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# Rodent Cancer Bioassay: Evaluating Mode of Action & Human Relevance

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*This talk represents the view of presenter & does not necessarily represent the decisions or the stated policies of the EPA.*

## How Do We Assess Human Health Risks?

- Relies heavily on laboratory animal data
- Relies on extrapolations, inference methods, safety factors, etc
  - Animal Biology = Human Biology
  - Effects found at high animal doses predict effects at environmental levels of exposure
  - Current animal assays provide adequate coverage for predicting effects on human health including susceptible groups

## Ten Most Prevalent Tumor Sites in Rodents (<http://potency.berkeley.edu/pathology.table.html>)

Rats (N=564 carcinogens)			Mice (N=442 carcinogens)		
Site	No. of Positive Chemicals	%	Site	No. of Positive Chemicals	%
Liver	222	40	Liver	254	57
Mammary gland	107	19	Lung	121	27
Kidney	94	17	Stomach	69	16
Stomach	88	16	Vascular system	64	14
Hematopoietic system	57	10	Hematopoietic	54	12
Lung	58	10	Kidney	27	6
Urinary bladder	52	9	Mammary gland	22	5
Nasal cavity / turbinates	50	9	Thyroid gland	21	5
Ear / Zymbal's gland	42	7	Urinary bladder	12	3
Esophagus	37	7	Uterus	12	3

## Quantitative Chemical Potency (QCP) Matrix\* Rat 2-Year Chronic/Cancer Data

Chemical Class	CAS No.	Chemical Name	Clinical Chemistry and Hematology					Body Weight	Organ Weight				NonNeoplastic Pathology				Neoplastic Pathology			
			ALT	AST	WBC	RBC	Chol		Liver	Kidney	Thyroid	Testes	Liver	Kidney	Thyroid	Testes	Liver	Kidney	Thyroid	Testes
Acylamino acid & Amide	57837-19-1	Metolachl																		
Acylephenoxypropionic	51238-27-3	Dichlof-methyl																		
Benzoyloxylohexanedione	104206-62-8	Mesotrione																		
Conazole (Triazole)	112281-77-3	Tetraconazole																		
Pyridylmethylamine	11988-49-9	Triadoprid																		
Pyrimidine	80168-88-9	Fenarimol																		
Diphenyl (bridged)	115-32-2	Dicofol																		
Phenylurea	330-55-2	Linuron																		
Conazole (imidazole)	35554-44-0	Imazali																		
Conazole & Dicarboximide	36734-19-7	Iprodione																		
Conazole (Triazole)	94381-06-5	Cyproconazole																		
Conazole (Triazole)	114389-43-6	Fenbuconazole																		
Pyrethroid	52945-53-1	Permethrin																		
Pyridine	1918-02-1	Picloram																		
Acetaldehyde	108-62-3	Metaldelyle																		
Chloroacetanilide	1918-16-7	Propachlor																		
Conazole (Triazole)	119495-69-3	Difenoconazole																		
Chitin synthesis inhibitors	116714-48-6	Hexacon																		
Cyclohexene oxime	148679-41-9	Tepraloxym																		
Conazole (Triazole)	4312-143-3	Triadimefon																		
Carbamate	63-25-2	Carbayl																		
Cyclohexene oxime	87820-88-0	Tralkoxydim																		
Moulting hormone agonists	161050-68-4	Methoxyfenozide																		
Methylthioiazine	834-12-8	Ametyrn																		
Phosphonate	52-88-6	Trichlorfon																		
Sulfonamide & Triazolopyrimidine	219714-95-2	Penoxsulam																		
Conazole (Triazole)	88671-99-0	Myclobutanil																		
Triazinyl sulfonurea	94125-34-5	Prosulfuron																		
Organothiophosphate (Phenyl)	55-38-9	Fenitron																		
Organophosphate (Pyrimidine)	96182-53-5	Phosteleptim																		
Conazole (Triazole)	55219-95-3	Triadimenol																		
Dinitroaniline & Sulfonamide	12044-98-3	Oryzalin																		
Spinosyn	131029-60-7	Spinosad																		
Dinitroaniline	40487-42-1	Pendimethalin																		
Organothiophosphate (Aliphatic)	1634-78-2	Milaxon																		
Carbamate (Oxime Carbamate)	50666-26-0	Thiodicarb																		
Carbamate (Oxime Carbamate)	23135-22-0	Oxaryl																		
Carbamate (Polymeric dithio)	9006-48-2	Metiram																		
Carbamate (Thio)	2212-67-1	Molinate																		
Carbazate	148877-41-8	Bifenazate																		
Chloroacetanilide	34255-82-1	Acetochlor																		
Chlorotriazine	1912-24-9	Atrazine																		
Herbicide Safener	135590-91-9	Mefapyr-diethyl																		
Imidazolone	81334-34-1	Imazapyr																		
Imidazolone	114311-32-9	Imazamox																		
Organophosphate	7798-34-7	Mevinphos																		
Organothiophosphate	65-39-2	Parathion																		
Organothiophosphate (Phenyl)	298-00-0	Methyl parathion																		
Phenoxyacetic	94-74-6	MCPA																		
Pyrethroid	62918-63-5	Deltamethrin																		
Pyrethroid	86359-37-6	Cyfluthrin																		
Pyrethroid	91465-08-6	lambda-Cyhalothrin																		
Pyrimidinyl sulfonurea	208495-21-8	Mesosulfuron-methyl																		
Pyrimidinyl sulfonurea	86209-51-0	Primsulfuron-methyl																		
Quinoline	12445-18-7	Quinoxifen																		
Quinolincarboxylic acid	84087-01-4	Quinclorac																		
Thiadiazinol	25057-99-0	Bentazon																		
Triazinyl sulfonurea	101200-48-0	Tribenuron																		

**ToxRef Database - Searchable relational database populated with detailed animal toxicity endpoints**

- Contains summary phenotype data from detailed animal studies gathered by on pesticide active ingredients.
- Will be used to support toxicity predictions from ToxCast

