCE or 0.02–2.0 μl DCE daily for 7d. Spleen cells were harvested and natural killer (NK) activity against ^{15}Cr labeled YAC1 (murine thymoma) cells measured. To evaluate the effect of the reduced NK activity in a host resistance model, C57B1/6 mice were treated for 7d with the above doses of TCE or DCE. Cells from the B16 melanoma tumor cell line were injected intravenously into the tail vein of the mice. After 7–14 days, continuing treatment with the halogenated hydrocarbons, mice were sacrificed and the lungs examined for B16 tumor cell colonies. Both TCE and DCE significantly reduced NK killing of YAC1 cells. There appeared to be a reduction in lung metastases in the mice treated with halogenated hydrocarbons. This may indicate an inflammatory response which compensated for the reduced NK response. An increase in the mixed lymphocyte response (MLR) to P815 cells was also caused by halogenated hydrocarbons. The T cell immune response and lung histology are being investigated. Contamination of water supplies with these immunomodulating agents is a potentially significant health issue. Some of their metabolites are also products of chlorination of municipal water supplies that contain natural organic material. (Supported by the Arizona Disease Control Research Commission Contract #9424.)

1214 MODULATION OF THE MURINE DTH RESPONSE TO PICRYL CHLORIDE BY SEROTONIN AND DRUGS OF THE SEROTONINERGIC SYSTEM

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The effects of the systemic administration of serotonin (5-HT) and several drugs of the serotoninergic system have been investigated on the contact sensitivity (CH) response to picryl chloride in Balb/c mice (ear swelling test). 5-HT (0.5, 1, 2.5, 5 or 10 mg/kg/d), DL-p-chlorophenylalanine (PCPA) (125 or 250 mg/kg/d), 5-hydroxytryptophane (5-HTP) (25 or 100 mg/kg/d), fluoxetine (5 or 25 mg/kg/d), paroxetine (5 or 20 mg/kg/d), buspirone (0.5 or 2 mg/kg/d), cyproheptadine (0.5 or 2 mg/kg/d), ketanserine (0.5 or 2 mg/kg/d), granisetron (1 or 4 mg/kg/d) were injected ip from day 0 (sensitization) to day 8 (24 hours after ear challenge). Additionally, 5-HT (5 mg/kg/d) was administered during 3 days prior to sensitization or prior to challenge. Whatever the dose, 5-HT induced a significant ($p < 0.05$) decrease (−25% to −52%) in ear thickness. The effect was more pronounced when 5-HT was administered prior to challenge than prior to sensitization. High-dose 5-HT significantly decreased (−55%) while high-dose PCPA increased (+21%) CH response. CH responses were decreased by fluoxetine (−32%), high-dose paroxetine (−58%), high-dose cyproheptadine (−47%) and high-dose granisetron (−34%). Buspirone and ketanserine had no effect.

Our results suggest that serotonin modulates CH which is further substantiated by the decreased CH responses induced by selective serotonin reuptake inhibitors. Therefore, assessing the effects of new serotoninergic drugs on the immune system may be warranted.

1215 SELECTIVE SUPPRESSION IN HUMORAL IMMUNITY TO TREATMENT WITH ISONIAZID IN B6C3F1 FEMALE MICE

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Isoniazid (INH) has been widely used as the antibacterial drug for tuberculosis. The purpose of these studies was to evaluate INH for immunosuppressive activity because of the current concern with its increasing use with other drugs in the treatment of tuberculosis in AIDS patients. B6C3F1 female mice were treated with INH in deionized water daily by gavage for 14 or 28 days using doses of 50, 100 and 150 mg/kg. The organ weights of the spleen, lung, thymus and kidneys were not altered during the 14 or 28 day treatment, however the liver weight significantly increased in both treatment times at the 150 mg/kg dose by 13% and 18%. The T-dependent antibody forming cell response (AFC) to s-RBC's (sheep red blood cells) showed a non-significant decrease in AFC/10^6 spleen cells for both treatment times. When serum from the same animals was evaluated using an ELISA to detect antibody production, the 14 day

titer was significantly decreased at 100 mg/kg by 33%. In the 28 day treatment all dose group titers were significantly decreased by 27%, 38% and 46% respectively. No alterations were seen in the mixed leukocyte response, the natural killer cell, the cytotoxic T cell or the fixed macrophage assays. No changes were seen in the host resistance studies using the B16F10 melanoma, *S. pneumoniae* and *L. monocytogenes*. These studies indicate a need to determine potential interactions between INH and AIDS drugs with respect to targeting the immune system.

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1216 THE EFFECTS OF TUMOR NECROSIS FACTOR BINDING PROTEIN (TNFbp) ON HOST RESISTANCE IN SPRAGUE-DAWLEY RATS

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Tumor necrosis factor binding protein (TNFbp) is being considered for clinical use in the treatment of inflammatory diseases, including rheumatoid arthritis (RA). As part of the toxicologic evaluation of this biopharmaceutical, host resistance parameters were evaluated. Host resistance assays using the bacteria *Listeria monocytogenes, Staphylococcus aureus,* and *Escherichia coli* were conducted. Rats were challenged with bacteria and treated with a single IV injection of a long-acting formulation of TNFbp in doses ranging from 0.25–25 mg/kg. Samples from blood, spleen, and liver were examined for bacterial colony forming units (CFU). Results indicated that no differences from control CFU occurred in the *S. aureus* or *E. coli* challenged rats. The *L. monocytogenes* treated animals displayed increased CFU at the 25 mg/kg treatment dose in the spleen tissue and blood, 3 days after bacterial challenge. Examination of the time of dosing relative to time of bacterial challenge showed there were significant increases in CFU in the blood and liver tissue at 25 mg/kg in animals treated with TNFbp 6 hours prior to, concurrently, and 6 hours post-bacterial challenge. When animals were exposed to drug 18–42 hours post-bacterial challenge, there was no change in CFU compared to controls. Day 3 liver samples were also tested for effects of TNFbp on primary and secondary challenges with *L. monocytogenes*. While primary challenge analysis demonstrated increased CFU in rats treated with TNFbp prior to bacterial challenge, no effects of TNFbp were seen after secondary challenge. To further evaluate cell-mediated immunity, the mixed-leukocyte response assay was also examined. In this murine study using TNFbp at concentrations of 0.11–11 μg/ml, a significant decrease in ^{3}H-thymidine incorporation was demonstrated at the highest concentration. The results suggest treatment effects at the highest dose of TNFbp examined, however, this dose is ten times the highest expected dose to be used in RA patients.

1217 MODULATION OF THE 12-O-TETRADECANOYL-PHORBOL-13-ACETATE (TPA) INDUCED DIFFERENTIATION OF HUMAN ML-1 CELLS BY DIBUTYRYL CYCLIC AMP (dbcAMP)

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DbcAMP has been shown to affect the differentiation of HL-60 cells and human peripheral blood monocytes. In this study, we have investigated the effect of various concentrations of dbcAMP on the TPA-induced differentiation of the human myeloblastic leukemia cell line ML-1 to monocytes/macrophages. Toward this end, we have assessed mitochondrial respiration, ATP content, NADPH oxidase activity, myeloperoxidase activity, cell surface marker expression and phagocytosis. While dbcAMP by itself did not induce the differentiation of ML-1 cells, dbcAMP did modulate TPA-induced differentiation. When ML-1 cells were differentiated in the presence of 0.25 and 0.50 mM dbcAMP, their differentiation was significantly inhibited, such that they did not exhibit any of the phenotypic changes observed in TPA-differentiated cells. However, when cells were differentiated in the presence of 0.05 and 0.10 mM dbcAMP, they exhibited an increased NADPH oxidase activity and expression of CD14, but not CD11b and C16. Conversely, the mitochondrial respiration and phagocytic activity of cells differentiated in the presence of 0.10 mM dbcAMP were significantly inhibited. These results indicate that low concentrations of dbcAMP can modulate the phenotype and function of the monocytes/macrophages resulting from the TPA-induced differentiation of ML-1 cells.

1218 TOXICOPATHOLOGY OF INDOMETHACIN IN THE SPLEEN OF OVARIECTOMIZED, ESTRADIOL-SUPPLEMENTED, NEW ZEALAND WHITE (NZW) RABBITS

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In an effort to elucidate the mechanism by which indomethacin (IN) lessens the immunostimulatory effect of estradiol (E_2) on rabbit splenic red pulp macrophages (RPM), 39 female, NZW rabbits were divided into 10 groups: ovariectomized (OVX), OVX/IN at 0.1 and 5.0 mg/kg body weight (bw)/day; sham OVX (SOVX), SOVX/IN at 0.1 and 5.0 mg/kg bw/day; OVX/25 mg E_2, OVX/25 mg E_2/IN at 0.1 and 5.0 mg/kg bw/day; intact Control. Effects of the drugs on the RPM were measured by a 0 to 4 histologic grade. E_2 treatment resulted in increased RPM grade in the OVX/E_2 groups when compared to the OVX groups. IN addition lowered mean RPM grade in the SOVX/IN 5.0 group when compared to its E_2 control group. IN had no significant effect on levels of PGE_2. Hematocrits were significantly reduced in the OVX and OVX/E_2 groups, this decrease was exacerbated by the high IN dose in both treatment regimens. The effect of IN on E_2-induced RPM activation appears to be mediated through a non-prostaglandin pathway. The observed hematocrit changes are possibly the result of direct action of the IN and E_2 on erythrocytes resulting in their accelerated clearance from the circulation by RPM.

1219 POSSIBLE IMMUNE MECHANISM FOR CADMIUM-INDUCED KIDNEY DAMAGE

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Cadmium (Cd) is a known nephrotoxin and anti-laminin antibodies are increased in the sera of workers chronically exposed to Cd. To determine if the induction of these antibodies is due to Cd-induced alterations of a self protein, CBA/J female mice were immunized (i.p.) to either Cd-treated laminin, native laminin, Cd or saline. Antibodies against laminin, glomerular basement membrane (GBM) and collagen IV (C IV) were assayed by ELISA in both serum and the supernatants of LPS-stimulated spleen cells. Increases in serum antibodies against laminin or GBM were not observed, however, increases in serum IgG and IgA antibodies against C IV were observed in mice immunized with Cd-treated laminin. Significant elevations of antibodies against laminin, GBM and C IV were observed in the spleen supernatants of animals immunized with Cd-treated laminin. In the glomerular basement membrane, laminin is bound to C IV. The increased levels of serum antibodies against C IV could be the result of this binding of laminin close to an epitope on C IV such as the Goodpasture's antigen which is highly immunogenic and close to the laminin-C IV binding site. Supported by Grant ES 04895.

1220 *IN VITRO* EVALUATION OF DMBA-INDUCED IMMUNOTOXICITY USING MURINE AND HUMAN LYMPHOCYTES

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The prototypic PAH 7,12-dimethylbenz(a)anthracene has been described to alter mitogen proliferation, mixed lymphocyte response (MLR), NK activity, cytotoxic T lymphocyte activity (CTL) and interleukine-2 (IL-2) dependent proliferation in vivo and in vitro in mice. In this work, we have compared the ability of DMBA to interfere with immune parameters, in vitro, using human and murine lymphocytes. The effects of DMBA on PHA-induced proliferation, MLR, NK activity, CTL activity and IL-2 dependent proliferation were assessed. DMBA induced a dose-dependent inhibition of all tests used in the two species. However, MLR and cytotoxic T lymphocyte activity were more affected in humans. On the contrary, PHA proliferation, NK activity and IL-2 dependent proliferation were more sensitive to DMBA when murine splenocytes were used. Finally, lymphocyte proliferation induced in response to ionomycin + PMA was completely suppressed in murine splenocytes by DMBA while this was not the case with human lymphocytes thus showing a different implication of the calcium pathway.

1221 SELECTIVE TARGETING OF B LYMPHOCYTE PRECURSOR CELLS BY T2 MYCOTOXIN

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Subchronic T2 toxin treatment of timed-pregnant B6C3F1 mice resulted in significant and selective depletion of fetal liver cells expressing low levels of surface CD44 and CD45 antigens, suggestive of possible lymphoid progenitor cell sensitivity to this agent. Evaluation of CD45R antigen expression in fetal liver supported such a hypothesis, demonstrating a significant reduction in fetal liver B lymphocytic cells in animals exposed to T2 toxin. Subsequent *in vitro* T2 toxin exposure of fetal liver cells enriched for prolymphocytes by differential density gradient centrifugation demonstrated the presence of a highly sensitive subpopulation of cells that was eliminated in a selective, and near-complete, manner by T2 toxin exposure. This sensitive cell population was observed to have light-scatter characteristics of $CD45R^+$ B-lineage lymphocytes. Additional studies in adult mice demonstrated a reduction in $CD44^{lo}$ and $CD45R^+$ bone marrow cells similar to that seen in fetal liver, indicating that T2 toxin may also target immature B lymphocytes in this hematopoietic compartment. Taken together, these data suggest that the precursors of B cells may represent, for unknown reasons, highly sensitive targets of T2 toxin exposure.

1222 IMMUNOSTIMULATION IN RATS FOLLOWING ORAL ADMINISTRATION OF A BACTERIAL EXTRACT

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Broncho-Vaxom (BV) is an immunobiotherapeutic product composed of lyophilized extracts of different bacterial species which cause respiratory infections. The mechanism by which oral BV administration in humans protects against lung infections has not been determined. The immunomodulatory effect of BV was studied in 30 adult male Lewis rats gavaged with 180 mg/kg daily for the first 10 days of 3 consecutive months (an effective treatment pattern in humans). The concentrations of secretory IgA in the saliva, and serum immunoglobulins were measured by radial immunodiffusion before and following BV treatment. Salivary IgA was maximally increased (250%) 15 days after the initiation of BV treatment and remained significantly ($p < 0.05$) increased, but at lower concentrations, after the second and third 10-day treatments. Significant ($p < 0.05$) increases in the serum concentrations of IgG were observed at day 30 after the initiation of BV treatment, and were maximally increased (>200%) after the second and third courses of treatment. A similar pattern of increases were observed for serum IgA concentrations following BV treatments. These data suggest that the BV regimen of oral treatment has both secretory and serum immunostimulatory effects in rats. (Supported by OM Laboratories and The Egyptian Culture Bureau).

1223 ACTIONS OF AZT ON LP-BM5 (MAIDS) INDUCED IMMUNOMODULATION

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AZT was used to confirm the utility of this model for studying the effects of drug and non-drug chemicals in this laboratory. Mice were infected with I unit of LP-BM5 MuLV by IP injection on day I. Infected and uninfected animals received 0.5% methyl cellulose, 60 mg/kg/day AZT or 125 mg/kg/day AZT in 0.5% methyl cellulose, via drinking water. The experimental period was 31 days. No effects were seen on body weights in noninfected, infected or drug treated groups. An increase in spleen weight was seen in infected versus uninfected animals. Among infected animals AZT treated groups had significantly lower spleen weights as compared to the non-treated infected animals. No significant differences were seen in thymus weights between any groups. Elevations in basal levels of IgM and IgG were seen in infected mice as compared to uninfected mice. Humoral immunity as measured by spleen antibody forming cell response to sRBC was significantly decreased in infected animals and the suppression was blocked by AZT. CTL activity was suppressed in infected versus uninfected controls. An increase in CTL activity was seen in the low dose AZT group but these levels did not reach statistical significance. NK activity was enhanced in virus infected non-treated animals and AZT blocked this effect. This study demonstrates the immunosuppressive action of LP-BM5 MuLV in C57Bl/6 mice and the ability of AZT administered in the drinking water to inhibit the immune suppression. Supported by NIEHS Contract ES 05288.

1224 ROLE OF THE PEROXYL RADICAL PATHWAY IN METABOLIC ACTIVATION AND IMMUNOSUPPRESSION BY BENZO(a)PYRENE *IN VITRO*

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Metabolism of benzo[a]pyrene, B(a)P, an ubiquitous environmental contaminant, by splenic macrophages has been shown to be mediated by P-450 and peroxyl radical pathways. Previous work has also demonstrated that the suppression of the *in vitro* T-dependent AFC response following exposure to B(a)P or B(a)P 7,8 diol is able to be attenuated by co-incubation with the P-450 enzyme inhibitor, alpha-napthoflavone(ANF). The objective of these studies was to determine if the *in vitro* immunosuppression was attenuated by cyclooxygenase and lipoxygenase enzyme inhibitors. Immunosuppression by acetylsalicylic acid, piroxicam, meclofenamic acid or indomethacin was evaluated using the *in vitro* T-dependent AFC assay to sRBC. Naive splenocytes from B6C3F1 female mice were incubated with vehicle or drug (0.1 nM–10 μM) with and without B(a)P for five days. No reversal of B(a)P induced immunosuppression was observed due to inherent immunosuppression of the inhibitors. No effect levels for indomethacin and acetylsalicylic acid were 1.0 nM for both drugs. Meclo-fenamic Acid inhibited the AFC response at all dose levels. Piroxicam significantly enhanced the AFC response at dose levels from 0.1 nM to 1 μM. As a result of inherent immunosuppression with five day exposure to drugs, an alternate experimental approach was undertaken. Ongoing studies are evaluating drug and B(a)P exposure for 30 min prior to incubation, in the same manner ANF attenuation was observed. Supported in part by NIEHS contract ESO 05288.

1225 TRICHLOROETHENE-INDUCED AUTOIMMUNE RESPONSE IN FEMALE MRL +/+ MICE

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Trichloroethene (TCE) has been implicated in the pathogenesis of autoimmune diseases such as lupus and scleroderma in humans. To clarify the role of TCE in autoimmune responses, the following subchronic studies were carried out in female autoimmune prone mice (MRL +/+). Three groups of mice (5 weeks old) received i.p. injections of 10 mmol/kg of TCE, 0.2 mmol/kg of dichloroacetyl chloride (DCAC) (one of the metabolites of TCE), or an equal volume (100 μl) of corn oil alone (controls). Animals were dosed every 4th day for six weeks and euthanized 24 hr following the last dose. Sera and major tissues were collected and analyzed; ELISA kits were used to measure autoantibodies in sera. Spleen weights in the TCE and DCAC groups increased 36% with a similar pattern of change in the spleen to body weight ratios. Serum IgG in the TCE and DCAC groups increased by 45 and 322%, respectively. The following pattern of autoimmune responses was observed in the sera of TCE and DCAC treated mice: anti-nuclear antibodies: controls, 0/4; TCE, 4/4; DCAC, 3/5; anti-ssDNA antibodies: controls, 0/4; TCE, 2/4; DCAC, 5/5; anti-cardiolipin antibodies: controls, 0/4; TCE, 0/4; DCAC, 3/5. An ELISA developed for the measurement of TCE-specific antibodies using DCAC-albumin as an antigen indicated the following: controls, 0/4; TCE, 0/4; DCAC, 5/5. Histological examination of spleen in TCE and DCAC treated mice showed capsular fibrosis, decreased cellularity of white pulp and increased cellularity of red pulp. Histopathological changes were also observed in kidney, lung and liver of treated mice. Our data suggest that TCE and its metabolite, DCAC, induce and/or accelerate autoimmune responses in female MRL +/+ mice.

1226 TOXIC ETIOLOGY OF MULTIPLE CHEMICAL SENSITIVITIES: REVIEW OF CASE HISTORIES WITH DOCUMENTED TOXIC EXPOSURE

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Three verified cases of Multiple Chemical Sensitivities (MCS) were assessed. In all cases, the subjects had a documented prolonged exposure to toxic agents via either environmental or occupational contamination. Two of the cases underwent legal challenge with resolution in support of the diagnosed MCS as being causally related to previous toxic exposure. In all 3 cases evaluated, clinical and environmental monitoring confirmed that toxic exposures to 2 or

more of the following agents had occurred (benzene, hexamethylene diisocyanate, vanadium, titanium dioxide, benzo[a]pyrene, carbon monoxide and high level uncontrolled auto-exhaust containing nitrogen dioxides). Direct ambient monitoring and/or blood analyses determined exposures to be in a range consistent with previously reported immunomodulatory effects. All of the agents assessed are associated with either alterations in serum immunoglobulin concentration, complement level alteration, T-cell and B-cell dysfunction, occupational asthma, antinuclear antibody formation or IgE mediation. Although the toxicological mechanism(s) responsible for MCS remain unclear, these studies suggest that multiple agents with established immunomodulatory effects together play a role in the etiology of MCS.

1226a PHYSICOCHEMICAL AND TOXIC INTERACTIONS OF CO-EXPOSURES TO PARTICULATE MATTER, SULFUR DIOXIDE, AND OZONE ON ALVEOLAR MACROPHAGE PHAGOCYTOSIS

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Particulate matter, sulfur dioxide (SO_2), and ozone (O_3) are common, simultaneously occurring air pollutants. Co-exposure to 10 mg/m^3 of respirable carbon black particles (CB) and 10 ppm sulfur dioxide (SO_2) at high (>85%) relative humidity (RH) results in the formation of particle-associated sulfate (SO_4^{2-}; 13.7 ± 0.4 μg SO_4^{2-}/mg CB) which suppresses alveolar macrophage (AM) phagocytosis (ARRD 149, 1994: A839). To increase particle-associated SO_4^{2-} yield, identical concentrations of CB and SO_2 were co-generated in a nose-only inhalation chamber in the presence of 0.1 ppm O_3, a powerful oxidant. Particle-associated sulfate levels were observed to increase to 21.6 ± 0.7 μg SO_4^{2-}/mg CB. To assess whether this increase in particle-associated SO_4^{2-} affected AM Fc-receptor mediated phagocytosis, mice were exposed for 4 hr to 10 mg/m^3 CB and 10 ppm SO_2 at high RH with or without 0.1 ppm O_3 and tested at 7 days after exposure. CB and SO_2 co-exposure alone significantly suppressed AM phagocytosis to 79.8 ± 1.1% unexposed controls. In the presence of 0,1 ppm O_3, AAM phagocytosis was further suppressed to 71.9% ± 1.3% ($p>0.05$). These data demonstrate that O_3 increases particle-associated SO_4^{2-} levels in the presence of airborne particles, SO_2, and high RH which, following acute exposure, augments dysfunctions in AM phagocytosis.

1227 EFFECTS OF UDP-GLUCURONOSYLTRANSFERASE (UDP-GT) INDUCERS ON THYROID CELL PROLIFERATION

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UDP-GT inducers are suspected to be thyroid tumor promoters. The proposed mechanism is their ability to reduce serum T_4 levels, which leads to elevated TSH and subsequent hypertrophy, hyperplasia (proliferation) and neoplasia of thyroid follicular cells. However, this mechanism has not been thoroughly investigated as only one UDP-GT inducer (phenobarbital, PB) is a known thyroid tumor promoter. Therefore, the purpose of the present study was to determine whether four well known UDP-GT inducers that reduce serum T_4 levels also increase serum TSH and thyroid cell proliferation. Rats were fed various doses of PB, pregnenolone 16-α-carbonitrile (PCN), 3-methylcholanthrene (3MC) or arochlor 1254 (PCB) for 7 days. Serum T_4 and TSH were measured by radioimmunoassay (RIA) and thyroid cell proliferation was quantified by proliferating cell nuclear antigen (PCNA) immunohistochemistry, and expressed as a labeling index (LI). PB, PCN, 3MC and PCB decreased serum total T_4 40, 43, 50 and 90%, respectively. PB and PCN increased TSH (65 and 90%, respectively) as well as the LI (300 and 600%, respectively), while 3MC and PCB had no effect on TSH or the LI. In conclusion, these data demonstrate that not all UDP-GT inducers produce thyroid cell proliferation, and suggests that only those UDP-GT inducers (PB and PCN) that increase TSH and thyroid follicular cell proliferation are probably thyroid tumor promoters. (Supported by an ILSI Risk Sci Inst Grant and NIH Grant ES-07079.)

1228 TOXICITY, CELL PROLIFERATION AND GST-P+ HEPATIC FOCI IN AFLATOXIN B_1 TREATED RATS

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The hypothesis that hepatotoxicity of aflatoxin B_1 (AFB_1) and compensatory cell proliferation are critical for the growth of putative preneoplastic (GST-P+) foci is under investigation. Also, the validity of urinary taurine excretion as a non-invasive marker of hepatotoxicity and a predictor of focal outcome is evaluated. Young male F344 rats were gavaged with 25 μg of AFB_1 per rat per day five times a week for two successive weeks. Control rats received only AFB_1 vehicle (trycaprylin). This carcinogenic protocol allows preneoplastic foci to appear within weeks of AFB_1 treatment. Five rats in each group were killed 24 hours after 2, 5, 7 and 10 doses of AFB_1 and 3 and 5 weeks post-treatment. Urine was collected at 12 hour intervals on dry ice from treated rats starting 5 days before the first dose of AFB_1 until 5 days after the last dose. Two hours prior to autopsy rats were treated with the cell proliferation marker BrdU. At autopsy, levels of serum alanine aminotransferase (ALT) and sorbitol dehydrogenase (SDH) were determined. The extent of necrosis was evaluated histologically. Livers were analyzed for GST-P and BrdU expression. Most of the toxicity of AFB_1 assessed by ALT, SDH and urinary taurine was observed after the first two doses. By the fifth dose of AFB_1 little toxicity was present. GST-P+ foci were seen in the first week of AFB_1 treatment while hepatocytes are still under the inhibitory action of AFB_1 except those resistant hepatocytes with altered phenotype. Volume per cent of the liver occupied with GST-P+ foci increased with time after the treatment. After two doses of AFB_1 cell proliferation in treated rats was mostly located in periportal region of the liver. Proliferation in parenchymal cells was completely inhibited. At later times in the AFB_1 treatment and subsequent to this treatment, GST-P+ foci were the predominant structures labeled. Supported by CA 39416.

1229 EXPRESSION OF TRANSFORMING GROWTH FACTOR ALPHA (TGFα) IN MALE F344 RAT EARLY HEPATIC FOCI

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TGFα is a positive growth regulator in epithelial cells. Its overexpression has been associated with increased growth and malignancy of endstage cancers including breast cancer, pancreatic cancer, ovarian carcinoma, and hepatocellular carcinomas in humans and rodents. The aim of this study was to examine the expression of TGFα in hepatic foci, adenomas, and carcinomas. Hepatic lesions were induced by diethylnitrosamine in 4 wk old male F344 rats, and lesions were allowed to develop for at least four months. One week prior to sacrifice, the rats received an osmotic minipump containing BrdU to allow measurement of DNA synthesis. Tissues were fixed in formalin, paraffin embedded, and serially cut onto glass microscope slides. Each slide was either stained with hematoxylin and eosin or antibody labeled for TGFα or BrdU. A total of 128 foci were classified by morphologic appearance. The most intense antibody label was observed in one subgroup of foci which also had the highest mean percentages of both TGFα and BrdU labeled cells. In contrast, another subgroup of the foci (designated ground glass) exhibited the lowest percentage of TGFα labeled cells suggesting that ground glass foci represent a separate population of foci. There was a positive association of TGFα overexpression and increased BrdU label in all preneoplastic foci. Hepatic adenomas and carcinomas all labeled positive for TGFα. In summary, TGFα is hetero-geneously expression in foci suggesting TGFα expression may be an important factor in determining which preneo-plastic lesions advance to cancer.

1230 ADMINISTRATION OF G2 ACTIVATING AGENTS MODULATES CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY

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Recent studies have shown that hepatocytes arrested in G2 phase of the cell cycle become activated to proceed through mitosis following administration of low doses of CCl_4. Elimination of the G2 response markedly enhances CCl_4 hepatotoxicity. Such findings suggest that low doses of CCl_4 induce a cellular adaptive response that prevents CCl_4 induced toxicity as well as enhances tissue repair. In a follow up series of experiments, two G2 activating agents were evaluated with respect to their capacity to modulate CCl_4 hepatotoxicity. Female Sprague-Dawley rats were pre-treated with either biliverdin or diatomaceous earth, and then exposed to CCl_4 either 16 or 44 hours later. The possibility that chemically induced liver mitogenicity may participate in protection was evaluated through the use of the anti-mitotic agent colchicine, which was administered at a time point coincidental with the peak of G2 mitotic activity. Hepatotoxicity, as measured by serum ALT and AST levels, was markedly reduced 48 hours after CCl_4 treatment in the pre-treated rats as opposed to controls. In addition, the use of colchicine eliminated a substantial portion of the protection afforded by biliverdin and diatomaceous earth, linking the protection to the G2 mitotic response.

1231 EFFICIENT TISSUE REPAIR UNDERLIES THE RESILIENCY OF POSTNATALLY DEVELOPING RATS TO CHLORDECONE + CCl_4 HEPATOTOXICITY

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Previous studies demonstrated that during early postnatal development rats are resilient to the lethal combination of chlordecone (CD) + CCl_4. The objective of this study was to further investigate the underlying mechanisms of this resiliency. Postnatally developing (20- and 45-d-old) and adult (60-d-old) male S-D rats were maintained either on normal diet (ND) or 10 ppm CD for 15 days. On day 16, rats from each dietary protocol received a single dose of CCl_4 (100 μl/kg, ip) or corn oil. Liver injury was assessed by serum enzyme (ALT & SDH) elevations as well as by histopathology during a time-course 0–96 hr. Hepatocellular regeneration was assessed by ^{3}H-thymidine (^{3}H-T) incorporation into hepatic nuclear DNA and proliferating cell nuclear antigen (PCNA) studies. The expressions of transforming growth factor-α (TGF-α) and proto-oncogenes (*c-fos & H-ras*) were measured in 20- and 60-d ND rats. In ND + CCl_4 rats, transient liver injury occurred regardless of age as indicated by ALT & SDH levels and histopathological lesions. In CD rats, CCl_4-induced toxicity progressed with time culminating in 25 and 100% mortality in 45- and 60-d rats, respectively, by 72 hr after CCl_4. Treatment of 45-d CD rats with antimitotic agent (colchicine, 1 mg/kg, ip) resulted in 75% mortality by 96 hr after CCl_4. ^{3}H-T incorporation and PCNA studies indicate delayed and attenuated DNA synthesis, indicating unrestrained progression of liver injury leading to death of the animals. In contrast, in 20-d rats CCl_4-induced DNA synthesis was efficient and substantial, the peak being between 24 and 72 hr after CCl_4 regardless of CD pretreatment. There were 3- and 3.5-fold increases in TGF-α and *H-ras* mRNA expressions, respectively, during the maximal DNA synthesis in 20-d ND rats, whereas only 2- and 2.5-fold increases were observed in 60-d ND rats, respectively. Increased expression of *c-fos* (10-fold) was observed only in 20-d rats, 1 hr after CCl_4. These findings strongly suggest that tissue repair mechanisms play critical role in the resiliency of rats during early postnatal development. (Supported by The Burroughs Welcome Fund and ORISE).

1232 HEPATOCYTE PROLIFERATION IN F344 RATS FOLLOWING LONG-TERM EXPOSURES TO LOW LEVELS OF A CHEMICAL MIXTURE OF GROUNDWATER CONTAMINANTS

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A six-month study was performed to investigate hepatocellular proliferation and histopathological changes in F344 rats after long-term exposures to low-levels of a chemical mixture of seven groundwater contaminants. The seven chemicals used are among the most frequently detected contaminants associated with hazardous waste sites; arsenic, benzene, chloroform, chromium, lead, phenol, and trichloroethylene. Male F344 rats were exposed to this mixture, or submixtures of the organic or inorganic chemicals, via drinking water for six months. The study design included a time course experiment (i.e., 3 days, 10 days, 1, 3, and 6 months) and a dose-response experiment. Hepatocellular proliferation studies were performed by subcutaneously implanting osmotic mini-pumps to continuously deliver 5-bromo-2′-deoxyuridine for 7 days which labelled nuclei of proliferating cells. In all groups, there were no differences in weight gain, body weight, liver weight ratios, or liver-associated plasma enzymes. Light microscopic evaluation revealed no lesions related to the treatments in any animals. However, significant increases in hepatocellular labelling were observed at the 3-day, 10-day and 1-month exposure time points, after treatment with the full mixture, as well as the organic or inorganic submixtures at 1X and 10X concentrations. Proliferating hepatocytes expressed a unique labelling pattern surrounding large hepatic veins (0.5–2.0 mm), but not central veins. This did not appear to be a regenerative response due to cytotoxic mechanisms as assessed by the absence of increased plasma enzyme activity and the absence of hepatocellular lesions. Ultrastructural changes in these specific hepatocytes will be presented.

1233 DETECTION OF PROLIFERATING CELL NUCLEAR ANTIGEN (PCNA) IN HEPATOCYTES IN TISSUE SECTIONS AFTER ADMINISTRATION OF TRICHLORO-ETHYLENE (TCE) TO MICE

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One potential mechanism of TCE-induced mouse hepato-cellular tumors is the formation of excessive active oxygen species (AOS), possibly during lipid peroxidation in hepatocytes. Target (proteins, lipids, DNA) interaction with free radicals can result in activation of signaling molecules, transcription factors and/or the induction of oxidative stress-responsive genes which are potential pathways for triggering cell proliferation, paramount to tumorigenesis. To test this hypothesis using our experimental approach, we preferred the non-invasive PCNA method over bromodeoxyuridine and H^3-thymidine, both which require invasive pre-administration that could potentially generate AOS. The purpose of this study was to determine the efficacy of PCNA antibody to detect TCE-induced S phase hepatocytes as a proliferation marker. Groups of mice were orally administered water, corn oil, or TCE (1200 mg/kg) in corn oil in equal volumes (once/day, 5 days/week) for 3, 6, 14, and 21 days. Histopathology, immunohistochemistry, and cell counts by image analysis revealed that PCNA antibody is an effective method for detection of TCE-induced S phase hepatocytes in tissue sections.

1234 EFFECT OF PIPERONYL BUTOXIDE (PBO) ON CELL REPLICATION AND XENOBIOTIC METABOLISM IN MOUSE LIVER

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We have compared the effects of PBO (a non-genotoxic pesticide synergist) and sodium phenobarbitone (NaPB) on markers of cell replication and xenobiotic metabolism in mouse liver. Male CD-1 mice were fed diets containing O(control), 10, 30, 100 and 300 mg/kg/day PBO and 0.05% NaPB (equivalent to 99 mg/kg/day) for periods of 7 and 42 days. Replicative DNA synthesis was studied by implanting 7 day osmotic pumps containing 5-bromo-2′-deoxyuridine during study days 0–7 and 35–42. Treatment with PBO (100 and/or 300 mg/kg/day) and NaPB for 7 and 42 days increased relative liver weight which was associated with, respectively, either a midzonal or a centrilobular hypertrophy. Hepatocyte Labelling Index (LI) values were increased 3.5 and 8.2 fold in mice fed 300 mg/kg/day PBO and NaPB, respectively, for 7 days. LI values were not increased in mice given 10–100 mg/kg/day PBO for 7 days or any treatment for 42 days. Treatment with NaPB for 42 days increased microsomal cytochrome P-450 content and 7-pentoxyresorufin and ethylmorphine metabolism. PBO also induced some markers of xenobiotic metabolism. These results demonstrate that PBO and NaPB induce xenobiotic metabolism in mouse liver but only produce a transient stimulation of cell replication. On a molar basis PBO is less potent than NaPB. Generally PBO produced effects only at high doses (100 and 300 mg/kg/day) at which liver nodules are formed in chronic studies, suggesting a role for enzyme induction/cell proliferation in nodule formation. (Supported by PBO Task Force II).

1235 UNLEADED GASOLINE AND METHYL TERTIARY BUTYL ETHER INDUCE SIMILAR SHORT TERM EFFECTS IN MOUSE LIVER

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PS-6 unleaded gasoline (UG) and methyl tertiary butyl ether (MTBE), an oxygenate added to UG, induced liver tumors selectively in female mice. Given that these mice exhibited uterine alterations, and estrogen inhibits liver tumorigenesis in mice, we propose that UG and MTBE are hepatocarcinogens secondary to their interaction with estrogen. In mouse liver PS-6 increases P450 activity and estrogen metabolism, and is mitogenic and a tumor promoter. To compare a newer formulation of UG (91-01) and similarity of responses to MTBE, we evaluated the effects of 91-01 and MTBE to PS-6. Mice were exposed to 2027 ppm PS-6, 2013 ppm 91-01 or 7813 ppm MTBE vapor for 3 or 21 days under the exposure conditions of the cancer bioassays. Liver weight increases and uterine weight decreases were seen in all treatment groups. P450 activity, assessed by 7-pentoxy-resorufin-O-dealkylase (PROD) and 7-ethoxyresorufin-O-dethylase (EROD) activities, were increased similarly in all exposed mice. In the absence of hepatoxicity, the hepatic labeling

index was increased 2–3 fold in all exposed mice at 3 but not 21 days, indicating that these agents are mitogenic. Treatment (i.g.) with PS-6, 91-01 or MTBE increased estrogen metabolism 2–3 fold in isolated mouse hepatocytes. These data demonstrate that a newer blend of UG and the UG additive, MTBE, elicit similar short term hepatic effects, responses seen in hepatic tumor promotion. Since the liver is under multihormonal control, the observed hormonal effects support a possible role for endocrine modulations in UG- and MTBE-induced hepatocarcinogenesis. (Supported in part by a grant from the American Petroleum Institute and National Institute of Environmental Health Science ES 05641-01).

1236 CELL PROLIFERATION IN UNLEADED GASOLINE-PROMOTED FOCI IN MOUSE LIVER

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Unleaded gasoline (UG) vapor induced female mouse liver tumors in a 2-year cancer bioassay. Exposure to UG under bioassay conditions increased the size, but not number, of N-nitrosodiethylamine (DEN)-initiated foci in mouse liver. To investigate potential mechanisms of action, cell proliferation was assessed in foci after 4 months of UG exposure. DNA synthesis as measured by hepatic labeling index (LI) was assessed following implantation of bromodeoxyuridine-containing osmotic pumps three days prior to euthanasia. LI in nonfocal hepatocytes was similar (1–2%) in control, DEN, UG, and DEN/UG groups (N = 9). LI was greatly increased in DEN-initiated control (DEN/Ct) hepatic foci (24.4% ± 4.4%). Although a wide range of LI was observed in all focal lesions, UG promotion resulted in a significant increase in mean focal LI (30.9% ± 8.5%) as compared to DEN/Ct foci. These data support our earlier results demonstrating the increased size of DEN-initiated/UG-promoted (DEN/UG) foci relative to DEN/Ct foci. Transforming growth factor-beta (TGF-B) is a negative growth regulator for rodent liver. Immunohisto-chemical analysis with antibodies directed to active TGF-B1 demonstrated decreased staining in hepatic foci relative to non-lesion hepatocytes. These data demonstrate a high rate of cell proliferation in chemically-induced hepatic loci in mice, which likely contributes to their neoplastic development. In addition, altered TGF-B growth regulation in DEN-initiated hepatic foci may afford them a selective growth advantage. (Supported in part by grants from the American Petroleum Institute and National Institute of Environmental Health Services ES 05641-01).

1237 NEPHRO- AND HEPATOTOXICITY OF INHALED CHLOROFORM IN MALE B6C3F1 MICE

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Cytotoxicity and cell regeneration with inhaled chloroform has not been characterized for long-term exposures. Male B6C3F1 mice were exposed for 6 hrs/day to chloroform vapors of 0, 0.3, 2, 10, 30, or 90 ppm 7 days/week (d/w) for 3 or 13 weeks or 10 or 90 ppm 5 d/w for 13 weeks. Bromodeoxyuridine was administered via osmotic pumps implanted 3.5 days prior to necropsy and the labeling index (LI), percent of cells in S-phase, was quantified immunohistochemically. Concentration-dependent increases in kidney histopathology primarily involved enlarged nuclei and regeneration in proximal tubules, with cortical mineralization at 90 ppm 7 and 5 d/w 13 week exposures. The LI in the cortex was elevated 1.5- and 2-fold over control (FOC) with 30 and 90 ppm 7d/w 13 week exposures. Interestingly, the LI in 10 and 90 ppm 5 d/w 13 week exposed mice was 4.8- and 3.5-FOC; thus a 5 d/w NOEL was not established. Hepatic changes were observed starting at 10 ppm, consisting of hepatocyte swelling, vacuolation and degeneration. Significant increases in the hepatocyte LI occurred only at 90 ppm, where the LI was 19-FOC with 7d/w and 6-FOC with 5 d/w 13 week exposures. On the premise that chloroform is a nonDNA-reactive/cytotoxic carcinogen and that differing degrees of cytolethality/proliferation may be required to drive tumor formation in specific tissues, these data suggest increased renal tumor induction may occur with 7 d/w exposures of 30 to 90 ppm and 5 d/w exposures of 10 to 90 ppm. Hepatic tumor induction might occur with 7 or 5 d/w exposures of 90 ppm.

1238 RENAL, HEPATIC, AND ESOPHAGEAL EFFECTS IN THE RAT INDUCED BY A SINGLE IV INJECTION OF FUMONISIN B_1

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Fumonisin B_1 (FB_1), a mycotoxin produced by *Fusarium moniliforme*, is hepato- and nephro-toxic as well as hepatocarcinogenic in the rat, and is associated epidemiologically with esophageal cancer in humans. Our objective was to investigate the effect of a single dose of FB_1 on major target organs. Male SD rats were injected iv with 0 or 1.25 mg FB_1/kg. They were then injected ip with 100 mg BRDU/kg 90 min prior to euthanasia at 12 hr, 1, 2, 3, or 5 days. In FB_1 treated rats, serum cholesterol and BUN were elevated, however, hepatic enzymes were unaffected. Hepatic and renal GSH were depressed at 12 and 24 hr but subsequently recovered. The most dramatic changes occurred in the kidney. In the proximal tubular epithelium of the pars recta, apoptosis began at 12 hours and was followed by progressive necrosis and by cell proliferation. Apoptosis also occurred in the pancreas and thymus beginning at 24 hrs. In the liver and esophagus, histologic changes consisted only of cell proliferation which peaked on day 1 in liver (0.6 ± 0.09% for FB_1 vs 0.1 ± 0.03% for control) and on day 3 in esophagus (251 ± 6 labeled cells/cm for FB_1 vs 131 ± 24 for control). The results indicate that the primary target organ of FB_1 in the rat is the kidney, and that both apoptosis and cell proliferation are induced by FB_1. These changes suggest that FB1 may trigger the tumor promotion pathway which may ultimately progress to cancer.

1239 CYTOTOXICITY AND CELL PROLIFERATION INDUCED BY THE NEPHROCARCINOGEN HYDROQUINONE AND ITS TRI-GLUTATHIONYL METABOLITE

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Hydroquinone (HQ) is non-mutagenic in short term tests, but a nephrocarcinogen in the 2-year NTP bioassay. Some chemicals may induce carcinogenesis by a mechanism involving cytotoxicity and sustained regenerative hyperplasia. Since glutathione (GSH) conjugates of several hydroquinones are nephrotoxic, we investigated the effect of HQ and tri-(GSyl)HQ on cytotoxicity and cell proliferation in male F344 rats. Tri-(GSyl)HQ (7.5 μmol/kg, i.v.) increased blood urea nitrogen (2X), urinary γ-glutamyl transpeptidase (γ-GT, 11X), alkaline phosphatase (ALP, 8X), GSH-S-transferase (GST, 25X) and glucose (G, 10X) 24 h after dosing. By 72 h, most of these parameters had returned to control levels. Light microscopy (LM) revealed the presence of tubular necrosis in the S_3M segment of the proximal tubule. Cell proliferation rates in this region, assessed immunohistochemically by incorporation of bromodeoxyuridine (BrdU) into newly synthesized DNA, were 2.4, 6.9, 15.3 and 14.3 % after 12, 24, 48 and 72 h respectively, compared to 1.6 % in vehicle controls. HQ (1.8 mmol/kg, po) was toxic in some rats but not others, as evidenced by LM and increases in urinary γ-GT (1–15X), ALP (1–7X) and GST (1–12X). BrdU incorporation correlated with the degree of toxicity in individual rats, and ranged from 4.3–9.2; 1.2–9.6 and 2.9–19.5 % after 24, 48 and 72 h respectively. These results indicate a possible role for HQ-thioether metabolite-mediated cytotoxicity in HQ-induced nephrocarcinogenicity. (GM 39338).

1240 A MULTI-STAGE CLONE GROWTH MODEL OF HEREDITARY RENAL CANCER IN THE EKER RAT

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Familial predisposition to renal cancer is well documented in humans. Risk assessment tools for these susceptible populations are needed. The Eker rat carries a single autosomal gene mutation that results in an inherited susceptibility to bilateral multicentric renal cell carcinomas (Eker and Mossige, *Nature*, 189: 858, 1961; Everitt, *et al., J. Urol.* 148: 1932–1936, 1992). We have developed numerical analytic (MVK) models of the type described by Moolgavkar *et al. (Risk Anal.* 8: 383–392, 1988) to predict the spontaneous appearance of pre-neoplastic and neoplastic lesions in the male Eker rat kidney. Data on kidney size and cell proliferation rates in 'normal' populations, atypical tubules, atypical hyperplasias, and tumors were used to specify proliferation rates in all stages of the MVK models and the size of the 'normal' population. Model parameters for all rates of mutation and malignant cell death rates, which have not been determined directly, were adjusted to obtain least-squares fits of the model predictions to data on the number of lesions as a function of age. Having specified all model parameters in this way, the appropriateness of the model was evaluated by comparing the fitted parameters to expected (realistic) ranges, and by comparing the model's prediction of the total number of transformed cells to experimental data. Models with either 1 or 2 *rate limiting* mutational stages between 'normal' Eker and malignant cell types were tested. Both model types describe the lesion number data adequately, with little difference between these predictions. Fitted values for mutation rates are within the expected range for the 1-stage model ($\approx 3 \cdot 10^{-5}$/cell division), but are rather high for the 2-stage model ($\approx 3 \cdot 10^{-3}$/cell division). The 1-stage model

provides a realistic description of tumorigenesis in the Eker rat kidney. This model can now be used to predict changes in the number of lesions resulting from chemically-induced changes in cell proliferation rates. Once validated, such a model can be used for human risk assessment.

1241 STRAIN-SPECIFIC FORMATION OF ABERRANT CRYPT FOCI IN MOUSE COLON

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Repetitive treatment with the organotropic colon carcinogen, 1,2-dimethylhydrazine (DMH), produces tumors in susceptible mouse strains that exhibit pathological features associated with the human disease. As in human populations, the genetic background of laboratory animals comprises a significant component to this organ-specific carcinogenesis, and several mouse lines, including AKR/J and DBA/2J are highly resistant to the tumorigenic effects of DMH. During the course of ongoing studies to establish phenotypic differences between susceptible (SWR/J and P/J) and resistant strains, we have examined the colonic mucosa of DMH-treated mice for the presence of aberrant crypt foci (ACF). ACF represent an early morphological lesion in stepwise progression of colon cancer. In experiment 1,5-month old SWR/J and DBA/2J were trealed s.c. with DMH (35 mg/kg) once a week over two weeks. Nine weeks later colons were removed and ACF were visualized at low magnification by light microscopy after methylene blue-staining. Only SWR/J mice revealed focal atypia indicative of preneoplastic change. To obtain additional information about their morphology, tissue sections containing ACF were sectioned and stained with H & E. ACF are larger and have a thicker epithelial lining than normal crypts. In experiment 2, SWR/J and AKR/J mice (6 weeks old) received two s.c. injections with DMH (35 and 20 mg/kg, respectively). After 5 weeks, colons were examined for ACF. Comparable to the first experiment, no ACF were observed in the resistant AKR/J epithelium. In contrast, ACF were readily identified in the middle and distal colons of treated SWR/J mice. The formation of ACF is discussed in terms of proximity to lymphoid follicles, colonic structures that may have a promotional role in colon cancer. This differential response between resistant and susceptible strains further supports an important role for ACF in the stepwise progression of colon cancer.

1242 DIBROMOACETATE (DBA) ACTS AS A PROMOTER OF ABNORMAL CRYPT FOCI IN THE COLON OF F344 RATS

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Dibromoacetate is produced when drinking water containing bromide is chlorinated or ozonated. Epidemiological studies have associated chlorination of drinking water with the development of colorectal cancer. Brominated byproducts of chlorination have been poorly characterized for their carcinogenic properties. Therefore, an initiation-promotion study of the development of aberrant crypt foci (ACF) in the colon of male F344 rats was conducted. A single i.p. dose of azoxymethane (AOM)(10 mg/kg) served as the initiator. DBA was administered at 1 g/L of drinking water for up to 20 weeks. At 9 weeks, the DBA treatment produced a few ACF, but these were not significantly greater than seen in control animals. However, the number of ACF were significantly elevated in AOM/DBA (78 ± 10) treated rats relative to animals receiving AOM alone (59 ± 5). The major effect, however, appeared to be an increase in the complexity of the foci. For example, the number of ACF with more than 5 crypts/foci was increased from a mean 7 per colon to more than 14. The increased number of abnormal crypts appeared to be increased primarily in a region 9–15 cm from the origin of the colon. Based on these preliminary data, DBA appears to be a potential promoter of colon cancer in the rat. Twenty week data will also be available at the meeting. (Supported by NIEHS RO1-ES04648).

1243 DIFFERENT MODES OF ACTION OF CHLORINATED AND BROMINATED HALOACETATES

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Chlorinated and brominated haloacetic acids (HAs) are by products of drinking water chlorination. Trichloroacetate (TCA) and dichloroacetate (DCA) produce hepatic tumors in mice. TCA appears to act primarily as a peroxisome proliferator. DCA induces severe hepatomegaly marked by extensive vacuolation and glycogen accumulation. DCA induces a transient increase in cell division that becomes inhibitory after 28 days. Preliminary data suggests that the bromochloro (BCA), dibromo (DBA), and bromodichloro (BDCA) analogs also induce hepatic tumors in B6C3F1 mice. Transient increases in CN-insensitive Acyl CoA oxidase activity was observed with DBA, but not BCA or BDCA. A small increase in glycogen accumulation was observed with BCA and vacuolations of the hepatocytes that occurred in the immediate vicinity of the nucleus was not apparent with the other HAs. DBA produced a 3-day labeling index of approximately 5 for up to 4 weeks, 2–3 X than that observed with the other HAs. The toxicology of the HAs involves diverse mechanisms and there is no rationale for treating these chemicals as a single toxicological class. (Supported by USEPA CR 819562 and NIEHS RO1-ESO4648).

1244 COMPARISON OF THE EPIDERMAL MITOTIC RESPONSE OF SENCAR, B6C3F1, FVB/N, AND TG.AC TRANSGENIC MICE FOLLOWING DOSING WITH AN EPIDERMAL TUMOR PROMOTER

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The TG.AC transgenic mouse strain was created from the FVB/N strain and carries the mutated v-Ha-*ras* transgene, therefore it responds in a mouse skin tumor model as if it were genetically initiated. A prior study with TG.AC mice demonstrated that these animals develop epidermal papillomas following as little as two weeks of twice weekly dermal dosing with 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (Spalding et al., *Carcinogenesis,* 14: 1335, 1993). In this study, the TG.AC mouse was compared to its parent strain (FVB/N) and to two other mouse strains (SENCAR and the B6C3F1 hybrid) that have been used for epidermal carcinogenicity studies. Mice were dosed twice weekly for 2 weeks with either acetone or TPA (either 2.5 or 10 μg/day) in acetone. Following the last dose, the skin at the dosing site was collected from groups of animals at 0, 6, 12, 18, 24, 36, and 48 hr. The histochemical methods used for strain comparison were: BrdU labeling index as an indicator of cell proliferation, *in situ* end labeling as an indicator of cell death, and H&E staining for histopathological evaluation. The histochemical and histopathological results of the study will be discussed relative to the sensitivity of these strains to chemically-induced epidermal carcinogenicity.

1245 EFFECT OF CELL PROLIFERATION ON MUTATION FIXATION *IN VIVO*

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Chemicals that interact with and modify DNA, thereby activating protooncogenes or inactivating tumor or suppressor genes, are thought to initiate the process of carcinogenesis. Damage caused by the interaction of environmental chemicals with DNA can be repaired by cellular repair processes. Unrepaired damage remaining during DNA replication could lead to mutation fixation. Thus cell proliferation could enhance the mutagenic potential of a given chemical by virtue of rapid fixation of the mutation before it is repaired. To study the effect of cell proliferation on mutation fixation *in vivo* we have utilized the Big Blue transgenic mouse system developed by Stratagene. This mouse carries an integrated bacteriophage lambda-based vector with the bacterial *lacI* gene as the mutagenesis target. Initially we chose to use the mouse dorsal skin as the target organ. Histological data following a single topical application of the potent tumor-promoting agent 12-O-tetradecanoylphorbol-13-acetate indicated that the compound enhanced proliferation resulting in a hyperplastic epidermis, peaking at 48 hours post-application; a single application of the promutagenic compound benzo[a]pyrene, at 64 μg/animal, does not result in cell proliferation. A two-fold increase in mutant frequency in the lacI target gone was observed in the skin of mice treated with both B[a]P and TPA, when compared to mice treated with B[a]P alone. These studies indicate that cell proliferation enhances mutation fixation *in vivo.* (Supported by ES06636-01).

1246 ROLE OF PROTEIN PHOSPHATASES 1 AND 2A IN SULFUR MUSTARD-INDUCED G_2/M BLOCK OF NORMAL HUMAN EPIDERMAL KERATINOCYTES

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Sulfur mustard (HD) is a vesicating chemical warfare agent, which produces crosslinks in cellular DNA. Low levels of DNA crosslinking agents have been

shown to produce a block at the G_2/M interface of the mammalian cell cycle. Studies were undertaken to determine the effect of low level HD on normal human epidermal keratinocytes (NHEK) derived from adult skin, and the role played by protein phosphatase 2A (PP2A) and protein phosphatase 1 (PP1), both implicated in the control of normal cell cycle kinetics. NHEK were exposed to HD, and whole cell lysates were separated on 10% SDS-PAGE gels and electrotransferred to PVDF. The cell cycle stage was determined by estimation of cellular cyclin B1 and cdc2 kinase phosphotyrosine levels through immunoblotting. A 1-hr exposure of NHEK to 5 μM HD was found to produce a profound G_2/M cell cycle block, characterized by markedly increased levels of cyclin B1 and cdc2 kinase phosphotyrosine. Okadaic acid and calyculin A, potent inhibitors of PP2A, were used to evaluate its role in HD-induced G_2/M block. The G_2/M block in NHEK was completely reversed by a 4-hr exposure to 500 nM okadaic acid or 10 nM calyculin A. Reversal was identified as a complete loss of cyclin B1 and cdc2 kinase phosphotyrosine. Dilutions (1:10 and 1:100) of okadaic acid or calyculin A produced little or no reversal of HD-induced G_2/M block. A 1-hr exposure of NHEK to 500 nM okadaic acid or 10 nM calyculin A was insufficient to produce reversal of the G_2/M block. The effect of 4-hr okadaic acid incubation on the G_2/M block was reversed by a 4-hr incubation in inhibitor-free medium. Tautomycin, an inhibitor of PP1, was used to evaluate its role in HD-induced G_2/M block. Tautomycin was ineffective, even at a concentration of 100 nM. In summary, HD induced a G_2/M block in NHEK, which was reversed by okadaic acid and calyculin A in a concentration- and time-dependent manner. These results suggest that PP2A is involved in the G_2/M block produced by exposure of NHEK to low concentrations of HD.

1247 EXPOSURE OF RATS TO ENVIRONMENTAL ESTROGENIC COMPOUND NONYL PHENOL ALTERS CELL CYCLE KINETICS IN THE MAMMARY GLAND

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Nonyl phenol is used in the preparation of lubricating oil additives, resins, plasticizers, surface active agents, etc. Recently, it has been shown that nonyl phenol is estrogenic in nature. p-Nonyl phenol mimics the action of estrogen both in vitro and in vivo. Whether nonyl phenol produces any adverse effect in the mammary gland is not clear. In this study we examined cell proliferation in the mammary gland of nonyl phenol treated (10 μg/24 hr) and untreated rats. Proliferating cell nuclear antigen (PCNA) expression was measured immunohistochemically after 11 days of treatment. Nonyl phenol significantly increased the conversion of mammary epithelial cells in Go into Gl- and S-phase cells by 2.5 and 1.5 fold, respectively, compared to that of control. Labeling index and growth fraction were 1.5% and 1.23%, respectively, compared to that of control. The alteration in cell cycle was mainly pronounced in lobule 1. Perturbation in cell cycle is considered as a risk factor for the development of cancer. Nonyl phenol-mediated perturbation in cell cycle may produce adverse effect(s) in the mammary gland.

1248 BACKGROUND CELL PROLIFERATION RATES IN COMMON TARGET TISSUES OF B6C3F1 MICE AND F344 RATS

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Increasing emphasis is being placed on mode of action of chemical carcinogens as an important consideration for risk assessment. Many rodent carcinogens appear to act through a nongenotoxic mode of action, such as induced cell proliferation. Information on cell proliferation rates based on species, sex, age, tissue and choice of marker will provide a foundation for incorporating such measurements into rodent toxicity studies. Cell proliferation was evaluated in liver, kidney, skin, and forestomach (the most common rodent tumor sites) of male and female B6C3F1 mice and F344 rats at 7, 10, 13 and 20 weeks of age. Proliferating cell nuclear antigen (PCNA), an endogenous cell proliferation marker, and bromodeoxyuridine (BrdU) given by pulse injection 2 hr before sacrifice were compared as markers of cell proliferation. In the liver and forestomach, BrdU and PCNA labeling indices (LIs; S phase only) were similar, whereas the PCNA proliferating index (PI; G1 + S + G2 + M phases) was consistently greater than the LI in all tissues examined. In the kidney and skin, PCNA LIs were greater than BrdU LIs in some instances. These results provide useful information for designing rodent toxicity studies that may involve species-, sex-, age- and tissue-dependent responses (NIH/NCI 1R43CA6280-01).

1249 THE EFFECT OF BENZO(a)PYRENE (BAP) AND MITOMYCIN C (MMC) ON HELA CELL DIVISION CYCLE

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Recently there has been significant progression in understanding the control of the cell division cycle. To investigate the effect of toxic substance on the cell cycle, we analyzed the effect of BAP and MMC on synchronized HeLa cells during the cell cycle. The rate of cell growth was measured by MTT assay. Fifteen percent of cell growth was decreased by incubating 30 μM BAP for 1 d and 12% by 0.5 mg/ml MMC. To synchronize HeLa cells, 10^6 cells were grown for 1 d and were treated with 1 mM hydroxyurea (Hu) for 14–16 h. The arrested cells were allowed to proceed through their cell cycle by removing Hu and resupplying fresh medium. The arrested cells in G1/S transition were proceeded to S at 4 h, G2/M at 8 h, and G1 phase at 12 h after resupplying fresh medium. To assay the effect of BAP and MMC, treated and untreated cells were harvested at desired time points, and their amounts of protein were normalized for further assays. The $p34^{cdc2}$ kinase activity measured by using $p34^{cdc2}$ specific peptide was peaked at 8 h and declined at 12 h. Treatment of 30 μM BAP delayed the peak of $p34^{cdc2}$ kinase activity to 2–4 h and 0.5 mg/ml of MMC significant reduced the kinase activity. The amount of $p34^{cdc2}$ kinase were measured by immunoblotting. The amount of $p34^{cdc2}$ was not changed either the untreated or BAP and MMC treated cells during the cell cycle. To further study the effect of BAP and MMC on cell cycle, cyclin B level was assayed by immunoblotting. Cyclin B level was peaked at 8 h in untreated cells corresponding to $p34^{cdc2}$ activity, but peaked at 10–12 h in BAP treated cells. There was no significant change of cyclin B level in MMC treated cells. It is suggested that BAP and MMC do not affect synthesis of $p34^{cdc2}$ but formation of cyclin B.

1250 THE EFFECT OF TCDD ON GENE EXPRESSION OF EPIDERMAL GROWTH FACTOR RECEPTOR IS MODULATED BY THE NUCLEAR TRANSCRIPTION FACTOR Sp1 IN NIH 3T3 MOUSE FIBROBLAST CELLS

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Changes in gene transcription are believed to be one of the key mechanisms mediating the toxic effects of TCDD through the TCDD/Ah receptor pathway. TCDD evoked changes in transcriptional regulation of the Epidermal Growth Factor Receptor (EGFr) gene were studied using CAT and DNA gel mobility shift assays. DNA pERCAT constructs, containing portions of the 5′ DNA regulatory sequence −771 to −16 of the first exon of the human EGFr gene ligated to the bacterial chloramphenicol acetyltransferase (CAT) reporter gene, were transfected into CCL 163.0 and CRL 1648 mouse fibroblast cell lines, and TCDD treatment was found to reduce CAT expression in all instances but one for all pERCAT constructs tested. This finding is consistent with down regulation of EGFr transcription, *in vivo*, by TCDD. By contrast, under identical conditions using a v-Ki-*ras* transformed cell line derived from CCL 163.0 (i.e. CCL 163.3) TCDD treatment was found to consistently stimulate CAT expression. By conducting a series of deletion analyses, we found the most responsive regulatory region for TCDD's actions (−150 to −16) contains several "GC boxes" positively responsive to Sp1, a negatively regulating T_3RE (T_3 response element) and a positive AP-1 response element sequence. The results of DNA gel mobility shift assays and studies on the effect of exogenously added T_3 and TPA showed that the influences of T_3RE (negative) or AP-1 response element (positive) are not marked in CCL 163.0 cells. On the other hand, the role of Sp1 on influencing CAT expression was quite significant. We conclude that the major cause for a suppressive affect of TCDD on EGFr transcription in this system is its action to decrease either the amount of nuclear Sp1 or to reduce DNA binding of this protein.

1251 A SINGLE GESTATIONAL TCDD EXPOSURE ALTERS PROTEIN EXPRESSION IN THE ADULT RAT EPIDIDYMIS

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Previous studies demonstrated that a single maternal dose of TCDD (0.064–1 μg/kg) on gestation day 15 results in a 50% decline in cauda epididymal sperm reserves in male rat offspring on day 63 despite only a slight reduction in testicular sperm numbers (10%). To examine the TCDD-induced effects on the epi-

didymis more thoroughly, pregnant Holtzman rats were given 1 μg/kg TCDD on day 15 of gestation and the epididymis (N = 8) was removed at necropsy 21, 32, 49, and 63 days after birth. The entire epididymis was analyzed at days 21 and 32, while only the caput/corpus region of the epididymis was analyzed in the older animals. The epididymal tissue was minced and homogenized in detergent-containing buffer. The postnuclear fraction was desalted and concentrated for protein assay and 2D gel electrophoresis. Epididymal protein (μg/mg tissue) was significantly different ($p < 0.01$) from control on day 63: 231.6 and 168.6 for control and TCDD-exposed males, respectively. Protein (70 μg) from day 49 and 63 samples was separated by 2D gel electrophoresis to determine if there was a TCDD-induced diminution in specific proteins. The integrated optical density of silver-stained protein spots was quantified using a Scanalytics analyzer. Spots that were common to control gels were used as a template for comparison with gels from TCDD exposures. While there was no TCDD-related decline in specific proteins at day 49, there was a significant ($p < 0.05$) decline in a 35 kD (pI = 5.8) protein in TCDD-exposed males at day 63. Since this protein was present on gels from day 49 samples and sperm are not found in the lumen of the epididymis until day 55, we believe the protein is of epididymal origin and not from sperm. Future work is necessary to identify this epididymal protein and determine whether this protein is related to the TCDD-induced decline in sperm numbers in the epididymis. (Funded in part by NIH grant ES01332)

1252 INHIBITION OF ESTROGEN-INDUCED PROGESTERONE RECEPTOR GENE EXPRESSION BY 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN: MECHANISTIC STUDIES

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2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) inhibits several 17β-estradiol (E2)-induced responses in the rodent uterus and mammary and in human breast cancer cell lines. Treatment of MCF-7 human breast cancer cells with 1 nM E2 increased (2- to 3-fold) cellular levels of immunoreactive progesterone (PR) protein and PR mRNA levels. Cotreatment of cells with 1 nM TCDD plus 1 nM E2 results in complete inhibition of the E2-induced response. Regulation of E2-induced PR gene expression was further investigated using a series of plasmid constructs containing the following oligonucleotide sequences from the PR gene: −2762/ + 788, −325/ +788, −165/ +788, −30/ +788 and +23/ +788 which were inserted in front of the heterologous chloramphenicol acetyl transferase (CAT) reporter gene. In MCF-7 cells cotransfected with the PR gene-derived deletion plasmids plus a human estrogen receptor (ER) expression plasmid, E2 induced CAT activity only with those plasmids containing the −689/+723 estrogen responsive element. TCDD significantly inhibited E2-induced CAT activity in transient transfection studies using all of the deletion plasmids indicating that DNA sequences required for TCDD-responsiveness may be located within the +23 to +788 region. Ongoing research is focused on identifying possible *trans*-acting factors and genomic sequences responsible for the inhibitory activity of TCDD.

1253 INHIBITION OF ESTROGEN-INDUCED *c-fos* PROTOONCOGENE mRNA LEVELS BY 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN (TCDD) IN MCF-7 HUMAN BREAST CANCER CELLS

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c-Fos protooncogene expression is induced by 17β-estradiol (E2) and other growth stimulating factors in the MCF-7 human breast cancer cell line. In this study, 1 nM E2 induced a > 8-fold increase in *c-fos* mRNA levels which were maximal 1 hr after treatment of the cells with E2; the induced mRNA levels rapidly decreased and returned to control levels after 24 hr. In MCF-7 cells treated with 10 nM TCDD alone, there were no significant effects in *c-fos* mRNA levels. However, in cells cotreated with E2 for 1 hr and TCDD for 1, 2, 4, 12 and 24 hr, TCDD significantly inhibited E2-induced *c-fos* mRNA levels after 2 hr and the levels were decreased for the 24 hr duration of the experiment. At the 2 hr time point, TCDD decreased E2-induced mRNA levels by approximately 50%. These data are similar to those previously reported in the female Sprague-Dawley rat uterus and demonstrates that TCDD blocks one of the early E2- and mitogen-induced responses in E2-responsive target cells. The mechanism of interaction between the Ah and estrogen receptor-mediated endocrine response pathways is currently being investigated.

1254 EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN ON EXPRESSION OF INSULIN-LIKE GROWTH FACTOR BINDING PROTEINS IN MCF-7 CELLS

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Insulin-like growth factor binding proteins (IGFBPs) are associated with insulin-like growth factors (IGFs) and modulate their mitogenic responses. Several IGFBPs are expressed in primary breast tumors and breast cancer cell lines and these proteins are secreted into conditioned media by various breast carcinoma cells. After culturing MCF-7 breast cancer cells in conditioned medium, IGFBPs 1, 2, 3, 4 and 5 could be detected by electrophoresis and transferred to nitrocellulose membranes by electroblotting and ligand blotting using [^{125}I]IGF-I. Treatment of the cells with 1 nM 17β-estradiol (E2) resulted in approximately a 2-fold increase in secretion of IGFBP-4. In cells cotreated with E2 plus the antiestrogen, ICI 182,780, there was a significant inhibition of E2-induced secretion of IGFBP-4 and, in parallel studies, it was shown that 10 nM 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) also inhibited E2-induced secretion of this protein. E2 also caused a time-dependent increase in IGFBP mRNA levels and a maximal 2-fold increase was observed after treatment with E2 for 24 hr. In cells cotreated with TCDD plus E2, a significant decrease in E2-induced IGFBP mRNA levels was observed within 2 hr after treatment with TCDD and the inhibitory response persisted for up to 24 hr. These data are consistent with other studies showing that TCDD inhibits diverse E2-induced responses in breast cancer cells and the mechanisms associated with inhibition of E2-induced IGFBP-4 gene expression are currently being investigated.

1255 INTERACTIVE EFFECTS OF INSULIN AND 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN (TCDD) ON PROLIFERATION AND GENE EXPRESSION IN MCF-7 HUMAN BREAST CANCER CELLS

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Treatment of MCF-7 cells with insulin (0.01 to 1 μg/ml) resulted in a concentration-dependent increase in cell proliferation and [^{3}H]thymidine uptake in MCF-7 human breast cancer cells with maximal responses observed at the 0.1 μg/ml concentration. Insulin (0.1 μg/ml) also caused a rapid 2-fold induction of both *c-fos* and *c-jun* protooncogene mRNA levels. In MCF-7 cells cotreated with 10 nM TCDD plus 0.1 μg/ml insulin, TCDD inhibited insulin-induced cell proliferation, and 1 μM α-naphthoflavone, an aryl hydrocarbon (Ah) receptor antagonist, blocked the TCDD-induced responses. TCDD did not affect the binding affinity of [^{125}I]insulin for the insulin receptor (IR) or the number of IR binding sites in MCF-7 cells. However, in cells cotreated with insulin plus TCDD, the K_D values for binding to the IR were decreased and the number of binding sites was increased. TCDD induced IR mRNA levels and inhibited insulin-induced *c-fos* protooncogene expression and phosphorylation of the insulin receptor and insulin receptor substrate-1 in MCF-7 cells. In contrast, TCDD did not inhibit insulin-induced *c-jun* protooncogene expression. The results suggest that TCDD inhibited insulin-induced cell proliferation in MCF-7 cells by interrupting the insulin receptor-mediated signal transduction pathway.

1256 MECHANISM BY WHICH TCDD REGULATES EPIDERMAL GROWTH FACTOR RECEPTOR IN RAT LIVER EPITHELIAL CELLS

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TCDD is a potent rodent liver carcinogen. It has previously been shown that TCDD caused a decrease in epidermal growth factor receptor (EGFR) levels in rat liver as well as in human keratinocytes in culture. This has led to the hypothesis that alterations in EGFR and related signal transduction pathways may play a role in TCDD's effects on cell growth and differentiation. The objective of the present study was to establish an *in vitro* model using liver cells for investigating the mechanism of TCDD induced changes in EGFR. We report here that the WB cell, a nontransformed rat liver epithelial cell line, expressed high levels of Ah receptor and was responsive to TCDD as determined by induction of CYP1A1, a target gene transcriptionally regulated by the TCDD-Ah receptor complex. The stimulation of cell proliferation by TCDD coincided with a decrease in EGFR protein and binding. The mechanism of EGFR decrease was investigated by assessing: 1) the rate of EGFR

synthesis and degradation, 2) EGFR mRNA steady-state levels and stability, and 3) EGFR activation status. There was no effect of TCDD treatment on the rate of EGFR protein turnover or activation. The results demonstrated that the mechanism by which TCDD decreased the EGFR protein in WB cells was by reducing steady-state EGFR mRNA at the transcriptional level thereby diminishing the rate of EGFR synthesis. We hypothesize that cross-talk from an altered growth regulatory pathway may contribute to the transcriptional repression of the EGFR.

1257 REGULATION OF ESTROGEN RESPONSIVE ELEMENT BINDING TO NUCLEAR PROTEINS EXTRACTED FROM MCF-7 CELLS TREATED WITH 2,3,7,8-TETRACHLORO-DIBENZO-p-DIOXIN (TCDD)

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It is well known that TCDD exhibits antiestrogenic activity in estrogen responsive organs and cell lines. We have investigated the molecular actions of TCDD on MCF-7 cells, using them as a model system for breast cancer. Under our experimental condition there was a rise in nuclear protein binding to the estrogen responsive element (ERE) in MCF-7 cells treated with TCDD (10 nM). The cause of this up-regulation was investigated. Nuclear proteins were extracted from MCF-7 cells treated with varying length of exposure with TCDD. Binding of NP to responsive elements (REs) was determined by gel shift assay. For competition assay, one of cold REs was added at 100-fold excess of 32P-labeled RE before the addition of labeled RE. Results were visualized on X-ray films, while the levels of binding activity were determined by using an AMBIS Radioanalytic Imaging System. A six hr treatment exhibited a tendency towards decreased binding of NP to ERE. Conversily, 1 and 24 hr treatments showed significant increases of NP binding to ERE. NP binding to ERE was moderately inhibited by the addition of cold AP-1, retinoic acid RE (RARE), and glucocorticoid RE (GRE). The levels of inhibition were very close to that of ERE's self competition. Addition of cold ERE led to a significant decrease in the binding of NP to labeled AP-1, RARE, thyroid RE (TRE), GRE and dioxin RE (DRE), but not to SP-1. AP-1 significantly decreased the binding to ERE, RARE, TRE and GRE, but not to DRE and SP-1. Our data suggested that estrogen receptor binding to ERE is not simple but regulated by other RE and/or DNA binding proteins.

1258 REGULATION OF GROWTH FACTOR EXPRESSION IN CULTURED CELLS IN RESPONSE TO 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is an environmental contaminant produced from various chemical syntheses and combustion processes. Research has lent insight into the effects of TCCD on growth factor gene expression in both SCC12F (skin cell carcinoma) and HEPG2 (hepatoma) human cell lines. In SCC12F cells, 10 nM TCDD treatment resulted in increased levels of mRNA for TGF-α, interleukin-1β (IL-1β) and interleukin-1 receptor antagonist (IL1-ra) with decreased levels of TGF-β_2 mRNA. No significant effect is observed for either TGF-β_1 or interleukin-1α (IL-1α) message levels following TCDD exposure. The increased mRNA levels observed for both TGF-α and IL-1β are due to an increase in stabilization of the mRNA rather than increase in transcription. The decreased level of mRNA for TGF-β_2 results from a decrease in transcription as detected by nuclear run-off analysis (SCC12F) and confirmed in the HEPG2 cell line by transfection of the TGF-β_2 gene promoter fused to the CAT reporter gene. Further analysis of the TGF-β_2 gene promoter activity in the HEPG2 cells demonstrated that the region of influence for TCDD is within 80 base pairs of the transcription start site, although DNA'se I footprinting of this region does not reveal marked differences between treated and untreated samples. A dose response curve generated using a TGF-β_2 construction containing 321 base pairs upstream and 24 base pairs downstream of the start site demonstrates maximal dose effects for TCDD on transcription occur between 1 and 10 nM TCDD. Time course experiments reveal that transcription of the TGF-β_2 is suppressed out to at least 48 hours following a single 10 nM TCDD dose. Experiments are currently in progress to determine mechanisms of TCDD regulated gene expression for the individual secreted or intracellular forms of IL1-ra message. The data obtained allows for a more thorough understanding of the mechanisms of TCDD-dependent gene regulation.

1259 ACUTE TCDD-DOSING INCREASES HEPATIC EXPRESSION OF THE CELL CYCLE CONTROL ENZYME CDK1 AND DECREASES ITS EXPRESSION IN SPLEEN OF Balb/c MICE

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Cyclin dependent kinases (CDKs) are a family of closely related serine/threonine kinases that control and coordinate passage through the cell cycle. CDK1 ($p34^{cdc2}$) is the best characterized of the CDKs. Regulation of the kinase activity of these enzymes is controlled through transcription, complexing with the cyclin proteins and by cycles of phosphorylation and dephosphorylation. TCDD is a hepatic tumor promotor and potent immunotoxicant. Previous work in our laboratory has shown that CDK1 can be utilized as a biomarker of xenobiotic-induced cell proliferation by tumor promoters. In this study we examined the CDK1 expression in liver and lymphoid tissue of TCDD-treated mice. Balb/c mice were given a single i.p. injection of TCDD at 0.25, 0.5, 1.0, or 2.0 μg/kg. Control animals received an equal volume of the corn oil carrier. Five days after dosing, livers and spleens were removed, homogenized, and centrifuged at 9000 $\times$ g to produce a supernatant fraction (S9). CDK1 expression in this tissue S9 was determined by ELISA assay and western blotting. TCDD dosing produced a dose-dependent increase in hepatic CDK1 (2-fold) as well as a dose-dependent decrease in spleen CDK1 expression (50%). The hepatic stimulation and splenic downregulation of cell division control enzymes provide evidence of a mechanism of action for hepatic tumor promotion as well as the immunotoxic effects of TCDD. Aberrations in expression of CDKs may represent a toxic manifestation of xenobiotic exposure.

1260 GESTATIONAL AND LACTATIONAL EXPOSURE TO TCDD IMPROVES SPATIAL LEARNING AND UP-REGULATES TYPE I CORTICOSTERIOD RECEPTORS IN THE HIPPOCAMPUS OF MALE RATS

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Recent studies have shown that visuospatial cognitive function is enhanced by corticosteroids acting through receptor systems in the hippocampus. This study tested the hypothesis that effects of perinatal exposure to Ah-receptor agonists on cognitive function might be mediated indirectly through altered CNS corticosteroid receptor expression. Pregnant Sprague-Dawley rats were dosed with 0.10 μg/kg/day 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) or corn oil vehicle on gestation days 10–16. Beginning at 80 days of age, a male and female from each litter were tested on an eight-arm radial maze for 20 sessions with all arms baited. Later the rats were gonadectomized and adrenalectomized under ketaminexylazine anesthesia to eliminate effects of endogenous steroids on corticosteroid receptor concentrations, and killed after 3 days. Type I and type II corticosteroid receptors were measured in serial 10 μm sections through the brain by quantitative *in vitro* autoradiography using [^{3}H]ZK91587 and [^{3}H]dexamethasone, respectively, as receptor-specific ligands. TCDD-exposed males made fewer errors on the maze than untreated males. Exposed females were unaffected. Similarly, type I corticosteriod receptors were up-regulated approximately 2-fold across all fields of the hippocampus in exposed males, but were not significantly affected in exposed females. Regression confirmed a negative correlation between hippocampal Type I receptors and maze errors. No changes in Type II corticosteroid receptors were observed in either sex. These data are consistent with the recent finding that effects of corticosteroids on hippocampal Type I receptors contribute significantly to visuospatial cognitive function (Vaher et al., 1994, *Brain Res.* 656: 71–77). Supported by Health Canada #4028 to SLS and NIH #ES01332 to REP.

1261 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN (TCDD) AS AN ANTIESTROGEN IN MCF-7 HUMAN BREAST CANCER CELLS: INHIBITION OF ESTROGEN-INDUCED HEAT SHOCK PROTEIN 27 GENE EXPRESSION

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Heat shock protein 27 (Hsp27) is highly expressed in estrogen receptor (ER)-positive mammary tumors and human breast cancer cell lines. Treatment of MCF-7 human breast cancer cells with 10 nM 17β-estradiol (E2) resulted in a 2-fold induction of Hsp27 mRNA levels whereas 10 nM 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) alone did not affect expression of this

gene. In cells cotreated with both E2 plus TCDD, the E2-induced Hsp27 mRNA levels were inhibited within 2 hr after addition of TCDD and the inhibitory response persisted for up to 24 hr. The 5'-proximal flanking region of this gene was further investigated to identify genomic sequences associated with both E2-responsiveness and the inhibitory effects of TCDD. An ER/Sp1 binding site, GGGCGC$(n)_{10}$GGTCA, was identified at −105 to −84 and the formation of the ER/Sp1 complex was investigated by *in vitro* assays using synthetic Hsp27-[^{32}P]ER/Sp1 oligos. The results of initial studies show that nuclear extracts from E2-treated MCF-7 cells form a complex with [^{32}P]ER/Sp1 in a gel electrophoretic mobility shift assay; preincubation of the extract with either ER or Sp1 antibodies or unlabeled estrogen responsive element inhibited formation of the retarded protein-DNA complex. In extracts from cells treated with E2 plus TCDD, the ER/Sp1 complex was also observed suggesting that the TCDD-induced inhibitory response is not related to disruption of the Hsp27-ER/Sp1 complex.

1262 USE OF COMPETITIVE PCR FOR DETECTION OF ALTERATION OF ESTROGEN RECEPTOR (ER) GENE EXPRESSION BY 2,3,7,8-TETRACHLORO-DIBENZO-p-DIOXIN (TCDD)

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Antiestrogenic effects of halogenated dioxins have been shown in many laboratories. These effects may be due to alteration of estrogen metabolism or down-regulation of ER gene expression or both. In this study we developed a competitive PCR procedure for measurement of changes of ER mRNA. The standard for the competitive PCR was created by PCR reactions using primers for the ER gene under following conditions: 94 C for 1 min, 37 C for 1 min and 72 C for 90 seconds, 30 cycles. A PCR product of about 170 bp was isolated and subcloned into a plasmid which was then used as the internal standard for competitive PCR. In the competitive PCR reaction total RNA, isolated from either mouse liver or hepatoma cell line, was reverse-transcribed into first strand cDNA and then distributed in equal amounts into tubes containing different concentrations of internal standard. The rest of PCR components were added in equal amounts to each tube and the PCR cycles were 1 min 94 C, 1 min 56 C and 2 min 72 C. By using the competitive PCR we showed that TCDD treatment caused more than 5 fold decrease of the ER mRNA in cells (1 nM TCDD, 48 hr) and CD-1 mice (100 μg TCDD/kg, 4 days). This technique is especially useful for detecting mRNA changes when only small amounts of samples are available. Supported in part by NIEHS Center Grant #ES05002.

1263 CLONING AND CHARACTERIZATION OF A NOVEL TCDD INDUCIBLE RAT cRNA XIRC9

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Persistent activation of the Aromatic hydrocarbon Receptor (AhR) by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) causes tumors in numerous species and in multiple tissues including the liver. Following activation by TCDD, the AhR complex is known to regulate the levels of specific mRNAs through transcriptional and post-transcriptional mechanisms. Using a subtractive hybridization procedure, we recently cloned a novel TCDD inducible cDNA XIRC9 (Xenobiotic Inducible Rat cDNA 9). The remaining 5' coding region of this cDNA was cloned using colony hybridization, and an anchored PCR amplification technique. Primer extension analysis indicates that the 5' end of XIRC9 contains sequence within 75 bp of the 5' terminus of the mRNA. The XIRC9 mRNA is induced in a dose dependent manner in the female Sprague Dawley liver, reaching levels five fold above control seven days after dosing by gavage with 50 μg/kg of TCDD. Levels of XIRC9 mRNA in the adrenal also increase markedly in some animals three days after 25 μg/kg TCDD treatment. Basal levels of XIRC9 mRNA are high in the liver, intestine, and testes and low levels were detected in the heart. Extensive searches suggest that there are no highly similar protein or nucleic acid sequences contained within the current public databases. Presently the function of the XIRC9 encoded protein is unknown. We expect that functional analysis of this protein and of those proteins with which it associates will help elucidate the mechanism by which TCDD causes its toxicity.

1264 THE ISOFORM-SPECIFIC EXPRESSION OF PKCs DURING INDIVIDUAL PHASES OF THE CELL CYCLE IN VASCULAR SMOOTH MUSCLE CELLS (SMCs) IS DISRUPTED BY TCDD

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Recent studies in this laboratory suggest that the deregulation of SMC growth and differentiation by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) may involve cell cycle-specific modulation of protein kinase C (PKC). To test this hypothesis, the profile of PKC isoforms was examined by Western blot analysis in synchronized SMC populations during progression through the cell cycle following TCDD challenge (10 nM). Five PKC subspecies were identified in vascular SMCs, namely PKC alpha (α), beta II (βII), delta (δ), epsilon (ε), and zeta (ζ) isoforms. TCDD challenge during the G0/G1 transition reduced expression of all 5 PKC isoforms. In contrast, PKCβII and PKCδ expression was enhanced in SMCs treated with TCDD during the G1/S transition, while PKCζ was slightly reduced and PKCα and ε were unaffected. The cell cycle-related modulation of PKC expression by TCDD was preceded by marked induction of tyrosine kinase activity. Flow cytometry studies revealed that reductions of DNA synthetic rates in SMCs treated with TCDD were due to impeded cell cycle transit as a result of deficient C-kinase signaling. This interpretation was supported by the inability of serum concentrations as high as 40% to overcome reductions in AP-1:DNA binding activity by TCDD. These data demonstrate that TCDD differentially modulates PKC isoforms as a function of cell cycle progression and that this effect impacts growth behavior in vascular SMCs. (Supported by NIH grant ES 04917).

1265 TCDD-INDUCED EXPRESSION OF A NOVEL GENE IN RAT LIVER

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In this study, we used the "Differential Display" (DD) technique 1) to identify new genes that may be up/down regulated by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and 2) to investigate novel aspects of TCDD action. Rat liver total RNA was isolated from animals that had been exposed to 0, 10 ng/kg, 1000 ng/kg (acute TCDD) or 0, 1 ng/kg/day, 125 ng/kg/day for 30 weeks (chronic TCDD). RNA was reverse transcribed into cDNA, PCR amplified using the DD kit, and separated on a sequencing gel. Comparison of two or more DD patterns obtained from untreated and treated samples allowed the identification of ≈30 candidate genes. The gene fragments were eluted from the DD gel, reamplified, and directly sequenced. 25 of these sequenced fragments had no homology to sequences in the GenBank database. Based on preliminary Northern analysis of total RNA, the expression of one of these unknown cDNAs (29-D) was increased 5- and 3-fold as a result of acute or chronic treatment with TCDD, respectively. The size of the mRNA coding for 29-D was about 2-kb. Screening of an expression library to identify the complete sequence of the 29-D cDNA is in progress.

1266 IDENTIFICATION OF NOVEL TCDD-RESPONSIVE GENES IN HEP G2 CELLS USING DIFFERENTIAL mRNA DISPLAY RT-PCR

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a well-known environmental contaminant and induces broad toxicological and biological responses in humans and animals. The search for TCDD-responsive genes is important to understand the mechanism of TCDD-induced responses. Using a conventional subtraction library to identify these genes can be very tedious and inefficient. Differential mRNA display RT-PCR (DD RT-PCR) offers the promise of a very powerful tool to identify genes which are regulated or responsive to certain receptors or chemicals such as TCDD. Treatment of Hep G2 cells with TCDD resulted, as expected, in expression of CYP1A1 mRNA which was differentially displayed on a gel with a homologous primer. Further TCDD treatment followed by DD analysis of a gel with a group of different primers revealed significantly different pattern from the control for a number of mRNAs. Twenty four differentially displayed mRNAs were isolated and reamplified. The reamplified PCR products were directly sequenced to give immediate DNA sequence information. A Genebank search revealed that only one had been cloned previously; the remaining 23 are currently unknown. Northern blot analysis revealed that at least 3 clones showed repression by TCDD in a time-dependent manner. These results show that the application of DD

RT-PCR to the study of TCDD-induced responses could be very useful in the discovery of as yet unknown genes important for TCDD toxicity. The further characterization of these genes will be discussed.

1267 INVESTIGATION OF MOLECULAR MECHANISMS OF TUMORIGENESIS IN BIVALVE GONADAL TUMORS

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Recent epidemiological investigations of germ cell tumors of Maine soft shell clams (*Mya arenaria*) and hard shell clams (*Mercenaria spp.*) from Florida demonstrate the prevalence of histogenically similar gonadal cancers as high as 40% and 60%, respectively. Human mortality rates due to ovarian cancer from the same areas are significantly greater than the national average and show a rise over the last two decades of the survey. This correlates with the increasing use of herbicides in these areas and in the appearance of significant numbers of tumors of the analogous reproductive system in the clams. We have investigated the molecular mechanism of tumor induction in the clams. Preliminary results of photoaffinity binding studies indicate the presence of two cytosolic proteins which specifically bind dioxin and may be candidates for invertebrate Ah receptors. Transfection studies indicate the presence of a transforming gene in tumor DNA from both species. Differential display PCR analysis has revealed the presence of differentially expressed messages.

1268 DISTRIBUTION OF THE ARYL HYDROCARBON RECEPTOR (AhR) IN THE POSTPUBERTAL MALE RAT REPRODUCTIVE SYSTEM

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Male rats exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) exhibit decreased testis, epididymis, and accessory sex organ weights, altered testicular and epididymal morphology, and decreased spermatogenesis. It was therefore of interest to identify cell types within male rat reproductive organs which might be direct targets of TCDD toxicity. Because the AhR is known to be involved in mediating TCDD toxicity, AhR distribution was immunohistochemically evaluated in reproductive organs of male Holtzman rats at 63 days of age. AhR was localized in Bouin's fixed, paraffin embedded tissue sections (N = 4) with affinity-purified polyclonal anti-mouse AhR; preabsorbed anti-mouse AhR was used as a negative control. Primary antibody was detected using a biotin/streptavidin/peroxidase system. In the ventral prostate, dorsolateral prostate, seminal vesicle, coagulating gland, and ductus deferens, both stromal and vascular smooth muscle were positive whereas stromal fibroblasts were negative. Epithelial staining was strong in the coagulating gland and ductus deferens, somewhat weaker in the dorsolateral prostate and seminal vesicle, and absent in the ventral prostate. In the epididymis, vascular smooth muscle was positive and stromal fibroblasts and spermatozoa were negative. Epididymal epithelial staining was strong in the corpus and cauda and somewhat weaker in the caput. In the testis, vascular smooth muscle was positive and developing germ cells were negative. Leydig cell staining showed interanimal variation and ranged from weakly to strongly positive. These results imply that all organs of the postpubertal male reproductive system may be directly affected by TCDD exposure and warrant further studies investigating the action of TCDD on the particular cell types which express AhR within affected organs. (Supported by NIH grant ES01332.)

1269 ISOLATION AND CHARACTERIZATION OF A UNIQUE BENZO[a]PYRENE-RESISTANT T47-D HUMAN BREAST CANCER CELL LINE

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T47-D human breast cancer cells were cultured in media containing 1 μM benzo[a]pyrene (BaP) for 3.5 months. Ten resistant clones were individually isolated and screened for inducibility of ethoxy-resorufin *O*-deethylase (EROD) activity by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). One of the BaP-resistant clones (C10) exhibited only minimal EROD induction and was chosen for further characterization. Sucrose density gradient analysis of nuclear extracts from cells treated with [^{3}H]TCDD detected the liganded nuclear Ah receptor in the wild-type cells. In contrast, C10 cells treated with [^{3}H]TCDD failed to accumulate significant levels of the 6S nuclear Ah receptor complex. Furthermore, nuclear extracts from TCDD-treated C10 cells failed to bind a [^{32}P]labeled dioxin responsive element (DRE). Aryl hydrocarbon receptor (AhR) mRNA levels were also investigated and only the wild-type cells constitutively expressed the AhR mRNA. Estrogen receptor (ER) mRNA levels, ligand binding and binding to the estrogen responsive element (ERE) were all decreased in the mutant cells; however, estrogen induced cell proliferation. In contrast, results from sucrose density gradient and gel shift assays indicated that progesterone receptor levels and binding to the progesterone responsive element in the BaP-resistant cells were not significantly different than observed in wild-type T47-D cells. A major difference between the BaP-resistant T47D and MCF-7 mutant cell lines were associated with the Ah receptor complex; in the latter cell line treatment with TCDD resulted in formation of a transcriptionally inactive nuclear Ah receptor complex whereas only minimal levels of this complex were detected in BaP-resistant T47-D cells.

1270 INDUCTION OF CYP1A1 GENE EXPRESSION IN HUMAN BREAST CANCER CELLS: A ROLE FOR THE ESTROGEN RECEPTOR

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In the MDA-MB-231 human breast carcinoma cell line, the estrogen receptor (ER) is not expressed and the Ah receptor (AhR) is non-functional. Transfection of the cells with the expression vector, ΔhER, encoding a human ER, restored Ah-responsiveness to these cells as indicated in a transient expression assay. Cells transfected with pRNH11c, containing the intact *CYP1A1* regulatory region in front of the *CAT* (chloramphenicol acetyl transferase) marker gene, expressed CAT activity when treated with 10 nM 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD); minimal induction was observed in control cells. In order to investigate whether the interaction between the ER and AhR was unique to the MDA-MB-231 cell line, MCF-7 human breast carcinoma cells were treated with 1 μM ICI 164,384, a pure antiestrogenic compound which destabilizes the ER-homodimer and increases the rate of ER degradation. Treatment with this antiestrogen resulted in a 65% decrease in levels of nuclear ER binding using [^{3}H]-estradiol as a radioligand. TCDD-induced CAT activity was significantly reduced in cells transfected with pRNH11c and cotreated with 1 μM ICI 164,384 and 10 nM TCDD. Likewise, the induction of ethoxyresorufin *O*-deethylase (EROD) activity by TCDD was significantly decreased in cells cotreated with TCDD plus ICI 164,384. In addition, ICI 164,384 also inhibited formation of the nuclear AhR-complex in these cells. The data indicate that the ER plays a role in AhR-mediated transactivation of the *CYP1A1* gene in human breast cancer cell lines and this may be related to expression of the AhR and aryl hydrocarbon nuclear translocation (Arnt) proteins.

1271 INTERACTIVE EFFECTS OF INDOLE-3-CARBINOL (I3C) AND 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN (TCDD) IN T47D HUMAN BREAST CANCER CELLS: I3C INHIBITS INDUCTION OF CYP1A1 BY TCDD

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Dietary / environmental compounds which exhibit aryl hydrocarbon (Ah) recep-
tor agonist and receptor binding activity include 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related haloaromatics, indole-3-carbinol (I3C) and derived heterocyclic aromatics found in vegetables, and polynuclear aromatic hydrocarbons (PAHs) found in cooked foods. TCDD binds with high affinity to the Ah receptor and is a potent agonist whereas I3C exhibits weak binding to the receptor and is a weak agonist. In Ah-responsive T47D human breast cancer cells, TCDD causes a concentration-dependent induction of ethoxyresorufin *O*-deethylase (EROD) activity and the maximal induced activity was 380 pmol/min/mg using 1 nM TCDD. In contrast, I3C did not significantly induce EROD activity at concentrations as high as 125 μM and this result was consistent with the reported low Ah receptor agonist activity of this compound. However, in T47D cells cotreated with 1 nM TCDD plus 1 to 125 μM I3C, the latter compound significantly inhibited TCDD-induced activity at concentrations from 31 to 125 μM with maximal inhibition (> 85%) observed at the 125 μM concentration. Treatment of T47D cells with 10 nM [^{3}H]TCDD resulted in formation of the nuclear Ah receptor complex; however, in cells cotreated with the radioligand plus 125 μM I3C, the radiolabeled nuclear Ah receptor complex was not detected. Thus, I3C exhibits Ah receptor antagonist activity. I3C and TCDD represent two classes of dietary Ah receptor agonists in which non-additive (antagonistic) interactions are observed.

1272 REGULATION OF CYP1A1 GENE EXPRESSION IN THE ESTROGEN RECEPTOR NEGATIVE Hs578T HUMAN BREAST CANCER CELL LINE

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Hs578T breast cancer cells are hormone (estrogen)-independent. Incubation of these cells with 10 nM [^{3}H]2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) resulted in formation of the radiolabeled nuclear aryl hydrocarbon receptor (AhR) complex which sedimented at 6.9S. Nuclear extracts from Hs578T cells treated with unlabeled TCDD formed a specifically-bound DNA-protein retarded band in a gel mobility shift assay using a [^{32}P] dioxin responsive element. This band only formed after treating the cells with TCDD. In contrast, TCDD did not induce CYP1A1 gene expression in Hs578T cells. The effects of human AhR, aryl hydrocarbon receptor nuclear translocator (Arnt) and estrogen receptor (ER) expression plasmids on restoring Ah-responsiveness to Hs578T cells was investigated by transient transfection studies. Ah-responsiveness was determined by cotransfection of the pRNH11c plasmid which contains the human CYP1A1 regulatory region (−1142/+2434) fused to the bacterial chloramphenicol acetyl transferase (CAT) reporter gene. In cells cotransfected with the pRNH11c plasmid plus the AhR or Arnt expression plasmids, constitutive CAT activity was not significantly increased and TCDD did not induce CAT activity. In contrast, there was a 2.7- to 5-fold or 15- to 20-fold increase in CAT activity in Hs578T cells cotransfected with hER alone or hER plus AhR plus Arnt expression plasmids. The increased CAT activity was observed in the presence or absence of TCDD. In estrogen-nonresponsive MDA-MB-231 breast cancer cells, TCDD significantly induced (9-fold) CAT activity only in cells cotransfected with the hER and pRNH11c plasmids. The differential regulation of Ah-responsiveness in Hs578T and other breast cancer cell lines is being further investigated using deletion plasmid constructs derived from the 5-flanking region of the human CYP1A1 gene.

1273 COMPARATIVE INDUCTION OF CYP1A1 FOLLOWING TCDD EXPOSURE IN RAT LIVER, KIDNEY, AND LUNG

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Cytochrome P-4501A1 (CYP1A1) induction is a sensitive Ah-receptor mediated response which may serve as a biomarker for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds. The relative sensitivity of this response was investigated in male Harlan Sprague Dawley rats at 24 hours following a single exposure to TCDD at doses of 0.002, 0.02, 0.1, 1, and 5 μg/kg, p.o. Tissue concentrations of TCDD ranged from 8 to 64,698 ppt (wet weight) for the liver, 4 to 1,177 ppt for the lung, and 2 to 1,701 ppt for the kidney in the low and high exposure groups, respectively. Western blot with enhanced chemiluminescence detection was used to quantitate CYP1A1 protein, while the O-deethylation of ethoxyresorufin (EROD) was used to assess CYP1A1 activity. A dose related increase in CYP1A1 protein levels was observed in each tissue with CYP1A1 protein being detected at the lowest TCDD dose (0.002 μg/kg). CYP1A1 protein levels for the respective low and high exposure groups ranged from 0.946 to 1,560 pmol 1A1/mg protein for the liver, 2.76 to 93.4 pmol 1A1/mg protein for lung, and 0.46 to 43.8 pmol 1A1/mg protein for kidney. EROD activity increased in a similar dose-related manner, with the high dose producing activities of 16,127, 4,482, and 3,933 pmol/min/mg for the liver, lung, and kidney, respectively. In conclusion, although less TCDD was distributed to the lung and kidney, all tissues were responsive to TCDD even at the lowest dose administered. In addition to the liver, the kidney and the lung appear to be sensitive tissues for biomarker studies for this class of compounds. (Supported in part by NIEHS grant RO1-ES06556-01).

1274 CELL-FREE ACTIVATION OF PROTEIN KINASES BY 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN (TCDD)

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2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) activates protein kinases after cell-free treatment of C57 mouse liver cytosol (100,000 × g supernatant). This action of TCDD was confirmed by using three different methods including SDS-polyacrylamide gel electrophoresis and trichloroacetic acid-precipitation of native ^{32}P-phosphoproteins, and phosphorylation of histone as an exogenous substrate. Increased protein phosphorylation by TCDD was dose-dependent with an EC_{50} of approximately 0.5 nM. In addition, this effect was found to be dependent on TCDD binding to the Ah receptor (AhR) since TCDD's activation of protein phosphorylation was blocked by two well-known AhR blockers, α-naphthoflavone and 4,7-phenanthroline. Increased protein phosphorylation was also observed after cell-free TCDD treatment of cytosol from Hepa 1C1C7 cells and from Hepa C4 cells, a mutant cell line that lacks the ARNT protein, a dimerization partner of the AhR required for DNA binding. Interestingly, genistein, a protein tyrosine kinase inhibitor, blocked TCDD's activation of protein kinases in this system. The present findings clearly show that: 1) TCDD activates protein kinase(s) in a cell-free system, 2) this effect of TCDD is dose-dependent and AhR-dependent, and 3) a protein tyrosine kinase appears to be involved. These effects might represent a novel mechanism to explain at least some of TCDD's diverse effects. (Supported by NIH grants ES03575 and ES02533).

1275 CHARACTERIZATION OF THE DNA BINDING OF TCDD: Ah RECEPTOR COMPLEXES TRANSFORMED *IN VIVO* AND *IN VITRO*

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The Ah receptor (AhR) is a ligand-dependent transcription factor which activates gene expression via a high affinity interaction with its specific DNA recognition site (the dioxin responsive element (DRE)). Hepatic cytosolic 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-bound guinea pig and C3H mouse AhR complexes, transformed *in vitro*, contain at least three protein subunits which appear to be contained within two distinct AhR complexes. However, the presence of these complexes in nuclear extracts from treated cells has not been demonstrated. Cytosolic and nuclear TCDD:AhR complexes (transformed *in vitro* and *in vivo*, respectively) prepared from C3H-derived mouse hepatoma and guinea pig epithelial cell lines were UV-crosslinked to a BrdU-containing DRE oligonucleotide. Denaturing PAGE revealed that both cytosolic and nuclear extracts contained three protein subunits which crosslinked to a BrdU-substituted DRE oligonucleotide in a TCDD-dependent manner. These results not only imply that *in vitro* transformation of cytosolic AhR mimics that which occurs in intact cells, but they suggest that both the multiple subunits and AhR complexes we observed utilizing crude cytosolic extracts also exist in intact cells and may be of toxicological significance. (Amer. Can. Soc. #CN-4C and Int. Life Sci. Inst.).

1276 FUNCTIONAL DOMAINS OF THE Ah RECEPTOR INVOLVED IN Ah RECEPTOR COMPLEX MEDIATED SIGNAL TRANSDUCTION

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The aryl hydrocarbon receptor (AHR) binds a variety of environmentally important carcinogens such as chlorinated polycyclic aromatic hydrocarbons (eg. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin) and polychlorinated biphenyls, and activates transcription of several genes involved in xenobiotic metabolism. The unliganded receptor is found in cytosol as a complex with the 90 kDa heat shock protein (HSP90) and perhaps other, as yet unidentified proteins. Upon ligand-binding, a dimer of AHR and the Ah receptor nuclear translocator protein (ARNT) is found in the nucleus where it binds to Xenobiotic Response Elements (XRE), which control transcription of the *CYP1A1* gene. ARNT and AHR both belong to the bHLH-PAS family of transcription factors. By means of site directed mutagenesis, we have identified functional domains in the AHR protein required for ligand activated signal transduction. Deletion of the first nine amino acids does not affect ligand binding capacity, however severely reduces heterodimerization and XRE-binding. As in other bHLH proteins, the basic region is primarily responsible for interaction with the DNA target, and the HLH domain directs heterodimerization with ARNT. Deletion of the PAS-A domain severely affects ligand binding, heterodimerization and XRE-binding. The PAS-B domain encompasses the ligand binding domain. A "minimal construct" lacking the C-terminal half of the full length AHR is functional in all *in vitro* assays, yet fails to activate transcription *in vivo*.

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1277 HEPATOCYTE PROLIFERATION AND PROMOTION OF ALTERED HEPATIC FOCI BY 2,3,7,8-TETRACHLORO-DIBENZO-p-DIOXIN

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Though not a known human carcinogen, 2,3,7,8-tetrachloro-dibenzo-p-dioxin(TCDD) is a prototypical rat liver tumor promoter. The objective of the current study was to investigate the dose-response relationship of TCDD mediated promotion of altered hepatic foci (AHF) and affects on cell proliferation in the two-stage model of rat liver carcinogenesis. Female Sprague-Dawley rats were administered a single dose of diethylnitrosamine (DEN, 10 mg/kg, p.o.) 24 hours after partial hepatectomy. Animals were administered TCDD biweekly at 0.0 μg/kg, 0.14 μg/kg, 0.014 μg/kg, or 0.0014 μg/kg. One week prior to sacrifice, minipumps containing bromodeoxyuridine (BrdU) were implanted sub-cutaneously. Animals were sacrificed at 1 month, and four serial liver sections were each stained for different markers of AHF, while one section was stained with an antibody to BrdU. The number and volume fraction of AHF was determined by quantitative stereology and the labeling index was determined by counting the number of labeled cells/thousand hepatocytes. Increases in the number and volume fraction of AHF were observed for each marker at the two highest doses of TCDD in the DEN-reated animals. TCDD reduced the proliferation rate of non glutathione S-transferase expressing hepatocytes at all doses in animals receiving TCDD. Thus, one mechanism of TCDD liver tumor promotion may be suppression of proliferation of non-focal hepatocytes.

