Mixtures Specialty Section Newsletter

Volume 2, Issue 2 February 2012

Letter from the President

Michael Dourson, PhD, DABT, ATS
MixSS President
Toxicology Excellence for Risk Assessment (TERA)

Dear Colleagues,

As many of us know, different scientific groups have been assessing and managing risk from chemical mixtures for quite some time. For example, the American Conference of Government and Industrial Hygienists (1) has guidance in this area that has been in place for decades. The U.S. Environmental Protection Agency (2-3) also has guidance and has various examples of mixtures assessments (e.g., the Integrated Risk Information System). Both groups first approach the assessment of mixtures by an analysis of the toxicity of the mixture of concern; if such data are not available, then by analysis of the toxicity of a sufficiently similar mixture; and without these data, then by analysis of the toxicity of component chemicals. Additionally, both groups recommend the development of a hazard index (HI) with an assumption of additivity as a default when analyzing the toxicity of component chemicals, with due attention paid to possible synergistic and antagonistic effects. EPA's guidance is more prescriptive, and indeed has been followed extensively in its environmental risk management decisions, most notably in its Superfund risk assessment work (4).

As risk assessment science has improved, new queries have been made as to whether these, and other, existing methods might be improved and further, whether such methods address non-chemical stressors, such as noise in the work place. The unequivocal response is a *qualified* yes, qualified if the non-chemical stressor can be analyzed individually and similarly as for chemicals, or addressed more appropriately as a cumulative exposure of concern, or as a sufficiently similar cumulative exposure. Furthermore, extrapolation from such mixed exposures to the human situation can be attempted using existing frameworks. However, additional algorithms may be needed if such stressors are not readily put into a chemical risk assessment framework. Stressors such as the loss of cultural benefits with the posting of fish consumption advisories due to chemical contamination might warrant a novel approach (5).

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MixSS in the City by the Bay – SOT 2012

Cynthia Rider, PhD, DABT
MixSS Secretary/Treasurer
NIEHS/DNTP

The SOT Meeting is just around the corner and there will be plenty of exciting opportunities to learn more about mixtures and cumulative risk, discuss the latest science, and socialize with colleagues. The what, when, and where of mixture-related program activities is provided below. We hope to see you at as many of them as possible!

Sunday, March 11

Continuing to offer opportunities for development of mixtures science skills is a priority for the MixSS. This year's **Advanced CE course** offering, titled **Specialized Techniques for Dose-Response Assessment and Risk Assessment of Chemical Mixtures (PM12)** will provide an in-depth discussion of tools available for cumulative risk assessment. The course will offer discussions of data quantity and quality, assessment of sufficient similarity of whole mixtures approaches, and a focus on component-based cumulative risk assessment approaches (toxic

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equivalency factors, relative potency factors, binary weight of evidence, hazard index, target-organ toxicity hazard index, interaction-based hazard index).
Chairs: Jane Ellen Simmons (US EPA, MixSS Past President) and Mike DeVito (NIEHS/NTP)
When: 1:15-5:00pm (registration required)
Where: CE Room (Moscone Convention Center)
Speakers: Dr. Jane Ellen Simmons (US EPA/NHEERL), Dr. Rick Hertzberg (Biomathematics Consulting), Dr. Bob Budinsky (Dow Chemical Company), Dr. Mike DeVito (NIEHS/NTP), Dr. Moiz Mumtaz (ATSDR)

Monday, March 12

Although the Officers' Meeting is generally a closed meeting, we would appreciate any input members have on improving our SS or ideas you want to submit for mixtures-related program items for SOT 2013. Contact the secretary at cynthia.rider@nih.gov.
What: MixSS Officers' Meeting
When: 6:30-8:00am
Where: Room 256

In addition to the CE course, there will be many scientific sessions that are likely to be of interest to the MixSS community.