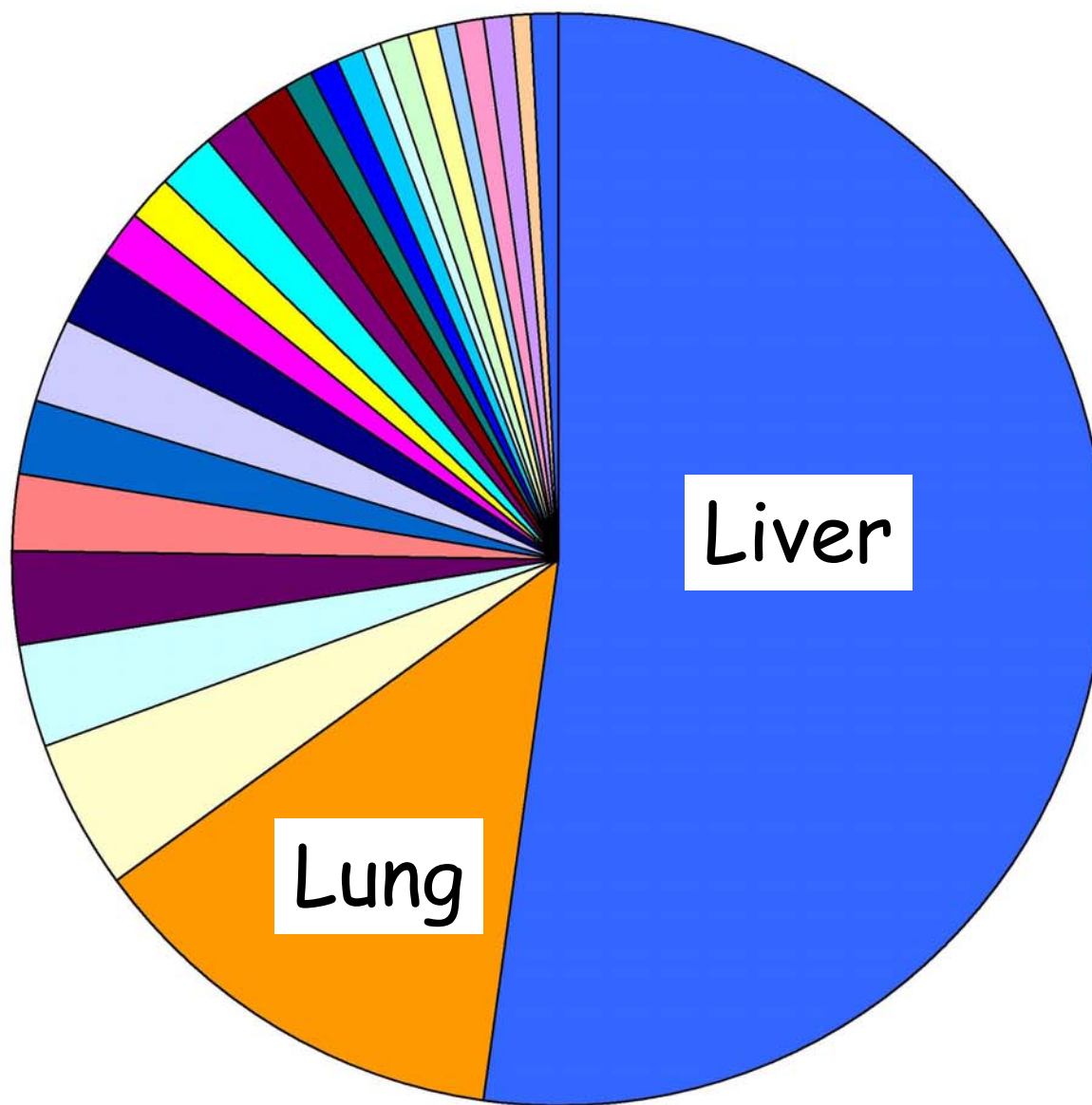
\*All data used in this analysis were derived from acceptable OPPTS/OECD health effects guideline studies but have not undergone internal QC

Legend	Increase	Decrease
No Effect Observed		
≤ 10 mg/kg/day		
≤ 100 mg/kg/day		
≤ 1000 mg/kg/day		
>1000 mg/kg/day		

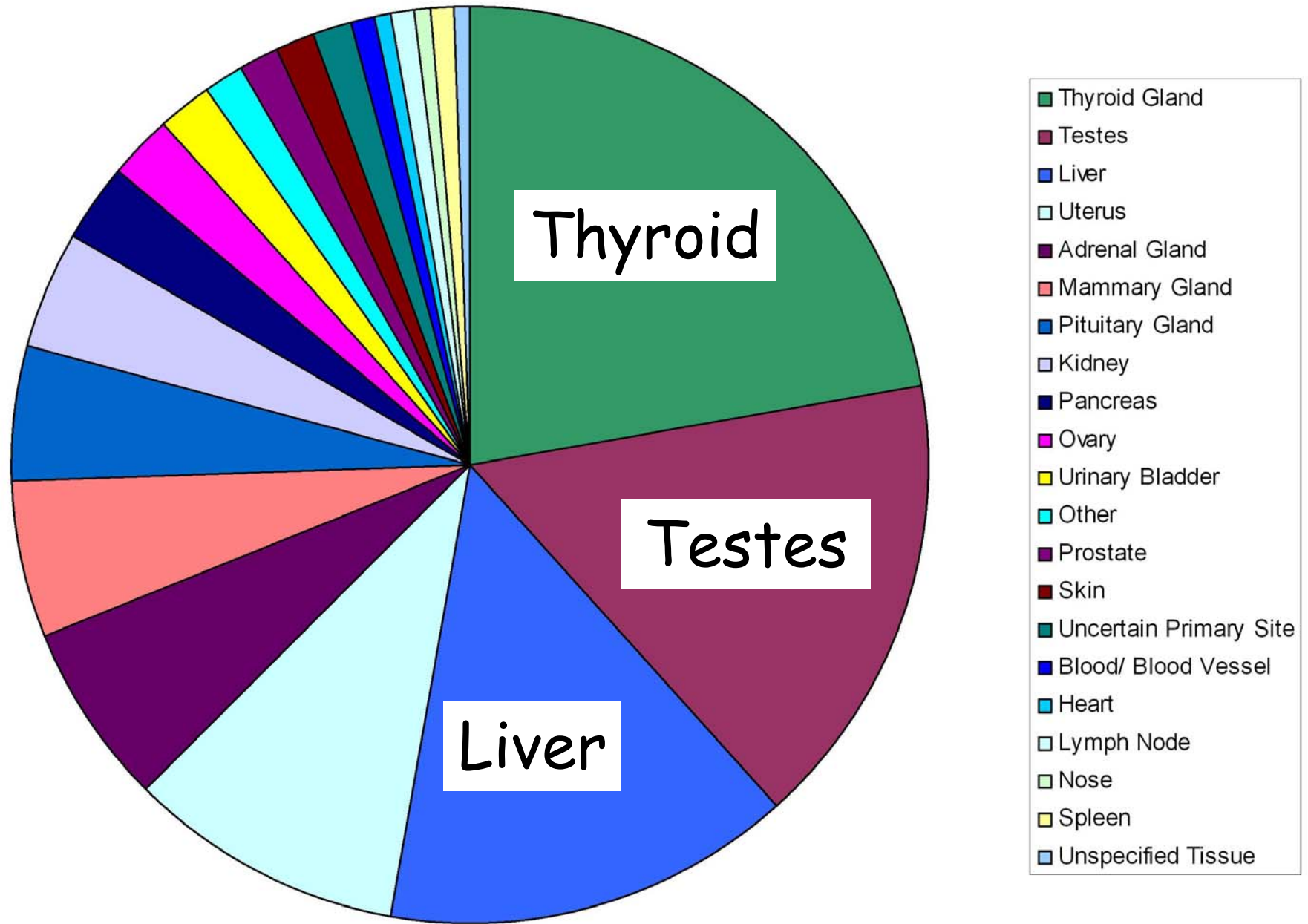
## Pesticide Rodent Tumor ToxRef Database