1278 SUBCHRONIC ORAL TOXICITY OF DI-N-OCTYL PHTHALATE (DNOP) AND DI-(2-ETHYLHEXYL) PHTHALATE (DEHP) IN THE RAT

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The objective of the present study was to investigate the systemic toxicity of DNOP and DEHP, two widely used plasticizers and ubiquitous environmental pollutants. Groups of 10 male and 10 female weanling rats were administered DNOP or DEHP in the diet at levels of 0, 5.0, 50.0, 500 or 5,000 ppm for 13 weeks. Growth rate and food consumption were not affected by either compound. Hepatomegaly was observed in the highest DEHP groups of both sexes but not in the DNOP treated ones. The highest dose DNOP groups had increased hepatic ethoxyresorufin-O-deethylase activity while the corresponding DEHP groups showed an increase in liver aminopyrine-N-demethylase activity. No hematological or other biochemical abnormalities were observed. Exposure to 5,000 ppm DEHP resulted in testicular atrophy as well as liver peroxisomal proliferation as examined by the EM. Treatment-related histological changes of various degrees were observed in the liver and thyroid of the top dose groups of DNOP and DEHP. The above data suggest that the two structurally similar phthalate esters appear to act by different mechanisms. The no observable adverse effect levels for DNOP and DEHP were judged to be 500 ppm in the diet or approximately 40 mg/kg b.w./day.

1279 KCN INSENSITIVE RESPIRATION FOLLOWING DIETARY EXPOSURE TO WY-14,643 (WY)

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WY increases basal rates of O_2 uptake in perfused livers. Because WY also uncouples oxidative phosphorylation, KCN, an inhibitor of mitochondrial cytochrome oxidase, was infused into livers of WY fed rats (0.1% WY in chow for 105 days) to determine if mitochondria are responsible for the increase in cellular respiration. As expected, the addition of KCN nearly abolished O_2 uptake. However, after about 15 minutes liver O_2 consumption returned to basal levels in WY treated rats but not in controls. Urea synthesis, a highly ATP dependent event, was decreased and remained low with KCN infusion in all groups indicating that mitochondria are not responsible for this unusual increase in O_2 uptake. Methanol metabolism, which requires peroxisomal O_2 to form H_2O_2, was decreased from 37 ± 5 to 6 ± 1 μmol/g/hr in both treatment groups; however, methanol metabolism increased subsequently to 25 μmol/g/hr in livers only from WY fed rats indicating that O_2 for peroxisomal H_2O_2 production is partly responsible for the KCN insensitive respiration. Fasting abolished the recovery of both O_2 uptake and methanol metabolism in WY fed rats, suggesting that ATP for acyl CoA synthetase, a peroxisomal enzyme which metabolizes fatty acids to acyl CoA compounds, is provided by glycolysis. Indeed, oleate significantly increased methanol metabolism from 8 ± 4 to 26 ± 3 μmol/g/hr in the presence of KCN indicating that fatty acid supply is rate limiting for peroxisomal acyl CoA synthetase activity. Taken together, these experiments show that in the absence of mitochondria livers from rats fed WY chronically have the unique ability to metabolize fatty acids through the peroxisome using glycolytic ATP. It is possible that when the normal cellular energy source is challenged (*e.g.*, hypoxia), peroxisomal fatty acid oxidation provides short chain fatty acids for the mitochondria thereby allowing accelerated cell growth upon the reintroduction of oxygen (ES-04325).

1280 COMPARISON OF THE EFFECTS OF WYETH-14,643 AND CHLOROFORM ON HEPATIC AND RENAL CELL PROLIFERATION IN THE MALE LONG EVANS RAT

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Wyeth-14,643 is a model peroxisome proliferator, a hepatocarcinogen and a mitogen causing increased hepatic cell proliferation in Fisher-344 rats. Chloroform ($CHCl_3$) is a hepatotoxicant which also stimulates cell proliferation but by a mechanism involving a regenerative response to hepatic damage. Detection of enhanced cell proliferation in target organs following subacute exposure to a test agent can provide important information about chemicals that stimulate mitosis. This study was conducted to compare hepatic and renal labelling indices (LI) in male Long Evans rats administered either 0.1% Wyeth-14,643 in the feed or 400 mg/kg/day $CHCl_3$ (days 1–5) or 180 mg/kg/day $CHCl_3$ (days 6–21) in corn oil. Animals were treated with Wyeth-14,643 or $CHCl_3$ for 4 and 21 days. LI, the percentage of cells in a tissue progressing through S-phase, was measured using immunohistochemical detection of 5-bromo-2′-deoxyuridine (BrdU) incorporation into newly synthesized DNA in proliferating cells. BrdU was administered for 3 days prior to sacrifice by s.c. implanted osmotic pumps. Both absolute and relative liver weights of Wyeth-14,643-treated animals were increased after 4 and 21 days of treatment. Although relative liver weights were unaffected in $CHCl_3$-treated animals, absolute liver weights were slightly increased at day 4 and decreased at day 21. There was an 11-fold increase in β-oxidation of Palmitoyl CoA in 600xg supernatant in rats administered 0.1% Wyeth-14,643 for 4 days. Liver LIs for control feed, Wyeth-14,643, corn oil and $CHCl_3$ treated livers were 2.73, 15.31, 3.36 and 47.09% for day 4 and 0.54, 3.39, 0.66 and 1.59% for day 21, respectively. Preliminary data indicate that Wyeth-14,643 and $CHCl_3$ had little effect on the LI of kidney at either 4 or 21 days. These data indicate that both Wyeth-14,643 and $CHCl_3$ increase hepatic cell proliferation in the Long Evans rat, but to different degrees and via different mechanisms.

1281 INVESTIGATION OF MECHANISMS FOR WYETH-14,643 INDUCTION OF PANCREATIC ACINAR CELL TUMORS IN THE RAT

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In a two-year feeding study with Crl:CD®BR male rats, the peroxisome proliferating compound Wyeth-14,643 (WY), at a dietary concentration of 50 ppm, increased the tumor incidence in the liver, testis (Leydig cells), and pancreas (acinar cells). The purpose of the current experiments is to examine the mechanisms of pancreatic tumor induction by WY in the rat. The primary regulator of pancreatic acinar cell growth is mediated by cholecystokinin (CCK), an intestinal peptide hormone. CCK_a receptor agonists and compounds that have trypsin-inhibition activity or increase gut fat produce pancreatic acinar cell hypertrophy and hyperplasia. We are investigating the effects of WY in a two-month study in which rats fed a diet of 100 ppm WY will be sacrificed at 7, 14, 28 and 56 days and assessed for alterations in pancreas weight, acinar cell proliferation and histopathology, and focal fat content. Serum CCK levels will be assayed by radioimmunoassay. WY will also be tested *in vitro* for its ability to compete for binding directly to cholecystokinin receptors. Using an *in vitro* assay WY and ammonium perfluorooctanoate (C8), another peroxisome proliferator which induces pancreatic cell tumors, were tested for trypsin-inhibition activity and both compounds were absent of this activity. Compounds which possess trypsin-inhibition activity generally increase pancreatic weight within 7 days of treatment. The time-course for WY-induction of pancreatic weight also suggest that it does not possess trypsin-inhibition activity since no increases were seen following 14 days of treatment. Aliphatic dicarboxylic acids are hypolipidemic and have been shown to increase gut fat content. Since WY is also a hypolipidemic agent and has been shown not to inhibit trypsin, this compound may alter gut fat and perturb CCK regulation.

1282 DISCORDANT CYCLIN DEPENDENT KINASE AND CYCLIN EXPRESSION IN RAT LIVER FOLLOWING ACUTE WY14,643 DOSING

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Cyclin dependent kinases (CDKs) and cyclins comprise two families of proteins that regulate cell cycle progression. They do so by forming complexes and initiating signal transduction cascades that control and coordinate the processes of cell division. Kinase activity and substrate specificity of the CDKs are controlled by transcription, complexing with cyclin proteins, and phosphorylation/dephosphorylation. Greater than 90% of the primary tumors and transformed cell lines studied in this and other laboratories demonstrated aberrant CDK or cyclin expression. In order to assess the potential of these proteins as biomarkers of carcinogen exposure, we examined the expression of several CDKs and cyclins in response to dosing with the hepatocarcinogen WY14,643. Male Sprague-Dawley rats were given daily i.p. injections of 45 mg WY14,643/kg or an equal volume of the corn oil carrier for 4 days. The livers were removed, weighed, homogenized and centrifuged at 9,000 × g to produce a supernatant fraction (S9). Cellular responses to treatment were analyzed using size exclusion chromatography and ELISA to assess formation of CDK/cyclin complexes *in vivo*. All hepatic CDKs and cyclins demonstrated a degree of enhanced, albeit discordant, expression compared to controls. Large differences were seen with CDK1 ($p34^{cdc2}$), CDK4, cyclin B, cyclin D3, and PCNA expression. These data represent further evidence that quantification of cell division control enzymes can be utilized as a biomarker for cell proliferation and risk assessment of nongenotoxic carcinogens.

1283 ACUTE DOSING OF RATS WITH THE NON-GENOTOXIC HEPATIC CARCINOGEN WY14,643 RESULTS IN INCREASED LEVELS OF A CDK1 COMPLEX WITH AN ABNORMAL MOLECULAR MASS

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Cyclin dependent kinases (CDKs) are a family of serine/threonine kinases that coordinate and control progression through the cell cycle. In normal asynchronously dividing cells, CDK1 (34 kD) forms a quaternary complex with cyclin B1 (63 kD) proliferating nuclear cell antigen (PCNA, 36 kD) and p21 (21 kD) while in transformed cells this complex disappears (Xiong et al., 1993, Genes Dev. 7: 1572–1583). The present study was done to determine if treatment with WY14,643 alters expression and complexing of CDK1 in liver. Sprague Dawley rats were injected IP daily with 45 mg WY14,643 / kg or an equal volume of corn oil vehicle for 4 days. The livers were removed, weighed, homogenized and centrifuged at 9000 × g to produce an S9 fraction. The S9 was subjected to gel filtration chromatography and the fractions probed with anti-CDK1 or anti-PCNA by direct ELISA. Gel filtration chromatography of WY14,643-treated rat S9 revealed a major peak of CDK1 with an apparent molecular mass of 70 kD that was 25-fold higher than in control S9. The CDK1 in this fraction had a molecular mass of 34 kD by western blot. Assay of treated S9 fractions by PCNA ELISA also revealed a major peak at 70 kD that was 15-fold higher than seen in control S9. The PCNA in this fraction had a molecular mass of 38 kD by western blot. Treatment with WY14,643 results in dramatically increased levels of CDK1 and PCNA. The CDK1 chromatographs as a complex with an apparent molecular mass inconsistent with reported CDK1 complexes and, together with the increased levels of CDK1, represents an abnormal expression of CDK1.

1284 INTRACELLULAR LOCALIZATION OF CYCLINS AND CYCLIN DEPENDENT KINASES (CDKs) IN WY14,643-TREATED RAT LIVER

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Previous studies have indicated that WY14,643 is involved in the modulation of cellular signaling via expression of the cell division control enzyme $p34^{cdc2}$ kinase (CDK1) and cyclin (PCNA). This study was designed to describe the expression and intracellular localization of CDKs and cyclins. Young adult male rats were given i.p. injections of 50 mg WY14,643/kg for 1 or 2 days. ELISA and immunoblotting methodology using anti-CDK and anti-cyclin antibodies were used to characterize hepatic CDK and cyclin expression. WY14,643 increased hepatic expression of CDK1, CDK2, CDK4, and CDK5 as well as cyclins A, B, E, and PCNA relative to controls. CDK1 was found, in descending order of magnitude, in cytosolic, microsomal and nuclear fractions. With WY14,643-treatment, CDK2, CDK4, and cyclin E were located principally in the cytosolic fraction, although smaller amounts were also found in the endoplasmic reticulum and nucleus. CDK5 was found in cytosol, but not in microsomes or the nucleus. Cyclins A, B, and PCNA were present in the cytosol and nucleus but absent in microsomal fraction. An understanding of the differences in the expression, localization and activation of cell division control enzymes between normal hepatic regeneration and carcinogen-stimulated proliferation can provide a mechanistic basis for risk assessment of nongenotoxic carcinogens such as WY14,643.

1285 REDUCTION OF EICOSANOID CONCENTRATIONS IN RAT HEPATOCYTE CULTURES TREATED WITH THE PEROXISOME PROLIFERATOR CIPROFIBRATE

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Several hypolipidemic drugs, plasticizers and other chemicals induce peroxisome proliferation and hepatic tumors in rodents. The carcinogenic activity of peroxisome proliferator may be related to alterations in gene expression, such as induction of peroxisomal β-oxidation enzymes or of the cytochrome P450 4A family. These enzymes metabolize lipids, including eicosanoids and their precursor fatty acids. In this study we used cultured hepatocytes to study if peroxisome proliferators alter the metabolism of prostaglandins (PG) and thromboxanes (Tx). Isolated rat hepatocytes were cultured for 4 days with 2 doses of ciprofibrate (CIP) (100 μM and 400 μM). Fatty acyl CoA oxidase activities of the 100 μM and 400 μM CIP treatment groups at the end of the experiment were increased 5.3 and 9.6 times respectively. TxB_2 concentrations in cultures treated with 400 μM CIP were significantly lower than the control at day 2, and treatment with both doses were lower than the control at days 3 and 4. Cultures treated with 400 μM CIP had lower $PGF_{2\alpha}$ concentrations than the controls at day 3, and also at day 4 with both doses. A lower concentration of PGF_2 was seen at day 4 only. These studies show that PG and Tx concentrations in cultured hepatocytes are lowered by the peroxisome proliferator CIP. (Supported by NIH grants CA-43719 and CA-01688)

1286 HEPATOCYTE PROLIFERATION INDUCED BY THE PEROXISOME PROLIFERATOR CIPROFIBRATE DOES NOT ACTIVATE α-FETOPROTEIN TRANSCRIPTION IN ADULT MICE

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The prototypical oncofetal protein α-fetoprotein (AFP) is expressed at high levels in fetal liver. A dramatic postnatal decline in AFP levels is seen and very low levels of AFP transcription are maintained in adult mice. However, repression is reversible as AFP can be reactivated in the adult liver during periods of renewed cell proliferation such as during liver regeneration, induced experimentally by partial hepatectomy or CCl_4. Although AFP transcription and DNA synthesis increase in parallel during regeneration, the relationship between these two events is unclear. To determine whether increased hepatocyte proliferation is sufficient for AFP induction, we have analyzed AFP levels in mice treated with the peroxisome proliferator ciprofibrate. Ciprofibrate greatly increases proliferation in hepatocytes in the first several days after it is administered. Mice were fed with 0.01% ciprofibrate in the diet and sacrificed 3, 5 and 10 days later. Control mice were sacrificed at the same times. The activity of cytochrome P-450 4A1 was induced 20–25 fold at all time points in liver microsomes from treated mice. Using a sensitive RNase protection assay, we observed no increase in AFP expression at any of the time points. These data show that ciprofibrate does not activate AFP transcription. We conclude that hepatocyte proliferation is not sufficient for AFP induction and argue that additional signals are required for AFP reactivation in adult mice. (Supported by NIH Grants ES05815 and CA01688).

1287 CELLULAR MECHANISMS OF NON-GENOTOXIC HEPATOCARCINOGENESIS: CO-ACTION OF PEROXISOME PROLIFERATORS AND EGF, TGFα AND TGFβ

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Peroxisome proliferators (PPs) are a class of non-genotoxic carcinogens and are potent rodent hepatocarcinogens and hepatomitogens. This induced hyperplasia may lead to the fixation and promotion of spontaneously initiated hepatocytes. We have generated showing that PPs act with physiological liver mitogens to cause the hepatocyte clonal expansion seen in the early stages of hepatocarcinogenesis. Using the soft agar cloning assay, only 5 spontaneous colonies were generated from 50,000 hepatocytes. However, an eight-fold increase in colony number was seen when the PP nafenopin and epidermal growth factor (EGF) were added together, suggesting that these factors synergize to promote the clonal expansion associated with the early stages of tumorigenesis. This response was concentration dependent with an optimum at

100 μM nafenopin and 25 ng/ml EGF. Interestingly, this effect was not common to all hepatomitogens since hepatocyte growth factor (HGF), one of the most potent of the liver growth factors, was unable to promote colony formation. However, TGFα could synergise with nafenopin whereas the synergy between EGF and nafenopin was suppressed by TGFβ.

To test the hypothesis that the factors synergize to promote the clonal outgrowth of initiated hepatocytes, rats were dosed *in vivo* with an initiating-dose of the genotoxic carcinogen diethylnitrosamine (DEN). Significantly, the number of both spontaneous and factor-promoted colonies was more than doubled. These data provide evidence to support the hypothesis that PPs act as non-genotoxic carcinogens by their ability to synergise with EGF or TGFα in the promotion of initiated hepatocyte clonal expansion. This finding is particularly significant when considered alongside our data demonstrating that PPs inhibit hepatocyte apoptosis that may remove DNA damaged, potentially initiated cells from liver.

1288 SPECIES DIFFERENCES IN HEPATIC PEROXISOME PROLIFERATION, CELL REPLICATION AND TRANSFORMING GROWTH FACTOR-β1 (TGF-β1) GENE EXPRESSION

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A variety of chemicals including methylclofenapate (MCP), ciprofibrate (CIPRO) and Wy-14,643 (WY) have been shown to produce hepatomegaly, peroxisome proliferation and liver tumours in the rat. Male Sprague-Dawley rats, Syrian hamsters and Dunkin-Hartley guinea pigs were given daily oral doses of either 0 (corn oil) or 75 mg/kg/day MCP for periods of 6 and 21 days. Groups of Syrian hamsters and guinea pigs were also given 25 mg/kg/day of either CIPRO or WY. Relative liver weights were significantly increased in peroxisome proliferator treated rats and Syrian hamsters, but not in guinea pigs. Hepatic peroxisomal and microsomal fatty acid oxidising enzyme activities and CYP4A1 mRNA levels were significantly increased in rats and Syrian hamsters, whereas only minor effects were observed in the guinea pig. Replicative DNA synthesis was studied by implanting 7 day osmotic pumps containing 5-bromo-2'-deoxyuridine during study days −1 to 6 and 14 to 21. Hepatocyte Labelling Index values were increased by MCP in the rat, but no significant effects were observed in the Syrian hamster and guinea pig. Although TGF-β1 (which is known to inhibit hepatocyte replication and trigger apoptosis) mRNA expression was increased by MCP in the rat, only a small effect was observed in the Syrian hamster and no change was noted in the guinea pig. These results demonstrate marked species differences in response to rodent peroxisome proliferators. Whereas peroxisome proliferators produce a wide spectrum of effects in rat liver, other species — such as the Syrian hamster and guinea pig — are clearly less responsive or refractory. (Supported by the UK Ministry of Agriculture, Fisheries and Food).

1289 FUNCTIONAL CHARACTERIZATION OF RAT PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR α (PPARα)

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Peroxisome proliferator-activated receptors (PPARs) are members of the steroid receptor superfamily which have been implicated in regulating cellular responses to a wide variety of peroxisome proliferators (PPs). The purpose of the present studies was to define the domains of PPARα which are involved in protein-protein interactions as well as possible sites of phosphorylation by protein kinases. Full length as well as putative functional domains (A/B, C, D, E/F) of rat PPARα were cloned into an expression vector. Maltose binding protein (MBP) fusion proteins were produced in *E. Coli* and affinity purified. The interaction of PPARα or PPARα-domains with rat hepatoma cellular proteins was initially examined using far-western blotting techniques and several binding partners were observed. These interactions appear to be localized to the A/B and E/F domains of PPARα. The identity of proteins which bind with PPARα will be determined using interaction cloning and screening of a rat hepatoma cDNA library. The functions of several members of the steroid receptor superfamily are regulated by phosphorylation. Incubating PPAR-MBP with rat hepatoma cytosol and ^{32}P-ATP followed by affinity purification indicated that the PPARα fusion protein is phosphorylated *in vitro*. The kinase responsible and the PPAR domain involved have not been determined. These preliminary studies provide evidence that PPAR function may be regulated via protein-protein interactions and/or phosphorylation. (Supported by Showalter Trust Grant 6056).

1290 COMITOGENICITY OF EICOSANOIDS AND THE PEROXISOME PROLIFERATOR CIPROFIBRATE IN CULTURED RAT HEPATOCYTES

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Several hypolipidemic drugs, and environmental contaminants induce hepatic peroxisome proliferation and hepatic tumors when administered to rodents. These chemicals increase the expression of the peroxisomal β-oxidation pathway and the cytochrome P-450IVA family, which metabolize lipids, including eicosanoids and their precursor fatty acids. We previously found that the peroxisome proliferator ciprofibrate (CIP) decreases the levels of eicosanoids in the liver and in cultured hepatocytes. In this study, we examined the effect of prostaglandins E_2 and $F_{2\alpha}$ (PGE_2 and $PGF_{2\alpha}$), leukotriene C_4 (LTC_4) and the peroxisome proliferator ciprofibrate on DNA synthesis in cultured hepatocytes. Primary rat hepatocytes were cultured on collagen gels in serum-free L-15 medium with increasing concentrations of CIP, eicosanoids, and the absence or presence of growth factors. The addition of PGE_2, $PGF_{2\alpha}$, and LTC_4 to cultures along with ciprofibrate greatly increased DNA synthesis, whereas treatment with CIP or eicosanoid alone resulted in a much smaller increase. The addition of epidermal growth factor (EGF) to the eicosanoid-CIP combination increased DNA synthesis more than EGF or the eicosanoid-CIP combination alone. Addition of both eicosanoids and CIP also blocked the growth inhibitory effect of transforming growth factor-β on DNA synthesis induced by EGF. These results show that eicosanoids and the peroxisome proliferator CIP are co-mitogenic in cultured hepatocytes. (Supported by NIH grants CA-43719 and CA-01688).

1291 THE DIRECT EFFECTS OF 11 HEPATIC PEROXISOME PROLIFERATORS (PPs) ON RAT LEYDIG CELL FUNCTION *IN VITRO*

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A review of the literature indicates that compounds which produce hepatic peroxisome proliferation in rats also appear to produce Leydig cell adenomas. The present study evaluated the direct effects of trichloroethylene (TCE), tibric acid (TIB), ciprofibrate (CIP), 2,4-dichlorophenoxyacetic acid (2,4-D), gemfibrozil (GEM), ammonium perfluorooctanoate (C8), tiadenol, Wyeth 14,643, trichloroacetic acid, clofibric acid, and acetylsalicylic acid on Leydig cell function *in vitro*. Leydig cells isolated from adult rats (12–16 weeks old) were incubated with PPs for up to 3 days with daily changes of medium. Each day, medium was retrieved and assayed for estradiol (E_2). Cells were then incubated for 3 hours in fresh medium ± human chorionic gonadotropin (hCG). Medium was assayed for testosterone (T), androstenedione (A), and 17α-hydroxyprogesterone (OH-P). Data are only reported for compound doses that were not cytotoxic. PPs reduced the hCG-stimulated release of T. With the exception of 2,4-D, GEM, and C8, this inhibitory effect was confirmed in a repeat experiment. A similar pattern of inhibition was generally seen for hCG-stimulated OH-P and A. Low doses of TCE, CIP, and TIB increased basal and stimulated T from cultures of up to 3 days. No common pattern in E_2 release could be established for all PPs. Of the 11 PPs, 3 decreased and 6 increased E_2 release, and 2 PPs showed inconsistent or no effects on E_2. No consistent relationship was seen between the E_2 and stimulated T responses. The results suggest that these PPs directly inhibit the stimulated release of T from Leydig cells *in vitro*, and exhibit selectivity in their effects on T and E_2. These direct effects provide a possible mechanism for Leydig cell tumor formation by some PPs.

1292 COMPARATIVE EFFECTS OF THE PEROXISOME-PROLIFERATORS, CLOFIBRATE, GEMFIBROZIL, AND BEZAFIBRATE ON THE CD RAT LIVER

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Male and female CD® *[Crl:CD® BR VAF/plus®]* rats were administered one of two dose levels (150 or 300 mg/kg; 150 or 300 mg/kg; and 50 or 100 mg/kg, respectively) of clofibrate, gemfibrozil, or bezafibrate daily for a period of 14 days. The objective was to compare β-oxidation changes indicative of hepatic peroxisome proliferation in this rat stock. Subsequent gross examination of the liver indicated dose-related increases in relative and absolute liver weights in males following clofibrate and gemfibrozil. In females, absolute and relative liver weights were elevated to a similar degree with either dose of gemfibrozil, and absolute liver weights were higher in clofibrate-dosed animals. Bezafibrate had no effect on female liver weights. Clofibrate and gemfibrozil increased hepatic palmitoyl CoA β-oxidation in both sexes; however, clofibrate had the greatest effect in males and gemfibrozil had the least effect in females. Bezafi-

brate treatment resulted in a very pronounced elevation of palmitoyl CoA β-oxidation in the males but had no effect in the females. These data demonstrate clear sex differences in the response to these peroxisome proliferators and indicate that bezafibrate is most potent and gemfibrozil is least potent in stimulating peroxisome-associated β-oxidation in the CD rat liver.

1293 PEROXISOME PROLIFERATION AND MICROSOMAL ENZYME INDUCTION BY THE HYPOLIPIDEMIC CI-924 IN PRIMARY RAT HEPATOCYTE CULTURE

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CI-924 (5,5'[[1,1'-biphenyl]-2,5-diylbis(oxy)]bis[2,2-dimethyl-pentanoic acid]), a lipid lowering agent, was previously shown to be hepatotumorigenic in male and female B6C3F1 mice but not in male and female albino Wistar rats. In *vivo* CI-924 causes significant peroxisome proliferation and microsomal enzyme induction in the livers of both B6C3F1 mice and Wistar rats. Experiments were designed to determine if CI-924 caused peroxisome proliferation and microsomal enzyme induction in primary cultures of rat hepatocytes. Although CI-924 is very well tolerated by rodents *in vivo*, in hepatocyte culture it was cytotoxic at concentrations above 200 μM. CI-924, up to 200 μM, induced concentration dependent increases in carnitine acyl transferase and peroxisomal β-oxidation activity. Increases in carnitine acyl transferase and acyl CoA oxidase activity were measurable 24 hours after addition of 100 μM CI-924 to the cells and activity continued to increase for up to 72 hours. Microsomal cytochrome P450 4A1 detected by Western blotting also increased with time relative to control. This was likely due to increased protein synthesis since, in CI-924 treated cells, increased levels of P450 4A1 mRNA were detected at earlier time points than the increase in protein. In conclusion, the effects of CI-924 in primary rat hepatocyte culture are similar to the effects observed in *vivo*.

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1294 CIPROFIBRATE-INDUCED REPRESSION OF α_{2u}-GLOBULIN EXPRESSION IN LIVER AND INHIBITION OF D-LIMONENE NEPHROTOXICITY

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Peroxisome proliferators induce hepatomegaly and peroxisome proliferation in rodent liver parenchymal cells. One of the earliest known effects of peroxisome proliferators is the rapid and coordinate transcriptional activation of the three genes responsible for the peroxisomal β-oxidation of fatty acids. Long term administration of these chemicals to rats and mice results in the formation of hepatocellular carcinomas. In an attempt to study the non-peroxisomal effects of these compounds, a 2 week ciprofibrate treated rat liver cDNA library was differentially screened using ^{32}P labelled cDNA obtained by reverse transcription of normal and 2 week treated liver RNA. One of the clones that was entirely repressed by ciprofibrate treatment was sequenced. Sequence comparison with the Genebank data base, identified the suppressed clone as α_{2u}-globulin. Northern blot analysis confirmed that α_{2u}-globulin which is normally present in very high levels in male rat liver was absent on treatment with ciprofibrate. This was further confirmed by immunoblotting and immunohistochemical methods. However, immunohistochemical staining showed the presence of a few cells that still stained positive for α_{2u}-globulin. Feeding normal rat chow after 2 weeks of ciprofibrate treatment, showed that α_{2u}-globulin mRNA started reappearing at day 14. Feeding of ciprofibrate for two weeks followed by simultaneous feeding of ciprofibrate and D-limonene for 5 weeks showed that ciprofibrate prevented the renal accumulation of α_{2u}-globulin and nephrotoxicity associated with the D-limonene α_{2u}globulin complex.

1295 HEPATIC TOXICITY OF A PERFLUORETHER ACID FLUORIDE FOLLOWING A TWO-WEEK INHALATION STUDY IN RATS

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Linear C_6–C_{10} perfluoroalkyl acids are liver toxins and possess relatively long biological half-lives. This study was conducted to evaluate whether similar effects occur following exposure to a C_9 perfluorether acid fluoride. Groups of 20 male, Crl:CD® rats were exposed nose-only to 5, 50 or 500 ppb for 6 h/d, 5 d/wk for 2 wk; to monitor temporal changes, subgroups of 5 rats were killed after recovery periods of 2, 6 and 12 wk. Clinical pathologic alterations were found only at 500 ppb and consisted of mild reductions in circulating red blood cell count, Hb, and Ht, increased serum enzyme ALT, AST and ALP activities, and increased serum concentrations of albumin, cholesterol and glucose. Except for cholesterol, these changes resolved by study termination. Concentration-related increases in relative liver weights of up to 140% were seen by wk 2. Liver weights remained 60% above control values in the 500 ppb group by wk 12. Compound-related histopathologic changes were seen only in the liver (hepatocellular necrosis and hypertrophy). Hepatocellular necrosis occurred at 50 and 500 ppb and resolved by wk 6. Hepatocellular hypertrophy, accompanied by a diffuse red cytoplasmic granularity, was found at 50 and 500 ppb and was most severe by wk 2. Except for the 500 ppb group, these microscopic changes resolved by wk 12. Biochemical changes consistent with peroxisome proliferation were noted in the 50 and 500 ppb groups. At 500 ppb, hepatic beta-oxidation activity was increased 5-fold compared to controls and 2-fold above controls by wk 12. The data shows that this perfluoroether acid fluoride produced marked hepatocellular hypertrophy and persistent peroxisome proliferation; the NOAEL was 5 ppb.

1296 HEPATIC EFFECTS OF THE NOVEL ACYL CoA:CHOLESTEROL ACYLTRANSFERASE (ACAT) INHIBITOR SK&F 98016

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Many hypolipidemic agents increase rodent liver size with accompanying proliferation of peroxisomes and increases in smooth endoplasmic reticulum (SER). This study was conducted to determine the effects of the novel ACAT inhibitor SK&F 98016 on peroxisomes and hepatic ultrastructure. Male and female Sprague-Dawley rats received vehicle or 200 mg/kg/day SK&F 98016 by gavage daily for 14 days. On day 15, animals were killed and livers were collected for (1) biochemical analyses of peroxisomal βoxidation activity and carnitine acetyltransferase activities and (2) electron microscopic morphometry. Drug-related increases in absolute liver weights of up to 83% were observed and liver to body weight ratios were increased from control values of 0.031–0.034 to 0.056–0.065. Total hepatocyte cross-sectional area was increased by 12% and thus contributed in part to drug-related increases in liver weight. However, no drug-related effects on peroxisomal β-oxidation and carnitine acetyltransferase activities were observed. In addition, the volume fraction of peroxisomes determined morphometrically was not affected by drug treatment, indicating that treatment with SK&F 98016 did not induce peroxisome proliferation. Volume fractions of nuclei and lipid droplets were not altered, but the volume fraction of SER was increased by 25%, consistent with known effects of SK&F 98016 on hepatic cytochrome P450 content. In contrast, the volume fraction of mitochondria was diminished about 40% by treatment with the agent. Taken together, the data demonstrate (1) drug-related increases in liver weight were due in part to increases in SER and cross-sectional area, and (2) SK&F 98016 does not induce peroxisome proliferation.

1297 CINCINNATI WATER MAZE AND PASSIVE AVOIDANCE PERFORMANCE OF RATS EXPOSED TO TRIMETHYLTIN

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As part of a validation program for neurobehavioral tests used in perinatal exposure studies done according to the new ICH guideline for reproductive and developmental testing for pharmaceuticals, we conducted a positive control study for the Cincinnati Water Maze (CWM) and Passive Avoidance (PA) as our tests of learning (CWM) and memory (PA). The neurotoxin, trimethyltin chloride (TMT), was given acutely by oral gavage to male and female Sprague-Dawley rats (≈10/sex/group) at doses of 3, 6 or 9 mg/kg. Controls received vehicle only (peanut oil). Animals were tested in CWM at 3 and 4 weeks and PA at 5 weeks postdosing. The CWM is an escape-motivated multiple T maze that uses a forward Path (A) and a reverse Path (B). Testing was for 2 trials/day for 3 days in each path. The step-through PA procedure used a single learning trial that resulted in a 2-sec, 0.8 mamp shock when the rat crossed from a lighted chamber into a darkened chamber and retention trials with no shock 1 and 2 days later. The only mortalities were in the 9 mg/kg group: 58% of males and 40% of females. The 9 mg/kg males and females made more errors and had longer latencies to complete CWM on both Path A and B and showed poorer retention in PA. The 6 mg/kg females made more errors and had longer latencies on Path B in CWM and showed poorer retention in PA. Both tests yielded a similar pattern of results, although each test likely measures different cognitive capabilities.

1298 DOMOIC ACID: BEHAVIORAL EFFECTS OF LOW DOSE EXPOSURE IN ADULT RATS

T J Sobotka, R Brown, D Y Quander, R Jackson, M Smith, S Long, C Barton, S Hall, A Scallet [1]. *CFSAN, FDA. 8301 Muirkirk Rd, Laurel, MD, 20708;* [1] *NCTR, FDA. 8301 Muirkirk Rd, Laurel, MD, 20708.* Sponsor: W L Roth

Experimental information is needed to help characterize the effects of low dose exposure to domoic acid. Groups of adult male rats (n = 12) were treated i.p. with domoic acid at doses of 0, 0.22, 0.66, or 1.33 mg/kg. On scheduled days during the week after dosing, animals were tested for passive avoidance (PA) performance and auditory startle (AS) response/habituation or for conditioned avoidance response (CAR). Body weights were routinely monitored. Hindlimb scratching, which occurred only in the highest dose group (1.33 mg/kg), was the most consistent clinical sign observed. Within 24 hours of dosing, the 1.33 mg/kg animals exhibited a significant decrease in body weight ($p < 0.0007$); over the next day, body weight changes significantly increased for the 1.33 mg/kg, as well as for the 0.66 mg/kg dose groups ($p < 0.007$). The behavioral results revealed a significant increase in AS response amplitude but only for the high dose group ($p < 0.02$). Latency to startle and response habituation were not affected. Performance measures for both the PA and the CAR tasks showed consistent but, generally, nonsignificant trends for performance to be enhanced by low dose exposure to domoic acid. The abrupt post-treatment decrease in body weight (indicative of hypophagia) together with the exaggerated startle responses suggests the presence of a domoic acid-induced behavioral hyperreactivity syndrome. This may be consistent with the finding in a subset of these animals of neuronal and axonal degeneration in the CA1 subregion of the hippocampus (Scallet et al., reported at this meeting).

1299 DOMOIC ACID: NEUROHISTOLOGICAL ANALYSES OF BEHAVIORALLY-TESTED RATS

A C Scallet, L C Schmued, R L Rountree, S Hall [1], J N Johannessen [1], T J Sobotka [1]. *NCTR/FDA, Jefferson, AR;* [1] *CFSAN/FDA, Laurel, MD.* Sponsor: G L Wolff

Four groups (n = 6/group) of adult male rats were treated i.p. with domoic acid (0, 0.22, 0.66, or 1.33 mg/kg). Eight days later, after behavioral testing (Sobotka et al., this meeting), rats were perfused. Hippocampal sections were stained with silver or with a novel fluorescent compound to label neurodegeneration. Other sections were immunostained for glial fibrillary acidic protein (GFAP). The silver stain revealed that only a subset (2/6) of the high-dose group had extensive damage, mainly in CA1. The novel fluorescent stain revealed the same pattern of neurodegeneration. GFAP-positive astrocytes in CA1 were larger (mean ± SEM of 6 cells per rat) in high dose rats than controls (107 ± 26 vs. 53 ± 7 sq microns; $t(11) = 2.18$, $p < 0.05$). Mean perimeter and mean length across astrocytes were also greater (p's < 0.01). The 2 rats with the greatest CA1 damage also had the most enlarged astrocytes. These same two rats were the most hypophagic after dosing, were poor at passive avoidance performance, and exhibited exaggerated startle responses, suggesting the presence of a behavioral hyperreactivity syndrome. Our data indicate a good correlation between certain neurobehavioral and neurohistological biomarkers of neurotoxicity during the week after exposure to domoic acid.

1300 USE OF A BEHAVIORAL TEST BATTERY TO ASSESS RHESUS MONKEY OFFSPRING EXPOSED TO COCAINE *IN-UTERO*

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In order to explore possible long-term effects of gestational cocaine (COC) exposure, pregnant rhesus monkeys were treated with COC chronically from about 1 month (mo) of gestation until term (either 0 (N = 3), 0.3 (N = 3), 1.0 (N = 3), or escalating doses up to 8.5 (N = 3) mg/kg (i.m.), 3 times/day, 5 days/week). Beginning at 6 mo of age, the behavior of offspring (N = 12) was monitored using an operant test battery with 4 food-reinforced tasks designed to model aspects of motivation, learning, color and position discrimination, short-term memory and attention. When assessed at 6, 12 and 18 mo of age, the acquisition of operant behaviors by juvenile offspring was not differentially affected by gestational COC exposure (18 mo: F = 0.83, 0.50, 0.23, 1.02; all $p > 0.05$). Possible long-term effects of gestational COC exposure were examined further by measuring the sensitivity of operant behaviors to acute COC administration (0, 0.3, 1.0, 1.8, or 3.0 mg/kg (i.v.)). When assessed at 19 mo of age, the operant behavioral responses of offspring to acute COC challenges were not differentially affected by gestational COC exposure (3.0 mg/kg: F = 0.05, 0.08, 2.82, 0.47; all $p > 0.05$). It was concluded that gestational COC exposure had no significant effect on the subsequent acquisition of complex behaviors by offspring, or the sensitivity of those behaviors to pharmacological challenge with COC. Supported by NIDA IAG #224-89-003.

1301 THE EFFECTS OF *IN-UTERO* METHANOL EXPOSURE ON EARLY COGNITIVE DEVELOPMENT IN INFANT MACAQUE MONKEYS

K S Grant-Webster, T M Burbacher. *Department of Environmental Health, University of Washington, Seattle, WA.* Sponsor: S Gilbert

The primary purpose of this study is to examine the maternal reproductive and offspring developmental effects of prenatal exposure to methanol, an alternative motor fuel being tested for future use in the United States. While known to be fetotoxic and teratogenic at high doses, the health effects of chronic, low dose methanol exposure are not well documented. Twenty-four adult female Macaca fascicularis were exposed to methanol for 2 hrs/day, 7 days a week, prior to and throughout pregnancy. Females were assigned to one of four dose groups; 0 ppm, 200 ppm, 600 ppm, or 1800 ppm methanol. Early cognitive development was assessed with measures of object permanence, visual recognition memory, and cross-modal recognition memory. Results from the object permanence test indicated that there were no differences between exposed and control infants on the cognitive-based portions of the test. There was evidence, however, that the high dose group performed more poorly on the motor portions of the test. Results from visual recognition memory tests showed that exposed infants were able to solve problems with abstract patterns as well as controls but failed to provide evidence for memory when more difficult, social stimuli were presented. Cross-modal recognition results showed no effect of methanol exposure on performance. At the doses studied, in-utero exposure to methanol was not related to gross developmental effects but subtle changes in early motor and memory skills were observed. Supported in part by grant #90-9 from the Health Effects Institute.

1302 BEHAVIORAL EFFECTS OF POSTNATAL EXPOSURE TO A PCB MIXTURE IN JUVENILE MONKEYS

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Epidemiological studies on the developmental effects of PCB exposure have suggested that *in utero* exposure is more important in producing behavioral impairment than is postnatal exposure. In order to further explore this issue, eight male monkeys (*Macaca fascicularis*) were dosed from birth to 20 weeks of age with 7.5 μg/kg/day of a mixture of PCB congeners designed to be representative of the PCB composition of human breast milk. Total blood PCB levels peaked at about 18 ppb by the end of the exposure period. When they were three years of age, these monkeys and a set of age-matched controls were tested on a series of three nonspatial visual discrimination tasks: form, color with irrelevant form cues, and form with irrelevant color cues. Fifteen reversals were run on each task. Monkeys then performed a delayed spatial alternation task, in which they were required to alternate responses between two pushbuttons. Delays between opportunities to respond were instituted to assess spatial memory according to two behavioral paradigms: a series of sessions with only one delay value within each session that increased across successive sessions to a maximum of 30 sec, followed by a number of sessions with delays varying between 0–60 sec within each session. Three of the treated monkeys made many more errors than controls over the first several reversals of the first discrimination reversal task, although the treated group as a whole did not perform statistically differently from controls. Treated monkeys made more errors than controls at the longer delays during both the fixed delay and variable delay portions of the delayed alternation experiment. These findings suggest that postnatal exposure to PCBs may result in behavioral impairment.

1303 NEUROCHEMICAL AND NEUROBEHAVIORAL DEFICITS PRODUCED BY PERINATAL EXPOSURES TO POLYCHLORINATED BIPHENYLS (PCB) OR PROPYLTHIOURACIL (PTU)

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Developmental neurotoxicity of PCB has been linked to the compound's thyrotoxic properties. This study compares the effects of PCB with those of the goitrogen PTU on maturation of central cholinergic neurons and performance of a spatial memory task (Morris maze) on PND90. Timed pregnant Long Evans rats were gavaged with PCB Aroclor 1254 (10 mg/kg) daily from GD6 to PND21, or given PTU (5 ppm or 25 ppm) in their drinking water from GD18 until PND21. At various age intervals, pups were sacrificed and hippocampi

removed for [^{3}H]hemicholinium-3 binding, a marker which labels the high affinity cholinergic transporter. Plasma thyroid hormones T_3 and T_4 were analyzed by RIA. PTU at 25 ppm markedly depressed plasma T_3 and T_4, but only T_4 was reduced in the 5 ppm group. PCB rendered the rats hypothyroid to a similar extent as 5 ppm PTU group. Hemicholinium-3 binding was lowered by PTU or PCB treatment. Behaviorally, marked deficits were seen in the 25 ppm PTU group, while the 5 ppm PTU or PCB group did not exhibit any alteration in spatial learning. Thus, PTU produced a spectrum of hypothyroid effects in a dose-dependent manner; PCB (at a dose which led to >30% mortality) only caused mild hypothyroid effects similar to those produced by low dose of PTU.

1304 DOPAMINERGIC (DA) AGONISTS SELECTIVELY EXERT DIFFERENTIAL EFFECTS ON FIXED INTERVAL (FI) SCHEDULE-CONTROLLED BEHAVIOR FOLLOWING LOW-LEVEL LEAD (Pb) EXPOSURE

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Changes in FI schedule-controlled behavior constitute a well documented behavioral effect of Pb exposure in experimental animals, one that previous work suggests may involve catecholaminergic system alterations. The current study sought to extend those findings using more selective DA D1 and D2/D3 receptor agonists. It assumed that an involvement of DA systems would be suggested by differential effects of DA compounds on FI response rates of control and Pb-treated animals. Changes in FI schedule controlled behavior of rats exposed from weaning to 0, 50 or 150 ppm Pb acetate were compared following the administration of drugs known to impact the various neurotransmitter systems altered by Pb exposure, including the selective D2/D3 agonist quinpirole, the partial D1 agonist SKF38393, the full D1 agonist SKF82958, the mu opioid agonist morphine, the glutamate agonist NMDA, the noncompetitive NMDA antagonist MK-801, and the muscarinic cholinergic agonist, arecholine. Although all drugs except NMDA altered FI performance, only the DA agonists induced differential effects in relation to Pb treatment. Decreases in response rate produced by D1 agonists were attenuated by Pb exposure. Changes in performance produced by low doses of quinpirole, particularly changes in interresponse times, were magnified by Pb exposure. These findings add further support to a DA basis for Pb-induced changes in FI performance, implicating both D1 and D2 receptors. ES01247, ES05017, ES05903.

1305 LEAD (Pb)-INDUCED CHANGES IN MUSCARINIC CHOLINERGIC SENSITIVITY

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This study was undertaken to determine whether the biochemical changes in cholinergic systems reported in response to Pb exposure also result in corresponding changes in cholinergic sensitivity in vivo. Rats chronically exposed from weaning to levels of 0, 50 or 150 ppm Pb acetate in drinking water were trained to discriminate the stimulus properties of a dose of 1.75 mg/kg of the cholinergic muscarinic agonist arecoline, from saline, using standard operant food reinforced drug discrimination procedures. Following acquisition of the discrimination, various doses of arecoline, another muscarinic agonist, oxotremorine, the nicotinic agonist nicotine, and the GABA modulator pentobarbital were substituted for the arecoline training dose, and the ability of various doses of the muscarinic agonist atropine to block the training dose of arecoline were examined. Both arecoline and oxotremorine, but not pentobarbital or nicotine, produced dose-related increases in arecoline responding, and arecoline stimulus properties were blocked by atropine. Pb exposure significantly increased sensitivity to oxotremorine, and attenuated the ability of atropine to block arecoline stimulus properties. These data may be consistent with a presynaptic M2 muscarinic supersensitivity and may be related to reports of decreased acetylcholine turnover after Pb exposure. These findings also lend credence to the possibility that changes in cholinergic system function may play a role in Pb-induced learning impairments. ES01247, ES05017, ES05903.

1306 LEAD (Pb) EXPOSURE INDUCES SUPERSENSITIVITY TO THE GLUTAMATE AGONIST NMDA

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Reports that Pb exposure inhibits NMDA receptor complex function, along with our findings of attenuated behavioral properties of the noncompetitive antagonist MK-801 and augmentation of NMDA's behavioral properties, indicate that Pb exposure has multiple effects on the NMDA receptor complex. The nature of the reported changes suggests a possible upregulation at the glutamate binding site, an effect which should increase glutamate agonist sensitivity in Pb-treated rats. To assess this contention, rats exposed from weaning to 0, 50 or 150 ppm Pb acetate in drinking water were trained to discriminate the stimulus properties of 30 mg/kg of NMDA from saline using standard operant food reinforced drug discrimination procedures. The number of sessions to learn the discrimination did not differ among control and Pb-treated groups. Following acquisition, lower doses of NMDA were substituted for the training dose to generate dose-effect curves. In addition, various doses of the competitive antagonist CPP were administered with NMDA to assess blocking efficacy, or were administered alone. NMDA dose-effect curves of Pb-treated rats were shifted to the left of the control curve, as indicated by mean ED50 values of 19.9, 15.9 and 15.2 mg/kg for the 0, 50 and 150 ppm groups, respectively, indicative of NMDA supersensitivity. CPP blocked NMDA stimulus properties, but did not do so differentially in relation to Pb exposure. CPP alone did not substitute for NMDA stimulus properties. These data provide additional support for an involvement of NMDA receptor complex changes in Pb-induced behavioral toxicity. ES01247, ES05017, ES05903.

1307 ADULT LEAD (Pb) EXPOSURE PRODUCES SUBSENSITIVITY TO THE NONCOMPETITIVE NMDA ANTAGONIST MK-801

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Previous findings from this laboratory, including alterations in behavioral properties and sensitivity to MK-801 and NMDA, suggest a role for the NMDA receptor complex in Pb-induced learning impairments. Since learning and memory impairments are also described in response to occupational Pb exposure, further evidence for this association would be provided if similar effects were noted in organisms exposed to Pb as adults. To evaluate this contention, adult rats were exposed to 0, 50, or 150 ppm Pb-acetate in drinking water for 3 months, and then trained to discriminate 0.05 mg/kg MK-801 from saline using standard operant food reinforced drug discrimination procedures. Following acquisition, lower doses of MK-801 were substituted for the training dose to generate MK-801 dose-effect curves. Because a statistically significant difference between the 3 Pb groups was not apparent (EC_{50} = 0.02, 0.023, 0.026 mg/kg), the rats were given an 8 week exposure to higher Pb concentrations, i.e. 500 or 1000 ppm, after which the dose-effect curves were redetermined. During this time, the blood Pb levels reached "occupational" levels (x = 0, 33, 60 μg/dl, respectively). Rats treated with 500 ppm Pb-acetate experienced subsensitivity to MK-801 as indicated by a significant right-shift of the dose-effect curve (EC_{50} = 0.018, 0.029, 0.02 mg/kg). These data provide additional support for the NMDA receptor complex as one site of action for Pb-induced learning impairments. Further, this mechanism may be operative across the life-span. ES01247, ES05017, ES05903.

1308 PHARMACOLOGICAL AND BEHAVIORAL PROBES TO ELUCIDATE PERSISTING EFFECTS OF LEAD EXPOSURE ON COGNITIVE PERFORMANCE IN RATS

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Persisting lead-induced cognitive effects were assessed in Long-Evans rats exposed to lead acetate via drinking water (0.2%) from gestational day 16 through postnatal day 85. Starting 12 days after the end of exposure, the rats (N = 6 control and 5 lead-treated litters) were trained on the radial-arm maze. No lead effect was seen on choice accuracy in win-shift (WS) radial-arm maze task. In contrast, a significant lead-induced accuracy deficit was seen on a delayed matching to position (DMP) task in the radial-arm maze. A series of drug challenges were then conducted. Scopolamine, a muscarinic acetylcholine (ACh) antagonist, caused a significantly greater impairment in the lead-treated rats, while pilocarpine, a muscarinic ACh agonist, significantly attenuated the lead-induced deficit. Mecamylamine, a nicotinic ACh antagonist, and pergolide, a dopamine agonist, significantly improved choice accuracy in both groups. In further behavioral challenges, the lead-exposed rats were significantly facilitated relative to controls after reversal back to the WS task but were significantly impaired relative to controls when they were subsequently switched back to the DMP task. These data show a lead-induced cognitive deficit after the end of lead exposure, specific to the DMP task. Muscarinic drugs had differential effects in the lead and control groups. Muscarinic, nicotinic and dopamine treatments were found that improved choice accuracy in the lead-exposed rats. (This research was supported by the March of Dimes and NIH grant #ESO 6253).

1309 PROSPECTIVE STUDY OF EFFECTS OF LEAD EXPOSURE ON CHILD BEHAVIOR PROBLEMS

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While clinical reports of children with elevated blood lead (BPb) often note difficulties in concentration, hyperactivity, and aggression, they do not control for potential confounding variables that may predict both exposure and behavior problems. Causal inferences are thus limited. To examine this association, pregnant women from a lead smelter town and a non-exposed town in Yugoslavia were recruited for a prospective study of Pb exposure and development. Venous blood samples were taken at 6-month intervals through age 3 years. Mean BPb's at 3-years in exposed and non-exposed children were 40.3 and 9.9 μg/dl, respectively. Maternal ratings of child behavior problems (CBCL/2–3) at age 3 (N = 283) were predicted from a set of risk factors including: child sex and intelligence; maternal education, parity and ethnicity; childrearing, residence type and town. These accounted for a significant portion (10–21%) of the variance in scales measuring Withdrawal, Sleep Problems, Aggressive and Destructive Behavior. Even after control for covariates, concurrent (and past) BPb accounted for a small but still significant portion (1–4%) of the variance in each CBCL scale. As 3-year BPb increased from 10 to 20 μg/dl, scores on each of the affected scales increased by 1 to 2 points. This prospective report provides the first systematic study of the relationship between BPb and early child behavior problems. It suggests that the adverse impact of Pb exposure on behavior is significant, but small in comparison to more powerful social determinants.

1310 ASSOCIATIONS BETWEEN BLOOD LEAD AND BEHAVIOR IN A SAMPLE AT-RISK FOR DISRUPTIVE BEHAVIOR

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Studies of children show an inconsistent association between lead (Pb) exposure and disruptive behavior. It is unclear whether such an association persists after control for potential socio-environmental confounders. Consistent with other psychiatric research examining biological risk factors for child disruptive behavior, associations between Pb and behavior occur predominantly among children with other risk factors for psychopathology. We attempted to extend these suggestive epidemiologic findings by examining the association between blood lead level (BPb) and disruptive behavior in 48 6–10 year old boys, at high risk for psychopathology, who are the younger brothers of adjudicated delinquents, recruited through NYC court records. Mean age of the study subjects was 8.4 years, and mean BPb was 4.8 μg/dl. Standardized psychiatric and social assessments were conducted at home prior to blood drawing. Multiple regression showed that age, pubertal stage, socioeconomic status and parenting skill accounted for a significant 12% of the variance in the Aggression subscale of the Child Behavior Checklist. After control for these confounders, BPb predicted a significant additional 12% of the variance. For each unit (ug/dl) increase in BPb, children exhibited a 1.5 point elevation in Aggression scores. Thus, among children already at risk for disruptive behavior, BPb is associated with substantially increased aggressive behavior.

1311 DEVELOPMENTAL LEAD EXPOSURE IMPAIRS ELECTRICAL KINDLING

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Lead (Pb) exposure during early development has been shown to produce postnatal deficits in cognitive function. We have previously reported an increase in the stimulus strength required to evoke long-term potentiation in adult rats exposed to Pb from birth. The present study examined the detrimental effects of early Pb exposure on another model of synaptic plasticity, electrical kindling. Female rats were administered 0.2% Pb-acetate in the drinking water beginning 2–3 wks prior to breeding (GSWean, GSLife) or on gestational day 16 (PNLife). Offspring were maintained on Pb-water until weaning (GSWean) or until completion of testing (GSLife, PNLife). Adult males were implanted with electrodes in the amygdala and stimulated (60 Hz, 1s, 40–200 μA) once daily to evoke an afterdischarge (AD). No differences were observed between control and PNLife groups in AD thresholds, durations, or kindling rate. Both gestationally-exposed groups (GSLife, GSWean) demonstrated shorter ADs over the course of kindling development. GSLife animals were also delayed in the development of kindled seizures relative to controls. These data suggest that gestational exposure to Pb compromises synaptic plasticity as measured in the kindling model.

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1312 LOW-LEVEL LEAD EXPOSURE ENHANCES p-CHLORO-D-AMPHETAMINE-INDUCED SEROTONIN BEHAVIOR IN RATS

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Lead exposure to rats has been shown to produce significant neurochemical changes on serotonergic central pathways. Therefore, we tested the effect of low-level lead exposure on p-chloro-amphetamine-evoked serotonin behavior, the well-known "serotonergic syndrome". We administrated lead acetate (2 mM) p.o. (though the drinking water) to male Wistar rats (150–200 g of weight) for 15 days. After lead exposure, animals were injected ip with 5 mg/kg of p-chloro-amphetamine. Thirty minutes after the injection, the components of the serotonergic syndrome were recorded in ten min epochs for 1 hour. Results indicate that lead exposure increased the scores of all serotonergic syndrome components: a) forepaw treading, 42.9%; b) hindlimb abduction, 67.7% c) tremor, 61.5% d) body and head shakes, 77.9% e) flat body posture, 69.4% vs control animals. Our results indicate that changes in serotonergic neurochemistry induced by lead exposure affect rat behavioral response to drugs.

1313 NEUROBEHAVIORAL TOXICITY FROM BRIEF EXPOSURE TO LOW LEVELS OF OZONE-DEPLETING SUBSTANCE REPLACEMENTS

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Little existing research has explored neurobehavioral toxicity from brief exposure to relatively low levels of replacements for ozone-depleting refrigerant, coolant and fire extinguishant gases. This subject is of special interest to the operational military where personnel must skillfully perform complex tasks during combat or damage control, when presence of these gases in enclosed spaces is very possible. Previous research in this laboratory indicated that brief exposure to increasing concentrations of R-134a, freon-12 or halon-1211 results in severe motor deficits and incapacitation in the rat. The current study examines performance deficits on a complex operant task resulting from exposure to concentrations insufficient to induce motor impairments. Brief exposure to 4,000 ppm halon-1211, 140,000–160,000 ppm R-134a or 180,000–200,000 ppm freon-12 induced significantly reduced levels of responding with, more importantly, significantly increased errors on the portion of the operant schedule requiring discrimination and response inhibition. Repeated exposure to the test gases (up to 32 times) resulted in no change in baseline performance or observable physiological effects. Exposure to the test gas during the first half of the daily operant session resulted in no deficit during the second half of the session conducted in room air. In some cases, mild performance enhancements were observed immediately following flushing of the test gas atmosphere with room air. We conclude that brief exposure to relatively low concentrations of the test gases can induce cognitive deficits at concentrations lower than required for motor or motivational impairments.

1314 BLOCKING THE EFFECTS OF CARBARYL ON THE REPEATED ACQUISITION AND PERFORMANCE OF RESPONSE SEQUENCES IN RATS

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Carbaryl has been shown to dose-dependently decrease the accuracy of response sequences emitted by rats trained under a repeated acquisition and performance task (Cohn and MacPhail, *Soc. Neurosci. Abstr.* 20(2):1563, 1994). This experiment determined the effect of pretreatment with scopolamine and methylscopolamine on carbaryl-induced deficits in the accuracy of response sequences. Adult male Long-Evans rats, maintained at 300 g, performed under a multiple schedule of repeated acquisition (RA) and performance (P). The RA component required rats to learn a new 4-member sequence of responses during each experimental session (Center Right Left Right, RLCL, *etc.*); the correct sequence of responses for the P component remained constant (CLRL). Each of these components alternated twice during a session. Prior to the present study, dose-effect functions were established for carbaryl (3.0–17.4 mg/kg, i.p.; Cohn and MacPhail, 1994) and scopolamine (0.25–2.0 mg/kg, i.p.). When given alone, a low dose of scopolamine (0.25 mg/kg) had no effect while a higher dose (0.5 mg/kg) decreased the accuracy of both acquiring and performing response sequences; methylscopolamine (0.5 mg/kg) was without effect. Pretreatment with either dose of scopolamine blocked the accuracy decreases produced by carbaryl (10 mg/kg). Pretreatment with methylscopolamine did not block the effect of carbaryl on response accuracy. These data

suggest carbaryl's effects on response accuracy are due to an action on central muscarinic receptors.

1315 DEVELOPMENTAL NEUROTOXICITY OF MN rgp120/HIV-1 ADMINISTERED SUBCUTANEOUSLY TO NEONATAL RATS

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The potential for neurotoxic effects in offspring after exposure to MN rgp120/HIV-1 (rgp120) *in utero* and/or during the neonatal period was evaluated. Thirty pregnant Fo generation female rats were given 300 μg/day rgp 120 with 600 μg/day Alum, and another 30 rats were given vehicle, once every three days from Day 1 of presumed gestation until parturition. One pup/sex/litter from treated and control group dams were given a daily subcutaneous injection, beginning on Day 1 through Day 22 postpartum (PP) of 1) vehicle; 2) 500 μg/kg rgp120; 3) 500 μg/kg rgp120 with 1 mg/kg Alum; or 4) 500 μg/kg rgp120 with 166 μg/kg QS-21. Neurobehavioral and physical development were evaluated in subsets of litters [preweaning reflex and development, sexual maturation, motor activity (PP 14, 18, 22, 61), acoustic startle (PP 23, 61), passive avoidance (PP 23–25, 30–32), functional observational battery (PP 42, 61), and watermaze testing (PP 59–63, 66–70)], and/or tissues were processed for anatomical examination [whole (PP 12, 70) and regional (PP 70) brain weights and neuropathology (PP 12, 70)]. Administration of rgp120 with or without adjuvant to pups did not cause any persistent effect on any parameter evaluated. Initial neurohistological examination (H & E sections) did not reveal any pathology related to treatment. Thus, rgp120 used as a vaccine does not cause neurotoxicity or developmental toxicity in neonatal rats after exposure *in utero* and/or during the neonatal period.

1316 ENHANCED ORAL ACTIVITY PERSISTS IN NEONATAL 6-HYDROXYDOPAMINE (6-OHDA)-LESIONED RATS WITHDRAWN FROM LONG-TERM HALOPERIDOL TREATMENT

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Because of the known association between long-term neuroleptic treatment and tardive dyskinesia, we studied the influence of haloperidol on induction of spontaneous oral movements in rats. Dopamine neurons in the brain were largely destroyed in half the rats by treating at 3 days after birth with 6-hydroxydopamine (6-OHDA; 134 μg icv, base form; desipramine pretreatment), in order to assess whether this alteration would influence the development of oral dyskinesias. Haloperidol (1.5 mg/kg per day) was added to the drinking water of half the intact and lesioned rats on a discontinuous schedule at 2 months (2 days per week for 4 weeks) and on a continuous schedule at 3 months. Spontaneous oral activity increased to its maximum level in each group after 15 weeks and persisted at that level for the next 33 weeks. Just prior to discontinuing haloperidol, there were 35.8 ± 4.9 vs. 18.4 ± 2.1 oral movements in lesioned vs. intact rats. Scopolamine did not reduce the level of spontaneous oral activity. Also, in rats lesioned with 6-OHDA, the high incidence of oral movements persisted for 7 months after withdrawal of haloperidol. The late-onset long-lasting stable high frequency of oral dyskinesias in these rats is an advantage in studying putative therapeutic drugs for tardive dyskinesia. (Supported by NS 29505).

1317 5,7-DIHYDROXYTRYPTAMINE (5,7-DHT) LESIONS OF SEROTONIN (5-HT) FIBERS ALTER DOPAMINE (DA) AGONIST-INDUCED BEHAVIORS IN RATS

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In adult rats, treated neonatally with 5,7-DHT (75 μg ICV; desipramine pretreatment), enhanced oral responses to the DA D_2 receptor agonist, quinpirole HCl (50–400 μg/kg), were observed. In rats lesioned neonatally with both 5,7-DHT and the DA neurotoxin 6-hydroxydopamine (6-OHDA; 134 μg ICV; desipramine pretreatment), oral responses to quinpirole and the DA D_1 receptor agonist, SKF 38393 (0.03 to 6.0 mg/kg), were much reduced vs. rats lesioned with either 5,7-DHT or 6-OHDA alone. When neonatal 6-OHDA-lesioned rats were treated as adults with 5,7-DHT (75 μg ICV; desipramine pretreatment), the enhanced oral activity response to the DA D_1 receptor agonist was attenuated, while the oral response to the DA D_2 agonist was enhanced. Spontaneous hyperlocomotor activity in rats co-lesioned with 5,7-DHT + 6-OHDA was attenuated by amphetamine (0.25 mg/kg). An HPLC/EC analysis verified that 5,7-DHT and 6-OHDA respectively depleted striatal 5-HT and DA contents. These findings indicate that 5-HT fibers have an important role in modulating behavioral responses to both DA D_1 and D_2 receptor agonists. (Supported by NS 29505 and the Fogarty Int'l Center).

1318 CORRELATING EXTRACELLULAR LEVELS OF AMPHETAMINE TO BEHAVIORAL SCORES DURING MICRODIALYSIS SESSIONS

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The question, as to whether behavioral scores are directly correlated with extracellular brain levels of amphetamine (AMPH) was addressed by simultaneously monitoring AMPH levels and behavior in the microdialysis bowl. Six mo. old Sprague-Dawley rats (4/dose) received single sc injections of 1 or 2.5 mg/kg AMPH. Microdialysate (MD) was collected every 20 minutes from Caudate/putamen (CPU) at 1 μl/min flow rate. AMPH levels were determined using HPLC (fluorescent detec-tion after derivatization with o-phthaldialdehyde/3-mercap-topropionic acid). Spontaneous behavior was rated during each MD collection period using the Observer^R software (i.e. duration of 8 behavioral elements and frequency of CIRCLING). Peak extracellular CPU levels (Mean ± SEM) were 2.8 ± 0.38 μM for 1 mg/kg and 5.1 ± 0.88 μM for 2.5 mg/kg, based on an estimated 10% *in vivo* microdialysis probe recovery. Individual AMPH-concentrations in were correlated with the number CIRCLINGs ($r_{30} = 0.6954$, $p < 0.001$) and with time spent in LOCOMOTION ($r_{54} = 0.733$, $p < 0.0001$). Present results suggest that extra-cellular brain levels of AMPH may be pivotal for individual differences in behavioral responses to this drug. In contrast such levels are of secondary significance for hyperthermia-induced AMPH-neurotoxicity according to our previous findings. (PCI supported by an ORISE/FDA postdoctoral fellowship).

1319 THE EFFECTS OF BOTH PSYCHOACTIVE DRUGS AND CONDITIONING ON ROTA-ROD PERFORMANCE

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Coordinated locomotor activity on the rota-rod is used to assess the behavioral neurotoxicity of psychoactive drugs. The animal's familiarity with the apparatus may affect the outcome of these tests. Rats were given one training trial on a rota-rod apparatus on the morning on day 1, and again on the afternoon on day 1. Animals that were able to remain on the rota-rod for at least 20 seconds (s) were kept for subsequent training and testing. These animals were given an additional trial on the morning of day 2. Then animals were randomly divided into 10 groups (n = 10/group) with the criterion that the mean time on the rota-rod in training session 3 was not significantly different. The results for the two training sessions indicated that a significant number of animals failed to reach the criterion as properly responding animals. On the afternoon of day 2, the animals received 3000 mg/kg of ethanol (or vehicle) PO. Ten minutes (m) later either cocaine (10–40 mg/kg) or morphine (20 mg/kg) (or vehicle) was administered IP. All drugs were given in pharmacological doses. Ten and 20 m after the second administration, each animal was given a single 60 s trial on the rota-rod. Morphine alone did not significantly alter the animal's ability to remain on the rota-rod; however, ethanol alone and in combination with morphine did significantly impair performance. Cocaine alone produced a graded decrease in performance and this effect was exaggerated when ethanol was administered together with cocaine.

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1320 BEHAVIORAL EFFECTS OF NON-CONVULSIVE DOSES OF THE NOVEL NEUROTOXICANT TRIMETHYLOL-PROPANE PHOSPHATE (TMPP) IN RATS

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Doses of TMPP lower than 0.25 mg/kg (ip) yield no convulsive behaviors or other casually observed behavioral indication of altered neural activity, although doses greater than or equal to 0.10 mg/kg produce reliable durations of epileptiform EEG in tested strains of rats. It was proposed that neurotoxicant

effects of TMPP should be detected using testing paradigms employing sensitive behavioral measures. Rats from either Fischer-344, Wistar, Sprague Dawley, or Long Evans strains were subjected to behavioral tests measuring conditioned place preference, social play behavior, plus-maze performance, radial-arm-maze performance, appetitive operant discrimination behavior, home cage activity, or behavioral activity sensitization. Doses of TMPP as low as 0.10 mg/kg were found to reliably disrupt play behavior indexed by a reduction in number of pins and social contacts and to sensitize behavioral activity to a 1.0 mg/kg amphetamine challenge. Doses as low as 0.20 mg/kg reliably increased task latency and number of errors in radial-arm-maze performance and reliably increased timidity in the plus-maze. Neither conditioned place preference, home cage activity, nor appetitive operant discrimination was affected by any dose tested. The results suggest that TMPP disrupts brain circuits related to social motivated and emotional responding but does not affect systems involved in general motor behavior, appetitive motivation, or sensory-motor integration.

1321 BEHAVIORAL PROFILE OF DITHIOBIURET NEUROMUSCULAR TOXICITY USING A FUNCTIONAL OBSERVATIONAL SCREENING BATTERY

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2,4-Dithiobiuret (DTB) produces a relatively specific and reversible neuromuscular toxicity exhibited behaviorally as a flaccid weakness. We examined the effects of DTB using a neurobehavioral screening battery (functional observational battery and motor activity) in order to establish the profile of a peripherally-acting neuromuscular toxicant. Male and female adult Long-Evans rats were administered 0, 0.25, 0.5, or 1 mg/kg DTB i.p. for 5 consecutive days; this dosing regimen has been shown previously to produce the muscular syndrome of interest. Rats were tested the day after the last dose, and at weekly intervals thereafter for a month. Female rats were more susceptible to DTB's toxic effects, with 50% lethality at the high dose compared to no lethality in males. DTB produced the expected cluster of motor effects: decreased forelimb and hindlimb grip strengths, decreased motor activity, gait changes, altered righting reflex, and loss of muscle tone, with no changes in landing foot splay. Chromodacryorrhea and diuresis were also seen in more severely-affected rats (mostly females). Analysis of the functional domains revealed significant effects in male rats on the Neuromuscular, Activity, Vestibular, and Autonomic domains on the day after the last dose, with persistent effects only on the Neuromuscular and Vestibular domains. Females showed generally the same profile with the lower, sublethal doses. These data indicate that DTB, a positive control for peripheral neuromuscular effects, produced a profile consisting of motoric alterations without concomitant sensory or excitability effects.

1323 FUNCTIONAL OBSERVATIONAL BATTERY (FOB) AND NEUROPATHOLOGY IN NEW ZEALAND WHITE RABBITS

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A neurotoxicity screening battery for the rabbit, composed of an FOB and neuropathology, was developed and evaluated in our laboratory. This battery was developed in order to provide neurotoxicity screening information on a species other than the rat to address regulatory requirements for a second species or questions regarding species sensitivity. In order to validate the FOB, male rabbits (5/group) were treated once with either chlorpromazine (CPZ; 0.8 mg/kg; i.v.), DDT (300 mg/kg; p.o.), d-amphetamine (AMP; 1.0 mg/kg; i.v.), or saline (control; i.v.) and evaluated on the day of treatment. CPZ produced gait abnormalities, a lack of some sensory responses, drooping eyelids, and decreased arousal, muscle tone, and pupil size. Treatment with DDT produced tremors, while AMP produced no clear treatment-related effects at the dose administered. An additional study was conducted to evaluate neuropathology techniques using 4 treated rabbits (1 sex/group), administered (p.o.) either iminodiproprionitrile (IDP; 125 mg/kg/day) for 6 or 7 days or acrylamide (ACR; 20 mg/kg/day) for 24 days (22 doses), and 1 untreated control animal/sex. All animals were sacrificed 7 or 8 days (IDP) or 25 days (ACR and control) after the initiation of treatment and the nervous system was examined microscopically using specialized tissue fixation, embedding, and staining procedures. Paranuclear axonal swelling of large neurons in the CNS and dorsal root ganglia was observed in IDP animals, and myelin swelling and fiber degeneration of the distal-most peripheral nerves were observed in ACR animals. In conclusion, alterations in nervous system function and morphology were detected in rabbits using the test battery developed by BRRC.

1324 TESTICULAR HISTOPATHOLOGY AND SPERM MORPHOLOGY TECHNIQUES TO ASSESS MALE REPRODUCTIVE TOXICITY IN RATS

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Male reproductive assessments such as sperm concentration, motility and morphology, testicular spermatid concentration and histopathological evaluation of the testes are currently used to evaluate reproductive toxicity in rodents. In order to comply with the International Conference of Harmonization and Environmental Protection Agency guidelines, techniques were developed to evaluate epididymal sperm parameters and methods of testicular embedding and sperm morphology evaluation were compared.

Adult Sprague-Dawley male rats were divided into two groups and the rats were sacrificed. One group had the right testes fixed in Bouin's fluid and embedded paraffin wax. For the other group, right testes were embedded in glycol methacrylate following fixation in neutral buffered 10% formalin. The left cauda epididymis was used for evaluation of sperm motility, concentration and morphology. Motility was assessed on a single count of 200 sperm following dispersion in medium. Sperm concentration was assessed from 2 counts of minced cauda epididymis. Morphology of 200 sperm (head and tail) was determined for stained and unstained sperm smears and compared to a sample from the suspension used to evaluate sperm concentration. Left testis of all animals was frozen (*ca* −80° C), thawed, homogenized and two counts were performed to assess testicular spermatid concentration. Inter-observer reliability was assessed for motility, sperm concentration, sperm morphology and testicular spermatid concentration.

Parameters were consistent between observers and with the published literature. Although testicular histopathology could be performed with both embedding media, reading was facilitated with the use of glycol methacrylate. All methods of sperm morphology evaluation provided slides of good quality with acceptable levels of artifacts but the unstained smear technique was preferred for efficiency and long term stability which would allow archiving.

Embedding of testes in glycol methacrylate and the unstained smear technique for sperm morphology were the methods chosen for standard battery of male reproductive assessments to provide accurate evaluation of epididymal sperm parameters and testicular histopathological variations in rats.

1325 CASA: THE MEASUREMENT OF SPERM MOTILITY USING CONTINUOUS TRACKING

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Epididymal semen samples from 100 rats were evaluated for motility using the Hobson Tracker. A number of different media were assessed (Moore's, Eagles and Hank's). Immediately after removal into pre-warmed (37°C) medium a sample was examined in a fixed depth chamber. The parameters measured included path velocity, straight line velocity, curvilinear velocity and beat cross frequency. The sperm were tracked continuously for up to 5 minutes. Fluctuations in the progressive motility were observed, with variations occurring every few seconds. In order to obviate against these changing characteristics it was necessary to maintain the sperm in focus for periods of up to 1 minute and to provide a wide field of view. Optimal viability and motility parameters were obtained using Moore's medium. This enabled sperm to be maintained for several hours with normal progressive motility and morphology. Variations in the depth of the observation chamber set limitations on the extent of movement by the sperm. Often the movement was more vigorous in restricted spaces. 50 μm appeared to be the optimal depth for maintaining the sperm in focus and enabling continuous tracking. With standardisation of the measurement environment path velocity is considered to be a robust aspect of motion. Beat cross frequency may also have some useful characteristics. It is suggested that changes in these parameters could be used as reliable measurements of the effects of toxic substances on male fertility. In order to obtain stable statistical results it is necessary to continuously track sperm for up to 1 minute.

1326 CASA: VALIDATION OF THE HOBSON CONTINUOUS TRACKING SYSTEM USING VIDEO SIMULATION AND MANUAL MEASUREMENTS OF RAT SPERM TRACKS

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The Hobson Tracker enables continuous tracking of motile sperm over an unlimited period of time. It measures various velocity parameters including

straight line (VSL) and actual path velocity (VAP), together with % of motile sperm and concentration. In order to evaluate its ability to produce accurate data a number of tests were carried out. In the first instance a calibration video of simulated waveforms was used. It had been programmed to move at predetermined amplitudes, frequencies and velocities. Four dots appeared on the screen for each set of waveforms. Each lasted 2 minutes, the first 3 consisting of sine waves and the fourth straight lines (Groups 1–4). These 4 groups moved at 4 different angles (horizontal, 0°; diagonal, 45°; vertical, 90°; diagonal, −45°). The values obtained for VAP and VSL were compared with the known values. Allowing for sampling frequency and pixellation errors the accuracy of the system varied between 95 and 100%. Tracing rat sperm tracks (on the VDU) from a pre-recorded tape, moving it along frame by frame, was used as the manual method of assessment. The tracks obtained were timed and measured with a ruler (VSL) and cartographer's measuring wheel (VAP). Following adjustments for imaging errors relating to the aspect ratio of the video system and allowing for the limits of manually derived values, the data obtained showed an accuracy rate of 85% or more. The % of motile sperm was checked visually from the VDU screen, by counting the number of motile and non-motile sperm. Accuracy was 99–100%. Sperm concentration using a haemocytometer showed good correlation (>90%) with the Hobson Tracking System. The data collection and measurements were carried out in compliance with internationally recognised Good Laboratory Practice regulations and guidelines. It was concluded that the System fulfilled all of the criteria and is now validated for use at Toxicol.

1327 SEMEN ANALYSIS AND SPERM COUNTS IN BEAGLE DOGS TREATED WITH THE HMG-CoA REDUCTASE INHIBITOR, ATORVASTATIN

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HMG-CoA reductase inhibitors have been suspected of having adverse effects on male reproduction due to their cholesterol lowering properties. Therefore, semen analyses were conducted during a 2-year toxicity study in beagle dogs with the HMG-CoA reductase inhibitor, atorvastatin. Oral doses of 0, 10, 40, and 120 mg/kg were given to 10 dogs/sex/grp for 52 weeks, and 3/sex/grp were sacrificed at Wk 52. Two reversal animals/grp received no treatment from Wk 52 until sacrifice at Wk 64. The remaining animals received treatment until sacrifice at Wk 104. Semen was analyzed during Wks 50, 51, 52, 64, 78, 91, and 104 for volume, color, and other cell types, and for sperm count, concentration, morphology, motility, and progressiveness. Testicular and epididymal sperm counts were determined at necropsy. Target organ toxicity, as well as 2 deaths, occurred at 120 mg/kg, and dose-related decreases (up to 63%) in serum cholesterol occurred at all doses. No treatment-related differences in semen parameters or tissue sperm counts were found at any time point. Means for semen volume ranged from 4.6 to 8.2 ml, total sperm counts ranged from 398 to 1037 × 10^6 per ejaculate, and sperm morphology ranged from 6% to 64% normal in all groups. Thus, atorvastatin had no adverse effects on semen or sperm parameters in dogs.

1328 INVESTIGATION OF FERTILITY AND MATURITY IN THE MALE CYNOMOLGUS MONKEY (*MACACA FASCICULARIS*)

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Detailed fertility investigations as a part of general toxicity evaluation are becoming increasingly important. This is also reflected in the final ICH Guideline on "Detection of Toxicity to Reproduction for Medicinal Products (June 1993)" and the draft US EPA Guideline on "Reproduction and Fertility Effects (July 1994)". The current/former guidelines of various countries and international organizations provide a rather fixed scheme for evaluation of reproduction toxicity where the commonly used species is the rat. There are, however, valid reasons to use the non-human primate as an alternative species because in man as well as in non-human primates, spermatogenesis is controlled by a similar complex hormonal interrelationship with testosterone acting directly on spermatogenesis in the Sertoli cells. The cynomolgus monkey (*Macaca fascicularis*) was investigated as a model for fertility studies due to its good comparability to man with regard to seminal and hormonal parameters and its lack of seasonality.

The data presented were achieved in accordance with the WHO "Manual for the Examination of Human Semen and Semen-Cervical Mucus Interactions (1987)" either during the course of regular subchronic and chronic toxicity studies or in special validation studies. Particular attention was paid to the comparison of feral monkeys with captive-bred monkeys. Sperm samples were obtained by rectal probe stimulation and more than 600 control and predose ejaculations were analyzed with regard to ejaculate weight (median: 0.3 g), sperm count/ejaculate (median: 75 million), normal sperm morphology (median: 75%) and progressive sperm motility (median: 74%). Serum concentration of testosterone (median: 27 nmol/l) was also determined.

The data presented support the assertion that the non-human primate, especially the cynomolgus monkey, is a valid and available model for hazard assessment of reproductive toxicants. There is a high degree of similarity to man with respect to the hormonal system and spermatogenesis. To allow proper judgement of effects on fertility, extensive background data are essential as considerable individual variability has to be taken into account.

1329 RISK OF INVALID TOXICITY STUDIES IN THE NON-HUMAN PRIMATE DUE TO IMPROPER SELECTION OF MATURE MALE ANIMALS

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The present study was undertaken to investigate the development of maturity in male cynomolgus monkeys (*Macaca fascicularis*) under laboratory conditions compared with natural conditions with a view to the suitability of the animals for toxicity studies. In the past, animals were generally selected on the basis of body weight (at least 3.5 to 4 kg) assuming maturity.

The captive-bred animals originated from the Guangdong Wildlife Breeding and Research Center in China and were born in the years 1989 to 1993. The feral animals were obtained from Indonesia and the Philippines in the years 1987 to 1991. The majority of the feral and captive-bred animals had a body weight of 4 kg or more. To supplement the data obtained at Hazleton Deutschland, extensive field trials were performed in the Chinese breeding facility.

The parameters evaluated were body weight, testicular weight, testicular volume and presence of sperm, and additionally for captive-bred animals age. Furthermore, testes biopsy data and data on testosterone levels were evaluated for a limited number of animals.

In this study, it was shown that the criterion "body weight" is insufficient to properly select mature captive-bred animals. Maturity was determined for the investigated animals on the basis of the presence of sperm. Relative to body weight, captive-bred males had a clearly lower testicular weight and showed a steeper increase in testicular weight within the body weight range of 3.5 to 5.5 kg than feral animals.

Testicular volume proved to be a rough indicator for maturity. However, ejaculates should additionally be examined for the presence of sperm. The following data were derived to serve as guidance for proof of maturity: testicular volume >10 ml; body weight >4.5 kg; age 4 to 5 years; serum testosterone >15 to 20 nmol/l; proof of sperm. It is concluded that captive-bred monkeys must be carefully examined for maturity before use in toxicity studies.

1330 A COMPARISON OF EFFECTS OF TWO DRINKING WATER DISINFECTION BY-PRODUCTS (DBPs) ON SPERM MOTILITY IN THE RAT

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Bromodichloromethane (BDCM) and chloral hydrate (CH) are commonly occurring DBPs. Previous studies have shown that parameters of epididymal sperm motility are sensitive indicators of DBP exposure. Here, the motility of epididymal sperm from F344 rats in cancer bioassay studies was analyzed during interim (52 week) necropsies. Rats either were exposed to 0, 0.33 g/l, and 0.62 g/l BDCM (N = 7) or 0, 0.78, and 2.7 g/l CH (N = 6) via drinking water. Exposures averaged 22 and 39 mg/kg/d for BDCM, and 55 and 188 mg/kg/d for CH. Sperm were collected from the cauda epididymidis by nicking the tubule with a scalpel blade and dilution into a modified, bicarbonate/HEPES-buffered Hanks Balanced Salts Solution, pH 7.4. An aliquot of the diluted sample was drawn into a 200 μm-deep glass cannula and approximately 200 sperm from each sample were videotaped and analyzed using a Hamilton-Thorn 2000 sperm motion analyzer. Exposure to 39 mg/kg BDCM resulted in significant decreases in both the straight-line velocity ($p < 0.01$) and average path velocity ($p < 0.05$) of sperm from the cauda epididymidis. Exposure to 188 mg/kg CH resulted in signifiant decreases in both the percentage of motile and progressively motile sperm ($p < 0.01$). While no individual measure of velocity was significantly affected by CH exposure, analysis of the frequency distribution of straight-line velocity of 750 tracks of epididymal sperm from the vehicle and 188 mg/kg CH animals revealed a shift in the median velocity of the population from 80–100 μm/sec to 60–80 μm/sec. Histopathologic evaluation revealed no

evidence of tumors in any of the treatment groups and there were no gross histologic alterations in the testis or epididymis. Thus, exposure to these DBP's in drinking water resulted in altered epididymal sperm motility in the absence of detectable carcinogenicity. More comprehensive studies of these DBP's on the male reproductive system are warranted.

1331 REPRODUCTIVE TOXICITY SCREEN OF 1,3,5-TRINITROBEN-ZENE ADMINISTERED IN THE DIET OF SPRAGUE-DAWLEY RATS

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Several Army installations targeted for restoration have measurable quantities of 1,3,5-trinitroben-zene (TNB). As part of the process to develop environmental and health effects criteria, a modified Screening Information Data Set (SIDS) reproductive study was performed. Male and female Sprague-Dawley rats received diet containing approximately 300, 150, or 30 mg TNB/kg diet. Mating occurred following 14 days of treatment. All dams, one-half the males, and representative pups were maintained for a total of 90 days of treatment. No mortality occurred during the study; however, a decrease in mean body weights was noted in both sexes of high-dose rats. A dose-related effect was noted in most measurements of sperm function/activity. Sperm depletion and degeneration of the seminiferous tubules were noted histopathologically. Methemoglobinemia was a common finding in the high- and mid-dose levels of both sexes. Splenic hemosiderosis was observed in the same dose groups at necropsy. No adverse effects occurred in mating or fertility indices. No significant treatment-related differences were noted in length of gestation, sex ratio, gestation index, or mean number of pups per litter. (Supported by DOD Contract No. F33615-90-C-0532)

1332 A REPRODUCTIVE TOXICITY SCREEN OF AMMONIUM DINITRAMIDE (ADN) ADMINISTERED IN THE DRINKING WATER OF SPRAGUE-DAWLEY RATS

R E Wolfe, E R Kinkead, M L Freedman, D J Caldwell, C R Miller, G B Marit. *Tri-Service Toxicology Consortium, Wright-Patterson AFB, OH*

The Department of Defense is currently considering replacing ammonium perchlorate with ammonium dinitramide (ADN). The ADN, a class 1.1 explosive oxidizer, will be used in solid rocket propellant mixtures and explosives. This study was intended to evaluate the potential of ADN to produce alterations in paternal fertility, maternal pregnancy and lactation, and growth and development of offspring. Male and female rats received drinking water treated with 200, 100, or 20 mg ADN/100 mL throughout the study. Mating occurred following 14 days of treatment. All dams, one-half the males, and representative pups were maintained for a total of 90 days of treatment. No mortality occurred in parental animals during the study. Treatment with ADN resulted in no adverse effects on mating as 92–100% of the animals mated. No treatment-related effects were seen in parental animals clinically or histopathologically. Adverse treatment-related effects were noted in the high- and mid-dose groups maternal and paternal fertility indices, gestational indices, and live birth indices. Litter sizes in the high- and mid-dose groups were significantly smaller than those of the low-dose and control groups. Mean pup weights showed no statistically significant differences between ADN-treated pups and controls. Gross and histopathological examination of the animals failed to identify the cause for the decrease in litter production in the high- and mid-dose dams. This study indicates that ADN is a reproductive toxicant. The NOEL is 29 mg/kg/day, the median dose of the low-level female rats. (Supported by DOD Contract No. F33615-90-C-0532)

1333 CONSEQUENCES OF ACETAZOLAMIDE-INHIBITED SEMINIFEROUS TUBULE FLUID SECRETION

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Disruption of the rate of seminiferous tubule fluid (STF) secretion is observed with many testicular toxicants (e.g., 2,5-hexanedione, phthalates, 1,3-dinitrobenzene). We hypothesize that inhibition of STF secretion is a final common pathway by which testicular toxicants result in germ cell necrosis. Here we describe the development of a method to determine the morphological and biochemical consequences of inhibited STF secretion in rat testis. We utilized acetazolamide (ACZ), an inhibitor of carbonic anhydrase, to rapidly inhibit STF secretion. Exposure of isolated segments of seminiferous tubules to ACZ (80 μM) rapidly inhibited STF secretion by 83% as compared to controls when STF secretion is measured using a previously described lumenal oil droplet transport assay. In order to produce a constant inhibition of STF secretion *in vivo* in testis over an extended time period, we developed a method which allows for a constant infusion of ACZ into the testis *in vivo*. A catheter is inserted through the tunica albuginea into the testis and secured using a purse string suture. The other end of the catheter is attached to an Alzet mini-osmotic pump which is secured in the abdominal cavity. To test the delivery of the mini-osmotic pumps, testis were infused (8 μl/hour) with FITC-labelled albumin (2 mg/ml) for 18 hours. Observation of FITC fluorescence in sections of testis frozen in OCT compound revealed that albumin was delivered throughout the testis. Using this delivery system to infuse ACZ into the testis, we will be able to identify population(s) of germ cells which are sensitive to STF inhibition.

1334 RETINOL PROTECTION OF ACUTE CADMIUM-INDUCED TESTICULAR TOXICITY

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Cadmium (Cd) is a toxic metal found in our environment. After acute parenteral exposure, germinal cells and vascular endothelium of the testis are severely damaged. Retinol (vitamin A, VA) is a food supplement, and an adjunct to chemotherapy. In rats, 1d and 7d of VA potentiate the hepatotoxicity of CCl_4. This study was to determine if VA would potentiate $CdCl_2$ toxicity. Adult rats were given VA (75 mg/kg/d, po) for 1d or 7d. Twenty four hrs later, $CdCl_2$ (2.5 mg/kg, iv) was dosed. Necropsies were performed 24h post-Cd. Testes were weighed, fixed, and stained. One day VA treatment had no effect on $CdCl_2$ toxicity. Seven days of VA treatment protected the testes from $CdCl_2$ toxicity. Testes from veh/$CdCl_2$ treated rats were swollen, dark purple, and weighed slightly more than VA/$CdCl_2$ testes. Histologically, there was massive, widespread necrosis of spermatogonia and spermatocytes with moderate spermatid degeneration. Testes from veh/$CdCl_2$ rats also had severe arteritis with hemorrhage and edema. In the VA/$CdCl_2$ rats, the testes had only mild degeneration and necrosis of spermatogonia and pachytene spermatocytes in stage VII and VIII tubules. Current studies investigate VA upregulation of protective proteins. Supported in part by NIEHS center grant, P30 ES 06694-01.

1335 AN *IN VIVO* AND *IN VITRO* INVESTIGATION INTO THE EFFECTS OF α-CHLOROHYDRIN ON SPERM MOTILITY AND CORRELATION WITH FERTILITY, IN THE HAN WISTAR RAT

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Following a recommendation from ICH, pharmaceutical companies are now monitoring possible drug effects on sperm motility in the rat during pre-clinical safety studies by assessing sperm motility (velocity). However, it is not known how changes in sperm motility relate to fertility. Therefore, the effects of α-chlorohydrin on sperm motility were investigated and related to fertility both *in vivo* and *in vitro*. α-Chlorohydrin was given orally to males at doses of 0, 5, 10 and 20 mg/kg for 5 consecutive days. Sperms were then assessed for motility using a standard scoring system (operators' observation of sperm) and computer assisted sperm analysis (CASA). The results showed a dose related decrease in sperm motility. The sperm also appeared to move with 'jerky' type action. When this was correlated to fertility, none of the females mated with treated males became pregnant although a dose related decrease in pregnancy would have been expected. There was no effect on sperm morphology and testicular and epididymal pathology were only seen after doses of 20 mg/kg. When sperms from untreated rats were incubated with α-chlorohydrin *in vitro* at concentrations of 0, 0.5, 1.0 and 1.5 mM, sperm motility and motion were affected as occurred *in vivo*. However, the fertilization capacity (*in vitro* fertilization) of the treated sperms showed a concentration related reduction in percentage fertilization and evidence of abnormal embryo development. The data obtained from the CASA system was very variable and did not characterise sperm motion effectively. These findings suggest that the present standard scoring systems used in pre-clinical safety studies (and CASA systems) are not comprehensive indicators of sperm function and/or fertility. A better understanding of sperm movement therefore is desirable so effects on sperm motility can be related to fertility.

1336 TESTIS SPECIFIC EFFECTS OF CARBENDAZIM (CBZ) ON MICROTUBULE (MT) ASSEMBLY

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Inhibition of MT assembly is thought to be central to the fungicidal activity

of benzimidazoles which bind to the colchicine (CLC)-binding site of fungal tubulin. The benzimidazole, CBZ causes testicular toxicity at much lower dose levels than that required to cause general toxicity suggesting a testis-specific target. The present study has compared the effect of CBZ on MT assembly using both MAP-containing and MAP-free tubulin prepared from brain and testis. Tubulin (along with microtubule associated proteins (MAPs)) was purified in a MES-glycerol buffer, using 2 temperature-dependent cycles of MT assembly/disassembly. MAP-free tubulin was prepared by DEAE Sephacel ion exchange chromatography followed by 2 assembly/disassembly cycles in glutamate (1 M) buffer. With MAP-containing tubulin, the IC_{50} for CBZ-induced inhibition of assembly was much lower in testis (0.05 μM) than brain (80 μM) preparations. Assembly reaction kinetics also differed for brain and testicular tubulin. Brain MTs assembled faster than testis and reached equilibrium within 10 min, whereas testicular MTs did not stabilize until approx. 20 min. The marked difference in sensitivity of testicular vs. brain tubulin to CBZ was abolished when the assembly reaction was carried out using MAP-free tubulin. Additional studies compared the inhibitory effect of CBZ on ^{3}H-CLC-tubulin binding in 100,000 g tissue extracts from brain and testis. ^{3}H-CLC-tubulin binding was competively inhibited in brain extracts but inhibition was noncompetitive in testis extracts. These studies indicate that testicular MT assembly may be uniquely sensitive to CBZ effects. Moreover, CBZ disrupts MT assembly through a process which involves testicular MAPs. Supported by NIH grant #ESO5707.

1337 ROLE OF CARBENDAZIM (CBZ) IN BENOMYL (BNL) INDUCED TESTICULAR TOXICITY

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It is well known that the fungicide benomyl (BNL) causes testicular toxicity in rats and mice. However, BNL undergoes rapid hydrolysis to form the metabolite carbendazim (CBZ). The present study has investigated the role of BNL vs. CBZ in BNL-induced testicular toxicity. In *in vivo* studies, BNL and CBZ were administered to rats intraperitoneally (in corn oil) or by direct injection into the testis (in DMSO). Dose levels were 25 and 50 mg/kg for the ip route and animals were killed 3 and 6 hr after toxicant administration. 100, 200, or 400 μg were administered intratesticularly and rats were sacrificed 1 hr later. Testis were immersion fixed in phosphate buffered formalin, embedded in methacrylate, stained with hematoxylin and PAS and examined for histopathological changes. After ip administration, BNL caused no significant testicular damage. In contrast, CBZ treatment resulted in sloughing of the seminiferous epithelium, which increased in severity with increasing dose and duration of exposure. For the it route, BNL caused testicular damage only at the 400 μg dose level whereas CBZ elicited a lesion even at the lowest 100 μg dose. Since it has been proposed that BNL causes testicular toxicity via inhibition of tubulin assembly into microtubules, the effect of BNL and CBZ on testicular microtubule assembly was investigated. Tubulin was purified by 2 cycles of temperature-dependent assembly and disassembly. CBZ was a much more potent inhibitor (IC_{50} approx. 0.05 μM) of the assembly reaction when compared with BNL (IC_{50} approx. 1 μM). These data indicate that the metabolite carbendazim may be responsible for the testicular damage induced by benomyl. Supported by NIH grant #ESO 5707.

1338 THE BINDING REACTION OF ^{14}C-NITRONITROSOBENZENE (NNB) WITH HEMOGLOBIN AND OTHER PROTEINS

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Exposure to 1,3-dinitrobenzene (DNB) has been associated with testicular toxicity and methemoglobinemia in a number of species. It has been proposed that the chemical species responsible for the toxicological effects of 1,3-DNB arise in the reductive metabolic pathway between the nitro and amino functional groups. A primary candidate for the reactive metabolite has been NNB, a product of 2 electron reduction of the nitro moiety. To facilitate investigation of the possible interaction between NNB and cellular proteins, ^{14}C-NNB was synthesized in two steps from ^{14}C-1,3-DNB. Reduction of ^{14}C-1,3-DNB with NaH_2PO_2 gave nitrophenylhydroxylamine which was reoxidized in the presence of ferric chloride to ^{14}C-NNB. Yield was approximately 69%. The product was purified by chromatography on Kesselgel 60 to give 93% purity by HPLC. The ability of both ^{14}C-1,3-DNB and ^{14}C-NNB to bind to hemoglobin and to rat erythrocytes as well as other proteins was investigated. A modified hydroxyapatite assay was used to quantify ligand binding and to examine the specificity and saturation kinetics of ^{14}C-NNB binding to hemoglobin. No binding of ^{14}C-1,3-DNB could be detected. In contrast, ^{14}C-NNB bound extensively to hemoglobin and to red blood cells (saturation at >20 mole NNB/mole hemoglobin. However, binding of ^{14}C-NNB to globin was relatively low suggesting an important role for heme iron in the binding reaction. Binding of ^{14}C-1,3-DNB and ^{14}C-NNB to testicular proteins is being further investigated after administration to whole animals and in cultures of whole testicular tubules. Supported by NIH grant #ESO5701.

1339 EFFECT OF NEFIRACETAM ON THE TESTIS IN RATS

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Nefiracetam [N-2, 6-dimethylphenyl)-2-(2-1-pyrrolidinyl) acetamide, DM-9384, CAS 77191-36-7]oxo is a newly developed cognition-enhancing agent for cerebrovascular dementia and senile dementia of the Alzheimer type. In a previous 13-week oral toxicity study in dogs, testicular toxicity had occurred in 30 and 100 mg/kg groups; however, 13 and 52-week dosing studies and reproductive study in rats had shown no testicular toxicity. Therefore, in the present study, testicular toxicity was investigated in loaded rats (Crj: CD, 5, 7 and 55-week-old, with and without unilateral nephrectomy) to determine whether the result was related to the deference in species. Testicular toxicity occurred 4 weeks after nefiracetam 1200 mg/kg and 800 mg/kg had been administered daily to 5 and 55-week-old rats, respectively. The changes observed were more pronounced in rats with unilateral nephrectomy. Degenerated spermatocyte and disappearance of spermatides were observed at 3 weeks of daily administration of 1500 mg/kg in 7-week-old rats. As for distribution of the spermatogenic stage, increases in Stages I to VI and decreases in Stages VII to VIII were observed at 2 weeks of treatment. In an experiment with BrdU, administration of BrdU a day before necropsy did not cause any difference between the dosing and the control groups at 3 weeks of treatment; however, another experiment with BrdU given a day before commencement of treatment showed that nefiracetam prevented the frequency of seminiferous tubules containing BrdU-labeled cells in rats, decreasing from 3 weeks of treatment. Electron-microscopic observations showed changes of mitochondria in Leydig cells. Increase in the number of luteinizing hormone (LH) cells in the pituitary was observed at 3 weeks by using immunohistochemistry. The above-mentioned observations demonstrated that nefiracetam induced testicular toxicity also in rats unilaterally nephrectomized or given excessively high doses, and that the changes were more conspicuous in aged rats. The present data indicate that prevention of the development of spermatocytes and spermatid cells in the spermatogenic process by the compound may account for its testicular toxicity. In addition, *in vitro* studies in testicular cells are now in progress.

1340 EFFECT OF GOSSYPOL ON PROSTAGLANDIN E_2 SYNTHESIS

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Gossypol, is a potential nonsteroidal male contraceptive, abortifacient, and antitumor drug particularly for endocrine-related neoplasia. Other tissues affected by gossypol include renal endothelial, and lung. Studies have suggested involvement of prostaglandins in mediating some effects of gossypol. This hypothesis has not be tested directly in any reproductive tissue. We examined the impact of gossypol (3–75 uM) on PGE_2 synthesis in seminal vesicle microsomes (0.4 mg) with arachidonic acid (150 uM), and glutathione (1.0 mM) as cofactor. PGE_2 was measured by RIA. The effect biphasic: inhibition up to 25 uM and stimulation at higher concentrations. Inhibitor and stimulatory potencies of gossypol were simile to indomethacin and hydroquinone respectively. Gossypol has both antioxidant and pro-oxidant effects often generating superoxide and hydrogen peroxide, both of which normally regulate PGE_2 by direct interaction with prostaglandin synthetase. Addition of the antioxidant, BHT, reversed these effects. Our observations support the hypothesis that some pharmacologic effects may be due to disruption of prostaglandin synthesis related to the pro-oxidant and antioxidant behavior of gossypol.

1341 EFFECTS OF TRANSFORMING GROWTH FACTOR-ALPHA (TGF-α) ON MORPHOLOGY AND GROWTH OF UTERINE LEIOMYOMA CELLS (LMC) *IN VITRO*

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Uterine leiomyomas (LM) "fibroids" are the most common tumors found in women over 30 years of age. The growth of uterine LM *in vivo* is estrogen-dependent, and it has been proposed that estrogen-induced cell growth may be mediated in part by peptide growth factors. In this study, we examined the

effects of TGF-α on the morphology and growth of uterine LMC maintained in serum-free (SF) or serum-supplemented (SS) media without phenol red indicator. The uterine LMC (5×10^4 cells/dish) were incubated with 0.0, 0.1, 1.0, or 10.0 ng TGF-α/ml for two weeks, and were evaluated on days 3, 5, 7, 10, 12 and 14. On days 3 and 5, LMC maintained in 0.0–10.0 ng TGF-α/ml of SF media were rounded, or formed stellate cell types that were individualized or in clusters. By day 10, many of the LMC cultured in SF media (with or without TGF-α) appeared dead. Uterine LMC incubated with 0.0–10.0 ng TGF-α/ml of SS media maintained the typical elongated morphology of smooth muscle cells throughout the duration of the study. Analysis of variance (ANOVA) procedures revealed a significant ($p < 0.0001$) increase in the growth of LMC cultured in SS versus SF medium. TGF-α had no effect on the growth of LMC maintained in SF media; whereas, LMC cultured in SS media had significantly ($p < 0.01$) increased growth on day 12 at 10.0 ng TGF-α/ml, and on day 14 at 1.0 and 10.0 ng TGF-α/ml, compared to controls. It appears that TGF-α alone is not sufficient to cause LMC to elongate and grow; however, in the presence of serum associated factors TGF-α stimulates the growth of uterine LMC *in vitro*.

1342 INFLUENCE OF LINDANE ON CYSTEINE (CYS) AND GLUTATHIONE (GSH) LEVELS IN BOVINE OVIDUCT EPITHELIUM CELLS CULTURED *IN VITRO*

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The oviduct is the site of fertilization and early embryonic development, and provides the controlled milieu in which these events occur. Therefore, any influence which may disrupt oviduct epithelium (OE) function could subsequently affect fertilization and embryonic development. In the present study, the effect of the organochlorine insecticide, lindane (γ-hexachlorocyclohexane), on toxicity and intracellular CYS and GSH levels in bovine OE cells cultured *in vitro* was investigated. Bovine oviducts were classified as luteal (L) or nonluteal (NL) according to the reproductive stage of the ovary. OE cells were harvested from the ampulla (AMP) and isthmus (ISTH) region of each oviduct, and cultured to confluency. Previous research conducted in this laboratory has shown that reproductive stage and region influence OE thiol status, in that a higher GSH turnover is observed in L compared to NL tissue and is also greater in the ISTH than AMP derived OE cells. Cysteine recovery and GSH synthesis are slow in OE cells. Cultured OE cells were exposed by direct addition of lindane (50–500 μM) to the culture medium and assessed for viability after a 30 hr exposure. A dose and time dependent toxicity was observed, with exposures of 250 and 500 μM concentrations causing the greatest cell death in confluent OE cell populations (250 μM, 24%; 500 μM, 43%). Cell survival, after lindane exposure, was greater in the NL-staged OE cells (71.5%) compared to L-staged cells (63%). In addition, OE cells from the AMP region survived lindane exposure better than ISTH (72% versus 62%, respectively). Cysteine levels were depleted in AMP OE cells (17%) when exposed to 100 μM lindane over a 48 hr time course, whereas ISTH OE cells exhibited a 48.5% increase over control levels. Reproductive stage did not appear to influence CYS content. Lindane treated OE cells showed a continued depletion of GSH over the incubation period, and by 48 hr were depleted by: 46%, NL-AMP; 40%, NL-ISTH; and 49%, L-AMP. However, L-ISTH OE cells exhibited a decline in depletion over the 48 hr exposure (29%, 12 hr; 13%, 48 hr). In general, results from this study show that the effects of lindane exposure on intracellular thiol status in bovine OE cells are dependent on the reproductive stage and region of the oviduct. This study provides further evidence that the response of this tissue to chemical insult is dependent on the reproductive cycle. (Supported by NIH grants ES05235 and ES07062).