Poster Session: Toxicity of Mixtures
When: 9:30am-12:30pm
Where: Exhibit Hall

Presentation: Assessment of Naturally Occurring Mixtures
Speaker: Timothy Adams (Flavor and Extract Manufacturers Association)
When: 9:52-10:24am
Where: Room 305

Presentation: Mixtures Risk Management: Moving Beyond TEQs and Hazard Indices
Speaker: Paul Price (Dow Chemical Company, MixSS Councilor)
When: 4:13-4:45pm
Where: Room 309

The MixSS Meeting/Reception is always a highlight of the Annual Meeting. Get involved in your SS and join us to socialize, see the awards presentation, meet the new officers, hear about what your MixSS has been up to this year, and provide your input. We hope you will be there!
What: MixSS Meeting/Reception
When: 6:00-7:30pm
Where: Yerba Buena Salon 10 (Marriott Marquis)

Tuesday, March 13

Workshop Session: Sufficient Similarity of Whole Representative Mixtures or a Relative Potency Factor Approach: Polycyclic Aromatic Hydrocarbons as a Case Study (sponsored by MixSS)
Session chairs: Cynthia Rider (NIEHS/NTP, MixSS Secretary/Treasurer) and Julia Gohlke (University of Alabama at Birmingham)
Speakers: Julia Gohlke (University of Alabama at Birmingham), Glenn Rice (US EPA), John Incardona (NOAA), Erica Bruce (Baylor University), Russell White (American Petroleum Institute)
When: 9:00-11:45am
Where: Room 304

Presentation: Contaminant Mixtures and Mixed Exposure Pathways: Using *In Vitro* Digestors to Tease out Human Exposure to Brownfield Soils and Identify Engineering Solutions that Reduce Risk
Speaker: Steven D. Siciliano (University of Saskatchewan)
When: 11:13-11:45am
Where: Room 305

Workshop Session: Novel Topics in Environmental Polycyclic Aromatic Hydrocarbon Metabolism Leading to Carcinogenesis (endorsed by MixSS)
Session chairs: Bhagavatula Moorthy (Baylor College of Medicine) and Danielle Carlin (NIEHS/DERT)
Speakers: Frederick P. Guengerich (Vanderbilt University), Stephen Nesnow (US EPA), David E. Williams (Oregon State University), Lynn Flowers (US EPA), Ruth A. Roberts (Astra Zenica)
When: 1:30-4:15pm
Where: Room 305

Presentation: Testing for Sufficient Similarity in Environmental Mixtures Using Exposure Data and Mixture Toxicology Data
Speaker: Chris Gennings (Virginia Commonwealth University)
When: 3:21-3:39pm
Where: Room 310

Wednesday, March 14

Symposium Session: The Toxicological Impact of Metals, Crude Oil and Chemical Dispersants from the Gulf of Mexico Oil Crisis on Human and Wildlife Health (endorsed by MixSS)
Session chairs: John P. Wise (University of Southern Maine) and Joe Griffitt (University of Southern Mississippi)
Speakers: Iain Kerr (University of Southern Maine), Samantha Joye (University of Georgia), Carys Mitchelmore (University of Maryland), Joe Griffitt (University of Southern Mississippi), Greg Mayer

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A Student's Perspective on the International Toxicology of Mixtures Conference

Julia Rager
MixSS Graduate Student Representative
University of North Carolina, Chapel Hill

The field of toxicology is constantly evolving, as the types of exposures studied are changing to represent those most pertinent to public health. Recent changes produced the study of mixture toxicology, since the majority of human exposure conditions are comprised of multiple compounds rather than single components. This new area of mixture toxicology requires leadership by individuals capable of understanding all the complex angles of mixtures-based research. Many of these leaders recently met at this year's International Toxicology of Mixtures Conference sponsored by Elsevier to discuss the current strategies and limitations surrounding the assessment of human health risk associated with exposures to chemical mixtures. Alongside these leaders were also graduate students, like me, seeking to learn as much as possible from this unique gathering.

My research focuses on the genomic and epigenomic responses to air pollutants within the respiratory tract. At the International Toxicology of Mixtures conference, I had the opportunity to present some of these findings during an evening poster session. Equipped with wine and cheese as sustenance, discussion surrounding my research provided me with exciting insight into new strategies that will greatly aid in my future project designs.

The presentations and round table discussions throughout the conference were extremely engaging and informative. However, perhaps the most invigorating discussion I participated in was during a catered lunch held at the hotel's top floor. Here, I had the pleasure of chatting with several influential leaders of the environmental toxicology field. Topics of conversation ran from the importance of evaluating toxicological responses across multiple doses to the politics underlying how scientists influence nationwide projects. As a systems biologist and an engineer-in-training, one of my favorite discussions related reverse engineering techniques to the construction of new molecular pathways altered upon exposure to toxicants.