# Mouse Tumor Distribution (219 Pesticides)

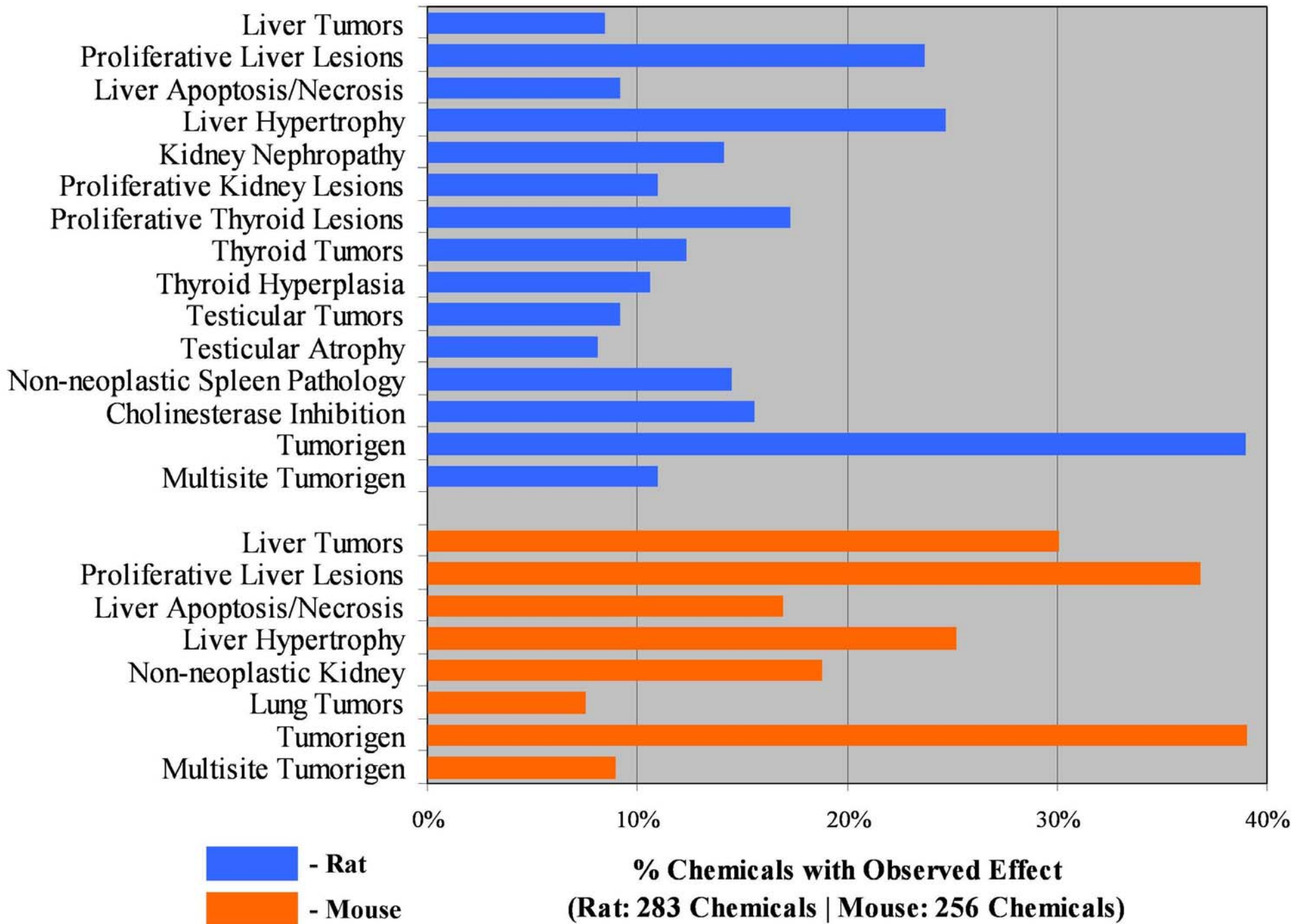
- Liver
- Lung
- Ovary
- Blood/ Blood Vessel
- Kidney
- Other
- Bone
- Harderian Gland
- Unspecified Tissue
- Adrenal Gland
- Intestinal Tract
- Mammary Gland
- Thymus Gland
- Urinary Bladder
- Brain
- Heart
- Lacrimal Gland
- Pancreas
- Pituitary Gland
- Salivary Gland
- Skin
- Spleen
- Stomach
- Testes
- Thyroid Gland



# Rat Tumor Distribution (219 Pesticides)



Pesticide Rodent Tumor ToxRef Database



## Ten Most Prevalent Tumor Sites in Humans (NCI SEER Cancer Statistics Review 1975-2005)

Site	Incidence/100,000
Prostate (male)	163
Breast (female)	126
Lung & Bronchus	79
Colon and rectum	59
Urinary bladder	37
Skin melanoma	25
Lymphoma**	24
Corpus uteri (female)	23
Kidney & Renal pelvis (male)	18
Oral cavity and pharynx	16

\*\*\*Non-Hodgkin's lymphoma; <sup>1</sup> age-adjusted to 2000 US population

# Mode of Action (MoA) Data in Risk Assessment

What have we learned?

Data from rodent assay in isolation is inadequate to predict human hazard

Value of  
Mode of  
Action Data



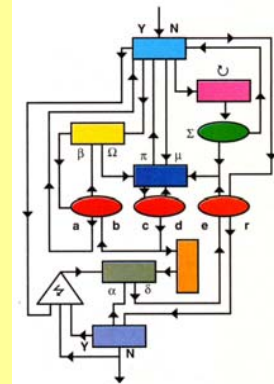
- Tumors predictive of human hazard?
- Significant species differences in response?
- Appropriate dose response extrapolation method?

# Approach: MoA/Human Relevance Framework

## History

- EPA & IPCS 1999-2001  
Adopts Conceptual Framework for Evaluating a Mode of Action for Chemical Carcinogenesis.
- ILSI 2003
  - Framework for human relevance analysis of information on carcinogenic modes of action
- ILSI 2005
  - Extends Framework to noncancer outcomes & life stage information
- IPCS 2006 & 2008
  - Adopts Human Relevance Framework

MoA = Plausible hypothesis with measured key events (vs detailed molecular description of causality)



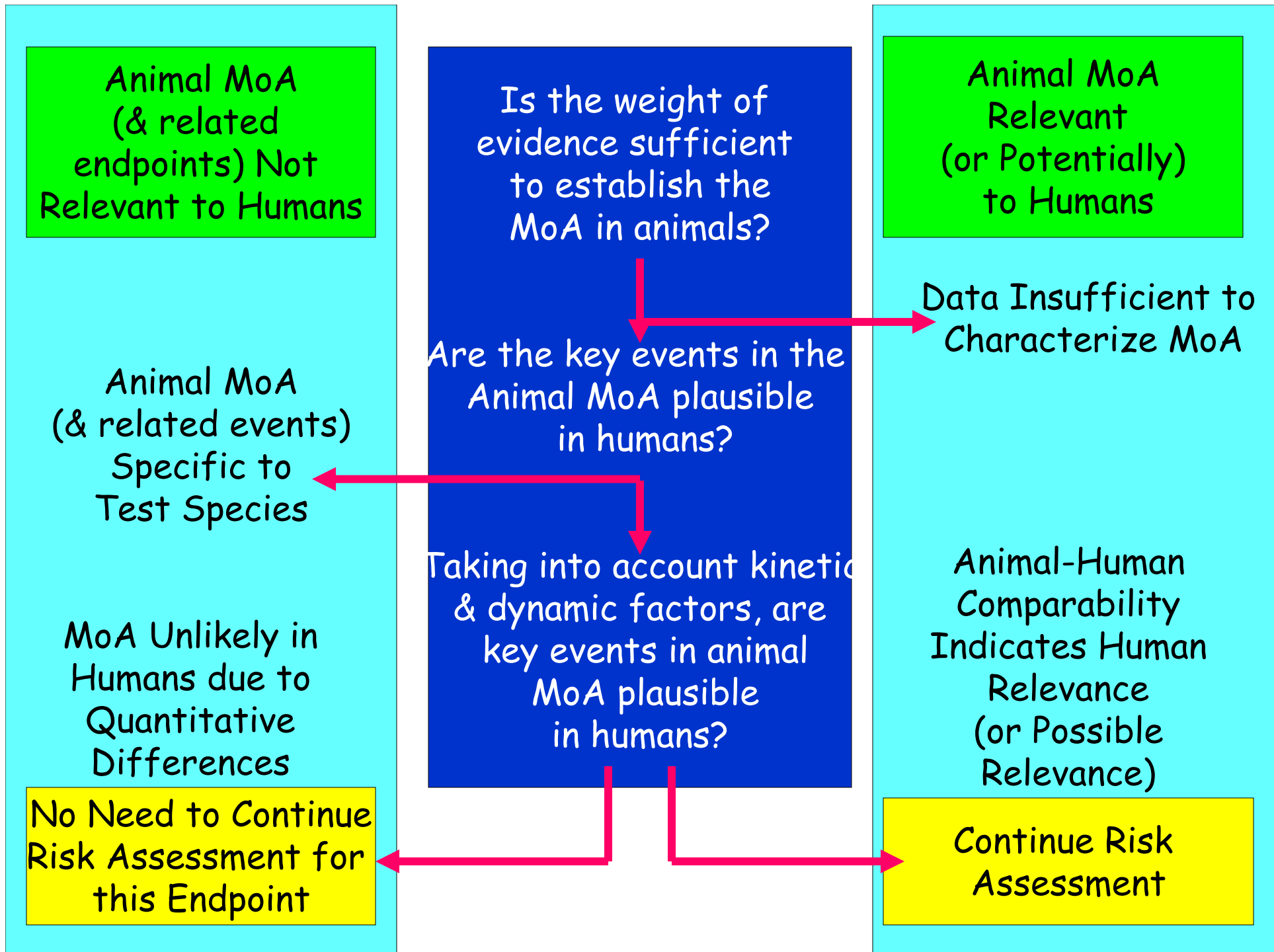
Key Event: Critical, Rate Limiting, Quantifiable

