Hello and welcome everyone to the first RSESS newsletter since our Society of Toxicology Annual Meeting in Nashville! I’m pleased to begin the year with a thank-you to our preceding leadership, a few updates and some good news for you about our Specialty Section, and a request for your input now (…and any time during the year) about future newsletter topics you think would be of interest to our membership.

First, let me provide thanks to Glenn Simon who has very ably served as our President during the ’01 -’02 year and at the Nashville meeting and now will continue to serve RSESS (very graciously) as Past President. Also, a thanks and farewell to Joe Tigner, who has provided us with timely and high quality news-letters this past year. Thanks Joe; you will be missed! Finally I want to thank Rita Rose in SOT Headquarters who has been a tremendous resource for Glenn and others in RSESS, and will continue to help us this year in providing information and timely answers from other SOT sources.

Looking forward to the coming year, Denise Robinson will now serve in Joe’s place and has assured my and others’ input for this first ’02 -’03 edition newsletter. As previously, we will be aiming this year at making the RSESS newsletter a sustained source of interesting and useful information and references to the members. Your other officers and councillors, Carol Auletta, Lin DePass, and now Frank Sistare and Ron Gerson will serve also as regular contributors.

Now for a ‘good news’ update on our success in program endorsements for the 2003 Annual Meeting in Salt Lake City. The following five sessions -- approved by the SOT Program Committee – carry RSESS endorsement:

- Cutaneous Toxicity – Current Methods and Concepts in Safety Evaluation: Relevance to Human Exposure
- Dose-dependent Transitions in Toxic Mechanism
- Choice and Application of Classical or Physiologically Based Pharmacokinetics for Chemical Assessment and Pharmaceutical Development
- Challenges of the Developmental Neurotoxicity Study
- Medicinal Herbals and Dietary Supplements

Also, as you will see inside on Page 2 of the newsletter, we awarded student travel awards to a number of worthy applicants at the Nashville RSESS meeting.

As a final note, please help us to help make RSESS a valuable resource to all of our membership by providing your input into the topics we include in upcoming newsletter articles. There are numerous emerging issues in our specialty section domain of probably high interest to members. I invite you please to send ideas -- or your interest in authoring future articles -- to Denise Robinson at denise_robinson-gravatt@groton.pfizer.com.

In the meantime my best wishes to you for an enjoyable summer. The next RSESS newsletter will come out in late September.

Starting to think about Salt Lake City!

Harry M. Olson
CONTINUING EDUCATION SESSIONS/SYMPOSIA/ROUNDTABLE SESSIONS/WORKSHOPS WHICH RSESS CO-SPONSORED IN 2002

Thank you to the co-chairs and speakers for making these sessions a success!

- An Overview of Toxicologic Pathology
- Alterations in Gene Expression as a Mechanism of Toxicant Action
- Challenges in Preclinical Development of Anticancer Drugs
- Strategies and Issues in Pre-Clinical Development of Intravenous Infusion Products
- Application of Genomics for Mechanism Based Risk Assessment
- Drug Induced Vascular Disease: Markers of Injury

2003 PROGRAM PROPOSAL APPROVED BY COUNCIL

RSESS endorsed a number of proposals for sessions for the 2003 annual meeting. We have just heard from SOT Council that the following sessions have been accepted. Congratulations to the Session Co-Chairs!

- W. Slikker and K. B. Wallace “Dose-dependent Transitions in Toxic Mechanism”
- J. C. Lipscomb and R. Dixit “Choice and Application of Classical or Physiologically Based Pharmacokinetics for Chemical Assessment and Pharmaceutical Development”
- S. Makris and M. Weiner “Challenges of the Developmental Neurotoxicity Study”
- A. Fuciarelli and W. Allaben “Medicinal Herbals and Dietary Supplements”

A CALL FOR ITEMS OF INTEREST TO OUR NEWSLETTER

As Harry Olson indicated in his President’s Message, we would like to make our newsletter a vital organ of our specialty section. If you have any items and/or short articles of interest to our group, please contact Denise Robinson or any of our other officers.
RSESS Presents Awards to Four Students at 2002 SOT Meeting

The Regulatory and Safety Evaluation Specialty Section of the Society of Toxicology presented Student Travel Awards to four students at the 2002 SOT meeting. The awards, of $500 each, were made at the specialty section meeting/reception on Tuesday, March 19. In addition, a representative from Taylor and Francis Publishers presented $100 gift certificates for the books of their choice to each student. The students and their academic advisors attended the awards ceremony and each student spoke briefly on his or her research and its relevance to regulation and/or safety evaluation. The section was pleased with the quality of the applications received and we look forward to continuing this program in future years.