1343 ROLE OF REACTIVE EPOXIDES IN 4-VINYLCYCLOHEXENE-INDUCED OVOTOXICITY IN B6C3F$_1$ MICE

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4-Vinylcyclohexene (VCH) depletes oocytes in pre-antral follicles. Ovotoxicity of VCH is associated with bioactivation to epoxides, with vinylcyclohexene diepoxide (VCD) being the most potent in terms of oocyte loss. Our laboratory has focused on an extensive structure-activity study to elucidate the epoxide metabolite of VCH responsible for its ovotoxicity. A striking difference in ovotoxicity between compounds with the potential of being bioactivated to monoepoxide metabolites, as compared to those bioactivated to diepoxides was observed. The former group, VCH analogues (vinylcyclohexane/VCHA, ethylcylohexene/ECHE and cyclohexene/CHE), were not ovotoxic; where as compounds in the latter group (butadiene and isoprene) significantly depleted ovarian follicles. These results suggested that VCD may be the ultimate ovotoxic metabolite of VCH. To further address this conclusion studies were conducted to determine: (1) the ability of VCH analogues to form monoepoxide metabolites, and (2) the chemical reactivity of the test compound epoxides. Metabolism of the VCH analogues was examined *in vitro* and *in vivo*, and the rates of monoepoxide formation of VCH and the VCH analogues compared. The analogues of VCH were converted to monoepoxide metabolites by hepatic microsomal preparations. ECHE was epoxidized at a rate similar to VCH of 3.61 ± 0.33 nmol/min/mg, VCHA at a slightly lower rate of 1.86 ± 0.12 nmol/mir/mg, and CHE at the highest rate of 5.34 ± 0.42 nmol/min/mg. A similar trend was observed *in vivo* following administration of a single dose of the analogues. The chemical reactivity of the epoxides, as determined by the rate of alkylation of nicotinamide, revealed a difference between the mono and diepoxide compounds. VCD had a threefold higher rate of alkylation, as compared to VCH monoepoxide and the VCH analogue monoepoxides. Similarly, butadiene diepoxide exhibited a fivefold higher chemical reactivity as compared to isoprene monoepoxide, butadiene monoepoxide or epoxybutane. Thus, a relationship may exist between epoxide formation, epoxide chemical reactivity, and ovotoxicity. Unlike analogues of VCH which form relatively non-reactive monoepoxides and are not ovotoxic, VCH and related compounds (butadiene and isoprene) form highly reactive diepoxides. The formation of these diepoxide metabolites may in turn be linked to oocyte depletion. (Supported by CMA, March of Dimes 15-FY93-0672, Center NIEHS Grant P30 ES06694)

1344 EFFECT OF 4-VINYLCYCLOHEXENE DIEPOXIDE ON DNA INTEGRITY IN RAT SMALL, PRE-ANTRAL OVARIAN FOLLICLES

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The industrial compound 4-vinylcyclohexene diepoxide (VCD) causes specific destruction of oocytes in rats. Previous studies showed a significant reduction in number of oocyte-containing small pre-antral follicles, in rat ovaries by day (d) 15 of daily dosing with VCD, reflecting oocyte destruction. Cell death results in DNA degradation and is characterized by the appearance of low molecular weight DNA fragments on agarose gel electrophoresis. The purpose of this study was to determine whether DNA fragmentation in small pre-antral follicles can be seen with *in vivo* exposure of rats to VCD and if so, to identify when this occurs. Female Fischer 344 rats (3/group) were injected daily (vehicle or 80 mg/kg VCD) for 6, 8, 10, 12 or 14 d. Rats were killed, and ovaries removed and gently dissociated with collagenase. Small pre-antral follicles (25–100 μm) were collected by mouth pipet, and DNA was isolated and end-labeled with [^{32}P] ddATP for agarose gel electrophoresis and autoradiography. Follicles obtained from rats 24 h after injection did not show DNA fragmentation on any day. However, VCD-specific random degradation of DNA ($\leq$6 kbp smearing) was observed on d 10 at 1 or 4 h following VCD injection and was also seen at 4 h on d 12 and d 14. VCD did not induce DNA fragmentation on d 6 or d 8. At no time was laddering of DNA (characteristic of apoptotic cell death) seen. However, certain compounds induce apoptosis at lower doses; therefore, whether an apoptotic pattern of DNA fragmentation can be produced by lower doses of VCD will be investigated. In summary, our results are consistent with VCD-specific cell death in small pre-antral follicles first appearing 10 d after the onset of daily dosing of rats with VCD. (Supported by 1-RO1-ES06999-01; NIEHS Center Grant #1P30ES06694-01).

1345 OVULATORY SUPPRESSION IN THE RAT BY LOCAL ADMINISTRATION OF THE NORADRENERGIC NEUROTOXICANT DSP4

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Although the process of mammalian ovulation is often viewed in an endocrine context, ovaries receive sympathetic noradrenergic input that may functionally contribute to one or more of the critical preovulatory events triggered by the proestrous luteinizing hormone surge (LHsur). Using ovarian intrabursal (IB) injections, these experiments investigated effects in L-E rats of a post-LHsur exposure (1900h, proestrus) to the noradrenergic toxicant DSP4 [N-(2-chloro-ethyl)-N-ethyl-2-bromobenzylamine] on preovulatory changes in ovarian norepinephrine (NE) and their relationship to oocyte release. Initial work showed that sealing the site of a unilateral IB injection with a surgical adhesive did not prevent changes in contralateral ovarian or cerebrocortical NE, supporting evidence (Alden, Anat Rec 83:421, 1942) for the existence of a

peritoneal-bursal foramen in the rat. While such IB DSP4 injections (0–10 μM/ovary in 10 μl H_2O) caused similar dose-related ovarian NE depletions on both sides, an ovulatory blockade was seen only for the injected ovary. The blockade was dose-related, as was a decline in the oocyte no. released by ovulating ovaries. Prevention of the marked NE decline by prior exposure to desipramine did not significantly affect the incidence of ovulation. Also, naloxone pretreatment was ineffective, even though opiate receptors may be involved in some DSP4-induced changes (Jacobson et al., Exp Br Res 59: 570, 1985). The results indicate that while IB DSP4 could block ovulation in an injected ovary, this was not linked to a reduction in levels of ovarian NE or to an opiate receptor effect.

1346 DIFFICULTY IN MATING HARLAN SPRAGUE-DAWLEY RATS IS RELATED TO ERRATIC LENGTHS OF ESTROUS CYCLE STAGES

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The rate of mating success in the Harlan Sprague-Dawley (HSD) rat is so low that this strain is rarely used in studies of reproductive toxicity. It has been suggested that sexually inexperienced male rats may fail to mate successfully. In this study, naïve male HSD rats were paired singly overnight with cycling HSD females whose vaginal smears indicated proestrus on the morning of cohabitation. Additional smears were obtained after 30 min of cohabitation and again the next morning. Results showed that all 14 females that were clearly in estrus (and therefore sexually receptive) on the evening of cohabitation were sperm-positive the next morning. Therefore, the sexual inexperience of the males did not affect mating success. However, although morning proestrus is considered a marker for evening estrus, only 47% (8/17) of these females mated successfully overnight. This finding suggests an abnormally long proestrus, and is consistent with our observation that, over 4–6 cycles, lengths of both proestrus and estrus were extremely variable in these rats. When a second group of 15 females was chosen for cohabitation on the basis of late afternoon estrus, all were sperm-positive the next morning. In conclusion, virgin HSD rats can be successfully mated overnight if time and manpower permit evaluation of vaginal smears in the late afternoon before cohabitation.

1347 THE MAXIMUM TOLERATED DOSE (MTD) FOR INHALATION BIOASSAYS: TOXICITY VS OVERLOAD

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High concentrations of inhaled particles can result in a state of lung particle overload characterized by decreased particle clearance and an excessive build-up of particles in the lung. Lung particle overload in rats is associated with adverse responses including inflammation, fibrosis and lung tumors, however, other changes indicating the MTD has been exceeded may not be apparent. In this respect, it needs to be decided if additional inhalation-specific MTD guidelines are needed or whether alternative approaches should be considered. The primary purpose of this workshop is to subject the concept of MTD for particulate inhalation studies to intensive discussion by a panel of experts. The first speaker will provide a historical and conceptual perspective of the MTD. This will be followed by two presentations discussing features of chronic inhalation study design and exposure-response relationships including implementation of the MTD. The final speaker will consider the problems of MTD interpretation for scientists and regulators. A panel discussion will be conducted to address key issues regarding inhalation MTD's including the relevance to low level exposures of responses (i.e. tumors) observed under conditions of lung particle overload and the development of guidelines for design of chronic particle inhalation studies.

1348 CELL CYCLE CONTROLS AND CARCINOGENESIS: INTRODUCTION

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Significant progress has been made in understanding the cellular and molecular basis of cell growth and division. Cell cycle progression is controlled by both positive and negative growth regulators. Factors regulating cell division (cyclins, kinases, growth factors) and a series of cell cycle checkpoints have been identified. Loss of maintenance of checkpoints and cell cycle controls leads to altered cell growth and genetic instability and gene mutation. It is increasingly apparent that many, if not all, toxicants exert many of their carcinogenic actions by directly or indirectly perturbing the cell cycle. To better understand toxicant-induced pertubations on the cell cycle and carcinogenic process, this symposium will focus on (1) DNA repair and cell-cycle specific gene mutation, (2) interference of cell cycle checkpoint control in preneoplastic cells, (3) G_2 cell cycle checkpoint pertubations, (4) altered expression of cyclins and kinases in chemical carcinogenesis, and (5) receptor-mediated and intercellular/extracellular growth factor signals in mitogenesis and carcinogenesis.

1349 DNA REPAIR, MUTATION INDUCTION AND CELL CYCLE STAGE SENSITIVITY

R J Preston. *CIIT, Research Triangle Park, NC.* Sponsor: T Goldsworthy

Recent advances in understanding the process of DNA repair of damages induced by chemical and physical agents has allowed for an assessment of how errors in the repair process can result in mutations. A number of human DNA repair defective syndromes have now been identified that allow for the error processes to be linked to specific cancers such as skin cancer and hereditary non-polyposis colon cancer. These alterations (mutations) in DNA processes have been shown to lead to mutator phenotypes, typically identified as instability of microsatellite sequences. DNA repair kinetics are also of importance for determining sensitivity, particularly the balance between repair and DNA replication. The control of repair vs. replication can be accomplished through the expression of cell checkpoint genes such as p53. An additional component of repair patterns and fidelity is the temporal order of gene repair, with housekeeping genes being more rapidly repaired than non-transcribed regions of the genome. The recently demonstrated association between DNA repair and transcription/transcription factors has helped in an understanding of this. Thus, a variety of cellular processes have already been shown to be closely linked in function and orchestrated temporally.

1350 CELL CYCLE CHECKPOINTS: G2 PERTURBATIONS

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The fate of a cell following DNA damage is determined largely by the cell's capacity to recognize lesions, delay progression through the cell cycle, and repair mutagenic, clastogenic, or lethal lesions before the genome is irreversibly altered during DNA replication or mitotic segregation. Signal transduction mechanisms have been identified that ensure proper cellular responses to the external environment and to the internal status of the cell and feed into checkpoints in the cell cycle. Defects in checkpoint control are associated with increased rates of mutation and chromosomal instability. We have investigated the G2 checkpoint response to ionizing radiation (IR) in normal human fibroblasts and fibroblasts derived from patients with the familial cancer syndromes ataxia telangiectasia (AT) and Li-Fraumeni syndrome (LFS). In normal human fibroblasts, exposure to IR resulted in a rapid reduction in the mitotic fraction and p34*CDC2* kinase activity, compared with sham-irradiated populations. G2 checkpoint function was degraded in both AT fibroblasts, as well as immortalized LFS cells lacking wild-type p53. However, studies with both human and mouse fibroblasts that focused on the effect of disruption of p53 function alone demonstrated that p53 was not required to elicit G2 delay in response to IR. Our results suggest that defects in G2 checkpoint function may arise as a consequence of genomic instability and may contribute to the increased incidences of cancers in individuals with these heritable syndromes.

1351 ABROGATION OF CELL CYCLE CHECKPOINT CONTROL IN PRENEOPLASTIC CELLS

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Genomic integrity is maintained by a network of cellular activities that assesses the status of the genome at a given point in time and provides signals to proceed with or halt cell cycle progression. Mutations in any part of these cellular pathways can have the ultimate effect of disrupting chromosomal integrity. We have used viral proteins involved in malignant transformation to investigate cellular pathways that may be perturbed during loss of genomic stability. Recent studies have identified cellular proteins which are targets for the viral oncoproteins, stressing the importance of these cellular proteins in controlling neoplasia. Among the targets of the viral oncoproteins are the products of the p53 and retinoblastoma (Rb) tumor suppressor genes. We demon-

strated that the expression of human papillomavirus type 16 E6 and E7 oncoproteins in normal, mortal cells disrupts the integration of the network of signals that maintain genomic integrity. E6-expressing cells, in which cellular p53 protein is bound and degraded exhibited alterations in cell cycle control and displayed the ability to amplify the endogenous CAD gene when placed in the drug PALA. Expression of E7, which complexes with a variety of cellular proteins, including Rb, resulted in a p53-independent alteration in cell cycle control, massive cell death and polyploidy upon PALA treatment. These results demonstrate that the viral proteins disrupt cellular processes that safeguard the genome and growth of normal cells.

1352 DISCORDANT EXPRESSION OF CYCLIN-DEPENDENT KINASES (CDKS) IN CHEMICAL CARCINOGENESIS

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The CDKs represent a family of cell division control enzymes that coordinate and control the major events of cell division. Once activated they initiate the visible events of mitosis such as chromosome condensation, nuclear envelope breakdown and spindle formation. They also play a critical role in the initiation of DNA synthesis and cell disassembly. Their activity is regulated by cycles of phosphorylation and dephosphorylation as well as periodic association with cyclin proteins. Additionally, recent research has indicated that transcription plays a major regulatory role in the control of CDK expression. Breast, prostate, stomach, and intestinal cancers have demonstrated high levels of expression of CDKs in comparison to surrounding normal tissues. Elevated serum concentrations of CDKs have been found in prostatic cancer in humans. In animals serum CDKs has been shown to be elevated in hepatocellular and pulmonary carcinoma, as well as osteosarcoma and lymphosarcoma. While the physiological and pathophysiological roles of the CDK are well described, little research has been done to elucidate the changes in CDKs with chemical carcinogenesis. CDKs are overexpressed in target tissues within several weeks when rats or mice are dosed with nonmutagenic carcinogens such as dioxins or peroxisome proliferators. Dose-response and structure-activity studies with carcinogenic peroxisome proliferator compounds indicate that the extent of CDKs expression correlates with the carcinogenicity of the compound. It is proposed that chemically induced, discordant expression of CDKs is a fundamental driving force for enhanced cellular proliferation.

1353 RECEPTOR INTERACTIONS AND MITOGENESIS IN CARCINOGENESIS: DIOXIN AND ESTROGENS AS EXAMPLES

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Forty percent of the chemicals that are positive for carcinogenicity in chronic bioassays are negative in tests for genetic toxicity. There is growing evidence that receptor-mediated events and cell cycle controls are critical events in the mechanisms responsible for the carcinogenic actions of many of these chemicals. Hormone interactions appear to be frequently involved in modifications of mitotic activity. For example, TCDD triggers cell proliferation in rodent hepatocytes *in vivo* only in the presence of ovarian hormones. Estrogen receptor, EGF receptor and Ah receptor act in concert to produce this response. Consistent with this finding, TCDD is a liver carcinogen in intact rats but not male or ovariectomized rats. In contrast to the liver, ovarian hormones appear to protect the lung against the carcinogenic actions of dioxin. In addition to the carcinogenic actions of dioxin, several separate lines of evidence support the hypothesis that breast cancer results from a complex interaction of altered hormone pathways including the estrogen receptor and intercellular/extracellular growth factor signals. For example, estrogen action in developing stromal cells leads to transcriptional activation of growth factor genes which interact with appropriate receptors in the rapidly dividing terminal end bud cells of the mammary epithelium, thereby activating the cell cycle. Current research is focusing on the significance of receptor-mediated responses in clarifying key issues in risk assessment such as dose-response and interindividual variation.

1354 ENDOCRINE MODULATION OF REPRODUCTION: INTRODUCTION

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Hormones are biologically active at exceedingly low concentrations, a characteristic that allows efficient endocrine signaling at distant target tissues. This characteristic may also render the target cells susceptible to exogenous agents that either mimic or block the action of an endogenous hormone. The nature of the response to these exogenous toxicants is target- and life stage-dependent. There has been a great deal of publicity recently regarding the potential for certain synthetic chemicals to act as estrogen mimetics. Recent research has expanded our understanding that other hormonal systems may also be affected by toxicants; that adverse responses are varied and may be permanent; and that there may be a large number of exogenous agents with the potential to act via hormonal disturbance. The purpose of this symposium is to present current information about the potential for interaction of various agents with hormonal systems controlling reproductive function and development. The first talk illustrates with results from a widely-used herbicide the role of endocrine disruption in mammary carcinogenesis. The second talk will describe the prevalence and effects of naturally-occurring estrogens in the diet. The third talk will describe new research demonstrating the existence of environmental agents with anti-androgenic activity. The fourth talk will document the influence of thyroid status on testicular development, illustrating that reproductive function may be controlled by endocrine factors not traditionally considered to be part of the reproductive system. Together, these talks will demonstrate the potential for toxicant-induced disruption of a variety of hormonal control points.

1355 ATRAZINE: MECHANISM OF HORMONAL IMBALANCE IN FEMALE SPRAGUE-DAWLEY RATS

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The carcinogenic potential of the chloro-s-triazine, atrazine, has been assessed using three strains of mice (two inbred and one outbred strain), and Fischer 344 (inbred strain) rats with no evidence of tumor formation. However, in outbred Sprague-Dawley (S-D) female rats atrazine administration yielded a variable mammary tumor response in 5 lifetime studies. Responses ranged from no increase in tumors to an increased incidence of fibroadenomas and adenocarcinomas and/or earlier onset of tumors. Examination of the duration and regularity of the estrous cycle, hormone levels, palpable masses confirmed as tumors, and galactocele formation indicated that lifetime dietary feeding of at least 400 ppm of atrazine results in a three month earlier onset of acyclicity with more days in estrus, prolonged exposure to estrogen, and an earlier appearance of mammary tumors. These changes were unique to the Sprague-Dawley female rat, and occurred only at a level $\geq$ the MTD. Atrazine is weakly anti-estrogenic based on results from uterine growth models and MCF7 cell cultures. Examination of the arcuate nucleus of the hypothalamus following exposure indicate that changes in this neurotransmitter pathway may be due to enhanced estrogen exposure. The mechanism for the earlier onset for mammary tumors appears to be associated with a premature loss of pituitary-hypothalamic control in the female S-D rat.

1356 DIETARY PHYTOESTROGENS

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Phytoestrogens are naturally-occurring constituents in many plants. Legumes are particularly rich in isoflavonoids and coumestans while grains are rich in estrogenic lignans. Estrogenic effects and potential risk or benefit of exposure depends upon dose, potency, duration and pattern of exposure. An additional critically important variable that must be considered is the developmental phase or life-stage of the individual at the time of exposure. Risk-benefit analysis regarding intake of dietary phytoestrogens is further complicated by potential mixed estrogenic agonist-antagonist properties of these agents. Furthermore, other data indicate that these compounds have important metabolic effects that are independent of the estrogen receptor altogether. These receptor-independent mechanisms include antioxidant properties and inhibition of certain protein kinases. In terms of human health implications, the emerging impression is a balance of concern (possible risk of adverse effects) regarding fetal/neonatal nervous and reproductive system development and adult female reproductive endocrine function but enthusiasm (potential benefit) regarding several chronic "Western" diseases including breast cancer, prostate cancer, colon cancer, arteriosclerotic cardiovascular disease and osteoporosis in contracepting premenopausal females, menopausal females and adult males.

1357 ENVIRONMENTAL ANTIANDROGENS

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The pattern of developmental malformations induced in the rat by exposure to vinclozolin (V) suggests that this fungicide has antiandrogenic activity. Our objective was to elucidate the biochemical mechanism responsible for these effects. To this end, we found that two V metabolites (M1 and M2), but not V itself, are pure competitive antagonists of rat androgen receptor (AR) binding. Molecular studies with the transfected human AR suggest that these metabolites bind human AR and inhibit AR action by inhibiting androgen induced transcription. The mechanism of transcriptional inhibition was not the result of altered nuclear import, but rather, inhibition of AR-DNA binding. Since 17β-estradiol is a potent competitive antagonist of AR binding and action, we examined the ability of several classical "environmental estrogens" to antagonize AR action. Many of the chemicals examined inhibited AR binding in the low micromolar range, however, the persistent DDT metabolite p,p'-DDE (DDE) was the most potent. Molecular studies suggest that DDE is a competitive antagonist of human AR binding and inhibits AR action via inhibition of androgen-induced transcription. Taken together, these results suggest that further studies be focused on the role and biochemical mechanism(s) of environmental antiandrogens.

1358 NEONATAL THYROID STATUS AS ONE DETERMINANT OF ADULT TESTIS SIZE

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Thyroid hormones are not considered to be major regulators of testicular function in the adult. This conclusion is supported by data showing minimal expression of thyroid hormone receptor in the mature testis. In contrast, neonatal hypothyroidism inhibits testicular growth and development, although it has been unclear if this effect is direct or indirect on the testis. Recently, it was shown that thyroid hormone receptors are expressed in the testis during early postnatal life, suggesting a possible direct role for this hormone in testicular development. However, the long-term effects of early exposure to hypothyroidism were not intuitive. We have shown that transient neonatal hypothyroidism results in unprecedented increases in both testis size and sperm production, accompanied by permanent reductions in serum LH and FSH concentrations, but without affecting testosterone. Neonatal hypothyroidism causes an increased period of Sertoli cell proliferation and a corresponding increase in the adult number of these cells. Since the number of Sertoli cells established during testicular development is the major determinant of adult sperm production, the increase in Sertoli cell number is believed to be the critical event in the development of this phenomenon. Recently, Sertoli cell cultures have displayed direct effects of thyroid hormone on both proliferation and maturation of neonatal Sertoli cells. These data suggest that thyroid hormone is an important regulator of testis growth and Sertoli cell differentiation. Thus, the various toxicants capable of causing hypo- or hyperthyroidism during the neonatal period have the potential to alter seminiferous tubule development and adult testicular function.

1359 [^{14}C]2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN (TCDD) PRETREATMENT INCREASES HEPATIC ACCUMULATION BUT NOT METABOLISM OF A SUBSEQUENT [^{3}H]TCDD DOSE IN RAINBOW TROUT

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Pretreatment of mice with low doses of TCDD (μg/kg) increases hepatic accumulation of a subsequent [^{3}H]TCDD dose (Fund. Appl. Toxicol. 14:523). This is explained by induction of cytochrome P450-1A2, which binds TCDD and sequesters it in liver. Cytochrome P450 1A1 but not 1A2 is widely reported as inducible in fish liver. Recently, mRNA for cytochromes P450s 1A1 and 1A2 are reported in liver of methylcholanthrene treated rainbow trout (Arch. Biochem. Biophys. 310:187). We ip injected 20–30 g rainbow trout with 20 ng [^{3}H]TCDD/kg. Hepatic [^{3}H]TCDD concentration increased with time from 1 to 10 days after injection. Liver contained approximately 15 pg [^{3}H]TCDD/g both 10 and 20 days after injection, however. Fish were injected with 0, 20 or 100 ng [^{14}C]TCDD/kg. A second injection of 20 ng [^{3}H]TCDD/kg followed after 10 days. Ten days after the second injection, hepatic [^{3}H]TCDD was 3-fold higher in 100 ng [^{14}C]TCDD/kg pretreated than control fish. Biliary excretion of [^{3}H]TCDD equivalents and hexane extractable hepatic [^{3}H]TCDD concentrations were not altered by [^{14}C]TCDD pretreatment. (Supported by NIEHS grant ESO5543).

1360 MURINE DIOXIN-INDUCIBLE CYTOSOLIC ALDEHYDE DEHYDROGENASE (Ahd4) GENE: STRUCTURE OF THE 5' FLANKING AND AROMATIC HYDROCARBON RESPONSE ELEMENTS (AhREs)

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The murine cytosolic aldehyde dehydrogenase ALDH-3c (encoded by the *Ahd4* gene) is highly inducible by 2,3,7,8,-tetrachlorodibenzo-*p*-dioxin (TCDD), negatively regulated by the CYP1A1/1A2 functional gene products (enzymes), and up-regulated by a gene on mouse chromosome 7. In order to study the regulation of *Ahd4* gene expression, we have cloned, sequenced and analyzed 3.2 kb of the 5' flanking region. Sequencing reveals several putative transcription factor-binding sites, which may be important in the positive and negative control of *Ahd4* gene expression; these include six AhREs, two electrophile response elements (EpREs), Sp1, NF-IL6 and AP-1 binding sites. Several deletion fusion genes containing regions of the *Ahd4* gene 5' flanking sequence linked to a CAT reporter gene have been constructed. Transfection experiments show that the 5' flanking region of the gene contains at least four distinct, functional domains: a strong promoter, a negative regulatory element (NRE), AhREs and EpRE. Gel mobility shift assays, in vitro and in vivo footprinting are in progress. (Supported by NIH Grants R01 AG09235, R01 ES06321 and P30 ES06096.)

1361 A PROTEIN FACTOR ESSENTIAL FOR RECOGNITION OF THE DIOXIN RESPONSIVE ELEMENT BY THE Ah-RECEPTOR/ Ah-RECEPTOR NUCLEAR TRANSLOCATOR COMPLEX

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We recently reported that the human Ah-receptor (AhR) and AH-receptor nuclear translocator (ARNT) can be overexpressed functionally in a baculovirus expression system (Chan *et al.*, J. Biol. Chem., manuscript in press). The recombinant AhR and ARNT proteins were designed to contain six histidines at their carboxyl termini. This allowed us to purify these proteins in one step using a nickel-nitriloacetic acid (Ni-NTA) sepharose affinity column. The Ni-NTA purified AhR could be photoaffinity labelled by 2-azido-3-[^{125}I]iodo-7,8-dibromodibenzo-*p*-dioxin and the labelling could be competed by the presence of 10 μM β-naphthoflavone. However, the Ni-NTA purified AhR and ARNT alone were not able to bind the dioxin responsive element (DRE) in the presence of an agonist in gel shift assay, but the AhR/ARNT/DRE complex could be observed when the sample was reconstituted with the uninfected *Sf9* soluble extract, uncharged reticulocyte lysate, wheat germ extract, rat liver cytosol, HeLa cytosol, or yeast soluble extract. Addition of the *E. coli* soluble extract, however, could not generate this complex. Heat treatment completely abolished the ability for the wheat germ extract to rescue the AhR/ARNT/DRE gel shift. Ammonium sulfate precipitation studies showed that the gel shift activity of the uninfected *Sf9* soluble extract resided in the 40–60% fraction. The results from ion exchange chromatography using DEAE cellulose and phosphocellulose, suggested that a negatively charged protein factor(s) was responsible for the generation of the AhR/ARNT/DRE complex. This protein factor could only generate this complex when the soluble extract was added prior to the *in vitro* activation step for AhR/ARNT heterodimerization to occur. We concluded that this factor acts by allowing the AhR to dimerize with ARNT.

1362 CELL CYCLE REGULATION OF ARYL HYDROCARBON RECEPTOR (AhR) FUNCTION

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Treatment of cultured 1c1c7 murine hepatoma cells with the microtubule inhibitor Colcemid (0.1–10 μM) resulted in a non cytotoxic cell cycle arrest in G_2/M (20 hr, 98%). The induction of cytochrome P450 *Cyp1a-1* by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), as quantified by measurement of CYP1A1 mRNA contents, was suppressed $\geq$85% in arrested cells. Neither changes in the relative cellular contents of the proteins comprising the aryl hydrocarbon receptor (AhR) complex (analyzed by Western blotting), nor inhibition of TCDD-activated AhR translocation (analyzed by indirect

immunofluorescence) were the basis of suppression. Suppression instead correlated with reductions in DNA-AhR complex formation, as assayed *in vitro* in gel retardation assays of nuclear extracts. Colcemid-arrested cells reentered the cell cycle within 6 hr of replating in drug free media as determined by measurements of DNA content and cyclin B-p34^{cdc2} kinase activity. Reentry into the cell cycle restored DNA-AhR complex formation and *Cyp1a-1* inducibility. Similar results have been found for the clinically important microtubule inhibitors Taxol and Estramustin. These studies demonstrate that AhR function is cell cycle-dependent.

1363 ORGANIZATION AND CHARACTERIZATION OF THE MURINE DIOXIN-INDUCIBLE NAD(P)H:MENADIONE OXIDOREDUCTASE (NMO1) GENE

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The murine NAD(P)H:menadione oxidoreductase NMO1 (encoded by the *Nmo1* gene) is highly inducible by 2,3,7,8,-tetrachlorodibenzo-*p*-dioxin (TCDD), negatively regulated by the CYP1A1/1A2 functional gene products (enzymes), and also up-regulated by a gene on mouse chromosome 7. We have recently reported [*Pharmacogenetics* (Dec 1994)] that the cDNA is 1,528 bp in length, excluding the poly(A$^+$) tail, and has 5′ and 3′ nontranslated regions of 108 and 595 bp, respectively. *Nmo1* encodes a protein of 274 amino acids, including the first methionine (M_r = 30,959). In order to study the regulation of *Nmo1* gene expression, we have isolated *Nmo1* genomic sequences from a mouse genomic library. Two overlapping fragments contain the entire *Nmo1* gene, along with considerable 5′ and 3′ flanking sequences. The *Nmo1* gene spans approximately 8 kb and comprises 6 exons. The sequence of more than 3 kb upstream of exon 1 reveals several putative transcription factor-binding sites, which may be important in the positive and negative control of *Nmo1* gene expression: aromatic hydrocarbon response elements (AhREs), an electrophile response element (EpRE), and AP-1 binding sites. (Supported by NIH Grants R01 AG09235, R01 ES06321 and P30 ES06096.)

1364 IDENTIFICATION OF A DNA MOTIF REQUIRED FOR TCDD-MEDIATED INHIBITION OF pS2 EXPRESSION

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TCDD and related compounds modulate endocrine systems by altering hormone synthesis, enhancing ligand metabolism and down-regulating receptor levels and binding activity. Previous studies have demonstrated that TCDD inhibits the 17β-estradiol (E2)-induction of pS2, a prognostic marker for breast cancer. This effect occurs at the transcriptional level and is Ah receptor-mediated. Computer analysis of the regulatory region identified three dioxin response element (DRE)-like motifs each containing a perfectly conserved DRE core. In order to identify the motif which mediates this effect, transient transfection experiments were performed in MCF-7 human breast cancer cells using pS2-regulated luciferase reporter genes. Deletion analysis indicates that the DRE-like motif (iDRE) located at −527 to −514 is required for TCDD-mediated suppression of E2-induced reporter gene activity. The introduction of a point mutation within this core motif (T to C at position-518) abolished the suppressive effects, further supporting a role for the iDRE. UV-induced protein-DNA cross-linking studies and competitive gel retardation assays have demonstrated that the iDRE can bind and compete with the consensus DRE for Ah receptor complex binding. These results suggest that Ah receptor complexes interact with a specific motif in the 5′ regulatory region to suppress E2-induction of pS2 expression. *Supported by funds from the MRC of Canada and the Pharmaceutical Manufacturers Association of Canada/Health Research Foundation.*

1365 POTENT TRANSACTIVATION DOMAINS OF THE Ah-RECEPTOR AND ARNT MAP TO THEIR C-TERMINI

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The Ah-Receptor (AhR) is a ligand activated transcription factor that is structurally related to its dimerization partner, the Ah-Receptor Nuclear Translocator (ARNT), and two Drosophila proteins, SIM and PER. All four proteins contain a region of homology now referred to as a PAS homology domain. In addition, the AhR, ARNT and SIM also harbor a bHLH motif in their N-termini whereas PER does not. Previous mapping studies of the AhR have demonstrated that the PAS domain contains sequences required for ligand recognition, dimerization and interaction with the 90-kDa heat shock protein. They also have confirmed that the bHLH domain plays a role in both dimerization and sequence-specific DNA binding. To identify domains involved in transactivation of target genes, we generated chimeras of AhR/ARNT deletion mutants with the DNA binding region of the yeast Ga14 protein, transiently expressed these in COS-1 cells, and monitored their capacity to activate the chloramphenicol acetyl-transferase reporter gene under the control of a minimal promoter driven by enhancer elements recognized by Ga14. Extensive analysis of these fusions revealed that the AhR and ARNT harbor potent transactivation domains within their C-termini. Importantly, the N-terminal halves of both the AhR and ARNT were found to be devoid of transactivation activity in this system. Our results suggest that both AhR and ARNT play a role in transcription regulation of their target genes.

1366 Ah RECEPTOR PHOSPHORYLATION: LOCALIZATION OF PHOSPHORYLATION SITES TO THE C-TERMINAL HALF OF THE PROTEIN

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The aryl hydrocarbon receptor (AhR) is a transcriptional enhancer activated by the binding of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related xenobiotics. Several studies have suggested that AhR phosphorylation may be instrumental in activating the AhR to a DNA-binding state. Treatment of the AhR with acid phosphatase resulted in the loss of DNA-binding activity. We determined whether TCDD binding altered total AhR phosphorylation, and identified phosphorylated regions by the examination of chemical cleavage patterns. The AhR was isolated by immunoprecipitation from $^{32}P_i$-labeled Hepa 1c1c7 cells grown in the presence or absence of TCDD. Examination of the amount of ^{32}P associated with the AhR indicated that the total level of AhR phosphorylation was not affected by ligand binding. Chemical cleavage with hydroxylamine and cyanogen bromide also revealed a similar pattern for liganded and unliganded AhR. The shortest regions of overlap determined by the chemical cleavage patterns localized phosphorylation sites to two regions in the C-terminal half of the AhR. One region is centrally located between amino acids 368 to 605 and within or adjacent to a DNA binding repressor domain. The other region is located at the glutamine-rich carboxyl terminus between amino acids 636 and 759. These data coupled with previous observations imply that total AhR phosphorylation is not altered by the ligand-elicited transformation to a DNA-binding form, but that phosphorylation nevertheless plays an important role in the ability of an active AhR-Arnt complex to associate with *cis*-acting regulatory elements. Support NIH grant ES02515, Center grant ES01247, and Training grant ES07026.

1367 SOYBEAN ISOFLAVONES ARE NATURAL Ah RECEPTOR ANTAGONISTS

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Agonists of the aromatic hydrocarbon receptor (AhR) include compounds such as 2,3,7,8-dichlorodibenzo-*p*-dioxin (TCDD), which are widely distributed and persistent in the environment, and produce disease conditions including immune deficiency, developmental anomalies and cancer. Antagonists of the AhR provide a potential mechanism of protection. While antagonists have been described, pharmacokinetic problems and concerns for toxicity limit their practical application. Herein we report that antitumor soybean isoflavones are antagonists of the AhR. In cultured mouse Hepa-1 cells, TCDD-inducible cytochrome P-450 form 1A1 (CYP1A1, assayed as benzo[*a*]pyrene hydroxylase) was inhibited by the isoflavones daidzein > daidzin > genistein ≫ genistin. The isoflavones are not general inhibitors of transcription or translation, since at the IC_{50} concentrations there is no effect on the rate of cell proliferation or the induction of NAD(P)H:menadione oxidoreductase (a Phase II enzyme of the [*Ah*] gene battery) by the electrophile *tert*-butylhydroquinone. Furthermore, gel mobility retardation assays indicate that the isoflavones compete effectively with TCDD for AhR complex binding to a DNA fragment containing a consensus Ah response element (AhRE3) sequence. The development of safe and effective AhR antagonists may provide a new approach for the intervention and treatment of AhR-related diseases, especially in high-risk populations. (Supported by Grants ES06096, ES06321, AG09235, ES06273).

1368 EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN (TCDD), 12-0-TETRADECANOYLPHORBOL-13-ACETATE (TPA), AND 17β-ESTRADIOL (E_2) ON ESTROGEN RECEPTOR REGULATION IN MCF-7 HUMAN BREAST CANCER CELLS

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TCDD exhibits remarkably potent antiestrogenic activity. To further elucidate the role of estrogen receptor (ER) regulation in this response, we examined the effects in human MCF-7 breast cancer cells of TCDD treatment on ER mRNA levels using the RNase protection assay, ER accumulation by using an estrogen receptor immunocytological assay (ER-ICA), and function by competitive binding assays under conditions of saturating E_2. Comparative studies were conducted with E_2 or TPA treatment, both of which are known to suppress ER expression. The results indicate that 10^{-9} M E_2 and 10^{-7} M TPA both suppress ER mRNA levels as early as 4 hr after exposure and to 33.6% and 16.5% of control levels respectively after 72 hr. In contrast, no significant effect on ER mRNA levels was attributed to 10^{-8} M TCDD exposure. A greater than 50% reduction in percent positive staining was observed by ER-ICA assay after 72 hr exposure to 10^{-9} M E_2 and 10^{-7} M TPA, while no effect was observed with 10^{-8} M TCDD. Specific binding of $[^3H]E_2$ at saturating conditions (10^{-8} M) in whole cells was reduced by 50% in cultures exposed to 10^{-7} M TPA, with no effect using 10^{-8} M TCDD. In conclusion, while TPA and E_2 effectively down-regulate the ER, TCDD has little if any effect on total ER levels in MCF-7 cells, and thus ER modulation is probably not necessary for suppression of estrogenic activity by TCDD (Supported by NIEHS ES-03561).

1369 MOLECULAR MECHANISMS IN THE REGULATION OF IMMUNOLOGICAL RESPONSES BY 2,3,7,8-TETRACHLORO- DIBENZO-p-DIOXIN (TCDD)

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2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD), a ubiquitous environmental pollutant, is known to act as a potent murine immunotoxin, inducing thymic atrophy, suppressing antibody responses, as well as profoundly inhibiting resistance to viral and parasitic infection. However, mechanisms by which TCDD induced these responses are not fully understood. Nitric oxide, a newly recognized reactive mediator, is produced by macrophages as well as epithelial cells in response to the inflammatory cytokine γ-interferon. Recent studies have demonstrated that nitric oxide is critical for cellular resistance to both viral and parasitic infection. We have found that the TCDD sensitive cell line Hepa 1c1c7 responds to γ-interferon by producing nitric oxide. Treatment with TCDD in the nanomolar concentration inhibited this response. Investigations into the mechanisms of the cellular alterations induced by this cytokine, have revealed that tyrosine kinases, activated by the interaction of γ-interferon with its receptor, mediate the induction of gene transcription. Tyrosine phosphorylation of unique proteins initiated by activation of the interferon receptor, results in formation of nuclear multimeric transcription complexes which bind cis-acting enhancer elements in the regulatory regions of interferon regulated genes, including nitric oxide synthase. We discovered that treatment of Hepa 1c1c7 cells with TCDD induces expression of a 48 kd protein antigenically related to this family of transcription factors. Although the cellular effects of alterations in the activity of these transcription factors are as yet undefined, TCDD-induced alterations in this pathway may disrupt cellular signaling initiated by γ-interferon leading to suppression of nitric oxide formation by Hepa 1c1c7 cells. These findings represent a potential mechanism in the immunosuppressive actions of TCDD. (Supported by ES 05022.)

1370 PULMONARY HOST DEFENSE ACTIVITIES IN RATS WITH AMIODARONE-INDUCED PULMONARY PHOSPHOLIPIDOSIS

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Amiodarone (AD) is a cationic, amphiphilic drug with class III antiarrhythmic activity. In humans and animals, AD causes pulmonary phospholipidosis. The functional consequences of this disorder are unknown. The objective of this study was to compare pulmonary host defense activities of rats treated with AD (100 mg/kg/day for 7d) with those of control rats which received vehicle. AD treatment resulted in a 5-fold increase in total phospholipid in alveolar macrophages (AMs). Pulmonary antibacterial activity against intratracheally instilled *Listeria monocytogenes* was not significantly different between AD-treated and control rats at 3 and 7 days post instillation. The generation of zymosan-stimulated or PMA-stimulated chemiluminescence (a measure of oxidant production) by isolated AMs from AD-treated rats was not significantly different from control AMs, nor was phagocytosis of yeast particles. The results indicate that the presence of phospholipidosis in the lungs of rats does not have an adverse effect on *in vivo* or *in vitro* aspects of host defense in the context of the activities studied. (Supported by a grant from Procter & Gamble Pharmaceuticals).

1371 EFFECTS OF SINGLE AND REPEATED EXPOSURE TO PHOSGENE ON PULMONARY BACTERIAL INFECTION IN RATS

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Acute phosgene exposure impairs resistance to streptococcal infection in mice. This study assessed effects of acute (0.1 or 0.2 ppm, 6 hr) and subchronic (4 or 12 wk, 0.1 or 0.2 ppm, 6 hr/day, 5 day/wk or 0.5 ppm, 2 day/wk) phosgene exposure on resistance to streptococcal infection in rats. Bronchoalveolar lavage (BAL) was assessed at intervals postinfection for bacteria and inflammatory cells. All exposures impaired bacterial clearance from the lungs and caused an increase in neutrophils in BAL. Effects of repeated exposure were similar to those observed after a single exposure. Effects in the 0.5 ppm repeated exposure group were greatest, and significantly different from those in the 0.2 ppm group although the concentration X time was equal. All parameters were normal in rats assessed 4 wks after the 12 wk exposure. The data indicate that streptococcal infection is the most sensitive endpoint for phosgene toxicity in rats. Effects of repetitive exposure were not additive nor was there attenuation of the response; however, recovery after exposure was complete. (This abstract does not reflect EPA policy.)

1372 DIFFERENTIAL MODULATION OF IMMUNITY TO HOUSE DUST MITE ANTIGEN BY EXPOSING RATS TO O_3 BEFORE OR AFTER IMMUNIZATION

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We have previously demonstrated that exposure to NO_2 after immunization and challenge with house dust mite antigen causes upregulation of immune responses and increased allergen-mediated lung disease in rats. This present study was designed to establish whether similar effects would occur with O_3 exposure, and to compare the effects of O_3 before or after administration of antigen. Exposure to 0.8 ppm O_3 for 3 hrs immediately after immunization and intratracheal challenge with antigen caused an increase in antigen specific IgG, IgA and IgE antibody, lymphocyte proliferation, and inflammatory cells in the lung 7 days post challenge compared to air-exposed controls. When animals were exposed to O_3 just prior to immunization, immune function and lung inflammation was significantly less than air controls. The data suggest that the timing of pollutant exposure in relation to immunization plays a critical role in determining whether immune function and subsequent lung disease is increased or decreased. (This abstract does not reflect EPA policy.)

1373 WOODSMOKE EMISSIONS: EFFECTS ON PULMONARY IMMUNE DEFENSE

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Epidemiological evidence suggests that inhalation of woodsmoke enhances the incidence, duration and, possibly, severity of respiratory illness in children. The hypothesis of this toxicological study is that woodsmoke exposure compromises pulmonary host resistance to infectious bacteria by altering immune defense mechanisms, particularly at the level of the macrophage. Rats were exposed nose-only to woodsmoke emissions generated from the burning of Red Oak (RO) and Douglas Fir (DF) in a furnace developed in this laboratory. Generated smoke was characterized in terms of respirable suspended particulate (RSP) matter, carbon monoxide (CO) levels, NO_x (NO and NO_2, respectively) concentration, and total PAH [measured as benzo(a)pyrene]. Under the present burning conditions, RSP concentration was maintained at a level of 750 $\mu g/m_3$ for 1 hr for RO; mass median diameter of the emitted particles was 0.15 μm ($\sigma g = 1.8$). CO levels emitted during combustion of DF and RO were 1 ppm, and all (100%) of the gaseous material produced was volatile. Levels of NO_x were 0.062 ppm for RO and 0.020 for DF. Following a single 1 hr exposure, rats were either anesthetized and intratracheally instilled with *Staphylococcus aureus* to assess effects on pulmonary clearance, or sacrificed

and their lungs lavaged to provide pulmonary macrophages. Inhalation of RO emissions reduced bacterial killing *in vivo* and diminished *in vitro* phagocytosis by macrophages recovered 1 and 2 h post-exposure. Results demonstrate that acute inhalation of woodsmoke generated from a model system that produces emissions comparable to those produced in homes using woodburning devices, compromises important immune defense mechanisms of the lung. Supported by Center Indoor Air Research. Contract No. CIAR 94-03.

1374 IMMUNOTOXIC EFFECTS IN THE RAT LUNG FROM INHALATION OF VANADIUM

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Male Fisher 344 rats (10 wk old, 200–250 g) were exposed to atmospheres containing 2 mg vanadium (V)/m^3 (as ammonium metavanadate NH_4VO_3, 0.32 μm diameter particles) for 8 hr/d for 4 d in a nose-only exposure system. In exposed rats, lung burdens of V increased in a time-dependent fashion. Nearly all (88%) V-exposed rats displayed markedly increased levels of bronchus-associated lymphoid tissue (BALT) after each exposure, though the effect was only transitory. Analysis of lung cells and lavage fluid 24 hr after the final exposure suggested that a strong inflammatory response was elicited; levels of free neutrophils and immature monocytes, as well as of lavage protein and lactate dehydrogenase, were greatly elevated as compared with levels observed in air-exposed controls. Vanadium also affected the capacity of pulmonary macrophages (PAM) to both produce and respond to important immunoregulatory cytokines. PAM production of tumor necrosis factor-α in response to lipopolysaccharide was significantly inhibited, as was their ability to synthesize/express MHC Class II/Ia molecules in response to interferon-γ (IFNγ). The PAM from V-exposed hosts were also inhibited in their ability to be primed by IFNγ to produce superoxide anion and hydrogen peroxide in response to opsonized zymosan stimulation. These studies indicate that subchronic exposure of rats to workplace levels of atmospheric V can cause strong immunomodulatory effects in the lungs, with a major effect occurring at the level of cytokine-related functions. These alterations may be underlaying mechanisms for the well-documented increases in bronchopulmonary infections and cancers in workers chronically exposed to V-containing atmospheres. This study was supported by NIOSH (Grant No. OH03064-01).

1375 VANADIUM ALTERS MACROPHAGE INTERFERON-γ-INTERACTIONS AND -INDUCIBLE RESPONSES

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Vanadium (V) impairs host resistance overall, and the antimicrobial activity and function of several intracellular enzymes of macrophages (Mø) in particular. Mouse WEHI-3 Mø-like cells were exposed overnight to subcytotoxic concentrations of ammonium metavanadate (NH_4VO_3) to determine whether this observed immunomodulation might be due, at least in part, to altered Mø interactions with interferon-γ (IFNγ) or IFNγ-inducible responses, i.e., increased reactive oxygen intermediate (ROI) production, Ca^{2+} ion influx, and surface Class II/Ia antigen expression. Binding studies performed at 22°C indicated that V-treated cells had ≅50% fewer actively-binding receptors, but binding affinities 450-times greater than that of control receptors. At 4°C, V-treated cells had 98% fewer functional receptors, but again higher (145-fold) affinities. IFNγ-receptor complex internalization was unaffected by V, although significantly higher in cells incubated at temperatures which minimize uptake. ROI production in IFNγ-stimulated V-treated (either NH_4VO_3 or V_2O_5 [vanadium pentoxide]) cells was decreased relative to spontaneous production, while control cells showed consistent increases due to IFNγ priming. Vanadium also reduced the Ca^{2+} ion influx rate into stimulated cells without affecting final cell Ca^{2+} burdens. Although V did not affect IFNγ-induced Ia expression, exposures resulted in increased numbers of Ia-bearing cells with lower maximal antigen densities than control cells. The results of this study show that V exposure may alter Mø-mediated functions by modifying cell interactions with IFNγ and subsequent IFNγ-dependent parameters. This study was supported by NIOSH (OH03064-01) and by EPRI (RP2155-1).

1376 EFFECTS OF INHALED NITRIC OXIDE (NO) ON RAT LUNG

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Inhaled NO has been shown to be a potent pulmonary vasodilator clinically useful in adult respiratory distress syndrome and persistent pulmonary hypertension of the newborn. However, little is known about the potential toxicity of this agent. In the present studies, we analyzed the effects of inhaled NO on the lungs. Exposure of female SD rats to 80 ppm NO for 3 hr resulted in a small increase in polymorphonuclear leukocytes in lung lavage fluid 24 hr after exposure. Superoxide anion production by lung lavage cells was also elevated, an effect which persisted for 48 hr. In contrast, although exposure of rats to 80 ppm NO for 3 days (6 hr/day) had no major effect on superoxide anion production, cells isolated from these animals produced significantly increased levels of NO *in vitro* in response to lipopolysaccharide. Interestingly, inhalation of NO prior to ozone resulted in partial reversal of ozone-induced increases in lavage protein levels and decreases in superoxide anion release. Taken together, these data suggest that inhalation of NO activates lung lavage cells to release increased quantities of reactive intermediates. Furthermore, NO may modify the response of the lung to pulmonary irritants. (ESO4738) and a fellowship from the American Lung Association (ALA).

1377 MODIFICATION OF AIRWAY EPITHELIAL PERMEABILITY AND NITRIC OXIDE RELEASE UPON OZONE EXPOSURE

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Results from epidemiological and laboratory animal studies have demonstrated that ozone (O_3) is an inducer of lower airway inflammation and nonspecific airway hyperreactivity. The airway epithelium is the first cell type inhaled O_3 will encounter. The production and release of mediators such as reactive oxygen intermediates (ROI) and nitric oxide (NO) as well as of chemotactic factors (i.e. IL-8) by epithelia in response to ozone might be contributory to these two observed responses. The present study was designed to investigate the effects of O_3 exposure on epithelial NO release, IL-8 production, as well as disruption of the epithelial permeability barrier. A Human type II-like cell line (A549) was grown to a monolayer in Costar Transwells (Costar, Cambridge, MA) and exposed to 0.4 ppm O_3 for 1 hour. Epithelial resistance was measured using a "chopstick" voltohmmeter (World Precision Instruments, Sarasota, FL). Resistance was decreased 72 hours after exposure (91.4 Ω^{1} cm$^{2-1}$) as compared to before the O_3 exposure (193.2 Ω^{1} cm$^{2-1}$) in unstimulated cells. No change was observed in non-exposed cell. Additionally, the effect of O_3 on NO release by epithelial cells was assessed. Immediately after exposure, A549 cells were stimulated with cytomix (IL-1β, TNFα, IFNγ, all at 10 ng/ml) for 72 hours and the nitrite content was determined in the basolateral media. O_3 exposed A549 cells showed an increased nitrite content (1.338 μM) as compared to non-exposed cells (0.846 μM). These results indicate the airway epithelium as a target as well as an effector cell of ozone toxicity in the lower airways. (Sponsored by EPA R819342).

1378 AIRWAY INFLAMMATION AFTER EXPOSURE TO OZONE — CYTOKINE mRNA PRODUCTION IN LUNG CELLS

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Animal and human studies have shown that acute inhalation of ozone causes airway inflammation and tissue damage, lung function decline, and airway hyperreactivity. Although these responses are also considered as important components in the pathogenesis of asthma, spirometric data following ozone exposure do not show that asthmatics are more susceptible than healthy people. Cytokines are thought to play a role in ozone-induced airway inflammation and structural tissue damage. Therefore, studies were initiated to investigate the airway inflammatory cell and mediator response to ozone and to develop (early) markers for (asthmatic) susceptibility.

In cells from lung lavages as well as in lung tissue from ozone exposed rats (6 hours, 2400 μg/m^3 O_3), changes in cytokine mRNA levels (IL-1α, IL-6, IL-12p40, TNF-α, TGF-β, and the chemokine KC) were assessed upon exposure by a semiquantitative RT/PCR using GAPDH as internal control. In cells from lung lavages increased levels were found for IL-6, IL-12p40, and KC mRNA, whereas decreased levels were found for IL-1α mRNA. No changes were found in TNF-α and TGF-β mRNA levels. In lung tissue increased levels were found for IL-1α and IL-6 mRNA, whereas decreased levels were found for IL-12p40 mRNA. No changes were found in TNF-α, TGF-β, and KC mRNA levels.

Data show that acute ozone exposure is able to induce pulmonary inflammatory mediator signals involved in cellular and immunological defense.

Future studies will include mRNA production of additional cytokines, in situ hybridization, and protein measurements.

1379 ASBESTOS-CYTOKINE STIMULATION AND OXIDATIVE STRESS

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Asbestos is a pneumotoxic dust associated with malignant and nonmalignant respiratory diseases. Studies have indicated that free radicals and other reactive oxygen metabolites are important mediators in asbestos-induced lung toxicity. Asbestos fibers are thought to stimulate cells to produce oxygen radicals and generation of reactive oxygen species via iron which is present in fibrous silicates. We examined whether asbestos-induced oxidative stress is also involved in mediating pro-inflammatory cytokine stimulation. This was investigated using various *in vitro* model systems. Treatment of alveolar macrophages cultures with asbestos stimulated dose-dependently TNF-α secretion. Crocidolite asbestos also stimulated a human epithelial type II cell line (A549) to elicit the chemotactic cytokine IL-8 in the absence of endogenious stimuli such as IL-1 and TNF. Consistent with the role of iron on asbestos fibers in catalyzing hydroxyl radical generation, membrane permeable hydroxyl radical scavengers (tetramethylthiourea, dimethylsulfoxide) inhibited asbestos-induced cytokine secretion in both cell types. The asbestos induced increase in TNF-α in alveolar macrophages and IL-8 in A549 cells and their inhibition by tetramethylthiourea occurred at the level of transcription. The role of ROS in signaling pro-inflammatory cytokines was confirmed by use of free radical generating systems (hypoxanthine-xanthine oxidase, H_2O_2, glucose-glucose oxidase and combinations with ferrous). Asbestos were shown by a DNA shift assay to influence directly transcription factors, the greatest effect on AP-1, in both cell systems. These data indicate that oxidative stress is responsible for asbestos-induced cytokine response and transcription factors can be early mediators of this response.

1380 EVALUATION OF THE TIME COURSE OF PULMONARY NEUTROPHILIC INFLAMMATION FOLLOWING EXPOSURES TO A VARIETY OF DUSTS

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This study was designed to identify the time course and mechanism(s) of dust-induced pulmonary inflammation following exposures to a number of different particle-types. Rats were exposed through intratracheal instillation to nonfibrogenic dusts such as titanium dioxide (TiO_2) and amorphous silica (AMO) particles, as well as fibrogenic crystalline silica (Si); lung fluids were evaluated for chemotactic factors and inflammation at several postexposure time periods. Biochemical and molecular biology methods were used to correlate the expression of the chemotactic cytokines (both at mRNA and protein levels) with the time course of chemotactic activity and the recruitment of neutrophils. All three dusts induced neutrophilic inflammation as early as 4 hours after exposure. Both Si and AMO elicited a higher degree of inflammation when compared to TiO_2 particles. Maximum infiltration of neutrophils into the lungs occurred 6 hours after intratracheal instillation of the dusts. The inflammatory response was transient for titanium dioxide and amorphous silica but was sustained through a 10-day period following exposures to crystalline silica. The chemotactic activity in lavage fluid was correlated with the influx and disappearance of neutrophils in spite of the continued presence of TiO_2 and AMO-containing macrophages. These results suggest that the presence of particles within macrophages does not necessarily induce a sustained inflammatory response.

1381 ASSOCIATION OF INTERCELLULAR ADHESION MOLECULE-1 (ICAM-1) WITH SILICA INDUCED LUNG INJURY IN C57BI6 MICE

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ICAM-1 is expressed on endothelial and epithelial cells. It serves as a ligand for CD11b/CD18$^+$ neutrophils and plays a role in their migration from the blood into the lung parenchyma. Intratracheal injection of silica (SI) in mice results in an influx of neutrophils into the lung parenchyma and alveolar space. Expression of ICAM-1 was measured in an ELISA of homogenized lung protein using anti-mouse ICAM-1 monoclonal antibody (Seikagaku, Rockville, MD). By 24 hours post-SI, lung tissue ICAM-1 was significantly elevated above saline injected mice. Alveolar protein, albumin, and neutrophils were also increased in SI exposed mice. Lung tissue ICAM-1 remained elevated at 72 hours post SI which could be associated with an increase in lung injury and neutrophil influx at this time. These data suggest an association between times of silica induced lung injury and neutrophil influx and lung tissue ICAM-1 expression.

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1382 STIMULATION OF MYELIN TRANSCRIPTS AND PROTEINS BY TRIETHYL TIN (TET) IN PRIMARY GLIAL AND C6 CELL LINE CULTURES

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Metals can both modulate the immune system at low level exposures and damage the nervous system at higher levels. The present experiments demonstrate this phenomenon in cell culture models exposed to the CNS myelinotoxicant, triethyl tin (TET). Cultures of mixed glial, pure oligodendroglia and C6 glioma cell line were exposed to this alkyl metal in a serum free culture medium (DMEM + ITS + 0.1% BSA). Baseline levels of myelin specific mRNAS and their proteins were established in these preparations using quantitative *in situ* hybridization and immunocytochemistry. The culture preparations were treated to various concentrations of TET-Br (0.01–10 μM) and sampled at 1, 3 and 24 hr post-exposure for changes in myelin basic protein (MBP) or 2'3'-cyclic nucleotide-3'-phosphohydrolase (CNP) myelin transcripts and proteins. TET (0.1 μM) significantly increased MBP transcripts in both mixed glia and pure oligodendroglial cultures after 3 hr exposure, but had to effect at higher doses. Concentrations of 10 μM TET also increased levels of MBP protein in pure oligodendroglial cultures. By contrast, CNP transcript was stimulated in C6 cell lines, but at relatively higher concentrations (5–10 μM) of TET. Another neurotoxic alkyl metal, Trimethyltin (0.1 μM) also stimulated CNP transcript in 4 DIV C6 cell lines. These cell culture models will be used to examine neuroimmune influences on this phenomenon.

1383 Pb DECREASES PHOSPHORYLATION OF TYROSINE HYDROXYLASE (TH) IN PC 12 CELLS

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Exposure to lead (Pb) impairs learning in children. Since learning appears to require a change in synaptic efficiency, lead (Pb) is known to disrupt signal transduction mechanisms such as those that utilize intracellular calcium (Ca) or cAMP. A common downstream effect of these mechanisms is the activation of protein kinases (PK). It is unclear whether protein phosphorylation is affected by Pb. In the experiments that will be presented, we asked whether the phosphorylation of TH is affected by Pb in PC 12 cells. TH is the regulatory enzyme in the biosynthesis of catecholamines. PC 12 cells were exposed to 10 uM for 1–3 d and the level of phosphorylated TH was determined. A 50% decrease in the level of phosphorylated TH was observed after a 2 or 3 d exposure but not 1 d. A change was not observed in the amount of TH protein or ATP. Furthermore, alterations in the phosphorylation of several other proteins, including the proteins that comprise the intermediate filaments, were not observed. Thus, the effect of Pb on phosphorylation appears to be relatively specific. Since TH activity is affected by phosphorylation, the production of catecholamines may be disrupted in neural cells exposed to Pb.

1384 ALUMINUM POTENTIATES TOXICANT-INDUCED CALCIUM ACCUMULATION AND REACTIVE OXYGEN SPECIES FORMATION IN NEURONAL CELL CULTURE

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Aluminum is a neurotoxic metal which may be involved in the progression of neurodegenerative diseases including Alzheimer's disease and amyotropic lateral sclerosis. Exposure of neuronal cell cultures to aluminum can result in the abnormal accumulation of neurofilament protein and cytotoxicity. While the mechanism of action is not known, aluminum has been shown to alter Ca^{2+} flux and homeostasis and facilitate peroxidation of membrane lipids. Since abnormal increases of intracellular Ca^{2+} and oxygen free radicals have both been implicated in pathways leading to cell degeneration, we examined the effect of aluminum on these parameters *in vitro*. $AlCl_3$ inhibited Ca^{2+}-sequestration in both mitochondria and microsomes from 7 day old rat cerebellum. The consequences of this inhibition on the intracellular accumulation of ^{45}Ca were examined in primary cultures of rat cerebellar granule cells. The excitatory amino acid glutamate (Glu) was used as a positive control. Exposure to $AlCl_3$

(10–1000 μM) or Glu (1–300 μM) caused a concentration-dependent accumulation of ^{45}Ca in granule cells to a maximum of 160 and 200 percent of control, respectively. The effects of $AlCl_3$ and Glu were additive, suggesting that they did not act by the same mechanism. The accumulation of intracellular Ca^{2+} resulted in the translocation of protein kinase C from the cytosol to the membrane as measured by ^3H-phorbol ester binding. The production of reactive oxygen species (ROS) was examined in granule cell cultures using the fluorescent probe dichlorofluorescin. By itself $AlCl_3$ had little affect on ROS production. However, $AlCl_3$ (100–1000 μM) potentiated the ROS production induced by 50 μM Fe^{2+}. These results suggest that aluminum may facilitate neuronal degeneration induced by other neurotoxicants.

1385 LOSS OF HIGH MOLECULAR WEIGHT MICROTUBULE-ASSOCIATED PROTEINS (MAPs) IN RODENT BRAIN FOLLOWING METHYLMERCURY ADMINISTRATION

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Development and maintenance of neural structure is dependent, in part, on microtubule stabilization by the temporally-regulated expression of microtubule-associated-proteins (MAPs). Changes in MAP expression appear to correlate closely in time with changes in microtubule polymerization efficiency. During development, expression of the juvenile forms of MAP promote plasticity of neurite outgrowth; adult MAPs are believed to mediate stable interactions between microtubules and other cytoskeletal elements. Chemical perturbation of MAPs may disturb neuronal function in both developing and mature CNS. Alterations of MAPs were examined following methylmercury (MeHg) administration by Western immunoblot and immunohistochemical analyses. Subacute treatment of 10 or 15 mg MeHg/kg i.p. to neonatal rats between days 2 and 6 resulted in cerebellar MAP2 loss, with appearance of proteolytic breakdown products. MAP2 reappeared in cerebellum of neonates within 48 hours of cessation of MeHg treatment. Chronic treatment of adult mice of 4.0 mg/kg MeHg i.p. on alternate days for two months caused persistent MAP2 loss in hippocampus. Regionally selective effects of MeHg on MAP2 in development may influence age-specific expression of neurotoxicity. (Supported by NIH ES 04976).

1386 NEURAL CELL ADHESION MOLECULES AND MICROTUBULES ARE DISTINCT TARGETS OF METHYLMERCURY *IN VITRO*

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We have previously demonstrated that both microtubules (MTs) and the neural cell adhesion molecule (NCAM) are cellular targets of methylmercury (MeHg). However it is not known whether these targets are affected independently or in a cooperative manner. The present study attempts to identify the relationship of MT integrity to the response of NCAM in MeHg-treated neurons in culture. Embryonal carcinoma cells (EC cells, P-19 line) were differentiated into neurons by exposure to 1 μM retinoic acid for 48 h, then cultured in serum free DMEM supplemented for neuronal growth until day 9. MTs were stabilized by pretreatment with taxol (5 μM, 4 h) prior to 2 h treatment with MeHg (2.0–10 μM). Cells were then double-labeled for MTs and NCAM using monoclonal primary antibodies and fluorescent conjugated secondary antibodies. In a separate study cells were treated for 4 h with the antimicrotubule agents colchicine (1 μg/ml) or nocodazole (2 μg/ml) and double-labeled as above. MeHg treatment of normal cells resulted in concentration-dependent loss of labile and most stable MT populations. NCAM staining in these cultures was also reduced, an effect which paralleled MT disruption. Taxol pretreatment protected most MTs against MeHg-induced disassembly. These cells also displayed near normal NCAM staining. While cytoskeletal stabilization with taxol prevented turnover and loss of NCAM following MeHg treatment, disassembly of MTs resulting from specific disruption by colchicine or nocodazole did not diminish NCAM staining. These data indicate that loss of MTs does not necessarily result in consequent loss of NCAM but rather that MTs and NCAM are distinct cellular targets of MeHg. (Supported by NIH ES04976).

1387 MANGANESE-INDUCED REACTIVE OXYGEN SPECIES: COMPARISON BETWEEN Mn^{2+} AND Mn^{3+}

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The present study was designed to determine the *in vitro* or *in vivo* exposure to Mn produced reactive oxygen species (ROS) and alterations of monoamines in different regions of the rat brain. Adult male Sprague Dawley rats were dosed ip, with O, 50 or 100 mg/kg of either $MnCl_2$ (Mn^{2+}) or MnOAc (Mn^{3+}) and were sacrificed 1 hr later. Brains were quickly removed and dissected for neurochemical analysis. ROS were measured using a molecular probe, 2,7-dichlorofluorescein diacetate (DCFH-DA) and monoamines and their metabolites by HPLC/EC. *In vitro* exposure to $MnCl_2$ (1–1000 μM) produced dose dependent increases in ROS in caudate nucleus (CN), whereas MnOAc produced similar increases at much lower concentrations (1–100 μM). *In vivo* exposure to MnOAc (Mn^{3+}) produced significant increases in ROS in CN and hippocampus (HIP), whereas only the high dose of $MnCl_2$ (Mn^{2+}) produced significant effects in the HIP. Concentrations of dopamine, serotonin and their metabolites did not change after acute injection of either $MnCl_2$ or MnOAc. These data suggest that both divalent and trivalent manganese induce ROS, although Mn^{3+} is more potent than Mn^{2+} and that Mn-induced neurotoxicity may be mediated via oxidative stress.

1388 METHYLMERCURY (MeHg) ALTERS INTRACELLULAR CALCIUM CONCENTRATION ($[Ca^{2+}]_i$) IN RAT CEREBELLAR GRANULE CELL CULTURES

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MeHg is known to alter normal Ca^{2+} homeostasis in NG108-15 neuroblastoma cells, and rat brain synaptosomes; however, the mechanism of this altered ion homeostasis is poorly understood. In the present study we sought to compare the susceptibility of cerebellar granule cells, a primary target of MeHg, with that of the NG108-15 cells on MeHg-induced alterations in Ca^{2+} homeostasis. Rat cerebellar granule cell cultures (7–10 DIV) were exposed to 0.2–5.0 μM MeHg and alterations in $[Ca^{2+}]_i$ were measured using fura-2. Similar to previous findings in NG108-15 cells, MeHg induced a multiphasic rise in $[Ca^{2+}]_i$. The changes in $[Ca^{2+}]_i$ were characterized by an initial small increase which, after several minutes was followed by a more precipitous rise in $[Ca^{2+}]_i$. However granule cells appear to be more sensitive than NG108-15 cells, showing alterations in $[Ca^{2+}]_i$ at 0.2 μM MeHg, whereas 2 μM MeHg was required to produce similar changes in NG108-15 cells. The early $[Ca^{2+}]_i$ rise was not dependent upon extracellular Ca^{2+} ($Ca^{2+}{}_e$) as it occurred with a similar time course in both the presence and absence of $Ca^{2+}{}_e$. In contrast, the later increase in $[Ca^{2+}]_i$ was not observed in the absence of $Ca^{2+}{}_e$. Concurrent with the initial rise in $[Ca^{2+}]_i$, the fluorescence of fura-2 was altered in a manner similar to that characterizing the non-Ca^{2+} phase in NG108-15 cells and synaptosomes. This response appears to occur simultaneously with the initial change in $[Ca^{2+}]_i$ and can be eliminated by treatment with the cell-permeant divalent cation chelator, TPEN. Thus cerebellar granule cells respond to MeHg in a highly sensitive manner with disruptions in Ca^{2+} homeostasis. Whether their enhanced sensitivity to MeHg reflects their unique sensitivity to this toxicant *in vivo* or specific differences in entry pathways and/or target sites for MeHg remains to be determined. Supported by NIH grants R01-ES03299 and T32-ES07255-05.

1389 INHIBITION OF PROTEIN KINASE C (PKC) ACTIVITY IN HIPPOCAMPAL FRACTIONS OF LEAD EXPOSED RATS

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We have previously shown that chronic Pb exposure causes a significant increase in the levels of membrane-bound PKC in all regions of the rat hippocampus at postnatal day (PN) 28 but not in PN14 or PN56 rats (Farmer and Guilarte, *The Toxicologist* 14(1) 143, 1994). These results suggested that developmental *in vivo* Pb exposure may have an effect on the redistribution of PKC from the cytosol to the plasma membrane and may alter intrinsic PKC activity. Using a selective PKC substrate, $Neurogranin_{28--43}$, we have performed PKC activity assays on fractions derived from the hippocampi of PN28 rats exposed to 0 and 750 ppm Pb acetate in the diet. The mean ± s.e.m. PKC activity in the cytosolic fraction of control rats was 0.57 ± 0.06 pmoles/min/ug protein at 30°C (n = 8) and 0.37 ± 0.04 (n = 8) for the cytosolic fraction of 750 ppm Pb exposed rats. The mean ± s.e.m. PKC activity in the particulate fraction was 0.27 ± 0.02 pmoles/min/ug protein at 30°C for control rats (n = 8) and 0.18 ± 0.02 (n = 8) for the 750 ppm Pb exposed rats. PKC activity was significantly inhibited by 34% ($p < 0.025$) and 35% ($p < 0.025$) in the cytosolic and membrane fractions, respectively, in the 750 ppm diet relative to controls. These preliminary findings suggest that developmental Pb exposure inhibits PKC activity both in the cytosolic and particulate fractions in the rat hippocampus as measured by the phosphorylation of the brain specific peptide $Neurogranin_{28--43}$. [Supported by grant No. ES06189, ES03819, and ES07141].

1390 Pb INCREASES PHOTO-ONCOGENE EXPRESSION IN GLIAL CELLS

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Exposure to lead (Pb) impairs learning in children. Since learning appears to require a change in synaptic efficiency, Pb may impair learning by directly affecting synaptic processes. Several investigators demonstrated that repetitive synaptic stimulation results in the production of mRNA that encodes for immediate early response genes (IERG), such as c-fos or c-jun. In this study we asked whether Pb alters the expression of c-fos and c-jun mRNA in glial cells. mRNA was isolated after exposing cells of the C6 glial cell line for 5 and 15 h to 10 μM Pb or sodium acetate and the level of c-fos, c-jun and β-actin mRNA was quantified by Northern Analysis and densitometry. A significant increase in the ratio of c-fow/β-actin mRNA and c-jun/β-actin mRNA was observed in cultures exposed to Pb acetate for 15 h. However, exposure for 5 h did not result in changes in the level of expression. Similarly, a significant increase in the expression of these IERG was also found in primary cultures of rat brain astrocytes exposed to 10 μM Pb acetate for 15 h but not for 5 h. We conclude that glial cells exposed to Pb upregulate the expression of c-fos and c-jun, Since the proteins encoded by c-fos and c-jun are transcriptional factors, this alteration by Pb may result in changes in the expression of other genes that adversely affect the molecular events of learning.

1391 INTRACELLULAR GLUTATHIONE $[GSH]_i$ LEVELS MODULATE MERCURIAL-INDUCED AMINO ACID RELEASE IN NEONATAL PRIMARY ASTROCYTE CULTURES

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Mercuric chloride (MC) and methylmercury (MeHg) were found to increase amino acid (AA) release from neonatal rat primary astrocyte cultures. This suggests interaction with sulfhydryl (-SH) groups which are controlled by $[GSH]_i$ levels. In the present study, we evaluated the effects of alterations in $[GSH]_i$ on the outcome of MC and MeHg treatment. $[GSH]_i$ were increased in a time-dependent fashion by incubating the astrocytes with 1 mM L-2-oxothiazolidine-4-carboxylic acid (OTC), a cysteine precursor. OTC attenuated the release of [2,3-^{3}H]-D-aspartic acid from astrocytes exposed to MC- (5 μM) and MeHg- (10 μM). MeHg-induced [^{3}H]-D-taurine release was also reduced by pretreatment of astrocytes with OTC. Treatment with BSO (50 μM) decreased $[GSH]_i$ in astrocytes, and increased [2,3-^{3}H]-D-aspartate release from MC- and MeHg-treated astrocytes, and [^{3}H]-D-taurine release from MeHg-treated cells. Neither OTC nor BSO when added to cultures in the absence of MC or MeHg had an effect on AA release by astrocytes. The current study underscores both the sensitivity of astrocytes to mercurials in terms of AA release and the relationship of these effects to astrocytic $[GSH]_i$. (Supported by NIEHS 05223 and EPA 89190).

1392 ROLE OF METABOLISM BY CYTOCHROME P450 IN ETHYL CARBAMATE-INDUCED IMMUNOSUPPRESSION IN FEMALE Balb/c MICE

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Ethyl carbamate (EC) is a potent carcinogen in rodents and is present at low concentrations in fermented foods and alcoholic beverages due to the reaction of ethanol with carbamyl phosphate. It has well been hypothesized that more carcinogenic forms are produced through the metabolism of EC by cytochrome P450 (P450). In the present study, a possible role of metabolism by P450 in EC-induced immunosuppression was investigated in female Balb/C mice. When mice were given EC intraperitoneally for 14 consecutive days at 0, 25, 50, 100, 200, and 400 mg/kg, the antibody response to sheep red blood cells (SRBCs) was significantly suppressed from 200 mg/kg without showing any elevation of SGOT and SGPT activities. To determine the role of metabolism, the ability of EC and its metabolites produced by P450, ethyl N-hydroxycarbamate (EHC) and vinyl carbamate (VC), to suppress the polyclonal antibody response to bacterial lipopolysaccharide was compared in splenocyte cultures. The results showed that EHC and VC were most suppressive compared to EC and methyl carbamate. These results indicated that metabolism of EC by P450 may produce more immunosuppressive metabolites. When mice were pretreated with 80 mg/kg of phenobarbital sodium for three days to induce P450 enzymes, followed by administration of EC intraperitoneally for 7 consecutive days, the antibody response to SRBCs was more suppressed when compared to saline-pretreated control, indicating an evidence that metabolic activation by P450 may be the major metabolic pathway of EC to its immunosuppressive forms. (Supported by a grant from Ministry of Science and Technology, Korea).

1393 DIFLUORONUCLEOSIDE TRANSFORMATIONS IN C3H MICE

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2′-Deoxy-2′,2′-difluororibo-2-aminoadenosine, LY207702, is a purine analog of gemcitabine (an oncolytic agent which has been submitted for registration in Europe). While LY207702 was found to have exceptional activity in a colon xenograft mouse antitumor model, it was also found to cause significant myocardial inflammation and necrosis in initial toxicology studies. The 6-amino substituent present in LY207702 makes it a substrate for adenosine deaminase (ADA), giving the guanosine analog LY223592. When the metabolism of LY207702 was studied in mice, using an LC/UV method to measure both products in plasma, it was found to also be rapidly converted to LY223592, which showed very similar activity and toxicity to LY207702 *in vivo*. In fact, a 20 mg/kg IV dose of LY207702 gave the same plasma levels of LY223592 as those observed after a 20 mg/kg dose of LY223592 itself. A number of similar purine analogs which exhibited good antitumor activity were then found to proceed through biotransformation pathways involving ADA, eventually forming LY223592 in those cases too. Also, one analog with no substituent at the 6-position but with good anti-tumor activity was found to nonetheless be metabolized to LY223592, apparently by oxidation with xanthine oxidase. In summary, a wide range of purine analogs were prepared in an attempt to retain antitumor activity and eliminate the cardiotoxicity seen with LY223592, but all those found to be active were also found to be metabolized to LY223592 or a similar guanosine derivative.

1394 URINARY METABOLITE ASSESSMENT IN F344 RATS FOLLOWING EXPOSURE TO α-METHYLSTYRENE

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Insufficient toxicity and toxicokinetic data are available to assess the health risks associated with inhalation exposure to α-methylstyrene (AMS), a phenylpropylene monomer used to give improved properties to plastics and resins. As part of a series of inhalation toxicity studies of AMS in rats and mice, biotransformation was also investigated to allow correlation with toxicity data and comparison to styrene metabolism. In the current study, male F344 rats (8 weeks old) were exposed to 0 or 500 ppm AMS for two weeks (6 hr/d, 5 d/wk). Following the eighth exposure, urine was collected from individual rats for approximately 15 hr. Urinary metabolites were determined using an ion pairing HPLC method, which has been reported to allow simultaneous determination of styrene metabolites (including mercapturic acids). Using this method and available chemical standards, the only quantifiable metabolite of AMS was atrolactic acid (2-phenyl-2-hydroxypropionic acid), the methyl analogue of mandelic acid. The total atrolactic acid collected was 90.5 ± 28.3 mg/kg body weight (mean ± s.d., n = 6). This acid was not detected in the urine of control rats. Hippuric acid was present in the urine of control rats and did not appear to be increased by AMS exposure. The presence of atrolactic acid, the expected product of epoxide formation followed by glycol production via hydrolase activity, suggests that an ethylene group derived epoxide, analogous to that formed from styrene, could be the initial oxidative metabolite of AMS.

1395 KINETICS OF *IN VITRO* CYP1A2 AND 3A4-MEDIATED AFLATOXIN B_1 OXIDATION IN HUMAN LIVER

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Previous studies have shown that two enzymes (CYP1A2 and 3A4) are primarily responsible for producing the mutagenic epoxide AFBO from AFB_1 in human liver microsomes (HLMs), while the AFB_1 detoxification products AFM_1 and AFQ_1 are produced by single enzymes (CYP1A2 and 3A4, respectively). The present study analyzed the kinetics of AFB_1 oxidation in HLMs and in human CYP3A4 and CYP1A2 cDNA-expressed lymphoblastoid microsomes to identify the CYP isoform(s) responsible for AFB_1 oxidation at

low substrate concentrations. Inhibition of CYP1A2 by furafylline eliminated detectable AFBO formation at the lowest AFB_1 concentration analyzed (0.5 μM). CYP1A2 was the predominant catalyst of AFB_1 production in all three HLMs analyzed at substrate concentrations below 30 μM AFB_1, but produced less AFBO at higher substrate concentrations. Application of the Hill equation to the rate data in furafylline-pretreated microsomes provided an excellent fit to the data and predicted that 99% of AFBO formation at 0.1 μM AFB_1 was contributed by CYP1A2. The mean apparent *Km* for CYP1A2-catalyzed AFBO formation in HLMs (38 ± 6 μM) was similar to the *Km* for AFBO formation in lymphoblastoid microsomes stably expressing human CYP1A2 cDNA (41 ± 4 μM). AFBO and AFQ_1 formation in CYP3A4 cDNA-expressed microsomes obeyed sigmoidal, rather than Michaelis-Menten, kinetics indicating that AFB_1 was capable of allosterically activating CYP3A4 at high *in vitro* substrate concentrations. Based upon these *in vitro* studies, CYP1A2 appears to be the primary catalyst for AFB_1 epoxidation in human liver at AFB_1 concentrations approaching those encountered in the diet. (Supported by NIH grants T32-ES07032, ES-05780, GM-47850, and GM-32165).

1396 *IN VIVO* AND *IN VITRO* METABOLISM OF [^{14}C]-DICHLOROACETIC ACID (DCA) IN THE MALE SPRAGUE-DAWLEY RAT

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DCA is an investigational drug for treatment of lactic acidosis in humans. In rodents, DCA can cause peroxisome proliferation and hepatocarcinogenicity. The pathways of metabolism of DCA are still poorly understood. We examined the in vitro metabolism and the in vivo fate of sodium [1-^{14}C]-DCA in rats. Groups of untreated rats or rats that had received 50 mg/kg unlabeled NaDCA 24 hrs previously were orally dosed with 50 mg/kg + 360 μCi/kg sodium [1-^{14}C]-DCA. Each [^{14}C]-DCA-dosed rat was kept in a glass metabolism cage and expired air, urine and feces were collected for 24 hrs and monitored for [^{14}C]. After 24 hrs, the distribution of [^{14}C] in rat tissues was determined by complete oxidation and scintillation counting. Urine was analysed by C_{18} reverse-phase HPLC with an ion-pair-containing-mobile phase. The distribution and elimination of ^{14}C was different ($p < 0.05$) in rats treated with two doses of DCA, compared with singly-dosed rats. The major route of excretion of DCA was in expired air as CO_2 (36.8 ± 1.2% dose, mean ± S.D., n = 5, for single dose and 43.6 ± 3.0%, n = 3, for repeat dose in 24 hrs). The 24 hr urine contained DCA and several metabolites (9.3 ± 0.6% dose for single dose and 15.2 ± 3.6% for repeat dose rats). More unchanged DCA was excreted in the urine of rats pretreated with DCA (3.3% dose versus 0.7% for single dose rats). Separate in vitro studies with microsomes from phenobarbital-induced rats failed to demonstrate NADPH-dependent monooxygenation or UDPGA-dependent glucuronidation of DCA. GSH and cytosol-dependent metabolism of DCA was demonstrated. In summary, this study showed that in rats, as in humans, one dose of DCA altered the elimination of a subsequent DCA dose. DCA was extensively metabolized by rats and one pathway was GSH-dependent.

1397 EFFECTS OF CHRONIC, LOW DOSE BENZENE ON LIVER ENZYMES INVOLVED IN BENZENE METABOLISM

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The bioactivation of benzene is believed to be required for the haematopoietic and carcinogenic effects of this prevalent environmental pollutant. Short-term, high dose benzene exposures have been reported to induce glutathione transferase (GST) and cytochrome P450 activities. However, little is known about the effects of chronic, low dose situations, such as those in occupational exposures, on the phase I enzyme (cytochrome P450) or the many phase II enzymes (GST, AIDH, UDPG and sulfotransferases) involved in benzene metabolism. We have recently found that hepatic GST activity was induced following a 6 week oral exposure to 1250 μg/day benzene. Our present objective was to extend this observation by determining if chronic benzene exposure alters the hepatic activity of aldehyde dehydrogenase (AIDH) towards muconaldehyde (MUC), an open-ring metabolite of benzene, or the activity of CYP-2E1. We exposed female CD-1 mice to the corn oil vehicle or benzene at 125 or 1250 μg/day, 5 days/week for 3 weeks by gavage. We found that GST activity toward CDNB and DCNB was increased by 1.2 and 1.4 fold, respectively, only in the 1250 μg/day group, while no changes were evident in CYP-2E1 or AIDH activity towards MUC or hydroxynonenal, a toxic product of lipid peroxidation. Thus, chronic, low dose benzene exposure has no evident effect on CYP-2E1 activity but has the capacity to induce at least one of the phase II, detoxifying enzymes, namely GST. Supported by: NIEHS GRANT T32ES07254 and UTMB SMALL GRANT.

1398 METABOLISM OF ALACHLOR BY HUMAN NASAL TISSUE

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Chronic feeding of alachlor results in the induction of nasal tumors in the Long-Evans rat, but not in the CD-1 mouse. The proposed mechanism for the nasal tumor formation by alachlor in the Long-Evans rat involves the metabolism of alachlor to the putative toxic metabolite 3,5-diethylbenzoquinone-4-imine (DEIQ). We have previously demonstrated significant species differences in the in vitro metabolism of alachlor and several alachlor metabolites (i.e. alachlor sec. sulfide and amide and diethylaniline) to DEIQ by rat, mouse and monkey nasal tissue. To determine the potential of human nasal tissue to form the DEIQ metabolite, we have studied the in vitro metabolism of alachlor and the selected metabolites to DEIQ by human nasal tissue. Human nasal tissue was shown to have a very low metabolic capability to convert alachlor and the selected metabolites to DEIQ. For example the metabolism of alachlor sec. sulphide was 750-fold less than with the rat and the metabolism of alachlor was over 20,000-fold less than with the rat. These results demonstrate that the potential for DEIQ formation in human nasal tissue is negligible. Furthermore, these and other results continue to support the view that the carcinogenicity seen in the rat as a result of chronic administration of alachlor is not relevant to humans.

1399 THE ROLE OF CYTOCHROME P450 1A1, 1A2, 3A4, 2D6 AND 2E1 IN THE OXIDATION OF CHLORINATED BENZENES

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The oxidation of chlorinated benzenes to their phenolic metabolites is a crucial step in their biotransformation, related to toxicity. The rate of phenol formation was investigated with microsomal preparations derived from cell lines, transfected with cDNSs encoding the human P450 isoenzymes 1A1, 1A2, 3A4, 2E1 or 2D6. Incubations were performed with 1,2- and 1,4-dichlorobenzene, 1,2,4-trichlorobenzene, 1,2,3,5-tetrachlorobenzene and pentachlorobenzene. In addition, the metabolism of 1,2-DCB and 1,2,4-TCB by 22 human liver microsomes as correlated with the O-dealkylation of 7-ethoxyresorufin (CYPIA), and the 6-hydroxylation of testosterone (CYP3A4) and chlorzoxazone (CYP2E1). CYP2E1 showed the highest activity towards all chlorinated benzenes, followed by CYP1A1/1A2 (1,2-DCB, 1,4-DCB, 1,2,4-TCB) and CYP3A4 (1,2,3,5-TeCB, PCB). Especially for the lower chlorinated benzenes. CYP2E1 was the most active isoenzyme. The 3A4/2E1-ratio with respect to the oxidation of chlorinated benzenes increased from 0.06 (1,2,4-TCB) to 0.6 (PCB). Incubations with liver microsomes and 1,2-DCB, showed a highly significant correlation with the 6-hydroxylation of chlorzoxazone. The metabolism of 1,2,4-TCB was both correlated with chlorzoxazone and testosterone hydroxylation. It was concluded that CYP2E1 is the major enzyme involved in the metabolism of 1,2-DCB and 1,4-DCB in human liver. Compared with CYP2E1, CYP3A4 showed an increasing activity towards chlorinated benzenes with increasing hydrophobicity. Because of its relatively high expression in human liver, the role of CYP3A4 towards chlorinated benzenes becomes more important with increasing number of chlorine atoms.

1400 TESTOSTERONE BIOTRANSFORMATION BY RAT LIVER MICROSOMES: DETECTION OF VARIOUS METABOLITES BY REVERSE-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

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Previous studies have demonstrated that microsomal enzymes contribute to anabolic steroid biotransformation. To evaluate the metabolism of steroids, an in vitro assay was developed which utilized hepatic microsomes from adult male rats and dogs. A liquid-liquid extraction, which produced neutral steroids free of acidic and basic compounds, was employed in order to increase testosterone and metabolite recovery. An HPLC system was interfaced to an on-line ultraviolet spectral detector and utilized an acidic aqueous/acetonitrile mobile phase. In this system, a characteristic steroid spectrum with a wavelength maximum band at 240 nm, allowed for the detection of testosterone metabolites. Metabolite formation was also investigated in both untreated and phenobarbital pretreated rats. Induction resulted in a two-fold increase in enzyme activity which enhanced the formation of testosterone metabolites. The developed

system can be used to determine the microsomal biotransformation profiles of steroids in various species.

1401 CHRONIC HIGH ARSENIC EXPOSURE ALTERS THE PROFILE OF URINARY ARSENIC METABOLITES IN HUMANS

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We have studied the urinary excretion pattern of arsenic (As) species in individuals chronically exposed to As *via* drinking water (400 μg/L), as compared with individuals exposed to 21 μgAs/L. The main findings of this study were significant increases in the relative proportion of inorganic As (IAs) and monomethylarsonic acid (MMA) to total As (TAs) excreted in urine (561 μgAs/gcreat), accompanied by significant decreases in that of dimethylarsinic acid (DMA) in the exposed individuals. These alterations caused a decrease in the MMA/IAs and DMA/MMA ratios, suggesting an impairment in the methylation of both IAs and MMA. Interestingly, exposed individuals suffering cutaneous signs of arsenicism showed significantly lower DMA/MMA values than the rest of exposed individuals. The relationships between the presence of cutaneous lesions and the changes in the ratios of As metabolites encourage further research on the relationship between As metabolism and overt signs of toxicity. (Supported by the Electric Power Research Institute, Palo Alto, CA).

1402 METABOLISM AND TOXICITY OF THE MAJOR METABOLITES OF 1,3-BUTADIENE IN FRESHLY ISOLATED RAT HEPATOCYTES

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BD is biotransformed to active metabolites, mainly in the liver, and these metabolites are thought to be responsible for the toxicity of BD. In an attempt to define metabolic mechanisms underlying the toxicity of BD, we have examined the metabolism and cytotoxicity of its major metabolites in isolated rat hepatocytes. The compounds used were the previously identified major metabolites of BD including 1,2-epoxy-3-butene (BMO), butadiene bisoxide (BBO), 3-butene-1,2-diol (diol), and 3,4-epoxybutane-1,2-diol (ED). 1,2-dichloro-3,4-epoxybutane (BCE), formed under physiological conditions as a solvolysis product of BMO, was also used. The compounds induced cytotoxicity, as defined by trypan blue exclusion, in the following order; BBO > BMO ≅ BCE > diol ≅ ED. Depletion of cellular reduced glutathione (GSH) occurred prior to toxicity and no generation of oxidized glutathione (GSSG) was observed. GSH analysis (HPLC) also detected the presence of additional products formed during BBO and BCE metabolism which may be GSH adducts. Metabolism studies, using GC analysis to detect products, showed that BD is converted to BMO in hepatocytes. Using BMO as a substrate the major product formed was diol. In summary, we have examined the metabolism of the biotransformation products of BD and characterized their GSH depletion and cytotoxicity profiles in freshly isolated rat hepatocytes. This will allow us to define metabolic mechanisms underlying the toxicity of BD. Supported by the Chemical Manufacturers Association.

1403 LINOLEATE DEPENDENT CO-OXYGENATION OF t-RETINOL ACETATE BY SOYBEAN LIPOXYGENASE (SLO) AND HUMAN TERM PLACENTAL LIPOXYGENASE (HTPLO)

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Several retinoids are potent teratogens in a number of animal species including human. Although, the importance of metabolism in retinoid toxicity has been stressed by many, no published data exist on the biotransformation of retinoids by human conceptal tissues. Here, we report linoleic acid (LA) dependent co-oxidation of t-retinol acetate (t-RAc) by SLO and HTPLO. Under optimal assay conditions, SLO exhibited a specific activity of 850 nmoles/min/nmole of enzyme. To observe the maximum rate of co-oxidation by HTPLO (375 nmoles/min/mg protein), incubation of 100 μM t-RAc, 2 mM LA and 50 μg/ml enzyme in Tris buffer pH 9.0 was essential. Both SLO and HTPLO catalyzed reactions were significantly inhibited by NDGA, BHT, BHA, gossypol and ETI. The results suggest that t-RAc is oxidatively metabolized in human term placenta via LO pathway and this may constitute an important part of puzzle worthy of consideration in the understanding of retinoid teratogenicity.

1404 LIPOXYGENASE (LO) MEDIATED METABOLISM OF ACRYLONITRILE (ACN) IN HUMAN LUNGS

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Increased incidence of lung tumors in workers exposed to ACN necessitates full understanding of its metabolism in human lung tissue. Here, we report linoleic acid (LA) dependent co-oxidation of ACN catalyzed by LO purified by Concanavalin A affinity chromatography from human lungs. LO converted ACN to a metabolite which reacted with p-nitrobenzyl pyridine to yield a purple adduct exhibiting a spectral peak at 560 nm suggesting the formation of 2-cyanoethylene oxide (CEO). ACN metabolism was dependent on concentrations of LA, ACN and the enzyme. The reaction was linear with time for approximately 30 – 45 min. The pH optimum for the reaction was 7.4. In the absence of the enzyme or in the presence of boiled enzyme, there was no appreciable ACN metabolism. The enzymatic ACN metabolism was inhibited by micromolar concentrations of classical LO inhibitors like nordihydroguaretic acid and 5,8,11-eicosatriynoic acid and by antioxidants BHT and BHA. Other polyunsaturated fatty acids e.g. arachidonic acid and γ-linolenic acid also supported ACN metabolism. These results suggest that LO purified from human lung cytosol is capable of ACN epoxidation to CEO.

1405 METABOLISM OF TRICHLOROETHANOL AND CHLORAL HYDRATE BY MOUSE LIVER SLICES

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Chloral hydrate (CH) and trichloroethanol (TCOH) are two major metabolites of trichloroethylene (TCE). TCE is a common ground water contaminant which induces hepatocellular carcinomas in rodents. In order to more clearly define the harmful effects of TCE exposure, its metabolites were examined. TCE is metabolized to form chloral hydrate through a P450 mediated metabolic pathway. Chloral hydrate is further metabolized to trichloroacetic acid (TCA), dichloroacetic acid (DCA), trichloroethanol (TCOH), and trichloroethanol-glucuruonide (TCOG). Liver slices were obtained from B6C3F1 male mice or human donors and incubated in various concentrations of CH and TCOH. Samples were analyzed by gas chromatography. Degradation of CH and TCOH and the appearance of subsequent metabolites TCOG, TCA, and DCA, were observed. The inhibitory effects of galactosamine on the glucuronidation of TCOH were also determined. No DCA formation was detected from the metabolism of TCOH, CH, or TCA. Metabolic rates of CH and TCOH were calculated from the rate of loss of parent compound. Kinetic parameters will be incorporated in the liver compartment of a physiologically based pharmacokinetic (PB-PK) model for TCE metabolism.

1406 NON-ENZYMATIC AND ENZYMATIC ACTIVATION OF MITOMYCIN C

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Non-enzymatic and enzymatic activation of mitomycin C (MMC) was studied. Non-enzymatic activation of MMC and DNA binding could be observed in the presence of water alone. Interestingly, non-enzymatic binding of MMC was not detected with the individual nucleosides. Human cytochrome P450 reductase transiently expressed from its cDNA in COS1 cells metabolised MMC as evidenced by the oxidation of NADPH and the generation of DNA adducts and interstrand DNA cross-links. Enzymatic activation of MMC was in addition to the non-enzymatic activation and resulted in binding of reactive metabolites to the deoxyadenosine and deoxyguanosine and cross-linking of the DNA. Co-chromatography of the P450 reductase and sodium borohydride generated MMC-DNA adducts indicated that MMC was metabolised by P450 reductase to produce 2,7-diaminomitosene(s) exhibiting very high affinity for binding to deoxyguanosine. The COS1 cells cytosol containing very high levels of cDNA derived human NAD(P)H: quinone oxidoreductase1 (NQO1) activity did not metabolise MMC. The cytosols from HT29, Hep-G2 and H4II and mouse liver and colon tissues containing varying levels of endogenous NQO1 activities catalyzed the metabolism of MMC. However, a relationship between the amount of NQO1 activities among various cells and tissues and MMC reduction was not observed. In addition, mouse kidney which contained high level of endogenous NQO1 activity failed to metabolise MMC and induce cross-linking of the oligomers. Further analysis using NQO1 activity purified from mouse liver, colon and kidney cytosol did not support the involvement of dicoumarol inhibitable NQO1 in the metabolic activation of MMC.

1407 A NOVEL RING HYDROXYLATED METABOLITE OF NALIDIXIC ACID IN RATS AND MICE

R J Griffin, L T Burka. *NIEHS, Research Triangle Park, NC.* Sponsor: H Matthews

Nalidixic acid is a prescription drug used to treat urinary tract infections. The National Toxicology Program has determined that nalidixic acid is carcinogenic in rats but not mice following chronic administration. A comparative metabolism study with rats and mice was conducted to gain a better understanding of species differences in metabolism that might contribute to differences in carcinogenicity. Male B6C3F1 mice and F344 rats were dosed with ^{14}C-nalidixic acid (p.o.) at 500 mg/kg and placed in glass metabolism cages. Over a 48 hour period urine and feces were collected. Urinary and fecal metabolites were analyzed by reverse phase HPLC and quantified by radiochemical detection. Peaks were isolated by HPLC and identified by NMR and mass spectrometry. Various previously reported glucuronide metabolites of nalidixic acid, 7-hydroxynalidixic acid and carboxynalidixic acid were identified. Another metabolite, present in large amounts in mouse urine and in much smaller amounts in rat urine was identified as a ring hydroxylated glucuronide. ^{1}H-NMR indicated only one aromatic proton and the FAB negative ion mass spectrum contained pseudomolecular ions at 423 (M-H) and 445 (M-H+Na). The data best fits 6-hydroxynalidixic acid glucuronide which has not been previously reported. The potential production of this metabolite in man and its relevance to the tumorigenicity of nalidixic acid remains to be determined.

1408 A SPECIES COMPARISON OF THE METABOLISM OF CHLORAL HYDRATE TO TRICHLOROETHANOL *IN VITRO*

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Trichloroethylene (TCE) has been widely used as an industrial degreasing solvent and consequently has become a common groundwater contaminant. TCE metabolism initially yields chloral hydrate (CH), a common human sedative agent, which is further metabolized to trichloroacetate (TCA) and trichloroethanol (TCOH). TCA has been shown to induce hepatocarcinomas in rodents. TCOH is of concern due to possible enterohepatic recirculation which may allow for the later formation of TCA. TCOH formation from chloral hydrate at pH 7.4 was measured by incubating rodent hepatic cytosol with limiting concentrations of CH at 37° C for 5 min. TCOH was extracted in ethyl acetate, analyzed from headspace by electron capture gas chromatography and quantified vs. an external standard curve of authentic TCOH. The Michaelis-Menten parameters, Km and Vmax, were determined from the data. Initial results reveal a Km of 52 and 33 μM and Vmax of 0.8 and 3.6 nmol/min/mg for the rats and mouse respectively, and that the formation of TCOH and TCA may be linked by exchange of oxidized/reduced cofactor. Information regarding rates of formation for relevant metabolites will allow a more accurate description of the pathway across species to be constructed. This will allow a more precise estimation of human risk due to the formation of carcinogenic metabolites. Funded by the Strategic Environmental Restoration Defense Program.

1409 ALKYLATION POTENTIAL AND METABOLISM OF 3,4-DICHLORO-1-BUTENE

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The alkylation potential of 3,4-dichloro-1-butene (3,4-DCB) was investigated by measuring the rate of reaction with 4-nitrobenzylpyridine (NBP). No reaction of 3,4-DCB with NBP was detected. Therefore, 3,4-DCB is unlikely to be a direct alkylating agent. The *in vitro* metabolism of 3,4-DCB with glutathione (GSH) was investigated with rat liver glutathione-S-transferase. No reaction was detected. *In vivo* metabolism was investigated by dosing rats i.p. with 400 – 500 mg/kg 3,4-DCB in corn oil. Bile and urine were collected 2, 4, 8, 16 and 24 hr after dosing. Analysis of the bile by LC/MS/MS revealed a metabolite identified to be a GSH conjugate of an epoxide intermediate. The metabolite was detected in the 2 hr sample, was maximal at 4 hr and then declined in magnitude over the next 20 hr. The parent ion was M + H = 412. The mercapturic acid analog of this GSH conjugate (M + H = 268) was found in the urine. The intermediate GSH-related derivatives (M + H = 283 and 226) were also identified in the bile of dosed rats. Mass spectral analysis suggests that 3,4-DCB was metabolized initially to an epoxide at the 1,2 position, followed by GSH conjugation of the epoxide moiety. Upon conjugation with GSH, the epoxide shifted to the 2,3 position releasing a chlorine atom. The resulting 2,3-epoxide was stable enough to be excreted in the urine as a mercapturic acid. Consistent with the *in vitro* data, no GSH conjugates of the chlorine atoms at the 3 or 4 positions were found in the bile or urine, indicating that potentially nephrotoxic episulfonium ions are not likely to be formed with this compound.

1410 DIFFERENCES IN HEPATIC CYTOCHROME P450-MEDIATED METABOLISM OF PARATHION AND CHLORPYRIFOS IN ADULT AND JUVENILE MALE RATS

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Quantitative and qualitative differences between cytochromes P450 are recognized in adults and neonates, with the enzyme composition changing with maturation. The developmental changes in the rates of *in vitro* activation (desulfuration) and detoxication (dearylation) of the phosphorothionate insecticides parathion and chlorpyrifos, and ethoxyresorufin *O*-deethylase (EROD) activity were investigated in hepatic microsomes of rats of several ages. There were no significant differences in chlorpyrifos dearylation among any of the ages studied. Activity was 3.7-fold greater at 80 days compared to 3 days for chlorpyrifos desulfuration, 5.5-fold greater for parathion desulfuration, and 3.0 times greater for parathion dearylation. There were no differences among the intermediate ages studied (12, 21, and 33 days) for either the desulfuration or dearylation reactions. EROD activity also increased with age, peaked at 21 days, and then declined. These data support the suggestion that as the cytochrome P450 enzyme composition changes during maturation, the amounts of activation and detoxication will also change but *in vitro* activities alone do not explain the greater toxicity of these insecticides to juveniles compared to adults. (Supported by NIH RO1 ES04394.)

1411 METABOLISM OF TRIHALOACETATE IN MALE B6C3F1 MICE

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Trichloroacetate (TCA), dichloroacetate (DCA) and bromodichloroacetate (BDCA) are by-products of the chlorination of drinking water. TCA acts primarily as a peroxisome proliferator, but DCA produces tumors at doses less than required for peroxisome proliferation. BDCA does not induce peroxisome proliferation even at high doses. This study attempts to determine whether differences in the metabolism of the trihaloacetates (THA) account for their differing toxicological properties. Studies were performed in male B6C3F1 mice given [$^{14}C_{1,2}$]-TCA, [$^{14}C_{1}$]-BDCA and [$^{14}C_{1,2}$]-DCA by gavage. The replacement of a Cl by a Br greatly enhances THA metabolism. Much less radiolabel from BDCA is retained in the carcass after 24 hours than from TCA. Radiolabel from BDCA is largely found in the urine, with oxalate being the major metabolite. TCA is largely eliminated unchanged in the urine. There are dose-related changes in the rate of CO_2 production from BDCA. The initial rate of CO_2 production is reduced from 4.1 ± 0.3 hour at 5 and 20 mg/kg, to 2.7 ± 0.6/hour at 100 mg/kg, but the net conversion to CO_2 in 24 hours is greater at the highest dose. The low activity of BDCA as a peroxisome proliferator appears to be related to its more rapid metabolism. This indicates that TCA rather than a metabolite is responsible for peroxisome proliferation. (NIEHS R01-ESO4648).

1412 COMPARISON OF PULMONARY AND HEPATIC MICROSOMAL METABOLISM OF BENZENE IN RATS

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Benzene is a widely used chemical with significant numbers of people exposed to low levels. The major concern is its bioactivation to metabolites responsible for its toxicity, primarily to bone marrow cells. While benzene metabolism has been studied extensively in liver, little is known about its metabolism in lung. The objective of this study was to compare the role of lung to that of liver in the microsomal metabolism of benzene (17.8 μM). ^{14}C-Benzene metabolites phenol, trihydroxybenzene, hydroquinone and catechol were separated by HPLC and quantified by liquid scintillation counting. As expected, phenol was the major hepatic metabolite. While the pulmonary metabolism of benzene to phenol was much less than in liver, on a per mg protein basis the formation of hydroquinone by lung was as great or greater than that by liver. Benzene (600 mg/kg/d × 3d, ip) did not induce its own metabolism in either tissue whereas pyridine (200 mg/kg, ip) induced benzene metabolism in liver but not lung. The CYP2E1 inhibitor diethyldithiocarbamate (300 μM) greatly inhibited the metabolism of benzene in the livers of both control and pyridine-induced rats

but was less effective in the lungs indicating that CYP2E1 is the major P450 isozyme involved but that other isozymes also play a role in the pulmonary metabolism of benzene. (Supported in part by NIH grant ES04362.)

1413 CYANOHYDROXYBUTENE, A CRUCIFEROUS NITRILE, INDUCES HEPATIC AND PANCREATIC GLUTATHIONE-S-TRANSFERASES IN RATS

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Glucosinolate derivatives from cruciferous plants have been proposed as chemoprotectants through their inductive effects on phase I and/or phase II detoxifying enzymes. Concurrent work has shown that the nitrile, 1-cyano-2-hydroxy-3-butene (CHB), is a monofunctional inducer of phase II enzymes in liver. We have further characterized the effect of CHB on glutathione-*S*-transferase (GST) activity and isoenzyme content. Adult male F344 rats (4/group) were gavaged once daily with a minimally toxic dose of CHB (100 mg/kg body wt) in corn oil or with corn oil alone for 7 days. Hepatic GST activities in treated animals toward 1-chloro-2,4-dinitrobenzene (CDNB), 1,2-epoxy-3-(*p*-nitrophenoxy)-propane, and *trans*-4-phenylbut-3-en-2-one were significantly elevated 1.7 fold, 2.1 fold, and 1.6 fold, respectively, in comparison to controls. Pancreatic GST activity towards CDNB was significantly elevated 2.7 fold. Cytosols were applied to *S*-hexylglutathione-Sepharose affinity columns, and isoenzyme subunits in eluted fractions were separated and semi-quantified by reverse-phase HPLC. Hepatic GST subunit concentrations paralleled the increased activities observed with the various substrates; both mu and alpha subunits were significantly induced nearly 2 fold. Despite affinity column inefficiencies, there was also an elevation of pancreatic isoenzyme subunits with a significant induction of the most preponderant subunit (RT equal to that of subunit 2) and a trend for induction of some of the minor GST subunits. These results suggest that a single mechanism for induction of all GST isoenzymes by CHB is present in a variety of tissues.