# Reasons for a Framework Approach to MOA & Human Rrelevance

- Provides Transparency
  - clarifies extent of weight of evidence as a basis for decision making
  - not prescriptive but designed to organize information
- Ensures Rigor of Evaluations & Consistency of Documentation
- Aids in Identification of Critical Data or Research Needs
  - basis for iterative dialogue between risk assessors/researchers

## Sufficient Weight of Evidence to Establish MoA in Animals?

- Weight of evidence for a toxicological response in experimental animals
- Postulated MoA (theory of the case)
- Experimental support for key events
  - Concordance of dose-response relationships
  - Temporal association
  - Strength, consistency and specificity of association of toxicological effect with key events
  - Biological plausibility and coherence
- Other possible MoAs
- Uncertainties, inconsistencies, & data gaps

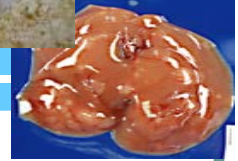


## Q2 & 3 Concordance Analysis: Melamine

<i>Key Event</i>	<i>Animals</i>	<i>Humans</i>
Urinary concentration adequate for precipitation	<i>Yes</i>	YES. data on toxicokinetics would help better define the likelihood
Formation of calculi	<i>Yes</i>	YES. But probability in humans unknown
Urothelial damage & regeneration	<i>Yes</i>	YES. But risk would be reduced due to anatomical differences
Urothelial tumor formation	<i>Yes</i>	No epidemiological evidence, but may well simply be that exposure is not sufficient to produce a response

# Mode of Action & Rodent Liver Tumors

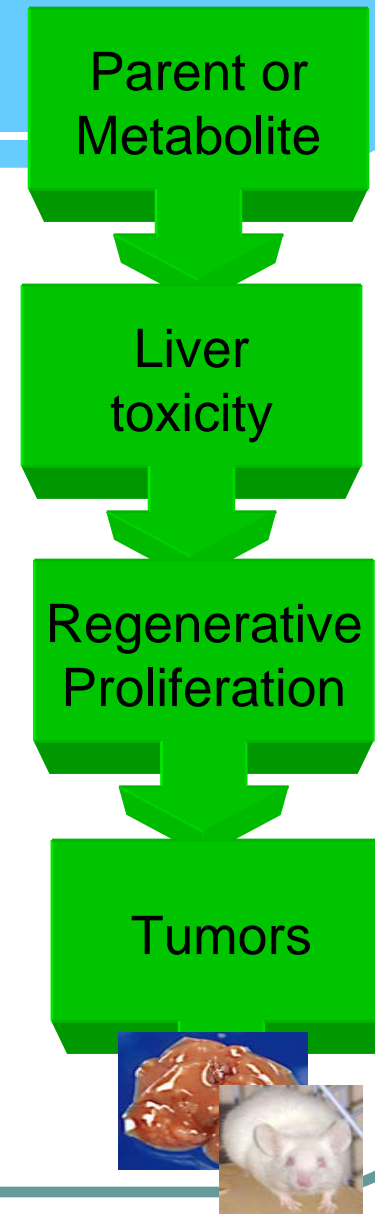
## Pesticide Database



- Types of non-genotoxic hepatocarcinogens typically encountered
  - Mitogenesis & clonal expansion of preneoplastic foci
    - Nuclear receptor activation (e.g., PPAR $\alpha$ , CAR)
    - Associated with P450 modulation (may not necessarily be a key event)
    - [perturbation of hormonal balance, which may or may not be due to a receptor-mediated process]
  - Chronic cell injury & death, regeneration, compensatory hyperplasia

# An Example of MoA Human Relevance Framework Approach

- Dose response & temporal concordance of key events & tumors
- Presence of sustained toxicity
  - histopathology (necrosis and/or apoptosis) with or without enzyme changes (possibility of other markers)
- Sustained increased cell divisions
  - measured by BrdU labeling index and/or cell number, may need to collect data in a zonal way
- Evaluation & elimination of other modes of action including DNA reactivity



## Thiamethoxam Induced Mouse Liver Tumors and Their Relevance to Humans Part 1: Mode of Action Studies in the Mouse

Trevor Green,<sup>\*1</sup> Alison Toghil,<sup>\*</sup> Robert Lee,<sup>\*</sup> Felix Waechter,<sup>\*</sup> Edgar Weber,<sup>\*2</sup> and James Noakes<sup>\*</sup>

<sup>\*</sup>Syngenta Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, United Kingdom

Received 11/10/04

Thiamethoxam, a neonicotinoid insecticide, was mutagenic either *in vitro* or *in vivo*, caused an increase in liver tumors in mice when fed in the diet for concentrations in the range 500 to 2500 ppm. A number of studies of up to 50 weeks duration have been conducted to identify the mode of action for the development of liver tumors seen at the end of the cancer bioassay. Both thiamethoxam and its major metabolites have been tested in these studies. The duration of a 50-week thiamethoxam dietary feeding study in mice, the earliest change, within one week, is a marked increase (by up to 40%) in plasma cholesterol. This was followed later by evidence of liver toxicity including single cell necrosis and an increase in apoptosis. After 20 weeks there was an increase in hepatic cell replication rates. All of these changes persisted from the time they were first observed until the end of the study at 50 weeks. They occurred in a dose-dependent manner and were only observed at doses (500, 1250, 2500 ppm) at which liver tumors were increased in the cancer bioassay. There was no effect level of 200 ppm. The changes seen in the cancer bioassay are consistent with the development of liver cancer in mice on the basis of the mode of action. When the major metabolites of thiamethoxam, CGA322704, CGA265307, and CGA265307, were tested in dietary feeding studies of up to 20 weeks duration, the metabolite CGA330050 induced the same changes as thiamethoxam in the thiamethoxam feeding study. It was concluded that thiamethoxam is hepatotoxic and hepatocarcinogenic in mice through its metabolism to CGA330050. Metabolite CGA265307 is also shown to be an inhibitor of inducible nitric oxide synthase and to increase the hepatotoxicity of carbon tetrachloride. CGA265307, through its effects on nitric oxide synthase, exacerbates the toxicity of CGA330050 in treated mice.