This international conference clearly fulfilled its goal to provide an open forum for the exchange of ideas regarding the understanding of mixture toxicology. The leaders in this field successfully communicated their viewpoints on effective strategies to evaluate chemical mixtures, while at the same time, encouraged and guided the next generation of scientists.

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(Texas Tech University), John P. Wise (University of Southern Maine)
When: 9:00-11:45am
Where: Room 308

Workshop: Advancing Food Safety in a Global Marketplace (endorsed by MixSS)
Session chairs: Nicola Stagg (Dow AgroSciences LLC) and Michael Bolger (FDA)
Speakers: Angelika Tritscher (WHO), Daniel Wilson (Dow Chemical Company), Alan Boobis (Imperial College, London), Bruce Chassy, (University of Illinois at Urbana-Champaign), Clark Carrington (FDA)
When: 1:30-4:15pm
Where: Room 303

Informational session: Evolution and Implementation of Combined Chemical Exposure Methods: International Perspectives (sponsored by MixSS)
Session chairs: Moiz Mumtaz (ATSDR, Past President of MixSS) and Bette Meek (University of Ottawa)
When: 4:30-5:50pm
Where: Room 303

Thursday, March 15

Workshop: Chemical Standardization of Botanical Medicines for Safe and Effective Use as Therapeutic Agents (endorsed by MixSS)
Session chairs: Madhu Soni (Soni & Associates Inc.) and Brinda Mahadevan (Abbott Laboratories)
Speakers: Cynthia Smith (NIEHS/NTP), Nandakumara Sarma (US Pharmacopeia), Ikhlas Khan (University of Mississippi School of Pharmacy), Craig Hopp (NIH/NCCAM), Bala Manyam (Penn State University)
When: 9:00-11:45am
Where: Room 307

President's Challenge

Question from August Newsletter: Toxicity testing in the 21st century will be much more focused on *in vitro* bioassays. What kinds of methods/guidelines will mixtures researchers need to develop to both analyze and assess risks from these bioassays?

Response from Gian Paolo Rossini
Dipartimento di Scienze Biomediche,
Università di Modena e Reggio Emilia,
Modena, Italy

The NRC report "Toxicity Testing in the 21st Century: A Vision and a Strategy" indicates the importance of a paradigm shift towards mechanistic based toxicity testing. The characterization of toxicity pathways, the use of (human) cells as model systems, the widespread exploitation of systemic, "omics" approaches, represent key items to pursue such a paradigm shift (1). The relevance of *in vitro* bioassays stems from these indications.

Given the extraordinary complexity of molecular signalling and control mechanisms in biological systems, which are characterized by networks of components (2), it seems likely that some methodological choices enabling, or else facilitating, the challenges posed by the paradigm shift in toxicity testing will be sought.
The call for concerted actions aimed at the characterization of toxicity pathways (1) in the mixtures toxicology community might be met by a network of research groups (Alliance?), organized by MixSS. The mission of this network could include the characterization of mixture toxicity pathways and their cross-talk, as well as the exploitation of the resulting mechanistic knowledge to develop reliable cell-based assays and predictive models to support risk assessment of chemical mixtures. Initiatives to obtain appropriate resources needed for the actions and research of this network would be part of its mission.

Establishing a framework for action would help in the approach to these scientific issues. For instance, the characterization of toxicity pathways and networks is sufficiently complex to suggest that finding some consensus regarding the model systems to be used in investigations might be a prerequisite to support effective action, as well as avoid unnecessary redundancy and consequent multiplications of costs at different levels. Following the same line of reasoning, it seems unlikely that "one choice may fit all" (3), and the identification of some set of experimental models to characterize the network could be found appropriate.