1414 METHADONE INHIBITS ZIDOVUDINE GLUCURONIDATION IN CULTURED HUMAN HEPATOCYTES

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Long-term methadone maintenance treatment is the most prevalent form of opiate addiction treatment among HIV seropositive drug users. Recent studies have demonstrated that methadone in combination with zidovudine (AZT) resulted in a 43% increase in the AZT AUC 14 days after therapy, however the basis for the effect is not clear. The present study evaluated the effect of methadone on AZT metabolism using primary cultures of human liver hepatocytes. Cells were exposed to 5.0 μM methadone for 72 hours during which time the media was replaced every 24 hours with fresh media containing drug. At 72 hr, the media was replaced twice at 1 hr intervals with fresh media containing no drug, AZT was then added at a final concentration of 10 μM. The extracellular compartment was analyzed at 8, 24 and 48 hr post AZT addition. The 5'-O-glucuronide of AZT, GAZT, represented 63.34 ± 1.60% of the total radioactivity in control cultures. In cultures pre-exposed to methadone, GAZT represented only 45.10 ± 1.84% of the radioactivity. This would suggest that prolonged exposure to methadone may affect the regulation of UDP-glucuronosyltransferase 2, the enzyme responsible for GAZT formation. Hence, long-term methadone treatment in combination with AZT therapy may potentially increase AZT induced side-effects by decreasing its elimination through glucuronidation. Further, enhanced formation of the toxic metabolite 3'-amino-3'-deoxythymidine probably due to increased AZT concentrations was also observed.

1415 TISSUE-DEPENDENT EXPRESSION OF FLAVIN MONOOXYGENASE (FMO) FORM1 AND FORM2 IN FETAL AND NEONATAL RABBIT

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FMOs comprise an important multi-gene family of enzymes that catalyze the oxidation of many xenobiotic compounds. There is evidence that mammalian isoforms 1 and 2 are expressed in different tissues as a function of development and/or hormonal manipulation. This study examined the relationships between mRNA levels, protein levels and catalytic activity of these distinct isoforms from day 25 of gestation through 21 days post-partum in rabbit liver and lung. Form-specific digoxigenin-labeled cDNA probes utilized for Northern and slot blot analyses provided both qualitative and quantitative profiles of steady-state mRNA levels. FMO protein levels were analyzed by western blotting. Catalytic activites were measured by S-oxidation of substituted thiocarbamides. Analysis of liver tissue showed a gradual rise in FMO1 levels for all parameters measured. In fetal lung, FMO2 levels were 60% of adult at the 25th day of gestation and gradually increased until parturition, with the notable exception of the 28th day, which was very low. Following parturition, FMO2 levels in lung rose dramatically and were 90% of adult levels in the oldest neonate examined (21 days). In both liver and lung a high degree of individual variation was observed. The early developmental appearance of FMO in rabbit suggests a significant role in the metabolism and subsequent target organ toxicity of xenobiotic compounds through fetal and neonatal exposures. Supported by PHS HL 38650.

1416 SUBSTRATE REACTIVITY OF FOUR EXPRESSED HUMAN CYTOSOLIC SULFOTRANSFERASES

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Conjugation of steroids, therapeutic drugs and xenobiotics with sulfate is an important metabolic process in human tissues. To characterize the kinetic properties of the individual isoforms of human cytosolic ST, our laboratory has cloned and expressed four human cytosolic STs in *E. coli*: the phenol-sulfating form of phenol ST (P-PST), the monoamine-sulfating form of PST (M-PST), estrogen-ST (EST) and dehydroepiandrosterone ST (DHEA-ST). For expression in bacteria, the human ST cDNAs were subcloned into the bacterial expression vector pKK233-2 which contains an IPTG inducible P_{trc} (trp/lac fusion) promotor and expressed in *E. coli* XL-1 Blue cells. For expression, the bacterial cells were grown to an $O\text{-}D_{\cdot 500nm}$ of 0.5 and induced with IPTG (0.3 mM) for 2 hours. The cytosolic fraction was isolated and the expressed STs were partially purified by DEAE-Sepharose CL-6B chromatography prior to kinetic characterization. The kinetic properties of the expressed STs were very similar to those of the enzymes purified from human tissues. The substrate reactivities of the purified expressed STs were examined using a variety of steroids, therapeutic drugs and xenobiotics as substrates. Expression of the human STs in *E. coli* provides a convenient method for obtaining the individual enzymes as well as an excellent system for screening the activity and properties of site-directed mutants. [Supported by NIH grant GM38953].

1417 THE EFFECT OF BIOACTIVE GLUCOSINOLATE BREAKDOWN PRODUCTS ON RAT LIVER GLUTATHIONE (GSH) AND GSH REDUCTASE ACTIVITY

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The mechanism whereby cruciferous vegetables appear to protect against carcinogenesis is not known. A number of bioactive crucifer components have been identified that alter drug metabolizing enzyme activity, but not all have been evaluated for their effects on the cellular system that protects against oxidative damage. We have evaluated the effect of four cruciferous components known to increase drug metabolizing enzymes on hepatic GSH and glutathione reductase (GR): Indole-3-carbinol (I3C), Cyanohydroxybutene (CHB), Iberin and Phenylethyl isothiocyanate (PEC). Doses were chosen which represent the relative proportions of these four agents in Brussels sprouts. Adult male F344 rats (6/group) received a semi-purified diet (AIN 76B-40) and distilled water *ad libitum*, and either corn oil alone (vehicle control), one of the agents: I3C, 56 mg/kg; Iberin, 38 mg/kg; PEC, 0.1 mg/kg; or CHB, 50 mg/kg; or *all* of the agents at the doses shown (as a mixture) given by gavage daily for 7 days. At these doses, hepatic GSH was significantly elevated in animals receiving CHB (182% of control), PEC (156%) and the mix (204%). GR activity was significantly elevated in animals receiving CHB (128%) and the mix (156%). Livers from animals receiving the mix showed increases in GSH that are accounted for by the sum of the effects of individual agents. However, increases in GR activity seen in livers from rats receiving the mix were significantly greater than could be accounted for by the effect of CHB alone. We conclude that even though CHB elevates hepatic GR activity individually, this proportional mixture of CHB with other bioactive glucosinolate breakdown products from cruciferous vegetables has a synergistic effect on increasing GR activity.

1418 RELATIVE CONTRIBUTION OF FOUR GLUCOSINOLATE BREAKDOWN PRODUCTS FROM BRUSSELS SPROUTS TO MONOFUNCTIONAL AND BIFUNCTIONAL INDUCTION OF DRUG METABOLIZING ENZYMES IN RAT LIVER

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Crucifers have been observed to have chemoprotective effects. One proposed mechanism of chemoprotection is that crucifer-specific glucosinolate breakdown products induce hepatic phase I and/or phase II detoxification enzymes. Four of these compounds, indole-3-carbinol (I3C), iberin (IBN), phenylethyl isothiocyanate (PEC), and cyano-hydroxybutene (CHB) are all known to alter drug metabolizing enzymes individually. Here we report the relative contribution of each to the effect of a mixture chosen to represent their relative proportions in Brussels sprouts. Adult male F344 rats (6/group) received a semi-purified diet (AIN 76B-40) and distilled water *ad libitum*, and either corn oil alone (vehicle control), one of the compounds (56 mg/kg I3C, 38 mg/kg IBN, 0.1 mg/kg PEC or 50 mg/kg CHB), or *all* of the compounds at the doses shown (as a mixture), by gavage daily for 7 days. There was a 10-fold induction of hepatic cytochrome P4501A1 and a 3-fold induction of cytochrome P4501A2 activities in both the mixture and the I3C groups, but no induction in the other groups. Glutathione S-transferase activity towards CDNB was induced 1.5-fold in the I3C and CHB groups and 2.5-fold in the mixture groups, suggesting a slightly more than additive effect. Quinone reductase was induced 3- and 2-fold in the I3C and CHB groups, respectively, but 7-fold in the mixture group, indicating a considerably greater than additive effect. No differences were observed between IBN, PEC, or pair-fed groups and *ad libitum* controls for any of the enzymes measured. These data suggest that at the relative doses found in Brussels sprouts, only I3C is bifunctional, responsible for both P450 induction and a portion of phase II enzyme induction by the mixture, while CHB is monofunctional, responsible for a portion of the phase II enzyme induction. A possible synergistic effect on phase II enzyme induction by IBN or PEC cannot be dismissed.

1419 PURIFICATION OF TWO RAT SERUM ENZYMES WITH A-ESTERASE ACTIVITY

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Phosphorothionate insecticide active metabolites can be detoxified via A-esterase hydrolysis. Two enzymes with A-esterase activity toward paraoxon and chlorpyrifos-oxon have been purified from rat serum. Whole serum was dialyzed against 0.05 M Tris buffer (pH 7.76 at 5°C) containing 1 mM $CaCl_2$ using a 100 kDa molecular weight exclusion limit membrane. The serum fraction was then applied to DEAE Sepharose Fast Flow gel and incubated 1 hr. The residual liquid was decanted and found to contain no A-esterase. Displacement of A-esterase from DEAE gel was achieved with 1.25 M NaCl in Tris buffer. Following desalting and concentration, further separation was achieved by preparative scale isoelectric focusing. Final preparations contained two separate proteins of similar molecular weight (greater than 100 kDa) according to Coomassie stained SDS-PAGE gels. Apparent pI range for the two enzymes was 4.5–6.5. Following native PAGE analysis, activity stains with β-naphthyl acetate and Fast Garnet GBC in the presence of paraoxon (10^{-5} M) verified that A-esterase activity was associated with both protein bands. Spectrophotometric assay detected A-esterase activity toward both paraoxon and chlorpyrifos-oxon in the final preparation. (Supported by NIH ESO4394.)

1420 KINETIC AND STEREOCHEMICAL DIFFERENCES IN BUTADIENE MONOXIDE HYDROLYSIS BY RAT AND MOUSE LIVER MICROSOMES

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The rate of hydrolysis of racemic butadiene monoxide by rat and mouse liver microsomes was examined by following appearance of 3-butene-1,2-diol. Quenching of the incubations with excess sodium metaperiodate instantaneously oxidized the diol formed to acrolein which was quantitated by hplc. The study revealed a Km of 0.68 ± 0.18 mM and Vmax 13.5 ± 1.2 nmol/mg of protein/minute for mouse liver microsomes and a Km 1.29 ± 0.16 mM and Vmax 35.9 ± 1.7 nmol/mg of protein/minute for rat liver microsomes. The V/K ratio for rat liver microsomes was 1.39-fold greater than that of mouse. In an investigation of stereochemical outcome of epoxide hydrolysis, rat and mouse liver microsomes were exposed to enantiomers of butadiene monoxide generated by treatment of (R)- or (S)-1-tosyloxy-3-buten-2-ol with sodium hydride in dimethylsulfoxide. The diol formed was extracted from the incubation mixture with ethyl acetate, converted to the corresponding bis-acetate, and the enantiomeric distribution determined by gas chromatographic analysis using a persilated b-cyclodextrin column. With either enantiomer of monoxide, hydrolysis by rat liver microsomes proceeded with 99% retention of configuration. However hydrolysis by mouse liver microsomes proceeded with 15% (S) to 30 (R)% inversion of configuration. These findings may be of potential importance in the differential toxicity of butadiene in the two species.

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1421 DISTRIBUTION OF CYTOCHROME P450 ISOZYMES IN HUMAN LIVER SPECIMENS

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Variation in the levels of cytochrome P450 isozymes in the liver of humans is substantial and is largely responsible for interindividual differences in disposition and in responses to chemicals. The availability of a bank of well-characterized specimens would greatly increase the value of these human liver preparations for drug metabolism studies. Microsomes were prepared from 26 human liver specimens (16 male and 10 female), acquired from organ donors. Total P450 levels and 7-ethoxycoumarin O-deethylase activities (ECOD) were measured. CYP1A CYP2E1, and CYP3A4 were estimated by Western blotting using polyclonal antibodies to rat P450s that cross-react with the human proteins. S-Mephenytoin 4′-hydroxylase (CYP2C19) and sparteine oxidase (CYP2D6) are also being assayed to detect specimens deficient in these polymorphic enzymes. Total P450 levels varied about 3-fold for all 26 specimens, and ECOD varied 4.5-fold with a mean activity of 285 ± 122 pmol/mg/min. Using cells that express a single form of P450 as standards (GENTEST Corp.), the amounts of CYP1A, CYP2E1, and CYP3A4 in each microsome preparation was estimated to be (mean ± SD): 28.4 ± 11.4, 69.5 ± 18.7, and 72.6 ± 37.8 pmol/mg, respectively. The greatest variation among the specimens was in CYP3A4 levels which varied from 19.6 to 148 pmol/mg (approximately 7.5-fold). There were no significant differences detected between male and female specimens for any of the assays, and no correlations found between P450 levels or activities and donor age. Previous drug exposures did seem to alter donor liver P450 levels as expected; e.g. the highest ECOD activity was found in microsomes from a donor treated with phenobarbital for 6 months prior to death and the lowest level of CYP3A4 was measured in microsomes from a patient treated chronically with corticosteroids. These characterized human liver microsomes allow screening of new chemicals for the range of activities likely to be encountered in the human population and preliminary identification of the isozyme involved in metabolism.

1422 SPECIES DIFFERENCES IN TOXICITY OF A HUMAN ELASTASE INHIBITOR

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WIN 63759 selectively inhibits *in vitro* human leukocyte elastase, an enzyme implicated in the pathogenesis of emphysema and cystic fibrosis. In preclinical studies minimal hepatotoxicity occurred at the highest tested oral doses in dogs (300 mg/kg bid) but not rats (500 mg/kg bid) after 2 weeks of daily dosing. However in 28 day studies hepatotoxicity, indicated by jaundice, hyperbilirubinemia, elevated liver enzyme levels and diffuse hepatocellular swelling with vacuolization, was seen in dogs following dosages at and above 30 mg/kg bid. Electron microscopic evaluation of hepatocytes (6 mg/kg) showed marked SER proliferation with an increase in lysosomes and membrane bound vacuoles. Only centrilobular hepatocellular hypertrophy was observed in rats with comparable toxicokinetic exposure. In both species WIN 63759 was extensively metabolised with a large first-pass effect. Two week metabolism studies in rats and dogs with 14C-WIN 63759 showed that the major metabolite in plasma was 4-isopropyl 6-methoxybenzisothiazalone (BIT), a product of esterase metabolism. Interestingly, plasma did not reflect tissue metabolism. The major component in liver was a morpholine ring opened oxidative metabolite, which accumulated to high levels (110 ug/g in dog and 470 ug/g in rat following 30 mg/kg and 500 mg/kg bid dosing, respectively). This metabolite was also produced *in vitro* with liver S9 and hepatocyte fractions from both species and its production was shown to be inducible by drug. The pathogenesis of hepatotoxicity in the dog is consistent with a dose duration-related storage dysfunction, possibly resulting in accumulation of a drug metabolite in hepatocytes. The greater mass accumulation of the metabolite in rat liver, in the absence of hepatocellular swelling with vacuolation was at variance with results in dogs and will require further evaluation.

1423 AMEIVA EXSUL: A POTENTIAL SENTINEL MODEL OF ENVIRONMENTAL CONTAMINATION

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The ground lizard *Ameiva exsul* is indigenous to Puerto Rico. Changes in the hepatic biotransformation enzymes caused by chemicals are being studied to evaluate their use as biomarkers of exposure and the potential use of the lizard as a sentinel model to monitor bioavailable contaminants and the extent of exposure. Hepatic microsomal enzyme activity has been determined in male animals. Mean values for cytochrome b5 and P450 were 0.151 and 1.342 nmoles/mg protein, respectively. Mean activity values per mg of protein were: NADH cyt b5 reductase: 44.05 nmoles/min, NADPH cyt P450 reductase: 34.08 nmoles/min, erythromycin-N-demethylase: 0.018 nmoles/min, p-nitrophenol hydroxylase: 0.348 nmoles/min, ethoxyresorufin-O-deethylase (EROD): 0.776 pmoles/min, glutathione-S-transferase (GST): 488.30 nmoles/min (cytosolic fraction), no pentoxyresorufin-O-deethylase (PROD) activity was detected. These levels are comparable to those in fish. Preliminary data suggest presence of a protein with epitope region at 59 kDa as recognized by Anti 2E1 rat by Western Blot. Also cross-reaction of Cytochrome P450 isoenzyme with Anti 1A1 rat suggest that *Ameiva exsul* possesses the ability to metabolize aromatic hydrocarbons. Initial studies indicate that exposure to chemicals such as β-naphthoflavone induce the levels of P450IA1 and may be used as a marker of exposure.

1424 BIOTRANSFORMATION OF BENZO(a)PYRENE TO POLAR METABOLITES BY COAL GASIFICATION FUNGAL ISOLATES

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Several species of fungi were isolated from samples of contaminated soil from a coal gasification site in Dover, OH. In order to determine the utility of these organisms in bioremediation, these fungi were screened for their ability to metabolize benzo(a)pyrene (BaP) and carbazole to polar metabolites and CO_2. Fungal cultures were sustained in 20% Sabouraud dextrose broth in a shaking water bath. 0.1 μCi BaP or 0.1 μCi carbazole were added to the culture medium. Experiments indicate that, in general, 66–100% of BaP and 0–30% of carbazole is taken up by the fungal isolates in the first week. Over a period of 8–9 weeks, a pattern of uptake and release of radioactivity into the media was observed for certain isolates. There was no evidence of CO_2 evolution. The media from these isolates was extracted with ethyl acetate; HPLC data indicate that polar metabolites of BaP were being formed in the media, while polar metabolites were not observed for carbazole. Based on representative isolates, these fungi may more readily take up and metabolize polycyclic aromatic hydrocarbons than N-heterocyclic aromatic hydrocarbons.

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1425 FUNGAL TRANSFORMATIONS OF ANTIHISTAMINES: METABOLISM OF DIPHENHYDRAMINE (DPH) BY THE FUNGUS *CUNNINGHAMELLA ELEGANS*

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DPH, a widely used non-prescription antihistamine is metabolized by the fungus *Cunninghamella elegans* (ATCC 9245) to produce four metabolites. After 96 hours incubation, 10 mg of DPH was metabolized to approximately 0.3 mg DPH-N-oxide, 3.0 mg mono-N-demethylated DPH, 1.2 mg N-acetylated DPH, and 1.3 mg N-demethylated-N-acetylated DPH. The amount of unmetabolized DPH remaining was about 2.6 mg leaving about 1.6 mg of the initial amount added unaccounted for. These metabolites were isolated by high performance liquid chromatography and characterized by mass spectrometry and nuclear magnetic resonance spectroscopy. DPH-N-oxide and mono-N-demethylated DPH have been previously identified as mammalian DPH metabolites. This study indicates that *Cunninghamella elegans* ATCC 9245 could be used as a microbial model of the mammalian metabolism of DPH. In addition, the microbial system provides an alternative to organic synthesis for providing DPH metabolites.

1426 COMPARATIVE DISPOSITION AND BIOTRANSFORMATION OF NAPHTHALENE IN FRESH- AND SEAWATER ACCLIMATED STRIPED BASS (*MORONE SAXATILIS*)

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The striped bass population in the San Francisco Bay and delta region is declining. Exposure to crude oil and petroleum products, in combination with increased water salinity encountered during seaward migration, could be a contributing factor. Therefore, striped bass acclimated to either fresh- or seawater were individually exposed to a sublethal water concentration (30 μg/L) of a model petroleum-based hydrocarbon, [^{14}C]naphthalene. Exposure occurred in a closed, flow-through system for 24h, after which some fish were allowed an additional 24h clean water period to depurate accumulated residues. Residue disposition was determined by tissue dissection, digestion, and liquid scintillation counting (LSC). Large concentrations of residue were found in the liver and brain of seawater acclimated fish, while in freshwater acclimated fish, large concentrations were found in the viscera/gonad and liver. Depurated metabolites were identified by high-pressure liquid co-chromatography and quantified via fraction collection and LSC. Although very little (<1%) naphthalene was metabolized by freshwater acclimated bass, a substantial amount (11.7%) was metabolized by seawater acclimated bass. In both cases, the main metabolites were 1,2-dihydro-1,2-dihydroxynaphthalene and 1- and/or 2-naphthylsulfate. Increased oxidation of accumulated naphthalene, as observed in seawater acclimated bass, could indicate an increase in activation of the aromatic hydrocarbon via epoxidation. This, together with the large residue concentrations found in neural tissues, may explain the increased sensitivity of seawater acclimated striped bass to petroleum hydrocarbons.

1427 ONTOGENY OF RAT HEPATIC SULFOTRANSFERASE (ST) mRNAs

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Sulfotransferases are a group of enzymes that sulfate endogenous hormones as well as xenobiotics. The present study was designed to examine the developmental expression of ST genes in liver of male and female rats. The mRNA levels of ST1A1, ST1C1, ST1E2, ST20/21, ST40/41 and ST60 were determined by Northern- and dot-blot analyses in livers of 1-, 3-, 7-, 15-, 30-, 45-, 60- and 90-day-old Sprague-Dawley rats. The expression of ST mRNAs during development exhibited four distinct patterns. The first pattern was seen with ST1A1. It increased gradually after birth in both sexes until 30–45 days of age, and then declined. mRNA levels of ST1A1 were slightly higher in males than females. The second pattern was seen with ST1C1 and ST1E2. Both were low for the first 30 days, then increased rapidly in males to adult levels, but remained low in females. The third ST developmental pattern was seen with ST20/21 and ST60. The mRNAs were lower in males, except at day 30 when there was a transient increase of mRNA, which was not seen in females. The mRNAs in females increased at 45 days of age and remained high thereafter. The fourth developmental pattern was seen with ST40/41, where a gradual increase was observed during the first 15 days of life, then decreased to a low level in male rats and an intermediate level in females. These results indicate that the expression of various ST isoforms differ markedly with age and sex of rats, which is probably responsible for the age and sex differences in the sulfation and toxicity of some chemicals. (Supported by NIH Grant ES-03192.)

1428 FACTORS INFLUENCING THE CONVERSION OF TRICHLOROACETIC ACID TO DICHLOROACETIC ACID IN BIOLOGICAL MATRICES

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Trichloroethylene (TRI) is an environmental contaminant in ground water. Trichloroacetic acid (TCA) and dichloroacetic acid (DCA) have been identified as metabolites of TCE. Analytical methods for the analysis of these metabolites are important for conducting pharmacokinetic studies. The derivatization of TCA and DCA to their respective methyl esters requires acidic conditions. In developing methods for derivatization it was seen that conversion of TCA to DCA could occur in blood samples under acidic conditions. Studies were conducted to investigate this conversion. Blood was drawn directly from a B6C3F1 mouse and spiked with 50 ug/ml TCA and sulfuric acid. The results showed that TCA was converted to DCA. The range of conversion of TCA to DCA varied from 0 – 90%. The amount of conversion decreased with increase in length of time after the blood was taken from the animal. This artifact was not seen in samples which were frozen for twenty-four hours prior to the addition of acid. The results reported here show that it is critically important to evaluate the ability of metabolites to interconvert during analytical procedures, particularly when collecting data for pharmacokinetic analysis and assessing the metabolite(s) of TRI responsible for its carcinogenicity. (Supported by DoD Contract No. F33615-90-C-0532 and funded by Strategic Environmental Restoration Defense Program (SERDP)).

1429 IMMUNOLOCALIZATION OF CYTOCHROME P-4502B IN TYPE I ALVEOLAR CELLS OF RATS

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Pulmonary cytochrome P-4502B1 (CYP2B1) has been shown to be responsible for the bioactivation and pneumotoxicity of O,O,S-trimethylphosphorothioate and methylcyclopentadienyl manganese tricarbonyl in rats. This activity is inhibited *in-vivo* by the organophosphate, bromophos, which results in destruction of the isoenzyme, and *in-vitro* by the incubation of lung microsomes with a polyclonal antibody to CYP2B. The same antibody has been used for the immunolocalization of the enzyme in lung slices and in sections of lung embedded in acrylic resin. In control rats the immunogold labelling was clearly evident, even by light microscopy, in Clara cells and in type II pneumocytes. Ultrastructural immunocytochemistry showed that antigenicity was also present in the cytoplasm of type I pneumocytes. These cells can only be resolved, with certainty, by electron microscopy. Immunogold was largely restricted to the apical cytoplasm of the Clara cells but distributed throughout the cytoplasm of the type I and type II cells. Immunolabelling in the Clara cells of lungs from rats dosed with bromophos (26 mg/kg) was only 50–70% of that in controls and labelling in type I and type II cells was abolished. The presence of CYP2B1 in type I cells probably contributes to their susceptibility to alveolar pneumotoxins.

1430 SYNTHESIS AND CHARACTERIZATION OF THE MERCAPTURIC ACIDS OF ACROLEIN

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The anticancer drug cyclophosphamide (CP) is metabolized to 4-hydroxycyclophosphamide which decomposes to form acrolein and phosphoramide mustard. Acrolein is the metabolite believed to be responsible for the organ-specific toxicity of CP. Glutathione (GSH) readily conjugates with acrolein, and mercapturates of acrolein have been found in the urine of animals and man given CP. The toxicity of these compounds has not been fully explored. The objectives of this study were to prepare and characterize, by mass spectrometry and NMR, synthetic standards of the GSH acrolein adduct 3-oxopropylglutathione (3-oxoPrGSH), the acrolein mercapturates 3-oxopropyl-N-acetylcysteine (3-oxoPrMCA) and 3-hydroxypropyl-N-acetylcysteine (3-hydroxyPrMCA), and the S-oxidation product of 3-oxoPrMCA (3-oxoPrMCA S-oxide). 3-OxoPrGSH and 3-oxoPrMCA were prepared by condensing acrolein with GSH and N-acetyl-cysteine, respectively. There was a 99% yield in both cases. 3-HydroxyPrMCA was prepared with a 63% yield by refluxing 3-chloropropanol and N-acetylcysteine in a basic medium. Oxidation of 3-oxoPrMCA with H_2O_2 was used to prepare 3-oxoPrMCA S-oxide. By decreasing the reaction time to 1 hr and adjusting the ratio of 3-oxoPrMCA to H_2O_2 the yield of 3-oxoPrMCA S-oxide was increased to 96%. This compound was found to exist as a mixture of two diastereomers. The anhydrous aldehyde, 3-oxoPrMCA, afforded characteristic aldehydic proton resonances (^{1}H-NMR) in deuterated dimethyl sulfoxide as solvent. However, in deuterated water new resonances were observed indicating a 75% hydration of the aldehyde to the corresponding geminal diol. This phenomenon was enhanced with 3-oxoPrMCA S-oxide where 100% hydration of the aldehyde to the corresponding geminal diol was observed. Thus, these mercapturate aldehydes exist mainly in the diol form in aqueous solution. This may affect the toxicity and ability of the aldehyde mercapturates to release acrolein by β-elimination reactions. (Supported by HL48035).

1431 PROBENECID (PRB) IMPAIRS THE GLUCURONIDATION OF VALPROIC ACID (VPA) IN RAT HEPATIC S9 FRACTIONS

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Previous investigations in the isolated perfused rat liver suggested that PRB altered the hepatobiliary disposition of VPA and valproate glucuronide (VG) by inhibiting VG formation and/or impairing VG biliary excretion. The purpose of the present study was to examine the effects of PRB on VPA glucuronidation in rat hepatic S9 fractions. Hepatic S9 fractions were prepared from male Sprague-Dawley rats (250–300 g; n = 3) and incubated in a shaking water bath at 37°C for 10 min with VPA (0.05 – 5 mM) and PRB (0 – 10 mM). VPA and VG (before and after alkaline hydrolysis, respectively) concentrations were estimated by gas chromatography. *In vitro* metabolic parameters (mean ± SD) for VG formation (Vmax = 0.907 ± 0.484 nmol/min/mg protein; Km = 0.888 ± 0.444 mM) were determined by nonlinear least-squares regression. PRB impaired VG formation in a concentration-dependent manner. Data from individual S9 preparations containing a fixed concentration of VPA and varying concentrations of PRB were fit with 3 different metabolic inhibition models. The Ki for PRB ranged from 0.076 to 2.18 mM, depending upon the profile analyzed and the model used. The estimated Vmax for VG formation (1.08 ± 0.07 nmol/min/mg protein) was compatible with the kinetics of VG formation in the absence of PRB. These data indicate that alterations in the hepatobiliary disposition of VPA and VG in the presence of PRB are due in part to inhibition of VG formation. (Supported by NIH grant GM41935 and NIEHS training grant T32 ES07126).

1432 PHASE II ENZYME ANALYSIS: GLUTATHIONE S-TRANSFERASE ISOELECTRIC POINT DETERMINATION BY CAPILLARY ISOELECTRIC FOCUSING

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Glutathione *S*-transferases (GSTs) are the principal detoxifying enzymes for electrophiles in mammalian cells. Four major classes of dimeric GSTs of similar physical properties, except for isoelectric point, occur. The large differences in pI make separation of these enzymes by capillary isoelectric focusing (CIEF) attractive. We separated and quantitated rat liver GSTs by glutathione-agarose affinity chromatography followed by CIEF. This two-step process provided good recovery and rapid quantitation at levels of dimers present in small amounts of cells. When the glutathione (GSH) content of the GSTs was reduced, the pIs of the homodimers of 1, 2, and 4 became more basic and approached the pIs calculated from their GenBank subunit sequences. Heterodimers of 1, 2, 3, and 4 also became more basic when their GSH content was diminished. GST pIs in cells are likely to be acid-shifted and more similar to those determined in 10 mM GSH than in micromolar or lower GSH levels. A major fraction of intracellular GSH is probably associated with GSTs. The function of GSH associated with the noncatalytic sites of GSTs is yet to be investigated. These results illustrate the utility of CIEF in structural studies of GSTs, for which analytical methods have been difficult and cumbersome.

1433 STRESS RESPONSE AND DRUG METABOLISM IN MICE

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We studied a modified stress model to investigate the relationship between the stress response and drug metabolism in mice. Stress was induced in male CD-1 mice by a daily i.p. injection of hypertonic (1.5 M) saline whereas control animals received isotonic (0.15 M) saline (n = 5). At the end of each experiment, serum corticosterone (CORT) and liver aminopyrine N-demethylase (AD) and aniline hydroxylase (AH) activities were determined. In the first experiment, stress was induced for three days. Two hrs after receiving the injection on the first, second (26 hrs.), or third (50 hrs.) day, animals were sacrificed. A second control group, to detect any effect of osmotic stimulation, was given 1.5 M saline as drinking water for 7 days, and the animals were sacrificed on day 8. There was no difference in CORT levels, AD activity or AH activity between untreated animals and 0.15 M saline treatment. Intraperitoneal injection of 1.5 M saline markedly increased serum CORT concentrations compared to 0.15 M saline regardless of the duration of the treatment. Injection of 1.5 M saline also decreased both enzyme activities at each time point. Osmotic stimulation alone after 7 days of 1.5 M saline in place of drinking water had no significant effect on either CORT levels, AD activity or AH activity. In other experiments, intact, sham-operated (SO), adrenalectomized (ADX), and aminoglutethimide (AG)-treated mice were treated with either 0.15 or 1.5 M saline. Two hours after saline injection, CORT levels and enzyme activities were significantly reduced in ADX mice. Injection of 1.5 M saline increased serum CORT levels in intact, SO, and low dose AG-treated mice, whereas high doses of AG inhibited the CORT response to stress. Administration of 1.5 M saline also decreased AD and AH activities in these various treatment groups, except for an amelioration of the stress response in high dose AG-treated animals. In conclusion, a suitable model was established to study interactions between the stress response and hepatic drug metabolism in mice.

1434 3-METHYLBENZYL ALCOHOL (3MBA) AND m-TOLUALDEHYDE (m-ALD) EFFECTS ON RAT LIVER AND LUNG CYP2B1 ACTIVITY

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Previous inhalation and ip studies showed that m-xylene (m-XYL) selectively

inhibited lung CYP2B1 but had no effect in liver. Others have suggested that the aldehyde metabolite is responsible for these MFO effects. We have shown that *in vitro* addition of m-XYL and the metabolite m-ALD to lung and liver result in similar MFO changes. The purpose of this study was to determine whether *in vivo* administration of the m-xylene metabolites 3MBA and m-ALD inhibited CYP2B1 and to identify the presence of m-ALD in tissue. In the present study rats were given 3MBA or m-ALD (0.5 g/kg, ip) for 1h or 3h. Liver and lung CYP2B1 activity was measured by the benzyloxy-resorufin dealkylase (BROD) assay. 3-MBA and m-ALD inhibited BROD in lung but not liver at both 1h and 3h. Tissue levels of m-ALD were measured by adduct formation with 2,4-dinitrophenylhydrazine and quantitated by HPLC. Blood m-ALD levels were decreased from 4.8 μg/g(1h) to 53 ng/g(3h) and from 164 ng/g to 94 ng/g after m-ALD and 3-MBA treatment, respectively. Hepatic m-ALD levels were between 100 and 200 ng/g liver at 1h and 3h after either m-ALD and 3MBA. In *in vitro* enzyme kinetic studies, m-XYL and m-ALD were non-competitive inhibitors of CYP2B1 in lung and, to a lesser extent in liver. The data show that m-ALD is detectable in tissue and further support the notion that metabolites of m-XYL are involved in the observed MFO effects.

1435 PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODEL FOR TRICHLOROETHYLENE WITH ENTEROHEPATIC RECIRCULATION OF METABOLITES

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Trichloroethylene (TRI) is a widespread environmental and workplace contaminant. TRI and its metabolites have been reported to be carcinogenic in rodents. This study quantitatively examines the role of trichloroethanol-glucuronide (TCE-g) and trichloroacetic acid (TCA) enterohepatic recirculation in F-344 rats, administered an IV dose (100 mg/kg) of trichloroethanol (TCE), and produces an upgraded PBPK model for TRI. Separate experiments are conducted with the enterohepatic recirculation pathway intact and with it removed. The differences in metabolite blood concentrations provide a measure of the contribution from enterohepatic recirculation. The results show that approximately three-fourths of the resulting TCE-g and TCA found in whole blood following an IV dose is attributable to enterohepatic recirculation. Model parameters describing the metabolism and enterohepatic recirculation of TCE-g and TCA are under development from the experimental data. Physiologic and TRI kinetic parameters are taken from the published literature. Allometric equations are used in the model where possible to simplify scaling of the model to humans. Using SimuSolv, sensitivity analyses and parameter optimization are conducted on selected model parameters to examine their relative importance and ability to predict experimental data. Such an optimized PBPK model provides a rational means for describing TRI, TCE-g, and TCA kinetics. (This work is supported by EPA Cooperative Agreement CR 819283-01.)

1436 GAS UPTAKE PHARMACOKINETICS OF VINYL FLUORIDE IN RATS

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Vinyl fluoride (VF) is a rodent carcinogen by inhalation, but little is known about its pharmacokinetics and metabolism. The whole-body pharmacokinetics of vinyl fluoride were investigated using a closed-chamber gas uptake system. Groups of 3 rats were exposed to VF at starting concentrations ranging from 60 – 180 ppm. Chamber concentrations of VF were measured every 10–12 minutes by gas chromatography. The uptake curves were characterized by a very short equilibration period (indicating poor body solubility) followed by a relatively slow decline in VF concentration. Saturation of metabolism seemed to occur at an inhaled concentration of approximately 90 ppm. Below this concentration the uptake rate became substantially faster. Partition coefficients for VF between air and blood, liver, lung, kidney and fat were determined by the vial equilibration technique. VF was found to be poorly soluble in blood (blood:air $\approx$ 0.8). Higher partition coefficients were determined for liver, fat and kidney (2.8, 2.2 and 2.4 respectively), and the partition coefficient for lung was 0.9. The partition coefficient for the slow perfused compartment was estimated to be 0.5. The chamber concentration versus time curves were fit to a physiologically-based pharmacokinetic model using SimuSolv®. A model assuming low capacity ($V_{max} \approx 0.2$ mg/hr-kg) and high affinity ($K_m < 0.1$ mg/L) kinetic constants best described the uptake curves. An improvement of the model is under investigation to account for lung uptake and metabolism, which are not included in the current model.

1437 COMPARISON OF GAVAGE VEHICLE-DEPENDENT CHLOROFORM ($CHCl_3$) TISSUE DOSIMETRY IN MICE AND RATS

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$CHCl_3$ causes mouse liver and male rat kidney tumors when administered in corn oil, but only male rat kidney tumors when administered *ad libitum* in drinking water. We have shown previously that $CHCl_3$ tissue dosimetry after gavage administration is vehicle-dependent in male F-344 rats. The objective of the current study was to investigate the oral absorption kinetics of $CHCl_3$ after gavage administration in corn oil, water or 2% emulphor vehicle using a previously described physiologically-based pharmacokinetic (PBPK) model. $CHCl_3$ was dissolved in corn oil, water or 2% emulphor vehicle and administered to male F-344 rats (15, 30, 90 and 180 mg/kg) and female B6C3F1 mice (70, 238 and 477 mg/kg) in dose volumes of 2 and 10 ml/kg for rats and mice, respectively. $CHCl_3$ was quantitated in blood, liver and kidney, 2 min to 3 hr post dosing, by gas chromatography using a vial headspace technique. Blood $CHCl_3$ concentration data were fit using the PBPK model originally described by Corley *et al.* (1990) with minor modifications by Borghoff *et al.* (1994). A two-compartment description of absorption from the gastrointestinal tract was also incorporated into the PBPK model with absorption rate constants from the first compartment to the liver, from the second compartment to the liver, and a transfer rate constant to the liver, from the second compartment to the liver, and a transfer rate constant from the first to the second compartment defined as k_{as}, k_{ai} and k_{si}, respectively. For rats, blood and tissue data were accurately predicted using k_{as}, k_{ai} and k_{si} values of 1.5, 0.5 and 1.8 hr^{-1} for corn oil and 2.5, 0.5 and 3.5 hr^{-1} water vehicle. For mice, poor fits to experimental data were obtained. The best fit was obtained for mice using k_{as}, k_{ai} and k_{si} values of 1.12, 0.18 and 1.55 hr^{-1} for corn oil and a one-compartment fit (k_{as} = 1.83 hr^{-1}; stomach directly into liver) for water. These data show that, for rats, a vehicle-dependent two-compartment description of $CHCl_3$ absorption is appropriate. For mice, vehicle-dependent absorption appears to be more complex.

1438 CROSS-SPECIES EXTRAPOLATION OF ETHYLENE OXIDE PHARMACOKINETICS

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Ethylene oxide (EO) is widely used as a gaseous sterilant and industrial intermediate with a high potential for human exposure. EO is a direct-acting mutagen, rodent carcinogen, and has recently been classified by IARC as a human carcinogen. A quantitative understanding of the exposure-tissue dose relationship for EO in rodents and humans is a critical prerequisite to dose-response modeling and subsequent risk assessment. We previously reported results describing in vitro and in vivo kinetics of EO metabolism in male and female F344 rats and B6C3F1 mice (Brown et al., *Toxicologist* 1994). We extended these studies by determining the in vitro kinetics of EO metabolism in human liver cytosol and microsomes. The results indicate enzymatically catalyzed GSH conjugation via cytosolic glutathione-S-transferase (cGST) and hydrolysis via microsomal epoxide hydrolase (mEH) occurs in both rodents and humans. A trend for decreasing metabolic capacity with increasing species body weight was found. The ratio of mEH/cGST activity (V_{max}/K_M) also increased dramatically across species: 0.1 (mice), 0.7 (rats), and 4.0 (humans). These results suggest that GSH depletion at high EO exposures likely occurs only in rodents, particularly mice, but not humans, where hydrolysis is the predominant metabolic pathway. For cross-species comparisons, the in vitro kinetic constants were scaled to account for cytosolic (cGST) and microsomal (mEH) protein content and incorporated into physiologically-based pharmacokinetic (PB-PK) descriptions for mouse, rat, and human. The models adequately predicted in vivo blood EO levels, disposition, and elimination kinetics determined experimentally in these species. The PB-PK models are currently being used to generate exposure-dose simulations under a variety of exposure scenarios. These models represent an essential step in developing a mechanistically-based EO exposure-dose-response description for estimating human risk from exposure to EO. Supported in part by CMA/EOIC and NIEHS 1 F32 ES05616-01.

1439 THE UTILITY OF PARTITION COEFFICIENTS IN DERMAL MODEL DEVELOPMENT FOR RAT AND GUINEA PIG

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The ability to extrapolate usable dermal penetration data from one species to another is fundamental to specific applications in risk assessment. The fundamental goal of this work is to quantitatively explain the movement and dispo-

sition of chemicals across the dermal barrier in differing species. The chemical absorbed during the exposure in amount per time per area (mg/hr/cm^2) is defined as the flux of the chemical. In order to estimate the total amount of chemical absorbed during the exposure, a physiologically-based pharmacokinetic (PBPK) model can be used if appropriate physiological and kinetic parameters are known. A primary data requirement for PBPK models is the partition coefficients for the chemical in blood and each of the tissue groups that display distinct kinetic behavior. In this study, blood, muscle, liver and fat harvested from male Fisher 344 rats and male Hartley Guinea pigs were exposed to three test chemicals. Using a vial equilibration method, the partition coefficient was determined for Chloropentafluorobenzene (CPFB), 1,2-Dichlorobenzene (DCB) and 1-Iodoheptafluoropropane (C_3F_7I). The partitions for CPFB in the rat ranged from 13.4 in blood to 766.0 in fat. Partitioning of DCB into rat tissue ranged from 218.8 in blood to 30,860.5 in fat, while Guinea pig average values varied from 162.7 in blood to 15,658 in fat. The partition coefficient of C_3F_7I into blood, muscle and liver of both species was less than one, with the partition coefficient for fat ranging from 10.6 to 8.6 in the rat and pig respectively. The tissue:air partition coefficients generated in this study covered a four order magnitude range and were incorporated into PBPK models that were used for describing species differences in organic chemical absorption through the dermal barrier.

1440 UNCERTAINTY, VARIABILITY, AND SENSITIVITY ANALYSIS IN PHYSIOLOGICAL PHARMACOKINETIC MODELS

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Physiologically-based pharmacokinetic (PBPK) models are now commonly used to predict the dose of the toxic moiety of chemical substances in target tissues. A typical PBPK model can involve twenty or more physiological, physicochemical, and biochemical parameters each of which is subject to some degree of error. The objective of the present study is to develop a methodological approach for the analysis of uncertainty and sensitivity in PBPK models, taking into consideration the natural constraints on model parameters. In this approach, distinction is made between inter-individual variation in the model parameters and inherent uncertainty about the values of those parameters. The proposed methods take into account the natural restraints on the parameters, including the upper bound on the sum of all tissue volumes imposed by total body volume, and rank ordering of the partition coefficients governing chemical uptake in different tissues. Provision is also made for correlation between maximum metabolic rate and the Michaelis affinity constant in modeling saturable metabolic process. Many of the model parameters are related to body weight, which is assumed to vary in accordance with a doubly truncated normal distribution. The present method has been applied to a five compartmental PBPK model for benzene. The application of this kind of approach based on the natural constraints and correlations among input parameters should be particularly useful in conducting sensitivity/uncertainty analysis of PBPK models.

1441 INCORPORATING TISSUE AND BLOOD COMPOSITION DATA INTO PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODELS

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The partitioning of chemicals between tissues and blood is determined mainly by their relative content of neutral lipids, phospholipids, and water. The objective of the present study was to investigate the feasibility of incorporating such tissue and blood composition data into PBPK models to conduct a "built-in" calculation of tissue:blood PCs of organic chemicals. The present approach involved characterizing each compartment as a mixture of neutral lipids, phospholipids, and water (instead of describing the model compartments as "empty" boxes as done conventionally). This approach enabled the automated calculation of tissue:blood PCs from log n-octanol:water (log $K_{o/w}$) PCs during the run, since the n-octanol and water solubilities are equated to solubility of chemicals in tissue/blood lipids and water. Using the present approach, the tissue:blood PCs could also be calculated within the model by providing chemical structural information as input. Accordingly, each chemical is introduced into the model as a set of fragments (e.g., methyl, carboxyl), each of which contributes a particular numerical value (i.e., fragment constant) to the log $K_{o/w}$ PC value of the chemical. This datum is then used in the algorithm based on tissue and blood composition data to predict tissue:blood PCs of chemicals. Incorporating the tissue composition data into the PBPK models also allows us to examine the impact of inter-individual differences in lipid levels on the tissue dosimetry predictions. Overall, the present approach not only enhances the biological and mechanistic basis of PBPK models but also provides a starting point for developing QSAR-based parametrization of PBPK models.

1442 PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODELING OF A TERNARY MIXTURE OF ALKYL BENZENES IN THE RAT

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The objective of the present study was to develop a PBPK model for a ternary mixture of toluene (TOL), m-xylene (XYL), and ethyl benzene (EBZ) in the rat. The approach involved the development of individual chemical models, and then linking them at the level of the liver via the metabolism term. The individual chemical models were developed by obtaining the physiological, biochemical, and physicochemical parameters either experimentally or from the literature. The mechanism of interaction for each pair of chemicals (i.e., TOL-XYL, XYL-EBZ, TOL-EBZ), including the inhibitory constants, was determined by modeling the blood concentration-time course curve of each solvent obtained following individual or combined exposures of male Sprague-Dawley rats to 100 or 200 ppm of these solvents. Of the four mechanisms of interaction (i.e., no inhibition, competitive, non-competitive and uncompetitive inhibition), competitive inhibition appeared to be the most pausible one at relevant exposure concentration for all binary mixtures. Incorporating the metabolic inhibition constants obtained with the binary chemical mixtures, the PBPK model for the ternary mixture simulated adequately the kinetics of TOL, XYL, and EBZ in rats exposed to a mixture containing 100 ppm of each of these solvents. This study represents the first attempt to model a ternary chemical mixture, by accounting for possible interactions among its components. (Supported by IRSST, Québec)

1443 INTEGRATION OF DATA ON PYRENE PHARMACOKINETICS IN THE TROUT AND RAT WITH A PHYSIOLOGICALLY-BASED MODEL

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The pharmacokinetic behavior of chemicals in mammalian and non-mammalian species could be described with a single modeling framework that accounts for their anatomical, physiological, and biochemical differences. In this context, physiologically-based pharmacokinetic (PBPK) modeling represents a potentially useful approach. The objective of the present study was to develop a PBPK model framework to integrate pharmacokinetic data of pyrene in the rainbow trout and the rat, which were collected previously in our laboratories. The approach involved inclusion of the essential parameters (i.e., physiological, physicochemical, biochemical) and the equations interrelating these parameters to describe the kinetics of pyrene in both rat and rainbow trout in a single model file. The data file accompanying this model file contained numerical values of parameters specific to rat and trout in separate procedural blocks. Simulations of pyrene pharmacokinetics in the rat (2–15 mg/kg, iv) and the trout (3–10 mg/kg, ia) were conducted by loading the appropriate procedural block. Regardless of the species, when the PBPK model is run, it is the same skeleton of equations that is solved to yield predictions of pyrene kinetics, but with quantitatively/qualitatively different physiological/mechanistic determinants specific to each species as specified in the data file. The application of the physiological modeling approach might allow the integration of the pharmacokinetic and toxicologic data in mammalian and non-mammalian species by accounting for their anato-physiological and mechanistic differences.

1444 DEVELOPMENT OF AN ANIMAL-ALTERNATIVE METHOD FOR PREDICTING THE TISSUE : BLOOD PARTITION COEFFICIENTS (PCs) FOR PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODELS OF ORGANIC CHEMICALS

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Tissue:blood PCs represent an important set of input parameters for PBPK models. The objective of the present study was to develop an animal-alternative method for predicting rat and human tissue:blood PCs of organic chemicals. The approach consisted of developing an algorithm that integrates data on tissue and blood (erythrocyte + plasma) levels of neutral lipids, phospholipids, and water with data on the solubility of chemicals in saline and vegetable oil, in order to predict tissue:blood PCs. The solubility of a chemical in (i) tissue (or blood) neutral lipids was assumed to correspond to that in

vegetable oil, (ii) in tissue (or blood) water was assumed to correspond to that in saline, and (iii) in phospholipids was described as an additive function of the solubility in vegetable oil (0.3) and saline (0.7). The predicted human and rat tissue:blood PCs were compared with experimental data reported in the literature for a variety of chemicals (alcohols, acetate esters, ketones, alkanes, haloalkanes, aromatic hydrocarbons). The predicted PC values corresponded to 0.7 to 1.5 times the experimental PC values, except for the rat tissue:blood PCs of alkanes, haloalkanes and aromatic hydrocarbons, which were improved by the consideration of binding in blood. The association constants for binding of most of these substances, estimated using a law of mass action approach, ranged from 1200 to 2500 M^{-1}. The use of this mechanistic, predictive approach should contribute to reduction of animal use for estimating tissue:blood PCs of organic chemicals.

1445 *IN VITRO* DATA BASED PHYSIOLOGIC PHARMACOKINETIC MODEL FOR METHYL tert-BUTYL ETHER (MTBE) IN THE RAT

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Current research in our laboratory focusses to develop and verify the potential usefulness of animal-alternative methods (*in vitro* and QSAR) for the parametrization of physiologically-based pharmacokinetic (PBPK) models. The objective of the present study was to develop a PBPK model for MTBE in the rat, based on the estimates of chemical-specific parameters obtained *in vitro*. The PBPK model for MTBE consisted of the following compartments: lung, liver, adipose tissue, slowly perfused tissues, richly perfused tissues, arterial blood, and venous blood. The data on physiological parameters were obtained from the literature. The plasma:air, whole blood:air and tissue:air partition coefficients of MTBE were estimated *in vitro* using a vial equilibration method. The metabolic parameters were obtained from an *in vitro* study with hepatic microsomes isolated from male Sprague-Dawley rat (Brady et al. 1990). Initially, the $Vmax_{invitro}$ (mg/mg protein/min) was scaled to $Vmax_{intactanimal}$ (mg/hr/100 g rat) based on the mass recovery of the microsomal fraction. Then, using an allometric scaling method, a $Vmax_c$ (mg/hr/kg) that can be used to generate the Vmax for rats of any body weight was calculated. The model simulations of plasma levels of MTBE were in agreement with the experimental data obtained in male and female Fischer-344 rats following inhalation exposure (400 and 8000 ppm) and intravenous administration (40 mg/kg) (studies previously conducted for MTBE Task Force). The ability of PBPK models to predict the *in vivo* kinetics of chemicals solely based on *in vitro* parameter estimates endorses the reliability of their use in conducting various extrapolations.

1446 THE USE OF HUMAN DATA FROM ACUTE EXPOSURE IN PREDICTING CHRONIC TOXICITY OF TRICHLOROETHYLENE: AN APPLICATION OF A PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODEL

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Acute exposures of previously unexposed human volunteers to trichloroethylene (TRI) have shown that initial symptoms of CNS dysfunction (drowsiness) occur when urinary trichloroethanol (TCE) rates of production reach 0.6 mg/hr (Nomiyama and Nomiyama, 1977). A PBPK model for TRI was developed based on that of Ramsey and Anderson (1984), which successfully simulated these acute data. Longer term TRI exposures, including both intermittent and continuous exposures, with and without various levels of physical activity, were simulated. Results suggest that accumulation of TCE does not occur under occupational exposure conditions. Also, under simulated continuous exposure conditions at 1 ppm, steady state for urinary TCE is reached in about 5 days. Simulation of long-term human exposure under various exposure conditions and levels of TRI indicate that exposure of humans to 1 ppm TRI results in steady state TCE urinary concentrations of less than 0.6 mg/hr. These results predict that under chronic exposure conditions, at least CNS symptoms are not likely to occur.

1447 AN A PRIORI PHYSIOLOGICALLY-BASED PHARMACOKINETIC/PHARMACODYNAMIC MODEL OF BENZO(a)PYRENE

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An a priori Physiologically Based Pharmacokinetic/Pharmacodynamic (PBPK/PD) model has been developed for Benzo(a)pyrene (BaP). The model has been developed to simulate the existing data on BaP. These simulations, along with model sensitivity analyses, are then used to determine what experiments should be conducted to verify the appropriateness of the PBPK/PD model for BaP. The extension of the PBPK/PD model is to be used as an extrapolation tool to model the risk associated with exposure to BaP in humans.

The pharmacokinetic (PK) aspect of the model describes the oral absorption of BaP and its subsequent blood and tissue concentrations. Included in this are tissue compartments representing the liver, lung, brain, fat, forestomach, glandular stomach, small intestine, colon, and the richly and slowly perfused tissue groups. Metabolism of BaP occurs in the liver, lung, and small intestine compartments. The metabolism of BaP is modeled with two separate metabolic routes, one for the activation of BaP and the other for the deactivation of BaP. The activation route produces reactive metabolites which then bind to DNA to form DNA-BaP adducts. The pharmacodynamic (PD) aspect of the model, which follows the principles set by the Moolgavkar, Venzon and Knudson (MVK) two stage cancer model, quantifies the number of DNA adducts, the transformation of normal cells to initiated cells and the transformation of initiated cells to tumor cells. The simulations of the model are consistent with the data in the literature. These results, along with the model, have given us a valuable tool to now initiate model directed experiments designed to reveal the principles underlying the pharmacokinetics and pharmacodynamics of Benzo(a)Pyrene.

1448 THE APPLICATION OF A PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) NICOTINE MODEL TO ESTIMATE NICOTINE UPTAKE IN SMOKERS

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A physiologically-based pharmacokinetic (PBPK) model for nicotine has been previously validated in the rat and human subjects. In this study, the model was evaluated with regard to predictability and study design. Nicotine uptake by 32 smokers was estimated by model simulation to best describe plasma nicotine concentration profiles observed after smoking a single cigarette. There is good correlation between nicotine uptake, from smoking cigarettes having different yields of nicotine, but similar "tar" yields, estimated by the model and nicotine yields per cigarette estimated by FTC nicotine measurement (R = 0.97) and human-mimic smoking machine measurement (R = 0.92). Plasma nicotine concentrations predicted by the model are in fair agreement with experimental data; however, there is some inconsistency. Model simulation of the plasma curve suggests sampling points that include the absorption phase of nicotine would permit better estimates of nicotine uptake. While the model was re-simulated by altering the body weight, the large interindividual variation in observed plasma nicotine concentration may have resulted from the differences in smoking behavior and clearances between the subjects. Overall, the PBPK-nicotine model appears useful for estimating nicotine uptake in smokers from plasma nicotine concentrations. Model predictions of plasma nicotine concentration can be linked with pharmacodynamic response measurements taken from the smokers.

1449 INFLUENCE OF BODY FAT ON PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODEL PREDICTIONS OF CARBON TETRACHLORIDE (CCL4) DEPOSITION AND METABOLISM IN FISCHER-344 (F-344) AND SPRAGUE-DAWLEY (S-D) RATS

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Body fat values may be important in toxicokinetic modeling, particularly for lipophilic chemicals such as CCl4. Differences in percent body fat due to age or strain could alter the internal dose of such chemicals, thereby affecting the extent of toxicity. The objective of this study was to measure fat content in 2-month-old F-344 (8.7% of body weight), 22-month-old F-344 (13.9%), and 2-month-old S-D (6.5%) rats and its effect on PBPK model output. Lipid content was ascertained by a 6 hr 2:1 chloroform:methanol extraction, drying, and gravimetric determination. For F-344 rats at both ages, but not S-D rats, this extraction method demonstrated an excellent fit with an equation developed for F-344 rats correlating body weight with fat content determined by whole body dissection (Anderson et al., 1993). Model simulations for constant inhalation were run at 1, 100, and 1000 ppm CCl4 for 8 hour/day/5 days. The output parameters were CCl4 concentration in the fat compartment (CF, mg/g fat) and mount metabolized (AM, mg/g liver) 16 hr after the 5th exposure. Comparisons of CF and AM were made between age, strain, and volume of the fat compartment. Comparing 2-month-old S-D and F-344 rats,

there was an ≈176% increase in CF in F-344 rats at all CCl4 concentrations. Age-extrapolation revealed an ≈345% increase in CF in the 22-month-old F-344 rats compared to 2-month-old F-344 rats. In contrast, differences in AM between strains (5.2–17.2%) and ages (.6–13.8%) were much smaller. In summary, these model predictions are consistent with the previously reported (Rikans et al., 1989) lack of age-mediated differences in CCl4 hepatotoxicity. (This abstract may not reflect EPA policy. Supported by EPA CR-820447.)

1450 PHYSIOLOGICALLY BASED PHARMACOKINETIC (PB-PK) MODELING OF INDOOR AIR POLLUTANT DEGRADATION BY HOUSEPLANTS

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In an effort to determine the extent to which houseplants and PB-PK modeling may be combined to provide a remedial approach to indoor air pollution, a closed chamber system linked to a computerized gas chromatograph was developed to monitor indoor air pollutant degradation by houseplants. Experiments using initial concentrations of 21–2100 ppm trichloroethylene (TCE, a common indoor air pollutant) with the houseplant *Spathiphyllum "Mauna Loa"* in soil removed between 27–34% of TCE during a 12-hour test period (system leak subtracted). Previous experiments with sterilized soil suggest that a portion of the TCE was degraded by soil microbes. A PB-PK plant model has been developed based on diffusion, contact area, sorption, membrane thickness, reflection, and first-order rate loss due to plant or microbial degradation and/or plant incorporation. Our PB-PK model, based on Boersma *et. al.* (1990), consists of five main compartments: soil, free root, root exterior, stem, and leaf. Both stem and leaf compartments are further divided into subcompartments. In this model, gas uptake of TCE occurs via soil and/or leaves.

1451 DEVELOPMENT, VALIDATION, AND APPLICATION OF A THREE-DIMENSIONAL QUANTITATIVE STRUCTURE–ACTIVITY RELATIONSHIP MODEL FOR DIOXINS AND RELATED POLYCYCLIC COMPOUNDS

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In the present study we have utilized comparative molecular field analysis (CoMFA), a three-dimensional quantitative structure-activity relationship (QSAR) paradigm, to explore the physicochemical requirements for binding to the Ah (dioxin) receptor. In contrast to previous QSAR attempts, including our initial CoMFA/QSAR model (Waller, C.L. and McKinney, J.D. *J. Med. Chem.* 1992, 36: 3660–3666.), the present model is based on a structurally-diverse training set of Ah receptor ligands ranging in size from halogenated naphthalenes to indolo[3,2-b]carbazole derivatives. An exhaustive validation process utilizing external test sets and hierarchical cluster analysis routines was employed during model construction and is outlined. Using partial least squares regression, a squared correlation coefficient (r^2) of 0.865 was obtained for the CoMFA/QSAR model. The internal predictive ability of the model was ascertained using a leave-one-out crossvalidation routine which yielded a squared correlation coefficient (q^2) of 0.624. The model accurately predicted the binding affinity of 3-methylcholanthrene, an external test set molecule, within 0.1 log unit. The limitations of this approach with respect to predictive ability of the CoMFA/QSAR models is demonstrated to be dependent on a balance between structural diversity and redundancy in the molecules comprising the training set. The results of our modified CoMFA/QSAR model are consistent with and unify all previously established structure-activity relationships established for less structurally-diverse training sets of Ah receptor ligands. The utility of the CoMFA/QSAR methodology in quantitative risk assessment of potentially hazardous chemicals and structure-based molecule design of therapeutic agents is explored using a series of diphenyl ether derivatives. (This abstract does not necessarily reflect EPA policy).

1452 CHROMIUM(III) AND Cr(VI) KINETICS IN THE RAT: A PHYSIOLOGICALLY-BASED MODEL

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A physiologically-based model of trivalent chromium (Cr(III)) and hexavalent chromium (Cr(VI)) kinetics in the rat has been developed. The general structure of the model is based on an existing model of lead kinetics in rats (E.J. O'Flaherty, *Toxicol. Appl. Pharmacol.*, 111, 313–331, 1991). The model describes chromium disposition following administration by intravenous or intratrachael injection, drinking water, inhalation, or gavage. Cr(VI) is rapidly reduced to Cr(III) in all tissues in the model as well as in pulmonary and gastrointestinal tract contents. Chromium exchanges between plasma and the bone surfaces in contact with plasma, and can also become incorporated into actively mineralizing bone. In accord with experimental observation, two pools of chromium are modeled in the lung, together with a time-dependent shift from the bioavailable pool into the non-bioavailable pool and clearance from both into the gastrointestinal tract. The performance of the model has been tested against data from several studies in which chromium salts were administered to rats by a variety of routes. Many of the data sets were well simulated, but some inconsistencies were also noted.

1453 A PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODEL FOR ^{133}Xe AND ^{222}Rn IN THE HUMAN

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A PBPK model was developed which describes the human kinetics of ^{133}Xe and ^{222}Rn for inhalation, ingestion, and intravenous infusion. The parameters in the model were based on human physiological data and measured tissue partitioning of the two radionuclides. The model was validated using whole body retention and exhalation data from the literature for human exposure to ^{133}Xe via inhalation and intravenous infusion, as well as for human ingestion of ^{222}Rn in drinking water. The PBPK model was then used to evaluate tissue distribution data for the inhalation and ingestion of ^{133}Xe in a radioimaging study which has been used by the EPA to estimate radiation doses to tissues from the ingestion of ^{222}Rn in drinking water. It was determined that the oral uptake rate and retention of ^{133}Xe in the ingestion study used by EPA was consistent with published data on the whole body activity profile for ^{222}Rn ingested from drinking water, and that the whole-body activity profile observed in the inhalation study was consistent with similar profiles from a published inhalation study with ^{127}Xe. However, the PBPK model was unable to reproduce the significant activities reported in the radioimaging study for liver, lung, and muscle, particularly at longer times. These data are difficult to explain in the face of the low partitioning of ^{133}Xe into these tissues and the rapid exhalation clearance of this element. The most likely explanation for the unexpectedly high and prolonged activities reported for these low-partition tissues is the inability of the radioimaging technique to unequivocally distinguish liver, lung or muscle tissue contributions from those of fat tissue or intestinal tract contents in the same region. (Supported by EPA/ODW)

1454 INHALATION UPTAKE AND METABOLISM OF PROPOSED HALON 1301 REPLACEMENTS: HFC-125 AND FC-218

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Preliminary pharmacokinetics and metabolism of Halon 1301 (trifluorobromomethane), HFC-125 (pentafluoroethane), and FC-218 (octafluoropropane) were investigated in male Fischer 344 rats. HFC-125 and FC-218 have been proposed as possible replacements for Halon 1301, the current total flooding fire extinguishant used by the USAF. Rats were exposed for six hours to Halon 1301, HFC-125, and FC-218 at starting concentration from 100 to 5000 ppm in a recirculating closed gas uptake chamber. Samples were taken from the chamber and gas chromatography was used to determine the concentration in the chamber atmosphere. A physiologically based pharmacokinetic (PBPK) model was used to estimate the first order and saturable metabolic constants K_f, K_m, and V_{max}. Tissue/air and blood/air partition coefficients of these chemicals less than 4 and 1 respectively, resulted in relatively low chemical uptake into the animal. The evaluation of the closed chamber data with the PBPK model demonstrated the low tissue solubility and relative biological inertness of HFC-125 and FC-218. Information obtained from this study will be used in selecting replacement chemicals for the currently used ozone depleting chemical, Halon 1301.

1455 A PHARMACOKINETIC/PHARMACODYNAMIC MODEL FOR THE REGIONAL INDUCTION OF HEPATIC CYTOCHROME P450 ISOFORMS BY TCDD

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TCDD induces hepatic cytochrome P4501A1 and 1A2 in a region specific

manner within the hepatic acinus. We have employed a multi-compartment geometric model of the liver acinus to describe the time-course of P450 induction in various portions of a 5-compartment liver. Sprague-Dawley rats were dosed with 300 ng TCDD/kg. Rats were killed at various times after treatment for determination of TCDD in multiple tissues, for estimation of hepatic levels of CYP 1A1 and 1A2 m-RNA and activity, and for protein visualization by immunohistochemistry (IMH). IMH slides were characterized by a sharp boundary between fully-induced and non-induced cells. In the induction model, the Ah-TCDD complex interacts with specific sites (presumably dioxin response elements on DNA) and augments rates of enzyme synthesis. The interaction is characterized by a binding constant between the TCDD-Ah complex and DREs (Kd) and a cooperativity constant (N) in a Hill-equation relating occupancy to Ah-TCDD complex concentration. To describe time course data for hepatic TCDD, 1A1 and 1A2 concentrations, and localized induction, the Kd in individual compartments varied by a factor of 3 (9, 3, 1, 0.33, 0.11 nM) from periportal to centrilobular regions and the N-value required was very high, 4 or greater. While the exact biological meaning of these model-based parameters is still uncertain, these models can be used to predict regional induction for various exposure concentrations and durations and to relate regional induction to biological effects, including cell proliferation and subsequent hepatic carcinogenicity. (This abstract does not necessarily reflect US EPA policy.)

1456 A PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PB-PK) MODEL FOR TCDD IN C57BL/6J MICE

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TCDD responsiveness is mediated by the Ah receptor. We have developed a 7-compartment PB-PK model for TCDD in two strains of C57BL/6J mice ($Ah^{b/b}$ and $Ah^{d/d}$) with high and low Ah receptor binding affinities, respectively. In the liver, the Ah-TCDD complex interacts with high affinity sites to enhance synthesis of cytochromes P4501A1 and P4501A2. Each has an associated enzymatic activity and the latter binds dioxin, leading to non-linear hepatic accumulation. Mice were intubated with 0.05, 2.5 or 50 μg TCDD/kg and killed at 1, 4, and 28 days post-dosing. TCDD was measured in liver, fat, muscle, kidney, and skin; hepatic CYP1A1 and CYP1A2 by both specific activity and protein determination; and excretion by monitoring daily elimination in urine and feces. All these data were successfully described with a single model with fat/blood partition coefficient of 100 and basal CYP1A2 binding of 0.7 nmole/liver (Kb = 30 nM) increasing 40-fold with full induction. Acetanilide-4-hydroxylase (ACOH) activity results indicated that basal ACOH activity was associated with enzymatic activities other than CYP1A2. A presumed 2-fold induction of metabolic clearance was also necessary to fit the excretion data. As expected, $Ah^{b/b}$ mice differed from $Ah^{d/d}$ mice only in the Ah binding constant (1.8 versus 15 nM); all other model parameters were the same. The ability of this PB-PK model to describe results in both these mouse strains increases our confidence in the model structure and in its ability to extrapolate across various species. (This abstract does not necessarily reflect EPA policy).

1457 A SIMPLIFIED PBPK MODEL DESCRIBING THE KINETICS OF TCDD IN HUMANS

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A PBPK model describing the long-term kinetic behavior of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in humans has been developed, and fitted to data from 343 veterans of Operation Ranch Hand. In the model it is assumed that TCDD in serum lipids is in dynamic equilibrium with TCDD in tissues, and that serum:tissue partitioning is directly correlated with the lipid content of the tissue. Partition coefficients estimated on this basis are consistent with published human data for TCDD. Tissue compartment sizes, most notably adipose tissue, are estimated from the height and weight of the subject, and can change over time. Clearance is modeled as directly proportional to the hepatic TCDD concentration, but the system as a whole exhibits nonlinear kinetics due to changes in body weight. For example, the model predicts a TCDD serum half-life of 17.1 years for a 170 cm/70 kg man; however, the serum half-life decreases to 9.8 years if one assumes a 5 kg weight gain over a 5-year simulation. The model also predicts that the serum TCDD concentration can increase in the absence of exposure, given rapid weight loss. The PBPK model is a better descriptor of the Ranch Hand data than the one-compartment models that have typically been used for human TCDD dose estimation, as judged by residual error plots, and is expected to be suitable for estimating TCDD body burdens and serum concentrations for purposes of quantitative risk assessment.

1458 A PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) RISK ASSESSMENT FOR VINYL CHLORIDE (VC)

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A PBPK model was developed to support a cancer risk assessment based on the pharmacokinetic and metabolic data available in the literature for VC. The initial metabolism of VC was hypothesized to occur via two saturable pathways, one representing low capacity-high affinity oxidation by CYP2E1 and the other representing higher capacity-lower affinity oxidation by other isozymes of P450, producing in both cases chloroethylene oxide (CEO) as an intermediate product. The percentage of CEO converted to CO_2 via reaction with H_2O was determined from published reports of radiolabeled VC whole body metabolism studies. Previous *in vitro* and *in vivo* studies support chloroacetaldehyde (CAA) as the major metabolite of VC through the breakdown of CEO, and this metabolite was modeled as the major substrate in glutathione (GSH) conjugation, with a lesser amount of CEO as the glutathione S-epoxide transferase substrate. Depletion of glutathione by reaction with CAA was also described. The parameter values for the two metabolic pathways describing the initial step in VC metabolism were determined by simulation of gas uptake data from mice, rats, hamsters, monkeys and controlled human inhalation exposures, as well as from data on total metabolism and glutathione depletion in inhalation exposures. The simulation of pharmacokinetic data from studies using multiple routes of exposure served as evidence that the PBPK model was valid over the exposure range of interest. Alternative dose surrogates were then compared with tumor incidences in animal and human studies using the pharmacokinetic model.

1459 PBPK MODELING/MONTE CARLO ANALYSIS OF METHYLENE CHLORIDE KINETIC CHANGES IN MICE IN RELATION TO AGING AND CHRONIC INHALATION EXPOSURE

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During a two-year chronic inhalation study on methylene chloride (2000 or 0 ppm; 6 hrs/day, 5 days/week), gas-uptake pharmacokinetic studies were conducted on female $B6C3F_1$ mice after 1 day, 1 month, 12, 22 (control mice only) and 24 months of exposure. For each group of mice at 1 day, 1 month and 12 months, kinetic studies were conducted at initial concentrations of 500 and 2000 ppm while an additional concentration of 1000 ppm was added for the 22- and 24-month groups. Using physiologically based pharmacokinetic (PBPK) modeling coupled with Monte Carlo analysis, computer simulated results were compared among exposure groups and time periods. A non-parametric analysis of variance revealed significant differences ($p < 0.05$) among exposure time points for apparent V_{maxc} and K_{fc} as well as significant differences between exposed and control groups ($p < 0.05$). Significant induction in V_{maxc} was seen at the 1 day and 1 month exposure points when compared to controls, but the difference was dramatically reduced in the 24 month exposure time point. Decreased K_{fc} activity was observed in the 1 day and 1 month exposed mice as compared to controls which was also not present in the 24 month exposure point. Furthermore, decreased enzyme affinity, represented by a higher K_m, was observed in the 22 month and 24 month time periods. While these effects were attributed to age related changes in metabolism, results suggest little metabolic effect over a life-time exposure to methylene chloride.

1460 A MULTI-EXPOSURE ROUTE PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODEL FOR CARBON TETRA-CHLORIDE IN RATS

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Human exposure to carbon tetrachloride (CCl4) typically occurs by inhalation or ingestion. Therefore, the objective of this study was to develop a physiologically-based pharmacokinetic model for CCl4 to describe time-course data in adult, fasted male SD rats (≈360 g) for various routes of exposure: i.e., 10 mg/kg iv; 100 or 1000 ppm by nose-only inhalation exposure for 2 hr; 17.5 or 179 mg/kg in an aqueous vehicle by oral bolus gavage; and,

17.5 or 179 mg/kg in an aqueous vehicle by constant gastric infusion for 2 hr. Blood was collected from an arterial cannula for 12 hr, with the exception of iv exposure where blood was collected for 32 hr post-exposure. All data sets were described with a single consistently parameterized model with 7 compartments: fat, liver, slowly perfused, rapidly perfused, brain, arterial blood and venous blood. Partition coefficients (PC), blood flows and metabolic rate constants were obtained from the literature (Delp et al., 1991; Evans et al., 1994) with the brain PC estimated to be 2.5. Oral uptake was assumed to follow first-order kinetics. While there was good agreement between the predicted and observed blood concentrations (determined by headspace gas chromatography), discrepancies were noted in the fit to the terminal elimination phase for iv dosing and in the steady-state blood concentration during inhalation exposure. It is noteworthy that the estimated absorption rate constant for gastric infusion (ka = 0.3/hr) was very much lower than that for oral gavage (ka = 3/hr). From this, one may speculate that the kinetics of chemical uptake associated with low volume oral administration (e.g., as would happen with sipping) may differ from those associated with high volume, short-time ingestion (e.g., as occurs with oral bolus administration). (This abstract does not necessarily reflect EPA policy. Supported by EPA Cooperative Agreement CR-820447.)

1461 DEVELOPMENT OF A PHYSIOLOGICALLY-BASED DESCRIPTION OF THE GI ABSORPTION OF ORALLY ADMINISTERED BROMODICHLOROMETHANE (BDCM)

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BDCM, a by-product of water chlorination, is carcinogenic to rats and mice. We have developed a physiologically-based pharmacokinetic (PBPK) model to examine the oral absorption kinetics ot BDCM administered in various dosing vehicles. The model consisted of six compartments (liver, kidney, fat, slowly and richly perfused tissues and gut) with liver metabolic rate constants of 0.5 (K_m; mg/l) and 12.0 (V_{maxc}; mg/hr). The gut compartment consisted of three to five sequential subcompartments. Each GI tract subcompartment has an absorption (KA; 1/hr) constant, a bioavailability (A; unitless) term, and time of compartment emptying (T; hrs). Blood and exhaled breath BDCM concentrations from 90d-old male F-344 rats gavaged with 50 or 100 mg BDCM/kg in different dosing vehicles (corn oil or 10% Emulphor®) were obtained to calibrate the model. Simulations of the complex, multi-peak blood concentration-time profiles after corn oil gavage resulted in KA, A, and T values for the subcompartments ranging, respectively, from 0.4–1.4, 0.3–1.0, and 0.45–6.0. Blood concentration-time data following aqueous BDCM gavage was described with a single subcompartment for the GI tract and KA values ranging from 0.45–1.4 with A = 1.0. Quantitation of exhaled breath concentrations following oral gavage were used as a noninvasive technique to provide further data on BDCM uptake from the GI tract. Analysis of exhaled breath and blood BDCM data using this absorption model indicates that BDCM absorption following corn oil gavage is a complex process which is more accurately described using a multi-compartment GI tract transport model while aqueous gavage is adequately simulated using a simpler model. These models should improve toxicity risk assessments for volatile organic compounds by aiding in extrapolations from studies using oral dosing vehicles to other exposure scenarios (This abstract does not reflect EPA policy)

1462 A MULTI-COMPARTMENT, SEQUENTIAL TRANSPORT MODEL OF GASTROINTESTINAL ABSORPTION

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The oral absorption of lipophilic chemicals administered in oil vehicles is complex. CCl_4 absorption from corn oil in fasted rats was delayed with multiple secondary peaks in the blood concentration-time profiles (Kim et al., Toxicol. Appl. Pharmacol., 102, 50, 1990). Similar profiles have been noted after oral oil dosing of methyl chloroform in mice in an exhaled breath system (Gargas, J. Amer. Coll. Toxicol., 9, 4, 1990). We analyzed this behavior by linking a PB-PK model for CCl_4 to a GI-tract model consisting of a series of sequential absorption-transfer compartments. Each GI compartment has a residence time, absorption rate constant (/hr), and bioavailability. Individual rat blood time-course curves were consistent with between 8 to 10 compartments. Absorption rate constants and bioavailabilities were varied for each compartment until agreement was achieved between simulated and experimental results of CCl_4 arterial blood concentration data in individual rats. Compartment bioavailability values ranged from 0.26–0.02 and absorption rate constants from 0.3 to 1.4/hr. During the first 5 hours transfer between consecutive compartments was characterized by short times (from 0.5 to 2.1 hr). Transfers occurred with more extended intervals, ranging from 3 to 4 hours, at longer times. Absorption from aqueous vehicles has been also investigated. Aqueous uptake is less complex, requiring fewer GT sub-compartments to describe the blood concentration profiles. These models should greatly aid in extrapolating toxicity results obtained in studies utilizing these vehicles to other exposure scenarios. (This abstract does not reflect US EPA policy).

1463 ROLE OF CELL COMMUNICATION IN MORPHOLOGICAL TRANSFORMATION IN HAMSTER EMBRYO FIBROBLASTS

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The objective of the present investigation has been to study the role of altered gap junctional communication in morphological transformation of SHE cells using different inducers and modulators of the carcinogenic process. The aim has been to obtain information on the mechanisms involved as well as the role of these two endpoints in screening for non-genotoxic carcinogens. Morphological transformation of early passage Syrian hamster embryo (SHE) cells is studied by seeding cells for colony formation on top of a X-irradiated feeder layer of the same cells. The cells are exposed during the colony forming period, and the colonies inspected for morphological transformation. Gap junction intercellular communication is measured as spreading of the microinjected fluorescent dye Lucifer Yellow to neighbouring cells growing in monolayer. Chemical carcinogens including chlordane, Aroclor 1260, DEHP, DDT, limonene, ethionine, anisidine and clofibrate, and different signalling pathway modulators have been tested. The data support SHE cell transformation as a screening test with a potential to detect compounds from different chemical classes, including non-genotoxic carcinogens. Inhibition of GJIC is not obligatory nor sufficient for morphological transformation of SHE cells, but could be a valuable supplement in screening.

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1464 DEVELOPMENT OF A CELL-TO-CELL COMMUNICATION ASSAY USING PRIMARY CULTURES OF FLOUNDER HEPATOCYTES

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The winter flounder *Pleuronectes americanus* has been used in field and laboratory studies to evaluate the effects of pollutants on survival and reproduction of marine fish. The effects of contaminants on the liver have reproductive implications in fish because liver cells are stimulated by estradiol to produce vitellogenin, the precursor of egg yolk protein. Gap junctional communication (GJC) has been implicated in the synchronization of tissue reactions and so may be important during vitellogenesis. For our studies on reproductive effects of environmental contaminants, we have established primary cultures of flounder hepatocytes that show a high degree of GJC. GJC is assessed in plated hepatocytes by fluorescence recovery after photobleaching using an ACAS laser cytometer. Measurable GJC varies with a number of factors including staining buffer, media composition, plating surface, and cell density. We tested GIBCO hepatocyte, L-15, DMEM, Williams', and DMEM/Williams' media. We assessed GJC in heptocytes plated on Nunc chambered glass coverslips as well as on 35 mm Falcon Primeria plates that are untreated or coated with poly-L-lysine, CELL-TAK, or flounder biomatrix. Strong, reproducible GJC occurred between hepatocytes plated on chambered glass coverslips for 2 to 3 days in serum-free DMEM media with no phenol red. Fluorescence was maximized using osmolality-adjusted Dulbecco's phosphate buffered saline for staining rather than seawater of Earles buffered saline solution. A cell density of 5×10^5 per 2 cm^2 surface area yields a monolayer with cells in close enough contact to give a reproducible assessment of GJC. This assay is currently being used to investigate the effects of estrogenic chemicals on GJC and vitellogenesis in cultured flounder hepatocytes.

1465 DETERMINATION OF THE EFFICACY OF OZONE TREATMENT SYSTEMS USING A GAP JUNCTION INTERCELLULAR COMMUNICATION BIOASSAY

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A non-genotoxic bioassay that monitors gap junction intercellular communi-

cation (GJIC) was used to assess the presence of potential tumor promoters after the ozonation of pyrene, a polycyclic aromatic hydrocarbon. Reverse-phase high pressure liquid chromatography (RP-HPLC) was used to monitor the chemical fate of pyrene. Water and ozone were not rate limiting in a solution containing 10% water:90% acetonitrile (v/v) and 5 mM pyrene during ozonation. Pyrene (5 mM) was oxidized at various ozone dosages and the resultant mixtures were assayed for their effect on GJIC. Approximately 4.5 mmol of ozone per mmol of pyrene was required to remove all the intermediate by-products found to be inhibitory to GJIC. Only 1.6 mmol of ozone per mmol of pyrene was needed to remove >90% of the pyrene but this ozonated prene mixture remained inhibitory to GJIC (approx. 60% inhibition). The mixture of by-products formed from the reaction of ozone (at a dosage of 2 mmol of ozone per mmol of pyrene) was fractionated into 6 components by RP-HPLC. Each component was found to be inhibitory to GJIC. The above results showed that monitoring only the removal of the parent compound was insufficient for the assessment of the treatment efficacy.

1466 EFFECT OF SEVERAL ENVIRONMENTAL POLLUTANTS PRESENTED AS A FOOD RESIDUE ON GAP JUNCTIONAL INTERCELLULAR COMMUNICATIONS IN NORMAL HUMAN BREAST EPITHELIAL CELLS

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Great Lakes pollutants has been found in breast tissue of woman with breast cancer. The objective was to examine if DDT, Dieldrin, Toxaphene, and congeners of polychlorinated (PCB) and polybrominated biphenyls(PBB), either sigularly or in mixture, would inhibit gap junctional intercelluar communication(GJIC) in normal human breast epithelial cells(HBEC) *in vitro*. Inhibition of GJIC has been used as a predictor for teratogens, tumor promotors, reproductive- and neuro-toxicants. The scrape-loading/dye transfer and fluorescent redistribution after photobleaching techniques were used to measure GJIC. DDT, Dieldrin and Toxaphene inhibited GJIC in dose-responsive manner after 90 min treatment. Dieldrin supressed GJIC within 30 min with no recovery after 24 hrs, while inhibition of GJIC by DDT was partially restored after 12 hrs and fully restored after 24 hrs. Several PCB and PBB congeners inhibited GJIC in a dose dependent and time dependent manner, GJIC was almost restored after 24 hrs. DDT, Dieldrin, 2,4,5-HCB and 2,4,5-HBB, alone, did not affect GJIC in normal HBEC at the concentrations used, yet significant inhibition of GJIC was observed in DDT and 2,4,5-HCB-, Dieldrin and 2,4,5-HCB-, and Dieldrin and 2,4,5-HBB-treated cells. Immunostaining of connexin 43(Cx43) in the human breast cells showed that the inhibitory concentrations and combinations caused a rapid loss of Cx43 from the cell membranes. These results suggest that DDT, Dieldrin, Toxaphene, 2,3,4-HCB, 2,4,5-HCB and 2,4,5-HBB might have tumor promoting potential in human breast tissue. Futhermore, the combination of these compounds could synergistically enhance the risk to breast cancer by acting as a tumor promotor. Research supported by grants from the Michigan Great Lakes Protection Fund, NIEHS(2942E504911) and U.S. AFOSR(F4960-92-0293) grant to J.E.T.

1467 INHIBITION OF GAP JUNCTIONAL INTERCELLULAR COMMUNICATION IN HEPTACHLOR/HEPTACHLOR EPOXIDE TREATED NORMAL HUMAN BREAST EPITHELIAL CELLS

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Cyclodiene pesticides, heptachlor and heptachlor epoxide, are organochlorines which are recognized as environmentally persistent and among the more toxic pesticides. These chemicals are found in elevated concentrations in breast milk in various countries. In this study, we examined whether heptachlor/heptachlor epoxide could inhibit Gap Junctional Intercellular Communication (GJIC) in normal human breast epithelial cells and initiated studies on various potential mechanisms by which these pesticides might inhibit GJIC. Cytotoxicity and GJIC were evaluated by lactate dehydrogenase assay and fluorescence redistribution after photobleaching analysis, respectively. Both heptachlor and heptachlor epoxide were non-cytotoxic up to 10 μg/ml. At this concentration, heptachlor inhibited GJIC of normal human breast epithelial cells after one hour treatment. However, GJIC completely recovered after a 12 hour treatment of 1 μg/ml heptachlor. After a 12 hour treatment with heptachlor and heptachlor epoxide at 10 μg/ml, resumption of GJIC began. No difference in connexin 43 mRNA levels was found between control and heptachlor-treated cells. Heptachlor-treated cells exhibited increased intracellular ionized calcium ($[Ca^{2+}]i$) concentration even in a low concentration (1 μg/ml). These data suggest that the inhibition of human breast epithelial cell GJIC by heptachlor correlated with an increase in ($[Ca^{2+}]i$). Given that heptachlor has been shown to be a liver tumor promoter and inhibitor of GJIC in rodent cells, the possibility exists these chemicals could be potential human breast tumor promoters. Research was supported, in part, from Mich. Great Lakes Protection Fund, and U.S.A.F.O.S.R. (FY9620-92-J-0293) to J.E.T.

1468 ACRYLONITRILE-INHIBITION OF GAP-JUNCTIONAL INTERCELLULAR COMMUNICATION IN RAT LIVER EPITHELIAL AND RAT GLIAL CELLS

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Gap junctional intercellular communication (GJIC) has been implicated in the regulation of cell proliferation, differentiation and adaptive responses of differentiated cells. Chemicals capable of inhibiting GJIC have been shown to be possible tumor promoters and neurotoxicants. In this study, we investigated whether potential toxic effects of acrylonitrile (AN) on cells of potential target organs might be mediated via inhibition of GJIC. After micro-injection with Lucifer Yellow, dye transfer between cells was observed using an Ultima laser cytometer. In rat liver epithelial (WB) cells, the ability to transfer dye was completely inhibited within 1 hour at a non-cytotoxic concentration of 1 mM AN. By two hours, partial restoration of GJIC was observed but never at the rate of the untreated cells. Cells treated with 3 mM AN, which was cytotoxic to 80% at the end of 24 hours, exhibited the ability to transfer dye at a similar rate as the untreated cells. In contrast, the rat glial cells treated with 3 mM AN showed a cytotoxic effect of only 20–30% by 24 hours. At this concentration, a significant reduction in the rate of communication was observed after 2 hours, followed by a partial recovery at a time interval of 4–24 hours. Lower concentrations at present yield no or little effect on GJIC in the rat glial cells. Studies to examine potential mechanisms underlying the differential response of rat glial and liver epithelial cells will include measurements of free calcium, peroxides and connexin m-RNA levels and immunostaining of connexin proteins. (Research was supported by a gift from the Acrylonitrile Group).

1469 GAP JUNCTION ASSAY IN RODENT HEPATOCYTES: A GOOD PREDICTOR FOR LIVER CANCER IN RODENTS FOR PHTHALATE ESTERS

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Ortho phthalate esters are an important class of industrial chemicals used as plasticizers. The best studied chemical is di-2-ethylhexyl phthalate (DEHP), a liver carcinogen and liver tumor promoter in rats and mice at high oral doses. Di-n-octyl phthalate (DNOP), a closely related analog of DEHP, is a liver carcinogen and liver tumor promoter in male rats but not in mice at high oral doses. The hepatic effects of phthalates appear to be mediated by the corresponding monoesters for DEHP (MEHP) and DNOP (MNOP). In the present study, we have studied and compared the effects of MEHP and MNOP on gap junctional intercellular communication (GJIC) in rat and mouse hepatocytes, a promising assay for tumor promoters. Noncytotoxic doses ($\leq$500 uM) were selected for the GJIC studies based on LDH release. MEHP inhibited GJIC in rat and mouse hepatocytes which is consistent with DEHP being a liver carcinogen/liver tumor promoter in rats and mice at high oral doses. MNOP inhibited GJIC in male rat hepatocytes, but not mouse hepatocytes, which is consistent with species differences seen in the liver cancer studies of DNOP. In conclusion, the results from this GJIC study in rodent hepatocytes offers promise as a potential tool for screening phthalate esters for liver cancer or liver promotion activity.

1470 LOSS OF GAP JUNCTIONS IN TUMOR PROMOTER-TREATED WB-F344 RAT LIVER EPITHELIAL CELLS BY CONNEXIN43 DEGRADATION AND DEPHOSPHORYLATION

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The mechanisms for the downregulation of gap junctional intercellular communication in tumor promoter-treated cells are not understood. We have reported that DDT, lindane, and 18β-glycyrrhetinic acid (18β-GA) treatment of WB-F344 rat liver epithelial cells resulted in the reduction of dye-coupling

and the disappearance of plasma membrane gap junction plaques. The major gap junction protein in these cells is connexin43 (Cx43) and junctional plaque assembly requires Cx43 phosphorylation to Cx43-P2. In the present study, we have examined whether gap junction loss was due to degradation or dephosphorylation of Cx43-P2. Western blot analyses of plasma membrane and whole cell extracts revealed that DDT (25 μM) and lindane (50 μM) treatments caused the loss of Cx43-P2 coincident with the appearance of a putative fragment at approximately 22 kDa. No increases in nonphosphorylated Cx43 (Cx43-NP) were detected. Treatment with 18β-GA (40 μM) also decreased Cx43-P2, but no fragment was seen and Cx43-NP levels increased. The protein phosphatase inhibitor, okadaic acid (50 nM), had no effect on the loss of Cx43-P2 in DDT and lindane-treated cells, but prevented its reduction in 18β-GA-treated cells. When Cx43 synthesis was inhibited with cycloheximide (25 μM), only phosphorylated Cx43 was detected. Treatment of these cells with 18β-GA, but not DDT or lindane, led to the loss of Cx43-P2 and appearance of Cx43-NP. These data indicate that the loss of gap junction plaques in tumor promoter-treated WB-F344 cells resulted from both Cx43-P2 degradation and dephosphorylation. (Supported by NCI grant CA57612).

1471 ACUTE INHIBITION OF UTERINE CONTRACTION BY 4-HYDROXY-2′4′6′-TRICHLOROBIPHENYL (4OHTCB) MAY RESULT FROM DISRUPTION OF GAP JUNCTIONAL COMMUNICATION

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Gap junctions are thought to be involved in oscillatory contractions in vascular smooth muscle and in the coordination of uterine contractions during parturition. In preliminary experiments 4OHTCB acutely inhibited spontaneous oscillatory contractions of midgestation rat uteri. In the present study we hypothesized that the inhibition of spontaneous oscillatory contractions by 4OHTCB was due to disruption of gap junctional communication. Standard muscle baths interfaced with a polygraph were used to record isometric contractions from longitudinal uterine strips excised from midgestation rats. Spontaneous oscillatory contractions decreased in response to increasing concentrations of 4OHTCB (0.3–30 μM). Contractions were nearly abolished at 30 μM 4OHTCB. Tetraethylammonium (TEA), previously shown to increase gap junction plaque size and gap junctional communication in smooth muscle, antagonized inhibition of uterine contraction by 10 μM 4OHTCB in a concentration-dependent manner. In contrast, the calcium ionophore A23187 (2 μM and the calcium-dependent potassium channel blocker apamin (1–100 nM) had no significant effects on the 4OHTCB response. In order to measure gap junctional communication, primary cultures of myometrial myocytes from midgestation rats were microinjected with the fluorescent tracer dye Lucifer yellow. The transfer of dye to cells adjacent to the injected cell was scored by epifluorescence microscopy. 4OHTCB (0.1–10 μM) decreased dye transfer in a concentration-dependent manner. Dye transfer was abolished at 10 μM 4OHTCB. The reduction of dye transfer by 10 μM 4OHTCB was reversed with increasing concentrations of TEA (10–40 mM). In conclusion, 4OHTCB inhibited spontaneous oscillatory contraction and disrupted gap junctional communication in a similar manner. Both effects were reversed by TEA. The data suggest that the disruption of gap junctional communication by 4OHTCB may contribute to the inhibition of spontaneous oscillatory contractions in the pregnant uterus.

1472 LINDANE HAS A DELAYED INHIBITORY EFFECT ON SPONTANEOUS RAT UTERINE CONTRACTIONS AND MYOMETRIAL CELL-CELL COMMUNICATION

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There is increasing concern that environmental contaminants may play a role in altering reproductive function. When uterine strips isolated from pregnant rats were treated with the pesticide lindane (30 μM) they retained the ability to spontaneously contract 24 h later but did so with a marked degree of irregularity compared to solvent-treated controls. Treatment for 24 h followed by removal of lindane eliminated the ability of strips to attain spontaneous contractions over a subsequent 8 h testing period. Because cell-cell communication is hypothesized to coordinate smooth muscle function, we subsequently examined whether lindane had a delayed effect on gap junctional communication between cultured uterine myocytes. Using dye transfer as a measure of cell-cell communication, it was observed that lindane (100 μM in ethanol) eliminated dye transfer. This inhibition completely reversed 15 min after lindane removal. However, inhibition re-occurred within 1 h (80% dye transfer), reached a maximum at 4 h (29%) and persisted for 24 h (31%). Concentration-response experiments were performed with lindane dissolved in DMSO in an effort to improve solubility. When cells were exposed to lindane for 1 h followed by a 23-h incubation without lindane, dye transfer was 94%, 58%, 53% and 54% at 0.1, 1, 10 and 100 μM lindane, respectively, compared to 95% in solvent controls. Similar results were obtained with cells continuously exposed to lindane for 24 h: 95%, 64%, 62% and 54%, dye transfer at 0.1, 1, 10 and 100 μM lindane respectively. Thus, inhibition reached a similar plateau at 1 μM lindane regardless of whether exposure was for 1 h or 24 h. However, the maximum level of inhibition appeared to differ depending on whether lindane was dissolved in ethanol or DMSO. Consequently, parallel dye transfer experiments were performed 24 h after exposure for 10 min, 1 h or 24 h to lindane (100 μM dissolved in either DMSO or ethanol. Although lindane significantly inhibited dye transfer under all conditions, greater inhibition occurred when lindane was dissolved in ethanol compared to DMSO. In cells pretreated with lindane for 10 min, 1 h or 24 h, dye transfer was 35%, 30% or 31% using ethanol as a solvent compared to 61%, 50%, or 53% using DMSO as a solvent, respectively. These data show an interactive effect of the solvent with lindane. In conclusion, the results suggest a mechanistic link between lindane's effect on gap junctions and alteration of uterine contraction. Furthermore, they indicate that a brief exposure to lindane may have a long term negative impact on the physiological processes essential to normal parturition.

1473 RAPID UPREGULATION OF INTERCELLULAR COMMUNICATION BY CYCLIC AMP IN LOW AND HIGH PASSAGE IMMORTALIZED RAT GRANULOSA CELLS

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High passage cultures (>300 passages) were generated from a spontaneously immortalized rat granulosa cell line (SIGC); gap junction-mediated intercellular communication (GJIC) was compared in these cells to low passage cultures (<30 passages). High passage cultures exhibited significant reduction of GJIC which correlated with reduced punctate surface immunoreactive Cx43. Treatment of low and high passage cells with 1.0 mM 8Br-cAMP resulted in rapid upregulation of GJIC, which was detectable within 1–2 min using a fluorescence recovery after photobleaching assay. In both low and high passage cells, gap junction channels were closed within 1 min by 1.0 mM 1-octanol. However, addition of 8Br-cAMP to these cultures resulted in rapid, partial restoration of GJIC. Analysis of immunoreactive Cx43 by digital imaging was performed in cultures treated with octanol and/or 8Br-cAMP for different time intervals. By 30 min, a reduction in cytoplasmic staining and increased punctate staining at the cell surface was obvious in 8Br-cAMP-treated cultures. In cells treated with octanol prior to addition of 8Br-cAMP, trafficking of Cx43 to the cell surface was accelerated. The increase in cell surface immunoreactive Cx43 correlates well with the rapid upregulation of GJIC in both low and high passage SIGC cultures. (Supported by NIH P42-ES04917 and a TAES REP).

1474 PHENOXYL RADICAL-INDUCED THIOL-DEPENDENT GENERATION OF REACTIVE OXYGEN SPECIES: IMPLICATIONS FOR TOXICITY OF PHENOLIC COMPOUNDS AND METABOLITES

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Mechanisms of phenoxyl radical-induced generation of oxygen radicals, involved in toxicity of phenolic compounds (PC) were studied. We hypothesized that phenoxyl radical intermediates formed from PC by oxidative enzymes (e.g. peroxidases, tyrosinase) are able to damage biomolecules via (i) oxidation of low molecular weight thiols and protein thiols, and (ii) thiol-dependent generation of oxygen radicals and subsequent oxidation of DNA, proteins and lipids. Phenoxyl radicals generated by oxidation of PC by horseradish peroxidase + H_2O_2 or by tyrosinase were reacted with GSH and dihydrolipoic acid (DHLA). Both thiols reduced the phenoxyl radicals back to their phenolic forms. The reactions were accompanied by the formation of thiyl radicals and superoxide radicals. Hydroxylation of 2′-deoxyguanosine occurred in the course of tyrosinase-catalyzed oxidation of phenol in the presence of DHLA and Fe(III)-EDTA. Formation of lipid hydroperoxides was detected in the system arachidonate/horseradish peroxidase/phenol/hydrogen peroxide/ Fe/EDTA. We conclude that redox-cycling of phenoxyl radicals by thiols produces oxygen radicals which may be responsible for oxidative damage of DNA and lipids.

1475 PHENYTOIN-INITIATED PROTEIN OXIDATION IN MURINE EMBRYO CULTURE: A POTENTIAL MOLECULAR MECHANISM MEDIATING PHENYTOIN TERATOGENICITY

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Phenytoin teratogenicity may result from embryonic peroxidase-catalysed bioactivation to a free radical intermediate that initiates protein oxidation. This study evaluated in murine embryo culture whether a therapeutic concentration of phenytoin could initiate embryonic protein oxidation, and whether this could be prevented by either the reducing agent dithiothreitol, or the antioxidative enzymes superoxide dismutase (SOD) or catalase. Embryos were explanted from CD-1 murine dams on gestational day 9.5 (vagina plug = day 1) and incubated for 4 hr at 37°C in medium containing either phenytoin (20 μg/ml, 80 μM) or its vehicle (0.002 NaOH). Embryos then were homogenized in 0.5 mM 2,4-dinitrophenylhydrazine, which reacts with protein carbonyl groups, and incubated for 1 hr at RT. Proteins were separated electrophoretically using SDS/PAGE and transferred electrophoretically to a nitrocellulose membrane which was incubated for 3 hr with anti-dinitrophenyl antisera, followed by a 1 hr incubation with peroxidase-labelled goat anti-donkey IgG. Using enhanced chemiluminescence, multiple oxidised protein bands were detected between 25–200 kD. Embryos incubated in the presence of phenytoin showed a substantial increase in protein oxidation compared to untreated controls. Addition of dithiothreitol, SOD and catalase all decreased protein oxidation in phenytoin-exposed embryos. SOD and catalase were differentially efficacious depending upon the protein band, suggesting compartmentalised cytoprotection. This study provides the first direct evidence that a therapeutic concentration of phenytoin can initiate the formation of reactive oxygen species that substantially oxidise proteins, which may constitute a molecular mechanism of teratogenesis. (Support: Medical Research Council of Canada).

1476 PARAQUAT-INITIATED HYDROXYL RADICAL (HO•) FORMATION: CHARACTERISATION *IN VIVO* BY SALICYLATE HYDROXYLATION AND GLUCURONIDATION

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3-Hydroxylation of salicylate forming 2,3-dihydroxybenzoic acid (2,3-DHBA) reflects HO• formation, as distinct from 2,5-DHBA formation catalysed by cytochromes P450. Since paraquat (PQ) toxicity is thought to be mediated via reactive oxygen species (ROS), we investigated the effect of PQ on in vivo salicylate hydroxylation and 2,3-DHBA biotransformation in female CD-1 mice. Following treatment with 2,3-DHBA, 200 mg/kg ip, blood was taken via cardiac puncture at 15, 30 min and 1 hr and analysed for glucuronide conjugates using high-performance liquid chromatography with electrochemical detection. Detector potentials were optimised for all substrates and glucuronide peaks were confirmed in vitro by peak disappearance with β-glucuronidase incubation (32 μg/μl for 20 hr), and by peak formation in microsomal incubations with 2,3-DHBA and UDPGA (10 mM). Two glucuronide peaks were detected, totaling no higher than 8% of 2,3-DHBA in plasma. Subsequently, mice were injected with ASA, 200 mg/kg ip, and 15 min later with either PQ, 30 mg/kg ip, or its vehicle. Blood was taken at 5, 15, 30 min and 1, 2 hr via tail tip amputation. PQ-treated mice had a 10-fold increase in the production of 2,3-DHBA, but not the 2,5-isomer, with a maximum at 3 hr. 2,3-DHBA formation appears to be a sensitive and readily measurable index of in vivo ROS-mediated oxidative stress. (Support: Medical Research Council of Canada).

1477 PHENYTION-INITIATED DNA OXIDATION IN MURINE EMBRYO CULTURE, AND CYTOPROTECTION BY THE ANTIOXIDATIVE ENZYMES SUPEROXIDE DISMUTASE AND CATALASE: EVIDENCE FOR REACTIVE OXYGEN SPECIES-MEDIATED DNA OXIDATION IN THE MOLECULAR MECHANISM OF PHENYTOIN TERATOGENICITY

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Oxidative damage to embryonic DNA may mediate phenytoin teratogenesis. A murine embryo culture model was used to investigate whether phenytoin could initiate embryonic DNA oxidation, and to assess the embryoprotective potential of two antioxidative enzymes, superoxide dismutase (SOD) and catalase. On gestational day 9.5 (vaginal plug = day 1), embryos were explanted from CD-1 murine dams and incubated for up to 24 hr at 37°C in culture medium containing either phenytoin (20 μg/ml, 80 μM) or its vehicle (0.002N NaOH). Embryos were then pooled (3–5), their DNA was isolated and purified, and DNA oxidation was determined by the formation of 8-hydroxy-2′-deoxyguanosine (8-OH-2′-dG), quantified by high-performance liquid chromatography with electrochemical detection. Control embryos showed an initial increase in 8-OH-2′-dG, followed by a small but significant decrease over 24 hr, suggesting embryonic DNA repair. Embryos incubated with phenytoin had substantially more 8-OH-2′-dG than controls within 4 hr, with a maximal 3-fold increase at 24 hr ($p < 0.05$). Embryos removed after 4 hr of phenytoin exposure, and subsequently cultured in phenytoin-free medium for an addition 20 hr, did not show a decrease in 8-OH-2′-dG. SOD and catalase virtually eliminated phenytoin-initiated 8-OH-2′-dG formation, indicating the involvement of a reactive free radical intermediate and reactive oxygen species. These results provide the first direct evidence that, with a therapeutic concentration of phenytoin, embryonic bioactivation, free radical-mediated oxidative stress and embryonic DNA oxidation may constitute a molecular mechanism mediating phenytoin teratogenesis. (Support: Medical Research Council of Canada).

1478 INDUCTION OF MICROBIAL OXIDATIVE STRESS MARKERS BY HYDROGEN PEROXIDE AND CARBAMIDE PEROXIDE

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Antioxidant defense enzymes are reduced in response to oxidant exposure in eukaryotic and prokaryotic organisms. To understand mechanisms of stress gene induction and determine reactivity profiles of xenobiotics, promoters from oxidative damage-inducible stress genes have been fused to the *lacZ* structural gene and introduced into *E. coli* (Xenometrix Pro-Tox assay). The objective of this study was to compare H_2O_2 and carbamide peroxide (CP) for induction of stress genes in this system, as CP releases H_2O_2 upon decomposition. Both peroxides were tested at concentrations of 0.16–10 μM for 90 min at 37°C. Induction of β-galactosidase activity following exposure to either compound was greatest with the *umuDC* promoter (7–8.6 fold), followed by the *recA* promoter (3–4 fold). With the *merR* promoter, β-galactosidase activity was induced 2.8 fold by H_2O_2, but only 1.3 fold by CP. In general, responses to H_2O_2 were greater than with CP, and the response decreased at the highest H_2O_2 concentration, probably due to toxicity. The stress-induced *umuDC* response seen with CP could not be solely attributed to the release of H_2O_2 as maximal responses were seen at equimolar levels. *RecA* induction, however, occurred at about a threefold higher molar concentration of CP than H_2O_2. Both *recA* and *umuDC* are part of the SOS response induced in bacteria following DNA damage.

1479 AGE RELATED CHANGES IN STRESS PROTEINS, LIPID PEROXIDATION AND ANTIOXIDANT DEFENSE IN FISCHER 344 RATS

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Oxidative stress is believed to be associated with the aging process, and changes in antioxidant defenses may be involved in aging. In the present study, age-related alterations in antioxidant capacity including superoxide dismutase (SOD) and catalase (CAT) activities, glutathione (GSH) content, lipid peroxidation (LP) and stress/heat shock protein were measured in the heart, liver, lung, retina and brain of Fischer 344 rats aged 4, 16, 22 and 29 months. LP was 2.0- to 2.6-fold higher in liver, brain, lung, heart and retinal tissues of 29 month old rats as compared to younger rats. CAT activity was 1.6- to 2.4-fold lower in liver, brain, heart and lung subcellular fractions in 29 month old rats. SOD activity decreased between 1.4- to 1.8-fold in liver and brain subcellular fractions of aged rats, but no changes were observed in the heart and lung. GSH content also decreased between 1.4- to 1.9-fold in liver, brain and heart of old rats. However, no changes were observed in the lung. Heat shock protein formation increased with age as confirmed with western blot by HSP 90 antibody in heart, liver, brain and retinal tissues. These results demonstrate that oxidative stress increases with age in Fischer 344 rats.