**Key Words:** thiamethoxam; liver tumors; mode of action

The authors acknowledge that they are employed by Syngenta Crop Protection who owns the patent on the compound that appears in this article.

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## Thiamethoxam Induced Mouse Liver Tumors and Their Relevance to Humans Part 2: Species Differences in Response

Trevor Green,<sup>\*1</sup> Alison Toghil,<sup>\*</sup> Robert Lee,<sup>\*</sup> Felix Waechter,<sup>\*</sup> Edgar Weber,<sup>\*2</sup> Richard Pepper,<sup>†</sup> James Noakes,<sup>\*</sup> and Mervyn Robinson<sup>\*</sup>

<sup>\*</sup>Syngenta Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, United Kingdom

Received 11/10/04

Thiamethoxam is a neonicotinoid insecticide that is a potent mutagen, but it did cause a significant increase in liver tumors in mice, but not rats, in chronic dietary feeding studies in mice have characterized a carcinogenic mode of action that involved depletion of plasma cholesterol and increases in cell replication rates. In a study reported in this article, female rats have been exposed to thiamethoxam in the diet at concentrations of 0, 1000, and 3000 ppm for 18 months in a study design directly comparable to the mouse study. The mode of action changes were characterized. In the mouse study, thiamethoxam had no adverse effects on either the biochemistry or the histopathology of the liver at any time point during the study. Replication rates were not increased, in fact they were decreased at several time points. The lack of effect on the liver was entirely consistent with the lack of liver tumor formation in the two-year cancer bioassay. Comparisons of the mode of action of thiamethoxam in rats and mice have shown that the mode of action of the parent chemical were either similar or higher than in mouse blood in both single dose and the diet studies, strongly indicating that thiamethoxam itself is unlikely to play a role in the development of liver tumors. In contrast, the major metabolites, CGA265307 and CGA330050, were shown to play a role in the development of liver tumors in rats. In the mouse study, the major metabolic pathways of thiamethoxam in the mouse, rat, and human liver fractions have shown that the rates in humans are lower than those in the rat study. Thiamethoxam is unlikely to pose a hazard to humans at this chemical at the low concentrations found in the

## Case Study: Weight of Evidence Evaluation of the Human Health Relevance of Thiamethoxam-Related Mouse Liver Tumors

Timothy Pastoor,<sup>\*1</sup> Patrick Rose,<sup>†</sup> Sara Lloyd,<sup>†</sup> Richard Pepper,<sup>\*</sup> and Trevor Green<sup>†</sup>

<sup>\*</sup>Syngenta Crop Protection, Inc., P.O. Box 18300, Greensboro, North Carolina 27455, and <sup>†</sup>Syngenta Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, United Kingdom

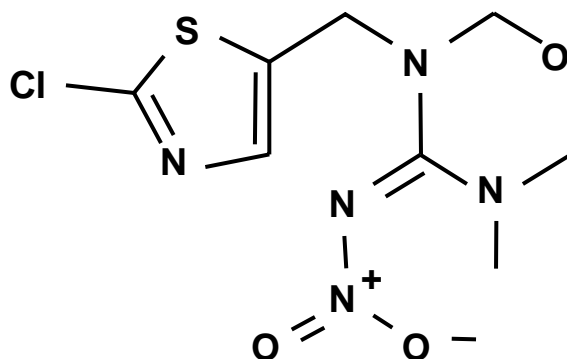
Received November 7, 2004; accepted January 23, 2005

Thiamethoxam (CGA293343; 3-(2-chloro-thiazol-5-ylmethyl)-5-methyl-[1,3,5]oxadiazinan-4-ylidene-N-nitroamine) was shown to increase the incidence of mouse liver tumors in an 18-month study; however, thiamethoxam was not hepatocarcinogenic in rats. Thiamethoxam is not genotoxic, and, given the late life generation of mouse liver tumors, suggests a time-related progression of key hepatic events that leads to the tumors. These key events were identified in a series of studies of up to 50 weeks that showed the time-dependent evolution of relatively mild liver dysfunction within 10 weeks of dosing, followed by frank signs of hepatotoxicity after 20 weeks, leading to cellular attrition and regenerative hyperplasia. A metabolite, CGA330050, was identified as generating the mild hepatic toxicity, and another metabolite, CGA265307, exacerbated the initial toxicity by inhibiting inducible nitric oxide synthase. This combination of metabolite-generated hepatotoxicity and increase in cell replication rates is postulated as the mode of action for thiamethoxam-related mouse

liver tumors. Thiamethoxam is a neonicotinoid insecticide that has been extensively tested in animal models for short- and long-term toxicological effects. An increased incidence of liver tumors was seen in male and female Tif:MAGf mice when fed in the diet for 18 months at concentrations up to 2500 ppm. In marked contrast, there were no increases in cancer incidences either in the liver, or at any other site, in rats fed on diets containing up to 3000 ppm thiamethoxam for two years. Furthermore, thiamethoxam was not genotoxic.

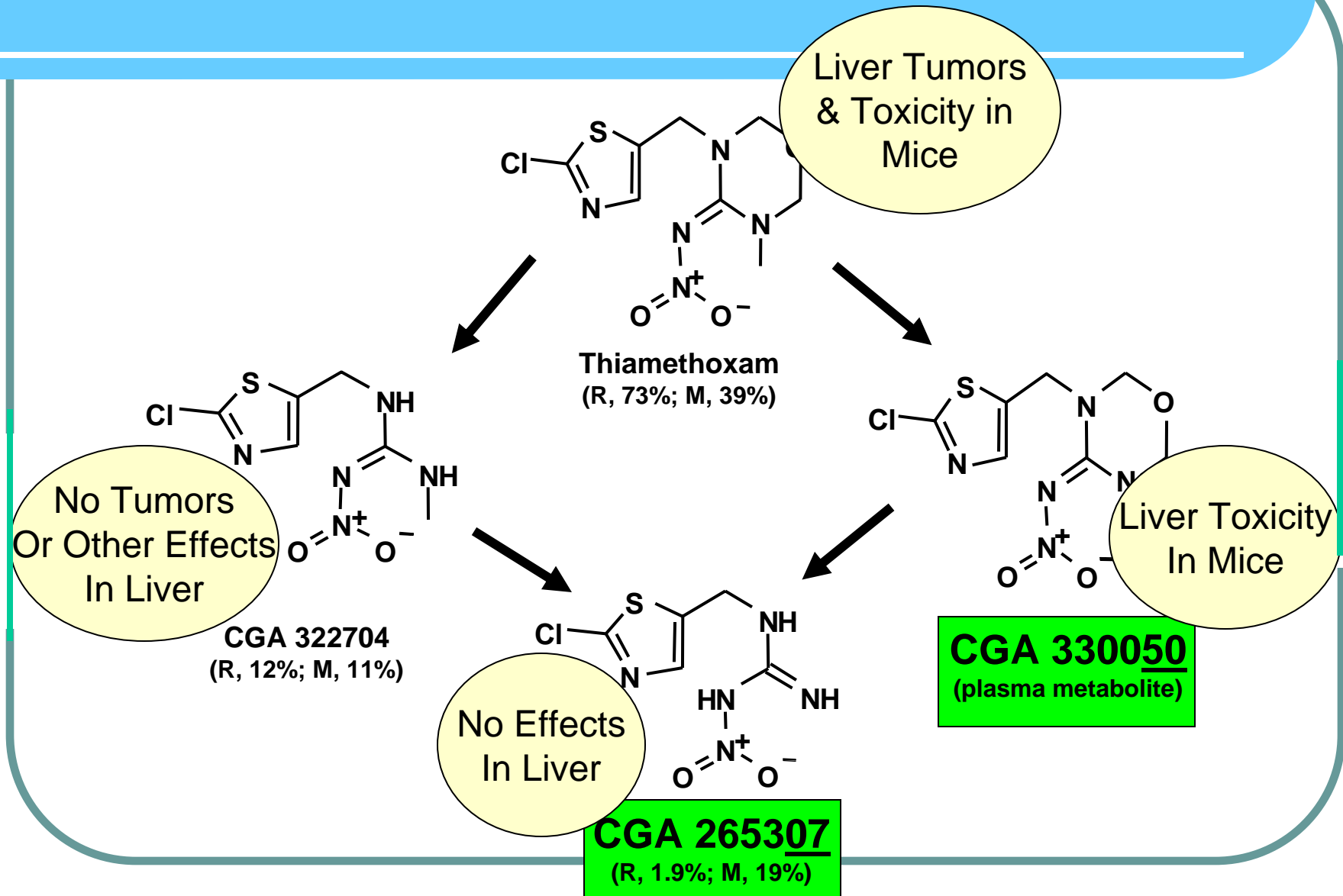
Previous articles in this series reported the development of a mode of action for the mouse-specific liver tumorigenesis (Green *et al.*, 2005a), as well as an explanation for the species differences in response and metabolism between the rat and the mouse and the significance of these species differences to human health (Green *et al.*, 2005b). Given that a well-defined mode of action can be described for the thiamethoxam-related