The four winners, their universities and advisors, and their presentations were:

S. Satheesh Anand, The University of Louisiana at Monroe, Advisor: Harihara M. Mehendale, Ph.D., Professor and Kitty DeGree Endowed Chair in Toxicology
Dose Dependent Liver Regeneration Following Co-Administration of Chloroform and Trichloroethylene-Induced Hepatotoxicity in Male Sprague-Dawley Rats. S.S. Anand, S. S. Devi, S. N. Murthy, M.M. Mumtaz and H.M. Mehendale. Department of Toxicology, College of Pharmacy, The University of Louisiana at Monroe, Monroe, LA

Melissa Bunderson, The University of Montana, Advisor: Howard D. Beall, Associate Professor of Medicinal Chemistry
The Formation of Peroxynitrite and Induction of The Inflammatory Mediator, Cyclooxygenase-2, are Important Factors in Arsenic-Related Cardiovascular Disease. M. Bunderson and H. D. Beall. The Department of Pharmaceutical Sciences, University of Montana, Missoula, MT

Deborah E. Burgin, University of North Carolina, Advisor: Linda S. Birnbaum, Ph.D., D.A.B.T., HSD, USEPA/ORD/NHEERL, Chapel Hill, NC
Comparing Mixtures of Dioxin-Like and Non-Dioxin-Like PCBS to TCDD. D.E. Burgin1, J.J. Diliberto2 and L.S. Birnbaum1. 1Toxicology, UNC Research Triangle Park NC 2ETD/PKB, USEPA/ORD/NHEERL, Research Triangle Park, NC and 3HSD, USEPA/ORD/NHEERL, Chapel Hill, NC

Catherine Lombard, Medical College of Virginia, Advisor: Prakash Nagarkatti, Ph.D., Professor and Director Department of Pharmacology and Toxicology
Evidence For the Induction of Apoptosis in Immune Cells by Delta-9-Tetrahydrocannabinol, R.J. McKallip, C. Lombard, B. R. Martin, M. Nagarkatti and P.S. Nagarkatti. Microbiology and Immunology and Pharmacology and Toxicology, MCV/VCU, Richmond, VA

SUMMARY OF THE ROUND-TABLE DISCUSSION:
“ALTERNATIVES TO ANIMAL TESTING: 20 YEARS OF FERTILITY OR FUTILITY?”

As an added feature to the specialty section receptions traditionally held during the SOT Annual Meeting, RSESS organized a roundtable discussion to engage the members and to stimulate debate. Roundtable Panelists were chosen with the goal of providing a provocative interchange and included:

- Shayne Gad, Gad Consulting Services, representing many years of designing, conducting and interpreting traditional and alternative toxicity assays
- Bill Stokes, National Institute of Environmental Health Sciences, representing the Interagency Coordinating Committee for Validation of Alternative Methods
- Neil Carmichael, Aventis CropScience, representing the perspective of the agricultural chemical industry
- Lutz Mueller, Novartis Pharmaceuticals, representing the pharmaceutical industry as well as his years as a European regulator

The topic, Alternatives to Animal Testing, is one we are all familiar with, although as the panelists demonstrated, a significant variety of experiences and perspectives exist. There continues to be increasing pressure to justify, reduce and eliminate the use of animals in traditional toxicity testing. Depending on your experience, you may agree with some of the panelists that the past 20 years have been fertile ground for the development of alternative assays. There has been a dramatic increase in knowledge about mechanisms and across-species understanding of various types of toxicity. Examples were given of the many modifications and refinements to traditional assays that have taken advantage of new knowledge. Examples of several non-animal alternative assays that have now been accepted by regulatory bodies were noted. On the other hand, you may have agreed with other panelists that a sense of futility is warranted, when it is considered how few alternative assays have actually made their way through the various regulatory approval and acceptance processes. One of the few points of consensus and take-home messages is that the process of development, evaluation, validation and acceptance of an alternative assay into common practice represents a significant hurdle.

This Roundtable was the first such activity for RSESS and we are interested to hear your feedback as to whether another provocative topic should be proposed for next year’s reception.

-Denise Robinson, RSESS Secretary/Treasurer
**Drug Metabolites in Safety Testing**

The advent of sophisticated bioanalytical technology such as LC-MS/MS has resulted in substantial improvements in our understanding of the in vivo disposition of pharmaceuticals. Specifically, these technologies not only allow the facile monitoring of parent drug within the blood but also allow the determination and monitoring of the Phase I and Phase II metabolites of these drugs as well.