1480 ANTIOXIDANT PROTECTION FROM CYANIDE-INDUCED APOPTOSIS IN DIFFERENTIATED PC12 CELLS

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PC12 cells terminally differentiate in the presence of NGF to resemble sympathetic neurons. Following withdrawal of NGF, differentiated cells undergo an apoptotic death. This model has been used to study the mechanisms of neuronal apoptosis. We have shown that cyanide induces oligonucleosomal DNA fragmentation and cell death in differentiated PC12 cells. Inhibition of the Ca^{++}/Mg^{++} dependent endonuclease with aurintricarboxylic acid protected the cells from cyanide-induced lethality. Based upon these findings we concluded that cell death in this model is apoptotic. In the present study the role of transcription and free radical generation in cyanide-induced apoptosis was determined. Treatment of cells with 100 μM KCN produced approximately 40% cell death within 24 hrs. as determined by trypan blue dye exclusion. In addition to DNA fragmentation, transmission electron micrographs demonstrated apoptotic nuclear morphology. Treatment of cells with the transcriptional inhibitor actinomycin D partially protected the cells from cyanide lethality. Treatment of cells with 5 mM ascorbate or 25 U/ml catalase was also found to be protective. These results suggest that KCN-induced apoptosis is mediated in part by an oxidative component and is transcription dependent. (Supported by NIH grant ES04140).

1481 LEAD AMPLIFIES GLUTAMATE-INDUCED OXIDATIVE STRESS

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The effects of lead acetate, L-glutamate, protein kinase C (PKC) inhibitor, and combinations of these compounds on the production of reactive oxygen metabolites (ROM) were studied in human SH-SY5Y neuroblastoma cells. We also measured the levels of cellular glutathione (GSH) and the cell viability. The dichlorofluorescin (ROM production) and monochlorobimane (cellular GSH) fluorescence was recorded at various time points for 180 min. Lead acetate (1 mM), L-glutamate (1 mM), or a PKC inhibitor Ro 31-7549 (1 μM) alone did not have an effect on the production of ROM in these cells. However, co-exposure of the cells to lead and L-glutamate, or to lead and a PKC inhibitor, always markedly increased ROM production. The maximal per cent increase in the relative fluorescence was 219 ± 62% for lead and L-glutamate, and 313 ± 71% for lead and PKC inhibitor, respectively. Moreover, 1 mM L-glutamate markedly increased ROM production if cellular GSH was depleted using 0.02% diethylmaleate (the maximal per cent increase was 85%). This increase was totally blocked by PKC inhibitor. The compounds alone did not affect cellular GSH levels. However, the GSH depletion by these substances together was 74 ± 5% (lead and glutamate) of control at 60 min and 69 ± 5% (lead and PKC inhibitor) at 180 min. The cell viability was decreased in 30 min to about 50% by both drug combinations (lead and glutamate or lead and Ro 31-7549). We propose that lead-induced neuronal damage is based on the production of ROM and that lead markedly amplifies glutamate excitotoxicity by increasing the ROM production elicited by L-glutamate. The results also suggest that PKC activation, primarily by lead, amplifies the harmful excitotoxic effects of mere L-glutamate on neuronal functions. Also L-glutamate alone seems to be excitotoxic through the production of ROM especially when neuronal GSH defence system is depleted. Supported by The Academy of Finland.

1482 ACTINOMYCETES ISOLATED FROM MOLDY HOUSES INDUCE NITRIC OXIDE PRODUCTION IN RAW264.7 MACROPHAGE CELL LINE

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Nitric oxide (NO) is a gaseous messenger molecule produced by activated macrophages as a nonspecific immune response directed against invading bacteria or microorganisms. NO regulates multiple biological functions including blood vessel relaxation, macrophage mediated cytotoxicity and neurotransmission. People living in moldy houses suffer from respiratory symptoms that may be caused by microbial strains present in these buildings. However, the specific causal relation and the cellular mechanisms behind the symptoms are not yet known. In the present study, we tested if any of the microbial strains, which are typical only for moldy houses, are able to activate inducible NO synthase and cause NO production in RAW264.7 macrophage cell line. The macrophages were induced 24 hrs by 0, 1×10^5, 5×10^5, 1×10^6, 5×10^6 or 1×10^7 spores/10^6 cells of Cladosporium, Aspergillus, Stachybotrys, Penicillium, Rhodotorula, and A4 or A91 Actinomycetes. NO-production was assessed as nitrite levels in the culture medium (Griess reaction) and the cell viability was measured by using the Trypan Blue exclusion test. Significant dose-dependent nitrite production was induced by Actinomycetes A91 (upto 17.10 uM/10^6 cells) or A4 (upto 15.14 uM/10^6 cells) while all the other tested microbes were without any effect or caused only slight increase in nitrite levels compared to the controls (0.72 uM/10^6 cells). The cell viability was decreased significantly only by Rhodorolula spores, but it was not related to the NO-production. These results suggest that Actimomycetes may be associated with the respiratory symptons observed in moldy houses. Supported by The Academy of Finland.

1483 PLASMA AND LYMPHOCYTE THIOLS AND DISULFIDES IN ASYMPTOMATIC HIV$^+$ AND AIDS PATIENTS: EVIDENCE FOR INCREASED OXIDATIVE STRESS AND PREDISPOSITION TO DRUG TOXICITY

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Plasma glutathione (GSH) is reported to be low in asymptomatic HIV$^+$ and AIDS patients, whose predisposition to drug hypersensitivity reactions may result from inadequate GSH-dependent detoxification of reactive intermediates. This study investigated the mechanism underlying low plasma GSH, its relation to other thiols (cysteine, CYS) and disulfides (GSH disulfide, GSSG; cystine, CYS-CYS; mixed disulfide, CYSSG; plasma protein disulfides, GSSPr, CYSSPr) and to intracellular lymphocytic concentrations. Thiols were stabilised by iodoacetic acid and 1,10-phenanthroline, derivatised with 2,4-dinitro-1-fluorobenzene and samples were analysed by high-performance liquid chromatography with UV detection. Both plasma thiols GSH and CYS were reduced by about 50% ($p < 0.05$) in HIV$^+$ (mean CD4$^+$ count = 400×10^6/L) and AIDS (mean CD4$^+$ count = 35×10^6/L) patients compared to controls, with corresponding increases of about 10-fold ($p > 0.05$) and 17-fold ($p = 0.0006$) in the respective oxidised products GSSG and CYSSG, and about a 70% decrease in GSSPr ($p = 0.0012$). Other disulfides were unchanged. Similarly, lymphocytic disulfides (CYS-CYS, CYSSG) were higher in HIV$^+$ and AIDS patients compared to reported control values, indicating intracellular oxidative stress. These results indicate a contribution of oxidative stress to the consumption of GSH, and possibly to predisposition to adverse drug effects, and to viral replication, supporting the evaluation of thiol rescue in these patients. (Support: Canadian Foundation for AIDS Research, Medical Research Council of Canada.)

1484 8-HYDROXY-2′-DEOXYGUANOSINE LEVELS IN THE LIVERS OF MALE B6C3F1 MICE EXPOSED TO TRICHLORO- ETHYLENE BY CORN OIL GAVAGE FOR 60 DAYS

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Trichloroethylene (RI) is a solvent which is a common contaminant in drinking water supplies and often a significant factor in environmental remediation efforts. TRI exposure has produced hepatocellular carcinomas in male B6C3F1 mice exposed by corn oil gavage in long term studies. To explore the oxidative stress to the hepatocyte nucleus and possible mutagenicity, we measured the ratio of 80hydroxy-2′-deoxyguanosine (8-OH-dG) to 2′-deoxyguanosine (dG) in liver tissue of male B6C3F1 mice exposed to 400, 800, and 1200 mg/kg daily doses of TRI as well as control mice. Animals were sacrificed at intervals throughout the 60 day exposure period. DNA was isolated from hepatocyte nuclei and digested to individual nucleosides. These were quantified by HPLC using electrochemical and ultraviolet detectors. Ratios of 800HdG/dG were elevated 2.3 fold above controls in the 1200 mg/kg/day. This increase was less in mice exposed for shorter periods of time. There were no statistically significant changes in the ratio in other groups.

1485 THE ROLE OF CALCIUM-INDEPENDENT PLASMALOGEN-SELECTIVE PHOSPHOLIPASE A_2 (CIPS-PLA_2) IN OXIDANT INJURY

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The goal of this study was to investigate the role of CIPS-PLA_2 in oxidant (0.5 mM t-butylhydroperoxide, TBHP) injury to rabbit renal proximal tubule

(RPT) suspensions. RPT were preincubated with for 30 min with 10 μM or 50 μM of HELSS, a specific inhibitor of CIPS-PLA_2 (Hazen et al., 1991). HELSS potentiated RPT lactate dehydrogenase release (LDH) after a 4 hr exposure to TBHP in a concentration-dependent manner (TBHP 40%, TBHP + 10 μM HELSS 57%, TBHP + 50 μM HELSS 71%, control 10%). HELSS alone had no effect on RPT LDH release. Preincubation with 10 uM or 50 uM HELSS had no effect on RPT cytosolic CIPS-PLA_2 activity but decreased microsomal CIPS-PLA_2 activity 63% and 76%, respectively. CIPS-PLA_2 activity was measured in RPT exposed to TBHP for 1 hr (prior to the initiation of LDH release). TBHP increased cytosolic CIPS-PLA_2 activity 24% but decreased microsomal CIPS-PLA_2 activity 30%. Pretreatment of RPT with 10 μM or 50 μM HELSS had no effect on TBHP-induced increases in cytosolic CIPS-PLA_2 activity. TBHP had no additional effect on HELSS-induced inhibition of microsomal CIPS-PLA_2 activity. These results show that oxidative stress increases cytosolic CIPS-PLA_2 activity but decreases microsomal CIPS-PLA_2 activity. Inhibition of microsomal CIPS-PLA_2 activity results in the potentiation of oxidant-induced cell death. Thus, in oxidant injury microsomal CIPS-PLA_2 may function to hydrolyze oxidized phospholipids and is therefore cytoprotective.

1486 QUINOLINIC ACID-INDUCED LIPID PEROXIDATION AS AN INDICATOR OF OXIDATIVE STRESS IN RAT BRAIN CAPILLARIES AND SYNAPTOSOMES

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Quinolinic acid (QUIN), an excitotoxic metabolite of tryptophan, was found to act as a potent lipid peroxidant in rat brain homogenates. Therefore, the generation of lipid fluorescent products (LFP) was studied in capillaries and synaptosomes derived from the entorhinal cortex, hippocampus and striatum of adult rats. We found that the generation of LFP was more than 10-times higher in brain capillaries in comparison to synaptosomes in which this process could be stimulated by QUIN (100 uM). The QUIN-induced lipoperoxidation was inhibited by reduced glutathione (50 uM). In contrast, high "basal" generation of LFP in brain capillaries was suppressed by desferoxamine (10 uM), suggesting an involvement of iron in brain endothelial cell lipoperoxidation. We conclude that brain capillaries are more sensitive to oxidative stress than synaptosomes in which some factors (QUIN, exogenous Fe^{2+}) can induce lipid peroxidation responsible for nerve cell damage in vulnerable rat brain regions.

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1487 CHROMIUM(VI) INDUCED LIPID PEROXIDATION AND PROTEIN OXIDATION IN CULTURED HUMAN LEUKEMIC T-LYMPHOCYTE MOLT4 CELLS

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Chromium(VI)-induced DNA-protein cross-links (DPCs) have been implicated in chromate carcinogenesis, although the exact mechanism of chromate-induced DPC formation has not been elucidated. Biologically generated intracellular chromium(III), has been suggested to mediate chromate-induced DPCs. Since, ·OH radicals are generated during intracellular reduction of chromate, we hypothesize that these radicals, in turn, may be responsible for formation of DPCs. The ·OH radicals are also known to generate malondialdehyde (MDA) and protein carbonyls (PC) during lipid peroxidation (LPX) and protein oxidation, respectively, which in turn, are thought to induce the formation of DPCs. We have studied the effect of potassium chromate (0–200 μM) in inducing LPX, PC formation, and DNA-protein cross-linking in MOLT4 cells. Lipid peroxidation was measured as thiobarbiturate-reactive products. Protein oxidation was measured by using 2,4-dinitrophenylhydrazine. DPCs were isolated by centrifugal sedimentation in the presence of 2% SDS and 5 M urea from ^{3}H-DNA and ^{35}S-protein labeled cells and quantitated based on their specific activities. No lipid peroxidation product was detected within 3 hrs of chromate treatment. After 4 hrs of treatment, a dose dependent increase in LPX was observed. A dose dependent increase in the formation of DPCs and protein oxidation was observed within 2 hrs of treatment. Since H_2O_2 is known to generate ·OH in a metal catalyzed reaction, we have studied the effects of various metals on the oxidation of BSA in the presence of H_2O_2. Reaction of H_2O_2 with Cr(VI), Cr(III), V, Cu, Fe and Co caused increased oxidation of BSA while Hg, Zn and Al were without effect. Treatment of MOLT4 cells with α-tocopherol or Tiron, prior to chromate treatment decreased formation of DPCs and protein oxidation. Pretreatment of cells with riboflavin, an intracellular chromate reductant, or aminotrizole, a specific catalase inhibitor, increased DPC formation and protein oxidation. These results suggest that MDA may not be involved in chromate induced DNA-protein cross-linking, at least in initiating DPCs, where as some of the chromate-induced DPCs may be formed via the formation of PC.

1488 OXIDATIVE LUNG INJURY IN A MODEL OF MULTIPLE ORGAN FAILURE IN RATS

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A short period of hepatic ischemia drastically enhances the susceptibility for endotoxin-induced liver injury during reperfusion (Liu et al, Circ Shock 43: 9, 1994). A priming of phagocytes for reactive oxygen formation seems to be critical for this effect. To investigate possible lung damage and the potential role of reactive oxygen in this model, male Fisher rats were subjected to 20 min hepatic ischemia followed by injection of 0.5 mg/kg *Salmonella enteritidis* endotoxin (ET) during reperfusion (RP). Lung wet-to-dry weight ratios (W/D) and broncho-alveolar lavage (BAL) protein content were determined after 4 h of reperfusion. Both parameters increased significantly during RP/ET (W/D: 4.4 ± 0.1; BAL: 639 ± 30 μg/ml) compared to controls (W/D: 3.5 ± 0.1; BAL: 332 ± 17) or ET-treated animals (W/D: 3.8 ± 0.1; BAL: 442 ± 18). The antioxidants Trolox (vitamin E-derivative) or 21-aminosteroid Tirilazad mesylate (U-74006F) reduced the BAL protein increase by 70% and 83%, respectively. There was no increase of TNF-α levels in BAL indicating no activation of alveolar macrophages. Isolation of neutrophils from the lung vasculature animals showed an increase of spontaneous superoxide formation from 1.4 ± 0.5 in controls to 5.5 ± 1.0 nmol O_2^-/h/10^6 in RP/ET cells in RP/ET animals. PMA-induced superoxide formation increased from 2.2 ± 0.4 to 8.7 ± 1.3 nmol O_2^-/h/10^6 cells. *Conclusion:* Hepatic ischemia-reperfusion and endotoxemia induced severe oxidant-dependent lung injury. Neutrophils sequestered in the lung appear to be the main source of the oxidant stress (ES-06091).

1489 EFFECTS OF U-74389 ON OXIDATION OF LUNG PROTEINS OF SD RATS EXPOSED TO HYPEROXIA

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Protection against hyperoxic lung injury by 21-aminosteroids has been reported. We investigated the effects of U-74389 on oxidative modifications of proteins in male SD rats exposed to >95% oxygen. We administered U-74389G, 10 mg/kg i.p. or vehicle at the onset and every 12 hr during continuous exposure to hyperoxia or air. At 60 hr, rats were sacrificed and blood, pleural fluid and lung tissue were collected. Samples were derivatized with 2,4-dinitrophenylhydrazine (DNPH), electrophoresed, transferred and cross-reacted with anti-DNPH antibody. Pleural fluid from hyperoxic rats showed apparent oxidative modifications of proteins of 137, 39 and 33 kD. These modifications in rats exposed to oxygen appeared to be attenuated in the U-74389 treated animals. Alterations in protein thiol status were assessed by derivatization of proteins with monobromobimane. Proteins in pleural fluid and lung tissue showed no significant changes in protein thiols resulting from exposure to hyperoxia, and administration of U-74389 caused no observable effects in either air-breathing or hyperoxia exposed rats. The support of a grant from Upjohn is gratefully acknowledged.

1490 ACETALDEHYDE ADMINISTRATION ALTERS TRACE ELEMENT METABOLISM AND SUPEROXIDE DISMUTASE ACTIVITY IN THE RAT

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Acetaldehyde (AA) is a known toxicant associated with alcohol intake and cigarette smoking. It has been suggested that ethanol-induced oxygen radical generation and resultant tissue injury may be mediated by AA. To investigate this hypothesis further, the present study examined tissue trace element (TE) concentrations and superoxide dismutase (SOD) activity in adult rats (n = 9/group) following 14-day administration of acetaldehyde (0.67 mg/kg/hr) or saline via osmotic minipump. AA-treated rats had nearly 2-fold higher serum Cu concentrations and significantly higher Zn concentrations in liver, kidney, heart and brain and higher Mg levels in liver, heart and brain than controls. Cluster analysis of TE revealed significant differences in TE relationships between the 2 groups. In addition, CuZn-SOD activity was significantly higher in liver and lung from AA-treated rats compared with controls. Since altered TE metabolism is indirectly related to antioxidant status and has been associated with various disease states, the present data support a role for AA in oxygen radical-mediated tissue injury.

1491 TISSUE LEVELS OF 8-HYDROXYDEOXYGUANOSINE (8-OHdG) IN SPRAGUE-DAWLEY RATS FED *AD LIBITUM* OR FED A CALORIE-RESTRICTED DIET

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In a two-year study conducted in-house, male and female Sprague-Dawley rats fed 65% of *ad libitum* intake had dramatically greater survival than animals fed *ad libitum*. Since 8-OHdG is a DNA modification that is produced as a consequence of normal cellular metabolism and is associated with aging and carcinogenicity, we analyzed DNA from several tissues to see whether caloric restriction would affect the steady state levels of 8-OHdG. DNA was isolated from brain, kidney and liver samples from rats after 52 or 106 weeks on the different dietary regimens; the purity of each DNA sample was assessed by UV spectral criteria. Following enzymatic hydrolysis of the DNA, 8-OHdG was measured by HPLC using UV and electrochemical detectors. In rats fed either a standard diet (Purina 5002) or a high-fiber diet (Purina 5002-9), the steady-state levels of 8-OHdG in these tissues were not changed by the 35% restriction of caloric intake after 52 or 106 weeks. Hepatic glutathione peroxidase activity was unaffected by the caloric restriction of the animals. At 52 weeks, calorically restricted rats had higher levels of glutathione and lower levels of malondialdehyde in liver, but these differences were not observed at 106 weeks. Thus, while caloric restriction of Sprague-Dawley rats dramatically increased the survival rate at 106 weeks, potential indicators of oxidative stress were essentially unchanged in the tissues examined at the conclusion of the study.

1492 ALLERGIC IMMUNE RESPONSE ACTIVATES CALCIUM-TRANSPORT IN MURINE LUNGS

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Exaggerated oxygen radical production by pulmonary cells is suggested to contribute to increased airway responsiveness and heightened smooth muscle constriction in asthmatic lungs. Smooth muscle cell contractility in the lung is regulated by Ca^{2+}-homeostasis. The contribution of inflammatory cells to these events is unclear. We have developed a murine model to study the role of Ca^{2+}-transport in allergic pulmonary reactions. Sensitization of mice was accomplished by injection with ovalbumin (OA) (1 μg or 50 μg) or OA (1 μg) plus aluminum hydroxide. Pulmonary responses were elicited by inhalation provocation challenge of mice with OA aerosol. Response was quantified by the extent of inflammatory cell infiltrate at 24 hours. We found increased Ca^{2+}-transport in microsomes and homogenates of the lung after antigen challenge. Activation of Ca^{2+}-transport correlated with the severity of allergic pulmonary response as evidenced by the inflammatory cell infiltrate and from the titer of OA-specific antibody. The greatest increase in Ca^{2+}-transport was noted in microsomes from mice sensitized with OA plus adjuvant. Ca^{2+}-transport in sensitized, but not in control mice, was sensitive to oxidative stress induced by addition of phenol and hydrogen peroxide. Lung homogenates from both groups of animals responded similarly to phenoxyl-radical induced oxidative stress induced by phenol plus exogenous tyrosinase. These results are the first to indicate heightened Ca^{2+}-transport in pulmonary microsomes following an allergic lung response and emphasize the role of aluminum hydroxide in enhancing allergic reactions in the lung. The responsiveness of the system to oxidative stress suggests that oxidative mechanisms may contribute to physiologic and pathologic manifestations, such as airway hyperreactivity, associated with allergic pulmonary disease. Supported by NIH ES05651 and American Heart Association (Pennsylvania Affiliate).

1493 NADH-DEPENDENT PROTECTION OF Ca^{2+} TRANSPORT IN SARCOPLASMIC RETICULUM MEMBRANES: POSSIBLE INVOLVEMENT OF VITAMIN E RECYCLING

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Membrane NADH- and NADPH-driven electron transport is known to initiate lipid peroxidation by activating oxygen in the presence of iron. This prooxidant effect can mask an antioxidant function of NAD(P)H-driven enzymatic reactions in membranes via vitamin E recycling from its phenoxyl radicals formed in the course of peroxidation. In the present work, we studied the effects of NADH on Ca^{2+} transport, content of vitamin E and lipid peroxidation induced in sarcoplasmic reticulum membranes (SRM) by an oxidation system independent of iron: an azo-initiator of peroxyl radicals, 2,2'-azobis(2,4-dimethylvaleronitrile) (AMVN) in the presence of an iron chelator, deferoxamine. We found that in the presence of deferoxamine NADH was able to protect against AMVN-induced: (i) inhibition of Ca^{2+} transport; (ii) loss of endogenous vitamin E, and (iii) lipid peroxidation. In contrast, NADH caused inhibition of Ca^{2+} transport in the presence of exogenously added iron and in the absence of deferoxamine. Thus NADH-driven enzymatic reactions may function both to protect or to inhibit Ca^{2+} transport in SRM dependent on the availability of transition metal catalysts. Supported by American Heart Association, Pennsylvania Affiliate and National Science Foundation (MCB-9322598).

1494 COMPARATIVE EFFECTS OF 3,3'-DICHLOROBENZIDINE (DCB) AND B-NAPHTHOFLAVONE (BNF) ON TISSUE LEVELS OF HEME OXYGENASE-1 (HO-1) mRNA AND METALLOTHIONEIN (MT) mRNA IN THE RAT

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Treatment of rats with DCB induces a pattern of P450 qualitatively similar to that induced by BNF and enhances tissue lipid peroxidation. In the present study, we compared the effects of DCB and BNF on tissue HO-1 mRNA and MT mRNA levels, which are reportedly increased by oxidative stress. A single ip dose (40 mg/kg) of DCB increased HO-1 mRNA abundance significantly in the kidney (from barely detectable levels in controls) and 25-fold and 3.5-fold, respectively in the liver and lung. The increased tissue HO-1 mRNA level was paralleled by increased microsomal heme oxygenase activity. In contrast with DCB treatment, treatment with BNF or another representative P450 inducer phenobarbital (PB) increased neither tissue HO-1 mRNA levels nor microsomal lipid peroxidation. DCB also elevated MT mRNA levels in the liver, kidney and lung as did BNF and PB. None of the compounds examined altered tissue GSH levels significantly. The data suggest that oxidative stress rather than P450 induction may be the major stimulus for HO-1 induction by DCB. Furthermore, HO-1 induction may be a more selective response to oxidative stress than MT induction, at least for the compounds we examined. (Supported by DK43135 and ES06414).

1495 PYRIDINE NUCLEOTIDE OXIDATION AND OXYGEN RADICAL FORMATION IN MITOCHONDRIA PRECEDE THE MITOCHONDRIAL PERMEABILITY TRANSITION (MPT) DURING OXIDATIVE INJURY

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Previously we showed that the oxidant chemical, *tert*-butylhydroperoxide (*t*-BuOOH), induces a MPT in intact hepatocytes leading to lethal cell injury. The purpose of this study was to visualize the critical events preceding the MPT induced by *t*-BuOOH using UV/visible laser scanning confocal microscopy of single cultured rat hepatocytes. Reduced pyridine nucleotides were imaged by autofluorescence excited at 351 nm. Formation of oxygen radicals was monitored using 2',7'-dichlorofluorescin. Loss cell viability was assessed by propidium iodide uptake into nuclei. *t*-BuOOH caused rapid oxidation of mitochondrial pyridine nucleotides: 50% was oxidized after 5 minutes. This event was closely followed by the generation of mitochondrial oxygen radicals. Trifluoperazine partially decreased pyridine nucleotide oxidation, completely prevented radical formation, inhibited the MPT, and preserved cellular ATP levels and viability. Desferal, an iron chelator, also prevented the oxidation of mitochondrial pyridine nucleotides, formation of mitochondrial oxygen radicals, and cell death. In conclusion, oxidation of mitochondrial pyridine nucleotides and subsequent formation of oxygen radicals may be critical events leading to the MPT during oxidative injury after oxidant chemicals and ischemia/reperfusion.

1496 DIVICINE-INDUCED HEMOLYTIC ANEMIA IN THE RAT

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Divicine (2,6-diamino-5-hydroxy-4-pyrimidinone) has long been thought to be a mediator of an acute hemolytic crisis, known as favism, in certain glucose-6-phosphate dehydrogenase (G6PD)-deficient individuals who ingest fava beans. In this study, the ability of divicine to induce a favic response in normal rats was investigated. Administration of divicine (300 mg/kg, ip) to rats preloaded with ^{51}Cr-labeled erythrocytes resulted in a severe hemolytic response that was characterized by the appearance of massive hemoglobinuria and jaundice. Further, over 80% of the red cells initially present were removed from the circulation within 48 hours of divicine administration, resulting in

a 50% loss in hematocrit. Survival of radiolabeled red cells in vivo was also reduced following in vitro exposure to divicine (1.0 mM). Prior depletion of red cell GSH with diethyl maleate (5 mM) exacerbated divicine hemotoxicity by about 10-fold, whereas preincubation with the G6PD inhibitor, epiandrosterone (200 μM), induced only a two-fold enhancement of divicine toxicity. These data indicate that favism can be reproduced in the rat, and substantiate the value of the rat as a model for human favism. In addition, the data suggest that the GSH levels may be the determining factor, rather than loss of G6PD activity, in susceptibility to favism. Supported by NIH grant HL-30038.

1497 MITOCHONDRIAL TOXICITY OF BHTOOH, THE HYDROPEROXYL METABOLITE OF BUTYLATED HYDROXYTOLUENE

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The food additive butylated hydroxytoluene can be oxidized to form a hydroperoxyl metabolite BHTOOH. The cytotoxicity of BHTOOH and its tumor promoting activity in skin may be mediated by further metabolism to its electrophilic quinone methide (BHT-QM), which occurs during incubation with cytosol or hematin. Previous investigations indicated that the earliest signs of BHTOOH toxicity in keratinocytes occurred in the mitochondria. The present research investigates the covalent binding of BHT-QM to subcellular fractions and effects on mitochondrial respiration and oxidative phosphorylation following BHTOOH. A plurality of radioactivity was found in the mitochondria following 30 or 120 min exposure of murine keratinocytes (PA cells) to [^{14}C]-BHTOOH. To examine further the toxicity of BHTOOH on mitochondria, oxygen consumption of isolated rat liver mitochondria was measured using an oxygen electrode, while oxidative phosphorylation was measured using luciferase-coupled bioluminescence. Incubation with micromolar doses of BHTOOH alone caused a decrease in mitochondrial respiration and a decrease in ATP production, while incubation of BHTOOH with hematin resulted in a greatly enhanced respiration and a decrease in ATP production. These data indicate that BHTOOH is toxic to mitochondria and that BHT-QM alkylates mitochondrial proteins which may result in uncoupling of oxidative phosphorylation. (Supported by NIH grants PO1 CA 44530 and ES 03819).

1498 BENZOYL PEROXIDE MEDIATED FORMATION OF 8-HYDROXY-2′-DEOXYGUANOSINE IN MURINE KERATINOCYTES

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Benzoyl Peroxide (BzPO) is a free radical generating compound that acts as a tumor promoter and progressor in mouse skin. BzPO is cleaved in the presence of Cu^{+1} to produce benzoyloxyl and phenyl radicals. Treatment of mutation reporter plasmids with BzPO and copper yields predominantly single strand breaks and G→T transversions. To explore the role of base modifications in the possible mutagenicity of BzPO, the formation of 8-hydroxy-2′-deoxyguanosine (8-OHdG) within the DNA of cultured murine keratinocytes was investigated. Treatment with 10 μM BzPO produced a maximum 3 fold increase in levels of 8-OHdG versus vehicle controls within 1–2 hrs with significant levels of 8-OHdG persisting 6 hours after initial BzPO exposure. The copper chelator, bathocuproinedisulfonic acid, reduced the levels of 8-OHdG generated by BzPO to near background. However, treatment with the iron chelator, desferal, did not. The metabolic product of BzPO, benzoic acid, was not effective in producing 8-OHdG. These results suggest that the free radical intermediate species of BzPO are responsible for the formation of promutagenic 8-OHdG in the DNA of keratinocyte cells. Supported by PO1 CA 44530 and Training Grant ES 07141.

1499 COPPER-REDOX-DEPENDENT ACTIVATION OF 2-*tert*-BUTYL(1,4)HYDROQUINONE (TBHQ)

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The metabolism of 2(3)-*tert*-butyl-4-hydroxyanisole (BHA), a carcinogenic food antioxidant, includes *o*-demethylation to TBHQ and its subsequent oxidation to 2-*tert*-butyl(1,4)paraquinone (TBQ). In this study, we have examined the capacity of copper, a nuclei- and DNA base-associated transition metal, to mediate the oxidation of TBHQ. In phosphate buffered saline, TBHQ underwent a slight autoxidation to TBQ, while the presence of Cu(II) strongly catalyzed the oxidation of TBHQ to TBQ. Oxidation of TBHQ by Cu(II) was also accompanied by the reduction of Cu(II) to Cu(I), the utilization of O_2 and the concomitant generation of H_2O_2. Using EPR spectroscopy, it was observed that copper mediated the one electron oxidation of TBHQ to semiquinone anion radicals ($SQ^{\bullet}$). The formation of $SQ^{\bullet}$, the utilization of O_2 and the generation of H_2O_2 and TBQ could be completely blocked by bathocuproinedisulfonic acid (BCS), a Cu(I) chelator. 4-Pyridyl-1-oxide-*N-tert*-butylnitrone (POBN)-spin trapping experiments showed that the reaction of TBHQ with Cu(II) resulted in the generation of POBN-CH_3 and POBN-CH(OH)CH_3 adducts in the presence of dimethyl sulfoxide (DMSO) and ethanol, respectively, suggesting the formation of hydroxyl radical or a similarly reactive intermediate. The formation of a POBN-CH_3 adduct from the TBHQ/Cu(II) plus DMSO system could be completely inhibited by catalase or BCS, indicating the reactive oxygen species is generated from the interaction of H_2O_2 with Cu(I). Incubation of supercoiled ϕX-174 plasmid DNA with TBHQ/Cu(II) resulted in extensive DNA strand breaks, which could also be prevented by catalase or BCS. The above results indicate that the activation of TBHQ by a copper-redox mechanism results in the formation TBQ, $SQ^{\bullet}$ and reactive oxygen species, and that the reactive oxygen formed may participate in DNA strand breakage. These reactions may contribute to the observed carcinogenic effects of BHA.

1500 REDUCTION OF GLUTATHIONE DISULFIDE IN OXIDATIVELY STRESSED RESPIRING RAT LIVER MITOCHONDRIA

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Factors affecting the reduction of glutathione disulfide (GSSG) by mitochondria after a *t*-butyl hydroperoxide-induced (t-BOOH) oxidative stress were studied. The amounts of ADP and mitochondrial protein were adjusted to consume less than 50% of the available oxygen during the 8 min experimental period using glutamate/malate (20 mM/2 mM), succinate (30 mM), or ascorbate/N,N,N′,N′-tetramethyl-*p*-phenylenediamine (TMPD) (30 mM/0.3 mM) as substrates. A four min treatment of mitochondria (25 mg protein/ml) with 60 μM t-BOOH oxidized 91% of total glutathione (6.3 ± 0.4 mmol/mg protein). In the presence of glutamate/malate, succinate or ascorbate/TMPD (state 4 respiration), 84, 84 and 28% of the GSSG formed during t-BOOH treatment was reduced after 4 min, respectively. A similar extent of reduction was seen during state 3 respiration (1.5 mM ADP) with glutamate/malate, but no reduction occurred during state 3 respiration with either succinate or ascorbate/TMPD. The succinate-supported reduction of GSSG was completely blocked by rotenone (0.1 μM), antimycin A (7 μM), carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) (0.4 μM), or 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) (0.1 mM). In contrast, oligomycin (5 μM) potentiated GSSG reduction using either glutamate/malate or succinate as substrates. Rotenone partially blocked glutamate/malate-supported GSSG reduction. Levels of individual pyridine nucleotides are being determined. The current data indicate that the reduction of GSSG in oxidatively-stressed isolated rat liver mitochondria occurs mainly during state 4 respiration and is independent of ATP synthesis. Mitochondrial NADPH is critical since the inhibition of glutathione reductase (an NADPH specific enzyme) by BCNU prevents the reduction of GSSG. Both the availability of mitochondrial NADH and the transmembrane proton gradient appear to be important in NADPH regeneration and consequent GSSG reduction. (Supported by HL51005.)

1501 IMMUNOCHEMICAL DETECTION OF OXIDIZED PROTEINS FORMED BY RADIOLYSIS OF FRACTIONS OF RAT RENAL AND HEPATIC TISSUE

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We have developed an immunochemical assay to detect carbonyl moieties that result from oxidative damage to proteins. Homogenates, microsomes and cytosol prepared from rat renal and hepatic tissue were exposed to hydroxyl radicals generated by a radiolysis mechanism. The resulting protein-derived carbonyls were reacted with 2,4-dinitrophenyl-hydrazine giving the corresponding hydrazones, which were detected by Western blot using anti-dinitrophenyl antisera. Analysis of the immunoblots using a densitometer indicated linear relationships between carbonyl group formation and increasing treatment from radiolysis in all fractions studied. In addition, distinct proteins stained more intensely in the immunoblots, particularly in the liver microsomes and cytosol, which demonstrated increased susceptibility of these proteins to oxidative damage. The characterization of susceptible proteins will increase our understanding of the mechanisms of oxidative toxicity, and may prove useful as early indicators of oxidative protein damage. (This study was supported in part by grant OH03061 from NIOSH and by grant R820970 from the U.S. EPA).

1502 METABOLISM AND CYTOTOXICITY OF MENADIONE AND ITS METABOLITES IN RAT PLATELETS

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Our previous studies demonstrate that menadione is cytotoxic to rat platelets by oxidative stress. In order to elucidate the mechanism of this toxicity, metabolism of menadione and cytotoxicity of its metabolite was investigated in subcellular fractions of platelets as well as platelet rich plama (PRP) isolated from female rat. When menadione was incubated in PRP, the primary metabolite was menadione glutathione conjugate (MEN-SG) which was excreted into the incubation medium. Incubation of microsomal or plasma membrane fractions of platelets with synthetic MEN-SG in the presence of NADH or NADPH led similar increases of oxygen consumption as its parent compound, menadione. However, exposure of MEN-SG to PRP resulted in neither cell lysis measured by lactate dehydrogenase nor turbidity change determined by platelet aggregometer. In addition, incubation of platelets with MEN-SG increased oxygen consumption to a lesser extent than menadione. These results suggest that even though MEN-SG was able to induce oxidative stress as potent as menadione within platelets, the MEN-SG formation from menadione in platelets may contribute to reduce the cytotoxicity of menadione due to its rapid excretion outside the cells.

1503 TIME AND CONCENTRATION DEPENDENCY OF PHORBOL ESTER TREATMENT ON MITOCHONDRIAL SUPEROXIDE GENERATION DURING DIFFERENTIATION OF HUMAN ML-1 CELLS

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The human myeloblastic leukemia cell line, ML-1, can be induced to differentiate to monocytes/macrophages by treatment with 12-O-tetradecanoyl phorbol-13-acetate (TPA). A component of the differentiation phenotype of the monocytes/macrophages is the development of mitochondrial respiration and accompanying superoxide generation. We have observed that maximal production of mitochondrial superoxide requires at least 2 to 3 days exposure to 0.3 ng/ml TPA followed by 3 days of differentiation after removal of TPA. Thus, generation of mitochondrial superoxide during the differentiation of ML-1 requires a signal that is evoked by sustained exposure to TPA. Increasing the TPA concentration to 1.2 ng/ml for the first 3 days of differentiation resulted in cells that do not exhibit mitochondrial superoxide production on day 6 of differentiation. In addition, these cells exhibit an altered morphology, were more adherent to the flasks, and have decreased surface marker CD14 expression. However, by day 9 of differentiation (i.e. 6 days after removing TPA) the cells treated with 1.2 ng/ml TPA were similar in biochemistry and morphology to cells differentiated with 0.3 ng/ml TPA. Thus, a high concentration of TPA delays the differentiation processs at a stage which lacks mitochondrial respiration. It is of interest to note that the effects observed with 1.2 ng/ml TPA are similar to what we have previously seen following treatment of ML-1 cells with 10 uM hydroquinone for 3 hours prior to induction of differentiation with TPA.

1504 COMPUTER SIMULATIONS OF LIPID PEROXIDATION IN PRECISION CUT MOUSE LIVER SLICES AND EXPERIMENTAL CALIBRATION OF THE MATHEMATICAL MODEL

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A computerized pharmacodynamic (PD) model was developed to simulate chemically induced lipid peroxidation in precision cut mouse liver slices, based on mathematical description by Tappel et al. (Free Radical Biol. Med. 1, 361, 1989). The model was written in Advanced Continuous Simulation Language (ACSL) and simulations were performed using SIMUSOLV software with optimization capabilities (DOW Chemical Co., Midland, MI) on a VAX/VMS mainframe computer. The PD model simulated formation of lipid hydroperoxides and thiobarbituric acid reactive substance (TBARS) in time as a function of remaining amounts of cytochrome P450 (CYP)-activated chemical inducer and remaining amounts of antioxidant. The rate of peroxidation was controlled by lipid peroxidizability, destruction of CYP, autooxidation, and activity of glutathione peroxidase. The PD model was initially calibrated with the literature data for TBARS formed during lipid peroxidation in rat liver slices induced with bromotrichloromethane and tert-butyl hydroperoxide (t-BOOH; Fraga et al. Free Radical Biol. Med. 4, 155, 1988). Then, the biochemical parameters were adjusted to reflect physiology of the mouse liver and the PD model was used to simulate TBARS formation during lipid peroxidation in precision cut mouse liver slices induced with t-BOOH. The PD model predictions were in agreement with the experimental data. (Supported in part by Dept. of A.F. Contract #F33615-90-C-0532.)

1505 THE EFFECT OF KETOCONAZOLE ON METABOLIC INTEGRITY IN RELATION TO ITS CYTOTOXICITY IN CULTURED RAT HEPATOCYTES

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Ketoconazole (KT) is an oral anti-fungal agent in a series of imidazole-derivatives with a broad spectrum of activity against both superficial and systemic mycoses. In many clinical studies, KT has been reported to cause liver damage. The mechanism of the KT-induced hepatic necrosis is unknown at the present time. In addition, KT has been reported to have inhibitory effects on mitochondrial respiration. The objective of this study was to evaluate the metabolic integrity of cultured postnatal rat hepatocytes after drug exposure to KT. Cytotoxicity was evaluated by measuring the leakage of the cytosolic enzyme, lactate dehydrogenase (LDH), into the medium; and by assessing mitochondrial reduction of 3-(4,5-dimethythiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT). The cultures were exposed to various concentrations of KT (5 μM to 188 μM) for 0.5 – 4 hr. Metabolic integrity was evaluated by measuring ATP and ADP levels by HPLC-UV detection methods. There was a significant increase ($p < 0.05$) in LDH leakage and decrease in MTT reduction at 4 hr for the higher concentrations (>80 μM) studied for KT. There was a 25% decrease in ATP/ADP ratios at 80 μM KT for 0.5 hr and a 30% decrease at 40 μM and 44% decrease at 80 μM after 1 hr. These results demonstrate that KT resulted in a time-and dose-dependent decrease in ATP/ADP ratios at concentrations lower than those seen for cytotoxicity. This decrease in production of ATP may be responsible for the observed cytotoxicity of KT. (Supported by NIDDK).

1506 COMPARATIVE CYTOTOXICITY OF UK-66,914 AND ITS METABOLITE, 1-(4-PYRIDYL)PIPERAZINE IN RAT, DOG, AND MONKEY HEPATOCYTES

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UK-66,914 is a class III antidysrhythmic agent that was intended for the comprehensive treatment of ventricular and supraventricular rhythm disorders. It was discontinued, however, because of potential human hepatotoxicity suggested by elevated serum enzymes of hepatic origin. Our purpose was to test both parent drug and the only urinary metabolite detected, 1-(4-pyridyl)piperazine (4-PP), in primary canine, cynomolgus monkey, or CD rat hepatocyte cultures to determine if any of these *in vitro* systems were predictive of human hepatotoxicity. After 20 hour exposure, concentrations producing 50% viability (via morphological examination) were 550, 1050, and 1950 μM for UK-66,914 versus 1950, 850, and >3200 μM for 4-PP in monkey, rat, and dog cells, respectively. When measured, LDH, ALT, and AST leakage increased and neutral red uptake decreased with increasing 4-PP exposure in rat hepatocytes. Within 15 minutes, conspicuous cytoplasmic vacuoles, were noted in all cell types at all concentrations of 4-PP $\geq$ 190 μM but only at UK-66,914 concentrations $\geq$ 1300, 700, or 2000 μM in monkey, rat, and dog cells, respectively. Vacuole formation was reversible in the absence of drug. These data suggest that cytotoxicity is more extensive with the parent compound than the metabolite in 2 of 3 species, detoxification via species-specific metabolism is not a factor in comparative hepatotoxicity, and that monkey hepatocytes may be more predictive of human liver than are rat or dog cells.

1507 ASSESSMENT OF CYTOTOXIC RESPONSES TO SELECTED AROMATIC HYDROCARBONS IN PRIMARY CULTURES OF ADULT RAT HEPATOCYTES

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The cytotoxic responses of cultured rat hepatocytes to selected aromatic hydrocarbons (AHs) were assessed to determine if class-specific differences in toxicity potential exist among this group of chemicals. Hepatocytes were exposed to benzo(a)pyrene (BaP, 0.3–300 μM), 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, 0.001–1 nM), 2,3,7,8-tetrachlorodibenzofuran (TCDF, 0.005–5 nM), naphthalene (NAPH, 0.1–1000 μM), 2-methylnaphthalene (2-MNAPH, 0.1–1000 μM), or pentachlorophenol (PCP, 0.1–1000 μM) for 24 hr. 3H-Thymidine incorporation into DNA, intracellular levels of glutathione (GSH) and lactate dehydrogenase (LDH) leakage were monitored as indices of cytotoxicity. BaP, TCDD and TCDF induced a concentration dependent inhibition of DNA synthesis, while NAPH, 2-MNAPH, and PCP were without

effect. There were significant increases of GSH levels in hepatocytes treated with BaP at all concentrations, while modest increases in GSH levels relative to controls were observed in cultures treated with TCDD or TCDF. The other hydrocarbons failed to modulate cellular GSH levels. Increased LDH leakage was only observed in hepatocytes treated with PCP at the 1000 μM concentration. Based on these data, we conclude that hepatocytes exhibit differential cytotoxic response profiles to aromatic hydrocarbons and that only aryl hydrocarbon receptor ligands are toxic to cultured hepatocytes. (Supported by NIH grant ES 04917).

1508 ASSESSMENT OF *IN VITRO* CYTOTOXICITY OF NOVEL BILE SALTS: EFFECT ON MEMBRANE INTEGRITY

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Bile salts are potent detergents capable of causing cholestasis and liver injury when delivered to the liver at high concentrations. This effect may be attributable in part to the ability of bile salts to disrupt cellular membranes by extracting membrane components. To investigate the potential of six novel bile salts to disrupt cellular membranes, primary rat hepatocyte cultures were exposed to varying concentrations of bile salts and membrane integrity was determined by measuring the release of cellular LDH or the incorporation of the fluorescent DNA probe, propidium iodide. LDH release and propidium iodide incorporation were both concentration and time dependent. The trihydroxy bile salts, CGS 26292A, CGS 27761A and CGS 29461A are more hydrophilic and thus were less cytotoxic than the dihydroxy salts, CGS 26291A and CGS 27762A. CGS 29462A, a taurine conjugated dihydroxy bile salt, was less cytotoxic than the dihydroxy bile salts due to the addition of the hydrophilic taurine moiety. All six compounds were shown to be less cytotoxic than the hydrophobic bile salts, deoxycholate and chenodeoxycholate. These assays provided a simple, rapid and sensitive method for evaluating the effect of subtle changes in bile salt structure on their potential to disrupt cellular membranes.

1509 ALLYL ALCOHOL TOXICITY IN FRESHLY ISOLATED AND CRYOPRESERVED HEPATOCYTES MAINTAINED IN COLLAGEN SANDWICH CONFIGURATION

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A simple procedure for cryopreserving isolated rat hepatocytes has been developed recently (Zaleski, et al., Biochem. Pharmacol. 46: 111, 1993). The purpose of the present study was to: (1) compare behavior of freshly isolated and cryopreserved hepatocytes in long term culture in a collagen sandwich configuration, and (2) determine whether sensitivity to hepatotoxic agents differ in freshly isolated and cryopreserved cells maintained in long term culture. Previous work indicated that while cryopreserved and freshly isolated cells are identical with respect to drug and energy metabolism, cryopreserved cells in suspension are more sensitive to the hepatotoxicants: allyl alcohol (AA), acetaminophen and carbon tetrachloride than freshly isolated hepatocytes. Accordingly, we compared the actions of AA on urea biosynthesis, albumin secretion and drug metabolism in freshly isolated and cryopreserved hepatocytes maintained in long term culture in a sandwich collagen configuration. AA (100 μm) did not affect basal urea biosynthesis, but decreased ammonia stimulated rates of urea biosynthesis in both freshly isolated and cryopreserved cells. The magnitude of inhibition of stimulated rates observed with both group of cells maintained on collagen sandwiches was comparable to that noted previously with cells kept in suspension. Production of conjugated metabolites using 7-ethoxycoumarin as a model substrate was maintained constant for at least 3 days; however, total metabolism was lower (about 20%) than of that seen in freshly isolated hepatocytes. Formation of phase II metabolites was not influenced by AA(100 μM) over the first two days of culture but was inhibited on the third day. In conclusion, the sandwich culture configuration enables freshly isolated and cryopreserved hepatocytes to maintain biological processes such as urea biosynthesis and drug metabolism and, thus, provides a useful system to study the action of hepatotoxic agents over relatively long periods of time. (Supported, in part, by a gift from Sandoz Pharmaceutical Corp. and NIEHS Center Grant ES-05052)

1510 AN ARTIFICIAL-LIVER BIOREACTOR FOR SHORT-TERM ^{31}P NMR AND MRI STUDIES ON ISOLATED RAT HEPATOCYTES

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We have developed a NMR-compatible bioreactor for non-invasive monitoring of ^{31}P and ^{13}C metabolites and near microscopic (64 μm resolution) magnetic resonance images (MRI). The bioreactor design is ideal for perfusion of hepatocytes because of (1) its similarity to liver ascinus architecture, (2) uniform perfusion of cells, (3) rapid and easy extrication of cell mass without destruction of bioreactor, (4) rapidity of preparation, (5) ability to use high resolution MRI's to check fiber integrity and cell distribution, and (6) its small volume which permits use of only one animal per study.

We demonstrate the ability to monitor bioenergetics by ^{31}P NMR of hepatocytes with 30 min timepoints and maintain viability in the bioreactor for at least 4 hrs using Krebs-Heinseleit (K-H) buffer. ^{31}P spectra obtained from hepatocytes perfused in the bioreactor were similar to those obtained from liver of intact rat. Hepatocyte extracts, obtained after 4 hrs of perfusion with K-H buffer revealed an ATP/ADP ratio of 3.1, indicative of normal energy charge. Staining with trypan blue revealed 80% viability of cells after 4 hrs of perfusion. Non-phosphate K-H buffer was also tested in order to obtain pH information, but hepatocytes did not remain viable. The MRI's revealed diverse information including (1) cell distribution, (2) flow dynamics, (3) fiber integrity, (4) magnetic susceptibility, (5) air bubbles and, (6) coil inhomogeneity. Various inoculation methods were used to increase cell packing, and thus increase temporal resolution. This is an ideal *in vitro* model for xenobiotic studies using non-invasive monitoring of bioenergetics as an early indicator of stress.

1511 ANALYSIS OF THE CHRONOLOGY OF PATULIN-INDUCED CELLULAR INJURY: MULTIPARAMETER FLUORESCENCE KINETIC ANALYSIS

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Vital fluorescence assays have previously been employed to identify cellular endpoints of patulin-induced toxicity (Burghardt et al., Toxicol Appl Pharmacol 112:235, 1992). In this study, kinetic analysis of a series of endpoints in a clonal rat liver line were monitored by laser cytometry. Endpoints evaluated during exposure to the mycotoxin (0.001, 0.01, 0.1, and 1.0 mM) included intracellular glutathione (GSH) levels, plasma membrane (PMP) and mitochondrial membrane potential (MMP), intracellular Ca^{2+} ($[Ca^{2+}]_i$) and pH, and production of reactive oxygen species (ROS). Typically, two fluorescent probes were preloaded into cells and employed in kinetic experiments in which two endpoints were monitored simultaneously with separate detectors. Each patulin dose was added directly to cultures and the intensity of fluorescence was recorded at 30 sec or 1 min intervals over 20 minutes. Depletion of intracellular GSH was the first change detected (20 min, 0.001 mM or 5 min 0.01 mM) which was followed by generation of ROS, altered $[Ca^{2+}]_i$ and MMP. Changes in intracellular pH and plasma membrane potential were the last alterations in the sequence. Major changes in all endpoints were detected with 1.0 mM patulin and no separation of injury was possible. These studies suggest that initial patulin-induced cellular injury involves GSH depletion and damage to sulfhydryl containing proteins. While plasma membrane is considered a major target of injury, changes in PMP and intracellular pH were late events which were preceded by production of ROS and alterations in $[Ca^{2+}]_i$ and MMP. (Supported by NIH P42-ES04917 and a TAES REP).

1512 CYTOTOXICITY AND MITOCHONDRIAL ACTIVITY OF WB 344 HEPATOCYTES WITH EXPOSURE TO AMMONIUM DINITRAMIDE: A LASER CONFOCAL MICROSCOPY STUDY

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Biological effects of ammonium dinitramide (ADN) were tested to determine toxicity in WB 344 hepatocytes. ADN is a high-energy compound under study as a replacement of environmentally unsafe rocket propellants. The WB 344 hepatocytes were incubated with ADN (0–10 mM) for 24h. Liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured to estimate membrane integrity. ADN increased leakage of both these enzymes at all concentrations studied. After normalization of results to % control, EC_{50} was determined: ALT EC_{50} = 0.842 mM and AST EC_{50} = 2.423 mM. To measure ADN-mediated action on mitochondrial potential 3

mM of ADN was exposed to the cell line for 24h. Rhodamine 123 (Molecular Probes, OR) was loaded into the cell line at the concentration of 1 μM and incubated for 30 min. The probe is used to detect the activity of the mitochondria. Preliminary results indicate that ADN decreases mitochondrial activity by 5.9%. Fluorescence signal was collected on an InSight IQ laser confocal microscope using the 488 nm excitation line of an argon laser and a 530/30 filter. Signal was quantitated by integrating fluorescence over a user defined area per cell number.

1513 DEXAMETHASONE INDUCES RESISTANCE TO THE LETHAL CONSEQUENCES OF MITOCHONDRIAL ELECTRON TRANSPORT INHIBITION IN CULTURED HEPATOCYTES

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Pretreatment with 1 μM dexamethasone protects cultured rat hepatocytes against cell killing by 5 μM rotenone and 1 mM KCN. Simultaneous treatment with this glucocorticoid is ineffective, as are other hormones without glucocorticoid activity. The protective effect of dexamethasone is blocked by the receptor antagonist, RU486, and by the inhibitor of protein synthesis, cycloheximide. Increasing the time of pretreatment with dexamethasone increases the degree of protection. Pretreatment with dexamethasone has no effect on either the decline of cellular ATP or the loss of the mitochondrial membrane potential produced by rotenone and KCN. In addition, dexamethasone does not prevent the mitochondrial permeability transition. By contrast, dexamethasone does prevent the increased release of [^{3}H]arachidonic acid from phospholipids produced by KCN. These data describe an inductive effect of dexamethasone in protecting cultured hepatocytes against electron transport inhibition. Pretreatment with dexamethasone prevents cell killing by inhibiting the mechanism that couples the mitochondrial permeability transition to the accelerated degradation of plasma membrane phospholipids. Supported by NIH grants DK38305 and AA07186.

1514 A LESION SUBSEQUENT TO LIPID PEROXIDATION IN THE GENESIS OF CELL DEATH

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Cultured rat hepatocytes are killed by *tert*-butyl hydroperoxide (TBHP) by a mechanism that depends on the peroxidation of cellular phospholipids. Accordingly,
antioxidants, such as vitamin E and *N,N'*-diphenylphenylenediamine, prevent this lipid peroxidation and protect against the cell death. We report here that strychnine, glycine and two nonsteroidal anti-inflammatory agents (niflumic acid and meclofenamic acid) protects the hepatocytes against the cell killing by TBHP without evident antioxidant action. Thus, there was no effect on the peroxidation of lipids, as measured by the accumulation of malondialdehyde in the culture medium. Schnellmann and his colleagues have reported protection by strychnine and glycine against the killing of suspensions of renal proximal tubular cells by hypoxia and a variety of chemicals (*Toxicol. Appl. Pharmacol.* 125: 102, 1994). Protection against this renal toxicity was interpreted as a consequence of blocking a chloride channel. Studies are currently in progress to determine whether the agents reported above to protect cultured hepatocytes act by a similar mechanism. Supported by NIH grants DK38305 and AA07186.

1515 FATTY ACIDS POTENTIATE CELL KILLING DURING MITOCHONDRIAL ELECTRON TRANSPORT INHIBITION IN CULTURED HEPATOCYTES

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The effects of saturated and unsaturated fatty acids (FA) on cell killing were studied in cultured rat hepatocytes treated with 5 μM rotenone. Linoleate (18:2) potentiates the killing of hepatocytes by rotenone, and 2 mM L-carnitine (LC) protects the cells against this potentiation. Palmitoleate (16:1), oleate (18:1) and arachidonate (20:4), but not palmitate (16:0) and stearate (18:0), also increase killing by rotenone. LC protects against the increased cell killing by all the unsaturated FA. Incubation of cultured cells with FA increases long chain acyl CoA both alone and with rotenone. Incubation with 1 μM of the antioxidant DPPD has little or no effect on the potentiation of cell killing with FA. Neither FA nor LC affects the decrease in ATP caused by rotenone. The induction of the MPT in isolated mitochondria is affected by the concentration of long chain Acyl CoA but not by chain length or degree of saturation, suggesting that the increased killing may not be a consequence of the ability of different fatty acid CoA moieties to induce the mitochondrial permeability transition (MPT). These data indicate that under conditions of mitochondrial deenergization, an increased FA content damages cultured hepatocytes by potentiating the induction of the MPT. Supported by NIH grants DK38305 and AA07186.

1516 CALCIUM LOADING POTENTIATES THE KILLING OF CULTURED HEPATOCYTES BY ITS EFFECT ON THE MITOCHONDRIAL PERMEABILITY TRANSITION

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The calcium ionophore, A23187, and the inhibitor of the calcium ATPase of the endoplasmic reticulum, thapsigargin (TG), both increase the intracellular calcium content. A23187 and TG potentiate the killing of cultured hepatocytes treated with 2 μg/ml rotenone or 0.1 μg/ml oligomycin. 5 μM cyclosporine A prevents the increased cell killing in the presence of both A23187 and TG. Both rotenone and oligomycin deplete cellular stores of ATP. Rotenone alone decreases the mitochondrial membrane potential, whereas oligomycin alone does not. A23187 and TG potentiate the loss of the mitochondrial membrane potential in hepatocytes treated with rotenone. By contrast, with A23187 and TG, there is no loss of the mitochondrial membrane potential in oligomycin-treated cells prior to the loss of viability. These data document two distinct mechanisms whereby calcium loading potentiates the mitochondrial permeability transition (MPT) and thus cell killing. With rotenone, there is increased mitochondrial de-energization, a condition that potentiates the MPT. With oligomycin, the MPT is potentiated by a mechanism that is not dependent on mitochondrial de-energization and likely represents pore opening by an increased mitochondrial matrix calcium content. Supported by NIH grants DK38305 and AA07186.

1517 UNCOUPLING OF OXIDATIVE PHOSPHORYLATION BY FURAN IN ISOLATED RAT HEPATOCYTES

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Furan is a potent rodent carcinogen which produces hepatic carcinomas and cholangiocarcinomas in male and female F-344 rats. Furan-induced carcinogenesis dues not occur via a DNA-reactive species, but instead appears to be linked to cytolethality followed by cell proliferation and tumor formation. Previous studies in our laboratory indicate that furan-induced cytolethality is related to changes in hepatic energy metabolism. Furan produced a dose-dependent ATP depletion and uncoupling of oxidative phosphorylation *in vivo* in male F-344 rats (Carfagna and Kedderis (1993) *Toxicologist* 13: 201). The present studies were conducted to determine the mechanism of furan-induced ATP depletion in isolated hepatocytes *in vitro*. Isolated F-344 rat hepatocytes were incubated with furan (2–100 μM) for 1 hr, and mitochondria were prepared. Furan produced a concentration-dependent depletion of hepatocyte ATP (>90%). Oxygen uptake measurements from furan-treated hepatocytes showed an increase in state 4 (succinate-stimulated) mitchondrial respiration, but no changes in state 3 (ADP-stimulated) mitochondrial respiration. These data indicate that furan produces the same uncoupling of oxidative phosphorylation in hepatocytes *in vitro* as is observed in intact rats. Future studies will investigate the toxic responses of furan-induced changes in hepatic energy metabolism such as the activation of calcium-dependent endonucleases which can damage DNA.

1518 KINETICS OF ACRYLONITRILE (ACN) BIOTRANSFORMATION BY ISOLATED RAT HEPATOCYTES

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The biotransformation kinetics of ACN were determined in freshly isolated F-344 rat hepatocytes and examined for their ability to predict ACN pharmacokinetics in intact rats. The solubility of ACN in heat-inactivated cells and Williams' Medium E was determined using vial bison methods. ACN disappearance from hepatocyte suspension (measured by gas chromatography) was due to saturable cytochrome P450 epoxidation and both enzyme-mediated and chemical reactions of ACN with glutathione (GSH). The second-order rate constant for the chemical reaction between ACN and GSH at pH 7.3 was 0.26 L/mmol/hr. The parameters for the random kinetic mechanism of ACN GSH conjugation were estimated from studies with hepatocytes from rats treated with aminobenzotriazole (2 × 100 mg/kg ip) to inactivate cytochrome P450. These parameters accurately simulated ACN hepatocyte biotransformation

in the presence of the P450 inhibitor phenylimidazole (1 mM). Using these GSH conjugation parameters, the parameters for the P450 epoxidation of ACN (V_{max} of 24.4 nmol/hr/10^6 cells and K_M of 3 μM) were estimated from hepatocyte kinetic data. Extrapolation of the hepatocyte kinetics to intact rats based on the cellularity of the liver provided reasonable simulations of previously obtained ACN pharmacokinetic data (Kedderis *et al.* (1994) *Toxicologist* 14: 43). Future studies will utilize isolated human hepatocytes as the basis for development of a ACN dosimetry description in people.

1519 PGE_2 AND cAMP STIMULATE OXYGEN UPTAKE IN RAT HEPATOCYTES

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Prostaglandin E_2 (PGE_2) has been reported to stimulate parenchymal cell oxygen consumption when added to the perfused liver; however, little is known about the mechanisms involved. PGE_2 added directly to parenchymal cells increased oxygen uptake in a dose-dependent manner from basal values of 35.6 ± 0.9 to 55.4 ± 6.6 μl/hr/10^6. Since PGE_2 elevates cAMP, we studied the effect of dibutyryl adenosine 3′:5′-cyclic monophosphoric acid (D-cAMP) and 8-bromoadenosine 3′:5′-cyclic monophosphate (Br-cAMP), cAMP derivatives which enter intact cells, on oxygen uptake in hepatic parenchymal cells. These agents caused a dose-dependent increase in oxygen consumption from basal values of 31.3 ± 1.0 to 44.5 ± 1.7 and 41.9 ± 2.2 μl/hr/10^6, respectively. Thus, cAMP and PGE_2 had similar effects on oxygen uptake in isolated parenchymal cells. The increase of oxygen uptake was blocked by KCN, suggesting involvement of the mitochondria. Further, we demonstrated that both PGE_2 and D-cAMP increased intracellular free Ca^{2+} concentration ([Ca^{2+}]i) about 4-fold in freshly isolated rat parenchymal cell using the fluorescent Ca^{2+} indicator, fura-2. When extracellular Ca^{2+} was omitted from the incubation medium and 1 mM EGTA was added, the increase in [Ca^{2+}]i caused by PGE_2 was only increased to about one-third of control values. Therefore, our working hypothesis is that PGE_2 interacts with receptors on hepatic parenchymal cells and increases cAMP levels, which in turn increases mitochondrial oxygen uptake by mechanism possibly involving intracellular free Ca^{2+} (AA-09156 and AA-03624).

1520 MECHANISM OF N-NITROSODIMETHYLAMINE (NDMA) HEPATOTOXICITY: CONTRIBUTION OF DENITROSATION AND α-HYDROXYLATION

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An understanding of the mechanisms of hepatocyte death and the concomitant compensatory cell proliferation is crucial to our understanding of hepatocarcinogenesis by NDMA. Previous studies of hepatocyte toxicity induced by NDMA showed that neither lipid peroxidation nor methylation of proteins can account for the cytotoxic effects of this compound. We have used monolayer cultures of primary hepatocytes to determine the contributions of each of the two pathways of NDMA metabolism to its cytotoxicity. NDMA produces a concentration-dependent increase in toxicity, with an $LC_{50} \approx 0.1$ mM, when measured 48 h after treatment. The metabolites formaldehyde, methanol and methylamine were not toxic at 0.1 mM. α-Acetoxy-NDMA (AcO-NDMA), a stabilized derivative of the unstable product of NDMA α-hydroxylation, produced a similar concentration-response curve to NDMA. Inhibition of O^6-methylguanine repair in DNA by O^6-benzylguanine resulted in a 40 ± 5% (n = 4) increase in AcO-NDMA toxicity, suggesting that DNA methylation contributes to NDMA cytotoxicity. Nitric oxide (NO) was generated spontaneously *in vitro* by the breakdown of a spermine/NO complex (Sper/NO) to give spermine and NO. When added to cultures, Sper/NO produced toxicity at concentrations $\geq$0.25 mM, with an $LC_{50} \approx 0.75$ mM. Spermine alone was not toxic at this concentration. Altogether, our results suggest that α-hydroxylation of NDMA resulting in DNA methylation is a major contributor to NDMA hepatotoxicity, though production of NO may also contribute.

1521 COVALENT BINDING OF THE DICHLOROMETHANE (DCM) METABOLITE, FORMALDEHYDE, TO NUCLEIC ACIDS IN RODENT AND HUMAN HEPATOCYTES

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The glutathione-S-transferase (GST)-dependent metabolism of DCM leads to formaldehyde (HCHO), which forms DNA-protein cross-links (DPX) in mouse but not in hamster liver. Mice but not hamsters developed hepatocellular carcinomas in inhalation bioassays of DCM. To compare human responses to DCM with those of rodents, metabolism of DCM via the GST pathway was examined in freshly isolated hepatocytes from B6C3F1 mice, F344 rats, and Syrian golden hamsters, and experiments using human hepatocytes were initiated. Hepatocytes were incubated with [^{14}C]DCM (0.5–5 mM), nucleic acids (DNA and RNA) were isolated, and covalently bound [^{14}C]HCHO was determined. Concentration-dependent formation of DPX (1–25 pmol/mg DNA) was observed only in mouse hepatocytes. Yields of DPX in mouse hepatocytes *in vitro* and in mouse liver *in vivo* were similar, when concentrations of DCM in hepatocytes corresponded to those calculated in liver using a physiologically-based pharmacokinetic model RNA-HCHO adducts were detected in hepatocytes from all species (mouse > rat ≅ human > hamster). Unlike DNA, soluble RNA containing covalently bound HCHO was extractable from proteins, indicating that at least a portion of the HCHO is not bound as RNA-protein cross-links. We propose that the HCHO bound to RNA may be used as a surrogate for the amount of DCM metabolized via the GST pathway in hepatocytes from different species. The use of human and rodent hepatocytes in conjunction with genetic analysis of human GST polymorphisms (D A Bell *et al., JNCI* 85: 1159 (1993)) may lead to more reliable estimates of human carcinogenic risks associated with exposure to dichloromethane.

1522 HUMAN HEPATOCYTES IN TOXICITY TESTING METHODS

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Interspecies variability in the toxic potential of chemicals has lead to an increased use of human cells in the toxicity testing methods. Present study describes the evaluation of human hepatocytes in nick translation assay, replication & sister chromatid exchange (SCE) studies and the lactate dehydrogenase (LDH) analyses. Positive control compounds such as cyclophosphamide, mitomycin C, dimethylnitrosamine and chlorambucil induced dose dependent increases of DNA damage when examined for genotoxicity. When four different semi purified extracts of barracuda samples were evaluated, two toxic samples could be distinguished from nontoxic samples by the induction of genotoxicity. Results obtained indicate that human hepatocytes can be used effectively in the toxicity assays and preliminary studies also show that ciguatoxin(s) can induce genotoxicity in human cells.

1523 INDUCTION OF CYP1A IN PRECISION CUT RAINBOW TROUT LIVER SLICES BY INDOLE-3-CARBINOL ACID CONDENSATION PRODUCTS

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We are investigating possible involvement of the Ah receptor in tumor promotion by indole-3-carbinol (I3C), a constitutive component in cruciferous vegetables, such as broccoli, cabbage, etc. Previous studies indicate that I3C itself is not the causative agent. I3C undergoes condensation reactions in the acidic stomach environment, leading to various condensation products. We used liver slices as an *in vitro* model to assess potential for CYP1A induction of I3C, I33′ (linear dimer of I3C), indolocarbazole (ICZ), as well as α- and β-naphthoflavone (ANF and BNF). Slices were prepared using a Krumdieck tissue slicer, and incubated in Hanks modified media for 48 h at 14° C. Test compounds were administered in incubation media for the first 24 h. Slices were incubated in 12-well tissue culture plates using an orbital shaker and their viability was monitored by assays of ATP levels and LDH leakage. After 48 h CYPIA protein levels were assessed by Western Blotting. As expected, I3C did not lead to any detectable induction. I33′ induced CYP1A significantly at concentrations as low as 2 μM. The effect of ICZ exposure was comparable to that of BNF, induction by which was evident at 0.1 μM and was maximum at 40 μM. ANF was a weaker agonist but was able to induce trout CYP1A at 2 μM and higher. The liver slice technique is a suitable model for assessing trout CYP1A induction by various Ah receptor ligands. I3C acid condensation products possess CYP1A inducing properties unlike the parent compound. Supported by PHS grant ES04766.

1524 LIVER SLICES IN DYNAMIC ORGAN CULTURE (LSDOC) AS A MODEL SYSTEM FOR INTEGRATED PHASE I–PHASE II DRUG METABOLISM

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Cytochrome P-450 (CYP450) metabolism-mediated toxicity, may be envisioned as a balance between the production of reactive species (typically phase

I) and bioprotective reactions (phase II). Using LSDOC we aimed to demonstrate the response of this system to phenobarbital (PB), a prototypic inducing agent. This methodology, unlike other cellular *in vitro* preparations, preserves the acinar architecture of the liver. Additionally, remnant tissue was be used for comparison of subcellular fractions.

Preliminary data using LSDOC demonstrated time-dependant increases in 7-Methoxy- and 7-Ethoxycoumarin (7-MC & 7-EC, respectively) metabolites. 7-EC metabolism proceeded at a faster rate than 7-MC. 7-Hydroxycoumarin (7-HC) glucuronidation and sulfation patterns of capacity-limited metabolism were also identified. The patterns of phase I metabolism from LSDOC correlated with rates determined from microsomal fractions from these tissues. Microsomal fractions from PB pretreatment revealed characteristic increases in CYP450 content, metabolism and glucuronosyl transferase activity. Similar increases in these parameters were observed using LSDOC. Phase I metabolism of 7MC, 7-EC and phase II glucuronidation of 7-HC was increased up to 3 fold. In contrast, sulfation rates were moderately increased by PB pretreatment.

Phase I-phase II drug metabolism may be studied in concert or in isolation using the same tissue. LSDOC's response to a prototypic inducing agent was found to correlate qualitatively with alternative *in vitro* preparations. Thus, LSDOC may provide a decided advantage in the study of bioactivation-toxicity relationships.

1525 FURTHER CHARACTERIZATION OF CULTURED RAT LIVER SLICES FOR TOXICOLOGICAL STUDIES

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Use of precision cut liver slices for predicting the toxicity of various compounds is increasing. We have studied cytochrome P450 1A1 activities in intact slices cultured up to 96 hrs in serum free Chee's essential medium supplemented with insulin, transferrin, selenium, dexamethasone and epidermal growth factor. Slices were incubated in 12 well plates on a shaker at 37° C in an air: CO_2 (95:5%) incubater. Induction of CYP 1A1 with 5 μM β-napthoflavone (βNF) was optimal at 72 hrs while the basal activity was fully maintained as measured by the metabolism of EROD in intact slices rather than in homogenates. Slices were also monitored for albumin secretion in order to confirm their hepatospecific function. After the initial sloughing of the cells albumin secretion remained stable. Slices were analyzed for mRNA expression of CYP 1A1 and albumin genes. After 48 hrs, 1A1 mRNA levels were higher in the βNF-induced slices, correlating with the level of enzyme activity. Histology of the slices revealed extensive degeneration except for a peripheral layer of hypertrophic cells irrespective of whether the shaker or a dynamic roller culture system was used. Efforts are in progress to improve the overall viability of the slices using a Matrigel coat so that they can be used for toxicity and related studies. Supported by The Johns Hopkins Center for Alternatives to Animal Testing (In Vitro Toxicology Program).

1526 UPTAKE-LIMITED TOXICITY IN RAT LIVER SLICES

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L-689,502 is a potent, rapid-acting hepatotoxin in rats, inducing cholestasis and periportal hepatocellular necrosis within 30 minutes after an i.v. dose (2.5 mg/kg). This agent caused increased LDH leakage in isolated hepatocytes (EC50 50 μM) but was not toxic in cultured rat liver slices at concentrations up to the limits of solubility (200 μM). This difference in susceptibility to toxicity appeared to be due to much greater accumulation of L-689,502 in rat hepatocytes compared to liver slices. L-689,502 was found to be taken up against a concentration gradient by a saturable, temperature-sensitive process in both systems. The uptake rate was significantly slower in cultured rat liver slices than in isolated hepatocytes. The uptake of taurocholate, mediated via the bile acid transport system, was also much slower in slices than hepatocytes. However, taurocholate and L-689,502 did not appear to be taken up by the same transport mechanism. Phalloidin is selectively hepatotoxic by virtue of active transport into hepatocytes via the bile acid transport system. Phalloidin toxicity was evident in isolated rat hepatocytes but much less pronounced in liver slices. These findings indicate that this phenomenon may be common to many agents taken up by carrier-mediated processes. Increased uptake rates were observed by changing from the conventional roller culture system to continuous immersion with orbital shaking. One interpretation is that the rate-limiting uptake step in slices is solute delivery rather than carrier-mediated transport. Freely diffusible solutes may enter the hepatocytes via transcellular as well as pericellular pathways. Compounds with limited diffusion may only be delivered via pericellular pathways. Without perfusion *in vitro*, sinusoidal spaces in slices may collapse limiting pericellular solute delivery to the parenchymal cells. These results present an important consideration in the use of cultured rat liver slices where active transport processes may be critical.

1527 EFFECT OF PIPERONYL BUTOXIDE (PBO) ON UNSCHEDULED DNA SYNTHESIS IN PRECISION-CUT HUMAN LIVER SLICES

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PBO is a pesticide synergist which is used in crop protection, environmental health, and for controlling body lice in children. In this study we have examined the effect of PBO on unscheduled DNA synthesis (UDS) in cultured human liver slices. Precision-cut liver slices (250 μm) were prepared with a Krumdieck tissue slicer and cultured for 24 hr in RPMI 1640 medium containing 5% fetal calf serum, [^{3}H]thymidine and 0–2.5 mM PBO using a dynamic organ culture system. As positive controls human liver slices were also cultured with 0.02 and 0.05 mM 2-acetylaminofluorene (2-AAF), 0.002 and 0.02 mM aflatoxin B_1 (AFB_1) and 0.005 and 0.05 mM PhIP (a heterocyclic cooked food mutagen). Liver slices were fixed in formalin and processed for autoradiographic evaluation of UDS. Compared to control cultures PBO had no effect on UDS expressed either as the net grain count (NGC) or as the percentage of centrilobular hepatocyte nuclei undergoing DNA repair with >5 and >10 net grains. In contrast, all three known mutagens produced a marked increase in UDS in human liver slices. For example, NGC values in control, 0.05 mM 2-AAF, 0.02 mM AFB_1 and 0.05 mM PhIP cultures were –0.4, 8.0, 10.8 and 30.8, respectively. These results provide further evidence that PBO is a non-genotoxic agent which does not damage DNA in human liver. (Supported by PBO Task Force II).

1528 EFFECT OF COOKED FOOD MUTAGENS ON UNSCHEDULED DNA SYNTHESIS (UDS) IN PRECISION-CUT RAT, CYNOMOLGUS MONKEY AND HUMAN LIVER SLICES

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Previous work has demonstrated that precision-cut liver slices are a valuable *in vitro* model system for investigating the metabolism and toxicity of xenobiotics. We have used this technique to examine the genotoxicity of two heterocyclic cooked food mutagens. Precision-cut liver slices (250 μm) were prepared from untreated and Aroclor 1254 (ARO) treated (single i.p. 500 mg/kg dose 5 days prior to necropsy) male Sprague-Dawley rats and from Cynomolgus monkeys and humans. Slices were cultured in RPMI 1640 medium containing 5% fetal calf serum, [^{3}H]thymidine and 0–0.05 mM of either MeIQ or PhIP using a dynamic organ culture system. After 24 hr liver slices were fixed in formalin and processed for autoradiography. UDS was quantified as the net grain count (NGC) and the percentage of centrilobular hepatocyte nuclei undergoing DNA repair with >5 and >10 net grains. MeIQ-induced UDS was more marked in liver slices from ARO treated than untreated rats, whereas PhIP had little effect. Although neither MeIQ nor PhIP produced any significant effect on UDS in Cynomolgus monkey liver slices, positive results were obtained with other known genotoxins. In human liver slices both MeIQ and PhIP produced concentration-dependent increases in UDS, the effect being particularly marked with PhIP. For example, NGC values in control, 0.02 mM MeIQ and PhIP treated cultures were –0.4, 1.9 and 23.2, respectively. Liver slices appear to be a useful alternative *in vitro* test system to cultured hepatocytes for UDS studies and the results demonstrate that cooked food mutagens can produced marked compound and species differences in response. (Supported by the UK Ministry of Agriculture, Fisheries and Food).

1529 METABOLISM AND DNA BINDING OF THE COOKED-FOOD MUTAGEN, 2-AMINO-1-METHYL-6-PHENYL-IMIDAZO[4,5-b] PYRIDINE (PhIP) IN PRECISION-CUT RAT LIVER SLICES

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Precision-cut liver slices prepared from Aroclor 1254 pretreated male rats

were used to investigate the metabolism of 2-amino-1-methyl-6-phenyl-imidazo[4,5-*b*] pyridine (PhIP). The acetyltransferase and sulfotransferase inhibitors pentachlorophenol (PCP) and 2,6-dichloro-4-nitrophenol (DCNP), and the cytochrome P450 inhibitor, α-naphthoflavone (ANF) were used to modulate PhIP metabolism and DNA and protein adduct formation. PCP and DCNP had similar effects on the formation of some PhIP metabolites. Metabolite formation as percent of controls was: 10% for 4'-PhIP-sulfate, 55% for N-hydroxy-PhIP-glucuronide formation, 100% for Peak B, and 150% for 4'-hydroxy-PhIP. PCP and DCNP had different effects on the formation of other PhIP metabolites. Metabolite formation as percent control for Peak A was 50% and 96% in incubations with PCP and DCNP respectively. Formation of 4' hydroxy-PhIP-glucuronide was 10% with PCP and 147% with DCNP. ANF decreased metabolite formation by 60–95%. The enzyme inhibitors had no statistically significant effect on PhIP-DNA binding. Covalent binding of PhIP to protein was slightly decreased in incubations containing DCNP or PCP. The lack of significant changes in covalent binding to either DNA or protein suggests that additional pathways may be important in PhIP bioactivation in rat liver slices. With ANF, there was a significant decrease (35%) in protein binding. These observations on the effects of PCP, DCNP and ANF on PhIP metabolism as well as on covalent binding of PhIP to tissue macromolecules are in close agreement with what was reported earlier in hepatocytes. This indicates that tissue slices from various target tissues for tumorigenesis will be a useful *in vitro* tool for future studies on heterocyclic amine metabolism.

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1530 VITAMIN A PRETREATMENT POTENTIATES VINYLIDENE CHLORIDE-INDUCED LIVER DAMAGE IN PRECISION-CUT RAT LIVER SLICES

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Administration of large doses of vitamin A (VA) is known to potentiate the hepatotoxicity of carbon tetrachloride (CCl_4). The exact mechanism of this potentiation is not fully known. However, Kupffer cells have been shown to participate in this potentiation. VA causes activation of Kupffer cells, increasing the release of cytokines such as tumor necrosis factor which may result in the increase in hepatotoxicity of CCl_4. Selective destruction of Kupffer cells by $GdCl_3$ 1 day prior to the administration of CCl_4 decreased VA potentiated CCl_4 hepatotoxicity. The present study was undertaken to investigate whether VA pretreatment causes similar potentiation of vinylidene chloride (VDC) hepatotoxicity in precision-cut rat liver slices. VA (250,000 IU) or the vehicle (water/propylene glycol/Tween 20, 83:10:7) was administered to male Sprague-Dawley rats (250–325 g) by oral gavage 24 hr prior to preparation of liver slices. In another experiment rats were given $GdCl_3$ (10 mg/kg iv) 4 hr after VA. Control rats received NaCl (iv) instead of $GdCl_3$. Liver slices were exposed to 0.25, 0.5 or 1.0 μl VDC vaporized in a total volume of 25 ml and incubated for 2, 4, or 8 hr. Hepatotoxicity was assessed by the leakage of intracellular K^+ from the liver slices. VA significantly increased the toxicity of all doses of VDC at 4 and 8 hr. Intracellular $K^+/\mu g$ DNA at 8 hr for 0.25 μl VDC alone was 25.62 ± 2.76 and 2.17 ± 0.39 for VA + 0.25 μl VDC vs 44.58 ± 2.64 for the control. However, $GdCl_3$ failed to reverse the VA induced potentiation of VDC hepatotoxicity. The results demonstrate that VA activated Kupffer cells do not participate in the potentiation of VDC hepatotoxicity and VA is acting via a different mechanism in increasing VDC hepatotoxicity. (NIH ES05790).

1531 LONG-TERM INCUBATION OF RAT AND HUMAN LIVER SLICES: A STUDY WITH ENALAPRIL MALEATE

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Enalapril maleate is a relatively new angiotensin-converting enzyme (ACE) inhibitor which is indicated for the treatment of renovascular hypertension and as therapy in the treatment of congestive heart failure. Unfortunately, however, a few cases of hepatotoxicity associated with enalapril therapy have been reported. This study utilizes rat and human liver slices to investigate whether enalapril hepatotoxicity can be reproduced in an *in vitro* system. Slices were produced using the Brendel/Vitron tissue slicer under oxygenated and ice cold V-7 preservation solution. Slices (200 um thick) were incubated in the dynamic organ culture system using Waymouth's medium + 10% FCS and $NaHCO_2$ and gassed with 95% O_2 and 5% CO_2. Enalapril (0.25–2 mM) toxicity was assessed every 24 hrs for 5 days using ATP content, K+ retention, and protein synthesis. Slices produced from non-induced rats exhibited no enalapril induced hepatotoxicity during the 5 days. Enalapril toxicity was observed, however, in liver slices prepared from phenobarbital-induced rats by the fourth day in culture. Liver slices produced from donated, non-transplantable human livers exhibited enalapril induced toxicity in only a few of the livers used. These results indicate that liver slices incubated in dynamic organ culture are able to maintain their viability for 5 days and can be used to predict the hepatotoxicity of drugs like enalapril maleate. Long term incubation of human liver slices might also be a suitable system to investigate the mechanism of ACE inhibitor hepatotoxicity. (Supported by NIEHS RO1-ES-05790).

1532 COMPARATIVE TOXICITY OF 2,6-DI-tert-BUTYL-4-NITROPHENOL AND OTHER NITRATED PHENOLS IN HUMAN AND RAT HEPATIC TISSUE SLICES

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2,6-Di-*t*-butyl-4-nitrophenol (DBNP) is a contaminant recently identified in submarine atmospheres. The toxicity of nitrated phenols to human and rat liver tissue slices was evaluated during a 4 hr exposure period. In addition to DBNP, the specific compounds studied were 2-butyl-4.6-dinitrophenol (DN*t*BP), 2,4-dinitrophenol (DNP) and *p*-nitrophenol (p-NP) at concentrations of 25, 50, 100 and 200 M. Indices of toxicity to liver slices were changes in K^+ content of slices, protein synthesis, ATP content and LDH release, relative to untreated slices. Indices most sensitive to treatment with the nitrated phenols were ATP content and protein synthesis, followed by potassium content and then LDH release. All indices were altered by DN*t*BP and DBNP at high concentrations. The onset of toxicity for nitrated phenols was concentration dependent, except for *p*-nitrophenol which was essentially non-toxic at the concentrations tested. Comparison of changes in indices, at different concentrations and different times to onset demonstrated the order of toxicity (highest to lowest) was DN*t*BP > DBNP > DNP > p-NP. Human liver slices were more resistant to insult from nitrophenols than rat liver slices. Enhanced toxicity of butylated nitrophenols may be a result of greater cellular uptake because of a higher lipophilicity.

1533 ACRYLIC ACID (AA) DETOXIFICATION RATES AND TISSUE/BLOOD PARTITION COEFFICIENTS IN RAT TISSUES

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Acrylic acid is a monomer used to produce acrylic esters and polymers. As part of an effort to develop physiologically based pharmacokinetic models for AA, we have measured the detoxification rate and tissue/blood partition coefficient for acrylic acid in several rat tissues. Rates of AA oxidation to CO_2 were measured in tissue slices obtained from male Fischer 344 rats. AA oxidation in kidney and liver was described by saturable kinetics with pseudo Vmax values of 3757 ± 189 and 2030 ± 884 nmol/hr/g tissue (mean ± sd, n = 3), respectively. AA oxidation rates in 11 additional tissues were less than 40% of the rate in liver. Common dosing site tissues possessed generally low rates. AA oxidation rates in Sprague-Dawley rat liver and kidney were similar to those in Fischer rats, and relative rates in homogenates of these tissues were similar to those measured in tissue slices. Tissue-to-blood partition coefficients were measured in homogenates of 12 tissues from Fischer 344 rats by micropartitioning and ranged from 0.90 ± 0.21 (mean ± sd, n = 4) in fat to 1.91 ± 0.36 in brain. These *in vitro* results may explain observations that despite the potential for acrylic acid to cause irritation in portal of entry tissues, it possesses low systemic toxicity. (Sponsored, in part, by the Basic Acrylic Monomer Manufacturers.)

1534 GUIDING PRINCIPLES FOR MANAGING THE CONDUCT AND QUALITY OF TOXICOLOGICAL STUDIES

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Guiding principles for managing the conduct and quality of basic research studies in toxicology and related disciplines have been developed. These principles provide a framework to assist scientists in adhering to good research practices. They are intended to (1) promote sound toxicology research through quality planning, conduct, and documentation; and (2) ensure the defensibility and reproducibility of study results, without inhibiting creativity. The principles include many of the concepts established in EPA and FDA Good Laboratory Practice (GLP) Standards, which address directly the quality of study

conduct and documentation, but not the scientific quality of a study. The GLP concepts, which were developed for application to EPA or FDA required testing of chemicals, have been integrated with observed good research practices to address both scientific quality and data quality. Specifically, these principles address adequate planning to establish scientifically defensible objectives and to ensure judicious use of resources; complete and accurate documentation of research plans, methods, and results; procedures to ensure data quality and integrity; appropriate biostatistical support; and effective peer review. The guiding principles were identified primarily from experience gained in providing support to develop and implement quality assurance (QA) programs for toxicology and health effects research programs. More detailed guidance on recordkeeping and internal quality control procedures was developed primarily from the results of several QA assessments of different types of toxicological research studies. These assessments were conducted by QA and scientific staff following a standard approach and using formal checklists to assess data accuracy, completeness, and integrity and to evaluate study-specific quality issues as well as more general areas of planning documentation and data management. The cooperative involvement of research scientists, managers, and QA personnel was essential in developing this guidance. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy).

1535 PRACTICAL APPLICATION OF NON-WHOLE ANIMAL ALTERNATIVES: SUMMARY OF IRAG WORKSHOP ON EYE IRRITATION TESTING

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In November of 1993, an ad hoc federal staff effort, the Interagency Regulatory Alternatives Group (IRAG) sponsored a workshop to examine the current scientific status of alternatives to the Draize eye irritation test by assesing the current practical application of methods used to predict in vivo eye irritation. Laboratories from around the world were invited to submit detailed in vitro and in vivo data in parallel according to a specific set of guidelines in a consistent format. In vitro scores were compared with individual tissue scores. Over 60 data sets from 41 laboratories were received for 29 different test methods. Methods were grouped into five categories and reviewed by expert working groups. Summary reports were prepared for the following method categories: organotypic models, chorioallantoic membrane-based assays, cell function-based assays, cytotoxicity assays and other systems. Data submissions and correlation analyses will be used to demonstrate the application of guidelines in method evaluations. Findings will be summarized and future directions will be presented. A significant outcome of the workshop was the cooperation demonstrated among representatives of industry, academia and government in sharing test data on more than 2,000 chemicals, products and product formulations for evaluation by their peers. Information obtained from this workshop will add to the weight of scientific evidence and scientific consensus about in vitro test methods and will establish credibility for regulatory acceptance of non-whole animal alternatives for ocular irritation.

1536 PROPOSED REVISIONS TO THE TESTING GUIDELINES FOR DEVELOPMENTAL TOXICITY AND TWO-GENERATION REPRODUCTIVE TOXICITY STUDIES CONDUCTED UNDER FIFRA AND TSCA — A PROGRESS UPDATE

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A USEPA inter-office Workgroup was formed to develop a set of testing guidelines which would be acceptable for developmental and reproductive toxicity testing of both pesticides (under FIFRA) and toxic substances (under TSCA). The resulting proposed draft guidelines, while recommending basic testing protocols similar to those currently in use, include additional requirements which will aid in the identification of effects on reproductive function and offspring development. For the two-generation reproductive toxicity study, these include cyclicity data and sperm evaluations for adults, vaginal opening and preputial separation times for offspring, ano-genital distance of offspring (if there is an indication of effect), the addition of macro- and microscopic postlife data on selected weanlings, and the enhancement of postlife data for adults. For the developmental toxicity study, it was proposed that the period of test substance administration be extended to termination of gestation instead of only during the period of major organo-genesis, the number of rabbits required for the study be increased from 12 to 20 per dose group, and evaluation of fatal cartilaginous development and coronal sectioning of rabbit fetuses be added. Public comments on the proposed guidelines have been received and are being addressed by the work group.

1537 PROPOSED REVISION OF THE USEPA's METABOLISM GUIDELINES FOR TESTING UNDER FIFRA AND TSCA

A Protzel [1], T F McMahon [1], Y M Ioannou [1], R P Zendzian [1], L C Keifer [2]. [1] *HED/OPP, Washington, DC;* [2] *HERD/OPPTS. U.S. EPA, Washington, DC*

U.S. EPA's Office of Prevention, Pesticides and Toxic Substances (OPPTS) is presently revising the Guidelines for conducting animal metabolism studies to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, et seq.), 40 CFR 158.340, and the Toxic Substances Control Act (TSCA)(15 U.S.C. 2601). This revision of the Metabolism Guidelines is intended to reflect the current state of the art regarding the conduct of metabolism studies of pesticides and other chemical substances. A major feature of this revision is the incorporation of a tiered approach in the conduct of metabolism studies. The proposed tiered approach will require a basic data set (Tier 1) to determine the routes and rates of excretion and to identify excreted metabolites. Additional studies (Tier 2) may be requested based upon the existing toxicology database and/or the results of Tier 1 testing which could impact upon the risk assessment process. For Tier 1 testing, the oral route will typically be required. However, if the use pattern of the chemical results in other types of exposure, other routes (dermal and/or inhalation) maybe required for initial testing of the disposition of the chemical substance. The proposed guidelines attempt to minimize the use of experimental animals and other resources.

1538 A PROPOSED METHOD FOR AMBIENT WATER QUALITY HUMAN HEALTH CRITERIA ON CANCER

J T Du, E V Ohanian. *U.S. EPA, Office of Water. Washington, DC*

The Ambient Water Quality Human Health (AWQHH) criteria methodology was published in 1980. In most cases, the risk for all carcinogens was estimated by applying the LMS (linearized multistage model) to animal tumor data. LMS assumes non-threshold and gives the 95% upper bound risk. The criterion gives a concentration range equivalent to cancer risk of 10^{-5} to 10^{-7}. Cancer biology has advanced substantially since 1980, EPA is in the midst of revising the existing cancer guidelines. In parallel, Office of Water is reviewing the 1980 AWQHH methodology, including cancer. In essence, the new approach will provide more narratives with rationale as to whether the agent is likely to be a human carcinogen. Risk assessment will integrate the whole database, including human and animal tumor data, mode of action, genotoxicity, pharmacokinetics, physical/chemical properties, structural analogues and the route and level of exposure on the agent. Also, depending on biological and physical/chemical considerations, either linear or nonlinear approach will be used for low dose extrapolation. Applications of the revised cancer guidelines to AWQHH criteria development will be presented.

1539 EPA GUIDELINE HARMONIZATION PROJECT

A Rispin, A Auletta. *Office of Prevention, Pesticides and Toxic Substances, The Environmental Protection Agency*

EPA has been engaged in a multi-year project to harmonize its testing guidelines for pesticides and industrial chemicals with those of the Organization for Economic Cooperation and Development. A systematic process has been put in place to identify and resolve significant issues, resolve them within EPA, and update test methods through agency workgroups. In the course of harmonization, many guidelines have been revised and updated in cooperation with the Organization for Economic Cooperation and Development and other interested organizations. This process culminates in peer review and publication of the harmonized guidelines.

1540 CONSIDERATION OF CHRONIC TOXICITY FOR RANKING OF "NON-THRESHOLD" POLLUTANTS UNDER SECTION 112(g) OF THE CLEAN AIR ACT AMENDMENTS

J C Caldwell, C Siegel Scott, C Shoaf, J Cogliano. *US EPA, Research Triangle Park, NC; US EPA, Washington, DC*

Under section 112(g) of the Clean Air Act amendments of 1990, the EPA is directed to rank 189 hazardous air pollutants (HAP) by hazard to human health and to distinguish between those with and without "a safety threshold of exposure". The hazard ranking is designed to help determine "emission increases of one HAP deemed to be more hazardous than that of another" and is a mixture of both scientific judgement (risk assessment) and risk management decisions. The ranking assigns HAP to 1 of 4 categories and establishes criteria for determining relative hazard between and within the categories. These categories are: "non-threshold", "high-concern", "threshold", and "unrankable".

Currently "non-threshold" HAP are those which are considered to be either known, probable, or possible human carcinogens. "High-concern" HAP are any HAP of high concern from acute exposure or "threshold" HAP of high concern for chronic exposure. However, the current hazard ranking system does not assign HAP which meet criteria as "non-threshold" to the "high-concern" category on the basis of hazard from chronic exposures.

This presentation focuses on the description of a data set, similar to that used to rank HAP for hazard from chronic exposure, that is available for HAP in which cancer was the initial concern for the proposed ranking. 59 HAP in the "non-threshold" category had such documents available. "Non-threshold" HAP for which there is not adequate information to do comparisons of non-cancer and cancer hazard will be identified. For HAP with appropriate data, general observations concerning level of exposure associated with cancer and chronic effects will be made.

This is an abstract of a proposed presentation and does not necessarily reflect U.S. EPA policy.

1541 ACUTE INHALATION EXPOSURE LEVELS FOR SOME SUBSTANCES RELEASED INTO THE ENVIRONMENT

R Das, J R Fowles, R Paige, G V Alexeeff. *OEHHA, Cal/EPA, Berkeley, CA*

We have developed one-hour inhalation exposure levels at or below which specified health effects are not expected to occur for 57 hazardous substances released into the environment. Of these, 51 are commonly released either as a result of routine or unplanned emissions from facilities or of transportation accidents and 6 are criteria air pollutants. There exist for most chemicals 3 acute exposure levels based on severity of toxicological response: Level I (discomfort or mild effect), Level II (disability or frank effect) and Level III (life-threatening effect). The Reference Exposure Level (REL), the concentration at or below which adverse health effects are not anticipated, is based on the most sensitive effect reported. If the most sensitive health effect for a chemical is a frank effect (e.g., reproductive or developmental endpoint), there are, at most, 2 exposure levels for that compound. RELs are used as indicators of potential adverse health effects from routine emissions. Other levels may have implications for emergency planning. All acute exposure levels are designed to protect the most sensitive individuals in the population by inclusion of margins of safety. Acute exposure level development proceeded in order: currently available exposure guidelines were adopted if appropriate, on the basis of appropriateness of toxicological endpoint, documentation supporting the choice of exposure dose, and incorporation of appropriate margins of safety; for acute exposure levels developed *de novo*, the Benchmark Dose approach, a log dose vs. probit response model to identify the concentration expected to produce a 1% increase in toxic response, was preferred if appropriate data existed; the most common procedure used was the No Observed Adverse Effect Level approach, based on the application of uncertainty factors to the maximum dose causing no observable adverse effects.

1542 ATSDR MINIMAL RISK LEVELS FOR HAZARDOUS SUBSTANCES

C-H S J Chou, M Fay, J Holler, C T DeRosa. *Agency for Toxic Substances and Disease Registry (ATSDR), Atlanta, GA.* Sponsor: M Mumtaz

The mission of Agency for Toxic Substances and Disease Registry (ATSDR) is to prevent exposure and adverse human health effects and diminished quality of life associated with exposure to hazardous substances from waste sites, unplanned releases, and other sources of pollution present in the environment. To support this mission, ATSDR prepares toxicological profiles for the hazardous substances on the Agency's priority list. The toxicological profiles contain information and interpretations of substance-specific health effects. When sufficient information is available, minimal risk levels (MRLs) are derived. An MRL is an estimate of the daily human exposure that is likely to be without appreciable risk of adverse noncancer effects over a specified duration of exposure. These estimates serve as guidelines that ATSDR health assessors use to determine contaminants of concern at hazardous waste sites. MRLs are derived for acute, intermediate and chronic durations via the oral and inhalation routes of exposure. MRLs are derived using the No-Observed-Adverse-Effect-Level/Uncertainty Factor (NOAEL/UF) approach. UFs that account for extrapolation from high to low doses, from animals to humans, and for intrahuman variation are applied. The default value for each UF is 10, however, a UF of 3 or 1 may be used when the database supports it. A modifying factor (MF) may also be used to reflect additional biomedical judgment on the database. To date, approximately 200 MRLs have been derived for 100 profiled substances. Representative MRLs will be discussed to explain the salient features and new trends that the Agency wants to incorporate in defining these guidance values.

1543 TOXICOLOGICAL APPROACHES TO THE HARMONIZATION OF MRLS FOR VETERINARY DRUG RESIDUES

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Harmonization of standards and procedures for establishing maximum residue levels (MRLs) for veterinary drug residues in edible animal products is a major goal of the international veterinary drug community. The MRL is the maximum concentration of residue resulting from the use of a veterinary drug that is legally permitted or recognized as acceptable in or on food. The MRL is normally calculated from the acceptable daily intake (ADI) determined for that compound. The ADI is defined as the amount of residue of that compound that can be ingested daily over a consumer's lifetime without appreciable health risk and can be considered the safety standard for that compound. In some instances countries or international organizations, using harmonized standards, will arrive at an identical MRL for a particular veterinary drug. Trade problems arise when there are differences between countries in the MRLs established for the same compound. This is often interpreted as the result of a difference in the human food safety assessment of the residues of that drug. However, differences in MRLs may also reflect differences in the use of that compound in a particular country, or in the choice of safety factors, food consumption values, or the analyte used in monitoring programs. An important question in deciding the acceptability of an MRL for harmonization purposes should therefore be whether use of a different MRL will result in dietary residues that exceed the particular country's established ADI. It is possible to predict, with varying degrees of conservatism, whether there is a realistic possibility that use of a particular MRL will result in a dietary intake of residues of a compound that exceeds its ADI. MRLs should be considered equivalent for purposes of trade and consumer safety if the use of the MRL does not result in residues above a country's ADI.

1544 VETERINARY DRUG USE IN ANIMALS: DEFINING THE "RESIDUE OF TOXICOLOGICAL CONCERN" AND ITS EXPOSURE IN MAN. NEW APPROACHES FOR THE TOXICOLOGICAL EVALUATION OF RESIDUES

A MacDonald, S C Fitzpatrick. *Hoffmann La Roche, Inc, Nutley, NJ/Veterinary Drug Consultant*

The risk assessment strategy for residues and contaminants currently used by the Food and Drug Administration is based upon the two assumptions; (1) all residue present in the edible food commidity has the same toxicity as the parent compound and (2) residue is present at its highest permitted use level daily in a food commodity over the lifetime of the consumer. Because of these assumptions, the majority of the compounds undergo extensive chronic toxicity and carcinogenicity testing prior to their approval for use. A more relevant risk assessment approach could be designed by first determining what the consumer is actually exposed to in his daily diet. This could be accomplished by first identifying what portion of residue remaining in food is still bioactive and bioavailable ("Residue of Toxicological Concern") to the consumer and then assessing the exposure of the consumer to residues in his diet on a chronic, or intermittent basis. Using the information from the exposure/bioavailability/bioacticity assessments, a toxicology testing strategy could be developed which would pinpoint those endpoints which were the most relevant to human exposure to the compound. For example, if the major consumer exposure was intermittent and/or short-term, testing would concentrate on defining the acute/subacute effects of a compound. This approach would enable the manufacturer to focus its time and resources on those toxicity tests which would be the most beneficial in identifying relevant toxic endpoints for the consumer while still be equally protective of public health and safety.

1545 INTERACTIONS BETWEEN IMMUNE CELLS AND NON-IMMUNE CELLS IN IMMUNOTOXICOLOGY

G J Rosenthal. *Somatogen, Inc., Boulder, CO*

It is increasingly apparent that cells and factors outside of the traditional definition of "immune" cell are intimately involved in both normal immune function and immunotoxicity. Evaluation of information derived from immunotoxicological studies, particularly *in vivo* studies, in the absence of an understanding of the potential non-immune influences, often lead to an incomplete understanding of the biological response in question. An obvious example of this is the role of overt stress on host immune function in which neuroendocrine influences play a significant role in the ultimate immune status of the host. This symposium will address the role of key cells not classically considered a part of the immune system, but intimately involved in immunological processes.

Specific topics which will be covered by the speakers will include: (1) the influence of leukocytes, particularly Kupffer cells, on hepatocyte function, (2) a discussion on the immunologic potential of keratinocytes in the context of skin pathophysiology, (3) the complex interactions between the central nervous system and the immune system, and lastly, (4) the global influence of endothelial cells in immune function. The specific goals of this symposium are to familiarize the audience with the inter-connective nature of immune processes, discuss the importance of exploring alternative hypothesis in interpreting immunotoxicological studies, and finally to stimulate collaborative research in the area of immunotoxicology.

1546 INTERACTIONS BETWEEN THE CENTRAL NERVOUS SYSTEM NEURONS AND IMMUNOLOGICALLY ACTIVE GLIAL CELLS

G J Harry. *National Institute of Environmental Health Sciences, RTP, NC*

Cytokines are produced in neural cells and demonstrate the capacity to regulate proliferation, survival, neurite outgrowth, neurotransmitter expression and play an active role in neurological disease and trauma induced pathophysiology. An injury response in the brain, gliosis, is characterized by an elevation of glia cells and has been proposed for use as an indicator of CNS perturbation. The major components of gliosis, microglia and macrophages, are derived from the cells of the monocyte lineage thus linking it to a "immune type" response. Following a viral or physical injury to the brain, the microglia assume an activated phenotype and upregulate their expression of MHC antigens. These recruited cells synthesize pro-inflammatory cytokines, interact with the resident astrocytes and initiate a cascade of responses. Under these conditions, the blood-brain barrier is compromised and systemic circulating substances contribute substantially to this response. The question remains as to whether a similar response cascade is initiated following chemical-induced injury to the brain. We have examined this question using a trimethyltin-induced model of hippocampal neuronal necrosis. Within hours, pro-inflammatory cytokine mRNAs are elevated suggesting a temporal association with the presence of microglia and an early modulatory role in the manifestation of TMT neurotoxicity not unlike the response to other forms of CNS injury.

1547 KERATINOCYTES AS IMMUNOLOGICALLY ACTIVE CELLS

J D Laskin. *UMDNJ – Robert Wood Johnson Medical School, Piscataway, NJ*

It has become increasingly apparent that there is a complex interplay between the different cell types in the skin and an array of inflammatory molecules that mediate xenobiotic toxicity. Initially, it was thought that skin inflammatory reactions were regulated to a large extent by immune cells. These include both resident immune cells of the skin (i.e., Langerhans cells) as well as infiltrating leukocytes. During xenobiotic induced inflammation, each of these cell types can become functionally active and release a variety of mediators. Recent studies have demonstrated that keratinocytes actively take part in xenobiotic-induced skin inflammatory reactions. Keratinocytes can be induced to process and present antigens, release highly toxic free radicals, and express and respond to a variety of inflammatory cytokines which affect dermal metabolism. Consequently, the progression and resolution of xenobiotic-induced toxicity is regulated by interactions between inflammatory mediators released by keratinocytes and leukocytes and anti-inflammatory influences. In addition to cytokines, the release of free radicals by both immune cells and activated keratinocytes is also an important mechanism by which xenobiotic-induced irritation and inflammation leads to skin tissue damage. Two major classes of free radical species produced by keratinocytes are reactive oxygen and reactive nitrogen intermediates, both of which may have positive and negative influences. This discussion will center on the variety of cellular/mediator interactions that culminate in the pathophysiology of xenobiotic-induced skin disease.