# Thiamethoxam

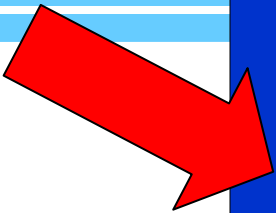


- Liver tumors at 18 months in male & female mice
- No liver tumors in rats
- Not genotoxic

# Major Metabolic Pathways of Thiamethoxam




# Thiamethoxam Mouse Liver Tumors



Is the weight of evidence sufficient to establish the MoA in animals?



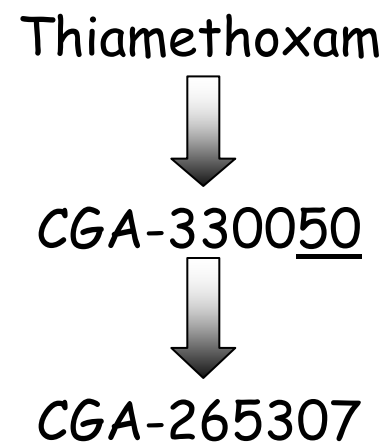
Are the key events in the Animal MoA plausible in humans?



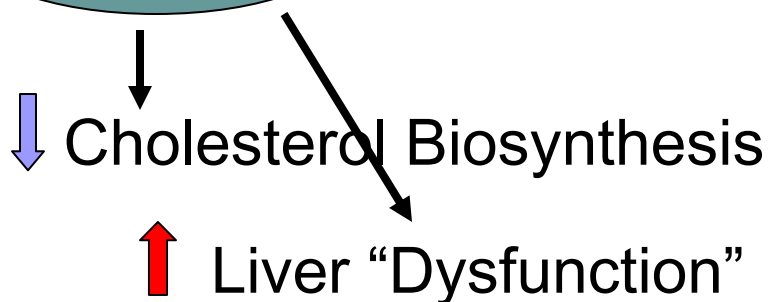
Taking into account kinetic & dynamic factors, are key events in animal MoA plausible in humans?

# Postulated Animal Mode of Action

Key Metabolic Pathway:



Metabolite 50



Metabolite 07

inhibition of  
iNOS

↑ Hepatotoxicity  
(sustained)

↑ Cell Proliferation ..... → *Neoplasia*  
(sustained)

# Human Relevance Framework: Q 1. Weight of Evidence Sufficient to Establish MoA


- Experimental Support: Dose Response Concordance (Thiamethoxam Dietary Study)

Key Event ( <i>Green et al., 2005</i> )	LOAEL
Generation of Critical Metabolites	
Generation of Liver "Dysfunctional" Changes	≥ 500 ppm
Sustained Hepatotoxicity	≥ 500 ppm
Sustained Cell Proliferation	≥ 500 ppm
Tumors	≥ 500 ppm

\*Only Metabolite "50" results in the same key events as found after thiamethoxam treatment

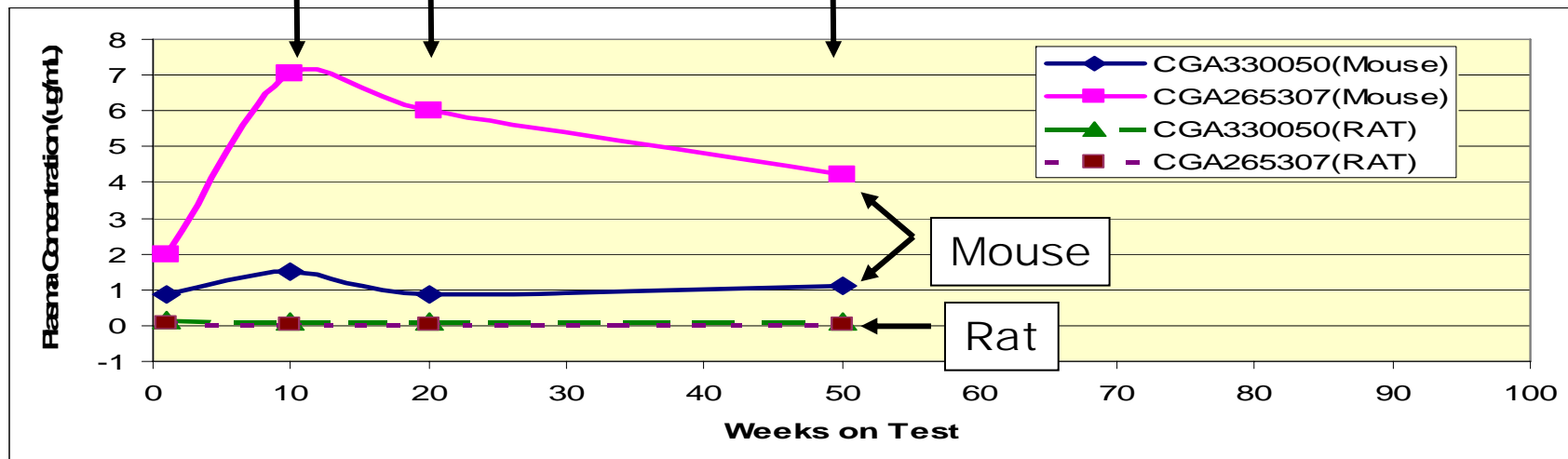
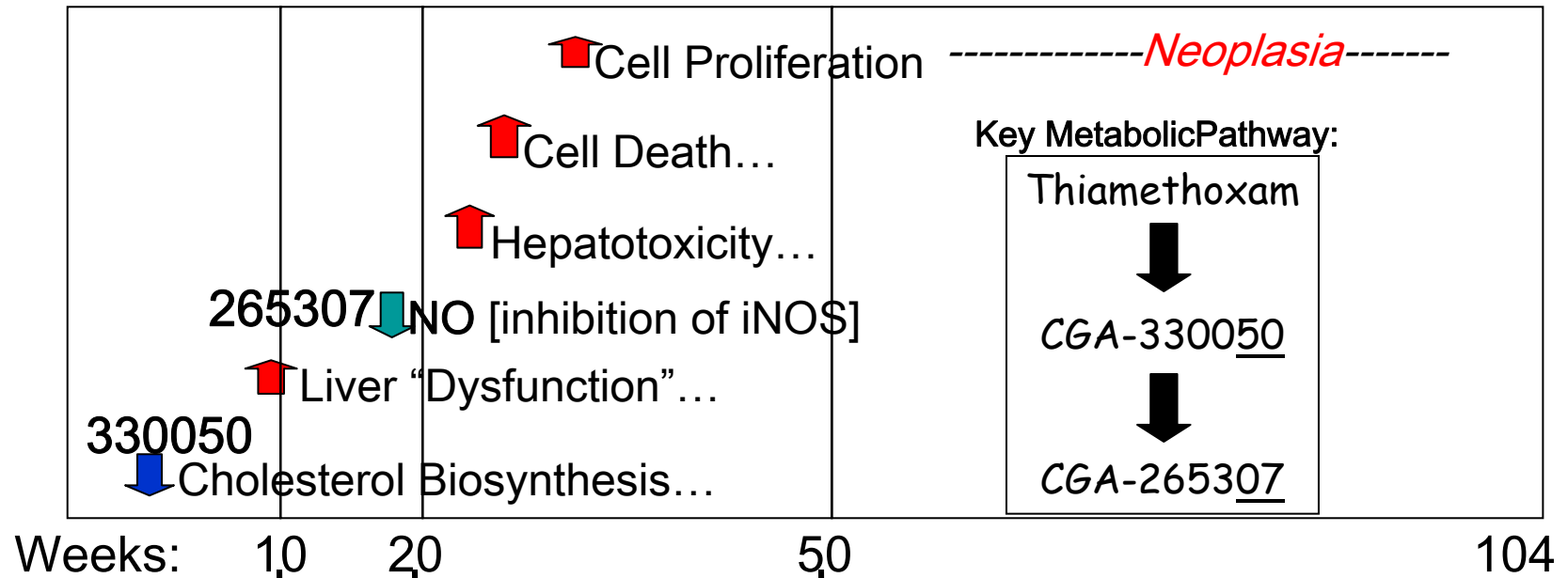
## Time Course of Changes in the Livers of Mice Fed on Diets Containing Thiamethoxam at Carcinogenic Dose Levels