An assumption inherent to most pharmaceutical preclinical toxicology programs is that the animal species used in these evaluations serve as biological surrogates for humans. However, analysis of drug metabolite profiles may reveal both quantitative and/or qualitative differences between the spectrum of metabolites formed in animal species and those formed in humans. Of particular interest are situations where:

1. Humans form a unique drug metabolite (or metabolites) not found in animals used in the preclinical evaluation or,
2. Humans form a metabolite found in only relatively minor amounts in animals.

Under such circumstances the animal species used for the preclinical toxicology evaluations do not adequately assess the potential toxicity of the drug candidate as humans are exposed to a molecular species for which sufficient animal data does not exist. In view of this possibility and because of the increased scientific and regulatory attention to circulating drug metabolites, the Pharmaceutical Research and Manufacturers of America (PhRMA) commissioned a task force in 1999 to review the topic of “Drug Metabolites in Safety Testing” (known as the “MIST” Task Force). This task force was comprised of members of the Drug Metabolism, Clinical Pharmacology and Safety Assessment (DruSafe) PhRMA subcommittees. A joint FDA - PhRMA workshop was subsequently held in Bethesda, Maryland in November 2000 to discuss the findings of the PhRMA MIST Task Force. A brief summary of key points discussed at the workshop is given below and contains some excerpts from a soon to be published paper entitled Drug Metabolites in Safety Testing authored by the members of the MIST Task Force. This paper will appear in the “Contemporary Issues in Toxicology” section of an upcoming issue of Toxicology and Applied Pharmacology. It is important to note that the recommendations of the MIST Task Force have not been sanctioned by the FDA or other

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**Recommendations on Statistical Analyses of Rodent Carcinogenicity Studies Published by Society of Toxicologic Pathology**

Traditionally, there have been diverse approaches to statistical analyses of rodent carcinogenicity studies. To help identify and develop a set of best statistical practices, the Society of Toxicologic Pathology (STP) convened a working group composed of pathologists, toxicologists and statisticians from industry, government and academia. The STP Peto Working Group reviewed statistical methods for rodent carcinogenicity studies and has recently published recommendations on how microscopic observations from these studies could be consistently and reliably categorized for analysis using existing statistical methods. The paper takes the form of comments to FDA’s draft Guidance for Industry – Statistical Aspects of the Design, Analysis, and Interpretation of Chronic Rodent Carcinogenicity Studies of Pharmaceuticals. The following topics are among those addressed in the published recommendations:

- Preference for routine use of a single statistical method, preferable a trend test
- Use of pairwise comparisons on a case-by-case basis
- Use of tumor incidence rates rather than prevalence rates
- Need to make adjustments for differences in mortality and the appropriate use of the Peto test
- Suggestions for consistently classifying neoplasms as Mortality Independent, Fatal or Incidental
- Challenges associated with classifying neoplasms as Rapidly Fatal vs Not Rapidly Fatal and the importance of classifying the cause of death, where possible, for interpreting carcinogenicity study outcome

Reference:
Morton et al., Toxicologic Pathology 30 (2): 415-418

-Denise Robinson, RSESS Secretary/Treasurer
Drug Metabolites in Safety Testing (Cont)

international regulatory agencies.

Two of the critical questions addressed by the MIST Task Force were:

1. What constitutes a unique human metabolite such that further safety evaluation of the metabolite is required?
2. If such a unique metabolite is identified, what should be done to provide a valid assessment of the metabolite?

It was recognized that current bioanalytical technology enables the identification of extremely small quantities of drug-derived products within the circulation. The MIST Task Force recommended that, in general, the toxicological evaluation of “unique human metabolites” (i.e., ones not formed in animals, or formed to a very limited extent in animals) be limited to “important or major” human metabolites. It was judged unlikely that a minor human drug metabolite would significantly contribute to the toxicological profile of a drug, unless there was reason to suspect that such a minor human metabolite was chemically reactive. In such a case, a minor human metabolite may also require subsequent evaluation as outlined below.

A consensus definition of what constituted an “important or major” human metabolite that could be applied to all drugs was not achieved among the participants of the workshop. However, it was agreed that the metabolite profile for each drug needs to be evaluated on a case-by-case basis. Some of the factors to be considered in determination of important or major human metabolites that may require additional evaluation are:

- The amount of exposure (AUC) to the drug metabolite relative to the exposure to parent drug found in humans. Such quantitative analysis can be performed during radiolabelled drug studies which are routinely performed in humans as part of the clinical development programs. In the absence of other scientific considerations, the MIST Task Force recommended that a major human drug metabolite be defined as one that accounts for 25% or more of the exposure (AUC) to circulating drug-related material.