1548 LEUKOCYTE–HEPATOCYTE INTERACTIONS

R E Billings. *Colorado State University, Ft. Collins, CO*

Liver macrophages (Kupffer cells) are increasingly recognized to play an important role in liver injury. This role may be either through propagation or amelioration of injury initiated by chemicals in hepatocytes. Key mediators released from stimulated Kupffer cells are reactive oxygen or nitrogen species and cytokines such as tumor necrosis factor-α (TNFα). To delineate mechanisms of leukocyte-hepatocyte interactions, our laboratory has investigated the cellular effects of inflammatory cytokines using recombinant proteins and primary cultures of hepatocytes. These studies have established that high concentrations of TNFα directly cause oxidative stress in hepatocytes. Additionally, the effects of TNFα (GSH depletion, GSSG accumulation/cellular efflux, and decreased ATP) are potentiated by IFN-γ. These adverse effects, as well as cytotoxicity, can be blocked by anti-oxidants. TNFα causes an oxidative stress response as indicated GSSG efflux, and induces the synthesis of proteins which protect hepatocytes from oxygen radical toxicity. Additionally, nitric oxide synthase (NOS) is induced in hepatocytes to TNFα and may play a protective role in cytokine-induced liver injury. Taken together, these results show that TNFα in concert with other cytokines, enhances the formation of oxygen radicals, which may participate either directly or indirectly in both a cytotoxic response or a protective response to hepatocytes under oxidant or xenobiotic-induced stress. Such data clearly show that immunostimulation or immunodepression will profoundly affect liver injury caused by chemicals.

1549 INTERACTIONS OF ENDOTHELIAL CELLS WITH THE IMMUNE SYSTEM: ROLE IN IMMUNOTOXICOLOGY

A L Baldwin. *University of Arizona, Tucson, AZ.* Sponsor: G Rosenthal

Venular endothelial cells play an important role in the immune system. Blood-borne immunological cells must traverse the endothelium to enter the tissue at sites of infection. Transendothelial migration occurs in three stages. First, cytokines, secreted into the blood stream after injury, induce expression of selectins and selectin-receptors on leucocytes and endothelial cells. Receptor-binding of selectins bothers the leucocytes to the endothelium, so they roll along the endothelial surface. Second, the leucocytes express integrins which bind to endothelial cell adhesion proteins and strengthen the leucocyte-endothelial adhesion. Third, leucocyte chemoattractants, produced by the endothelium, stimulate transmigration of adherent leucocytes into the tissue, possibly by creation of a concentration gradient. Endothelial cells may become damaged during leucocyte adhesion by production of active oxygen species. These species have very short lifespans so the leucocytes must adhere closely to the endothelium in order for this cytotoxicity to be expressed. Cell-cell adhesion is influenced by the relative surface charges on the two cells. Leucocytes and endothelial cells both bear negative charges and so cell-cell adhesion is opposed by charge repulsion and by the loss of entropy involved in interdigitating the surfaces of the contacting cells. Changes in cell surface charge and/or in the molecular conformation of cell surface glycoproteins could alter the repulsive barrier between cells and affect cell-cell adhesion. In intestinal mucosal microvessels the endothelial surface charge is radically altered within minutes of arrested blood flow or after perfusion with free hemoglobin. This change is partially mediated by removal of nitric oxide from the glycocalyx. These findings will be discussed regarding the effects of cell surface charge alteration on cell-cell adhesion and on subsequent endothelial cytotoxicity and leucocyte migration.

1550 GAP JUNCTIONAL COMMUNICATION FOR DRUG DISCOVERY AND DEVELOPMENT

J E Trosko. *Michigan State University, East Lansing, MI*

The rationale for this symposium is to present a new conceptual strategy for both toxicity testing of new chemicals and the development of new chemopreventive/chemotherapeutic drug development for cancer prevention and management. It is based on a series of experimental observation over the last 30 years that dysfunctional gap junctional inter-cellular communication appears to correlate with the neo-plastic phenotype, as well as other toxicological endpoints. With recent developments in the cloning and characterization of the family of highly conserved gap junction genes and their protein products (connexins), as well as in the development of new techniques to measure, functionally, gap junctions, it appears that GJIC is a crucial process needed for the regulation of normal growth control, differentiation and adaptive functions of differentiated cells. Chemical tumor promoters, growth factors, oncogenes and hormones have been linked with the *down-regulation* of GJIC, while several tumor suppressor genes, anti-tumor promoters and anti-oncogenic inhibitors have been shown to *up-regulate* GJCI. In addition, transfection with connexin genes into non-communication tumor cells have been shown to up-regulate GJIC and restore growth control. Three different, but inter-related, presentations will address how the study of GJIC could be useful in toxicology studies of non-genotoxic chemicals, in drug development for chemotherapeutic and chemoprevent of cancers and in potential gene therapy of cancer.

1551 ALTERATIONS OF GAP JUNCTIONAL INTERCELLULAR COMMUNICATION BY XENOBIOTICS: MECHANISMS AND ROLE IN DRUG DEVELOPMENT AND TOXICITY TESTING

R J Ruch. *Medical College of Ohio, Toledo, OH*

Gap junctional intercellular communication (GJIC) has been implicated in a variety of physiological processes including growth regulation, embryogenesis, electrical synapsing, and maintenance of cellular homeostasis. Many toxic and carcinogenic agents such as pesticides, barbiturates, phorbol esters, peroxides, heavy metals, and oncogenic viruses inhibit GJIC *in vivo* and *in vitro* and this effect may contribute to their toxic and carcinogenic properties. With the cloning of several mammalian gap junction proteins (connexins) and the development of specific anti-connexin antibodies, the mechanisms of inhibition of GJIC by xenobiotics are being defined rapidly. Xenobiotic effects on connexin expression, posttranslational phosphorylation, and turnover as well as gap junction function (cell-to-cell permeability) and assembly have been described and could be monitored in the regulatory setting. Such approaches would enable the rapid identification of xenobiotic effects on gap junctions, their mechanisms of action, and the development of non inhibitory analogs.

1552 GENE THERAPY FOR GAP JUNCTIONALLY-DEFICIENT CELLS: POTENTIAL THERAPEUTIC APPLICATIONS

C C Naus. *Departments of Anatomy and Clinical Neurological Sciences, The University of Western Ontario, London, Ontario, Canada.* Sponsor: J Trosko

Many studies have suggested that gap junctional intercellular communication (GJIC) plays an important role in controlling cell growth. This has led to the design of approaches to manipulate the level of GJIC in tumour cells, with a view to possible therapeutic applications. While several reports have described the use of gene therapy via transfection of gap junction (connexin) cDNAs, this approach has been complicated by the variety of tumour cells used, the expression of endogenous connexins in these cells and the ever expanding family of connexin genes as candidates for transfection. We have focused our attention on glioma cells as a model for connexin gene therapy. These cells grow rapidly and express limited amounts of connexin43, functionally reflected in a low degree of GJIC. Transfection of either connexin43 or connexin32 into these cells results in increased GJIC, reduced proliferation and some differentiation. Ongoing studies have revealed that GJIC can affect expression of genes implicated in growth control. While increased GJIC slows the growth rate, it appears that other factors are necessary to arrest or reverse tumour growth. Thus it may be more appropriate to combine such manipulations of GJIC with other therapies.

1553 THE RELEVANCE OF ALTERED GAP-JUNCTIONAL COMMUNICATION TO NEOPLASIA

P P Mehta, G A Howard, B A Roos. *GRECC, VA Medical Center, and University of Miami School of Medicine, Miami, FL.* Sponsor: J Trosko

Direct communication between cells is altered in cancer; these alterations have biological significance and their elucidation should enlarge the pathogenetic understanding and approach to cancer. Evidence is mounting that direct communication mediated via cell-to-cell channels embodied in gap junctions may provide a homeostatic mechanism to curb the growth of tumor cells. A number of genes that code for the channel-forming proteins called connexins have now been found to be regulated, both transcriptionally and posttranscriptionally, by agents that are known to affect the outcome of neoplasia. Expression of a specific connexin in a particular tumor tissue, as well as lack of heterotypic communication between tumor and surrounding normal cells, may provide hints about its pathological state. The fact that inhibition of the growth of many tumor cells in coculture with normal cells is contingent upon the communication between the two cell types implies that the interface of normal and tumor cells is critical in determining the outcome of neoplastic phenotype. By using agents that alter cell surface topology of the gap-junction-forming cells, we have developed new strategies to induce communication between normal and tumor cells. These strategies should provide a new path to characterizing and possibly preventing cancer.

1554 TIME AND DOSE RELATIONSHIP BETWEEN THE CELLULAR EFFECTS OF FUMONISIN B_1 (FB_1) AND THE UPTAKE AND ACCUMULATION OF [^{14}C]FB_1 IN LLC-PK_1 CELLS

R T Riley, H-S Yoo. *USDA-ARS, Athens, GA*

There is a time and dose-response correlation between FB_1-induced disruption of sphingolipid biosynthesis and its cellular effects in LLC-PK_1 cells. The cellular effects (inhibition of proliferation and cytotoxicity) appear after a dose-dependent 24–72 h lag time preceded by inhibition of *de novo* sphingosine biosynthesis, increase in free sphinganine (S_a), and decrease in complex sphingolipid (CSL). LLC-PK_1 cells were exposed to FB_1 at 10 μM (minimally cytostatic, non-cytotoxic), 20 μM (cytostatic, minimally cytotoxic), and 50 μM (cytostatic and cytotoxic). All concentrations caused marked increases in intracellular S_a at 24 h peaking at 48 h and decreases in CSL beginning at 24 h peaking at 48–72 h. The changes in S_a were proportional to the FB_1 dose and the accumulation of [^{14}C]FB_1 up to 48 h. However, S_a concentration decreased markedly at 72 h. This decrease in S_a was not due to a decrease in intracellular FB_1. [^{14}C]FB_1 uptake was passive and reached a stable equilibrium with extracellular FB_1 ([FB_1] out/[FB_1] in = 1) after 4 to 8 h with no apparent metabolism or lag time.

1555 PARTITIONING OF INCREASED SPHINGANINE (S_a) AND DECREASED COMPLEX SPHINGOLIPIDS (CSL) AS CAUSATIVE FACTORS IN FUMONISIN B_1 (FB_1)-INDUCED CYTOSTATIC AND CYTOTOXIC EFFECTS IN LLC-PK_1 CELLS

H-S Yoo, J L Showker, R T Riley. *USDA/ARS, Athens, GA*

FB_1 is a specific inhibitor of ceramide synthase, a key enzyme in CSL biosynthesis in LLC-PK_1 cells. The purpose of this study was to determine the relative contribution of increased intracellular free S_a and decreased CSL to the cytostatic and cytotoxic effects of FB_1. FB_1 induced an increase in intracellular free S_a which preceded depletion of CSL at a FB_1 concentration which was both cytostatic and cytotoxic. β-chloroalanine (BCA), an inhibitor of the first enzyme in CSL biosynthesis reduced the free S_a in FB_1-treated cells to an S_a concentration which was shown not to be cytotoxic and minimally cytostatic. While the combination of BCA plus FB_1 reduced intracellular free S_a, the decrease in CSL was greater than when either FB_1 or BCA was added alone and the cytostatic and cytotoxic effects of FB_1 were only partially reversed ($\cong$50%). The results indicate that in LLC-PK_1 cells, neither the elevation of free S_a nor decrease in CSL alone, is sufficient to account for the cytostatic and cytotoxic effects of FB_1.

1556 EFFECTS OF *FUSARIUM MONILIFORME* ISOLATES ON SPHINGOLIPID (SL) LEVELS IN HORSES

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Disruption in SL metabolism is a biomarker of exposure to fumonisins. However, the role of SLs in the pathogenesis of diseases caused by fumonisins is not understood. Toxicity (reported elsewhere) of two strains of *F. moniliforme* was determined in a 27 day feeding trial in horses. *F. moniliforme* strains *AU 2/3* (isolated from feed associated with duodenitis/ proximal jejunitis [DPJ] in Alabama) and *MRC 826* (isolated from corn in South Africa and shown to cause equine leukoencephalomalacia [ELEM]) were cultured on corn, diluted with clean corn and sweet feed, with final concentrations of fumonisin B1 (FB1) of 130 and 200 ppm, respectively. None the horses developed DPJ, but horses fed MRC 826 had intestinal lesions consistent with DPJ and horses in AU 2/3 and MRC 826 groups developed ELEM. Tissue SL levels were analyzed in horses fed *F. moniliforme* culture material and control horses euthanized as part of another ongoing study. Sphingosine (S_o) levels were significantly elevated in proximal duodenum and ileum of MRC 826 horses. Sphinganine (S_a) levels were significantly elevated hypothalamus/dentate gyrus in horses fed AU 2/3. However, no significant differences in S_a to S_o ratios were observed in the brain or intestinal tissues of the control AU 2/3 or MRC 826 horses. Results indicate that the tissues (brain) with the most severe pathological changes did not have consistent increases in S_a to S_o ratios that have been observed in livers and kidneys from animals exposed to FB1.

1557 CHRONIC TOXICITY OF FUMONISIN IN PIGS

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Fumonisins are mycotoxins commonly produced in *Fusarium moniliforme*-infected corn, are hepatotoxic in several mammalian species, induce hepatocarcinomas in laboratory rats, leukoencephalomalacia in equine species and acute pulmonary edema in swine. In this study, the chronic toxicity of fumonisin (FB_1 plus FB_2) in pigs was characterized by: biochemical and histopathological changes consistent with liver disease, medial hypertrophy of small- to medium-sized pulmonary arteries, and right ventricular hypertrophy and dilatation. Five gilts were fed a ration *ad libitum* containing 100 mg FB_1/kg for 10 days followed by a ration containing 150–170 mg FB_1/kg for up to 210 days. The concentration of fumonisin B_2 was 25% ± 4% that of the FB_1. Weight-matched controls were used for cardiac parameter comparisons. Gross cardiac measurements revealed significant ($P < 0.02$) decreases in left ventricular plus septal weight/right ventricular weight (LV + S/RV) and left ventricular plus septal weight/total cardiac weight (LV + S/TC) ratios in pigs fed fumonisin-containing diets. Histopathologic examination of four fumonisin-fed pigs revealed medial hypertrophy of the pulmonary arteries. Lesions were restricted to the muscular layer with no evidence of endothelial lesions in the affected arteries. Evidence from the microscopic examination of the lungs and gravimetric measurements of cardiac tissues from 3 pigs exposed to dietary fumonisins for 6 months indicates that the pulmonary vasculature is a target of chronic exposure.

1558 EXPERIMENTAL PODOPHYLLOTOXIN (BAJIAOLIAN) INTOXICATION: PATHOLOGICAL EFFECTS AND BIOCHEMICAL BASES

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Bajiaolian, one of the species in the Mayapple family has been used widely as a traditional Chinese herbal medicine as well as lay-medicine in Western countries. Outbreaks of human poisonings have been reported. In our previous study, we had reported neuronal edema, chromatolysis, and axonal swelling (axoplasmic stasis) various parts of the nervous system. In the present study, rats were dosed orally with 0, 1.67, or 5 mg/kg/day of podophyllotoxin (PTX). Pathological changes in the liver, kidneys, pancreas, intestines, and testis were evaluated after 7 days of treatment. Biochemical changes in DNA, RNA, and protein syntheses in primary hepatocyte cultures, treated with 0, 1, 5, 25, 50, and 100 uM of PTX for 48 hrs, were also examined. In animal study, liver and intestines appeared to be most sensitive to PTX, showing degenerative changes even at the lowest treatment dose. Nerve cells and their axons, pancreas, and testis also exhibited pathological changes at higher dose level. In-vitro biochemical studies revealed dose-dependent suppression on DNA, RNA, and protein syntheses. Protein synthesis was most sensitive and appeared to be unrelated to RNA inhibition.

1559 RICIN-INDUCED LIPID PEROXIDATION IN MICROSOMES AND MITOCHONDRIA

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Previous studies in our laboratory indicate that ricin induces an oxidative stress in mice *in vivo* as indicated by increased hepatic lipid peroxidation, glutathione depletion, DNA damage, and urinary excretion of carbonyl compounds. Little is known, however, about the mechanism of this oxidative stress, or the effects of ricin on *in vitro* systems. In this study, lipid peroxidation was measured in hepatic microsomes from animals treated with either ricin (2.5 μg/kg) or vehicle. In addition, the effects of adding ricin (50 μg/mL) to microsomal or mitochondrial lipid peroxidation systems were determined. The effects of Fe^{2+}, desferrioxamine (DFX), or both were determined in all experiments. Microsomes from ricin-treated mice exhibited lipid peroxidation levels 83% higher than those from control animals. Fe^{2+} increased MDA production almost 15-fold in microsomes from control animals and over 5-fold in microsomes from ricin-treated animals. DFX decreased lipid peroxidation by 26% in microsomes from control animals and by 45% in microsomes from ricin-treated animals. The addition of ricin to mitochondria from untreated animals resulted in a 26% and a 17% decrease in lipid peroxidation in the presence and absence of Fe^{2+}, respectively. Levels of lipid peroxidation in the DFX-treated mitochondrial systems suggest that ricin reduces the effectiveness of the chelator. The results indicate that ricin induces oxidative stress *in vivo* by an indirect mechanism involving Fe^{2+}.

1560 TOXICITY AND EFFECTS ON BLOOD GLUCOSE OF TREHALOSE IN THE MOUSE AND DOG

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The simple disaccharide of glucose, α,α-trehalose, shows outstanding stabilizing effects on dried proteins, peptides and other biological molecules. It has potential as an ideal excipient in pharmaceutical preparations. This study was designed to investigate the toxicity associated with daily administration by the oral, intravenous or subcutaneous routes in mice and dogs. Dogs (3/sex/group) received trehalose daily for 14 days at doses of 1 g/kg/d, i.v., 5 g/kg/d, oral, or 0.25 g/kg/d, s.c.; mice (10/sex/group) received doses of 1, 5, or 2.5 g/kg/d by the i.v., oral or s.c routes, respectively. All dogs survived the study; one female mouse treated i.v. with trehalose died on day 6. Trehalose had no effect on body weights or food consumption over the 14-day study. No treatment-related macro- or microscopic changes were noted for mice or dogs. Mice had no discernible clinical signs of toxicity; several dogs had diarrhea and occasional vomiting after oral administration. Hematological and clinical chemistry profiles for dogs were comparable to baseline. Minimal changes from control values were noted in male mice for lymphocytes and serum electrolytes. Transient elevations were noted for serum glucose in mice during a 24 hour sampling period after 14 days of dosing for all routes. No effects were apparent on glucose by the s.c. route in dogs and transient elevations were noted for the oral and i.v. groups. It is concluded that Trehalose administered orally, intravenously, or subcutaneously is not associated with overt toxicity. Further, baseline glucose was not dramatically affected which suggests that trehalose does not accumulate after repeat dosing.

1561 BILIARY EXCRETION OF PYRROLIC METABOLITES OF [^{14}C] MONOCROTALINE IN THE RAT

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The biliary excretion of amphoteric pyrroles was studied using *in situ* isolated rat liver preparations perfused in a recirculatory manner for 60 to 90 min with ^{14}C-monocrotaline (MCT) (40 μmol/120 ml of Krebs-Henseleit buffer). After 90 min, 20% of the administered ^{14}C, and an amount of pyrrole equivalent of 9 μmol of dehydroretronecine was recovered in the bile. Bile collected between 15 and 30 min contained the highest levels of ^{14}C and pyrrolic metabolites. Isoelectric focusing (IEF) and electrophoretic separations on matrices of polyacrylamide and silica were used to isolate and estimate levels of pyrrolic conjugates. IEF and electrophoretic separations on silica revealed the presence of six pyrrole positive metabolites. Reactions of monocrotaline pyrrole with glutathione produced three conjugates which had the same pIs as components found in bile. Electrophoretic separations on silica and polyacrylamide followed by reverse phase chromatography removed sufficient biliary matrix contaminants to permit identification of glutathione and cysteinylglycine conjugates of 6,7-dihydro-7-hydroxy-1-hydroxymethyl-5H-pyrrolizine and 1-formyl-7-hydroxy-6, 7-dihydro-5H-pyrrolizine with FAB MS/MS. This study has revealed a complexity in the biliary pyrrolic excretion profile that has not previously been realized and has provided additional metabolites to be tested as potential pulmonary, renal or hepatic toxins.

1562 90-DAY ORAL (DIET) TOXICITY STUDY OF AZATIN-TECHNICALT™ IN RATS

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Azatin-Technical™ is a biological insecticide containing approximately 9% Azadiractin, a tetranortriterpenoid extracted from the seeds of the neem tree, which is a highly active insect feeding deterrent and growth regulator. Sprague-Dawley rats (10/sex/group) were fed Azatin-Technical™ at levels of 500, 2500 and 10,000 ppm in the diet for 90 days to evaluate its potential toxicity. A control group of equal size was fed basal diet alone. Animals were observed daily and their body weight and food consumption were measured weekly. Hema-

tology and serum chemistry parameters were evaluated after 90 days of treatment. All animals were sacrificed and subjected to a gross necropsy. Kidney, liver and gonads from all animals were weighed at necropsy. Histopathological examination was performed on tissues from the control and high dose animals. The lungs, liver and kidneys from the low and medium dose animals were also examined microscopically. None of the animals died during the study. No signs of overt systemic toxicity were seen at any dose level after 90 days of feeding. Mean body weight was significantly decreased in the 10,000 ppm males and females at week 3 (males) or week 4 (females) and persisted for the duration of the 90-day feeding period. Food consumption was significantly decreased in the same animals at the same time and, with a few intervals excepted, persisted for the duration of the study. These changes were attributed to decreased palatability of the test diet at the 10,000 ppm dose level. Treatment-related changes in clinical chemistry parameters consisted of increased gamma glutamyl transpeptidase in high-dose males and in mid- and high-dose females, and increased urea nitrogen and creatinine in high-dose females. Treatment-related alterations in hematological parameters consisted of increased total erythrocyte count (high-dose males) and decreases in hematocrit (high-dose females), hemoglobin (high-dose females), mean corpuscular volume (high-dose males and females), mean corpuscular hemoglobin (high-dose males) and mean corpuscular hemoglobin concentration (high-dose males). These changes are indicative of a possible slight hypochromic, microcytic anemia in the high-dose animals. Absolute and relative liver weights were significantly increased in the mid- and high-dose females. No treatment-related gross lesions were observed at necropsy. Microscopic tissue evaluation revealed no treatment-related effects in any organ or tissue at any dose level. Based on the results of this study, the no-observed-effect level was 2500 ppm in males and 500 ppm in females.

1563 THIRTEEN WEEK ORAL TOXICITY STUDIES OF THE CHMOPREVENTIVE AGENT INDOLE-3-CARBINOL IN RATS AND DOGS

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These studies examined the subchronic oral toxicity of indole-3-carbinol in CD• rats and Beagle dogs by the oral route. Dose levels were 0, 4, 20 and 100 mg/kg/day for both species. Dosing was by capsule in dogs and by gavage in rats. In dogs (high dose), anorexia, dehydration and a significant decrease in body weight were observed. One high dose male lost >30% of its body weight and was sacrificed moribund in week 4. Accordingly, high dose animals were not dosed in week 5, and recieved 50 mg/kg/day from week 6 through week 13. Other toxic effects included diarrhea and vomiting primarily in high dose males. Vacuolation of gall bladder epithelium was observed in all dose levels, whereas vacuolation of renal cortical and gastric parietal cells in addition to stress-related histologic changes were only seen in high dose animals. In rats, a biologically, but not statistically significant decrease in body weight was observed in high dose males. Liver relative weight (% brain weight) was significantly increased in the high dose rats, and was associated with centrilobular hepatocytic hypertrophy (males > females). However, only females demonstrated significant hyper-bilirubinemia. In mid and high dose rats, an increase in relative kidney weights was not supported by clinical chemistry changes. In dogs, the NOEL was not defined, however, it was 4 mg/kg/day in rats. Supported by NCI contract No. NO1-CN-15352-02.

1564 THE FATAL AND SEVERE TOXIC EFFECTS OF THE SAND VIPER (CERASTES CERASTES) BITES ARE SECONDARY TO VENOM EFFECT ON HAEMOSTASIS

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Two fatal and 3 severely envenomed cases are presented. The two fatal cases (42 and 90-year old males) developed right and left sided hemiplegia secondary to massive left and right cerebral infarction that occurred 5–24 hr following *C. cerastes* bite. Both died 18 hr and 17 weeks after the bite. A 40-year old male had upper left limb palsy and a 29-year old male developed dissiminated intravascular coagulation with acute tubular necrosis. A 2-year old boy developed right hemiparesis 6 days following the bite. Two months later he developed tonic clonic seizures that progressed as he grew older. The seizures were not adequately controlled by phenytoin or phenobarbitone but more effectively controlled by carbamazepine and sodium valproate. All victims received 20–40 ml of polyvalent antivenom in addition to the appropriate therapy, In rabbits, *C. cerastes* venom caused a significant increase in activated partial thromboplastin time and fibrin degradation products and a marked decrease in platelet count 1–4 hr following injection. On i.v. injection, the venom caused very rapid death with massive clot formation in the right side of the heart, pulmonary artery and vena cavae. It is concluded that the severe toxic effects of *C. cerastes* bites are likely due to the coagulant effect of its venom.

1565 DEVELOPMENTAL TOXICITY OF DIOXIN IN THE FEMALE RAT: EXTERNAL GENITALIA MALFORMATIONS WITHOUT ALTERATIONS IN PRIMORDIAL FOLLICLE NUMBER

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In utero and lactational TCDD exposure decreases testicular, epididymal and ejaculated sperm, decreases accessory sex organ weights and delays preputial separation in male rats. We sought to determine if such exposure would affect the female reproductive system. Primordial follicle number and time to vaginal opening were assessed as equivalent female parameters. Pregnant Holtzman rats were treated with 1.0 μg TCDD/kg or vehicle, po, on gestation day (GD) 11, 15 or 18 and offspring were weaned on postnatal day (PND) 21. TCDD did not change primordial follicle number on PND 7 (treated on GD 11 or 18) or PND 20–25 (treated on GD 15). However, although time to vaginal opening was not delayed, malformations of the external genitalia were observed, TCDD exposure starting on GD 11, 15, or 18 produced a superficial thread of tissue, running anterior to posterior across the vaginal orifice in 36, 44 and 38% and cleft prepuce with cleft clitoris in 96, 55 and 63% of the female offspring, respectively. Thread and cleft frequencies in female offspring of control dams ranged from 0 to 7%. *In utero* and lactational TCDD exposure does not appear to impact gamete number or delay an indicator of puberty in females as it does with males, however TCDD treatment resulted in a significant increase in external genitalia malformations. (Supported by NIEHS 01332 and HD27194)

1566 REPRODUCTIVE EFFECTS OF 2,3,7,8-TETRACHLORO-DIBENZO-p-DIOXIN (TCDD) IN FEMALE RATS: OVULATION, HORMONAL REGULATION, AND POSSIBLE MECHANISM

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A previous study has shown that exposure of adult rats to TCDD alters estrous cyclicity and ovulation. To further explore the mechanisms involved we employed the gonadotropin-primed immature female rat model. Single doses (0.3–60 μg/kg) of TCDD, dissolved in corn oil, were given orally to 22–24 day old rats. Equine chorionic gonadotropin (eCG) (20iu) was injected 24 hr later to induce follicular development. Rats were killed at various times after eCG, blood collected, and ovarian weights obtained. Serum concentrations of estradiol-17β (E2), luteinizing hormone (LH), follicle stimulating hormone (FSH), and prolactin (LTH) were measured by radioimmunoassay. Ovulation was determined 72 hr after injection of eCG by counting ova flushed from oviducts. TCDD reduced dose-dependently the increase in ovarian weight gain induced by eCG and also decreased the rate of ovulation and the number of ova recovered; the ED50 was about 5 μg/kg of TCDD. The increase in serum E2 induced by eCG was enhanced in animals treated with TCDD. Peak serum levels of FSH and LH, but not of LTH, were decreased by TCDD. E2, as expected, decreased dramatically in control animals between 60 and 72 hr after injection of eCG, concomitant with an ovulatory surge in LH. This response was absent in TCDD-treated animals. None of these effects were secondary to overt toxicity of TCDD since they were absent in pair-fed controls. In hypophysectomized rats treated with eCG and exogenous LH the rate of ovulation and the number of ova recovered were also significantly reduced by TCDD (10 and 60 μg/kg). These results suggest that TCDD alters reproductive function in the immature female rat model via effects on the hypothalamic-pituitary axis as well as by direct effects on the ovary.

1567 TCDD IS NOT ANTIESTROGENIC IN SPRAGUE-DAWLEY RATS

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Previous investigators have shown that 2,3,7,8 tetrachlorodibenzo-p-dioxin

(TCDD) can be antiestrogenic *in vivo* and *in vitro*. In the present study, the antiestrogenicity of TCDD was tested in immature female Sprague-Dawley rats. Postnatal day (PNd) 19 rats were weaned and separated into 5 groups: control, 10 μg/kg estradiol (E2), or E2 plus 20, 40, or 80 μg/kg TCDD. Rats received a single s.c. injection of olive oil or TCDD on PNd19 and 2 consecutive s.c. doses of E2 or olive oil on PNd21 and PNd22, and were sacrificed on PNd23. Total estrogen receptors (ER) and progesterone receptors (PR) were measured in uterine whole cell extracts. Three experiments were performed for a total of 12–20 animals per group (4–8 animals per group per analysis). E2 increased uterine wet wt./body wt. ratios to 219% of control (1.60 ± 0.23 mg/gm vs. 0.73 ± 0.19 for controls; $P < 0.001$), and caused keratinization of the vaginal epithelium. Neither uterine growth nor vaginal keratinization were affected by TCDD at any doses tested. E2 tended to reduce uterine ER levels ($P = 0.062$), whereas ER levels for the E2 plus TCDD groups were between, and not significantly different from those in the control and E2 groups. Uterine PR levels were not affected by E2 or TCDD in the present study. In contrast to its effects on estrogen-dependent processes. TCDD significantly decreased body weight at the 40 and 80 μg/kg level by PNd21 ($P < 0.008$), significantly decreased thymus/BW ratios ($P < 0.001$), and significantly increased liver/BW ratios ($P < 0.001$), indicating that TCDD was properly delivered, and could cause other well-documented toxic effects. In conclusion, TCDD, at doses which affected body, thymus and liver wts., had no effect upon uterine ER, PR, estrogen-induced uterine enlargement or vaginal keratinization, indicating that immature female Sprague-Dawley rats are resistant to the antiestrogenic effects of TCDD. These data suggest that the effects of TCDD on estrogen-dependent processes may be tissue, species and strain specific. Supported by NIH ES05549, NIH ES01247, DAMD17-94-J-4379.

1568 EFFECTS OF PERINATAL EXPOSURE TO 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN (TCDD) ON SERTOLI CELL NUMBER AND TESTICULAR FUNCTION

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TCDD reduces the steroidogenic capacity of the adult Sprague Dawley testis without altering spermatogenesis (*Toxicol* 89:49, 1994). Perinatal exposure to TCDD decreases spermatogenesis and alters androgen status in Holtzman rats (*Tox Appl Pharm* 89:97, 1992). The objective of this study was to determine if perinatal exposure to TCDD would reduce Leydig cell function or spermatogenesis via a reduction in the number of Sertoli cells. On day 15 of gestation timed-pregnant Sprague Dawley females were given a single oral dose of TCDD (0.0 or 0.40 μg/kg in corn oil/acetone). Control ($n = 4$) and treated ($n = 17$) males were terminated at 49 days of age to assess testicular function. TCDD reduced ($p < 0.01$) body weight on days 4 through 49 (242 ± 10 vs 217 ± 3 g; day 49). Weights of left (1.19 ± 0.05 vs 1.10 ± 0.02 g; $p < 0.05$) and right testicular parenchyma ($p < 0.05$), tail of the epididymis ($p < 0.05$), and number of spermatozoa in the head of the epididymis (1539 ± 245 vs 701 ± 96 × 10^6; $p < 0.01$) were reduced by TCDD. However, there was no difference in weights of the prostate, seminal vesicles (0.20 ± 0.01 vs 0.17 ± 0.03 g; $p > 0.05$), or total epididymis. The effect of perinatal exposure to TCDD on serum testosterone (3.75 ± 0.31 vs 3.01 ± 0.43 ng/ml) or human chorionic gonadotropin stimulated *in vitro* testosterone concentration (21.36 ± 1.86 vs 21.33 ± 1.18 ng/ml), daily sperm production (DSP) (22.2 ± 1.5 vs 19.6 ± 1.7 × 10^6), or Sertoli cell number per testis (46.2 ± 2.7 vs 40.7 ± 2.9 × 10^6) was not statistically significant. While Leydig cell function, Sertoli cell number, and DSP were not significantly affected by TCDD, the 13% reduction in Sertoli cell number and significant reduction in testicular parenchymal weight are consistent with detrimental effects of perinatal exposure of TCDD on spermatogenesis. (NIH R01 AG 11093-09 and NIH T32 ESO 7273.)

1569 EFFECTS OF PRENATAL TESTOSTERONE EXPOSURE ON RAT PROSTATE ANDROGEN SENSITIVITY

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Elevated circulating androgen levels during prenatal development may alter hormonal sensitivity of androgen target tissue and have been implicated in the high risk for prostate cancer among African-Americans. To address this question in an animal model, pregnant female WU:Hsd rats were given s.c. testosterone (T)-containing Silastic implants. Implants (ID 0.2 cm, OD 0.31 cm) 3.75 cm given before 12d of gestation interfered with implantation indicated by decreased gestation index. Implants given at 12d did not change gestation and viability indices, but birth weight and litter size were decreased. In female offspring, ano-genital distances were increased by 20% at birth (indicative of androgenization), and body weight was suppressed by 17% for up to 4 wks after birth. Based on these observations this implant length was selected for further study. Male offspring were sacrificed at an age of 4 (immature), 8 (immediately after puberty), and 16 wks of age. Body weights at these time points were not different from control, but relative weights of dorsolateral and ventral prostate were significantly increased (45% and 67%, respectively) by 16 wk; absolute testes weight at 4 and 8 wks were decreased (29% and 24%, respectively) but reached control levels at 16 wk. These data are interpreted to suggest that prenatal testosterone exposure at a dose that reaches the fetus delays the onset of puberty, but at the same time increases the androgen sensitivity of the target tissues in the male genital tract. This effect of prenatal androgen exposure is being verified by determining effects on androgen-regulated prostatic gene expression and changes in accessory sex gland weights of androgen-substituted castrated rats. (NIH grant CA58088).

1570 ENDOCRINE AND REPRODUCTIVE TOXICITY OF VINCLOZOLIN (VIN) IN MALE LONG-EVANS HOODED RATS

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Vinclozolin (CAS 50471-44-8), a fungicide, is an environmental anti-androgen that inhibits morphological sexual differentiation in rats (Kelce et al., TAAP 126:276, 1994). Experiment (Exp) 1 tested the influence of 0 and 100 mg/kg/day (M/K/D) vinclozolin ($n = 20$ per group) on basal and corticotropin-stimulated plasma LH, testosterone (T) and corticosterone (C4) in adult male rats. Exp 2 tested 0, 5, 15, 25, and 100 M/K/D vinclozolin ($n = 12$ per group) on reproductive development and testicular function. For both exps, dosing began at 3 weeks of age (WA) and continued until 14 WA (Exp 1) or 25 WA (Exp 2). In Exp 1, basal secretion patterns were assessed in samples collected every 10 min for 2 hours ($n = 6$–9) on D1; and responsiveness to corticotropin Releasing Hormone (CRH) (100 ng, iv; $n = 8$) or saline (100 μl, iv; $n = 5$) in samples collected at −15, 0, 2, 15, 30, 60, 120, and 1440 min. from CRH on D2. Organs were weighed in both Exps. In Exp 1 and 2, vinclozolin (100 M/K/D) significantly enlarged adrenals, testes, kidneys, and liver; decreased cauda epididymal weights and caudal sperm number; and increased mean basal testosterone and LH at 100 M/K/D; the cauda epididymides were also smaller at 15 and 25 M/K/D. After CRH, C4 increased 4-fold in controls but only 2.7-fold for the vinclozolin group. In Exp 2, age at preputial separation was delayed at 100 M/K/D (48 ± 3D vs 41 ± 4D in controls; $p < 05$). Fertility was not affected by vinclozolin. Thus, vinclozolin disrupted endocrine function in the pituitary gonadal axis and interfered with sexual maturation and adult androgen-dependent functions in rats (CR820335-01-0; conclusions do not necessarily reflect EPA policy).

1571 MECHANISTIC BIOASSAY WITH LINURON

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In a previously conducted two-year study with linuron, a concentration-dependent increase in Leydig cell adenomas was observed in Crl:CD®BR(CD) rats. In a two-week mechanistic study it was shown that linuron acts as an antiandrogen. To bridge the data from the two-week mechanistic study with tumor formation a two-year mechanistic bioassay was conducted using male CD rats. Rats were fed 625 ppm linuron, a concentration that was previously shown to produce tumors. In addition to the *ad libitum* control group, a second pair-fed control group was included. Rats were sacrificed at 1, 3, 6, 9, 12, 15, 18, 21 and 24 months. Organs were weighed and organ weight/final body weight ratios were calculated. Leydig cell proliferation was analyzed. Additionally, at these timepoints blood was collected from 10–20 rats/group and serum was analyzed for estradiol, testosterone, prolactin, luteinizing hormone (LH) and follicle stimulating hormone (FSH). Over the first year of the study, linuron produced endocrine alterations consistent with that of an antiandrogen (increased testosterone and FSH levels), and elevated relative testicular weights and decreased accessory sex organ unit weights were observed. At the end of the two years, linuron had induced elevated incidences of Leydig cell hyperplasia and adenomas. These data support the hypothesis that linuron possesses antiandrogenic properties and produces Leydig cell tumors via an antiandrogenic mechanism. The results of this study also demonstrated some general points which should be considered when investigating endocrine-mediated tumorigenesis: 1) FSH may be a better marker than LH for gonadotropin release, and thus androgen status, due to the long half-life of FSH; 2) the focal Leydig cell hyperplasia can be detected by light microscopy without doing cell

proliferation; and, 3) endocrine-mediated data collected over the first year of the two-year study appears to be far more consistent than data collected over the second year. These data suggest that short term mechanistic studies following bioassays appears to be sufficient to link mechanism of action with eventual tumor formation.

1572 EFFECT OF ATRAZINE ON THE HORMONAL CONTROL OF THE OVARY

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The effect of atrazine on the neuroendocrine control of ovulation was evaluated. In ovariectomized Long-Evans hooded rats, 300 mg/kg Atrazine (i.p.) reduced the E^2-induced surge of luteinizing hormone (LH) and markedly suppressed the prolactin (prl) surge. In intact females, a single dose of 300 mg/kg atrazine (p.o.) on vaginal proestrus did not effect on ovulation, although sustained treatment with this dose eliminated ovarian cycles within 4 days. A dose-dependent disruption of ovarian cycles was observed in females exposed to 150 and 75 mg/kg atrazine (p.o.) for three weeks. This exposure resulted in a significant suppression of the pituitary (LH, FSH, & prl) and ovarian (estradiol and progesterone) hormones. In Sprague Dawley rats a similar three-week exposure to atrazine also altered ovarian cycles. Hypothalamic dopamine and norepinephrine concentrations were not affected by either single or continued dosing suggesting that a depletion of CNS catecholamines was not responsible for altered pituitary hormone secretion. Since serum LH and prl were rapidly depleted, it is suggested that atrazine is a reproductive toxicant in the female and that this effect is mediated by a yet unidentified CNS-pituitary mechanism.

1573 THE EFFECT OF THIRAM ON THE CNS CONTROL OF LUTEINIZING HORMONE IN THE MALE AND FEMALE RAT

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The dithiocarbamate thiram (T) inhibits norepinephrine (NE) synthesis. We reported 50 mg/kg T suppresses the ovulatory surge of luteinizing hormone (LH) presumably because it alters hypothalamic (HYP) control of pituitary LH release. In this study, we determined the dose of T required to alter LH secretion in proestrous (PRO), ovariectomized (OVX) females and the male rats. Long-Evans hooded rats were exposed to T (i.p., 0–100 mg/kg at 1300 h and killed 1, 3, or 6 h). In PRO females, 50 mg/kg significantly decreased serum LH (at 3 h), but 12 mg/kg had the same effect in the OVX-estrogen primed rat at this time. To examine the putative CNS mechanism of T on LH secretion, we measured HYP NE and dopamine (DA). NE concentration decreased 3 h after 25 mg/kg T in PRO females. However, 12 mg/kg T decreased NE in OVX rats. HYP DA increased 3 h after 25 mg/kg in the PRO female and 12 mg/kg in OVX. By comparison, basal LH in males at 3 h decreased by 25 mg/kg. At 3 h male HYP NE decreased and HYP DA increased by 12 mg/kg. DMDC, T's primary metabolite, was also evaluated in males. LH and HYP NE & DA measures after DMDC reflected the same dose and time response differences seen after T. These data indicate that (1) the effect of T on serum LH and HYP NE & DA concentrations in the OVX female and male can be observed at a lower dose than in the PRO female; and (2) that CNS NE and DA changes occur at doses lower than those required to alter LH. The functional significance of this difference remains to be demonstrated.

1574 DIFFERENTIAL EFFECTS OF ENVIRONMENTAL TOXICANTS ON STEROID RECEPTOR BINDING

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Much attention has focused on environmental toxicants which disrupt the reproductive system by mimicking the action of estrogen by an interaction with the estrogen receptor (ER). Here we evaluate the ability of environmental toxicants to inhibit ligand binding to steroid receptors (SR). Using *in vitro* competitive receptor binding assays, the relative binding affinities of a series of environmental toxicants for ER, progesterone (PR) and androgen (AR) receptors were determined. The putative estrogenic compounds, chlordecone and o,p'-DDT, were equally effective as competitors for ER and PR (IC_{50} 3–5 μM) and less effective for the AR (IC_{50} 95 μM). While the major metabolite of DDT, p,p'DDE, exhibited little ability to bind ER, it was a potent inhibitor of AR binding (IC_{50} 5 μM). The active metabolites of vinclozolin, M1 (2-[[(3,5-dichlorophenyl)-carbamoyl]oxy]-2-methyl-3-butenoic acid) and M2 (3'5'-dichloro-2-hydroxy-2-methylbut-3-enanilide) inhibited AR binding (IC_{50} 92 and 9.7 μM, respectively), moderately inhibited PR binding (IC_{50} 400 and 68 μM, respectively), but had no affinity for the ER. Lindane, endosulfan, and atrazine (0.01–100 μM) were not effective SR antagonists at any of the concentrations tested. We suggest that environmental toxicants are promiscuous inhibitors of SR binding because they compete with endogenous ligand for binding to AR, ER and/or PR.

1575 NARINGENIN: A WEAKLY ESTROGENIC BIOFLAVANOID WHICH EXHIBITS ANTIESTROGENIC ACTIVITY

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Treatment of 21 day-old female Sprague-Dawley rats with naringenin at doses of 15, 30 or 40 mg did not result in uterine hypertrophy whereas 17β-estradiol (E2) caused an increase in uterine wet weight. In rats cotreated with E2 + naringenin (30 or 40 mg/rat), there was a significant decrease in E2-induced uterine hypertrophy. The binding of uterine nuclear extracts to a [^{32}P]-labeled estrogen responsive element (ERE) or progesterone responsive element (PRE) was determined using eletromobility shift assays. Treatment of rats with naringenin or E2 resulted in formation of specifically-bound estrogen receptor:ERE complexes; a higher mobility complex was dominant in the extracts from E2-treated rats whereas a lower mobility complex was observed in extracts from naringenin-treated animals. EMSA of uterine nuclear extracts from rats cotreated with E2 + naringenin showed that naringenin inhibited formation of the E2-induced band. Cytosols from the same animals showed that E2 induced formation progesterone receptor:PRE complex and naringenin inhibited this E2-induced response. In MCF-7 cells transiently transfected with a plasmid containing the promoter region from the *pS2* gene and a luciferase reporter gene, naringenin exhibited weak estrogenic activity. In cells cotreated with naringenin + 1 nM E2, naringenin inhibited the E2-induced response. Comparable results were obtained with other naturally-occurring flavonoids in the female rat uterus and MCF-7 human breast cancer cells. These results are consistent with the reported antitumorigenic activities associated with dietary uptake of vegetables which contain phytoestrogens such as the bioflavonoids.

1576 ACCUMULATION OF CEPHALORIDINE IN NORMOGLYCEMIC AND DIABETIC FISCHER 344 RATS

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Previous work has shown that the acute renal toxicity of cephaloridine was reduced in a diabetic rat model. The mechanism for reduced toxicity may be due to decreased renal accumulation of cephaloridine. Diabetes was induced in male Fischer 344 rats (190–220 g) by injection of 35 mg/kg streptozotocin (STZ). Normoglycemic animals were injected with buffer (1 ml/kg). Cephaloridine (750 or 1500 mg/kg) was injected (intraperitoneal) into normoglycemic and diabetic rats. Blood, urine and kidneys were collected 1–12 h after cephaloridine administration. Serum levels following 1500 mg/kg cephaloridine were lower ($p < 0.05$) in the diabetic compared to normoglycemic group 2 and 4 h after treatment. Renal excretion of cephaloridine was greater ($p < 0.05$) in diabetic compared to normoglycemic rats. Maximal accumulation of cephaloridine was quantitated 1 h after cephaloridine injection in both groups. Renal cortical accumulation of cephaloridine was diminished ($p < 0.05$) 4, 8 and 12 h after cephaloridine administration in the diabetic group relative to normoglycemics. These results indicate part of the mechanism for decreased toxicity in diabetic rats can be attributed to diminished renal accumulation of cephaloridine. (Supported by NIH grant DK45099).

1577 STUDIES ON NEPHROTOXICITY OF ASPIRIN AND GENTAMICIN IN COMBINATION IN RATS

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Aspirin and Gentamicin are widely used by many patients. Aspirin; 100 mg/kg orally for 14 days, and Gentamicin; 40 mg/kg by intramuscular injection in two divided doses for 10 days alone and in combination in rats was studied. Plasma Creatinine (PCr), Blood Urea Nitrogen (BUN), Urine Creatinine

(UCr), Urine Urea Nitrogen (UUN) and urine volume were measured after one and two weeks of treatments. In the Aspirin group, no significant changes were noticed in all the indices measured for nephrotoxicity. However, in the Gentamicin group, the parameters of renal effect which may indicate nephrotoxicity were observed. PCr. and urine volume significantly increased, and in the same time a significant decrease in both UCr. and UUN was noticed compared to control group. When Aspirin and Gentamicin were administered in combination, a marked increase in nephrotoxic parameters was detected compared to the Gentamicin group alone. This effect appeared in the form of a significant increase in PCr., BUN and urine volume, in addition to a concomitant significant decrease in both UCr. and UUN. So the data suggest that the nephrotoxic indices in the combination of both drugs were significantly higher than the results from Gentamicin alone.

1578 EFFECT OF ETHANOL ON FOLATE BINDING BY RAT RENAL BRUSH BORDER MEMBRANES

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Folate deficiency is known to be a physiologic effect of chronic alcoholism, partly due to an increased urinary excretion of folates. An Isolated Perfused Rat Kidney model in our laboratory has shown that ethanol must be present in the perfusate to increase urinary excretion of folate, demonstrating that ethanol has a direct effect on folate reabsorption in the proximal tubule. A specific Folate Binding Protein (FBP) is localized in the renal brush border membranes (BBM) and is believed to be the first step in reabsorption. Using BBM preparations from rats, these studies have determined if ethanol reduces the binding of folic acid (FA) and 5 Methyl tetrahydrofolate (5M, the physiologically active form) to FBP. BBM were pretreated with 100–500 mg/dl ethanol for 30 min, then ^{3}H-FA or ^{3}H-5M (0.1–100 nM) was added for a 60 min incubation. Ethanol did not reduce the binding of either of these forms of folate. Since ethanol is known to reduce folate reabsorption in the rat, then the lack of an effect on 5M binding by rat kidney BBM suggests that ethanol is affecting some step in the reabsorption process other than the initial binding to FBP. Supported by NIH Grant R01-AA05308.

1579 EFFECT OF PHLORIZIN ON THE NEPHROTOXICITY AND RENAL DISPOSITION OF β-CYCLODEXTRIN IN THE RAT

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Cyclodextrins are cyclic oligosaccharides which can increase the solubility and bioavailability of lipophilic molecules. Parenteral administration of β-cyclodextrin (βCD) results in nephrotoxicity associated with cytoplasmic crystals in the proximal tubule. Little is known about the mechanism of injury. The effect of phlorizin-induced glucosuria on the nephrotoxicity and renal accumulation of βCD was examined. Plasma urea nitrogen levels (BUN) and renal cortical slice uptake of p-aminohippurate (PAH) were used to assess nephrotoxicity. A HPLC method was also developed to estimate βCD in biological samples. Toxicity and renal βCD levels were quantitated 24 h after dosing. Administration of βCD (397–1000 mg/kg, ip) resulted in dose-dependent nephrotoxicity as judged by increased BUN levels and decreased PAH uptake. Renal levels of βCD increased in a dose-dependent manner. A 10 min pretreatment with phlorizin (130 mg/kg, ip), protected against the nephrotoxicity of βCD. Phlorizin pretreatment of the βCD group diminished renal βCD levels. The results indicate that the renal accumulation of βCD is probably involved in the expression of injury and that glucosuria can reduce the renal uptake and nephrotoxicity of βCD. (Supported by Higuchi Biosciences Center and NIH grant SO3 RR03053).

1580 A CHEMICAL INITIATION/PROMOTION STRATEGY INDUCES FEATURES OF A TRANSFORMED PHENOTYPE IN GLOMERULAR MESANGIAL CELLS

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Repeated *in vitro* challenge of cultured glomerular mesangial cells (GMCs) with benzo(a)pyrene (BaP) alone or in combination with methoxamine (MeoA) has been associated with acquisition of a highly proliferative phenotype. In the present study, experiments were conducted to assess the transformation potential of these cells by conventional *in vitro* approaches. In contrast to the spindle shape morphology characteristic of normal GMCs, BaP cells, but not BaP/MeoA cells, displayed an epithelial-like morphology. Both BaP and BaP/MeoA cells exhibited reduced serum requirements for growth relative to control cells, or GMCs treated with MeoA alone. No differences were seen in cell numbers during density arrest among any of the treatment groups. BaP and BaP/MeoA cells proliferated in an anchorage-independent manner, but the extent of colony formation by BaP/MeoA GMCs was more pronounced than BaP cells and comparable to H4IIE rat hepatoma cells. Total cellular glutathione (GSH) levels were doubled in BaP and BaP/MeoA GMCs relative to control counterparts. These data suggest enhanced transformation potential in GMCs following a chemical initiation/promotion strategy *in vitro* and lend support to emerging evidence implicating the kidney as an important target of toxicity for aromatic hydrocarbons. (Supported by NIH grant ES 04917).

1581 PARA-AMINOPHENOL-INDUCED NEPHROTOXICITY *IN VIVO* AND *IN VITRO*: ROLE OF EXTRARENAL METABOLISM

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Para-aminophenol (PAP) produces acute proximal tubular necrosis and elevations in blood urea nitrogen (BUN) concentrations within 24 hours following administration. Very little is known concerning early functional changes following PAP administration *in vivo* or during incubation of isolated renal proximal tubule suspensions with PAP *in vitro*. We assessed PAP toxicity *in vivo* and *in vitro* using female Sprague-Dawley rats. At 2 hr after PAP administration (300 mg/kg ip), renal GSH content was depleted 68%, O_2 consumption by renal cortical slices was inhibited 23%, and ATP, ADP and AMP content in kidney were decreased 29%, 20%, and 58%, respectively. At 4 hr after PAP administration (300 mg/kg ip), renal GSH content was depleted 62%, O_2 consumption by renal cortical slices was inhibited 24%, and ATP, ADP, and AMP, content in kidney were decreased 26%, 30%, and 64%, respectively. Decreases in adenine nucleotide content were accompanied by increases in renal xanthine content. In contrast to toxicity *in vivo*, incubation of renal proximal tubules with up to 1 mM PAP for up to 2 hr produced no significant changes in GSH content, adenine nucleotide content, O_2 consumption, or organic ion accumulation such as para-aminohippuric acid and tethaethylammonium uptake. Lack of *in vitro* toxicity suggests that the nephrotoxicity of PAP *in vivo* is due to bioactivation of metabolites formed in extrarenal organ(s) rather than by PAP in the kidney.

1582 IMMUNOHISTOCHEMICAL LOCALIZATION OF THE CYTOPROTECTIVE GLYCINE/STRYCHNINE RECEPTOR IN RABBIT KIDNEY CORTEX

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We have shown that glycine and strychnine protect renal proximal tubules (RPT) from a variety of nephrotoxicants. Furthermore, strychnine binds specifically to a site on the plasma membrane of RPT. Since immunoblotting identified proteins corresponding to the β-subunit of the neuronal strychnine-sensitive glycine receptor and the associated protein gephyrin in homogenates of kidney cortex, we proposed that glycine and strychnine exert their cytoprotection through a novel receptor related to the neuronal glycine receptor (Life Sci. 55:27, 1994). The purpose of this study was to determine the subcellular distribution of the receptor complex in rabbit kidney cortex. Immunohistochemical studies were performed using the mAb GlyR 4a, directed against the β-subunit and GlyR 7a, directed against gephyrin. Fresh frozen sections (15 μm) of kidney cortex were cut, mounted and representative sections stained with H&E. Sections were fixed in acetone and blocked in 9% sheep serum. Following incubation with GlyR4a or GlyR7a, the slides were processed using the peroxidase, antiperoxidase technique or incubated with a FITC-conjugated secondary antibody. Specific staining was identified along the cytoplasmic face of the basolateral membrane of the RPT cells. There was no staining found along the cytoplasmic face of the apical membrane, in the brush border or nuclei, or in the glomeruli. Thus, the identification of the basolateral distribution of the β-subunit and gephyrin supports previous immunoblotting and functional data indicating the existence of a novel cytoprotective receptor complex in RPT. (Supported by NIH ES-04410 and a Procter & Gamble Co. Graduate Fellowship).

1583 PROTEIN PHOSPHATASE INHIBITORS PREVENT ANTIMYCIN-A INDUCED RENAL CELL DEATH

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Glycine and strychnine protect renal proximal tubules (RPT) from a variety of toxicants, including the mitochondrial inhibitor antimycin A (AA). We have previously shown that protein kinase inhibitors reverse the cytoprotective properties of glycine to RPT exposed to AA (J. Am. Soc. Nephrol. 2:658, 1991). The goal of this study was to determine if protein phosphatase 1 and 2A inhibitors are cytoprotective. Rabbit RPT suspensions were pretreated with the protein phosphatase inhibitors calyculin A (3–100 nM) or okadaic acid (100–10,000 nM) for 5 min prior to AA (200 nM) addition. RPT lactate dehydrogenase (LDH) release was determined 30 min later. LDH release in control suspensions was 6%. Calyculin A decreased AA-induced LDH release in a concentration-dependent manner (AA 29%, AA + 3 nM calyculin A 26%, AA + 10 nM calyculin A 16%, AA + 30 nM calyculin A 12%, AA + 100 nM calyculin A 14%) Okadaic acid was less potent but also decreased AA-induced LDH release in a concentration-dependent manner (AA 29%, AA + 100 nM okadaic acid 31%, AA + 1,000 nM okadaic acid 26%, AA + 10,000 nM okadaic acid 11%). These results suggest that protein phosphatases 1 and 2A play an important role in cell death produced by inhibition of mitochondrial function. Furthermore, cell death from ATP depletion may be due to the inability of protein kinases to maintain critical proteins in a phosphorylated state in the presence of phosphatase activity.

1584 MODULATION OF SODIUM-COUPLED UPTAKE AND BRUSH-BORDER MEMBRANE FLUIDITY BY CISPLATIN IN PROXIMAL TUBULAR CELLS IN PRIMARY CULTURE AND BRUSH-BORDER MEMBRANE VESICLES

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It has been previously demonstrated that cisplatin (CDDP) decreased Na^+-coupled uptake of Pi and α-methylglucopyranoside (MGP) in rabbit proximal tubular (RPT) cells in primary culture. To determine whether these alterations could result from direct mechanisms or modified brush-border membrane (BBM) fluidity, the effects of CDDP on BBM fluidity and on Na^+-coupled uptake of Pi and MGP were examined in RPT cells and BBM vesicles from the renal cortex. At 17 μM CDDP, Na^+-coupled uptake of Pi and MGP in RPT cells was reduced by 30%, and the cholesterol content of BBM vesicles prepared from RPT cells in primary culture declined by 28% whereas their fluidity was increased. The increased-BBM fluidity was not associated with a rise in lipid peroxidation or permeability of tight-junctions. In contrast, CDDP decreased Na^+-coupled uptake of Pi and MGP in BBM vesicles isolated from the outer rabbit renal cortex whereas their fluidity remained unchanged. This was associated with a significant decrease in sulfhydryl groups of BBM vesicles. We conclude that (i) CDDP increased BBM fluidity in RPT cells by indirect mechanism which could depend on an alteration in cholesterol metabolism, (ii) the CDDP-induced inhibition of Na^+/Pi and Na^+/glucose cotransport were not due to the concomitant change in membrane fluidity but may be mediated through chemical interactions with sulfhydryl groups.

1585 DISSIMILAR ALTERATIONS OF SODIUM-COUPLED UPTAKE BY PLATINUM DERIVATIVES IN RENAL PROXIMAL TUBULAR CELLS IN PRIMARY CULTURE

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Cisplatin (CDDP) interferes early with electrolyte transport by the renal proximal tubule. The effects of increasing concentrations of CDDP, transplatin (TDDP), and carboplatin (CBDCA) on Na^+-coupled uptake of Pi, methyl-α-D-glucopyranoside (MGP) and L-alanine by rabbit proximal tubule cells in primary culture were studied. At 17 μM CDDP and 540 μM CBDCA, (i) cell viability and ATP content were unaffected, (ii) Na^+-K^+-ATPase activity was reduced by 40%, (iii) Na^+-coupled uptake of MGP and Pi were reduced, while (iiii) Na^+-coupled uptake of alanine rose to twice the control value. Alterations of Na^+-coupled uptake of Pi, MGP and alanine were due to changes in K_m, with no significant change in V_{max}. At 333 μM TDDP, Na^+-dependent Pi and MGP uptake decreased, whereas Na^+-independent uptake increased markedly and was associated with a decline in cell viability and ATP content. We conclude that (i) the TDDP-induced decrease in Na^+/Pi and Na^+/glucose cotransport was associated with reduced cell viability, (ii) both CDDP and CBDCA had different effects on Na^+/Pi, Na^+/glucose and Na^+/alanine cotransport, arguing against an alteration of the Na^+ gradient due to reduced Na^+-K^+-ATPase activity, (iii) CBDCA induced alterations of Na^+-coupled uptake similar to those of CDDP at concentrations 20 to 30 times higher by possible interactions with essential sulfhydryl groups of transport proteins and/or changes in brush-border membrane fluidity.

1586 TIME COURSE AND POTENTIAL MECHANISMS FOR VANCOMYCIN ACCUMULATION *IN VIVO* AND *IN VITRO*

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Vancomycin (VAN) a glycopeptide antibiotic used to treat gram-positive infections can cause serious nephrotoxicity. VAN accumulation into the kidney may be a prerequisite to the development of toxicity. The purpose of this study was to monitor the accumulation of VAN *in vivo* and *in vitro* and to begin to evaluate potential mechanisms for VAN accumulation in renal tissue. VAN was administered i.p. to female Sprague-Dawley rats (225, 450 and 750 μmol/kg). Animals were terminated at 15, 30, 60, 90 min, 2, 3, 6, 12, 24, & 48 hrs following dosing. *In vitro,* renal cortical slices were incubated with 1 mM VAN for 5, 15, 30, 45, and 90 min. Incubation conditions were: 1) under O_2 as control; 2) under O_2 plus cyanine 863 (a cation transport inhibitor); 3) under N_2 and without substrates (to inhibit active transport); 4) under N_2 and with substrates. Serum and renal tissue were analyzed for VAN content by HPLC. Serum VAN concentration peaked (793.1 μg/ml) 90 min after injection of 225 μmol/kg VAN and cleared from the serum by 24 hrs. For 450 and 750 μmol/kg, VAN peak serum concentrations (1317.7 μg/ml and 1771.5 μg/ml respectively) were reached by 3 hrs and cleared from the serum by 48 hrs. Renal VAN concentrations peaked at 2 hr (225 μmol/kg) and 6 hr (450 and 750 μmol/kg) after dosing. Renal cortical slice VAN accumulation increased with time (1.29, 1.63, 1.89, 2.06, 2.40 mg/g for 5, 15, 30, 45, and 90 min, repectively). Incubation without substrate and under N_2 decreased VAN accumulation by approximately 40% within the first 5 minutes and remained depressed through 45 minutes of incubation. These results suggest that renal VAN accumulation may occur via an active transport pathway. (Supported by NIGMS R29-GM47549).

1587 EFFECTS OF VANCOMYCIN ON CELLULAR PROTEIN IN PRIMARY CULTURES OF RENAL CORTICAL EPITHELIAL CELLS

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Previous work has demonstrated that vancomycin (VAN) administered *in vivo* to Sprague-Dawley rats at dosages of 450 and 750 μmol/kg results in a significant increase in kidney size within 48 hours post-dosing. This increase in kidney weight is due to an increase in protein, water and phospholipid content. The purpose of this study was to develop an *in vitro* model to evaluate the effects of VAN on renal protein synthesis. Primary cultures of renal cortical epithelial cells were prepared from Sprague-Dawley rats and grown on collagen I — coated 35 mm culture dishes in DMEM-F12 medium supplemented with 10% newborn calf serum. Cells were grown to confluency and treated for 4, 12 and 24 hours with 1, 2.5 and 5 mM VAN. Morphological changes following 5 mM VAN were observed after 12 and 24 hours of incubation with VAN. These changes included a decreased definition of cell boundaries with a shrinking of colony size. No morphological changes were observed at the two lower concentrations of VAN. Cellular protein was measured at the end of each time point. A concentration-dependent increase in total cellular protein was observed at each time point measured. Cellular protein increased by 34, 44 and 66%, respectively for 1, 2.5 and 5 mM VAN after 4 hrs of incubation. Incubation with 1, 2.5 and 5 mM VAN resulted in a 10, 48 and 60% increase, respectively, in cellular protein after 12 hrs of incubation. Cellular protein was increased by 37, 40 and 65% following 1, 2.5 and 5 mM VAN after 24 hrs of incubation. These results suggest that the increase in renal size observed after VAN exposure may be due to an increase in renal protein synthesis. In addition, primary cultures of renal cortical epithelial cells may be useful in elucidating the mechanisms of VAN's effect on renal protein synthesis. (Supported by NIGMS R29-GM47549).

1588 DIRECT EFFECTS OF *IN VITRO* VANCOMYCIN (VAN) ON RAT RENAL CORTICAL MITOCHONDRIAL FUNCTION

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Vancomycin (VAN) is a nephrotoxic cationic glycopeptide that is used to treat gram-positive bacterial infections. We have demonstrated VAN-induced accumulation in renal tissue and nephrotoxicity following *in vivo* and *in vitro*

exposure. The purpose of this study was to investigate potential mechanisms of VAN-induced renal injury by examining the direct effect of VAN upon rat renal cortical mitochondria (RCM). RCM were isolated from female Sprague-Dawley rats and exposed to control or VAN (1×10^{-3}M, 2.5×10^{-3}M or 5×10^{-3}M) for 3 minutes and parameters of mitochondrial respiratory function were measured in the presence of site I substrates (pyruvate/malate). Respiratory control ratios (RCR) were 5.5 ± 0.94, 3.6 ± 0.75, 1.64 ± 0.23 and 1.045 ± 0.045 for control 1×10^{-3}M, 2.5×10^{-3}M and 5×10^{-3}M VAN, respectively. State 4 values were 29.5 ± 1.9, 40.9 ± 4.5, 81 ± 18.3 and 90.8 ± 15.9 and ADP/O ratios were 3.06 ± 0.18, 2.6 ± 0.48, 2.56 ± 0.33 and 0.435 ± 0.435, respectively, with state 4 rates expressed in terms of nmoles-O_2-mg protein^{-1} min^{-1}. There was a statistically significant concentration-dependent decrease in RCR following VAN exposure paralleled by a pronounced stimulation of state 4 respiration at VAN $\geq 2.5 \times 10^{-3}$M. Effects upon state 3 rates exhibited an obvious downward concentration-dependent trend. The results strongly suggest that VAN is a potential uncoupler in rat renal cortical cells that accumulate VAN to concentrations $>1 \times 10^{-3}$M. Further studies are necessary to define this effect in the presence of site II and site III substrates. Whether or not mitochondrial dysfunction plays a central causal role in producing VAN nephrotoxicity will be examined in future studies. (Supported by NIGMS R29-GM47549).

1589 PHOSPHORAMIDON(PRM), AN ENDOTHELIN SYNTHESIS INHIBITOR, PROTECTS AGAINST HEXACHLORO-1,3-BUTADIENE (HCBD) NEPHROTOXICITY

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HCBD causes a dose-dependent acute renal failure, characterized by decreases in renal blood flow and renal function. Endothelin, a 21 amino acid peptide, is an intense renal vasoconstrictor. We examined the role of endothelin in HCBD nephrotoxicity by using PRM to block endothelin synthesis. Male Sprague Dawley rats were pretreated with PRM (5 mg/kg) or saline followed by an i.p. injection of HCBD or oil. At 24 hours, HCBD increased BUN in the saline pretreated rats (40 ± 5 mg/dL vs. 21 ± 0.79 mg/dL) but not in the PRM pretreated rats (27 ± 3 mg/dL vs. 23 ± 0.6 mg/dL). At 48 hours, BUN levels were increased in the saline pretreated rats (59 ± 7 mg/dL vs. 22 ± 1 mg/dL), and PRM pretreatment brought the BUN level back down toward normal (37 ± 4 mg/dL vs. 23 ± 1 mg/dL). HCBD increased glucose excretion in saline pretreated rats (at 48 hours — 77 ± 46 mg vs. 2 ± 0.3 mg). PRM pretreatment decreased glucose excretion (at 48 hours — 21 ± 10 mg vs. 0.4 ± 0.1 mg). HCBD increased protein excretion in saline pretreated rats (at 48 hours — 330 ± 67 mg vs. 126 ± 18 mg), while PRM pretreatment reduced protein excretion to 223 ± 44 mg vs. 107 ± 10 mg at 48 hours. This data shows that blocking endothelin synthesis with PRM offers some protection from HCBD nephrotoxicity. This research was supported by the Allegheny-Erie regional chapter, WVU School of Medicine Research Grant and NIH Training Grant GM 07039-20.

1590 HEXACHLOROBUTADIENE EXCRETION IN URINE BY BINDING TO α_{2u}-GLOBULIN

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Hexachlorobutadiene (HCBD) is nephrotoxic in rats causing damage to the proximale tubules. Renal toxicity is presumed to be due to bioactivation by glutathione *S*-conjugate formation and further processing by the enzymes of the mercapturic acid pathway to reactive intermediates. Recent studies revealed major sex-dependent differences in the pattern of urinary metabolites and gave evidence for the excretion of unmetabolized HCBD in the urine of male, but not female Wistar rats. Object of this study was to investigate the basis for HCBD-excretion in the urine. We administered ^{14}C-HCBD (200 mg/kg b.w., p.o.) to male and female Sprague-Dawley and NCI-Black-Reiter rats (NBR), an α_{2u}-globulin-deficient strain. No significant differences in the disposition and in the rates of excretion of ^{14}C-derived radioactivity were observed between male and female animals of both strains. 8.2% (±3.3) of dose were excreted in the feces, 3.1% (±0.7) in the urine and 1.7% were exhaled unmetabolized. The major metabolite excreted by female rats was *N*-acetyl-*S*-(1,2,3,4,4-pentachlorobutadienyl)-L-cysteine (N-AcPCBC) and small amounts of *S*-(1,2,3,4,4-penta-chlorobutadienyl)-L-cysteine (PCBC) were detected. In the urine of male rats *N*-acetyl-*S*-(1,2,3,4,4-pentachlorobutadienyl)-L-cysteine sulfoxide and small amounts of PCBC and N-AcPCBC were found. ^{14}C-HCBD (6% of total urinary metabolites) was identified in the urine of male Sprague-Dawley rats. No HCBD was observed in the urine of male NBR rats and of female animals. Anion-exchange HPLC showed radioactivity associated with the α_{2u}-globulin fraction in urine and renal cytosol, identified as HCBD bound to the protein. The results indicate, that the male specific urinary excretion of HCBD is associated with binding to α_{2u}-globulin. A role of α_{2u}-globulin-binding in the nephrotoxicity of HCBD has still to be elucidated. *Supported by Hauptverband der Berufsgenossenschaften and Deutsche Forschungsgemeinschaft (SFB 172).*

1591 S-[(1 AND 2)-PHENYL-2-HYDROXYETHYL] CYSTEINE (PHEC)-INDUCED RAT KIDNEY MITOCHONDRIAL DYSFUNCTION

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Previous studies suggest that mitochondrial dysfunction may be an important early event in PHEC-induced cytotoxicity in isolated rat renal proximal tubules (Toxicologist 14: 64, 1994). This study was therefore designed to obtain more detailed information about the effects of PHEC on rat renal cortical mitochondrial dysfunction. Both State 3 and State 4 respiration rates as well as the respiratory control ratio were inhibited by PHEC in concentration- and time-dependent manner with either succinate or α-ketoglutarate/malate, but not with ascorbate/N,N,N^1,N^1-tetramethyl-p-phenylene diamine as a substrate. The same phenomena were observed 4 and 24 hr following an i.v. administration of 500 μmol PHEC per kg in rats. Studies of the time course of PHEC-induced nephrotoxicity *in vivo* have shown that the decreased respiration at 4h may be causally related to the onset of nephrotoxicity at 24h, as measured by urinary excretion of glucose and N-acetyl-β-D-glucosaminidase. PHEC caused a marked dose-dependent inhibition of mitochondrial succinate dehydrogenase and NADH cytochrome c reductase activities. PHEC also inhibited 2,4-dinitrophenol-activated mitochondrial ATPase activity. Pretreatment with aminooxyacetic acid failed to prevent mitochondrial dysfunction of PHEC, suggesting an absence of further metabolism of PHEC by β-lyase to a toxic thiol species. These results suggest that PHEC exerts its effect on the mitochondrial respiration and oxidative phosphorylation through the action on the mitochondrial electron transport chain. (Supported by MRCC, MA-9705).

1592 ORGANIC ION TOXICANTS DISRUPT ORGANIC ION TRANSPORT IN RABBIT RENAL PROXIMAL TUBULE (RPT) SUSPENSIONS

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The ability to inhibit the accumulation of the organic ions p-aminohippurate (PAH) and tetraethylammonium (TEA) is often used as an indicator of toxicity. However, the mechanism(s) responsible for the toxicant-induced inhibition of organic ion accumulation have been largely ignored. Although toxicant-induced mitochondrial dysfunction and depletion of ATP levels might be expected to produce a significant inhibition of transport function, this link has not been demonstrated. The organic ion toxicants dichlorovinyl-cysteine (DCVC), N-acetyl-dichlorovinyl-cysteine (NA-DCVC) and celiptium (Cel) are believed to target the mitochondria in the nephrotoxicity produced by these agents. Thus, we examined the relationship between the mitochondrial dysfunction and inhibition of organic ion transport produced by these agents in RPT suspensions. In digitonin permeabilized tubules, 100 μM DCVC decreased ADP-stimulated respiration by 60% after 15 min which was followed by a 50% decrease in TEA accumulation after 30 min. Similarly after 30 min, NA-DCVC (200 μM) decreased ADP-stimulated respiration by 54% which was followed by a 40% decrease in TEA accumulation after 60 min. ADP-stimulated respiration was inhibited by 38% and PAH accumulation reduced by 50% after 30 min in 100 μM Cel-treated tubules. However, Cel-induced mitochondrial dysfunction paralleled, rather than preceded, the inhibition of PAH uptake. These observations suggest that DCVC and NA-DCVC induced mitochondrial dysfunction leads to disruptions in organic ion transport, but mechanisms other than mitochondrial dysfunction may contribute to Cel effects on organic ion transport. (NIH DK 41006)

1593 *IN VIVO* AND *IN VITRO* RENAL EFFECTS INDUCED BY N-(3,5-DICHLOROPHENYL)-2-HYDROXYSUCCINIMIDE-O-SULFATE (NSC), A PUTATIVE METABOLITE OF N-(3,5-DICHLOROPHENYL)SUCCINIMIDE (NDPS)

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NDPS was developed as an agricultural fungicide, but proved to be a nephro-

toxicant. Metabolites are known to contribute to NDPS nephrotoxicity, but the ultimate nephrotoxicant chemical species has not been identified. The purpose of this study was to examine the renal effects induced by the putative sulfate conjugate (NSC) of a potent nephrotoxicant metabolite of NDPS, N-(3,5-dichlorophenyl)-2-hydroxysuccinimide (NDHS). In the in vivo studies, male Fischer 344 rats (4/group) received a single administration of NSC (0.1 or 0.2 mmol/kg, ip; 0.05 or 0.1 mmol/kg, iv), NDHS (0.1 or 0.2 mmol/kg, ip) or the appropriate vehicle, and renal function was monitored at 24 and 48 hr. NSC administration (ip or iv) did not result in any marked changes in the renal functional parameters monitored, while NDHS induced marked nephrotoxicity at both doses tested. In in vitro studies using renal cortical slices, p-aminohippurate accumulation was reduced by bath concentrations of NSC as low as 0.001 mM, but no effects were observed on tetraethylammonium accumulation (0.001–0.1 mM NSC), gluconeogenesis (0.01–1.0 mM NSC) or lactate dehydrogenase release (0.001–0.1 mM NSC). These results suggest that NSC does not contribute to NDPS nephrotoxicity. Supported by NIH grant DK31210.

1594 EFFECT OF DIMETHYL SULFOXIDE ON N-(3,5-DICHLOROPHENYL)SUCCINIMIDE (NDPS) AND NDPS METABOLITE NEPHROTOXICITY

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The purpose of this study was to determine the suitability of dimethyl sulfoxide (DMSO) as a vehicle for administering the nephrotoxicant N-(3,5-dichlorophenyl)-succinimide (NDPS) and two nephrotoxicant NDPS metabolites; N-(3,5-dichlorophenyl)-2-hydroxysuccinimide (NDHS) and N-(3,5-dichlorophenyl)-2-hydroxysuccinamic acid (NDHSA). Male Fischer 344 rats (4/group) were administered a single intraperitoneal injection of NDPS (0.4 or 0.8 mmol/kg), NDHS (0.1 or 0.2 mmol/kg), or NDHSA (0.1 or 0.2 mmol/kg) dissolved in 25% DMSO in sesame oil or 100% sesame oil (2.5 ml/kg), while control rats received vehicle only. Renal function was then monitored at 24 and 48 h. DMSO markedly attenuated NDPS 0.4 mmol/kg-induced nephrotoxicity and reduced NDPS 0.8 mmol/kg-induced renal effects. Thus, the magnitude of the attenuating effect of DMSO depended in part on the nephrotoxicant dose of NDPS. In addition, NDHS nephrotoxicity was not altered by DMSO and only slight effects on NDHSA nephrotoxicity were observed. These results suggest that DMSO is capable of attenuating NDPS nephrotoxicity, and that the primary mechanism of this interaction appears to be due to an inhibition of the biotransformation of NDPS to NDHS. Supported by NIH grant DK31210.

1595 NEPHROTOXIC POTENTIAL OF N-(3,5-DICHLOROPHENYL)GLUTARIMIDE (NDPG) AND N-(3,5-DICHLOROPHENYL)GLUTARAMIC ACID IN FISCHER 344 RATS

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N-(3,5-Dichlorophenyl)succinimide (NDPS) was developed as an agricultural fungicide, however, subsequent testing showed that it was nephrotoxic in rats. The potential renal toxicity of NDPG, a structural analogue of NDPS, and its probable major metabolite NDPGA was determined in these experiments. Male Fischer 344 rats were divided into groups (N = 4) and housed in metabolism cages. After a control day, the animals were administered NDPG or NDPGA (0.4 or 1.0 mmol/kg, ip in corn oil) or corn oil alone. Renal function was monitored over 48 hours. NDPG and NDPGA had no effect on urine volume, urine protein content, or body weight. Two rats in both NDPGA groups exhibited hematuria and one rat in each NDPGA group exhibited glucosuria 6 hr post injection but all values returned to normal by 24 hr. The animals were sacrificed at 48 hr post injection and the kidneys were removed. Uptake of [^{3}H]p-aminohippurate and [^{14}C]tetraethylammonium by renal cortical slices was not different from control values. Blood urea nitrogen levels were normal. Kidney weights and food and water consumption values were unaffected when compared to control animals. These results suggest that NDPG and NDPGA are not nephrotoxic and that a succinimide ring is necessary for toxicity to ocgur. Supported by USPHS grant ES05189.

1596 PHENOBARBITAL MODULATION OF THE *IN VIVO* METABOLISM AND DISTRIBUTION OF N-(3,5-DICHLOROPHENYL)SUCCINIMIDE

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Pretreatment of rats with the enzyme inducer phenobarbital (PB), was previously shown to potentiate *N*-(3,5-dichlorophenyl)succinimide (NDPS)-induced nephrotoxicity. In the present work, PB modulation of the metabolism and distribution of a non-nephrotoxic dose of NDPS was studied. Naive and PB-pretreated (80 mg/kg; ip in saline) male Fisher 344 rats were administered 0.2 mmol/kg ^{14}C-NDPS (10 μCi; ip in corn oil). Rats were sacrificed 6 hours postinjection, and the liver, kidneys, heart, urine, and serum were removed. Liquid scintillation counting was used to determine tissue radiolabel content and covalent binding. Aliquots of tissue samples were deproteinized and prepared for HPLC injection. Between 40 and 45% of the administered radioactivity was recovered. In both the control and treatment groups most of the radiolabel was excreted in the urine with detectable amounts also in the liver, kidneys, and serum. Less than 1% of the recovered radioactivity was found in the heart. PB-pretreatment elevated the concentration of *N*-(3,5-dichlorophenyl)-2-hydroxysuccinamic acid (2-NDHSA), an oxidative metabolite of NDPS, while decreasing the level of a nonoxidative product, *N*-(3,5-dichlorophenyl)succinamic acid (NDPSA), in the urine. Compared to controls, PB induction also increased renal covalent binding by greater than 3-fold and elevated urine volume, giving evidence for possible renal toxicity. These results suggest that the formation of extrarenal metabolites contributes to NDPS-induced acute nephrotoxicity. Supported by USPHS grant ES05189.

1597 *IN VITRO* NEPHROTOXICITY ASSOCIATED WITH g-HEXACHLOROCYCLOHEXANE OR LINDANE

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Lindane (LIN) or g-hexachlorocyclohexane, an organochlorine insecticide, functions as g-aminobutyric acid (GABA) antagonist within the central nervous system. Peripheral GABA-like receptors have been localized to the distal portion of the nephron of the kidney. LIN may have potential to act as a distal tubular toxicant. Recently, our laboratory has investigated the use of urinary kallikrein (UK) as a marker for distal tubular injury both *in vivo* and *in vitro*. Using UK as a marker for LIN-induced renal injury, renal cortical slices were incubated with 1×10^{-4}, 5×10^{-5}, 1×10^{-5} M LIN dissolved in 75% acetone/25% ethanol for 2 hours and UK excretion into the medium was determined. Slice viability was determined through lactate dehydrogenase (LDH) leakage. Leakage of alkaline phosphatase (AP), a proximal tubular enzyme, into the incubation medium was also assayed to determine proximal damage. LDH leakage (units/mg tissue) was 9290 ± 808, 8141 ± 922, 7659 ± 139, 7617 ± 189, and 7534 ± 312 for control, vehicle control, 1×10^{-4}, 5×10^{-5}, 1×10^{-5} M LIN, respectively. UK activity (units/mg tissue) was 22.69 ± 1.66, 25.08 ± 3.48, 42.18 ± 1.29, 37.9 ± 5.98, and 31.88 ± 1.11 for control, vehicle control, 1×10^{-4}, 5×10^{-5}, 1×10^{-5} M LIN, respectively. There was no change in AP leakage from control in either the vehicle control, 1×10^{-4}, 5×10^{-5}, or 1×10^{-5} M LIN. These data suggest that LIN may produce distal injury *in vitro* and UK leakage might be a sensitive marker for this injury.

1598 BIOCHEMICAL EVIDENCE OF APOPTOSIS IN RENAL TISSUE RESULTING FROM TISSUE PREPARATION

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Apoptosis is a process that occurs as part of the normal cell turnover and in tissue remodelling. Apoptosis has been reported to increase in renal tissue following renal artery stenosis, ischemia/reperfusion hydronephrosis, radiation nephropathy, and after analgesic drugs. One of the frequently used biochemical markers of apoptosis is the appearance of a ladder pattern of DNA extracted from renal tissue. The processing of renal tissue for subsequent DNA extraction and analysis varies from laboratory to laboratory. As for many other biochemical measurements, tissue processing may potentially affect DNA fragmentation and make it difficult to compare DNA fragmentation in control and treated tissues. Therefore we conducted experiments to examine the effect of perfusion, clamp freezing, drop freezing, and time of processing on DNA ladder formation and apoptosis. Our results indicate that DNA purified from renal tissue perfused and drop frozen in liquid nitrogen resulted in the highest degree of detectable ladder patterns of DNA fragmentation as assessed by agarose gel electrophoresis. Similarly, a 3 min delay before freezing resulted in increased internucleosomal DNA fragmentation. Histo-

logic examination of the processed tissues revealed apoptotic bodies in various segments of the nephron. In situ end-labeling further identified the presence of DNA fragments in many cells. Conversely, DNA from tissues prepared by immediate clamp freezing showed no evidence of DNA fragmentation. These results provide evidence that apoptosis and DNA fragmentation can be increased in untreated tissues as a artifact of tissue processing. This increase can be minimized in toxicologic studies of apoptosis by immediate clamp freezing. It is recommended that clamp freezing be used as the method of renal tissue processing in toxicologic evaluations which utilize apoptosis as a toxicologic endpoint. (Sup NIH DK15440 & Navy N00014-91-J-1863).

1599 ASSESSMENT OF THE APOPTOTIC EFFECTS OF OKADAIC ACID IN PRIMARY CULTURES OF RENAL PROXIMAL TUBULE CELLS

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We recently reported that the phosphatase 1 and 2A inhibitor okadaic acid induces apoptosis in a normal rat kidney epithelial cell line (NRK-52E). Established cell lines are frequently used to identify mechanisms of cellular toxicity. However, it has been argued that freshly isolated PTE cells may differ from the continuous cell line in their cytotoxic response due to the presence of specialized cellular functions. Therefore we conducted this study to determine the response of primary cultures of proximal tubule (PTE) cells to okadaic acid and compare the response to that of the NRK-52E cell line. Exposure of PTE cells to 0.5-1 μM okadaic acid resulted in morphologic changes characteristic of apoptosis. Ultrastructurally, 1.0 μM okadaic acid caused cytoplasmic bleb formation, cellular fragmentation, condensation of heterochromatin into clumps, and segregation of nucleoli. Marked cell shrinkage, cytoplasmic fragmentation, nuclear fragmentation and cell detachment occurred in a dose-dependent fashion. The attached, rounded cells also effectively excluded propidium iodide dye demonstrating maintenance of membrane integrity despite pronounced morphologic alterations. Transverse alternating field electrophoresis revealed the appearance of large DNA fragments of approximately 300 kbp. The appearance of these 300 kbp fragments correlated temporally with the onset of morphologic alterations. Although the DNA of PTE cells fragmented into 300 kbp units, and the cells exhibited classical apoptotic morphology, no detectable internucleosomal fragmentation was observed. These studies support the hypothesis that during apoptosis, chromatin condensation reflects chromatin release from chromatin loop domains independent of internucleosomal cleavage. These results also indicate that the morphologic and biochemical response of NRK-52E cell line primary culture PTE cells are similar. (Sup NIH DK15440).

1600 PHASE I AND PHASE II METABOLIC ACTIVITY IN RABBIT RENAL PROXIMAL CONVOLUTED (PCT) AND STRAIGHT (PST) TUBULES

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Many nephrotoxicants express their toxicity asymmetrically within either the proximal convoluted (PCT) or proximal straight tubules (PST). These asymmetric toxicity patterns may be determined in part by a differential distribution of phase I and II metabolic enzymes between these segments. In these studies chemical probes were used to measure specific phase I and phase II metabolic enzyme activities in homogenates of PCT and PST segments isolated following collagenase digestion and Percoll centrifugation of the cortical corticies and the outer medullary stripe of rabbit kidneys. When homogenates were evaluated with testosterone, the 6b-, 16b-, and 2α-hydroxytestosterone metabolites were formed at faster rates in PST compared to PCT indicating that the cytochrome P450 (CYP) activities of 3A, 2B and 2C(11) are greater in PST than in PCT. CYP2E activity measured with chlorzoxazone was greater in PCT (350 $\pm$ 50 pmole/mg/hr) as compared to PST (220 $\pm$ 20 pmole/mg/hr) homogenates. No PCT vs PST differences were observed in CYP1A activity measured with ethoxyresorufin. The phase II UDP-glucuronyl transferase (UDPGT) and sulfotransferase activities measured with p-nitrophenol and 7-hydroxycoumarin were greater in PST as compared to PCT, regardless of the substrate used. These results suggest that differential distributions of phase I and II metabolic enzyme activities in PCT and PST segments may partly be responsible for the innate differential susceptibilities of PCT and PST segments to nephrotoxic agents. (Supported by NIEHS grant ES08217).

1601 CYTOTOXICITY, ZINC PROTECTION AND STRESS PROTEIN INDUCTION IN RATS PROXIMAL TUBULE CELLS EXPOSED TO CADMIUM CHLORIDE IN PRIMARY CELL CULTURE

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Cadmium is a common pollutant in some industrial areas, and has long been studied for its nephrotoxicity which has been observed in humans and animals. Previous studies have shown that zinc pretreatment protects against Cd toxicity but the underlying mechanisms are unknown. In this study, the rat renal proximal tubule cells were used to study the relationship between the stress protein response and cytoxicity of $CdCl_2$ in vitro. Rat kidney primary cell cultures were exposed for 2, 4 or 8 hours over a concentration range of 43–172 μM $CdCl_2$. The cellular effects were evaluated by the alamarBlue assay, a newly developed fluorescence method for evaluation of cell viability, morphology of cells by transmission electron microscopy and the expression of stress protein gene products demonstrated by ^{35}S-two-dimensional gel electrophoresis. The results of the alamarBlue assay and ultrastructural evaluations showed high cytotoxicity of $CdCl_2$ to the cells at or above concentrations of 129 μM (14.55 μg Cd/ml medium) in the medium after 4 hours of exposure. Prior administration of zinc in vivo significantly protected these cells in vitro with improved cell viability, increased incorporation of ^{35}S-methionine and preservation of cellular ultrastructural morphology. The ^{35}S labelling of gene products induced by cadmium exposure clearly demonstrated that distinct gene products with approximate molecular masses of 12, 30, 46, 70, and 90 kDa were induced in cells from zinc pretreated rats relative to cells not pretreated with zinc. These data provide further evidence that the observed zinc-induced increases in expression of the major stress protein groups in addition to metallothionein correlated well with protection of cell viability and that this in vitro model system is suitable for the study of Cd-related tubule cell toxicity.

1602 COMPARISON OF STRESS PROTEIN INDUCTION PATTERNS AFTER HIGH-DOSE *IN VITRO* EXPOSURE OF HAMSTER AND HUMAN KIDNEY CELLS TO ARSENIC, GALLIUM AND INDIUM

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Previous studies from this laboratory have demonstrated marked chemical-specific alterations in gene expression patterns of hamster kidney epithelial cells following *in vivo* exposure to either gallium (Ga), indium (In), arsenite (As), indium arsenide undertaken to compare alterations in gene expression patterns of male and female hamster kidney epithelial cells with human male and female kidney epithelial cells following high-dose exposures to these semiconductor elements *in vitro*. Primary cultures of hamster and human kidney epithelial cells were exposed to 100 μM As, Ga, or in respectively or 50 μM and 100 μM combinations of In + As. Incorporation of 35S-methionine into specific gene products was determined by 2-dimensional gel electrophoresis. There were similarities between the two species with regard to induction of the 60 and 70 kilodalton stress protein gene families but differences in expression patterns between males and females of both species were noted. The combination of Ga + As and In + As showed marked inhibition of the stress protein response indicating that the combination of these elements alters normal gene responsiveness to an individual element. Results of these studies indicate the potential value of *in vitro* studies form comparing the gene responsiveness of rodent and human kidney epithelial cells with regard to evaluating mechanisms of cellular toxicity for these elements in experimental animals.

1603 SUBCELLULAR TARGETS OF CISPLATIN AND RELATED ANALOGS IN RAT RENAL PROXIMAL TUBULE CELLS

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To assess the relative *in vitro* toxicities of platinum analogs to organelles, biochemical and ultrastructural analyses were performed in rat renal proximal tubule cells (PTC). Neutral red (NR) uptake was used to measure lysosomal damage and conversion of MTT to formazan used to assess mitochondrial function. NR uptake was a more sensitive indicator of damage than MTT conversion: 100 μM cisplatin (CIS), 1 mM carboplatin and 1 mM CI-973 reduced NR uptake by 67%, 58% and 57%, and MTT by 42%, 0% and 30% compared to control, respectively, at 24 hr. Exposure of PTC to 100 μM CIS for as little as 1 hr inhibited NR uptake and protein synthesis; these functions were decreased 60% and 90%, respectively, at 6 hrs. Fluorescent dyes which detect

lysosomes (acridine orange), mitochondria (rhodamine 123) and cell death (propidium iodide) were used to follow the development of toxicity in PTC treated with CIS. Lysosomal changes appeared to precede loss of mitochondrial function. ER and lysosomal changes were seen by electron microscopy and may precede mitochondrial swelling. These data suggest that platinum agents may initially damage the protein synthetic machinery and lysosomes of rat PTC, while mitochondrial damage occurs later.

1604 CHARACTERIZATION OF THE LIGAND BINDING SITE IN α2U-GLOBULIN BY X-RAY CRYSTALLOGRAPHY

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The rate-limiting step in the development of hyaline droplet nephropathy (HDN) is the reversible binding of a chemical to α2u-globulin. Chemicals that cause HDN do not bind to other proteins in the α2u-globulin superfamily. The goal of this work was to determine the nature of the ligand binding pocket in α2u-globulin by x-ray crystallography. α2u-Globulin was purified from male rat urine and protein crystals were grown and evaluated using standard techniques. The binding site inside the protein was characterized as: (1) closed off to water; (2) lined by extremely hydrophobic amino acid residues; (3) having a probe-accessible volume of 84.0 ± 0.9 $AÅ^3$, and (4) very similar to mouse urinary protein (MUP) in both hydro-phobicity and volume (75 $AÅ^3$). However, subtle differences in amino acid sequence between α2u-globulin and MUP affect the shape of the binding pockets, so that the overlap between the cavities in these homologous proteins is only about 42$AÅ^3$. The cavity in α2u-globulin is also more spherical in shape, whereas the MUP cavity is flattened and cannot accomodate HDN inducing agents such as d-limonene-1,2-epoxide. Other structurally-related proteins, such as retinol-binding protein and bilin-binding protein also have flattened binding cavities and have less than 20$AÅ^3$ overlap with α2u-globulin. Thus, the specific binding of chemicals to α2u-globulin, which accounts for the specificity of HDN in male rats, can be explained by the unique nature and shape of its ligand binding site.

1605 NEPHROTOXICITY OF 2-BROMOHYDROQUINONE CAN BE REDUCED BY THE ADRENERGIC ANTAGONIST PHENTOLAMINE

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Bromobenzene-induced liver injury can be dramatically reduced with the adrenergic antagonist phentolamine administered either simultaneously or following a delay of up to 6 h. This protection does not appear to relate to alterations in bromobenzene metabolism. One metabolite of bromobenzene, 2-bromohydroquinone (BHQ), is specifically nephrotoxic and was thus useful to test whether adrenergic modulation of toxicity occurs outside of the liver. B6C3F1 male mice were treated with 1 mmol/kg BHQ with or without phentolamine (10 mg/kg) cotreatment. Nephrotoxicity was evaluated by γ-glutamyl transpeptidase leakage into urine and renal histopathology. The animals that received BHQ without phentolamine exhibited clear enzymuria at 8 h (0.367 U/mouse ± 0.047 SEM) and at 24 h (0.460 U/mouse ± 0.147 SEM). These levels were significantly higher than both the phentolamine/BHQ group (0.174 U/mouse ± 0.041 SEM at 8 h and 0.139 U/mouse ± 0.012 SEM at 24 h) or mice treated with phentolamine alone (0.106 U/mouse ± 0.004 SEM at 8h and 0.116 U/mouse ± 0.022 SEM). Analysis was by ANOVA and Student-Newman-Keuls multiple comparison tests with the significance level set at 0.05. With phentolamine cotreatment, the BHQ-induced enzymuria was not significantly higher than controls. Pathology scores for acute tubular necrosis also showed that BHQ alone caused substantial cellular damage while phentolamine/BHQ cotreatment yielded minimal damage with scores similar to control. These observations suggest that the nephrotoxicity of BHQ can be reduced by phentolamine and, thus, cellular damage in the kidney, as well as in the liver, may be modulated by adrenergic agents. (Supported by ES05216).

1606 ROLE OF COMPLEX II OF THE MITOCHONDRIAL RESPIRATORY CHAIN IN CELLULAR DEFENSE AGAINST OXIDATIVE STRESS

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Coenzyme-Q_{10} is an ubiquitous lipid which is primarily present in the mitochondrial membrane, both in the reduced form (QH_2) and the oxidized form (Q). In submitochondrial particles it was demonstrated that the redox-state of endogenous coenzyme-Q_{10} (QH_2/Q) is of importance for susceptibility to oxidative membrane damage. It remains, however, unclear whether the ratio QH_2/Q is of importance in oxidative cell damage. To investigate this, freshly isolated rat renal proximal tubular cells (PTC) were incubated with the nephrotoxin dichlorovinyl cysteine (DCVC). We showed previously that DCVC causes oxidative stress in PTC which precedes cell death. Inhibition of this oxidative stress by the antioxidant diphenyl phenylenediamine completely protected against cell death caused by DCVC. The oxidative stress caused by DCVC, was preceded by a decrease of QH_2/Q. Inhibition of succinate:ubiquinone reductase (SQR) of complex II potentiated toxicity of DCVC. The role of QH_2/Q was investigated in more detail, by incubating PTC with an inhibitor of cytochrome-c-oxidase, KCN. KCN increased the ratio of QH_2/Q in untreated PTC, and, in addition, prevented the decrease of QH_2/Q caused by DCVC. The effect of KCN on QH_2/Q was associated with an inhibition of the oxidative stress and cell death induced by DCVC. However, inhibition of complex I of the respiratory chain with rotenone in combination with KCN and DCVC treatment resulted in a low QH_2/Q ratio but the toxicity of DCVC was prevented. Inhibition of complex II with oxaloacetate and KCN and DCVC resulted in higher QH_2/Q values but DCVC toxicity was not prevented. We conclude that the activity of SQR of complex II rather than the QH_2/Q ratio is essential in the defense against oxidative stress.

1607 HPLC ANALYSIS OF GLUTATHIONE (GSH) AND RELATED BIOLOGICAL THIOLS AND DISULFIDES

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Measurements of GSH, GSSG and related species can be used as biomarkers of metabolic activity and provide useful information in studies of mechanisms of toxicity. Derivatization with iodoacetic acid (IAA) and dansyl chloride (DnsCl), HPLC separation and fluorescence detection is sensitive and detects both thiols and disulfides. We observed that GSSG concentrations measured by DnsCl and HPLC appeared to be significantly higher than when measured by Teitze-derived methods (102 ± 26 nmol/g tissue verses 26 ± 3 nmol/g tissue). Derivatization of samples with N-ethylmaleimide (NEM) followed by DnsCl for HPLC analysis indicated concentrations (31 ± 11 nmol/g tissue) that were comparable with the levels estimated by enzyme recycling methods. The lower estimates of GSSG in the samples treated with NEM are best explained by the more rapid reaction of GSH with NEM than with IAA, more effectively limiting the oxidation of GSH during sample preparation. Effective inhibition of γ-glutamyl transpeptidase (GGT) is also important in sample processing. Serine borate, in the concentrations we currently use (375 mM final concentration), was found to inhibit >97% GGT (28 U/L) in 1–2 seconds whereas AT-125, even at 10 mM final concentration, inhibited GGT <50% by 2 minutes. Supported by NIH GM44263.

1608 AN ANIMAL MODEL FOR NON-INVASIVE MONITORING OF GLUTATHIONE, GLYCINE, BIOENERGETICS AND pH IN LIVER OF INTACT RAT BY *IN VIVO* ^{13}C AND ^{31}P NMR

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For the first time, we have succeeded in non-invasively monitoring liver glutathione (GSH) of intact rat, by *in vivo* ^{13}C NMR and infusion of 2-^{13}C-glycine. In conjunction with *in vivo* ^{31}P NMR we are also able to monitor intermittently early indices of stress such as ATP levels, pH, and glycine metabolism. We found the half-life of glycine to be 50 min, but GSH levels continued to increase linearly even after 4 hrs of intravenous infusion of 4.3 mM/kg/hr 2-^{13}C-glycine. ATP and pH did not significantly change over the course of the experiment. In addition to GSH, the other major 2-^{13}C-glycine metabolite was doubly-labeled 2,3-^{13}C-serine.

High resolution NMR analysis of the liver extracts revealed that the glycine and GSH pools were 81 ± 3% and 76 ± 8% ^{13}C-enriched, respectively. There was a GSH metabolite that co-resonated with GSH *in vivo* and was identified to be γ-glutamylglycine in extracts. Liver extracts from rats given intraperitoneal injections of 4.3 mM/kg 2-^{13}C-glycine revealed a γ-glutamylglycine/GSH ratio of 1.54 and 1.26 at 2 and 6 hrs, respectively.

We will describe our *in vivo* ^{13}C and ^{31}P NMR animal model that is ideal for xenobiotic studies and demonstrate that administration of 2-^{13}C-glycine permits non-invasive monitoring of GSH in addition to other indicators of stress: bioenergetics, pH, and glycine metabolism. This is also an ideal model to study the role of GSH in the γ-glutamyl cycle.

1609 UTILIZATION OF EXOGENOUS GLUTATHIONE (GSH) BY THE SMALL INTESTINE

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Glutathione (GSH) is found in food as well as bile; however, the action of extracellular GSH in the intestine has not been fully investigated. Our previous data have shown that GSH added to either the lumen of the rat small intestine or to isolated cells from the rat small intestine can increase the formation of conjugate with the electrophile 1-chloro-2,4-dinitrobenzene (CDNB) above that formed without added GSH. Current results show that this increase in conjugate is due to two mechanisms, one intracellular and another extracellular. The intracellular mechanism utilizes the GSH inside the cell, as well as transported GSH to conjugate via glutathione S-transferases (GST) inside the cell. The extracellular mechanism appears to involve the efflux of GSTs and extra-cellular conjugation of CDNB with the exogenous GSH. About 20% of the stimulated activity seen from exogenous GSH was due to uptake of GSH and the remainder (about 80%) was due to GST release and extracellular conjugation. Extracellular conjugation could be very beneficial physiologically. Intestinal cells have a very rapid turnover rate, especially the cells located on the tips. As these cells are sloughed, release of their GSTs into the mucosal layer could allow the GSH from bile and food to be used to conjugate the electrophilic compounds present in food.

1610 ISOLATION OF NUCLEI THAT ARE FUNCTIONAL IN THE NUCLEO-CYTOPLASMIC EXCHANGE OF GLUTATHIONE AND SMALL MOLECULES

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Numerous studies have shown rapid exchange between the nucleus and cytoplasm of cellular information and material. However, these studies were mainly based on whole cell models which often times complicate the interpretation of experimental data. We have developed a reliable method of isolating functional nuclei by modification of existing methods. The resuspending medium for nuclei is more physiological and the time required for isolation is significantly reduced. The purity of the nuclear fraction is assessed by marker enzymes and the morphology is examined by microscopy. ATP-dependent nuclear calcium uptake is consistently demonstrated by mouse liver nuclei isolated by this method. But the exchange of glutathione between isolated nuclei and the surrounding medium is not ATP dependent at any concentration of glutathione tested; a passive uptake system is indicated.

1611 DIFFERENCES IN THE RATE OF GLUTATHIONE (GSH) RESYNTHESIS IN LUNG SUBCOMPARTMENTS OF MICE AND MONKEYS

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Injury to pulmonary epithelial cells from chemicals which undergo P450 dependent activation and from gases such as ozone is highly focal. These studies examined the rate of GSH resynthesis in subcompartments (trachea, minor daughter/respiratory bronchiole, parenchyma) of the mouse and monkey lung to determine if differences in the rate of GSH resynthesis are, in part, responsible for wide variations in susceptibility of lung subcompartments to insult. GSH was maintained at control levels in incubations of subcompartments with complete Waymouth's medium. In 4 hr incubations containing no methionine, cysteine, or GSH, monkey lung subcompartments lost less than 30% of the GSH present at 0 time while GSH in mouse airways decreased by 40–60%. Addition of diethylmaleate (DEM) for 30 min. to airway incubations decreased GSH levels by varying amounts depending on species and subcompartment. Rapid resynthesis of GSH was observed when GSH-depleted airways were incubated with medium containing cysteine but not methionine. Substantial differences were observed in the rate of GSH resynthesis between the monkey and mouse and between trachea and minor daughter/parenchymal subcompartments of the mouse. In all airways of the monkey GSH levels returned to those at T = 0 within 2–4 hours after addition of cysteine containing medium. In contrast, in minor daughter and parenchyma of the mouse, GSH returned to control levels within 1 hour of incubation with cysteine supplemented medium while trachea required 2 hr for recovery. These studies show striking species and airway level differences in the rate of GSH resynthesis and suggest that focal injury to respiratory epithelium may, in part, be mediated by regional differences in the ability to supply GSH for protection against electrophiles and reactive oxygen species. Supported by NIEHS 04311, 04699 and 00628.