### Study Week



1-10	<b>Liver dysfunction:</b> Decreased protein synthesis, glycogen and lipid accumulation, reduced cholesterol
10-50	<b>Sustained Hepatotoxicity:</b> Increased ALT/AST, fatty change, pigmentation. Inflammatory cell infiltration. Single cell necrosis, increased apoptosis.
20-50	<b>Sustained Increase in Cell Proliferation</b>
50-80	<b>Neoplasia</b>

# Metabolic & Hepatic Events Leading to Mouse Liver Tumors



Thiamethoxam Treatment at a Tumorigenic Dose

# Thiamethoxam

- **Strength, Consistency, Specificity of Association**
  - Well-defined measured effects with dose response concordance
  - Role of specific metabolites & time-dependent progression of hepatic lesions consistently seen including two strains of mice but not in rats
  - Metabolite 7 shown to inhibit iNOS & exacerbate iNOA-dependent carbon tetrachloride hepatotoxicity
- **Biological Plausibility & Coherence**
  - Regenerative proliferation associated with persistent toxicity well established MoA for different agents & tissues
- **Other MoAs**
  - Not a DNA reactive mutagenic
  - Cytochrome P-450 induction, peroxisomal beta oxidation, and oxidative stress were considered experimentally & shown not to be viable

## Thiamethoxam Mouse Liver Tumors

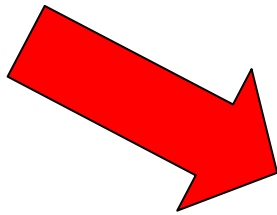
Is the weight of  
evidence sufficient  
to establish the  
MoA in animals?



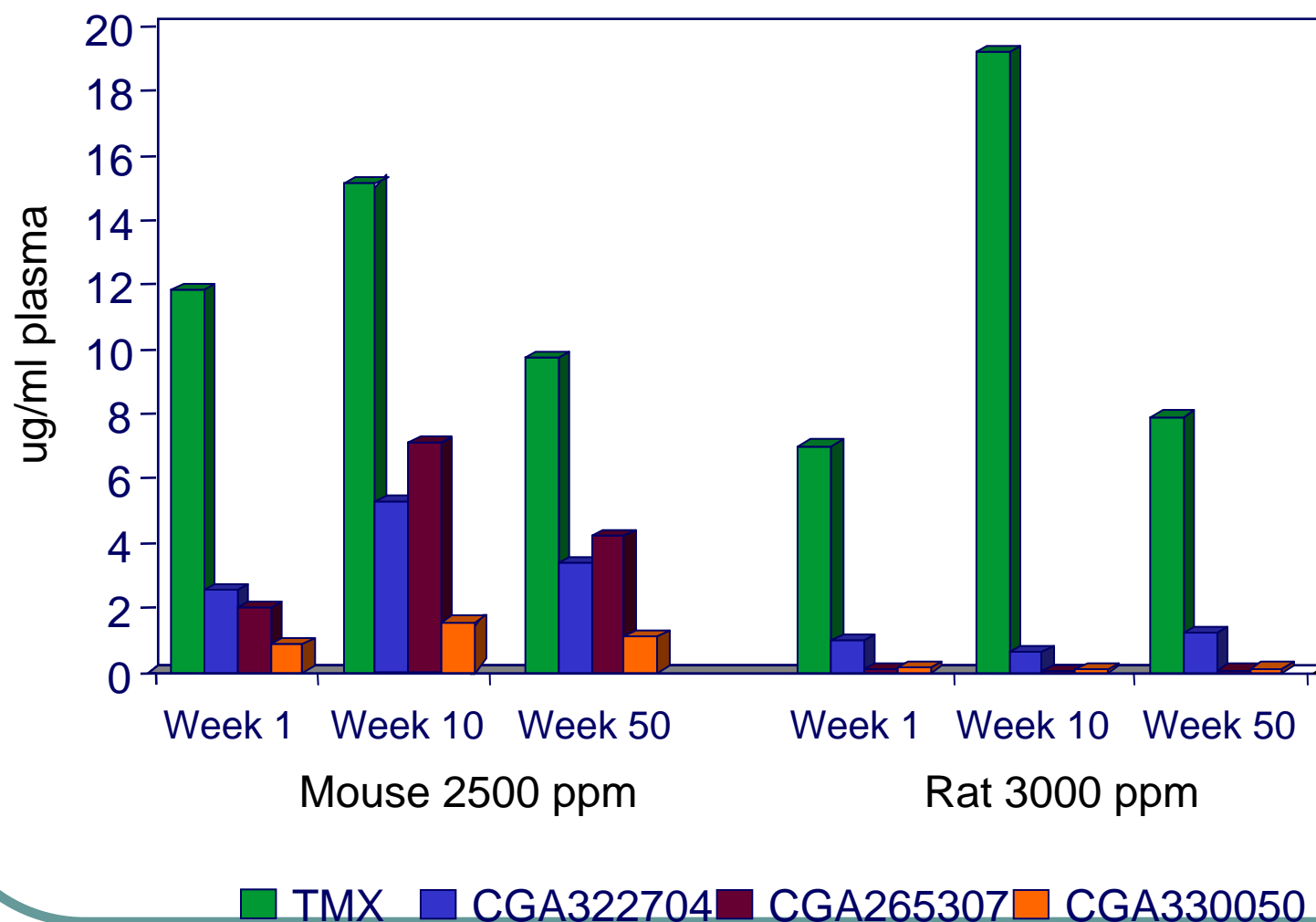
Are the key events in the  
Animal MoA plausible  
in humans?