- Whether the metabolite is pharmacologically active or not. This is particularly important if the toxicity is linked to the pharmacological activity of the compound. It should be recognized however, that the toxicity of the drug and/or metabolites may be completely unrelated to the pharmacology of the drug.

- If the metabolite contains any structural alerts that would suggest the metabolite may have toxic liabilities.

- The intended indication of the drug. With certain indications (such as oncology) there may be less concern relative to potential human toxicity than with other indications.

It was also recognized that unique conjugated (Phase II) metabolites circulating in plasma (i.e., glucuronides, sulfates, etc), would generally not require evaluation (even if they qualified as “major” metabolites based upon plasma AUC) in light of the low propensity of these polar metabolites to cause adverse events. Exceptions may arise where there is reason to suspect that a specific conjugate may pose a toxicological concern (e.g. reactive acyl glucuronide conjugates of a carboxylic acid-containing drugs or certain glutathione conjugates).

If a major or important human metabolite requiring further evaluation were identified, the use of an alternative animal species in which the metabolite of concern was formed could be considered. Alternately, the conduct of dedicated toxicology tests on the unique human metabolite could be considered. It was emphasized that an underlying concern associated with any study in which a metabolite is administered to an animal is that the disposition of that metabolite dosed exogenously may not accurately reflect its disposition when formed endogenously from the parent drug.

In developing a plan to evaluate the toxicity of a major or important human metabolite, consideration of the following points was recommended:

1. Genotoxicity studies - If deemed necessary, a minimum screen for potential genotoxicity should be considered. In vitro studies to detect point mutations and chromosomal aberrations would be an example of a minimum screen.

2. General toxicity studies - Study duration should be based on available relevant information and performed in the species likely to express the
Drug Metabolites in Safety Testing Cont.

potential toxicity (studies of the drug metabolite would not be required in two species). In general, a minimum of 14 and a maximum of 90 days duration would be considered appropriate study durations.

3. Studies to address specific toxicity endpoint – These would be appropriate if deemed necessary from general toxicity studies.

4. Reproductive toxicology studies – These would be appropriate where the parent drug may be used in populations that include women who are pregnant or are of childbearing potential.

The proposed plan should incorporate the following considerations:

- Physicochemical characteristics of the unique human metabolite as they relate to solubility, permeability, absorption and thus route of administration;
- Extent of exposure to metabolite at projected clinical dose of parent;
- Target patient population and duration of use;
- Drug class effects;
- Clinical safety concerns given the human data available;
- Implication of impurities in synthesized metabolites;
- Potential for further biotransformation in animals of the unique human metabolite;
- Interpretation and relevance of data from any in vitro assay that includes an animal-based metabolic activation system; and
- Single dose pharmacokinetic profile of the unique human metabolite in animals as a measure of the feasibility of conducting repeat-dose toxicity studies.

Carcinogenicity Testing – Consideration of the need for carcinogenicity testing of the unique human metabolite would depend on the following:

- Whether carcinogenicity testing is required for the parent drug (based on the intended indication for the parent drug). If carcinogenicity testing is not necessary for approval of the parent compound (normally where the cumulative human lifetime exposure to the compound would not be expected to exceed 6 months or for use in life-threatening conditions) then testing of the metabolite likewise would not be required;
- If there were any evidence in the metabolite toxicity study that the metabolite caused lesions that might be expected to progress to neoplasia, and such lesions were not observed with the parent compound (i.e. they were metabolite-specific). In such a case then consideration should be given to testing of the metabolite for potential carcinogenicity. Use of validated alternative short-term carcinogenicity assays may be considered for this assessment.

The basis for setting doses in carcinogenicity studies of the metabolite would be similar to those applied to the testing of drug candidates as described by the International Conferences on Harmonization (ICH).

In conclusion, should a metabolite be present in significant amounts in human plasma but not present, or present in only trivial amounts in the plasma of all toxicity species tested, additional studies may be warranted in order to determine whether the metabolite poses an unidentified risk to humans. It was recognized that there are often practical limitations to a sponsor’s ability to collect such information, and that its interpretation may not be straightforward.

Carcinogenicity studies on an administered major metabolite are generally not recommended, unless there is evidence in separate metabolite toxicity studies that the metabolite caused novel (i.e. dissimilar to parent) lesions that might be expected to progress to neoplasia.

In support of developmental and reproductive toxicology assessment, it is recommended that efforts be made to establish that all major circulating human metabolites also are present in the plasma of at least one of the animal species (rats and rabbits) used for such studies.

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