1612 PROPERTIES OF GLUTATHIONE UPTAKE AND EFFLUX IN RENAL CORTICAL MITOCHONDRIA

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Renal cortical cells contain separate pools of glutathione (GSH) in cytosol and mitochondria. Maintenance of mitochondrial, rather than cytosolic, GSH content appears to be important for cellular function. Little or no GSH synthesis activity has been detected in renal mitochondria. Thus, it is transport of GSH from cytosol into mitochondria that primarily determines intramitochondrial GSH content. Isolated renal cortical mitochondria were preincubated with 0.25 mM acivicin for 15 min at 20°C to inhibit the γ-glutamyltransferase and were then incubated with GSH; aliquots of mitochondria at different time points of incubation were then centrifuged at 13,000g × 40 sec. After removing the supernatant, pellets were resuspended in ice-cold buffer and recentrifuged to wash out the extramitochondrial medium that was trapped in the pellet. We estimated that by this method, contamination of the mitochondrial pellet with extramitochondrial medium was approximately 1% by volume and at most 10% by GSH content. GSH content in the pellets was determined by HPLC analysis. GSH uptake was time- and concentration-dependent. The uptake reached equilibrium (approx. 7 nmol/mg protein) in 5 min with 5 mM exogenous GSH. Mitochondria lost functional integrity, as indicated by respiratory control, quickly when incubated at 20°C, and addition of dithiothreitol or GSH helped to maintain mitochondrial function. Efflux of GSH from freshly isolated and GSH-preloaded mitochondria was very slow and was temperature-dependent. Analysis of mitochondrial GSH content versus the inverse of osmolarity showed that nearly 60% of mitochondrial GSH was bound and 40% was free. GSH uptake into mitoplasts exhibited properties similar to that of intact mitochondria, suggesting that the mitochondrial outer membrane has little role in the transport process.

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1613 CLONING AND SEQUENCING OF THE MOUSE cRNAs ENCODING THE LIGHT AND HEAVY SUBUNITS OF γ-GLUTAMYLCYSTEINE SYNTHETASE

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The antioxidant tripeptide glutathione (GSH) is synthesized in a two step process. The first step catalyzed by gamma-glutamylcysteine synthetase (γ-GCS), forms a very stable atypical peptide bond between the gamma carbon of glutamate and cysteine. The second step catalyzed by glutathione synthetase completes the tripeptide by adding a terminal glycine. In the presence of ample amino acid precursor concentrations, γ-GCS is the rate limiting enzyme in GSH synthesis. Rat and mouse (and presumably human) γ-GCS are composed of two subunits, a light subunit with 274 amino acid residues (M_r 30,000) and a heavy subunit with 640 amino acid residues (M_r 73,000). The heavy subunit has all the catalytic activity and exhibits feedback regulation from GSH. The light subunit appears to regulate the heavy subunit's affinity for glutamate and feedback inhibition by GSH in a redox-responsive manner. We designed primers encompassing the open reading frame of both subunits, based on the rat sequences reported by Meister and colleagues. Using RT-PCR, the mouse cDNAs for both the light and heavy subunits were synthesized from total kidney RNA, subcloned into a plasmid vector and sequenced. Homology between the mouse and rat cDNAs is 98% for the heavy subunit and 92% for the light subunit. Homology between the mouse and human heavy subunit is 89%. This is comparable to the 86% homology found between rat and human heavy subunits (Gipp, et.al.). Amino acid sequence homology is 98% and 87% between the mouse heavy subunit and the rat and human heavy subunits respectively, while the mouse light subunit is 90% homologous with the rat light subunit. Expression vectors are being constructed to overexpress both subunits of mouse γ-GCS in mammalian cell lines. Supported by NIH grants AG01751, ES04696 and AG00057.

1614 CYLINDROSPERMOPSIN (CY): A SPECIFIC INHIBITOR OF GLUTATHIONE SYNTHESIS

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CY is a naturally occurring alkaloid produced by the cyanobacterium *Cylindrospermopsis raciborskii* which has been linked to an outbreak of hepatoenteritis in man. We previously showed that CY was cytotoxic to primary cultures of rat hepatocytes and that CY lowered cell GSH at nontoxic doses. Lower cell GSH also potentiated CY-induced cytotoxicity (B.B.R.C. 201:235–241, 1994). Here we examined the mechanism of the fall in cell GSH induced by CY. We excluded several possible explanations for the loss in GSH, namely

increased formation of GSSG, increased GSH efflux, hidden forms of GSH, decreased GSH precursor availability or decreased cellular ATP level. To address whether the fall in GSH was due to decreased GSH synthesis or increased GSH consumption, we examined the rate of fall in total GSH after 5 mM buthionine sulfoximine (BSO, an irreversible inhibitor of GSH synthesis) treatment. The rates of fall in total GSH (nmole/10^6 cells/hr) were 8.2 ± 2.5, 6.0 ± 1.7 and 5.9 ± 1.3 for control, 2.5 μM and 5 μM CY pretreated cells, respectively. These results show that the fall in GSH induced by CY was due to inhibition of GSH synthesis rather than increased consumption because in the latter case the rate of fall in GSH would have been accelerated by CY pretreatment. Furthermore, excess GSH precursor (20 mM N-acetylcysteine), which supported GSH synthesis in control cells, did not prevent the fall in GSH or toxicity induced by CY. Inhibition of GSH synthesis is most likely an important factor in the cytotoxicity of CY. Support NIEHS Grant ES05678.

1615 EFFECT OF CELLULAR GLUTATHIONE DEPLETION ON FUMONISIN B_1 TOXICITY IN PIG KIDNEY (LLC-PK_1) CELLS

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Fumonisin B_1 (FB_1) has been shown to be a causative agent of equine leukoencephalomalacia, porcine pulmonary edema, and rat liver tumors and chronic nephritis. To investigate mechanisms by which FB_1 causes toxicity, the effect of cellular glutathione (GSH) depletion on FB_1 cytotoxicity in LLC-PK_1 cells was studied. LLC-PK_1 cells were cultured in DMEM/F-12 medium supplemented with 5% fetal calf serum. Cellular GSH levels were measured using a high-performance liquid chromatography (HPLC) method. The FB_1 cytotoxicity was determined by an MTT and a long-term survival assay. Treatment of LLC-PK_1 cells for 12 hr with 0.1 mM buthionine sulfoximine (BSO), a selective inhibitor of the enzyme γ-glutamylcysteine synthetase that catalyzes the rate-limiting reaction in *de novo* GSH synthesis, markedly decreased cellular GSH levels to about 20% of that found in the non-BSO treated cells. This BSO treatment, however, did not affect the cell viability. The cells pretreated with 0.1 mM BSO for 12 hr and controls treated with the same volume of saline were then exposed to varying concentrations of FB_1. Both MTT and long-term survival assays revealed that the LC_{50} value of FB_1 for the BSO-treated cells was significantly lowered ($p < 0.01$). Therefore, depletion of cellular GSH sensitizes the cells to FB_1 toxicity. Because GSH is an important antioxidant participating in cytoprotective responses to oxidative injuries, the results obtained suggest that generation of toxic free radicals may be a mechanism by which FB_1 induces cell injury. Supported in part by USDA grant 93-37208-9277.

1616 STRAIN SPECIFIC EFFECTS OF SUB-CHRONIC, LOW LEVEL METHYLMERCURY EXPOSURE ON GSH LEVELS IN MICE

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It has been previously reported that acute methylmercury (MeHg) exposure results in an elevation of glutathione (GSH) levels. This study evaluated the effects of sub-chronic, low level MeHg exposure on GSH levels in liver, kidney, brain, lung, splenocytes and thymocytes, in two different strains of mice. Male DBA and C57Bl/6 mice were exposed to MeHg in their drinking water at 0, 3, or 10 ppm for 4 weeks, and the above tissues were collected and analyzed for GSH levels by Tietze assay. In addition, splenocytes and thymocytes were analyzed for alterations in GSH levels by monochlorobimane fluorescence and flow cytometry. Tietze analyses showed an elevation of GSH in kidney, liver, lung, brain, and spleen of DBA mice at both MeHg doses. C57 mice showed an elevation in GSH levels of brain, liver and splenocytes at both doses, but GSH was increased only at the high dose in kidney and lung. Analysis of GSH levels in splenocytes and thymocytes by MCB fluorescence showed a dose dependent decrease in MCB fluorescence of all subsets of splenocytes and a dose dependent increase in MCB fluorescence of all subsets of thymocytes. These data indicate potential mouse strain-specific differences in GSH metabolism in kidney and lung after MeHg exposure. This was supported by NIH grants ES04696 and RR07019.

1617 RELATIONSHIP OF METHIONINE/GLUTATHIONE DEPLETION AND BROMOBENZENE-INDUCED DNA DAMAGE

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Liver and kidney necrosis caused by a single high dose of bromobenzene (BB) is known to be associated with impairment in O- and S-methylation of 4- and 5-bromo-2-hydroxythiophenol in the Syrian hamster. Impairment in methylation is due to methionine (MET) depletion which occurs as a consequence of glutathione (GSH) depletion. MET deprivation and/or depletion is known to divert folate from purine and pyrimidine biosynthesis. The present study was designed to test the hypothesis that BB toxicity is associated with DNA damage. When a single ip dose of thymidine (200 mg/kg) labeled with [2-^{14}C]thymidine (5 μCi) was given to Syrian hamsters at 5 hr after BB (800 mg/kg), the result was striking. Intrahepatic hemorrhage which is a typical characteristic of liver necrosis in the Syrian hamster was significantly decreased in the thymidine-treated animals. Liver toxicity, however, was still evident as indicated by plasma GPT activity and histological examination. When DNA was isolated from liver cells, the BB and thymidine treated hamsters showed approximately a two-fold increased in thymidine incorporation when compared with those observed from thymidine treated alone. The protective effect by thymidine presumably occurs through the assisting of DNA repair/unscheduled DNA synthesis. BB-induced DNA damage could due to the diversion of folate from *de novo* synthesis of purine and pyrimidine to the resynthesis of MET. MET resynthesis is due to MET depletion which occurs as a consequence of GSH depletion. (Supported by ES-04857).

1618 DIFFERENCES IN XENOBIOTIC DETOXIFYING ACTIVITIES BETWEEN BONE MARROW STROMAL CELLS FROM MICE AND RATS: IMPLICATIONS FOR BENZENE-INDUCED HEMATOTOXICITY

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Mice have been shown to be more sensitive to benzene-induced hematotoxicity than rats. In this regard, we have observed that bone marrow stromal cells from mice were significantly more susceptible to the cytotoxicity induced by the benzene metabolites hydroquinone (HQ) and benzoquinone than cells from rats. Since cellular glutathione (GSH) and quinone reductase (QR) are known to play critical roles in modulating HQ-induced cytotoxicity, we have measured the GSH content, and QR and GST activity in stromal cells from both species. In rat cells, the GSH content and QR activity were 2 and 44 times as much as those from mice, respectively. GSH and QR in both mouse and rat stromal cells were inducible by 1,2-dithiole-3-thione (D3T). D3T pretreatment of both mouse and rat stromal cells resulted in a marked protection against HQ-induced toxicity. Pretreatment of both mouse and rat stromal cells with GSH ethyl ester also provided a dramatic protection against HQ-induced toxicity. Conversely, dicoumarol, an inhibitor of QR, enhanced the HQ-induced toxicity in stromal cells from both mice and rats, indicating an important role for QR in modulating HQ-induced stromal toxicity in both species. Buthionine sulfoximine (BSO), which depleted GSH significantly in both species, potentiated the HQ-induced toxicity in mouse but not in rat stromal cells. Surprisingly, incubation of stromal cells with BSO resulted in a significant induction of QR, with the net QR induction being ≈20 nmoles/min/mg protein in mice and ≈150 nmoles/min/mg protein in rats. The failure of BSO to potentlate HQ-induced toxicity in rat stromal cells may be due to the concomitant induction of QR by BSO. Overall, this study demonstrates that the differences in stromal cellular GSH content and QR activity between mice and rats contribute to their respective susceptibility to HQ-induced cytotoxicity in vitro, and may be involved in the greater in vivo sensitivity of mice to benzene-induced hematotoxicity.

1619 12-O-TETRADEOCANOYLPHORBOL-13-ACETATE (TPA)-INDUCED DIFFERENTIATION OF ML-1 CELLS TO MONOCYTES/MACROPHAGES IS ACCOMPANIED BY INCREASED XENOBIOTIC DETOXIFYING ACTIVITIES AND DECREASED SUSCEPTIBILITY TO HYDROQUINONE (HQ)-INDUCED TOXICITY

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Previously, we have characterized the glutathione (GSH), quinone reductase (QR) and GSH-S-transferase (GST) activity of undifferentiated ML-1 cells, a human leukemic cell line used in studying myelopoietic cell differentiation

(*Life Sci.* 54:901, 1994). Since the alteration of cellular xenobiotic detoxifying activities during differentiation ML-1 cells by TPA has not been investigated, we have characterized the induction of GSH, QR and GST during TPA-induced differentiation of ML-1 cells to monocytes/macrophages. Differentiation of ML-1 cells by TPA was accompanied by significant increases in cellular GSH content, QR and GST activities. 24 h after incubation of ML-1 cells with TPA, cellular GSH content, QR and GST activities exhibited a ≈2-fold increase over undifferentiated cells, which lasted for 72 h until removing TPA from the culture medium. 24 h after removing TPA, cellular GSH, QR and GST were further induced, being 6–7, 7–8 and 3.5–4 fold over undifferentiated cells, respectively. The GSH content and GST activity remained constant 72 h after removing TPA, whereas the QR activity continued to increase, with the activity being ≈11 fold over undifferentiated cells at 72 h after removing TPA. In addition, the TPA-differentiated cells exhibited a ≈50% decrease in myeloperoxidase activity. Further studies examining the sensitivity of TPA-differentiated cells to HQ toxicity showed that differentiation of ML-1 cells to monocytes/macrophages was accompanied by a dramatic resistance to the HQ-induced toxicity, with the LC_{50} of HQ in undifferentiated and differentiated cells being ≈40 and ≈200 μM, respectively. Studies are underway to assess the contributions of the above cellular factors to the resistance to HQ toxicity, and to explore the mechanism involved in the elevation of cellular GSH content, QR and GST activities during TPA-induced differentiation of ML-1 cells.

1620 EXPRESSION AND ACTIVITY OF GLUTATHIONE S-TRANSFERASES (GST) ASSOCIATED WITH TOLERANCE DUE TO MULTIPLE DOSES OF NAPHTHALENE (NA)

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Marked architectural rearrangements and altered expression of cytochrome P-450 protein is associated with tolerance after multiple doses of NA (200 mg/kg). Expression of cytochrome P450 2F is markedly diminished in mice made tolerant to NA. However, activation of NA to reactive intermediates by isolated bronchioles does not differ from control bronchioles. We investigated whether expression of cytosolic detoxification enzymes (GST) or cofactors (glutathione; GSH) were altered in the lungs of tolerant mice. Activity of GST in cytosol of tolerant mouse lungs towards CDNB was two-fold higher than in corn oil treated control lungs. Immunohistochemical detection of GST in mouse lungs indicated that GSTμ was expressed in greater quantities in the NA tolerant mice than in CO treated controls. In spite of these differences, metabolism of naphthalene oxide (NO) by isolated dissected airways was approximately 2-fold higher in target areas of the lungs of vehicle treated compared to tolerant mice. Cellular GSH in isolated airways was determined by HPLC. In tolerant mice, 2-fold greater levels of cellular GSH in target areas (bronchioles) than in non-target or control mice were detected. We conclude that tolerance due to NA occurs in part by increased expression of cytosolic GSTμ and increased concentrations of GSH in distal bronchiolar epithelium. Other GST isoenzymes may play an important role in NO metabolism in bronchioles. Supported by NIEHS ESO5578, and ES04311.

1621 IRON INDUCED LIPID PEROXIDATION IN RAT LIVER IS ACCOMPANIED WITH PREFERENTIAL INDUCTION OF GLUTATHIONE S-TRANSFERASE 8-8 ISOZYME

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Since previous studies from our laboratory have suggested that glutathione S-transferases (GST) 8-8 of rat belongs to a distinct subgroup of GST isozymes which may be involved in the detoxification of the products of lipid peroxidation, during the present studies we examined the effect of iron induced lipid peroxidation on the expression of GST 8-8 in rat liver. Rats treated with 100 mg/kg body weight iron showed a significant increase in lipid peroxidation in liver. This was accompanied with a concomitant increase in the expression of GST 8-8 in liver as observed in isoelectrophoretic profiles of rat liver GSTs, and an increase in GST activity towards 4-hydroxynonenal, a preferred substrate for GST 8-8. Western blot studies also indicated that rat liver GST 8-8 was preferentially induced upon iron treatment. This was further confirmed by purifying and quantitating GST 8-8 protein from the controls and iron treated rats. Significant differences in the specific activities of GST 8-8 purified from the controls and iron treated rats were observed, indicating that more than one GST isozymes related to GST 8-8 may be present in rat liver. This observation is consistent with the observed heterogeneity in mouse mGSTA4-4 which is an ortholog of rat GST 8-8. Iron treatment also caused significant increase in GSH-levels probably because of *de novo* synthesis as indicated by an increase in γ-glutamyl cysteine synthetase activity. The results of these studies suggest that GST 8-8 isozymes may play an important role in defense mechanisms against lipid peroxidation. Supported by NIH Grant CA27967.

1622 ROLE OF GLUTATHIONE S-TRANSFERASES IN THE INTERACTIONS OF MELPHALAN WITH GLUTATHIONE

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L-phenylalanine mustard, melphalan (MEL) is used in chemotherapy of multiple myeloma and ovarian cancer. Over-expression of glutathione S-transferases (GSTs) has been reported in cells with acquired resistance to MEL implying that GSTs mediate the detoxification of MEL by conjugating it to glutathione (GSH). However, the role of GSTs in the conjugation of MEL to GSH is not established. In present studies, we evaluated the effect of GSTs on GSH and MEL interactions using reversed-phase HPLC to isolate and quantify the reaction products of GSH and MEL in the presence or absence of GST. Non-enzymatic formation of monoglutathionyl-melphalan and consumption of MEL appeared to be second order processes with respect to MEL concentration, but were independent of GSH. These findings were consistent with an S_N1 reaction mechanism and implied that increased GSH alone in MEL resistant cells may not lead to increased GSH-conjugation. Equine liver GSTs (primarily α-class GSTs) were found to significantly accelerate consumption of MEL. This activity of GST was linear with respect to GST protein concentration. The rate of MEL consumption in the presence of GST was saturable with respect to GSH and MEL concentrations. GST activity towards MEL was inhibited by both the mono- and diglutathionyl-melphalan which were found to be non-competitive inhibitors of GST activity towards CDNB (K_i 2.6 and 35.0 μM, respectively). It is thus concluded that GSTs can increase the rate of MEL consumption and render the rate of MEL consumption sensitive to GSH concentration. Supported in part by NIH grant CA27967 and T32ES07254.

1623 INVESTIGATION OF ISOCYANATE S-CONJUGATE STABILITY

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Sulfhydryl reactions with isocyanates have been known to yield an unstable thiolic ester linkage which, upon hydrolysis, can cause the regeneration of the isocyanate species under certain conditions. This investigation sought to determine the stability of two isocycanate S-conjugates, involving p-tolyl isocyanate and n-butyl isocyanate, and to identify the products of the hydrolysis. Isocyanate S-conjugates of glutathione (GSH) and its metabolite, N-acetyl cysteine (NAC), were synthesized. The kinetics of the reversibility of the formed thiolic ester linkage in these adducts were determined under different pH conditions at 37°C, using HPLC methods. The results indicate that the thiolic ester linkage formed by both the aryl and alkyl isocyanate is very stable under acidic conditions, yielding <20% hydrolysis after 5 days. At pH 10, an initial phase of rapid hydrolysis occurred, followed by an equilibrium phase which varied from 2–4 hr for the p-tolyl adduct, ending in total hydrolysis before 8 hr. Under physiological conditions, the p-tolyl S-adduct was hydrolyzed at a rate 4 times faster than the butyl S-adduct, both following first-order kinetics with half-lives of 4.2 hr and 17 hr, respectively. These studies suggest that S-conjugation of isocyanates may serve as a physiological transport mechanism of these compounds to remote sites, and thus give rise to systemic effects such as formation of hemoglobin adducts. This work is supported by NIH grant GM16441.

1624 EVALUATION OF POTENTIAL PROTECTIVE EFFECT OF PROCYSTEINE® OR PROCYSTEINE CONGENER TREATMENT ON CYCLOPHOSPHAMIDE-INDUCED ALOPECIA IN NEONATAL RATS

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Objective: Alopecia is a distressing adverse effect of the alkylating, cancer chemotherapeutic agent Cyclophosphamide (CP). The objective of this study was to evaluate the potential protective effect of Procysteine® (L-2-oxothiazolidine-4-carboxylate), an intracellular L-cysteine precursor, or Procysteine Congener treatment on CP-induced alopecia.

Methods: Nine treatment groups of eight neonatal rats each were compared: 1) Untreated control, 2) CP only, 3) CP + Procysteine® (subcutaneous), 4) CP + Procysteine-ethyl ester (topical), 5) CP + Glutathione-ethyl ester (topical), 6) CP + N-acetyl-cysteine (NAC, subcutaneous), 7) CP + Procysteine® (topical), 8) CP + NAC (topical), 9) CP + Petroleum Jelly (topical). All animals (except untreated controls) received an intraperitoneal injection of CP (30 mg/kg) on Day 0 to induce alopecia. Beginning one day prior to CP treatment, subcutaneous treatments (2 g/kg) were administered 3 times per day for 3 consecutive days and topical treatments (1.5 g/kg) were applied once per day for 5 consecutive days. Hair loss was evaluated on study Day 10.

Results: The untreated control animals had normal hair growth. Predominately, moderate to severe alopecia was observed in animals that were treated with CP only and animals treated subcutaneously with NAC. One animal in the NAC (subcutaneous) group died prior to alopecia evaluation. No protective activity was observed with topical applications of the vehicle (petroleum jelly). Relative protective activity was observed as follows: Procysteine-ethyl ester (topical) > Procysteine® (topical) > NAC (topical) > Procysteine® (subcutaneous) > NAC (subcutaneous) > Glutathione-ethyl ester (topical).

Conclusion: Under the conditions of this study, topical applications of the Procysteine-ethyl ester, Procysteine®, and NAC appeared to offer protection against severe hair loss. Additional protection from alopecia may be achieved by optimization of the topical medium and treatment regime.

1625 THE REACTION OF GLUTATHIONE WITH THE ANTITUMOR AGENT DIAZIQUONE: FREE RADICALS AND CONJUGATE FORMATION

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The antitumor agent diaziquone (AZQ, 2,5 diaziridinyl-3,6-bis(carboethoxy-amino)-1,4-benzoquinone) reacts with reduced glutathione (GSH) in aqueous solutions to give rise to the glutathionyl and hydroxyl free radicals, as well as the AZQ semiquinone as detected by Electron Spin Resonance Spectroscopy using the spin trap DMPO (5,5-dimethyl-1-pyrroline-N-oxide). Under anaerobic conditions, the only radical observed is the glutathionyl radical. Aerobic conditions result in the formation of hydroxyl radicals and the AZQ semiquinone in addition to the glutathionyl radical. The reaction of AZQ with GSH also leads to a variety of conjugates detected by mass spectrometry. These conjugates include addition of glutathione to both aziridines, or displacement of the aziridines by GSH, and a combination of both. The majority of the conjugates are formed by nucleophilic attack of GSH to the AZQ aziridines or by 1,4 Michael addition to the AZQ quinone or a combination of both. The majority of the free radicals observed are from redox reactions that involve the reduction and oxidation of AZQ to produce oxygen radical and the semiquinone, concomitant with the oxidation of GSH. Supported by NIH grant CA-53491.

1626 BOTH GLUTATHIONE AND PROTEIN THIOLS REDUCE PHENOXYL RADICALS OF ANTITUMOR DRUG, ETOPOSIDE (VP-16), IN CELL HOMOGENATES

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Phenoxyl radicals are inevitable intermediates in the oxidative enzymatic metabolism of a phenolic antitumor drag, Etoposide (VP-16). One-electron reduction of the VP-16 phenoxyl radical by ascorbate and thiols prevents/delays its oxidative metabolism both in model systems and in cell homogenates. To elucidate the role of endogenous thiols in the reduction of VP-16 phenoxyl radicals, we used K562 human leukemia cells grown in DMEM cell culture medium which does not contain vitamin C (ascorbate) thus excluding the ascorbate-dependent reduction of VP-16 phenoxyl radicals. We found that VP-16 phenoxyl radicals were reduced by endogenous reductants in K562 cell homogenates and that intracellular thiols were mainly responsible for the reduction. Three systems were employed to evaluate the contribution of GSH and protein thiols into overall thiol-dependent reduction of VP-16 phenoxyl radicals: (i) K562 cell homogenates depleted or supplemented with GSH *in vitro*, (ii) K562 cells with a decreased level of endogenous GSH (using a specific inhibitor of gamma-glutamyl cysteine synthetase, buthionine-S,R-sulfoximine, BSO), and (iii) cells with increased content of endogenous thiols as a result of treatment with cadmium. We demonstrated that endogenous GSH and protein thiols each account for half of K562 cell's ability to reduce VP-16 phenoxyl radicals in thiol-dependent reactions when cells were untreated. Depletion or supplementation of K562 cells or cell homogenates with GSH proportionally decreases or increases their ability to reduce the radicals. Induction of endogenous thiols by cadmium increased their ability to reduce VP-16 phenoxyl radicals but did not reveal any significant difference in the ability of the two types of cells to interact with the radicals. Supported by grant 94A45 from the American Institute for Cancer Research and grant DHP-125 from the American Cancer Society.

1627 RELATIONSHIP BETWEEN TOXICITY OF GLUTATHIONE-ACROLEIN CONJUGATES AND THIOL STATUS OF A549 CELLS

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Acrolein, an α,β-unsaturated aldehyde, is prevalent in the environment and is formed during the metabolism of several xenobiotics. It is highly electrophilic and will readily bind to glutathione (GSH), presumably leading to detoxification. However, our previous work showed that the acrolein mercapturic acid [S-3 oxopropyl N-acetylcysteine (oxoPrMCA)] was toxic to A549 cells. In the current study, a 2 h exposure of A549 cells to acrolein (500 μM) or oxoPrMCA (1 mM) produced 32 and 20% leakage of lactate dehydrogenase, respectively. The GSH conjugate of acrolein S-3 oxopropyl glutathione (oxoPrGSH) was non-toxic after a 2 h exposure, but caused significant inhibition of cell growth after a 24 h exposure in the presence of serum. Although γ-glutamyl transpeptidase activity was measurable in A549 cells (14.1 units/mg protein), acivicin (600 μM) failed to block the effect of oxoPrGSH indicating that toxicity was not due to metabolism via this enzyme. Treatment of A549 cells with 1 mM acrolein, oxoPrMCA or oxoPrGSH for 2 h under serum-free conditions decreased cellular GSH levels by 97, 84, and 43%, respectively. Glutathione disulfide (GSSG) levels were unaffected by these treatments. Exposure of A549 cells to 1 mM oxoPrGSH for 24 h in the presence of serum decreased GSH levels by 85%. Depleting cellular GSH stores with diethyl maleate prior to the addition of these metabolites enhanced their toxicity as assessed by measuring cell growth. In summary, although somewhat less potent than acrolein, both oxoPrMCA and oxoPrGSH were toxic to A549 cells and significantly decreased GSH concentrations. Treatment of GSH-depleted cells with these metabolites caused greater inhibition of cell growth than did similar treatment of non-thiol depleted cells. Toxic effects of these acrolein metabolites seem to be linked to their direct actions on cellular thiol levels and not to mechanisms involving oxidative stress, since GSSG levels did not increase. (This work was supported by NIH grant HL48035.)

1628 ACROLEIN MERCAPTURATES AND THE BLADDER TOXICITY OF CYCLOPHOSPHAMIDE

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The anticancer drug cyclophosphamide (CP) is bioactivated to phosphoramide mustard and acrolein. Acrolein is believed to be responsible for CP-induced bladder damage. The mechanism by which this reactive intermediate is delivered to the bladder following bioactivation of CP in the liver and/or other tissues is not known. Glutathione (GSH) readily conjugates with acrolein, and mercapturates of acrolein have been found in the urine of animals and man given CP. The objective of this study was to investigate the release of acrolein from, and the bladder toxicity of, the GSH-acrolein adduct (3-oxoPrGSH), the acrolein mercapturates 3-oxopropyl-N-acetylcysteine (3-oxoPrMCA) and 3-hydroxypropyl-N-acetylcysteine (3-hydroxy-PrMCA), and the S-oxidation product of 3-oxoPrMCA. Synthetic standards of each of the compounds were prepared. When incubated for up to 20 min at 25 °C in 100 mM K_2HPO_4 buffer containing 1 M KCl, pH 8.0, 3-oxoPrMCA released ≈6% of the theoretical maximum yield of acrolein as indicated by an increase in absorbance at 210 nm and confirmed by trapping this aldehyde as a semicarbazone. 3-OxoPrMCA S-oxide released ≈11% of the theoretical maximum yield of acrolein. In contrast, there was less than a 2% yield of acrolein from 3-hydroxyPrMCA or 3-oxopropyl GSH. Acrolein, 3-oxoPrMCA and 3-oxoPrMCA S-oxide, but not 3-hydroxyPrMCA or 3-oxopropyl GSH, damaged the bladder when instilled intravesically in mice at concentrations of 10 to 20 mM. Toxicity correlated with the ability of these compounds to provide a positive response in a fluoresence assay for reactive aldehyde groups. These data support the possibility that a mercapturic acid rather than 4-hydroxyCP (the initial unstable metabolite that releases acrolein) is responsible for the bladder toxicity of CP. This may provide a mechanism for transport of acrolein from its site of formation in the liver (or possibly kidney) to its site of action in the bladder. (This work was supported by NIH grant HL48035.)

1629 2,3,5-(TRIGLUTATHION-S-yl) HYDROQUINONE INDUCES PROSTAGLANDIN E_2 SYNTHESIS AND gadd 153 AND hsp70 EXPRESSION IN LLC-PK_1 CELLS

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Certain prostaglandins (PG's) can modulate gene expression and regulate cell proliferation and differentiation. Recent *in vivo* studies have shown that the nephrocarcinogen hydroquinone and its metabolite 2,3,5-(triglutathion-*S*-yl)hydroquinone [(triGSyl)HQ] induce renal tubular cell proliferation in male Fisher 344 rats. In the present study we therefore investigated the effects of (triGSyl)HQ on PG formation and gene expression. Renal proximal tubular epithelial cells (LLC-PK_1) were exposed to (triGSyl)HQ. Analysis of the media by radioimmunoassay revealed a 2.5-fold increase in PGE_2 after a 2-h exposure to (triGSyl)HQ (1 mM). Pretreatment of the cells with arachidonic acid (20 μM) increased basal PGE_2 levels 27-fold, but had no effect on (triGSyl)HQ-mediated toxicity determined by neutral red accumulation. Under these conditions (triGSyl)HQ increased PGE_2 synthesis (2–3-fold) dose dependently. A 1.5-fold increase in PGE2 was observed after 15 min exposure to (triGSyl)HQ (200 μM). The levels continued to increase up to 2-fold of the control levels after 2 h. Increases in the expression of the growth arrest and DNA damage inducible gene, *gadd 153*, were detected after a 2-h exposure to (triGSyl)HQ (200 μM; 19-fold). In addition, *hsp70* expression increased after exposure to (triGSyl)HQ (100 μM, 3.9-fold). The relationship between PG synthesis and gene expression mediated by (triGSyl)HQ requires further investigation. (GM 39338.)

1630 MODULATION OF INTRACELLULAR pH BY 2-BROMO-6-(GLUTATHION-S-yl)HYDROQUINONE

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Intracellular pH (pH_i) plays an important role in a variety of cellular processes including signal transduction, energy metabolism and gene expression. It has been shown that exposure of renal tubular epithelial cells to injury by oxidant stress causes an acidification of cytosolic pH. In the present study we investigated the effect of 2-Br-6-(glutathion-S-yl)-hydroquinone [2-Br-6-(GSyl)HQ] on pH_i in renal proximal tubular epithelial cells (LLC-PK_1). Exposure of LLC-PK_1 cells to 2-Br-6-(GSyl)HQ resulted in a transient, time- and dose-dependent decrease in pH_i. pH_i reached a nadir about 45 and 70 min after exposure of cells to either 200 μM or 100 μM 2-Br-6-(GSyl)HQ, respectively ($\Delta pH \approx 0.5$–0.6 units). Subsequently, pH_i gradually returned to neutral and this occurred faster in cells treated with the higher concentration of 2-Br-6-(GSyl)HQ. Cells treated with 200 μM 2-Br-6-(GSyl)HQ were more alkaline than control cells at-≈110 min whereas alkalinization was delayed in cells treated with 100 μM 2-Br-6-(GSyl)HQ. Cell blebbing occurred concomitant with the alkalinization of cells treated with 200 μM of 2-Br-6-(GSyl)HQ. The transition from an acidic to alkaline pH was also accompanied by a change in the permeability characteristics of the nuclear membrane, evidenced by the ability of the fluorescent probe to access the nucleus at an alkaline pH. Inhibition of the Na^+/H^+ exchanger with 5-(*N,N*-dimethyl)-amiloride prolonged the phase of intracellular acidification, thus suggesting that this exchanger has a role to play in this process. The relationship between 2-Br-6-(GSyl)-HQ-mediated changes in pH_i and cell death is under further investigation. (ES 04662).

1631 REGIONAL NEUROCHEMICAL ALTERATIONS AND MERCAPTURIC ACID FORMATION FOLLOWING ADMINISTRATION OF 5-(GLUTATHION-S-yl)-α-METHYLDOPAMINE

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5-(Glutathion-S-yl)-α-methyldopamine (5-GSyl-α-MeDA) is a metabolite of the serotonergic neurotoxicant methylenedioxy-amphetamine (MDA). Since glutathione conjugates of many polyphenols are biologically (re)active, we investigated the role of 5-GSyl-α-MeDA in the acute and long term neurochemical changes observed after administration of MDA. Intracerebro-ventricular (icv) administration of 0.72 μmol 5-GSyl-α-MeDA to Sprague-Dawley rats produced behavioral changes and short term alterations in dopaminergic, serotonergic, and noradrenergic neurotransmitter systems which paralleled those observed following subcutaneous administration of 87 μmol/kg MDA. However, a single icy injection of 5-GSyl-α-MeDA did not produce long-term deficits in the serotonin system. We also investigated the regional CNS metabolism of 5-GSyl-α-MeDA. We confirmed regional differences in the distribution of γ-glutamyl transpeptidase which correlated with the formation of 5-(cystein-S-yl)-α-MeDA. Interestingly, we also observed the presence of 5-(N-acetylcystein-S-yl)-α-MeDA in all brain regions, suggesting the brain has the ability to synthesize mercapturic acids. Substantial differences were seen in the ability of different brain regions to synthesize 5-(NAC)-α-MeDA. Maximum 5-(NAC)-α-MeDA levels were found in the order: hypothalamus > midbrain/diencephalon/telencephalon > pons/medulla > hippocampus > cortex > striatum. Because the mercapturic acid pathway can regulate the reactivity of quinones, our data may provide a biochemical basis for the heterogeneity in response of the brain to certain neurotoxicants.

1632 CARRIER-MEDIATED TRANSPORT OF QUINONE-THIOETHERS IN RAT LIVER MEMBRANE VESICLES

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Utilizing the *in situ* perfused rat kidney we have shown that infusion of 2-bromo-3-(glutathion-*S*-yl)HQ [2-Br-3-(GSyl)HQ] and 2-(glutathion-*S*-yl)HQ [2-(GSyl)HQ] *via* the renal artery results in the appearance of the conjugates in bile. This suggests that the liver has the ability to transport GSH conjugates from the circulation into hepatocytes, and subsequently into bile. The present study was designed to characterize the transport of quinone-thioethers across the basolateral and canalicular domain of male rat liver using highly purified basolateral (bLPM) and canalicular (cLPM) membrane vesicles. Inhibition of uptake of [^{3}H]-estradiol-17β-(D-glucuronide) (^{3}H-$E_2$17G) in bLPM was examined. (GSyl)HQs inhibited the initial uptake of ^{3}H-$E_2$17G at 15 sec, but had no effect on the uptake at equilibrium (1 hr). The transport of ^{3}H-$E_2$17G in the absence and presence of the HQ-(GSyl)HQs, expressed as pmol/mg/15sec, was; control, 23.6 ± 2.6; 2-(GSyl)HQ 11.3 ± 1,2; 2,5-(di-GSyl)HQ, 14.9 ± 3.1; 2,5,6-(tri-GSyl)HQ, 14.3 ± 1.9; and 2,3,5,6-(tetra-GSyl)HQ, 14.9 ± 2.1. In contrast to the HQ-(GSyl)HQs no effect was seen with the Br-(GSyl)HQs. Interestingly, both HQ-(GSyl)HQs and Br-(GSyl)HQs inhibited ATP dependent transport of ^{3}H-$E_2$17G (10 μM) in cLPM. These results suggest that HQ-(GSyl)HQs and Br-(GSyl)HQs are transported into hepatocytes by different transporters, in that (GSyl)HQs compete with the $E_2$17G transporter in bLPM. Both (GSyl)HQs and Br-(GSyl)HQs compete with the ^{3}H-$E_2$17G transporter in cLPM. Further characterization of these transporters is under investigation. (GM39338, ES04662, HD 13250).

1633 METABOLISM AND TOXICITY OF TRICHLORO-ETHYLENE (TRI) BY GSH CONJUGATION IN MALE RATS

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Metabolism of Tri by GSH conjugation and cellular and mitochondrial toxicity of Tri, several cytochrome P-450-derived metabolites (tri- and dichloroacetate, trichloroethanol, chloral hydrate), *S*-(1,2-dichlorovinyl) glutathione (DCVG), and *S*(1,2-dichlorovinyl)-L-cysteine (DCVC) were studied in hepatocytes, kidney cells and subcellular fractions from male Fischer 344 rats. Formation of DCVG in cells and subcellular fractions was protein concentration-, substrate concentration- and time-dependent. Although hepatic GSH-*S*-transferases were approximately 10-fold more active towards Tri than the renal enzymes, DCVG formation was detected in isolated kidney cells. Liver microsomal GSH *S*-transferases were more active than cytosolic isozymes. This suggests that primarily liver microsomal GSH *S*-transferase, but also the renal enzymes, are responsible for the first step of Tri bioactivation by this pathway *in vivo* leading to nephrotoxicity. Tri was not cytotoxic to isolated hepatocytes or kidney cells, as assessed by lactate dehydrogenase release, although DCVG and DCVC were nephrotoxic and DCVC was hepatotoxic *in vitro*. Absence of hepatotoxicity due to Tri and DCVG may be due to efficient detoxification of Tri, limited hepatic activity of γ-glutamyltransferase and absence of hepatic uptake of GSH *S*-conjugates. All chemicals produced hepatic and renal mitochondrial toxicity. Depletion of mitochondrial GSH content and oxidative stress in liver and kidney mitochondria may play important roles in hepatic and renal mitochondrial dysfunction and in *in vivo* toxicity induced by Tri and its metabolites. Supported by NIDDK (R01-DK40725 to LHL) and US EPA (CR-822240-01-0 to LHL and AAE).

1634 PROTECTIVE EFFECT OF AMINOOXYACETIC ACID ON 1-CYANO-3,4-EPITHIOBUTANE INDUCED NEPHROTOXICITY IN FISCHER 344 RATS

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1-Cyano-3,4-epithiobutane (CEB), a naturally-occurring nitrile, is bioactivated by glutathione (GSH) conjugation with resultant nephrotoxicity and karyomegaly in F344 rats. Treatment with CEB results in early depletion of hepatic GSH followed by a rebound elevation; however, no phase of depletion was observed in renal GSH which was elevated at all time periods studied. To further elucidate the metabolism and toxicity of CEB in F344 rats, eight week old, male F344 rats were orally gavaged with 125 mg CEB/kg body weight or deionized, distilled water and received an IP injection of saline or aminooxyacetic acid (AOAA). Hepatic and renal GSH were significantly elevated at 24 and 48 hours in rats treated with CEB alone or CEB + AOAA. Significant elevations in renal GSH were also observed in rats treated with AOAA alone at 24 hours; however, the magnitude of the elevation of renal GSH in rats receiving CEB in addition to AOAA was at least 2 times greater than those rats receiving only AOAA. Epithelial cell necrosis in the pars recta of the renal proximal tubule and karyomegaly were observed in rats treated with CEB alone at both 24 and 48 hours. At 24 hours, however, pretreatment with AOAA decreased histopathological lesions and karyomegaly as measured by histomorphometric methods. Rats pretreated with AOAA had equivalent karyomegalic measurements as compared to controls, which received only saline injections and water gavages, and were significantly lower than CEB controls. By 48 hours AOAA treated rats exhibited significantly more severe histopathological changes and karyomegaly as compared to saline controls. These results indicate a protective effect of AOAA against CEB-induced nephrotoxicity at 24 hours. This effect is diminished by 48 hours following CEB administration. AOAA inhibits renal β-lyase, suggesting that the reactive thiol, produced from the cysteine conjugate, is at least partially responsible for the CEB-induced nephrotoxicity.

1635 THE TOXIC INTERACTIONS OF ARSENIC AND CADMIUM IN RATS DEPEND UPON THE SEQUENCE OF EXPOSURE

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Arsenic and cadmium are both priority hazardous substances and human carcinogens. Although there is the potential for simultaneous exposure to both metals, the interactions of cadmium and arsenic are not well defined. We examined the toxicity of these metals when given alone or in alternating sequence to adult male Fischer rats. In the first study, a non-toxic dose of arsenic (22.5 μmoles $NaAsO_2$/kg, sc) was given 24 hrs before cadmium (10, 20, or 30 μmoles $CdCl_2$/kg, sc) and toxicity was assessed 24 hrs later. Arsenic pretreatment markedly reduced mortality in rats given the high dose of cadmium (9 survivors/10 treated) compared to rats given cadmium alone (2/10). Arsenic pretreatment also reduced cadmium-induced hepatotoxicity, as indicated by serum glutamic oxalacetic transaminase (SGOT) activity, and markedly reduced cadmium-induced testicular hemorrhagic necrosis. Arsenic pretreatment produced an 8-fold increase in hepatic levels of metallothionein (MT), a metal-binding protein often associated with cadmium tolerance. In the second study, a non-toxic dose of cadmium (3 μmoles $CdCl_2$/kg, sc) was given 24 hrs before arsenic (68, 79, 84, or 90 μmoles $NaAsO_2$/kg, sc) and toxicity was assessed 24 hrs later. Cadmium pretreatment did not alter the lethality of the high dose of arsenic and had no effect on arsenic-induced hepatotoxicity. Although cadmium pretreatment had no effect on arsenic toxicity, it produced large increases in hepatic MT (26-fold) before the arsenic challenge and greatly enhanced MT induction after the challenge. Thus, even though both arsenic and cadmium induce MT synthesis, only arsenic pretreatment protects against cadmium intoxication. The toxic interactions of arsenic and cadmium appear to depend on the sequence of exposure.

1636 EFFECTS OF LONG-TERM ORAL CADMIUM ADMINISTRATION TO RENAL FUNCTION AND BONE MINERAL DENSITY IN RATS

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Wistar mail rats (6 weeks old) were given Cd (as CdCl2) at a various dosage of 0, 2, 5, 10, 20, 30 and 60 mg/kg with gastric tube daily for 6 consecutive days a week. Animals were sacrificed at 0, 3, 5, 10, 20, 30 and 60 weeks after Cd administration. Cd concentration in kidney, urinary enzyme (GST, NAG, AAP) excretion and bone mineral density were measured. More Cd accumulated in the kidney than in the liver of lower Cd dosage groups without hepatic damage (2, 5 and 10 mgCd/kg). While Cd concentration in liver of high dosage groups was contrary higher than in kidney. The increase of urinary enzyme excretion (NAG, AAP and GST) reflecting the renal dysfunction and the decrease of bone mineral density were found in the high dosage groups of Cd. Remarkable renal dysfunction was not observed in the lower Cd dosage groups, although the Cd concentration in the kidney was similar levels to those of the high dosage groups of Cd. These results suggest that the critical concentration of Cd in kidney may be changeable according to the dosage and the exposure period of Cd. Also the manifestation of decrease of bone mineral density may be different considering the renal dysfunction by chronic Cd toxicity.

1637 SERIAL RIB BIOPSIES TO STUDY EFFECTS OF CADMIUM ON BONE FORMATION AND RESORPTION IN AGED, OVARIECTOMIZED BEAGLES

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The effects of ovarian hormone depletion and cadmium (Cd) exposure on bone formation and resorption were studied in 7- to 9-y-old beagles. Early results showed that Cd at 2–5 μg/L in blood increased bone resorption (skeletal ^{45}Ca release) within 24 h (Sacco-Gibson et al., 1992). In addition, loss of vertebral and tibial bone mineral density was greater after OV + Cd — 8.5 mo of ovariectomy (OV) and 5.5 mo of continuous Cd (15 ppm in drinking water) — than after OV or Cd alone (Bhattacharyya et al., 1992). Results reported here are from serial rib biopsies taken from each OV and sham-operated (SO) dog at the time of surgery (T_0), just before the start of Cd administration (1 mo after surgery) (T_1), and after 1 mo (T_2) and 5.5 mo (T_3) of continuous Cd exposure (4 mo and 8.5 mo after surgery, respectively). (Lag time between surgery and start of continuous Cd is due to initial 3 mo of low, noncontinuous Cd). Tetracycline (T_1), calcein (T_2), and xylenol orange (T_3) were administered by a fluorochrome labeling procedure (2-10-2) to provide markers of bone formation. Histomorphometric analyses showed that, at 4 mo after surgery (T_2), ovariectomy alone strikingly increased bone formation rates (BFR_{ms}, %/y: T_1, 6.6 ± 2.7; T_2, 48.7 ± 4.5; OV group, mean ± SE, n = 4) and cortical porosity (CP, %: T_1, 1.39 ± 0.32; T_2, 4.89 ± 1.26); Cd had little or no effect on these OV-induced increases. Resorption porosity was low at T_1 (0.2–0.3%) and increased gradually in all groups to 0.8–1.0% by T_3; however, in the OV + Cd group, resorption porosity had already increased substantially by T_2, 1 mo after continuous Cd (RP, %: T_1, 0.23 ± 0.06; T_2, 0.78 ± 0.13; T_3, 1.08 ± 0.16). The percentage of cortical porosity accounted for by resorption osteons (RP/CP × 100), 15 ± 4% in the SO group, did not increase with time due to OV or Cd alone. In contrast, OV + Cd caused steady increases (RP/CP, %: T_1, 10 ± 3; T_2, 22 ± 2; T_3, 41 ± 6). Results are consistent with the hypothesis that Cd increases bone resorption in vivo and that ovarian hormone deficiency exacerbates this effect.

1638 EFFECTS OF CADMIUM ON BONE RESORPTION *IN VITRO*

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Chronic exposure to cadmium (Cd) has been linked to bone loss, low bone mass, and increased incidence of fracture. A previous investigation into the effects of Cd exposure and estrogen depletion on the skeletons of aged beagles, whose skeletons are comparable to those of humans, indicated that Cd increases bone resorption. To study the cellular mechanism of Cd-induced bone resorption, we cultured normal dog bone marrow cells containing the progenitor cells for osteoclasts (bone-resorbing cells). Cultures were evaluated for the number of osteoclast-like cells (MNOC); cells that are multinucleated, contain tartrate-resistant acid phosphatase and express the vitronectin receptor. Under appropriate culture conditions, MNOC formed in response to the osteotropic hormones 1.25 $(OH)_2$ Vitamin D_3 and parathyroid hormone within 12–14 days. In contrast, exposure to Cd (10–1000 nM) did not increase the number of MNOC formed over control values. The MNOC formed by the bone marrow cultures were functional, as evidenced by pits excavated on 100 μm-thick, devitalized bone slices included in the cultures. By 12 d, MNOC formed in the presence of 50 nM Cd excavated significantly more pits than untreated MNOC (70 ± 14 vs. 6 ± 3; mean ± SE, n = 4). The area excavated was also increased over that for untreated cultures (28.0 ± 3.9 vs. 1.8 ± 1.2 $\mu m^2 \times 10^{-3}$). By 14 d, the number of pits and the area excavated by untreated MNOC were similar to those of Cd-exposed MNOC. However, pit formation was still greatly enhanced by exposure to Cd; the ratio of pit complexes to single pits was increased two fold over that for untreated cultures (3.2 vs. 1.7). These data suggest that Cd increased the activity of MNOC formed. This ob-

servation was confirmed directly by culturing mature osteoclasts (OCl), isolated from the long bones of rat neonates, for 1–3 d on bone slices. Exposure of the OCl to 100 nM Cd increased the number of pits excavated over that for untreated OCl by a factor of 3.8 ± 0.9 (mean ± SE; 6 separate experiments), the area excavated by a factor of 4.0 ± 0.7, and the area excavated per OCl by a factor of 1.8 ± 0.3. These data, taken together, suggest that Cd activates or increases the activity of mature OCl but does not induce formation of new OCl.

1639 CADMIUM ACTIVATES PROTEIN KINASE C (PKC) IN RAT OSTEOSARCOMA (ROS 17/2.8) CELLS

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The mechanism of the toxic effects of Cd^{2+} on normal bone growth and bone cell function is not completely understood at this time, but Cd^{2+} perturbs the intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) in ROS 17/2.8 cells. Ca^{2+} activates PKC, and PKC is a known mediator of $[Ca^{2+}]_i$. Therefore, PKC is a possible loci of Cd^{2+} effects on Ca^{2+} metabolism and Ca^{2+} regulated processes. The purpose of this study was to characterize the activation and/or inhibition of PKC by Cd^{2+} in ROS 17/2.8 cells. PKC activity was measured as Ca^{2+} and Cd^{2+}-dependent activation of phosphatidyl-serine/diolein-dependent incorporation of ^{32}P from ATP into histone and endogenous TCA precipitable proteins in the 100,000 X g supernatant from homogenized ROS 17/2.8 cells. The specific free concentrations of both Cd^{2+} and Ca^{2+} were set using the divalent cation chelator and indicator 1,2-bis(2-amino-5-fluorophenoxy) ethane N,N,N′,N′-tetraacetic acid (5F-BAPTA). Free $[Ca^{2+}]$ and $[Cd^{2+}]$ in the PKC reaction mix were directly confirmed using 5F-BAPTA and ^{19}F NMR. Free Cd^{2+} activates PKC in the range from 10^{-12} to 10^{-7} M, with an activation constant of 7.5×10^{-11} M, while Ca^{2+} activates PKC in the range from 10^{-8} to 10^{-3} M, with an activation constant of 3.6×10^{-7} M. These data suggest that PKC is a sensitive target of Cd^{2+} toxicity in ROS 17/2.8 cells, and Cd^{2+} may interfere with normal Ca^{2+} activation of PKC.

1640 THE EFFECT OF CADMIUM ON IMMUNOLOGIC FUNCTION AND LYMPHOCYTIC CALCIUM HOMEOSTASIS IN MICE

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The present study examines that Cadmium might have an impact on the relationship between immunologic function and Ca-homeostasis in lymphocyte. This study would be expected to explore the toxic mechanism of Cadmium. In this study, $CdCl_2$ has been given to female LACA mice by oral administration for fourteen days. The results illustrate that when oral administration dosage is at 0.3 mg/kg per day, (1) the number of IgM plaque-forming cells in lymphocyte is significantly inhibited ($p < 0.001$); and (2) there is an increase of the concentration of Ca^{2+} with a decrease of the content of Calmodulin in lymphocyte. Moreover, the test in vitro shows that when the concentration of $CdCl_2$ is increased to 50 u mole, the transformation of T-cells is inhibited with an increase of the concentration of Ca^{2+}. The above results have indicated a significant dose-response relationship. This study has revealed that Cadmium could cause dysfunction of Ca-homeostasis in lymphocyte. One might consider that the increase of the concentration of Ca^{2+} could be related to the closing and/or reducing of the N-channel.

1641 CADMIUM-INDUCED INTERLEUKIN-6 FROM GLOMERULAR MESANGIAL CELLS ENHANCES KIDNEY TUBULAR EPITHELIAL CELLS REGENERATION

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Chronic or subchronic exposure to cadmium (Cd) produces renal damage characterized by degeneration of tubular epithelial cells followed by interstitial inflammation and finally regeneration of proximal tubular cells. Since chronic kidney diseases are usually associated with the presence of cytokines and Cd affects cytokine expression in monocytes and the Kupffer cells of the liver, we investigated the role of proinflammatory cytokines in Cd-induced nephrotoxicity. Low-to-moderate increases in inflammatory cytokine transcription and secretion was observed following exposure of LPS-primed mice to a total of 21 mg/kg body weight Cd administered over a 14-week period. IL-6 was the predominant cytokine expressed and was produced in mesangial cells. Proliferative cell nuclear antigen (PCNA) staining revealed increases in regeneration of tubular epithelial cells following Cd exposure. Furthermore, the renal tubular epithelial cell line, LLC-PK_1 responded to IL-6 by marked proliferation. Taken together, these data suggest that Cd-induced IL-6 secretion in the kidney may act as a growth factor supporting regeneration of renal tubular epithelial cells.

1642 Na^+,K^+-ATPase, GLUTATHIONE (GSH) AND HYDROXYL FREE RADICALS IN CADMIUM-INDUCED TESTICULAR TOXICITY

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Cadmium-induced biochemical changes were characterized in male, CD-1 mouse testes. Cadmium inhibited the testes microsomal Na^+,K^+-ATPase activity in vitro and in vivo. The inhibitory range was 30–150 μm and the concentration for half maximal inhibition (IC50 value) was 90 μm. $CdCl_2$ (2 mg/kg s.c.) for 2 days significantly inhibited testes Na^+,K^+-ATPase (near 90% inhibition). The content of testicular GSH and the ratio of GSH/GSSG decreased in cadmium treated groups. Using salicylate as a trapping agent and high pressure liquid chromatography with electrochemical detection (LCED), we measured the OH production in vivo. 2,5-DHBA and 2,3-DHBA as indices of hydroxyl free radicals formation significantly increased after 5 days cadmium exposure. Pretreatment with vitamin E (20 mg/kg, s.i.d., i.m., 7d) protected cadmium-induced increase in OH· generation in testes. From this study, we were able to demonstrate that cadmium induced testicular toxicity could possibly mediated by a significant increase in hydroxyl free radicals formation and a reduction in GSH content and Na^+,K^+-ATPase activity. Vitamin E seems to prevent cadmium induced increase in hydroxyl free radicals generation.

1643 Cd^{2+} INDUCES UTP POOL EXPANSION THROUGH ACTIVATION OF UMP SYNTHESIS IN CULTURED CELLS

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Exposure of Swiss 3T3 fibroblasts to Cd^{2+} results in a marked elevation in UTP pool concentration, while the ATP pool is only slightly diminished. To determine the mechanism of the Cd^{2+}-induced UTP expansion, cells were labeled with a mixture of two precursors of UMP synthesis, [^{14}C]-orotic acid and [^3H]-uridine, to assess Cd^{2+} effects on cellular UTP synthesis (through either *de novo* or salvage pathway) as well as UTP utilization in synthesis of nucleotide sugars and RNA. Time course studies with 50 μM Cd^{2+} showed a significant increase in incorporation of both UMP precursors into UTP, reaching 198.8% and 157.0% of the control for uridine and orotic acid, respectively, after 3 h treatment. This increase in UMP synthesis coincides with a twofold rise in UTP pool concentration as determined by HPLC analysis. Incorporation of [^3H]-uridine into the three UDP-sugar derivatives, UDP-acetylglucosamine, UDP-glucose and UDP-glucuronic acid, was decreased to around 40% of the control after 5 h treatment with Cd^{2+}. Similar Cd^{2+} effects were observed in RNA synthesis, which was decreased to 22.5% at 5 h, with no significant changes at earlier timepoints. These results suggest that the Cd^{2+}-induced UTP pool expansion results mainly from an increase in UMP synthesis through the salvage pathway. Furthermore, the observed decreases in cellular UTP utilization could possibly contribute to accumulation of UTP at later timepoints. (Supported by NIH ESO3543 and Boston Univ. Student Res. Award).

1644 LYSYL OXIDASE DEFICIENCY IN CADMIUM-RESISTANT (CdR) CELLS

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Lysyl oxidase (LO), a copper-dependent metalloenzyme, plays a central role in crosslinking collagen and elastin in the extracellular matrix. To further understand the mechanism of Cd toxicity, the cellular distribution, mRNA expression, and posttranslational processing of LO were examined in CdR cells isolated from Swiss mouse 3T3 cells. CdR cells expressed elevated levels of metallothionein (Mt), a known marker for Cd resistance, but synthesized low levels of an apparently variant LO proenzyme (51.9 kDa) inside the cell, rather than the 50 kDa species found in their parental counterparts, and produced very little extracellular 32 kDa mature LO. As a result, enzyme activity was markedly decreased in the conditioned medium of CdR cells (11% of the parental basal level). CdR cells displayed a distinct antibody staining pattern of LO distribution characterized by numerous powdery enzyme "particles" crowded in the cytoplasm, as opposed to a morphology of antibody stained LO appearing as small, curving "fragments" in parental cells. The 51.9 kDa protein remained associated with the CdR cells during a 5 h-chase period, suggesting a defect in

LO secretion. These cells also showed down-regulation of LO mRNA expression. The LO activity in CdR cells was partially restored by incubating the cells with $CuCl_2$, implying that LO deficiency in CdR cells may be related to abnormal copper metabolism, i.e., unavailability of cellular copper as a result of its binding to the increased levels of Mt. (Supported by NIH grant HL46902).

1645 Ni^{2+}-STIMULATED INCREASE IN ACETYLATED α-TUBULIN, A MARKER FOR STABLE MICROTUBULES (MT), IN CULTURED CELLS

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Stable MT are well known to contain acetylated α-tubulin (Ac-tubulin). Ni^{2+}-induced MT bundling may result from such tubulin posttranslational modification. To explore this possibility as a mechanism of Ni^{2+}-induced cytoskeletal injury, we monitored Ac-tubulin in cultured 3T3 cells by fluorescent staining of MT and immunoprecipitation assay using anti-Ac-tubulin antibody. MT in growth-arrested 3T3 cells contained very little Ac-tubulin, localized in a few short, brightly-stained fragments at the perinuclear MT organizing center (MTOC) area. In contrast, treatment of cells with $NiCl_2$ (0.25–2 mM) for 20 h resulted in accumulation of intensely stained Ac-tubulin distributed along the Ni^{2+}-induced MT bundles. Cell extracts prepared from [^{35}S]methionine labeled cultures in the presence or absence of Ni^{2+} were immunoprecipitated and analyzed by SDS PAGE followed by autoradiography. Consistent with the above morphological findings, a predominant protein band (50 kDa), representing Ac-tubulin, appeared in Ni^{2+} (1 and 2 mM)-treated cells in a dose-dependent manner whereas the corresponding labeled band was only barely detectable in control cells. These results indicate that Ni^{2+} induces MT bundling probably by promoting tubulin acetylation, resulting in enhanced MT stability. (Supported by NIH RO1ES03543).

1646 Mg^{2+} ANTAGONISTIC EFFECTS ON Ni^{2+}-INDUCED CYTOSKELETAL INJURY IN CULTURED CELLS

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Since Mg^{2+} exhibits a resisting effect for some endpoints of Ni^{2+} actions such as carcinogenesis, we examined its antagonistic potential on several parameters related to Ni^{2+}-induced cytoskeletal injury in cultured 3T3 cells. Exposure of cells to 2 mM $NiCl_2$ for 20 h resulted in perinuclear bundling of microbtubules (MT), redistribution of microfilaments (MF) to the cell periphery, and decreases in cytoskeletal and cytosolic protein sulfhydryls (PSH) and cellular glutathione (GSH) levels. Presence of $MgCl_2$ (1.25–20 mM) markedly diminished the Ni^{2+}-induced perturbations of MT and MF. Under these conditions Ni^{2+} interference with PSH was blocked such that both the cytoskeletal and cyosolic PSH levels were essentially in the range of control cells. Treatment of cells with low doses of Mg^{2+} for 20 h produced an increase of cellular GSH while higher Mg^{2+} doses (>2.5 mM) decreased cellular GSH. Importantly, in Ni^{2+}-treated cells, Mg^{2+} elevated GSH levels to 224, 230, 214, and 198% at 1.25, 2.5, 5, and 10 mM Mg^{2+}, respectively, relative to the level in cells treated with Ni^{2+} alone. Because of the essential role of tubulin PSH for MT assembly and the GSH modulation of PSH, cellular GSH may play an important role in Mg^{2+} antagonistic effects on Ni^{2+}-induced cytoskeletal injury (supported by NIH RO1ES 03543).

1647 CADMIUM LEVELS IN MATERNAL, CORD AND NEWBORN BLOOD IN MEXICO CITY NEWBORNS

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Cadmium exposure is a health hazard to human populations. High cadmium levels has been associated to low birth weight in newborns. Therefore, it is important to determine the sources of human cadmium exposure. We obtained a sample of n = 50 women at the time of delivery, from a Hospital of México city. One-ml blood samples were obtained from the mother, the newborn and the cord, and stored in metal-free tubes until analysis. A questionnaire was applied to the mothers to record potential determinants of cadmium exposure. Birth weight was also recorded. Multiple regression analysis applied to the data indicate a significant association between cord and newborn blood and between cord and maternal blood, but not among maternal and newborn blood. Smoking habits of the mother was found associated to high levels of cadmium only in the maternal blood. Birth weight of the newborns was found associated to the frequency of yoghurt consumption and inversely associated to cord blood cadmium levels and smoking habits.

1649 ALTERED URINARY PORPHYRIN EXCRETION IN HUMANS CHRONICALLY EXPOSED TO AS IN MEXICO

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A study on the profile of urinary porphyrins and its relationship to cutaneous signs of arsenicism was performed in humans chronically exposed to As *via* drinking water (400 μg/L), as compared with individuals exposed to 21 μgAs/L. There were no significant differences in total porphyrin excretion. However, exposed individuals showed a small but significant increase in URO excretion, accompanied by a significant decrease in urinary COPRO III, both findings contributed to an inversion of the COPRO/URO ratio (1.58 in controls vs 0.79 in exposed, $p < 0.001$). The decrease in COPRO III also contributed to the inversion in the COPRO III/COPRO I ratio (1.31 in controls vs 0.48 in exposed, $p < 0.0001$). A lower URO-D activity in the exposed individuals and/or a diversion of COPRO from the urinary to the biliary route of excretion may explain these findings. Both alterations and the prevalence of cutaneous signs of arsenicism were directly related to time-weighted exposure to As. Our data suggest that porphyrin alterations occurred earlier than As-skin lesions and that they may be useful in the process of identifying individuals at high risk of suffering skin lesions.

1650 ARSENIC METHYLATION IN RAT LIVER CYTOSOL

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The mono- and dimethylation of arsenic was examined in rat liver cytosol *in vitro*. Up to 40% of carrier-free ^{73}As-arsenite was methylated during 60 minutes incubation of rat liver cytosol at 37°C in presence of 1 mM S-adenosylmethionine (AdoMet) and 10 mM glutathione (GSH), yielding monomethylarsenic (MAs, 2%) and dimethylarsenic (DMAs, 38%). MAs concentration peaked after 5-minutes. DMAs concentration peaked between 30 and 60 minutes. MAs production increased with increasing concentration of arsenite in the range of 0.1–50 μM. DMAs production was inhibited at 50 μM arsenite. MAs formation was not dependent on the addition of AdoMet. Optimal DMAs production required addition of 0.1 or 1.0 mM AdoMet. In the presence of 0.1 mM AdoMet, 0.1 or 1.0 mM S-adenosylhomocysteine inhibited formation of both MAs and DMAs. Omission of GSH from the incubation system nearly abolished methylation of arsenite. With increasing GSH concentration (1–20 mM), production of both MAs and DMAs increased; however, the DMAs/MAs ratio decreased. Addition of GSH decreased binding of As to cytosolic proteins. A negative correlation was found between production of both methylated metabolites and amount of protein-bound As at different GSH concentrations. Selenite (10 μM) or EDTA (1–10 mM) inhibited production of both MAs and DMAs. $HgCl_2$ (1–10 μM) inhibited only DMAs formation. Addition of 1 mM *p*-anisic acid an inhibitor of cytosolic methyltransferases, or 1 mM 2,3-dichloro-α-methylbenzylamine, an inhibitor of microsomal methyltransferases, had no effect on methylation of arsenite. The enzymes catalyzing the methylation of arsenic may be members of a novel enzyme family. (This abstract does not necessarily reflect EPA policy).

1651 ENZYMATIC ASSAY AND PURIFICATION OF ARSENIC METHYLTRANSFERASE FROM RABBIT LIVER

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We have developed an assay for arsenic methyltransferase activity in rabbit liver cytosol, using [^{3}H]-S-adenosyl-methionine as the methyl donor. Chloroform extraction was used to isolate the methylated arsenic products. The optimal pH of either methylation activities was 8.0. Arsenite as the substrate produced primarily monomethylarsonic acid (MMA), while MMA as the substrate resulted in only dimethylarsinic acid (DMA) formation. HPLC, cation exchange and TLC were used to confirm the identities of these methylated products. Although neither reaction had an absolute requirement for glutathione, addition of monothiols or dithiols was necessary to achieve full activity. Both arsenic methyltransferase activities co-purified from cytosol through anion exchange chromatography, ammonium sulfate precipitation and gel filtration chromatography. The enzyme(s) have been purified 2000-fold. The most purified preparation containing methyltransferase activities corresponds to 60 kDaltons. The 2000-fold purified enzyme preparation does not methylate selenium compounds, although there is such activity in less purified fractions. Selenite inhibits purified methyltrasferease activity. As yet, the question as to

whether there are two separate proteins catalyzing the conversion of arsenite to MMA and of MMA to DMA has not been resolved. (Supported in part by the Superfund Basic Research Grant NIEHS #ES-04940)

1652 METABOLISM OF ARSENIC BY THE LUNG

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Arsenic is recognized as a human pulmonary carcinogen. The mechanism of this toxicity is unknown, but it may be related to metabolism of arsenic in the lung. Inorganic arsenic is metabolized by two processes: reduction/oxidation and methylation. Rat lung homogenates incubated at 37°C with arsenic reduce approximately 3–4% of the arsenate present within 5 hours. Oxidation of arsenite occured in rat lung homogenate incubations, but at a much slower rate than reduction. Glutathione (GSH) is capable of reducing arsenite in solution. This led us to investigate GSH levels in lung homogenates and cells treated with arsenic. In rat lung homogenates, arsenite produced a dose dependent decrease in GSH concentrations without concomitant increases in GSSG. Concentrations of arsenate up to 2 mM had little effect on concentrations of GSH or GSSG. In L132 cells, a human embryonic epithelial cell line, treated with 2 mM arsenite, GSH concentration was greater than control at 0.5, 1, and 3 hours. GSH levels returned to normal by 6 hours. In L132 cells treated with 2 mM arsenate, GSH concentrations were less than controls at 30 minutes; then became greater than control at 1 and 3 hours and returned to control levels by 6 hours. We have also investigated the methylation capacity of rat lung cytosolic preparations using ^{3}H S-adenosyl-L-methionine (SAM) in an assay developed by Aposhian et al. Rat lung cytosol incubated with 20 μM arsenite produces monomethylarsonic acid (MMA) at a rate of 0.878 ± 0.09 pmol/min/mg (mean ± SE). Cytosol incubated with 1 mM MMA produces dimethylarsinic acid (DMA) at a rate of 0.132 ± 0.015 pmol/min/mg (mean ± SE). Our data indicate that the lung is capable of reducing and methylating arsenic; that GSH may be involved in the lung's response to arsenic; and that the lung produces methylated arsenic metabolites. We are further investigating lung arsenic metabolism using primary and established cell lines. This grant was supported by grants ES 5561 and ES6694.

1653 TOXICOLOGICAL AND DISPOSITIONAL EFFECTS IN MICE FOLLOWING A 4 WEEK EXPOSURE TO ARSENATE (As^{+5}) IN DRINKING WATER

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The carcinogen arsenic, primarily in the form of As^{+5}, is a common constituent of drinking water, present from natural sources and pollution. Thus, exposure to As^{+5} occurs daily. The objective of this study was to examine in mice the effect of a 4 week exposure to As^{+5} in drinking water. Female B6C3F$_1$ mice (3/cage) were placed in metabolism cages and given water and food *ad libitum*. Water was either control (C), 56.8 μg/L (L) or 5.68 mg/L (H) As^{+5}. Animals were divided into 2 groups (A,B) with 4 cages/treatment/group. Group A was sacrificed by cardiac puncture after collecting their urine on day 28. Clinical chemistry analysis on the urine and serum and histopathological analysis on liver and kidney was done. Reduced thiol content was measured in liver, lung and kidney. Group B was gavaged with a tracer dose of [^{73}As]-arsenate on day 28 and continued the As^{+5} exposure for 48 hr. Urine and feces were collected and analyzed for ^{73}As. The animals were then sacrificed and tissues removed and analyzed for ^{73}As. No dose effects on the mean daily amount of water and food consumed, or reduced thiol content in tissues examined were observed. The mean daily urine volume excreted was significantly greater in the H treated animals. A dose-related non-specific vacuolar degeneration in the liver was observed. Clinical chemistry analysis of urine showed no effect on the parameters examined. In serum, glucose levels in C and L animals was significantly greater than H, triglyceride levels were significantly greater in C than L and H, and creatinine levels were significantly greater in H than C. Urinary excretion was the main route of elimination of ^{73}As, but no dose effects on the 48 hr ^{73}As cumulative excretion were detected. The kidney had the highest ^{73}As content (%dose/gm). Only the blood showed a dose effect, with C significantly greater than H. The overall effects of the doses of As^{+5} examined in this study appeared to be minimal. (This abstract does not reflect EPA policy.)

1654 VARIATION IN HUMAN SENSITIVITY TO CLASTOGENIC EFFECTS OF ARSENIC

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The induction of sister chromatid exchanges (SCES) in vitro by sodium arsenite was studied in peripheral lymphocytes and lymphoblast lines from normal donors. Although interindividual variation was large, the pooled data showed a clear dose-related increase in SCE frequency over the concentration range of 5×10^{-7} M to 5×10^{-6} M. At higher concentrations the arsenite was cytotoxic. When the arsenite-induced SCE frequencies were compared on an individual basis, there appeared to be at least a bimodal distribution in sensitivity to the clastogenic effects of arsenite, with over 1/2 of the subjects showing little or no effect of arsenic, while the others showed a significant response. Treatment of arsenic-insensitive lymphocytes with the strong clastogen 4-nitroquinoline-1-oxide (4NQO) increased the SCE frequency, and this response was markedly enhanced when arsenic was also present. These results indicate that humans are highly variable in their susceptibility to the genotoxic effects of arsenic and that arsenic is co-genotoxic with certain other genotoxic agents.

1655 UPTAKE AND REDUCTION OF SODIUM ARSENATE IN THE RED BLOOD CELL

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To characterize the role of the blood in the disposition of sodium arsenate (As(V)), its transport and reduction were determined in rat red blood cells. Previous work indicated that high concentrations (10 mM) of arsenate were reduced to arsenite (As(III)) in the blood. This process was relatively slow (hours) and *in vivo*, arsenate reduction is relatively fast (minutes). In these experiments reduction of As(V) to As(III) was measured as a function of dose and was inversely related to the initial dose of arsenate. An important consideration in these experiments was the transport kinetics of arsenate into red cells. The rate of uptake of [^{73}As]-sodium arsenate into red blood cells was both saturable and temperature dependent. Steady-state arsenate levels were reached within 0.5 hours for very low initial concentrations (1 μM) but could take up to 2.5 hours for higher levels (100 μM and greater). Uptake was compared at 37 °C and 4 °C and found to be greatly diminished by the decrease in temperature. These data are not consistent with a simple diffusion model, and arsenate transport may be a mediated process. Further, the mode of uptake may be different at higher concentrations of arsenate. In conclusion, the red blood cell is able to take up and reduce physiological levels of arsenate at speeds which could contribute to the overall metabolism of arsenate *in vivo*. (This work supported by ES4940 and ES6694.)

1656 REACTIVE OXYGEN SPECIES ARE NOT RESPONSIBLE FOR ARSINE-INDUCED HEMOGLOBIN DAMAGE

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Previous work in our lab suggests that arsine (AsH_3)-induced hemoglobin (HbO_2) damage leads to hemolysis. The purpose of the work presented here was to determine if reactive oxygen species are formed by AsH_3 in hemoglobin solutions (0.04 mM HbO_2 and 0.12 mM AsH_3) and if these species are responsible for the observed hemoglobin damage. We have shown previously that hydrogen peroxide (H_2O_2) was detected in AsH_3-treated HbO_2 solutions. However, we show here that high activity (19,200 U/ml) catalase added to solutions of HbO_2 and AsH_3 has only a minor protective effect by reducing slightly the amount of HbO_2 precipitation. Further, the differences between the visible spectra of AsH_3-treated HbO_2 and H_2O_2-treated HbO_2 indicate that two different degradative processes occur. These results indicate that AsH_3 does not damage HbO_2 through H_2O_2 formation. The presence of superoxide anion (O_2^-) was measured by O_2^--dependent reduction of nitro blue tetrazolium (NBT). The results were negative for O_2^- but did indicate a small amount of *non*-superoxide-dependent reduction of NBT in solutions containing both AsH_3 and HbO_2. Exogenous superoxide dismutase (50 μg/ml) had no effect on AsH_3-induced spectral changes. Mannitol (20 mM), a hydroxyl radical scavenger, also had no effect in these samples. These results indicate that the superoxide anion, the hydroxyl radical (·OH), and H_2O_2 are not involved in the mechanism of AsH_3-induced HbO_2 damage. Two other antioxidants, butylated hydroxyanisole and ascorbate, had no effect on AsH_3-induced HbO_2 damage at low millimolar concentrations. (Supported by grants ES07091, ES06644 and ES06694.)

1657 DMPS-MERCURY CHALLENGE TEST: URINARY MERCURY AFTER EXPOSURE TO MERCUROUS CHLORIDE

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The DIMAVAL (DMPS) challenge test (300 mg, po after an 11 hr fast) was given to 8 users of a skin lotion that contains mercurous chloride, 11 factory workers who make the skin lotion and 9 controls who had no known occupational exposure to Hg in Tampico, Mexico. Urines were analyzed for total Hg using cold vapor AAS. The μg Hg excreted for 6 hrs before and 6 hrs after DMPS treatment was 16.2 ± 3.43 and 1410 ± 346 for skin lotion users (n = 8); 113 ± 26 and 5037 ± 682 for factory workers (n = 11); and 1.08 ± 0.61 and 28.6 ± 12.1 SE for controls (n = 9), respectively. The increases in urinary Hg resulting from the DMPS challenge test were 87, 45 and 26-fold, respectively. Urinary coproporphyrin, precoproporphyrin and pentacarboxylporphyrin levels in all 3 groups decreased after DMPS administration. Coproporphyrin concentration before DMPS challenge was strongly correlated with urinary Hg excretion after DMPS challenge for the factory workers (r = 0.73) and the lotion users (r = 0.83). The results show the value of DMPS in humans for increasing urinary excretion of Hg and the value of the DMPS/MERCURY challenge test and urinary porphyrins for determining mobilizable Hg. (Supported in part by the Superfund Basic Research Program NIEHS Grant #ES-04940.)

1658 HUMAN AND RABBIT RENAL CORTICAL SLICES IN AERATED SUBMERSION CULTURE AS A MODEL TO EVALUATE HEAVY METAL CHELATORS

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Heavy metal chelators have been used for many years as therapeutic agents against metal poisonings by mercury, lead and arsenic. A submersion culture system has been developed to evaluate the efficacy of heavy metal chelators in removing metals from renal tissue. Slices were exposed to non-toxic loading doses of $HgCl_2$ (10 uM) and $NaAsO_2$ (50 uM) for 4 hrs prior to being transferred to the media (DME/F-12) containing the chelators 2,3-dimercapto-1-propane sulfonic acid (DMPS) or meso-dimercaptosuccinic acid (DMSA). DMPS and DMSA (0.5–10 mM) removed $NaAsO_2$ and $HgCl_2$ in a dose-dependent manner. Over 80% of the metals could be removed by 10 mM DMPS in 4 hrs of incubation. DMPS routinely removed more metal than DMSA. Even the lowest concentration of chelator (0.5 mM DMPS) removed 60% of the $HgCl_2$ from the renal slice, as compared to slices not exposed to chelators. The chelators were equally effective with either human or rabbit renal slices. The results of these experiments indicate that human and rabbit renal cortical slices are efficient models to evaluate heavy metal chelators as potential therapeutic agents. (NIEHS P42-ES-04940 and RO1-ES-05790).

1659 CHELATING AGENTS IN THE TREATMENT OF EXPERIMENTAL MANGANESE INTOXICATION

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The effects of seven structurally different metal chelators in the treatment of semichronic manganese (Mn) intoxication were assessed in male Swiss mice. Groups of 15 animals received daily s.c. injections of Mn (II) chloride tetrahydrate (32 mg/kg) for four weeks. Forty-eight hr after the last injection, chelation therapy was initiated and continued for 5 days. Mice received daily i.p. injections of 0.9% saline (control group), or cyclohexanediaminetetraacetic acid (CDTA), ethylenglycol-bis-(alfa-aminoethylether)-N,N'-tetraacetic acid (EGTA), N-(2-hydroxyethyl) ethylendiamine triacetic acid (HEDTA), isonicotinic acid hydrazine (INH), L-dopa, sodium 4,5-dihydroxy-1,3-benzenedisulfonate (Tiron) and p-aminosalicylic acid (PAS) at doses approximately equal to 1/8 of their respective LD_{50} values. Animals were housed in metabolic cages, and urine and feces were daily collected for 5 days. After this period, the animals were killed and tissues removed. Although none of the chelators increased the fecal Mn excretion, CDTA, EGTA, and HEDTA significantly enhanced the excretion of Mn into urine. Tiron, INH, and EGTA had no beneficial effect on tissue Mn concentrations, whereas PAS, HEDTA, and L-Dopa were only partially effective in reducing the tissue levels of Mn. In contrast, CDTA caused a significant decrease in the concentration of Mn in bone, brain, kidney and liver, the main target tissues of Mn accumulation. CDTA was the most effective chelator of those studied in the removal of Mn in Mn-loaded mice.

1660 RESPONSES OF LACTATING COWS TO MESO-2-3-DI-MERCAPTO SUCCINIC ACID (DMSA) USED TO INCREASE CLEARANCE OF HEAVY METALS

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DMSA has shown promise as a chelator to reduce burdens of metals in humans. Neither efficacy nor safety of this therapeutic agent has been demonstrated for cattle. Four lactating Jersey cows (521 ± 12 kg) were given 2 iv doses at 24 hr intervals of 0, 5, 15, or 45 mg/kg BW of DMSA in 400 ml of 5% $NaHCO^3$. Blood, urine, and milk were collected to determine changes in heavy metals. Unanticipated clinical signs of toxicosis, anorexia and depression, were evident within 24 hr of the first 15 and 45 mg/kg DMSA doses. For the 0, 5, 15, and 45 mg/kg treatments, urine production decreased by 22, 47, 74, and 85%, respectively, during the second 24 hr. By 72 hr, daily milk production decreased 18, 45, 100, and 100% from the mean of the 7 day predose period. Peak blood creatinine/BUN (mg/dl) was 0.8/9.0, 1.0/11.0, 15.7/192.0, and 10.2/127.0, respectively. The 15 mg/kg treated animal was moribund 9 days after the first dose. Necropsy revealed severe acute renal tubular necrosis with protein and cellular casts. The cow dosed with 45 mg/kg was recovering by day 15, with blood creatinine and BUN approaching normal and limited production of milk returning. Kidneys of cattle appear to be a target organ for DMSA and the threshold of toxicity should be determined.

1661 ROLE OF CALCIUM AND PHOSPHOLIPASE IN POTASSIUM ANTIMONYL TARTRATE-INDUCED CARDIAC MYOCYTE TOXICITY

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Exposure of rat cardiac myocytes to potassium antimonyl tartrate (PAT) has been shown to cause oxidative stress and toxicity. This study investigates the roles of intracellular calcium (Ca^{2+}i) and phospholipase (PLPase) activation in PAT-induced toxicity. Myocytes isolated from rat neonates were cultured on plastic culture dishes or glass coverslips and were used after 2–3 days in culture. Toxicity and lipid peroxidation were assessed by release of lactate dehydrogenase (LDH) and thiobarbituric acid reactive substances (TBARS), respectively. Ca^{2+}i was monitored on an inverted phase contrast microscope with the fluorescent probe fura-2. ATP content was measured by extraction and HPLC analysis. Exposure to 200 μM PAT for 4 hrs resulted in a 50–60% release of LDH (% of total) and a 10-fold increase in TBARS release. In individual fura-2 loaded myocytes, 200 μM PAT increased Ca^{2+}i by approximately 75% by 3 hours, whereas, exposure to 500 μM PAT resulted in Ca^{2+}i overload (greater than 10-fold increase). Preloading of myocytes with the Ca^{2+} chelator BAPTA (10 μM) prevented the effects of PAT on LDH and TBARS release, but PAT exposure in calcium-free/EGTA medium did not prevent toxicity, suggesting release from intracellular Ca^{2+} stores. Co-exposure to the PLPase inhibitor mepacrine also prevented PAT toxicity. After 6 hrs exposure to PAT (200 μM) in the presence of mepacrine (50 μM) LDH release was still prevented, but myocytes were hypercontracted, suggesting an elevated Ca^{2+}i. PAT-induced ATP loss was partially prevented by BAPTA and mepacrine. These results link elevated Ca^{2+}i and activation of PLPase with PAT-induced myocyte cell death and suggest that increased Ca^{2+}i precedes phospholipase activation.

1662 EFFECTS OF ANTIMONY ON MITOCHONDRIAL FUNCTION AND PROTEIN THIOL AND ADENINE NUCLEOTIDE STATUS IN CULTURED CARDIAC MYOCYTES

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Antimony and antimony containing compounds are used in the manufacture of paints, pigments, ceramics, pyrotechnics, fire retardants and glass. Exposure to antimony containing compounds has been associated with cardiac toxicity in man and experimental animals. In a previous study in our laboratory, we demonstrated that potassium antimonyl tartrate (PAT) depletes cellular glutathione and induces oxidative stress and toxicity in rat cardiac myocytes. In the present study, cardiac myocytes isolated from neonatal rats and cultured for two days were used to further examine the mechanism of PAT-induced

toxicity. Exposure to 100 μM PAT for 4 hrs reduced the mitochondrial membrane potential by about 30% as measured by rhodamine 123 retention. The effects of PAT on cellular ATP levels were assessed by measuring total adenine nucleotides by HPLC with uv detection. PAT produced a concentration-dependent reduction in ATP levels. Concentrations of 50 and 100 μM PAT reduced cellular ATP levels by 20% and 70% respectively after 4 hrs. However, ATP was not depleted after a 2 hr exposure to these concentrations of PAT. PAT-induced losses of ATP could not be accounted for by reciprocal increases in either ADP or AMP concentrations. The effects of PAT on protein thiol levels were examined by reacting cellular proteins with the fluorescent thiol labeling probe monobromobimane. At a concentration of 100 μM, PAT produced a 40% reduction in protein thiols after 4 hrs. A significant reduction (15%) in protein thiols was also seen following a 4 hr exposure to 50 μM PAT. These results suggest that PAT has deleterious effects on mitochondrial integrity, cellular thiol status and the energy status of the cell.

1663 EFFECTS OF ALUMINUM INGESTION ON BEHAVIOR IN YOUNG, ADULT, AND OLD RATS

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Both aluminum (Al) and aging have been associated with neurobehavioral changes in mammals. In the present study, the effects of prolonged Al exposure on behavior were assessed in young (21 day old), adult (8 months), and old (16 months) male Sprague-Dawley rats. Each age group received Al nitrate nonahydrate in drinking water at doses of 0, 50, or 100 mg Al/kg/day for 6.5 months. During that period, citric acid (0, 355, or 710 mg/kg/day) was also added to drinking water. Solution concentrations were prepared daily to adjust the dose to achieve a constant intake by taking into account the differences in body weight and fluid intake. Behavioral testing consisted of assessments of horizontal and vertical activity in an open-field (OP) and passive-avoidance conditioning (PA). There were no Al effects on OF behavior in any age group. Also, there were no significant differences among dose groups in PA test latencies in the young rats. Irrespective of Al treatment, adult and old animals showed low PA conditioning. In conclusion, Al ingestion at levels of 50 and 100 mg/kg/day would not influence neurobehavioral function as indexed by OF activity in young, adult or old rats and by PA conditioning in young rats.

1664 DIFFERENTIAL TOXICITY OF TRIMETHYLTIN IN FOUR INBRED STRAINS OF MICE

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Trimethyltin (TMT) is a relatively selective neurotoxin that affects primarily the granule cells of the hippocampal fascia dentata in mice, resulting in performance deficits in various behavioral tasks used to assess learning and memory. In addition to this selective effect, TMT also causes systemic toxicity which is manifested by malaise, cessation of eating and drinking, and death in severe cases. Four-month-old male mice from four inbred strains, AKR/J, BALB/cByJ, C57BL/6J and DBA/2J, were compared for their relative sensitivities to the toxic effects of TMT following a single i.p. injection of TMT chloride at 1.5, 1.8, 2.0, 2.3, 2.5, 2.7 and 3.0 mg/kg. The systemic toxicity of TMT was assessed using body weight changes, clinical signs and mortality. Behavioral effects were assessed through Morris water maze swim performance and passive avoidance test. Neurohistopathological effects were determined through silver staining for neurodegeneration. TMT produced a dose- and strain-dependent decrease in body weight, ranking in severity as follows: AKR/J > BALB/cByJ > (C57BL/6J = DBA/2J). Clinical signs were most severe in AKR/J mice and included hyperexcitability, tremors and tonic-clonic convulsions. The lethal dose of TMT was 3.0 mg/kg. The order of death was: C57BL/6J (< 24 h), AKR/J and DBA/2J (24–48 h) and BALB/cByJ (> 48 h). No behavioral or neurohistopathological changes due to TMT were seen in any strain at the dose levels tested, perhaps because the mice were too young to bring the neurotoxic range down into the sublethal systemic range. Toxicokinetic evaluation of TMT at 2.3 mg/kg revealed that blood levels of TMT were significantly lower ($p < 0.001$) and volume of distribution greater ($p < 0.0001$) in C57BL/6J mice than other strains.

1665 ERG CHANGES IN RATS EXPOSED TO TRIMETHYLTIN

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Trimethyltin (TMT) is an organotin known to be neurotoxic to many structures in the central nervous system. We measured electroretinograph (ERG) changes in male and female Sprague Dawley rats administered vehicle, 3, 6, or 9 mg/kg of TMT chloride as a single dose via oral gavage. ERG changes were assessed using the UTAS 2000 ERG measurement system. Needle electrodes were placed at the lateral canthus and in the sclera near the limbus of both eyes. A protocol was developed to measure a-wave, b-wave, c-wave, oscillatory and flicker potentials. Amplitudes were generally lower in the left eyes compared to the right eyes. Effects of treatment were more pronounced in the right eyes compared to the left. A-wave amplitudes were significantly lower than vehicle controls in rats exposed to 9 mg/kg TMT (−139.2 +/− 61.6 vs. −67.6 +/− 35.9 mV, control vs. treated, mean & sd). The amplitude of the second oscillatory potential was also lower in the high dose group (75.7 +/− 31.6 vs. 32.6 +/− 10.8 mV). The amplitudes of flicker potentials measured at 20, 25 and 30 Hz were all lower in the high dose group compared to vehicle controls. None of the measured endpoints in the 3 and 6 mg/kg dose groups were significantly different from vehicle controls. These data indicate that rats receiving a single dose of 9 mg/kg of TMT show measurable changes in ERG amplitudes 6 weeks after exposure. Changes in sensory systems should be considered when assessing the effects of TMT on cognitive or behavioral tests that rely on visual cues.

1666 NEUROTOXICITY OF INTRATHECAL MANGANESE^{2+} IN THE RAT

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We investigated the inhibition of motor activity in young male Sprague-Dawley rats (225 g) and the time course of Mn accumulation and dopamine depletion after the intrathecal administration of $MnCl_2$. One day after the intrathecal administration of 1 or 2 mg Mn^{2+}/kg, motor activity (n = 5 rats) was decreased to 10.6% or 8.9% of pre-manganese activity, respectively. Control animals (equimolar Cl-, n = 5 rats) were unaffected. Mn levels in the caudate-putamen, ventral masencephalon, occipital pole, frontal cortex and cerebellum were determined at 3, 6, 12, 24, 48, 72 and 120 hours after intrathecal Mn2+ (250 μg Mn/rat as $MnCl_2$, n = 4 rats) by graphite furnace atomic absorption. Six hours post-intrathecal Mn, the ventral mesencephalon had the largest mount of Mn (59.3 μg Mn/g); the next largest amount was in the occipital pole (12.1 μg Mn/g). The caudate-putamen gradually accumulated Mn with a maximum level at 120 hours post-injection (6.1 μg Mn/g). Dopamine, DOPAC, HVA and serotonin levels in the caudate-putamen and ventral mesencephalon were determined by HPLC-ECD at 3, 6, 12, 24 and 120 hours after intrathecal Mn^{2+} (n = 3 rats). Dopamine levels in the caudate-putamen at 6 and 120 hours were 52% and 41% of controls, respectively. Dopamine, DOPAC and HVA were not different from controls in the ventral mesencephalon. Serotonin levels in the caudate-putamen and the ventral mesencephalon were unaffected by intrathecal Mn^{2+}. (Supported in part by Superfund Basic Science Grant NIEHS #ES-04940.)

1667 CHROMIUM INDUCES A PERSISTENT ACTIVATION OF MITOGEN-ACTIVATED PROTEIN KINASE (MAPK) BY A PROTEIN KINASE C-INDEPENDENT MECHANISM

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Chromium is an important industrial metal and an environmental pollutant. Although the hexavalent form of chromium [Cr(VI)] is well established as a carcinogen, the paucity of epidemiological data involving human exposure has made it difficult to determine the manner by which this element acts to induce and/or promote the growth of tumors. The ability of this metal to cause various types of DNA lesions has supported the notion that chromium functions as an initiating agent in carcinogenic mechanisms. However, it is possible that some of the epigenetic effects of this element contribute to the induction of human neoplasms. We have found that Cr(VI) induces the tyrosine phosphorylation of several cellular proteins in rat hepatoma cells and alters the pattern of phosphoproteins produced in response to various growth factors. Furthermore, Cr(VI) induces the phosphorylation and (persistent) activation of MAPK, a key regulatory kinase in numerous growth factor signalling cascades. Many of the Cr(VI)-dependent effects were also observed in cells treated with the protein kinase C (PKC) agonist, phorbol 12-myristate 13-acetate (PMA). However, the bioeffects of Cr(VI) were determined to be independent of PKC activity since Cr(VI)-dependent phosphorylations/MAPK activation were observed in PKC-depleted cells. The ability of Cr(VI) to influence phosphoprotein production and MAPK activity in this manner suggests a mechanisms of

chromium-induced carcinogenesis which involves the persistent stimulation of growth regulatory pathways. (Supported by DE&RF (#B11/I-93) & NJCCR (#690-043) to E.J.Y.).

1668 EFFECTS OF ANTIOXIDANTS ON CHROMIUM(VI)-INDUCED ROS PRODUCTION IN A549 HUMAN LUNG CELLS

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The production of reactive oxygen species (ROS) during the intracellular reduction of Cr(VI) was studied in A549 cells using flow cytometry analysis. Cr(VI) produced dose-related ROS with addition of $K_2Cr_2O_7$ (20 and 100 uM) in A549 cells. Cr(III) as $CrCl_3$ (200 uM) caused no increase in ROS. Chromium picolinate (200 uM) caused fewer ROS than controls. Cells preincubated with 200 uM vitamin C (C) (20 hr) followed by $K_2Cr_2O_7$ (100 uM) treatment produced fewer ROS than $K_2Cr_2O_7$-treated cells. Vitamin E (20 uM) preincubation (20 hr) caused no changes in ROS with $K_2Cr_2O_7$ (100 uM). However, there is increased production of ROS when C is added only 60 min prior to the Cr(VI) addition to the cells. Catalase (1000 U/ml) and/or superoxide dismutase (100 U/ml) preincubation (90 min) did not cause any change in ROS production caused by $K_2Cr_2O_7$ (100 uM) treatment. $K_2Cr_2O_7$ treatment (1, 5, 10 and 20 uM) for 24 hr caused S-phase cell cycle specific changes in DNA. The effect was maximal at 10 uM.

1669 SEX AND ORGAN SPECIFIC EFFECTS OF HEXAVALENT CHROMIUM (Cr(VI)) ON CYTOCHROME P450 (P450) ISOZYMES

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Recent studies have shown that Cr(VI), a putative carcinogen, affects the mixed function oxidase system. These studies characterize the effects of Cr(VI) on hepatic and pulmonary P450 isozymes from male and female Sprague-Dawley rats. Microsomal fractions were isolated from rats after IP injection with 130 μmoles Cr(VI)/kg/48 hours as $K_2Cr_2O_7$ (pH 7.4) for 12 days. Control animals received saline. P450 isozyme catalytic activities were determined using the *in vitro* metabolism of testosterone to its hydroxylated metabolites. The levels of P450 isozymes were measured by Western blot analysis using monoclonal antibodies (values = % of control). In male rats, Cr(VI) induced pulmonary CYP2B1 (191 ± 9%), whereas, in the liver it decreased CYP2C11 (31 ± 4%) and CYP3A2 (60 ± 9%) levels. In female rats, induction was seen in pulmonary CYP2B1 (179 ± 4%), as well as hepatic CYP2B1 (180 ± 13%) and CYP3A2 (351 ± 20%). In all tissues examined, the changes in isozyme levels were correlated with similar changes in catalytic activities. (NIEHS Training Grant ES07148, ECF; R01-GM44982, PET).

1670 DIFFERENTIAL EFFECTS OF GOLD THIOMALATE IN LIVERS AND KIDNEYS OF RATS AND MICE

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Gold thiomalate (AuTM) is used chronically in the treatment of rheumatoid arthritis with variable response between individuals and is often associated with transient proteinuria. In these studies, the effect of AuTM on livers and kidneys of rats and mice were examined. Benzo(a)pyrene hydroxylase activity was significantly decreased in kidneys of AuTM (100 mg/kg)-treated rats whereas no significant change was observed in the kidneys of Swiss-Webster mice treated with the same dose of AuTM. Blood urea nitrogen, an indicator of kidney dysfunction, was significantly increased in AuTM-treated rats whereas no significant difference was observed in AuTM-treated mice. Liver benzo(a)pyrene hydroxylase activity was not significantly altered in rats or mice treated with AuTM. Both liver and kidney metallothioneins were induced significantly in AuTM-treated rats and mice. Since susceptibility to metal toxicity differs among inbred strains of mice, two strains of mice were examined for AuTM toxicity. C3H/HeJ and DBA/2J inbred strains have been shown previously to be sensitive and resistant to cadmium-induced hepatotoxicity, respectively. However, AuTM did not cause any significant change in the benzo(a)pyrene hydroxylase activity in the kidneys of either strains of mice. These data indicate AuTM, a nephrotoxic agent in rats, showed no nephrotoxic effects in the mice strains studied here.

1671 ROLE OF MONOCYTE-DERIVED CYTOKINES IN THE INDUCTION OF α1-ACID GLYCOPROTEIN-2 GENE EXPRESSION BY HEAVY METALS

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α1-Acid glycoprotein-2 (AGP-2) is a liver specific, acute-phase gene which is highly inducible by bacterial lippopolysaccharride (LPS) and heavy metals. The induction of AGP-2 by LPS involves the stimulation of monocytes to produce cytokines (primarily IL-6 and TNFα) which act at the liver to induce transcription of the AGP-2 gene. This research was conducted to investigate the possibility that heavy metals induce AGP-2 gene expression via a similar mechanism. Treatment of cultured human monocytes with 20 μg/ml LPS resulted in the secretion of IL-6 (2313 ± 20 pg/ml) and TNFα (482 ± 41 pg/ml) into the media. However, treatment of monocytes with 1 μM $HgCl_2$, $CdCl_2$, or $ZnCl_2$ resulted in no statistical change in secreted IL-6 or TNFα. Human hepatoma (Hep G2) cells were transfected with a plasmid, p(−532)AGP2LUC, containing the firefly luciferase gene under the control of the AGP-2 proximal promoter. Treatment of these Hep G2 cells with media from $HgCl_2$-, $CdCl_2$-, or $ZnCl_2$-conditioned monocyte cultures resulted in no transient luciferase activity. In contrast, treatment of the transfected Hep G2 cells with LPS-conditioned monocyte media produced a 23-fold induction of luciferase activity. In addition, injection of Balb/c mice intraperitoneally with 50 μg/kg LPS resulted in a 33- and 78-fold induction of serum IL-6 and TNFα levels, respectively. However, injection of 0.5 mg/kg $HgCl_2$, $CdCl_2$, or $ZnCl_2$ resulted in no statistical change in circulating IL-6 or TNFα levels. Results from these experiments indicate that the induction of AGP-2 gene expression by heavy metals is not mediated by monocyte-derived cytokines. (Supported by DHHS-5T32ES07254-04 and the Shriners Burn Institute, Galveston, TX).

1672 DIETARY IRON LOADING GENERATES HYDROXYL RADICAL IN RATS: AN ESR SPIN TRAPPING INVESTIGATION

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Although iron is an essential nutrient, the pathological processes associated with various forms of iron overload demonstrate that the metal can also be toxic. We have already provided ESR evidence for the generation of hydroxyl radical during acute iron poisoning. In this study we investigated the possibility of hydroxyl radical generation by chronic dietary iron loading. A secondary-radical spin-trapping technique was employed where hydroxyl radical forms methyl radical upon reaction with DMSO. The methyl radical adduct was then detected by ESR spectroscopy as its adduct with the spin trap phenyl-*N-t*-butylnitrone (PBN). This adduct was detected in the bile of rats 10 weeks after being on an iron-loading diet and 40 minutes after the injection of the spin trap PBN dissolved in DMSO. Bile samples were collected into solution of the ferrous stabilizing chelator dipyridyl in order to prevent the generation of radical adducts *ex vivo* during bile collection. The analysis of radical adducts excreted *via* the biliary route provides ESR evidence for the generation of the hydroxyl radicals during chronic iron-loading intake. No radical adducts were detected in rats fed the control diet for the same period of time. This is the first report of hydroxyl radical generation detected by ESR in by chronic iron-loaded rats.

1673 THE ROLE OF FERROUS IRON IN DUST-INDUCED CARCINOGENESIS

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Increasing evidence demonstrates that an excess of lung cancer and stomach cancer has been observed in iron ore and steel factories workers. It has been shown that the incidence of cancer is related to the exposure to mineral dusts. Moreover, many studies have shown that the iron content of the asbestos fibers has an important role in the generation of reactive oxygen species (ROS) and in the induction of mesothelioma. In the present study, we found that $FeSO_4$ in aqueous solution and aged pyrite (FeS_2) which contained a coating of $FeSO_4$ produced ROS following interaction with O_2, as detected by the spin trapping agent DMPO with ESR. We have also found that $FeSO_4$ in solution is toxic to Syrian hamster embryo (SHE) cells, but not transforming. Aged pyrite with a coating of $FeSO_4$ was not very toxic, but transformed SHE cells. These results

suggest that very little Fe^{2+} ions can get into cells and ROS resulting from interaction of water soluble $FeSO_4$ and O_2 may damage cell membrane which causes cell toxicity. The aged pyrite which was phagocytized and subsequently released Fe^{2+} in SHE cells induced transformation of cells. Therefore, the role of reactive Fe^{2+} in airborn dusts is a parameter which is worth considering in order to predict which dusts will lead to a higher incidence of cancer. The mechanism of Fe^{2+}-containing dust-induced cell transformation is being studied.

1674 MEASUREMENT OF URANIUM IN BONE USING AN X-RAY FLUORESCENCE TECHNIQUE

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Transuranic elements such as those present in depleted Uranium (U) can pose a serious health hazard. Current approaches to the measurement of U include evaluation of its concentration in urine and whole body counting. Since U is accumulated in bone and there are both theoretical and practical problems associated with measurement of daughter products there is need for more accurate assessments of U exposure. The objective of this study was to measure U by an X-ray fluorescence (XRF) technique *in situ*. We excited the K shell electrons in U which have a binding energy of 115.6 KeV with 122 and 136 KeV gamma rays from a Co-57 source. A liquid nitrogen cooled intrinsic Ge detector was employed to measure the characteristic K fluorescence from the U as well as coherently scattered gamma rays. The quantity of U-238 in the bone was determined from the number of K fluorescence events extracted from the measured scattered photon spectrum. The bone mineral content was estimated by measuring the number of coherently scattered gamma rays. In this way we were able to relate the U concentration to the bone mass. Using this procedure it was possible to measure micromolar concentrations of U with high precision and reproducibility. This new technique overcomes limitations that are commonly seen with both urine analysis and whole body counting.

1675 TISSUE REPAIR AND INJURY AS A RESPONSE TO THIOACETAMIDE HEPATOTOXICITY: A NOVEL DOSE-RESPONSE RELATIONSHIP

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Stimulated cell division and tissue repair are known to a play a pivotal role in the final outcome of toxicity. The objective of this study was to measure tissue repair and injury as simultaneous but opposing biological responses to a model hepatotoxicant in a dose-response paradigm. Male S-D rats were treated with a 12-fold dose-range of thioacetamide (TA, 50, 150, 300 and 600 mg/kg). Serum ALT elevation and liver histopathology were used as markers of injury while ^{3}H-T incorporation and proliferating cell nuclear antigen (PCNA) study were used as markers of cell division and tissue repair. All responses were measured over a time course of 0–96 hr. Significant ALT elevation was noted after 48 hr only in the group receiving 600 mg/kg. Tissue repair indicated by ^{3}H-T incorporation peaked at 36 hr after administration of a low dose of TA (50 mg/kg). With increasing doses a greater but delayed stimulation of cell division was observed till a threshold was reached (300 mg/kg). Above this threshold (600 mg/kg), tissue repair was both delayed and attenuated leading to animal death. Therefore, timely and adequate tissue repair response restrained the progression of injury in a 6-fold dose-range (50, 150, and 300 mg/kg) while too little and too late repair response led to an unrestrained progression of injury in the group receiving 600 mg/kg. If tissue repair is a critical determinant of the final outcome, then intervention with this process should result in lethality even from non-lethal doses. Antimitotic administration of colchicine (CLC) to inhibit cell division in the 150 and 300 mg/kg treated groups led to 60% and 100% lethality, respectively. Thus, intervention with the repair response resulted in lethality with ordinarily non-lethal doses. These findings underscore the pivotal role of tissue repair as the determinant of the ultimate outcome of liver toxicity. Measuring both tissue repair and injury might increase the usefulness of dose-response paradigms in predictive toxicology. (Supported by The Burroughs Wellcome Fund).

1676 STRAIN DIFFERENCES IN TISSUE REPAIR RESPONSE TO A TOXIC INSULT BY 1,2-DICHLOROBENZENE

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Fischer 344 (F344) rats are reportedly 75-fold more sensitive than Sprague Dawley rats (S-D) to 1,2-dichlorobenzene (o-DCB) hepatotoxicity. However, no information is available regarding the ultimate consequence of this sensitivity on animal survival. Lethality studies were performed in the 2 strains. LD50 values in male rats for o-DCB were 1.66 ml/kg (1.18–2.355 ml/kg: 95% C.I.) in F344 and 1.76 ml/kg (0.856–3.652 ml/kg: 95% C.I.) in S-D rats. Previous studies have shown the importance of tissue repair which occurs as an endogenous response to injury leading to animal survival. Therefore, the objective of this study was to investigate if increased cell division and tissue repair underlie the observed recovery of F344 rats despite higher liver injury. Age-matched male S-D rats (250–300 g; 8/9 weeks old) and male F344 rats (160–190 g; 8/9 weeks old) were used in the study. A dose response study was performed in which injury and tissue repair occurring as two dynamic but opposing events were measured over a time course (0–96 hr). Liver injury was studied by measuring plasma ALT and SDH levels and liver function was evaluated by measuring plasma bilirubin levels. Higher plasma ALT elevations were observed in F344 rats around 36–48 hr. Plasma glucose and hepatic glycogen were used as a measure of energy status of the liver. Greater depletion of hepatic glycogen was seen in F344 rats which returned to normal by 96 hr. Liver regeneration was observed by measuring ^{3}H-thymidine incorporation into hepatonuclear DNA. Increased incorporation of ^{3}H-thymidine was seen in the F344 rats around 36–48 hr signifying an increase in S-phase synthesis. This differential in tissue repair may play a vital role in equalizing the ultimate outcome of toxicity in the two strains of rats. (Supported by the Burroughs Wellcome Fund).