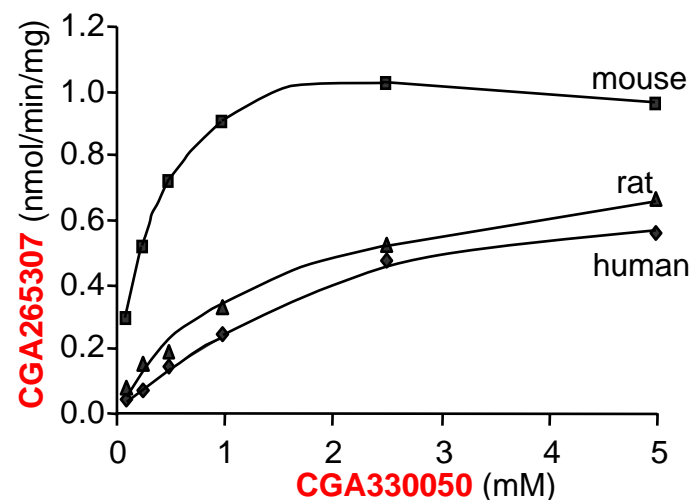
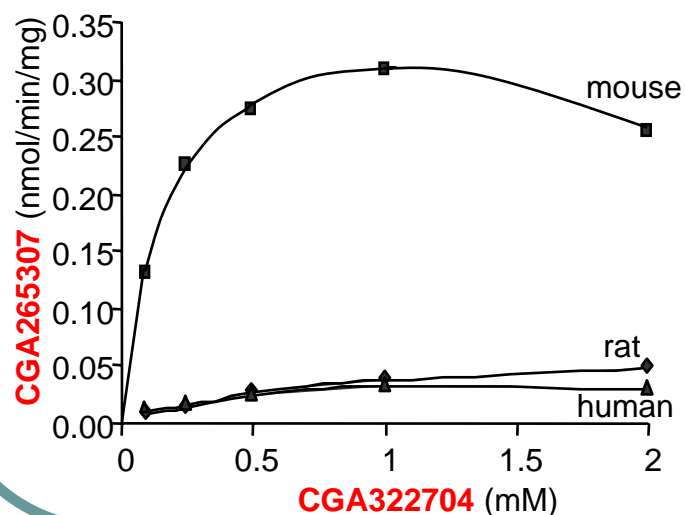
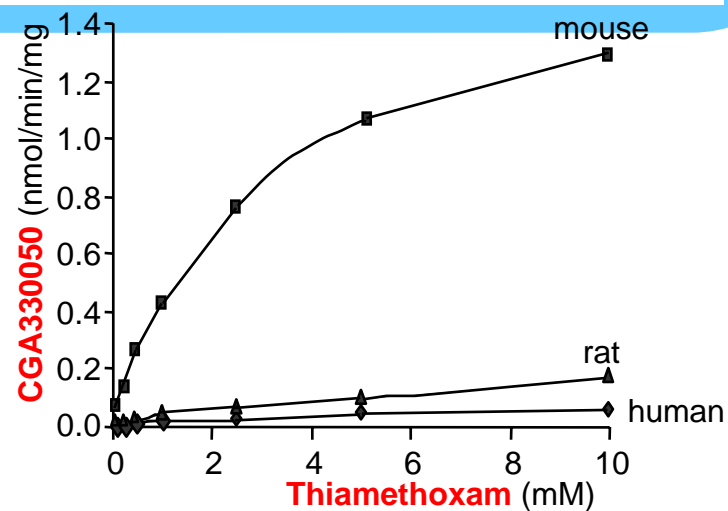
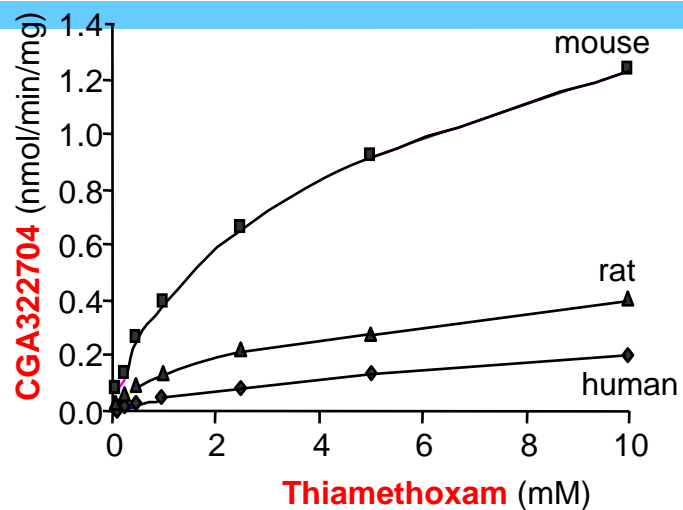
Taking into account kinetic  
& dynamic factors, are  
key events in animal  
MoA plausible  
in humans?



# Comparative Metabolism of Thiamethoxam in Rats & Mice Following Dietary Administration - Plasma metabolites



# Metabolism of Thiamethoxam in Rat, Mouse & Human Liver Microsomal Fractions



# Concordance Table for Thiamethoxam

Key Events	Mouse	Rat	Human
Generation of Critical Metabolites	Yes	Yes but quantitative differences	Yes but quantitative differences
Generation of Liver "Dysfunctional" Changes	Yes	No, insufficient Metabolite "50"	Insufficient "50" (in vitro)
Inhibition of Inducible Nitric Oxide Synthase (iNOS)	Yes	Insufficient Metabolite "07"	Insufficient "07" (in vitro)
Sustained Hepatotoxicity	Yes	No	No data but not likely
Sustained Cell Proliferation	Yes	No	No data but unlikely
Tumors	Yes	No	No data but unlikely



## Contribution of MoA Human Relevance Framework

- Promotes Use of All Relevant Data
  - Helps Identify Kinetic & Dynamic Differences Between Species
  - More appropriate predictions & dose response extrapolation methods
  - Delineates Types of Data that are Preferred over Defaults
- Supports moving toward a more mechanistic approach

## For more information: Mode of Action & Human Relevance Framework

- **ILSI RSI Website**

<http://www.ilsa.org>

- 2003: Meek *et al.* A framework for human relevance analysis of information on carcinogenic modes of action. *Crit Rev Toxicol* 33:591-653
- 2005: Seed *et al.* Overview: Using mode of action and life stage information to evaluate the human relevance of animal toxicity data. *Crit Rev Toxicol* 35:664-672

- **IPCS Harmonization Website**

<http://www.who.int/ipcs/methods/harmonization/index.html>

- 2001: Sonich-Mullin *et al.* IPCS conceptual framework for evaluating a mode of action for chemical carcinogenesis. *Regul Toxicol Pharmacol* 34:146-152
- 2006: Boobis *et al.* IPCS framework for analyzing the relevance of a cancer mode of action for humans. *Crit Rev Toxicol* 36:781-792
- 2008: Boobis *et al.* IPCS framework for analyzing the relevance of a non-cancer mode of action for humans. *Crit Rev Toxicol*. 38:87-96