1677 RETINOL PROTECTION OF ACUTE CADMIUM CHLORIDE-INDUCED HEPATOTOXICITY IN THE MALE SPRAGUE-DAWLEY RAT

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Cadmium is a toxic heavy metal which is an environmental contaminant found in meats, fishes, and fruits. The major toxicologic effects of cadmium are to the pulmonary and renal systems during chronic exposure, but when injected parenterally, it can cause acute hepatic, testicular, and cardiac injuries. In the liver, cadmium causes primarily damage to the parenchymal hepatocytes. Recently, pretreatment with retinol for 1 or 7 days has been shown to potentiate the hepatotoxicity of carbon tetrachloride in rats. Therefore, the objectives of these experiments were to evaluate the ability of retinol to modulate the liver toxicity associated with intravenous administration of cadmium chloride ($CdCl_2$) using both a 1 and 7 day retinol pretreatment regimen. Male Sprague-Dawley rats were pretreated with *all-trans*-retinol (75 mg/kg/d; po) for 1 or 7 days. Twenty-four hr after the last dose of retinol, $CdCl_2$ (2.5, 3.0, or 4.0 mg/kg; iv) was administered. Rats pretreated with retinol vehicle and given $CdCl_2$ exhibited liver injury as measured by increased plasma ALT activity and liver histology at 24 hr after receiving $CdCl_2$. Histologically, there was mild, diffuse hepatocellular degeneration and necrosis. Retinol pretreatment for 1 day had no effect on $CdCl_2$-induced hepatotoxicity. However, seven days of retinol pretreatment resulted in protection from $CdCl_2$-induced liver damage, significantly decreasing plasma ALT activity and associated liver histopathology. The mechanism(s) by which retinol protects the liver from $CdCl_2$ is unknown. However, current studies are focusing on retinol-induced increases in protective proteins. (Supported in part by NIEHS Center Grant, P30 ES 06694 and ES 06095).

1678 KUPFFER CELL ACTIVATION AFTER TRAUMA

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Kupffer cells (KC) may play an important role in the immune response after injury. This study tested the following hypotheses: 1) femur fracture (FF) activates KC leading to increased hepatic O_2 uptake, 2) KC activation leads to altered responses to lipopolysaccharide (LPS), and 3) KC depletion reduces mortality associated with FF and cecal ligation and puncture (CLP).

Methods: Under Ketamine and Xylazine anesthesia, Sprague-Dawley rats were subjected to closed FF with and without $GdCl_3$ (Gd, 7 mg/kg) pretreatment to deplete KC. Subsequently, livers were perfused for measurement of O_2 uptake or for KC isolation. Isolated KC were stimulated with LPS, and nitric oxide (NO) formation was measured. In other experiments, CBA/J mice were subjected to FF and/or CLP 72h later, with or without Gd pretreatment.

Results: Hepatic O_2 uptake increased 17% and 19%, respectively, 2 h and 48 h after FF. Gd pretreatment reduced O_2 uptake to levels in sham-operated controls. FF also caused a 59% decrease in KC NO formation. In mice, CLP caused 44% mortality, which increased to 60% after CLP plus FF. Gd reduced mortality to 13% and 5%, respectively, after CLP and CLP plus FF.

Conclusion: FF caused activation of KC, which led to increased hepatic O_2 uptake and decreased NO formation after LPS stimulation. KC activation, therefore, may contribute to the increased vulnerability to lethal sepsis seen clinically after trauma.

1679 INHIBITION OF NITRIC OXIDE (NO) SYNTHESIS ENHANCED REPERFUSION INJURY AFTER HEPATIC ISCHEMIA AND ENDOTOXEMIA (I/RP+ET)

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Short periods of hepatic ischemia prime Kupffer cells and neutrophils for reactive oxygen formation and enhance the susceptibility for endotoxin (Liu et al., Circ Shock 43: 9, 1994). Because NO may scavenge oxygen radicals, a potential role of NO in the pathophysiology of I/RP+ET was investigated. Male Fisher rats, subjected to 25 min of hepatic ischemia followed by reperfusion and injection of 0.5 mg/kg *Salmonella enteritidis* endotoxin at 30 min of RP, developed liver injury as indicated by increases in plasma ALT activities from 35 ± 5 U/l before ischemia to 1328 ± 113 at 4 h RP. Treatment with the NO synthase inhibitor nitro-L-arginine methyl ester (30 mg L-NAME/kg, sc), which reduced plasma levels of NO_2 + NO_3 by 44%, significantly increased liver injury (2808 ± 204 U/l). Administration of L-arginine (300 mg/kg) or infusion of SIN-1 (0.5 mg/kg/h) to stimulate NO formation had no effect on basal RP injury, however, partially restored plasma NO_2 + NO_3 conc. and completely reversed the increase of liver injury during L-NAME treatment. Mass spectrometric analysis of 8-, 9-, 11-, and 12-hydroxy eicosatetraenoic acid levels in the liver as index of lipid peroxidation (LPO), showed only a minor increase during RP but no change after L-NAME. It is concluded that inhibition of NO synthesis aggravated liver injury by a mechanism independent of LPO. Lack of NO may enhance microcirculatory failure during I/RP and endotoxemia.

1680 GALACTOSAMINE HEPATOTOXICITY: EFFECT ON NITRIC OXIDE FORMATION

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Galactosamine hepatotoxicity *in vivo* has long been associated with rapid and extensive depletion of hepatic uracil nucleotides (30 min; >90%), with protection from toxicity afforded by uridine. However, the exact mechanism of galactosamine-induced hepatic necrosis is still unknown. We have previously shown that galactosamine is cytotoxic in rat primary hepatocyte cultures, and that this toxicity is associated with rapid loss of hepatocyte UTP, with protection afforded by uridine. Using this rat hepatocyte culture system we examined the effect of galactosamine on nitric oxide production by the hepatocytes. A cytotoxic concentration of galactosamine (4 mM) was added to the medium of cultured hepatocytes at the time of cell attachment Twenty-four hours later the levels of accumulated nitrite in the medium were determined as an estimate of nitric oxide production. Galactosamine addition caused a dramatic decrease (≈90%) in nitric oxide production as compared to control cells (30 μM). The decrease in nitric oxide production was partially (≈30%), but not completely, attenuated by co-addition of 3 mM uridine. This concentration of uridine did protect the cells from galactosamine cytotoxicity. These data suggest that the decrease in hepatocyte nitric oxide production alone does not lead to galactosamine hepatocyte toxicity. The decrease in nitric oxide could, however, in association with other key events (*i.e.* UTP depletion), be contributing to overall damage to the hepatocytes. Supported by NIH grant DK 47185.

1681 STIMULATION OF RAT LIVER NITRIC OXIDE SYNTHASE BY ALPHA-TOCOPHEROL *IN VITRO*

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It has been established that alpha-tocopherol (Vit E), a fat-soluble compound, has potent antioxidant effects in various biological systems. Vit E dietary deficiency in animals is associated with cellular injury in a number of tissues, including the liver. Nitric oxide (NO), a highly reactive molecule, is synthesized by a number of cell types ranging from neurons to hepatocytes and can be produced in large quantities by an inducible NO synthase (NOS) during host defence. The aim of this study was to determine the effects of Vit E on rat liver NOS activity *in vitro*. NOS activity of both the inducible (iNOS) and the constitutive (cNOS) isoforms, was measured in rat liver homogenates by monitoring the enzymatic conversion of [^{3}H] arginine to [^{3}] citrulline. The results demonstrated that Vit. E increased both cNOS and iNOS in a concentration dependent manner. In conclusion: Alpha-tocopherol stimulates both inducible and constitutive NOS of rat liver *in vitro*.

1682 EARLY EFFECT OF DICHLOROACETIC ACID (DCA) ON SPONTANEOUS APOPTOSIS IN THE MALE B6C3F1 MOUSE LIVER

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Dichloroacetic acid (DCA) is a complete hepatocarcinogen and tumor promoter in the male B6C3F1 mouse. DCA is considered non-genotoxic. Thus, the mechanism(s) of its carcinogenicity is unknown. The purpose of this study was to examine the early effect of DCA on spontaneous apoptosis in hepatocytes. Male B6C3F1 mice were given 0, 0.5 or 5.0 g/L DCA in their drinking water for up to 30 days. Groups of animals (5/group) were sacrificed at 5 day intervals. Histologic liver sections were H&E stained and subjected to histopathologic analysis. Apoptosis was evaluated by *in situ* end-labeling analysis in up to 4 histologic sections/animal. This assay employs a standard avidin peroxidase diaminobenzidine (DAB) procedure to detect biotinylated dUTP molecules specifically inserted into apoptotic cells by terminal deoxynucleotidyl transferase. The absolute number of apoptotic (DAB-positive) cells in each section was counted microscopically and total area of tissue was determined by planimetry using a Zeiss IBAS image analysis system. The average number of apoptotic cells/mm^2 tissue was calculated for each group and subjected to statistical analysis by the Student's *t*-test. A *highly significant* time and dose related inhibition of spontaneous apoptosis was found in animals receiving drinking water containing 0.5 or 5.0 g/L DCA for 5, 10, 15 or 30 days. Hepatocyte apoptotic frequencies of 0.84, 0.53, and 0.24 were found after 5 days of treatment in mice receiving 0, 0.5 or 5.0 g/L DCA, respectively, and these frequencies were 0.61, 0.26, and 0.14 after 30 days of treatment. This time and dose related downregulation of apoptosis in animals exposed to DCA suggests that the non-genotoxic hepatocarcinogenicity of this agent may arise in part through a reduced ability to apoptotically clear spontaneously initiated cells. (This abstract does not reflect EPA policy.)

1683 DICHLOROACETIC ACID (DCA) ALTERATIONS OF HEPATIC GLUCOCORTICOID RECEPTOR BINDING ACTIVITY (GR) IN MALE B6C3F1 MICE

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DCA, a chlorine disinfection by-product, increases liver cancer in male B6C3F1 mice. Glucocorticoids exert a significant effect on cellular metabolism, differentiated function and proliferation. Studies examining the effects of DCA treatment on the levels of GR activity were done. Mice were exposed 0.05, 0.5, 1.0, 2.0 and 3.5 g/l DCA in the drinking water for 6, 12, 21, and 31 days. GR activity in the cytosolic and nuclear fractions was measured using equilibrium binding techniques. GR Kd and Bmax were determined for 0.5 g/l or 2 g/l DCA at 28 days. DCA decreased the GR activity in the cytosolic fraction at doses greater than 0.5 g/l DCA. GR activity in the nuclear fraction was increased. Scatchard analysis showed no significant differences in the Kd of the cytosolic GR; a decline in the Bmax with dose was seen. Dot-Blot analysis showed that DCA decreased cytosolic GR protein levels. The dose response for GR correlated with that for liver tumors and suggest that GR might play a role in DCA enhanced liver cancer. (This abstract may not necessarily reflect EPA policy.)

1684 EFFECTS OF DICHLOROACETIC ACID ON CONTROL OF GLYCOGEN METABOLISM IN MALE B6C3F1 MICE

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Dichloroacetate (DCA) is a by-product of chlorination. In mice, DCA has been shown to be a potent hepatocarcinogen. DCA treatment results in large accumulations of glycogen in the liver of B6C3F1 mice. Therefore, an examination of how DCA-treatment alters control of glycogen metabolism has been undertaken. DCA-treatment appears to inhibit glycogen synthase (GS) activity, but does not affect glycogen phosphorylase (GP). However, the normal increase in GS activity seen in fasted animals challenged by glucose was not significantly affected by DCA treatment. Glycogen that accumulates with DCA-treatment becomes increasingly resistant to mobilization by fasting. These data suggest that increases of glycogen, in the liver of DCA treated animals, is not due to changes in its synthesis, but to accumulation in a compartment that is not readily accessible for degradation. (This project was supported by NIEHS grant #R01-ES04648).

1685 INFLUENCE OF CHEMICALS ON HEPATIC UROPORPHYRIA IN IRON LOADED Ah-NONRESPONSIVE SWR MICE

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The development of hepatic uroporphyria in iron loaded mice in response to chemicals has been associated with *Ah*-responsiveness, so that C57BL mice respond whereas those of the DBA/2 strain are resistant. On the other hand, iron loaded Ah-nonresponsive SWR mice eventually develop porphyria without any chemicals, especially given the heme precursor 5-aminolevulinic acid (ALA) in the drinking water (2 mg/ml) for 5 weeks, but DBA/2 still apparently do not. This suggests that genes besides those controlled via the *Ah* locus are involved in the onset of porphyria, perhaps associated with iron metabolism. We determined the influence of inducers of cytochrome P450 isoenzymes on the development of porphyria in iron loaded SWR mice (single dose 600 mg/kg) also given ALA for 4 weeks. Single doses (all 60 mg/kg) of 3-methylcholanthrene and isosafrole were without effect whereas hexachlorobenzene markedly potentiated the response and inhibition of uroporphyrinogen decarboxylase (UD). Four doses of phenobarbital had only a small effect. Three doses of iron decreased the response to ALA. In addition, exposure to ALA without any iron or treatment with chemical eventually also caused UD inhibition and uroporphyria to develop in SWR (after eight weeks) but not in DBA/2 mice. After 96 h uroporphyrin accumulated to a greater extent with isolated SWR hepatocytes exposed to ALA than with DBA/2 hepatocytes. These results illustrate that the *Ah*-nonresponsive strain will respond to HCB and that there are gene variations between SWR and DBA/2 mice which are revealed by the heme precursor ALA and potentiated by iron and polychlorinated aromatic hydrocarbons.

1686 IRON OVERLOAD IN RATS FED ETHANOL AND CARBONYL IRON

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It has been reported that 30–50% of alcoholic individuals have elevated hepatic iron stores. However, the mechanisms behind this iron accumulation and its precise role in mediating alcoholic liver disease remain obscure. In order to study this phenomenon an animal model was developed with male Sprague Dawley rats using a liquid high-fat (35% fat) diet deficient in Vit. E (HFD). Treated animals (EI + HFD) received the HFD with added ethanol (35% ethanol derived calories) and carbonyl iron (0.02%–0.04%). Control animals received the ethanol (E + HFD), carbonyl iron (I + HFD), or HFD without added ethanol or iron (HFD). At 8 weeks, liver non-heme iron, serum non-heme iron, total iron binding capacity (TIBC), alanine aminotransferase (ALT), and glutathione levels were determined. Liver iron stores were highly elevated in EI + HFD animals (1,110 ug Fe/g liver dry weight) compared to normal chow fed rats (295 ug Fe/g), and were also increased in I + HFD and E + HFD fed animals (976 and 920 ug Fe/g respectively). Serum non-heme iron values also followed the same trend with EI + HFD at 86.1 umol Fe/L, followed by E + HFD, I + HFD, HFD, and normal chow with values of 65.1, 60.0, 43.0, and 21.3 umol Fe/L respectively. The TIBC of serum from all animals except untreated controls (HFD and normal chow) was lower than the serum non-heme iron concentration, indicating serum transferrin saturation and potential iron toxicity. Furthermore, ALT values indicated hepatic injury with EI + HFD at 80.1 U/L, then decreasing approximately 2 fold in the following order; I + HFD > E + HFD > HFD. Although no significant glutathione perturbations were observed in whole liver homogenates and sub cellular fractions, the trends observed provide evidence for a synergistic effect between ethanol and iron in causing liver injury, presumably by lipid peroxidation. Equally interesting, is that the iron concentrations in E + HFD animals are very similar to that found in alcoholics, hence, making the model relevant for investigating the association between increased hepatic iron stores and alcoholism. (Supported by a supplement to NIH/AA9300).

1687 BINDING OF HEPATIC FATTY ACID BINDING PROTEIN TO A 33 kDa NUCLEAR PROTEIN

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Fatty acid binding protein (FABP) is a 14.3 kDa cytosolic protein that is thought to play a role in lipid transport between the various compartments of the cell. It binds many hydrophobic molecules including long chain fatty acids, cholesterol, and peroxisome proliferators. Recently, a role for it has been hypothesized in the regulation of gene expression and cellular growth. To further evaluate its cellular function, interactions of FABP with other proteins were analyzed by far-western blotting. We have identified a 33 kDa protein in rat hepatocytes that binds strongly to biotinylated FABP. This FABP associated protein (FAP) was isolated from rat liver nuclei by a 0.5 M NaCl extraction. Treatment with high NaCl concentrations or Triton X100 did not affect the assocation significantly. Also, pretreatment of FABP with heat had no effect on the FABP:FAP interaction. Hydroxylamine treatment to cleave any ester linked acyl-modifications of the protein did not influence the interaction, suggesting that FABP does not bind to a ester-linked fatty acid modification of the FAP. The addition of SDS prevented the FABP:FAP interaction. When an excess of unlabelled FABP was present, the binding signal was decreased by approximately 90%, suggesting a specific interaction. Sequence analysis is now underway to determine the identity of this protein, which will improve our understanding of the significance of this interaction. Also, the affect of fatty acids and peroxisome proliferators on the interaction is actively being investigated to determine the role FABP may have in fatty acid/peroxisome proliferator signal transduction from the cytosol to the nucleus.

1688 HEPATIC FATTY ACID BINDING PROTEIN FACILITATED NUCLEAR TRANSPORT OF FATTY ACIDS

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Long-chain fatty acids are known to modulate gene expression, presumably by interacting with receptors in the nucleus. Fatty acid binding protein (FABP) is a cytosolic protein which is thought to solubilize fatty acids in the intracellular aqueous environment. This protein is found in lipid responsive cells and has been shown to transport fatty acids to microsomes and mitochondria. We hypothesize that the transport of fatty acids to their nuclear receptors is mediated by FABP. We found that ^{3}H-palmitate complexed to FABP associated with rat liver nuclei in a concentration-dependent manner. Free ^{3}H-palmitate did not bind nuclei with specificity. The ^{3}H-palmitate:FABP association was linear with respect to DNA in the sample. This association was inhibited with an excess of unlabelled palmitate:FABP, but not by palmitate complexed to bovine serum albumin (BSA). Fatty acid-free BSA decreased the nuclear binding of FABP complexed ^{3}H-palmitate, probably due to the ability of the fatty acid to exchange from FABP to BSA. The association of ^{3}H-palmitate:FABP with nuclei was independent of temperature, suggesting a binding phenomenon and not an uptake process. Whether or not the ^{3}H-palmitate bound to the nuclei remains complexed to FABP has not yet been determined. We conclude that FABP delivers fatty acids to rat liver nuclei in a specific manner and may, therefore, be involved in fatty acid signal transduction between the cytosol and nucleus.

1689 SUBACUTE 2,6-DI-t-BUTYL-4-NITROPHENOL (DBNP) EFFECTS ON THE RAT LIVER FATTY ACID BINDING PROTEIN AND BILE SALT/DOPAMINE SULFOTRANSFERASES

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DBNP, an atmospheric contaminant in Navy submarines, has been shown in a previous study to be toxic to mammalian species. The cumulative subacute toxicity (60 days of daily, i.p. injection) from DBNP occurs in liver, kidney, heart and spleen. Particularly significant are fatty liver, renal dysfunction and myocardial degeneration. To study the basis for these phenomena, we explored (for 33–58 days) subacute DBNP effects in male Fischer 344 rats on the liver fatty acid binding protein (FABP), bile salt (BA), and dopamine (D) sulfotransferase (ST) enzymes. Alteration of FABP and BAST levels could cause fatty liver, DST level alteration was seen as capable of changing catecholamine metabolism and cardiovascular function. We observed the following average changes in levels of FABP, BAST, and DST/g liver, FABP, −40%; BAST, 11%; DST, 17%, Effects per 100 g BW were FABP, −23%; BAST, 70%; DST, 31%. Altered levels of FABP/g liver, BAST and DST per 100 g BW were statistically significant. These data are consistent with alterations of the test parameters that participate in production of fatty liver and alter cardiovascular function. Specific DST and BAST enzyme levels were altered. The data support additional study.

1690 HEPATOBILIARY DYSFUNCTION IN RATS CHRONICALLY EXPOSED TO A STEROIDAL ANTIANDROGEN

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Oral exposure of male, orchiectomized and female Sprague-Dawley rats to 400 mg/kg/d of Zanoterone (Z, a steroidal antiandrogen with 17αethynil and 17β-hydroxyl moieties) for 3 months resulted in multifocal deposits of pigments (Hall's stain positive) in the lumen of hepatic biliary ducts and surrounding macrophages, leading to biliary epithelium degeneration, chronic inflammation and bile duct proliferation. Hepatocellular canaliculi were not affected. The lesion was only observed in male and orchiectomized rats. Bile flow of male, orchiectomized and female rats treated with Z was higher (2-fold) than for controls while biliary concentration of bile acids, but not cholesterol, was lower (2-fold). Sulfobromophtalein clearance was increased for males only. After 3 months of recovery, pigment deposits accompanied by bile duct proliferation were still observed while functional parameters returned to control values. The biliary composition of Z metabolites (hydroxylated derivatives and glucuronoconjugates) measured at the end of the 3-month exposure period differed between male (orchiectomized or not) and female rats. The deposits observed in portal areas may represent precipitates of Z metabolites secondary stained with biliary pigments.

1691 LOW DOSE METHYLENE DIANILINE SELECTIVELY INJURES BILIARY EPITHELIAL CELLS (BEC)

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Our objective was to develop a model which causes biliary epithelial cell (BEC) injury without evident injury to hepatocytes. We examined methylene dianiline (DAPM) at a dose of 25 mg/kg which is below the reported threshold of hepatic damage (Bailie et al., Envir. Health Persp. 101:130, 1993). Male SD rats (350 g) had biliary cannulas implanted under pentobarbital anesthesia. Control bile was collected for 1 hr; DAPM was given po in 35% ethanol; vehicle was given to controls. Bile was collected for 6 hr; then serum parameters, liver and bile ducts were evaluated. In bile, elevations of glucose and inorganic phosphate began in hr 3, were 13- and 30-fold, respectively, higher by hr 4, and remained stable from hrs 4 to 6. Protein increased in hr 1 but decreased to basal levels by hr 4. SDS-Page indicated that only certain proteins were elevated in hr 1. Biliary bilirubin, bile salt and electrolytes were not altered while bile flow was transiently elevated in hr 1. This dose of DAPM had no effect on serum indices of liver injury at 6 hr. Histology showed necrosis and exfoliation of BEC in the largest bile ducts with the effects of DAPM diminishing as bile duct size decreased; hepatocytes consistently appeared normal. The stable 2 to 3 hr elevation of biliary glucose, a constituent normally removed from bile by BEC, indicates BEC impairment. These results demonstrate the utility of low dose DAPM as a model to investigate BEC functions in bile formation.

1692 LYSOSOMAL AND CANALICULAR MEMBRANE ENZYME ACTIVITIES INCREASE IN BILE OF RATS TREATED WITH 1,1-DICHLOROETHYLENE (DCE)

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Background: DCE (50 mg/kg po) causes selective damage to the bile canalicular membrane of centrilobular hepatocytes within two hours. Ultrastructural changes include dilation of the canalicular lumen and increased numbers of lysosomes in the pericanalicular region. Also at two hours, total protein in bile is diminished by 40%, which could reflect impairment of canalicular transport functions. *Objective:* Assess the early effects of DCE on the biliary excretion of proteins of lysosomal and canalicular membrane origin in freely moving male Sprague-Dawley rats equipped with permanent biliary, duodenal, and jugular cannulas. *Results:* The biliary activity of lysosomal enzymes β-glucuronidase and N-acetyl-β-D-glucosaminidase transiently increased to 500% basal by approximately 40 minutes after DCE treatment and subsequently decreased to 25% basal levels by 3 hours after DCE. In contrast, biliary excretion of canalicular membrane enzymes leucine aminopeptidase and 5'-nucleotidase progressively increased to 8–10-fold basal levels by approximately three hours after DCE. DCE did not alter bile flow. Total biliary protein declined progressively during the first hour after DCE. *Conclusion:* These observations are consistent with an initial increase in lysosomal exocytosis due to a DCE-induced increase in hepatocyte autophagy and a latter decrease in lysosomal exocytosis due to impaired function of the DCE-damaged canaliculus. (Supported by an Amaco Fellowship and DK 34806).

1693 EFFECT OF KETONE EXPOSURE ON THE LIPID CONTENT OF HEPATIC MEMBRANES IN THE PATHOGENESIS OF CHOLESTASIS

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Ketones can potentiate experimental cholestasis. To see if altered membrane lipids may be a possible mechanism in the potentiation phenomenon, male Sprague-Dawley rats were exposed 4hr/day for 3 days to methyl isobutyl ketone (MiBK) vapors (150 or 600 ppm). Eighteen hr later, manganese (Mn, 4.5 mg/kg) was given i.v. 15 min before bilirubin (BR, 25 mg/kg) to induce cholestasis. Rats were killed 60 min after BR. Liver cell plasma membranes [bile canalicular (BCM) and non-bile canalicular plasma membranes (PM)], were isolated by differential centrifugation. Lipids were extracted and measured colorimetrically. With MIBK 600 ppm exposure, results indicate a marked increase in BCM cholesterol content (2–3 fold) compared to controls. The cellular origin of BCM cholesterol was determined by ^{3}H-cholesterol i.p. injection followed 16 hr later by 2-^{14}C-mevalonic acid. MiBK enhanced the *de novo* synthesis of cholesterol. Phospholipid content in BCM- and PM-enriched fractions was not affected by the MiBK pretreatment. Thus, altered lipid composition is a possible mechanism for ketone-potentiated cholestasis. (supported by the Medical Research Council of Canada and the Canadian Network of Toxicology Centres).

1694 HEPATIC PLATELET SEQUESTRATION AFTER LIPOPOLYSACCHARIDE ADMINISTRATION IS NOT ALTERED BY HEPARIN

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Lipopolysaccharide (LPS) is a component of the cell wall of gram-negative bacteria and mediates many of the pathophysiologic alterations observed during systemic sepsis. After iv administration to female, Sprague-Dawley rats, LPS produces hepatic parenchymal cell injury and cholestasis. Both blood platelets and activation of the coagulation system are required for the expression of liver injury, but any interaction between platelets and coagulation as it relates to liver injury is not fully understood. Heparin is an anticoagulant that prevents liver injury in this model. In this study, we tested the effect of heparin on the hepatic accumulation of platelets during endotoxemia. To measure platelet sequestration, platelets were isolated, radiolabeled with ^{111}In-oxine, and administered to rats prior to administration of LPS (4 mg/kg, iv). Hepatic radioactivity was elevated 1 hr after LPS administration. Elevations in hepatic radioactivity continued throughout the 6 hrs after LPS administration, at which time hepatic radioactivity was 4.5 times greater than in saline-treated rats. No treatment-related alterations in radioactivity were observed in the spleen, kidneys or lungs after LPS administration. Heparin did not prevent the LPS-induced hepatic platelet sequestration. These results indicate that platelets accumulate within the liver after LPS administration and suggest that the platelet accumulation is independent of coagulation. (Supported by NIH grant ES04139).

1695 THE EFFECT OF GAVAGE VEHICLE AND VOLUME ON BROMODICHLOROMETHANE TOXICITY

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Bromodichloromethane (BDCM) ranks second in concentration only to chloroform (CHCl3) among known chlorination disinfection byproducts in finished drinking water. CHCl3 toxicity is modulated greatly by gavage vehicle with administration of high doses in corn oil producing greater hepatic and renal toxicity than aqueous administration. The volume of oil vehicle also modulates CHCl3 toxicity with substantially greater toxicity seen with the higher volume. Following oral exposure to BDCM in oil, greater hepatic damage resulted relative to administration in an aqueous vehicle (Lilly et. al, 1994). However, the effect of vehicle on BDCM-mediated nephrotoxicity was dependent on the dose of BDCM. To study the effects of gavage vehicle and volume on BDCM toxicity, adult male Fischer-344 rats (n = 6, ≈70 days old) were gavaged with 0, 150 or 300 mg BDCM/kg in corn oil or 10% Alkamuls solution at a total gavage volume of either 2.5 or 10 ml/kg. Body weight was significantly decreased when 300 mg BDCM/kg was administered in oil in both low and high gavage volumes. At 48 hr, there were no significant elevations in serum hepatotoxicity indicators (aspartate aminotransferase, alanine aminotransferase, and sorbitol dehydrogenase) from administration of BDCM in the aqueous vehicle at either gavage volume. In oil, serum hepatotoxicity indicators were significantly elevated at 300 mg BDCM/kg in the high gavage volume. At 48

hr, there were no significant elevations of serum indicators of nephrotoxicity, blood urea nitrogen and creatinine. In summary, at these BDCM dose levels, the only evidence of hepatotoxicity was in the high volume oil administration. In conclusion both gavage vehicle and volume affect BDCM hepatotoxicity. (This abstract does not necessarily reflect EPA policy.)

1696 EXAMINATION OF THE MECHANISM UNDERLYING STRAIN DIFFERENCES BETWEEN FISCHER-344 (F-344) AND SPRAGUE-DAWLEY (SD) RATS IN CARBON TETRACHLORIDE (CCL4) HEPATOTOXICITY

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F-344 and SD rats are commonly used in hepatotoxicology studies. Previously, we have shown that both age-matched (44, 64, and 120 day old) and weight-matched (≈200 g) F-344 rats are more sensitive to CCl4 hepatotoxicity than SD rats based on serum sorbitol dehydrogenase and hepatic histopathology. In this study we examined the contribution of metabolism to the mechanism underlying these strain differences. Microsomes (n = 6/day/2 days) were isolated from the livers of 64 day old F-344 and SD rats. CYP2E1 activity was determined by measuring p-nitrophenol hydroxylase activity (nmol/mg/min). There was little difference in CYP2E1 activity between the two strains, with F-344 rats having ≈15% higher levels than SD rats ($p \approx 0.05$). Although we plan to confirm these findings in the weight-matched and other age-matched rats, it is doubtful that this small difference accounts for the observed difference in toxicity. In conclusion, the data suggest that another mechanism may underlie the strain difference in toxicity. (This abstract may not reflect EPA policy.)

1697 THE EFFECTS OF REPEAT DOSING AND REPEAT BLOOD WITHDRAWAL ON CARBON TETRACHLORIDE TOXICITY

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Carbon tetrachloride (CCl_4) hepatotoxicity increases with repeat doses and/or daily repeat blood withdrawal. Serum enzyme levels (serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) were measured 24 and 48 hour after the following treatments: Oil-CCl_4, oil-bleed-CCl_4, CCl_4-CCl_4, and CCl_4-bleed-CCl_4 where bleed groups were bled at baseline, 24, 48, 72, and 120 hours. The ratio of median 48 hour SGPT levels after the second dose of CCl_4 of the above mentioned groups was 1:3:6:10, respectively. Mechanisms accounting for this phenomenon are currently being explored.

1698 THE SWIFT INCREASE IN ALCOHOL METABOLISM (SIAM) CAUSES HYPOXIA IN RAT LIVER *IN VIVO*: ASSESSMENT WITH THE 2-NITROIMIDAZOLE HYPOXIA MARKER, PIMONIDAZOLE

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Hypoxia markers, such as pimonidazole (pimo) have been used extensively in radiation research to predict the hypoxic fraction of tumors to index radioresistance. Pimo accumulates in cells containing less than 10 mmHg O_2, but few studies have used this technology to assess O_2 tension in normal tissues. It has been shown that acute ethanol (EtOH) treatment ("SIAM") increases O_2 consumption in isolated perfused rat liver and liver slices which could lead to pericentral hypoxia. However, it has been suggested that hypoxia does not occur *in vivo* because EtOH also increases blood flow to the liver. To address this apparent paradox, 100–125 g Sprague-Dawley rats simultaneously received pimo (120 mg/kg i.p.) and one large dose of EtOH (5 g/kg i.g.) to produce SIAM. After 2 hr, animals were anesthetized with pentobarbital (50 mg/kg i.p.), livers were surgically removed and homogenized. ELISA was performed on liver homogenate from sham control (n = 8) and SIAM (n = 12) animals using a monoclonal antibody to protein bound pimo. The average tissue concentration of pimo in SIAM rats was significantly greater than controls by a factor of about 2.0, indicating that EtOH produced hypoxia. Since the accumulation of pimo occurred in intact awake animals in this study, these results present compelling evidence in support of the hypothesis that hypoxia is involved in the mechanism of early alcohol-induced liver injury. (ES-07126, AA-03624, CA-50995).

1699 COMPARISON OF HALOTHANE, ISOFLURANE AND DESFLURANE ON HEPATIC BLOOD FLOW AND OXYGENATION IN CHRONICALLY INSTRUMENTED SWINE

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One of the roles inhaled anesthetics play in hepatotoxicity can be attributed to their effect on hepatic blood flow (HBF) and oxygenation. Chronically instrumented domestic swine (22–25 kg) were exposed to a relatively new anesthetic, desflurane, and two established anesthetics, isoflurane and halothane. Isoflurane has been shown not to significantly affect HBF and oxygenation while halothane has been shown to depress these parameters. Ultrasonic flow probes were placed around the hepatic artery and portal vein, catheters were inserted in the aorta, hepatic and portal veins for blood flow measurements, pressure monitoring and determination of O_2 and CO_2. After control measurements, anesthetics were randomly selected and administered at 1.0, 1.5, and 2.0 minimum alveolar concentrations (MAC; 1.0 MAC = ED_{50} for lack of stimulus response). Halothane produced the greatest reduction in hepatic arterial blood flow, portal vein flow and O_2 delivery. Isoflurane showed a minimal depression of hepatic arterial blood flow, portal vein flow and O_2 delivery. Desflurane slightly decreased portal vein blood flow, maintained hepatic arterial blood flow and O_2 delivery. Contrary to halothane, isoflurane and desflurane do not decrease hepatic blood flow and oxygenation. Thus, they are less prone to hepatotoxicity via this method. (NIHGM 467873).

1700 STYRENE INHALATION CAUSES A TRANSIENT HEPATOTOXICITY IN FEMALE B6C3F1 MICE

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Inhalation studies were conducted to investigate the susceptibility of B6C3F1 and Swiss mice to hepatotoxicity caused by exposure to styrene at concentrations that do not saturate metabolism. Male and female B6C3F1 and Swiss mice were exposed to 0, 150, or 200 ppm styrene 6h/d, 5 d/wk for up to 2 wks. Serum ALT and SDH were significantly increased only in female B6C3F1 mice after 3 exposures to 200 ppm styrene; enzyme levels had returned to control levels when measured after 5 and 10 exposures. Degeneration and necrosis of centrilobular (CL) hepatocytes were observed in female B6C3F1 mice after 2, 3, and 5 days exposure to 150 or 200 ppm styrene; incidences of these lesions were greater in the 200 ppm than in the 150 ppm dose group. Microscopic examination after 10 days exposure to 150 or 200 ppm indicated no continuing hepatotoxicity. Degeneration of CL hepatocytes was observed in 1 male B6C3F1 mouse after 3 exposures to 200 ppm; no significant lesions were observed in Swiss mice. Dose-related decreases in hepatic GSH were observed in all groups throughout the study, and were greatest in female B6C3F1 mice. Blood styrene and SO levels increased in all groups with the number of exposures. Styrene levels were significantly higher in B6C3F1 mice than Swiss mice; however, within each strain, gender differences were not significant. Hepatotoxicity in female B6C3F1 mice appeared to be related to greater GSH depletion and/or slower GSH regeneration in these animals. Hepatotoxicity was transient with mice recovering in spite of continuing styrene exposure.

1701 METABOLISM AND HEPATOTOXICITY OF INHALED STYRENE IN B6C3F1 MICE

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Inhalation studies were conducted to investigate the role of cytochrome P-450 enzymes (P-450) and glutathione (GSH) in styrene hepatotoxicity in male B6C3F1 mice. Styrene is metabolized by P-450 to styrene-7,8-oxide (SO), the primary toxic metabolite. SO is detoxified in part by conjugation with GSH. Mice were treated with either phenobarbital (PB), SKF-525A (SKF), N-acetylcysteine (NAC), or saline prior to styrene exposure. Induction of P-450 by PB may increase SO production and hepatotoxicity, whereas inhibition of P-450 by SKF may decrease SO production and hepatotoxicity. NAC, a GSH precursor, may prevent GSH depletion and decrease hepatotoxicity. After exposure to 500 ppm styrene or air for 6 hours, hepatotoxicity was evaluated by measurement of serum ALT and SDH, and histological evaluation. Styrene caused a significant increase in relative liver weights and serum ALT and SDH levels only in mice pretreated with PB. Exposure to styrene caused hepatocellular necrosis in all pretreatment groups; however, these histological changes were more extensive and severe in mice pretreated with PB. Styrene exposure caused greater than 95% depletion of liver GSH in all pretreatment

groups. Pretreatment with NAC did not prevent GSH depletion or hepatotoxicity. Blood styrene and SO levels and SO/styrene ratios were not significantly different between the pretreatment groups. In fat, styrene levels were significantly higher in the SKF-pretreated mice suggesting that metabolism was decreased. The SO/styrene ratio was significantly higher in the fat of PB pretreated mice indicating increased styrene metabolism.

1702 METABOLISM OF HEXADECANEDIOIC ACID (HA) AND RE-EVALUATION OF ITS TOXICITY IN RATS

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HA, the dicarboxylic metabolite of palmitic acid (PA), is formed via a pathway comprising cytochrome P-450 dependent ω-oxidation and alcohol and aidehyde dehydrogenases. The metabolic role of long-chain dicarboxylic acids and toxicity of these acids accumulating in the blood in certain pathologies are unclear. Mortensen and Gregersen (Biochim. Biophys. Acta 710:477–484, 1982) reported that intragastric delivery of HA (0.2 mmol/100 g of body weight), but not other dicarboxylic acids, to 48-hr fasted rats was lethal during the next 24 hr of fasting. In this study, no mortality was observed after treatment of rats with HA according to the above protocol. Thus, the effects of HA *in vivo* could be studied. Rats were maintained on an AIN-76A diet containing 5% beef tallow as a source of palmitate-rich fat because HA inhibited by ≈50% ketogenesis from PA, but not from oleate, in isolated rat hepatocytes. Rats were treated with HA as described above and after 2 hr their blood was analyzed. HA decreased [3-hydroxybutyrate] by ≈20% while [acetoacetate] was not changed. In hepatocytes isolated from fed rats, the total rate of oxidation of [^{14}C]HA (1 mM), quantified as a cumulative formation of $^{14}CO_2$ and [^{14}C]acid soluble metabolites, amounted to ≈40% of the rate of oxidation of 1 mM [^{14}C]PA. These observations support our concept that [HA] may modulate the rate of palmitate oxidation in the liver and other organs. The absence in this study of acute toxicity of HA *in vivo* may be related to an effective elimination of HA by peroxisomal β-oxidation. (Supported by NIEHS Center Grant, ES-05022).

1703 SUBCHRONIC DOSED-FEED TOXICITY STUDY OF ANTHRAQUINONE IN F344 RATS AND B6C3F$_1$ MICE

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Anthraquinone was given *ad libitum* in dosed-feed to F344 rats and B6C3F$_1$ mice (10 animals/sex/dose group/species) at concentrations of 0, 1875, 3750, 7500, 15000 and 30000 ppm for 13 consecutive weeks. There were no exposure-related clinical observations nor early deaths. Group mean body weight values decreased in a dose-dependent fashion for female rats but were unchanged for male rats and mice of both sexes. Group mean food consumption values were similar to control group values, except for a slight decrease only during Week 1 for the rats. Absolute and relative liver weights (rats and mice) and kidney weights (rats) exhibited dose-dependent increases for both sexes. There were no chemical-related gross necropsy findings. In rats, microscopic changes included a dose-related centrilobular hypertrophy and bone marrow hyperplasia (both sexes), hyaline droplet accumulation and RBC-engorged spleens (all treated rats), thyroid follicular hypertrophy (males) and inflammation/urothelial hyperplasia (high dose females). In mice, microscopic changes included a dose-related centrilobular hepatocyte hypertrophy and accumulation of eosinophilic granules in urinary bladder epithelial cells (both sexes). (Supported by Contract No. N01-ES-25337).

1704 HEPATOTOXICITY OF THE CYANOBACTERIAL ALKALOID, CYLINDROSPERMOPSIN (CY) IN MICE

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In mice, the major target organs of CY, an alkaloid isolated from *Cylindrospermopsis raciborskii,* are liver and kidney. Ribosomal detachment and lipid droplet accumulation in liver was reported by Terao et al. (1994) 48 hr after CY. Our objective was to examine the early hepatic ultrastructural changes to gain an understanding of the mechanism of toxicity. Male Swiss Webster mice were injected ip with 0 or 830 ug CY/kg and killed at 1, 3, 6, 12 or 24 hr. In CY-treated mice, serum hepatic enzymes progressively increased starting at 12 hr. Histologically, at 6 hr, there was scattered single cell necrosis in centrilobular hepatocytes and in tubular renal epithelium of the pars recta. These changes became more severe over time with panlobular hepatic necrosis at 24 hr. Ultrastructurally, at 1 hr, there was progressive lipid accumulation and detachment of ribosomes from RER in the liver. Additionally, CY induced progressive dilation of bile canaliculi with loss of microvilli, vesiculation of RER following detachment of ribosomes, and loss of glycogen. In more severely affected regions, endothelial cell phagocytosis of dead cells was observed. This study shows that ribosomal detachment and lipid accumulation are very early lesions of CY. Ribosomal alteration could block protein synthesis, thereby blocking lipoprotein assembly, decreasing lipoprotein secretion, and causing lipid accumulation.

1705 CHRONIC HEPATIC LESIONS IN MICE INFECTED WITH *HELICOBACTER HEPATICUS* MIMICKING TOXIC HEPATIC LESIONS INDUCED BY CHEMICALS

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Helicobacter hepaticus, a newly identified helical bacteria, produces a slow bacterial hepatic and enteric infection in mice. Natural and experimental infections are associated with a sequence of hepatic lesions from acute nonsuppurative inflammation and focal necrosis to chronic hepatitis with oval cell hyperplasia, bile duct hyperplasia, hepatocytomegaly, intranuclear pseudoinclusions, increased levels of cell proliferation and liver tumors. Bacteria can be identified within bile canaliculi with silver stains, by immunohistochemistry using antibodies to *H. pylori,* a human pathogen, and by ultrastructural examination of the liver. The characteristics of the chronic liver lesions varies somewhat with sex and the strain of the infected mouse. Some strains have severe cholangitis while others have marked oval cell hyperplasia. Chronic lesions resembling those induced by mouse liver toxins usually develop by ten months of age. The disease may have spread from NCI-FCRDC research and production colonies to other mouse colonies in the USA.

1706 MORPHOMETRY OF THE LIVER FROM RATS FED 3,3′,4,4′-TETRACHLOROBIPHENYL: AN ULTRASTRUCTURAL STUDY

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Many PCB (Polychlorinated biphenyls) congeners are demonstrated environmental pollutants. We reported [*Histol. Histopathol.*, 9:453–459, 1994], male and female Sprague-Dawley rats that were fed 3,3′,4,4′-tetrachlorobiphenyl or PCB congener #77 at a dose of 10,000 ppb for 91 days revealed alterations including an augmentation of smooth endoplasmic reticulum (SER) profiles in the hepatocytes. In the present study, the same liver samples were subjected to stereology. Resin-embedded sections were obtained from the samples of 12 rats: three animals of each gender from the control and from 10,000 ppb groups. Up to four cells from zone III were selected for thin sectioning [*161st Ann. Mtg., AAAS.*, Atlanta. Abstr., 1995]. SER volume per volume of the cytoplasm was estimated by placing a 1-cm^2 grid on the micrographs that were photographed at 5,000 diameters. There was circa fourfold elevation of SER profiles in the hepatocytes of the treated males in comparison with those in the females. We conclude that the female rats, which were more severely affected than the males, needed relatively more enzymes that are housed on SER to nullify. the ill effects of congener 77 on the liver.

1707 *IN VITRO* KINETICS FOR NON-STEADY-STATE UPTAKE OF CHLORINATED SOLVENTS FROM DILUTE AQUEOUS SOLUTION INTO HUMAN SKIN

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Kinetics of *in vitro* dermal uptake of ^{14}C-labeled chloroform (CF) and trichloroethylene (TCE) from dilute (≈5-ppb) aqueous solutions were examined using alternative mathematical models fit to data obtained using accelerator mass spectrometry (AMS) analysis of human cadaver skin exposed for relatively brief periods (≤1 hr). We found that short-term, non-steady-state dermal uptake for both compounds are consistent with first-order pharmacokinetics, i.e., that total uptake is initially an approximately linear function of exposure time. Estimated initial, normalized uptake rates (K_p) of 0.079 (±7.4%) and 0.32 (±10%) cm/hr we obtained for CF and TCE, respectively, are within a factor of 2 of previous corresponding estimates based on *in vivo* uptake studies involving human subjects and/or hairless guinea pigs. In contrast, uptakes we measured at or beyond typical bathing-exposure times (e.g.,

±0.2 hr) were significantly greater than values predicted by models specifying that uptake is proportional to the square root of exposure time, such as one recently proposed by the U.S. Environmental Protection Agency (EPA). Our findings support the hypothesis that the EPA model may systematically underpredict short-term, non-steady-state dermal uptake of organic chemicals from water, and indicate the importance of acquiring additional experimental data to test alternative uptake models. [This work was performed under the auspices of the U.S. Department of Energy at Lawrence Livermore National Laboratory under Contract W-7405-ENG-48.]

1708 THE MECHANISMS OF TEST METHODS FOR ASSESSMENT OF ALLERGIC CONTRACT DERMATITIS

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Allergic contact dermatitis is a widespread problem in which sensitization develops to chemicals used industrially and to consumer products. We are attempting to utilize a computer-assisted structural alert system to enable identification of chemicals with the potential to cause allergic contact dermatitis. To develop the data learning set, a search was made of the scientific literature for the past 35 years. In reviewing the articles, it was apparent that numerous test methods have been employed in both experimental and clinical trials of contact sensitivity, including the modified Draize test, guinea pig maximization test, optimization test, Freund's complete adjuvant test, and the repeated insult patch test, among others. To establish the data set and assign a potency to chemicals, consideration must be given to the sensitivities of the various test methods. Increased sensitivity results from use of: chemical adjuvants, occlusive covering of test sites, increased dosage, intradermal vs. epicutaneous application of material, repetitive applications, etc. Mechanistic considerations have not been a major factor in the design or use of the various test methods. This study attempts to evaluate the methods routinely used for contact sensitivity testing by examining the mechanisms likely involved at each phase of the induction and elicitation stages of sensitivity. Consideration is given to activities of adjuvant classes, such as Freund's adjuvant, aluminum hydroxide, and *Bordetella pertussis* vaccine, as well as to contributions from irritation, inflammation, cell proliferation, cell migration patterns, expression of adhesion molecules and cytokines. Results will allow a comprehensive approach toward evaluation of potencies obtained from the various sensitization tests and compilation of a robust data set from which structure-activity relationships can be concluded. Supported by the Center for Indoor Air Research and NIH ES05651.

1709 USE OF 2-MERCAPTOBENZOTHIAZOLE AS A POSITIVE CONTROL IN GUINEA PIG SKIN SENSITISATION TESTS

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Sensitisation testing has been a standard feature of regulatory safety evaluation programmes for many years. Whilst many study designs have been included in regulatory guidelines (OECD, 1981; EEC, 1984; US-EPA, FIFRA, 1984; US-EPA, TSCA, 1985; JMAFF, 1985), 2 methods have tended to dominate in regulatory toxicology — the guinea pig maximisation test (Magnusson and Kligman 1969 and 1970) and the occluded patch test (Buehler 1965). The former has gained favour in Europe, whereas in the United States the latter remains pre-eminent.

Meanwhile, recent guideline revisions (OECD, 1992; EC, 1992) have required that animal numbers be reduced, and a revised approach to the selection of positive controls has been recommended. Previously published studies of these "new" materials (Basketter *et al*, 1993) have demonstrated their suitability. One such material, 2-mercaptobenziothiazole (MBT), was chosen for evaluation within the authors' laboratory. The results of these investigations confirm the applicability of MBT for use as a positive control on sensitisation studies in Guinea Pigs. Data obtained are supported by histopathological evidence.

1710 EFFECT OF AN ANALGESIC ON DOSE AND TIME RESPONSE CURVE TO T-2 TOXIN IN AN *IN VIVO* RABBIT SKIN ASSAY

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T-2 toxin is a trichothecene mycotoxin that produces a severe vesicant response when applied to the skin. This dermal irritation is dose dependent and can be used as a bioassay to evaluate the efficacy of various protective treatments against this group of fungal toxins. To reduce any minor pain or suffering by animals that may be associated with this bioassay, an analgesic (Buprenex) was tested for its affects on the dose and time response in the rabbit skin assay. On the day before the study, eight rabbits were sedated with a s.c. injection of Buprenex; (0.1 mg/kg) and a i.m dose of ketamine (10 mg/kg). The dorsal area of the hair coat was clipped from the withers to the rump. The next day, rabbits were injected with Buprenex (0.1 mg/kg) and samples of 0.01, 0.1, 1, 10 and 100 μg of T-2 toxin/2 μl of methanol were applied to 2.5 × 5 cm sites on the skin. At 8, 24 and 30 hr after exposure to the toxin, half of the rabbits received an analgesic dose of Buprenex (0.03 mg/kg) and the other half were injected with saline. Skin lesions were measured at 24, 30 and 48 hr postexposure. The rabbits were killed after the last reading. No significant difference in the dose or time response for the skin lesions were observed between the rabbits receiving the analgesic and the saline controls. Thus, an analgesic can be used to alleviate pain associated with the skin bioassay.

1711 THE PRECLINICAL TOXICOLOGIC PROFILE OF BMS-181162

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BMS-181162, a topical nonsteroidal phospholipase A_2 (PLA_2) inhibitor, is being developed as an anti-inflammatory drug for the treatment of dermatitis. A series of single- and repeat-dose dermal studies using a 1, 2.5, or 5% solution of BMS-181162 was conducted in mice, rats or rabbits. Studies were also performed to investigate the genotoxic, and dermal irritation or sensitization potential of topically administered BMS-181162.

The minimal lethal single oral dose of 5% BMS-181162 was greater than 250 mg/kg (5.0 ml/kg) in rats. In rabbits, BMS-181162 (5% solution) produced moderate skin irritation following 24-hour patch occlusion. BMS-181162 was found to be non-sensitizing and non-phototoxic in hairless guinea pigs.

Topical administration of BMS-181162 at doses up to 150 mg/kg/day produced dose-related minimal to mild dermal effects in mice after 1 month; however, no treatment-related systemic effects were seen. In rats, dermal application of BMS-181162 for 1 month at dose levels up to 100 mg/kg/day resulted in dose-related dermal irritation at all dose levels and slight changes in serum enzyme levels and increases in liver and kidney weights only at the high-dose.

BMS-181162 was non-mutagenic in a bacterial reverse-mutation study (Ames test), and non-clastogenic in an *in vitro* cytogenetic study in human lymphocytes. Additionally, a micronucleus evaluation of rat bone marrow cells revealed no potential of BMS-181162 to cause *in vivo* chromosomal or mitotic spindle damage.

In conclusion, the results provided evidence for the general safety of BMS-181162 solutions. BMS-181162 is currently being evaluated in human clinical trials.

1712 FLUOROQUINOLONE ANTIBIOTICS (FQA) INDUCE PHOTOSENSITIZED SKIN REACTIONS IN HAIRLESS MICE EXPOSED TO SIMULATED SUNLIGHT

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Recently, concerns have arisen about the potential of FQA to elicit adverse photobiological responses. We have conducted preliminary studies to assess the phototoxic potential of three of these compounds. Single oral (gavage) administrations of Nalidixic Acid (NA) and of three FQA were tested in hairless mice (2 to 5 per group); the doses ranged from 10 to 1000 mg/kg body weight. Approximately one hour after FQA (or vehicle) administration, restrained mice were exposed to full spectrum simulated sunlight from a long-arc xenon lamp (an exposure dose of approximately one half of the amount needed to elicit erythema in previously untanned human skin). Graded responses attributable to phototoxicity occurred in back skin (e.g., erythema and edema) and the pinnae ("ear swelling") of sensitized mice. Based on the test article dosages that elicited responses, the phototoxic potential was ranked as lomefloxacin > nalidixic acid > ciprofloxacin = ofloxacin. A repeated dose study conducted only with lomefloxacin (6 mice per group) at 20 or 200 mg/kg (once daily, 5 days weekly for two weeks) plus simulated sunlight, elicited early signs of erythema, flaking and epidermal melanization which subsequently resolved. However, at 10 weeks after this two-week regimen, 0 and 6 mice in the 20 and 200 mg/kg dosage groups, respectively, developed cutaneous papillomas.

1713 SAFETY EVALUATION OF AZELAIC ACID WITH PLASMA CONCENTRATIONS IN RATS AND DOGS: COMPARISON WITH HUMAN DATA

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Azelaic acid 20% cream is being developed as a topical anti-acne drug. Azelaic acid is a naturally occurring dicarboxylic acid found in cereal grain. Safety studies with azelaic acid were conducted in rats and dogs and the margin of safety was determined when compared to the human exposure. In a 6 month topical study, 20 rats/sex/group were dosed with vehicle and azelaic acid 20% cream at doses of 250 and 500 mg/kg/day applied as divided doses BID, and 1500 mg/kg/day as divided doses TID. Expressed as active compound, these doses are 50, 100, and 300 mg azelaic acid/kg/day. Azelaic acid concentrations in plasma obtained after six months of treatment were 0.126, 0.255, and 0.791 μg/ml for the 250, 500, and 1500 mg/kg/day azelaic acid treated rats, respectively. In a 6 month oral study, 6 dogs/sex/group were dosed with 0, 10, 100, and 800 mg/kg/day of azelaic acid in capsules as divided doses BID. These doses produced mean peak plasma concentrations of 2.19, 20.8, and 135 μg/ml, respectively, at the end of the study. In humans the intended dose is approximately 2 to 5 mg azelaic acid/kg/day. 12 healthy volunteers were treated with 20% azelaic acid cream topically for 7 days at a dose of 2.5 mg azelaic acid/kg. The baseline plasma concentration of azelaic acid in this study was 0.052 μg/ml, whereas after 7 days of dosing the mean peak plasma concentration was 0.136 μg/ml. In another human study, 56 patients with mild to moderate acne were treated with 20% azelaic acid cream applied topically twice daily for 12 weeks at a dose of 3 mg azelaic acid/kg/day. After 12 weeks of treatment, the mean plasma concentration of azelaic acid, 0.090 μg/ml was not significantly different than the baseline, 0.084 μg/ml. In the rat topical study, the plasma concentrations were 6 times higher than the human topical study, and in the dog oral study, the plasma concentrations were 1000 times that seen in the human study. These data show that azelaic acid topical cream is very well tolerated and also confirm the excellent safety profile of azelaic acid.

1714 TWENTY-EIGHT DAY REPEATED CUTANEOUS DOSE TOXICITY STUDY IN FISCHER 344 RATS USING GLUTARALDEHYDE (GA)

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Fischer 344 rats (15/sex/group) were treated by occluded cutaneous application with 2.5, 5.0, or 7.5% aqueous solutions of glutaraldehyde (CAS No. 111-30-8) at a dose volume of 2 ml/kg/day (50, 100 and 150 mg/kg/day) for 20 applications over 28 days. Controls were treated with distilled water. Five rats/sex (control and 7.5% groups) were held for a 4-week recovery period. Treatment-related clinical signs of skin irritation included barely perceptible erythema, color change, exfoliation, excoriation, crust and skin necrosis, which resolved in the 7.5% group after 4-weeks recovery. Reduced food consumption, body weight and body weight gains were noted in the 7.5% group during the dosing period. Treatment-related increases in reticulocyte and platelet counts observed in females were attributed to skin irritation and necrosis. Males had dose-related increases in BUN, considered to be related to skin irritation effects. Dose-related increases in absolute and relative adrenal weights observed in males and females are believed to be due to stress from repeated application of an irritant material to the skin. Microscopic evaluation of the skin revealed treatment related acanthosis, dermatitis, hyperkeratosis (females), epidermitis and dermal fibrosis (males) of the superficial layers. Dermal fibrosis persisted in these animals after 4-weeks of recovery. Under the conditions of this study, the clinical findings were considered to be secondary to localized cutaneous irritation, with no indication of systemic toxicity. A no-observed-effect level (NOEL) was not determined in this study, although the effects were minimal at 50 mg/kg/day.

1715 DERMAL ABSORPTION KINETICS OF LIQUID CHLOROPENTAFLUOROBENZENE (CPFB) AND 1,2-DICHLOROBENZENE (DCB) IN RATS AND GUINEA PIGS

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The potential for occupational or accidental skin exposure to chemicals requires a full understanding of chemical absorption through the skin. Two model chemicals (CPFB and DCB) were chosen to provide a range of water & lipid solubilities for *in vivo* study. To determine whether species differences in dermal absorption are due to physical and physiological dissimilarities, Male F-344 rats and Hartley guinea pigs were chosen to provide different characteristics in the skin. 24 hrs prior to exposure each animal was fitted with a jugular cannula and the glass cell was attached to the animal's back. All the animals were exposed to pure liquid CPFB or DCB for up to 8 hrs in a 3.14 cm^2, septum sealed glass cell. Blood was serially drawn and analyzed for these chemicals. The blood concentration of the absorbed chemicals increased rapidly during the first ½ up to 2 hrs of exposure; it reached the maximum value and stayed constant up to 8 hrs. Additionally, in each species with each chemical, a dermal permeability constant was estimated with a PB-PK model. These studies suggest that anatomical species differences are important to understand in skin penetration.

1716 SKIN ABSORPTION OF FORMULATED 2,4-D, DIAZINON, AND DDT IN RATS, GUINEA PIGS AND HUMANS

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Percutaneous absorption of pesticides is a major route of occupational exposure to these compounds and accurate models of the absorption process are needed in order to conduct hazard/risk assessments. *In vitro* skin absorption assays have previously been reported to provide good models for nonformulated pesticides dissolved in acetone and applied to the skin, however the effect of the pesticide formulation constituents has not been well-detailed. The present study examines the *in vitro* skin absorption of two commercial formulations of 2,4-D, two formulations of Diazinon, and three formulation concentrations of DDT when applied to rat, hairless guinea pig and human skin. The *in vivo* skin absorption of these formulations was also tested in rats. The *in vitro* rat assays overestimated *in vivo* dermal absorption of 2,4-D and Diazinon but underestimated *in vivo* skin absorption of DDT. There was no apparent effect of the pesticide concentration (% active ingredient in the formulation) on the total amount of pesticide absorbed. A marked enhancement of pesticide skin absorption was observed *in vitro* subsequent to a soapy water wash of the skin conducted at 24 hours postexposure. This 'wash-in' effect may have important toxicological ramifications for hazard/risk assessments.

1717 TERTIARY BUTYL HYDROPEROXIDE TOXICITY AS A MODEL OF OXIDATIVE STRESS IN PRIMARY NEONATAL RAT KERATINOCYTE CULTURES

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Tertiary butyl hydroperoxide (tBHP) has been used a model compound for inducing oxidative stress in many different cell types. The purpose of this investigation was to establish a model of oxidative stress in primary neonatal rat keratinocyte cultures. Primary cultures of rat keratinocytes were established from 2–3-day-old neonatal Sprague-Dawley rat pups. After 3 days in culture, the cells were exposed for 1 to 4 hr to tBHP in concentrations ranging from 0.001 to 2.0 mM. In addition, cells were exposed for 1 to 4 hr to diamide, a glutathione depleting agent, in concentrations ranging from 25 to 2000 μM. Toxicity was assessed by measurement of propidium iodide uptake using a fluorescent plate reader. After 2, 3, and 4 hr exposure to 0.5 mM and higher concentrations of tBHP, a significant increase in propidium iodide uptake was shown when compared to untreated control keratinocyte cultures. Diamide, at exposure concentrations of 400 μM and above, significantly increased propidium iodide uptake after 2, 3, and 4 hr of exposure. When keratinocytes were simultaneously exposed to various concentrations of tBHP and 100 μM diamide, there was no significant change in the tBHP dose-response curve through 4 hr of exposure. The results indicate that tBHP may be used as a model compound for inducing oxidative stress in primary keratinocyte cultures. This model will be valuable in understanding the role of oxidative stress in keratinocyte growth, differentiation, and senescence. (Supported by a grant from R. J. Reynolds).

1718 PERCUTANEOUS ABSORPTION OF TRINITROBENZENE. ANIMAL MODELS FOR HUMAN SKIN

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The percutaneous absorption of trinitrobenzene (TNB), a potentially toxic chemical used in munitions, was studied in viable hairless guinea pig (HGP), Fischer 344 rat, and human skin. Skin was dermatomed and assembled in flow-through diffusion cells followed by TNB application in either an acetone or a water vehicle. Skin absorption was expressed as the percent of applied dose absorbed (skin and receptor fluid) in 24 hr. Rapid absorption of TNB by rodent skin was obtained with both vehicles. For HGP skin, TNB absorption was

73% (acetone vehicle) and 83% (water vehicle). For rat skin, TNB absorption was 61% (acetone) and 67% (water). Interestingly, absorption of TNB from acetone was significantly reduced (36%) in human skin, but absorption from water remained high (72%). TNB is well absorbed even through human skin because of its high solubility in both water and lipids (octanol/water partition coefficient = 8.4). There is little tendency for TNB to form a reservoir in skin when a thin (200 μm) dermatome section is used (HGP and human skin). With haired rat skin a thicker dermatome section is required (350 μm), and 13–21% of the absorbed radioactivity remained in the skin at 24 hr.

1719 *IN VITRO* PENETRATION OF ETHYLENE GLYCOL (EG) THROUGH MOUSE AND HUMAN SKIN

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EG is commonly used as an antifreeze and a deicing fluid as 50% aqueous solutions as well as being an important industrial solvent. A single oral dose of 1.5 g/kg can be fatal to humans, while repeated oral doses of 500 mg/kg/day can cause developmental toxicity in mice. However, the most common route for human exposure to EG is by skin contact. Therefore, the cutaneous penetration of EG was evaluated with excised, full-thickness skin from female CD-1® mice and humans using a flow-through *in vitro* skin penetration apparatus. Approximately 25 mg/cm^2 skin of undiluted or a 50% aqueous solution of ^{14}C-EG was applied to each skin preparation and the amount that penetrated was measured as a function of time to determine various penetration characteristics. Comparing mouse and human skin, the steady-state rates of penetration (0.52 and 0.013 mg/cm^2/hr, respectively) and permeability constants (4.68×10^{-4} and 0.12×10^{-4} cm/hr, respectively) were greater for mouse skin treated with undiluted EG. For the 50% diluted EG, the steady-state rates of penetration (0.22 and 0.007 mg/cm^{2}hr, respectively) and the mean permeability constants (4.36×10^{-4} and 0.14×10^{-4} cm/hr, respectively) were also greater for mouse skin. In addition, the lag times to reach steady-state rates of penetration were significantly shorter for mouse skin (0.90 – 1.02 hr) than for human skin (3.07 – 3.10 hr). Comparing the effect of diluting EG by 50%, the steady-state rates of penetration were reduced by approximately half, while the permeability constants remained similar for both species. These results show that human skin was 30 to 40 times less permeable to EG than mouse skin. Therefore, the potential toxicity resulting from cutaneous exposure to EG would likely be significantly less for humans than that predicted by dermal experiments in mice. Additionally, the total absorbed dose of EG would be less for cutaneous exposures to water solutions of EG, like antifreeze mixtures, than for similar exposures to undiluted EG.

Sponsored by the Ethylene Glycol Panel of the Chemical Manufacturers Association.

1720 THE RELATIONSHIP BETWEEN STRATUM CORNEUM DIFFUSION COEFFICIENTS AND TEMPERATURE FOR HALOGENATED HYDROCARBONS

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The relationship between diffusion coefficients and exposure temperature was evaluated in isolated stratum corneum (SC). Due to the difficulty in measuring a diffusion coefficient directly, the movement of chemicals into and across the dermal barrier is often described as a function (the permeability constant) of the diffusion coefficient. The permeability constant also depends on the skin:chemical partition coefficient and the pathlength of diffusion. In the idealized expression of diffusion given in the Stokes-Einstein equation, diffusion is described in terms of kinetic energy and the viscous resistance of diffusion. If this relationship holds for the diffusion of halogenated chemicals in isolated stratum corneum then the observed diffusion coefficients will be dependent on the temperature of the exposure system. Rat or human stratum corneum was removed from whole thickness skin by trypsin treatment and stored in a dessicator jar. Approximately 5 mG of SC was placed into the weighing pan of a thermogravimetric analyzer and exposed to vapor of halogenated hydrocarbons. The increase in tissue weight over time for temperatures ranging from 27–40°C was evaluated. Diffusion coefficients were estimated by firing the pre-equilibrium data with a non-steady state diffusion equation. The observed diffusion coefficients were dependent on the temperature of the exposure, indicating the importance of careful temperature control in dermal absorption studies using halogenated hydrocarbons.

1721 TEMPERATURE EFFECTS ON STRATUM CORNEUM: AIR PARTITION COEFFICIENTS FOR HALOGENATED HYDROCARBONS

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The relationship between temperature and stratum corneum:air partition coefficients for dibromomethane, tetrachloroethylene and methylene chloride was evaluated. The permeability coefficient used to describe dermal absorption kinetics is a function of the stratum corneum:air partition coefficient. The partitions were determined using a Thermogravimetric Analyzer (TGA) modified for continuous flow through of the chemical of interest. A sample of approximately 5 mG of rat or human stratum corneum (separated enzymatically) were used in all determinations. The sample was placed into the TGA and exposed to a dry air flow of 2L/min. Once equilibration was obtained, the dry air was simultaneously stopped and replaced with a 10,000 ppm concentration of the chemical of interest. The mass of the sample was recorded by the TGA until a chemical equilibrium was reached and the mass appeared constant. The change of weight in the sample size was used to determine the stratum corneum:air partition coefficient. Samples were exposed at temperatures of 27, 30, 32, 35, 37, and 40°C. Stratum corneum:air partition coefficients decreased linearly with increasing temperature. For the chemicals evaluated, the magnitude of the change was highly significant and demonstrated the potential impact of temperature on chemical flux across the dermal barrier.

1722 DERMAL ABSORPTION KINETICS OF HALOGENATED HYDROCARBONS APPLIED IN FINITE DOSES

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The dermal absorption kinetics of finite doses of dibromomethane (DBM) and bromochloromethane (BCM) were evaluated in rats. The dermal penetration was assessed by estimating a steady state permeability coefficient, Kp, for DBM and BCM. Kp has units of cm/hr and is a composite measure of permeability that contains the diffusion coefficient, D, the pathlength of diffusion, L, and the skin:chemical partition coefficient, P. The relationship between these terms is $Kp = D/L \times P$. The Kp was incorporated into a physiologically based pharmacokinetic model (PBPK) that combined kinetic terms for absorption, distribution, metabolism and metabolism with physiological descriptions of organ volumes and flows. Using the PBPK model, simulations of DBM and BCM blood levels following finite dermal exposure were compared to experimentally derived blood data. The blood data was derived from male rats which had their fur closely clipped and each was fitted with a jugular cannula and a 3.8 cm^2 stainless steel dermal exposure cell to the mid back. 250 μl of DBM or BCM were placed into the exposure cell and 0.1 mL blood samples were drawn and analyzed at 2, 5, 10, 13, 16, 19, 25, 30, 45, and 60 minutes. The PBPK model using the steady state Kp adequately predicted DBM and BCM blood concentration following finite dermal exposures.

1723 COMPARATIVE *IN VITRO* DERMAL PENETRATION OF SALICYLIC ACID FROM COMMERCIAL VEHICLES

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The time-dependent percutaneous absorption of salicylic acid (SA) was measured *in vitro* from 4 commercial formulations (two creams, a hydroalcoholic liquid, a hydroalcoholic gel) and 2 simple vehicles (ethanol/water and propylene glycol) using human skin and occluded infinite-dose conditions. Comparison of the experimentally-derived permeability coefficients (Kp cm/hr) demonstrated that absorption of SA was greatest from the liquid hydroalcoholic vehicle (6.6×10^{-3} cm/hr) and the ethanol/water vehicle (6.3×10^{-3} cm/hr). The Kps for the hydroalcoholic gel (3.6×10^{-3} cm/hr) and for the cream vehicles (5.3×10^{-4} and 3.5×10^{-4} cm/hr) were significantly less than those of the hydroalcoholic liquids (commercial formulation and simple vehicle). Moreover, the Kp for SA in propylene glycol (7.5×10^{-5} cm/hr) was approximately 100 times less than that found for the hydroalcoholic liquids. Separately, Kps determined *in vivo* for absorption of SA from one cream and one liquid hydroalcoholic vehicle under nonoculuded conditions were found to be 3.0×10^{-4} cm/hr and 7.0×10^{-4} cm/hr, respectively. Thus, the *in vitro* Kp was predicative of the *in vivo* data for the cream vehicle but not for the hydroalcoholic vehicle. Further, comparison of these *in vivo* and *in vitro* skin penetration studies illustrates the importance of *in vitro* study design when such data are to be applied to human risk assessment decisions.

1724 SUITABLE ANIMAL MODEL FOR *IN VITRO* SKIN PENETRATION STUDIES USING ^{14}C-ETHANOL AS A STANDARD CHEMICAL

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In vitro skin penetration studies are performed to determine the extent of chemical penetration through skin and to estimate the potential human health risk from dermal exposure. For these studies, different species are used for various reasons, but little information is available as to which animal species represents a suitable model for human skin. This question was investigated using excised, full-thickness skin from male and female Fischer 344 and Sprague-Dawley rats, CD-1 and B6C3F1 mice, New Zealand White rabbits, and humans. These skin samples were compared for *in vitro* skin penetration using ^{14}C-ethanol as a model compound. A flow-through skin penetration apparatus was used and approximately 50 mg was applied as a 25% solution. The amount that penetrated was measured as a function of time to determine various penetration characteristics. The results showed that rabbit and mouse permeability constants (Kp in cm/hr) were significantly higher than rat and human for both sexes. In addition, there were statistically significant differences for Kp values between males and females for both mice and rabbits, but no sex difference for rats and humans. The lag time to reach steady state rates of penetration was also compared among species, with humans having the longest lag time (approx. 2 hrs) and mice having the shortest lag time (0.6 hr). For all species, males showed a longer lag time than females. Mouse and rabbit skin were the most permeable skin types of the species tested. For *in vitro* skin penetration studies and among the species tested, rat skin appears to be the most suitable animal model for human skin. Although rat skin tends to reach steady-state at a faster rate, the Kp values are very similar to human skin.

1725 VARIABLES AFFECTING THE PERCUTANEOUS ABSORPTION OF BENZOPHENONE-3 SPECIES VARIATION AND VEHICLE EFFECTS

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Benzophenone-3 (BP-3, oxybenzone, 2-hydroxy-4-methoxybenzophenone) is a commonly used topical sunscreen ingredient. The absorption kinetics of BP-3 in acetone vehicle or sunscreen lotion was examined *in vitro* using human, rat and mouse skin. Mouse skin was obtained from the dorsal region of 4 SKH-1 hairless females, rat skin was from the dorsal region of 4 CD hairless females, and human skin was from the trunk region of 4 donors. Duplicate skin sections, less subcutaneous fat and a portion of the reticular dermis, were mounted onto Franz diffusion chambers and studied using the finite dose technique. 4% BP-3 in acetone and lotion was applied topically at a dose of 22 μl/cm^2 for 24 hours. Receptor solutions were collected at time intervals over 48 hours at which time the epidermis and dermis were also collected. BP-3 was assayed by HPLC. For all species evaluated, BP-3 showed, on average, a two fold greater total penetration and peak flux from acetone than from the lotion vehicle. In human skin 5.5% (acetone) and 3.2% (lotion) of the applied dose penetrated the stratum corneum. Rat skin was 4.8X more permeable and mouse skin 8.0X more permeable than human skin from the acetone vehicle. The flux profiles for each species were similar for acetone and lotion, however, between species they greatly differed. In addition, BP-3 distribution within the skin varied with vehicle and species tested. The data demonstrate that the vehicle has an important impact on the penetration of BP-3 and that rodent skin is more permeable to this chemical than human skin under the conditions of this study.

1726 COMPARISON OF THE *IN VITRO* HUMAN SKIN PENETRATION OF THREE NITROSAMINES

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The human skin permeation of three ^{14}C-labelled nitrosamines (N-dimethylnitrosamine [DMN], N-nitrosodiethanolamine [NDELA] and N-dodecylmethylnitrosamine [NDOMA]) has been assessed *in-vitro* following application as an infinite dose in isopropyl myristate (IPM). IPM was chosen since it has good solvation properties for each of the penetrants investigated. Heat-separated human epidermal membranes were mounted on Franz-type diffusion cells, and membrane integrity evaluated with a suitable marker compound, Permeation of the individual nitrosamines was subsequently followed over a 48 hour period. Mass balance experiments were also conducted. The percentage of the applied dose recovered from skin samples at 48 hr amounted to 0.78 ± 0.46 (DMN), 45.9 ± 5.3 (NDELA) and 0.79 ± 0.21 (NDOMA). The percentage of the applied dose absorbed at 48 hr amounted to 2.6 ± 1.2 (DMN), 23.6 ± 6.4 (NDELA) and 0.10 ± 0.01 (NDOMA) although the value for DMN was significantly affected by permeant evaporation from the donor phase (in fact, over the first hour the rate of DMN permeation was greater than that of NDELA). These results demonstrate the importance of considering evaporation when determining the extent of skin absorption of volatile nitrosamines such as DMN. Permeability coefficients and lag times for NDELA ($kp = 4.1 \times 10^{-3}$ cmh^{-1} and $t_{lag} = 5.4$ hrs) and NDOMA ($Kp = 9.0 \times 10^{-6}$ cmh^{-1} and $t_{lag} = 4.5$ hrs) were calculated by application of linear regression to the appropriate regions of steady-state (a value for DMN could not be calculated because steady-state was not achieved). The shorter chain, more hydrophilic compound (NDELA) was absorbed to a much greater extent than the more lipophilic NDOMA. This effect may have been due to the relative affinities that these compounds possess for the lipophilic donor phase solvent (IPM). This type of effect can only be assessed properly through experimentation, and the use of *in-vitro* human skin diffusion measurements is the method of choice.

1727 *IN VITRO* PENETRATION OF ISOPROPANOL (IPA) THROUGH RAT, MOUSE AND HUMAN SKIN

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IPA is an important industrial solvent and is commonly used in household products. The most common route for consumer exposure to this compound is by skin contact. Therefore, the cutaneous penetration of IPA was evaluated using excised, full-thickness skin from male and female Fischer 344 rats, B6C3F1 mice and humans using a flow-through *in vitro* skin penetration apparatus. A 70% aqueous solution of ^{14}C-IPA (250 μl) was applied to each skin preparation and the amount that penetrated was measured as a function of time to determine various penetration characteristics. For rat, mouse and human skin, steady-state rates of penetration (1.30, 1.88 and 1.65 mg/cm^2/hr, respectively) and permeability constants (2.39×10^{-3}, 3.37×10^{-3} and 3.00×10^{-3} cm/hr, respectively) for occluded doses of IPA were similar among the three species. However, as a percent of dose applied, more IPA penetrated through mouse skin (11.6%) than through rat (6.4%) or human (7.1%) skin over the 6-hr treatment period. This was probably because the lag time to reach steady-state penetration was shorter for mouse skin (1.0 hr) than for rat (2.0 hr) or human (2.5 hr) skin. When unoccluded doses were used, the percentages of IPA that penetrated rat, mouse and human skin were 0.7, 3.0, and 0.6%, respectively; 9, 4 and 12 times less than for occluded doses respectively. These results indicated that mouse skin was the most permeable of the three species tested, while rat skin appeared to be a good human skin model for IPA penetration. The amount and rate of IPA penetration through skin, in general, were relatively low and cutaneous exposures would probably result in most of the IPA evaporating instead of being absorbed.

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1728 *IN VITRO* IRRITATION AND CORROSIVITY STUDIES ON ORGANOSILICON COMPOUNDS AND CORRELATION WITH THEIR *IN VIVO* PROPERTIES

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There is a growing impetus towards the development of alternatives to animal testing for humane, scientific and economic reasons. This study describes recent experiments in this area employing *in vitro* models to assess skin and eye irritation and skin corrosion. Three commercially available *in vitro* assays (Skin^2 1200, Skin^2 1350 and CORROSITEX) were assessed using a range of siloxane polymers and silanes and other chemicals, in order to evaluate their accuracy to predicting the skin irritation/corrosivity and eye irritation potential of these materials.

The Skin^2 1200 and 1350 models were generally applicable for the *in vitro* assessment of eye and skin irritation and skin corrosivity of liquid silicone polymers and other materials, although some effort will be required to overcome the problems of working with awkward test materials (volatile, low surface tension materials, viscous pastes, etc.) for which variable results were observed.

The correlation of the Skin^2 *in vitro* skin and eye irritancy results with known *in vivo* irritancy was generally good, although alkoxysilanes do not appear to give results which correspond well with their *in vivo* irritant for silicone polymers properties. Both the Skin^2 1350 and CORROSITEX models

performed well in detecting corrosive and non-corrosive materials. The Skin[2] 1350 was however more reliable in its overall performance and specificity (correct identification of non-corrosive substances) although CORROSITEX appeared to be more sensitive (correct identification of corrosive materials and degree of corrosivity).

1729 DNA ADDUCTS ISOLATED FROM HUMAN KERATINOCYTES EXPOSED TO BIS(2-CHLOROETHYL) SULFIDE (BCES)

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Topical exposure of human skin to BCES results in vesication. This blistering phenomenon, not seen in rats similarly exposed, is thought to involve BCES alkylation of DNA. Experiments designed to isolate and identify DNA adducts in primary human keratinocyte cultures exposed to BCES indicate bis(2-guanin-7-yl-ethyl) sulfide (GSG) as the major adduct observed. Isolated [^{3}H]guanosine-labelled rat keratinocyte (RK) and human keratinocyte (HK) DNA exposed to 50–5000 μM BCES, hydrolyzed under mild acidic conditions (70°C, pH 3.5, 30 min.) and then eluted from a Bio Rad P2 column, show radioactivity in fractions of a molecular weight (390 daltons) consistent with GSG. RK-DNA and HK-DNA exposed to 300 μM BCES and then hydrolyzed, show 25% of available guanine akylated. 50% and 80% of these residues coincide with GSG on P2 for RK-DNA and HK-DNA respectively. Identification of these adducts with a GSG standard on HPLC shows that while 86% of the radioactivity in these fractions co-elute with GSG standard for HK-DNA, only 33% co-elute with the GSG standard for RK-DNA. These results indicate an inherent difference between HK and RK DNA in regard to alkylation by BCES. Supported by USAMRICD, Contract No. DAMD17-90-C-0031 and ARO Grant No. DAAL03-92-G-0074.

1730 MECHANISMS OF DNA REPAIR IN HUMAN KERATINOCYTE CULTURES AFTER EXPOSURE TO LOW DOSES OF BIS-(2-CHLOROETHYL)SULFIDE (BCES)

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Ribeiro, *et al.* (Toxicol. Appl. Pharmacol., 111, 342–351, 1991) reported the dose-responsive appearance of single-strand breaks (SSB) in the DNA of cultured rat keratinocytes immediately after exposure to 0.1–15 μM BCES. By 22 hrs. post-exposure to as much as 5 μM BCES, no SSB were found. During this period of repair, when replication of DNA was inhibited, [^{3}H]deoxyguanosine, but not [^{3}H]thymidine, was incorporated into the repaired nucleic acid post-exposure to as much as 20 μM BCES. Since SSB are a consequence of depurination/depyrimidination followed by specific excision of the apurinic-/apyrimidinic-"nucleotide" and guanine is the major alkylation site in DNA exposed to BCES, it was considered that repair was occurring by a replacement of damaged guanine residues by a "base replacement" mechanism. Human keratinocytes in submerged 3 day old cultures have now been exposed to 0.1–1.0 μM BCES and assayed for the incorporation of [^{3}H]guanosine (as a measure of "base replacement") or [^{3}H]thymidine (to monitor excision repair or DNA replication) between 0 and 24 hrs. post-exposure. At all levels of exposure, the incorporation of [^{3}H]guanosine was 5 times higher then [^{3}H]thymidine at 0 time, was highest within the first hour after exposure and decreased rapidly thereafter. These data are consistent with the postulated specific replacement of damaged guanine residues and the absence of excision repair or DNA replication at the low levels of BCES utilized. (Supported by USAMRICD Contract No. DAMD17-90-C-0031 and ARO Grant No. DAAHO4-93-G-0095).

1731 FORMATION OF INTERSTRAND DNA CROSS-LINKS BY BIS-(2-CHLOROETHYL)SULFIDE (BCES) IN RAT KERATINOCYTES

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Keratinocytes in the basal layer of the epidermis are a major target in BCES-induced cutaneous injury. BCES is a bifunctional alkylating agent and its effects on cell viability in synchronized keratinocytes was determined at 24 hr post-exposure. When cells at the G1/S border were exposed to 50 and 100 uM BCES, cell viability was 36% and 26% of control, respectively. Comparatively, viability of cells exposed in the G1 phase was 56% and 43% of control. Interstrand DNA cross-links are believed to be responsible for the cell death caused by bifunctional alkylating agents. The formation of interstrand DNA cross-links was measured in BCES-exposed keratinocytes by an ethidium bromide-fluorescence assay. At 0 and 24 hr post-exposure, a dose-dependent formation of interstrand DNA cross-links was observed. When cells at the G1/S border were exposed to BCES, 1.7 times more cross-links were observed immediately after exposure than in cells exposed in the G1 phase. At 24 hr post-exposure, the level of cross-links in cells exposed at the G1/S border was 45% of that immediately after exposure. In cells exposed in the G1 phase the value was 27–30%. These results suggest that keratinocytes synchronized at the G1/S border are more sensitive to BCES than cells in the G1 phase because (a) cellular DNA is more accessible to BCES at the G1/S border than in the G1 phase and (b) cells in the G1 phase have more DNA repair capability than cells at the G1/S border. It is likely that the presence of interstrand DNA cross-links is related to the cytotoxicity observed in keratinocytes subsequent to exposure to BCES. (Contract No. DAMD 17-90-C-0031).

1732 APPEARANCE OF INTERLEUKIN-1α RELATES DNA INTERSTRAND CROSS-LINKS AND CYTOTOXICITY IN CULTURED HUMAN KERATINOCYTES EXPOSED TO BIS-(2-CHLOROETHYL)SULFIDE

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The utility of an increase in the level of interleukin-1α (IL-1α) as an indicator of cytotoxicity from exposure to bis-(2-chloroethyl)sulfide (BCES) was evaluated in submerged monolayer cultures of human cutaneous keratinocytes. Four-day old cultures were exposed to 1–100 μM BCES at 35°C for 30 minutes. The amounts of IL-1α in the medium at 24, 48, and 72 hr and in cells at 72 hr post-exposure were measured immunologically with an ELISA using monoclonal antibody to human IL-1α. The antibody was conjugated with peroxidase for visualization. Cell viability was measured concomitantly using the trypan blue dye-exclusion technique. The degree of interstrand cross-linking as a measure of damage in the cellular DNA was determined by measuring the fluorescence resulting from the intercalation of ethidium bromide into double-stranded molecules that remained in heat-denatured DNA isolated from cells that had been exposed to BCES. A high correlation was observed between the dose-responsive increase in the level of IL-1α in the medium and in the cells, and the dose-responsive decrease which took place in the fraction of viable cells in exposed cultures. The dose-responsive increase in the interstrand cross-linking found in the DNA of cells immediately after exposure to BCES also correlated with the increase in IL-1α at 72 hr post-exposure. These data suggest that the appearance of IL-1α can be used to quantify the cytotoxicity resulting from BCES-mediated damage to cellular DNA and that the degree of cross-linking in the DNA immediately after exposure to BCES is predictive of the level of cytotoxicity in an exposed culture 3 days later. (Contract. DAMD 17-90-C-0031).

1733 IMMUNOHISTOCHEMICAL LOCALIZATION OF TUMOR NECROSIS FACTOR-α IN PORCINE SKIN EXPOSED TO CONTACT IRRITANTS

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Tumor necrosis factor-α (TNF-α) is a primary mediator of inflammation. It induces a biochemical cascade responsible for localizing tissue damage to the site of injury and coordinating the wound healing mechanism of the body. Keratinocytes have been proven to produce TNF-α in addition to several other inflammatory cytokines. In the present study, TNF-α was localized in porcine skin by immunohistochemistry (anti-swine monoclonal TNF-α antibody) after barrier perturbation with 20% sodium lauryl sulfate (SLS), absolute acetone (A) and controls with occlusion for 1, 4, or 8hrs. In addition, TNF-α staining was observed after tape stripping (TS) and a betadine scrub (B). TNF-α staining was found intracellularly in the viable epidermal cell layers in both A and SLS which increased at 4hrs and decreased at 8hrs while, 1hr controls and TS and B showed slight staining in the stratum basale. This study demonstrates a baseline distribution of TNF-α in porcine epidermis. Changes in TNF-α localization secondary to various perturbations may make it a valuable biomarker for evaluating cytotoxicity, irritation and inflammation in pig skin.

1734 MECHANISTICALLY DEFINED CHEMICAL MIXTURES (MDCM): A NEW EXPERIMENTAL PARADIGM FOR RISK ASSESSMENT APPLIED TO SKIN

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Risk assessment of chemical mixtures is bewildering. Since exposure with re-

sultant absorption is the rate-limiting determinant of risk, the testing process could be simplified through selecting components of a defined mixture possessing specific activity toward modulating primary toxin absorption. These identified mechanisms would then serve as a template for screening of field mixtures. Because skin is both a route of exposure and a target organ, we tested this paradigm with 5 component topical mixtures in isolated perfused porcine skin using parathion and the benzidine based dye Direct Red 28. Cutaneous toxicity was quantitated using biomarker assays and histology. Chemical flux data was integrated into a comprehensive mathematical model construct to quantitate percutaneous penetration, disposition and absorption of a topically applied chemical on the basis of biophysical and physiological parameters. Mixtures of chemicals are treated by the model in parallel, with each chemical component as a substruct consisting of the basic construct. Each substruct then can account for interactions among the other substructs (chemical components).

1735 HISTOCHEMICAL DISTRIBUTION OF FIVE EPIDERMAL-DERMAL JUNCTION EPITOPES IN PORCINE SKIN TREATED WITH BIS (2-CHLOROETHYL) SULFIDE

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Sulfur mustard (bis (2-chloroethyl) sulfide, HD) is a potent cutaneous vesicant that causes separation of the epidermal-dermal junction (EDJ). Isolated perfused porcine skin flaps (IPPSF) were dosed topically with 0.2, 5.0, and 10.0 mg/ml HD or ethanol (control) for 8 hr (dose response study) and 10.0 mg/ml HD or ethanol for 1, 3, 5, and 8 hr (time response study). Successful EDJ mapping was carried out in normal pig skin (NPS), ethanol-treated IPPSFs, and HD-treated IPPSFs using antibodies to fibronectin, epidermolysis bullosa acquisita (EBA), and GB3 (Nicein). Two mouse anti-human monoclonal antibodies, L3d and 19-DEJ-1 (Uncein), did not cross-react with the EDJ of the pig. Histochemical staining with fibronectin and GB3 was more discrete in NPS than in the IPPSF. No differences in staining were noted between the ethanol and nonblistered areas of the HD-treated IPPSFs. In HD-blistered areas, fibronectin and GB3 stained primarily the dermal interface with some fragment staining of the basal pole of the stratum basale cells, while EBA stained only the dermis. Mapping of these three epitopes further reinforces previous studies that the precise plane of EDJ separation in HD-treated skin occurs within the upper portion of the lamina lucida. The conservation of human epitopes in the EDJ of the pig further emphasizes the similarities between human skin and pig skin. (Supported by USAMRDC Contracts DAMD17-87-C-7139 and DAMD17-92-C-2071).

1736 AN AUTORADIOGRAPHY STUDY TO EVALUATE THE DISTRIBUTION OF BIS (2-CHLOROETHYL) SULFIDE IN THE ISOLATED PERFUSED PORCINE SKIN FLAP

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Bis (2-chloroethyl) sulfide (sulfur mustard, HD), a potent cutaneous vesicant and bifunctional alkylating agent, causes macroscopic and microscopic vesicles in the isolated perfused porcine skin flap (IPPSF). Six IPPSFs were topically dosed with 300 μl of 10.0 mg/ml ^{14}C-HD in ethanol (9.36 μCi) and perfused for 8 hrs. Skin samples were frozen and autoradiography (sandwich technique) performed on 4 μm cryosections to minimize diffusion of the ^{14}C-HD. The emulsion was exposed to sections containing ^{14}C-HD from 50–384 days at −20°C and processed to stabilize the silver halide grains. Autoradiographs revealed a high concentration of ^{14}C-HD within the upper epidermis which decreased with dermal depth. Specific binding was present within the capillaries of the dermis. Morphometric analysis of the autoradiographs demonstrated that ^{14}C-HD occurred predominantly within a tissue depth of 80 μm, which incorporates the entire epidermis and the superficial dermis. (Supported by USAMRDC, Contract DAMD17-92-C-2071).

1737 PROTECTIVE EFFECTS OF SODIUM THIOSULFATE, CYSTEINE, NIACINAMIDE, AND INDOMETHACIN ON SULFUR MUSTARD-TREATED ISOLATED PERFUSED PORCINE SKIN

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Sulfur mustard (HD), a bifunctional alkylating agent, causes dark basal cells (DBCs), vesication, and an increase in vascular resistance (VR). The isolated perfused porcine skin flap (IPPSF) was used to test the efficacy of pharmacological intervention of 4 compounds on HD dermal toxicity. IPPSFs were infused with 10^{-3}M of sodium thiosulfate, 10^{-2}M of cysteine, 10^{-3}M of niacinamide, or 2×10^{-4}M of indomethacin for 2 hrs, treated with 5.0 mg/ml HD, then perfused for an additional 6 hrs. Mean DBCs for HD alone was 19.7%, but decrease to 3.9% sodium thiosulfate, 4.2% cysteine, 5.8% niacinamide, or 1.0% indomethacin. Niacinamide and indomethacin caused a decrease in VR, while only indomethacin partially prevented microvesicles. These compounds alone would not be efficacious antivesicant agents. Blocking of DBCs did not prevent vesication, indicating that different mechanisms are involved in HD-induced DBCs, microvesicles, and VR. Therefore, there is a multistep, biochemical process which finally culminates in blister formation. (Supported by USAMRDC, DAMD17-92-C-2071).

1738 QUANTITATIVE PERCUTANEOUS ABSORPTION AND DISTRIBUTION OF BINARY MIXTURES OF TWO PHENOLS IN THE ISOLATED PERFUSED PORCINE SKIN FLAP

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A total of 62 8-hr isolated perfused porcine skin flap (IPPSF) topical experiments studying binary mixture (solute/solvent) absorption and distribution were conducted on ^{14}C-labeled phenol vs *p*-nitrophenol (PNP); concentration: 4 μg/cm^2 vs 40 μg/cm^2; vehicle: acetone vs ethanol; occluded vs nonoccluded. All IPPSFs were analyzed for absorption, depth of penetration, compound location, and total recoveries. Total recoveries were much greater in all cases for PNP than phenol. Absorption, penetration depth, and total recoveries of phenol were greater under occluded conditions than nonoccluded. Absorption, penetration depth, and total recoveries of PNP were greater under occluded conditions than nonoccluded in the low concentrations, but the opposite was observed with the higher concentrations of PNP. Absorption and penetration depth of phenol were greater with ethanol than with acetone under nonoccluded conditions, but the opposite was observed under occluded conditions. Absorption and penetration depth of PNP was greater with acetone than with ethanol under either occlusion condition. Percent absorption was greater in PNP in acetone than in phenol in acetone occluded or nonoccluded. There was a lower percentage from the higher dose of phenol absorbed, penetrated, and recovered under occluded and nonoccluded conditions and in PNP under occluded conditions, but the opposite was observed (lower percent dose in lower concentration) under nonoccluded PNP conditions. These results suggest that comparative absorption of phenol and PNP are vehicle-, occlusion-, and penetrant-dependent. Occlusion enhanced the vehicle effect on absorption, but reversed the distribution pattern. These findings suggest that single chemical data is not predictive of even binary mixtures and that exposure conditions further modulate disposition. (Supported by EPA Cooperative Agreement CR-818129).

1739 PERCUTANEOUS ABSORPTION OF PARATHION APPLIED IN MECHANISTICALLY DEFINED CHEMICAL MIXTURES ON ISOLATED PERFUSED PORCINE SKIN

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Attention is being directed to the risk assessment following cutaneous exposure of chemical mixtures. Using acetone (A) or dimethyl sulfoxide (DMSO, 80%) as a vehicle, percutaneous absorption and disposition of parathion (PA) and its metabolites were studied following PA (40 μg/cm^2) dosing on isolated perfused porcine skin flaps as chemical mixtures consisting of sodium lauryl sulfate (SLS), methyl nicotinate (MN), and stannous chloride ($SnCl_2$). A 2^4 factorial design was used to asses treatment differences. Preliminary studies indicated that: PA was absorbed more with DMSO than with A; SLS enhanced PA absorption with DMSO and A; MN, $SnCl_2$, or MN + $SnCl_2$ did not show effects on PA absorption; MN increased SLS's effect with DMSO and A, indicating a synergistic interaction between MN and SLS. Higher level interactions were also noted. These studies indicated multiple levels of interactive effects on PA absorption which will be incorporated into a dermatopharmacokinetic model to identify critical mechanisms which affect risk assessment of topically-exposed chemical mixtures.

1740 PERCUTANEOUS ABSORPTION AND TOXICITY OF THE BENZIDINE DYE DIRECT RED 28 IN A MECHANISTICALLY DEFINED CHEMICAL MIXTURE USING ISOLATED PERFUSED PORCINE SKIN FLAP

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Humans are more likely to be exposed to chemical mixtures than to a single chemical at hazardous waste sites. Mechanistically defined chemicals (MDCM) can potentially consist of a marker chemical and 1 to 4 other chemicals, each of which can have different mechanistic actions on dermal penetration and absorption of the marker chemical. Sixteen mixtures, consisting of a marker chemical (direct red 28, DR28), a solvent (acetone or dimethyl sulfoxide, DMSO), a surfactant (0 or 10% sodium laurel sulfate, SLS), a vasodilator (0 or 180 μg methyl nicotinate, MNA), and a reducing agent (0 or 2% stannous chloride, $SnCl_2$) were selected. Isolated perfused porcine skin flaps (IPPSF) were utilized. For each of the 16 skin flaps, 50 $\mu g/cm^2$ DR28 was topically applied. Perfusate samples were collected for 8 hrs and skin biopsies were obtained for histology. Preliminary data suggest that little or no DR28 or its carcinogenic benzidine metabolites were absorbed. Light microscopic evaluations depicted minor alterations (inter- and/or intracytoplasmic edema) with DR28 mixtures containing SLS and/or DMSO. Use of an MDCM experimental design will identify if additive, synergistic, or inhibitory interaction occur.

1741 CYTOKINE PROFILE IN NORMAL HUMAN EPIDERMAL KERATINOCYTES FOLLOWING *IN VITRO* EXPOSURE TO ANTHRALIN AND ITS DERIVATIVE 9-ANTHRONE

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The antipsoriatric and tumor-promoting biological actions of anthralin are believed to be a result of autooxidation and the generation of reactive oxygen species. Elucidation of this mechanism could provide insight into the inflammatory response of the skin and allow the study of the role of proinflammatory cytokines, chemokines and growth factors in inflammatory pathology. Anthralin and seven derivatives were studied to determine structure-activity determinates in their ability to affect Il-1a, IL-1b, Il-1r, Il-6, Il-8, GM-CSF, TGFa, TNFa mRNA transcription and protein secretion. Dose response relationships were demonstrated for Il-1r, Il-6, Il-8, GM-CSF and TNFa transcript induction by RT-PCR at 1.5 and 25 μM. Protein secretion will be measured by ELISA. The *in vitro* inclusion of intra and extra cellular antioxidants resulted in inhibition of cytokine transcription implicating reactive oxygen intermediates as second messengers for gene activation. Utilizing the mouse ear swelling assay the ability of exogenously administered antioxidants to reduce anthrone inflammation *in vivo* will be studied. Cytokine profiles of keratinocytes in response to 9-anthrones will be used to define structure activity relationships with respect to the biological properties of the compounds.

1742 CONSTITUTIVE CYTOKINE GENE EXPRESSION IN SKIN^2™ HUMAN EQUIVALENT SKIN CULTURES

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SKIN^2™ is a three-dimensional human skin model consisting of stratified human dermal fibroblasts and differentiated layers of epidermal keratinocytes with an attached functional stratum corneum. The presence of the stratum corneum in this full barrier skin model allows simulation of human topical exposures and application of non-aqueous materials. In order to better characterize this model, constitutive levels of cytokine gene expression were determined using untreated SKIN^2™ cultures and Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR).

IL-1α, IL-1β, IL-6, IL-8, IL-10, TNFα, GM-CSF, TGFβ1, and TGFβ2 were found to be constitutively expressed. IL-12 p35 was also expressed in untreated cultures, but the inducible IL-12 p40 was not. IL-10 was the least abundant constitutive transcript, detectable in 200–400 ng of total RNA and 30 cycles of PCR. IL-8 was the most abundant cytokine transcript, being easily detected in 5 ng of total RNA and 24 cycles of PCR. The level of IL-8 expression was comparable to that of the G3PDH housekeeping gene.

These studies demonstrate constitutive expression of a panel of cytokine genes in this *in vitro* human skin model. This knowledge will be useful in future evaluation of cytokine message modulation by chemical irritants and sensitizers.

1743 INTERLEUKIN-1α AND INTERLEUKIN-8 RELEASE FROM HUMAN KERATINOCYTE CELL CULTURE TREATED WITH SURFACTANTS

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Effects of cosmetic surfactants on interleukin-1α (IL-1α) and interleukin-8 (IL-8) release from human keratinocytes(HK) were tested *in vitro* for prediction of irritation potentials of chemicals. After exposure of surfactants, an amount of IL-1α and IL-8 released into culture supernatant was measured by enzyme-linked immunosorbent assay. Cytotoxicity of surfactants was evaluated for residual cells by neutral red uptake assay(NRU assay). Sodium laurylsulfate(SLS), lauryltrimethylammonium chloride(LTAC), alkylamidobetaine (AAB), and polyoxyethylenealkylether(POAE) were used as test materials. Half effective concentrations of cytotoxicity (EC_{50s}) by NRU assay were 35, 5.8, 220, and 0.58 μg/ml for SLS, LTAC, AAB, and POAE, respectively. At the lethal concentration of test materials, IL-1α release from HK was increased 7 to 15 times as high as non-treated control. SLS and LTAC did not stimulate IL-8 release while 100 μg/ml of AAB and 1 mu;g/ml of POAE increased the release 3 to 4 times as compared to the control. These results indicate that IL-1α release, but not IL-8 release, reflects cytotoxicity of the test materials. On the contrary, increased IL-8 release by AAB may account for the irritation potential of this chemical, of which cytotoxicity was weak. We conclude that measurement of IL-8 release from HK may predict irritation potential of materials which cannot be detected by cytotoxicity testing.

1744 ALCOHOL-INDUCED TOXICITY, ARACHIDONIC ACID RELEASE AND CYTOKINE mRNA PROFILE OF CULTURED HUMAN KERATINOCYTES

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The release of cytokines and inflammatory mediators from chemically injured keratinocytes (KCs) are believed to play a role in irritant allergic dermatitis and their induction may represent *in vitro* endpoints that are predictive of the *in vivo* toxicity. Multiple endpoints were utilized to test toxicity of three alcohols of different potencies: Ethyl alcohol, Isopropyl alcohol and 2-Butoxyethanol. Tetrazolium MTT incorporation was used to evaluate cell toxicity. MTT_{50} ranked 2-Butoxyethanol > Isopropyl alcohol > Ethyl alcohol in toxicity. Prelabelled KCs with ^{3}H arachidonic acid (AA) exposed to the alcohols induced minimal release at concentrations up to 1%. To investigate the changes in cytokine profile, we used RT-PCR technique to assess cytokine mRNA signals. Preliminary results demonstrated that ethyl alcohol upregulated IL-6 and IL-8. Isopropyl alcohol upregulated IL-8 and 2-Butoxyethanol upregulated IL-1α and IL-8. Cytokine TNFα was not upregulated with all 3 alcohols at the concentrations tested. These results indicated that these 3 alcohols exhibit cell toxicity and that this toxicity is not related to the release of arachidonic acid from cell membrane. However, these alcohols do seem to stimulate various proinflammatory and chemotactic cytokine message. Cytokine and mediator profile may thus provide an *in vitro* assay to evaluate the potential toxicity of a alcohol.

1745 UVB IRRADIATION OF AN ORGANOTYPIC SKIN MODEL, EpiDerm™, RESULTS IN SIGNIFICANT RELEASE OF CYTOKINES

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Ultraviolet light especially UVB (290–320 nm) is believed to be responsible for most of the immediate and long term effects of excessive exposure to sunlight. In many cases, the initial stages of these UV induced changes are the release of cytokines. Although cytokine release from human keratinocytes in monolayer culture following UV exposure has been studied, the results of UVB exposure to keratinocytes in fully differentiated epidermal models have not been adequately delineated. UVB irradiation of EpiDerm, a commercially available, highly differentiated model of the human epidermis, resulted in significant increases in PGE-2 and IL-1α, even at UV fluxes which were not cytotoxic, as determined using the MTT viability assay. At UVB doses of up to 100 mj/cm^2, MTT viability remained at or above 100% whereas PGE-2 and IL-1α release increased to 3.4 and 2.7 fold of baseline cytokine levels, respectively. Hence, EpiDerm may prove to be a useful tool in understanding the immunological and inflammatory reactions associated with UV irradiation and other related skin pathologies.

1746 TECHNIQUES FOR QUANTIFYING UNCERTAINTY IN RISK ASSESSMENT

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The various steps performed in conducting a risk assessment are accompanied by differing degrees of variability (e.g. randomness in a population) and uncertainty (e.g. lack of fundamental knowledge). This workshop will focus on approaches that can be used by a risk assessor for quantifying these uncertainties. The first speaker will discuss quantifying the uncertainty and variability in the concentrations of toxicants in the environment, their fate and transport, and the parameters of the exposure assessment. The second speaker will describe techniques of Monte-Carlo simulation and sensitivity analysis for for characterization of estimates of tissue dose from toxicokinetic models. The next speaker will elaborate on uncertainty in dose-response by describing the relationship of tissue dose to short-term toxic effects such as mutagenicity, cytotoxicity, and induced cellular replication. The last speaker will describe the process of decision analysis in which Bayesian concepts of subjective probability can be used with probability trees and elicited expert judgments to address uncertainties in modeling carcinogenic potencies. Following the formal presentation, a discussion session will be directed at several key issues concerning the imprecision of risk numbers. Issues to be discussed include extrapolation of animal data to humans, Bayesian concepts compared to classical analysis, probabilistic analysis versus point estimates and resolution of conflicting data.

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