On a more general ground, the networks of molecular reactions in a living system are such that cross-talk and interactions at a variety of levels should be expected (2), and the chains of events in the responses to toxic agents would not depart from this general condition (4). When mixtures of toxicants are investigated, increased complexity is to be expected. Within this framework, systemic approaches and global analyses of molecular domains seem necessary to tackle this level of complexity, and these methodological tools may demand attention by the mixtures toxicology community. For instance, a study on the effects mixtures of two biologically active agents has shown that multiple patterns of response are found with any pair of agents, when many molecular endpoints/effects were analyzed, and this could be explained by cross-talk between different signalling pathways in a context-dependent fashion (5). In keeping with this study, when molecular responses induced by a mixture of toxicants possessing distinct molecular mechanisms of action were studied by analyses of cell proteomes, several patterns of response were detected, including both independent and interacting actions, among which synergistic, similar and antagonistic effects were observed (6).

These studies focused on mixtures of biologically active components raise methodological/general questions, waiting for proper answers. Some of them are:

- Is detection of multiple patterns of response a common feature, whenever a variety of endpoints/effects are analyzed in the same *in vitro* system exposed to any toxicant mixture?
- To what extent would multiple patterns of response for any pair of agents be at variance with the current classification of actions of toxicant mixtures (for instance: Bliss, 1929), and would this represent an obstacle to the use of *in vitro* assays for risk assessment of chemical mixtures ?
- Should the experimental design or methodology involved in system level analyses of effects of toxicant mixtures be reconsidered/refined?
- Should the classification of actions of toxicant mixtures be refined?

In a perspective of mechanistic based batteries of *in vitro* bioassays, old and new questions may get mechanistic-based answers. Concerted actions by a network of investigators can capitalize on complementary expertise, thereby mastering the extreme complexity inherent in a mechanistic-based risk assessment of chemical mixtures.

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Very challenging and exciting times are coming for investigators in the area of mixtures toxicology!

1. NRC: *Toxicity Testing in the 21st Century: A Vision and a Strategy*. (2007), National Academies Press, Washington, DC,
2. Buchanan M., Caldarelli G., De Los Rios P., Rao F., Vendruscolo M., Eds. (2010), *Networks in Cell Biology*, Cambridge University Press, Cambridge.
3. Rossini G.P., Sala G.L., Ronzitti G., Bellocci M. (2011), The use of proteomics in the study of molecular responses and toxicity pathways in biological systems. In "*Advances in Molecular Toxicology*", Vol. 5, J.C. Fishbein, Ed., Elsevier, Amsterdam, 45-109.
4. Rossini G.P. (2005), Functional assays in marine biotoxin detection. *Toxicology*; 208, 451-462.
5. Natarajan M., Lin K.-M., Hsueh R.C., Sternweis P.C., Ranganathan R. (2006), A global analysis of cross-talk in a mammalian cellular signalling network. *Nature Cell Biol*. 8, 571-580.
6. Sala G.L, Ronzitti G., Sasaki M., Fuwa H., Yasumoto T., Bigiani A., Rossini G.P. (2009), Proteomic analysis reveals multiple patterns of response in cells exposed to a toxin mixture, *Chem. Res. Toxicol.* 22, 1077-1085.

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We all live in a constantly changing "sea" of exogenous and endogenous chemicals. Because of this, some folks have mentioned that addressing background exposures is perhaps a forgotten, and now necessary, part of risk assessment (6). Fortunately, addressing background exposures to the chemical of interest is a routine part of any experimental or observational situation (i.e., the control group). In addition, exposures to similarly acting chemicals, or chemicals that may synergize or antagonize the toxicity of the target chemical, are already addressed, perhaps not fully, by the methods mentioned above. However, it may be that background disease, not addressed by control groups, needs to be better integrated into the risk assessment equation. Moreover, it is gratifying to see additional research and analysis in this critically important area. For example, recent publications (7-10) bode well for further advances. I encourage all of us to read these latest publications for additional insights.

1. ACGIH (2006), Threshold Limit Values and Biological Exposure Indices. Appendix E: TLVs for Mixtures.
2. EPA (1986), Guidelines for the Health Risk Assessment of Chemical Mixtures.
3. EPA (2000), Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures.
4. EPA (1989), Risk Assessment Guidance for Superfund, Volume I - Human Health Evaluation Manual Part A: Baseline Risk Assessment. Office of Emergency and Remedial Response.
5. Dourson, M.L. (2002), Comparative Dietary Risks: Balancing the Risks and Benefits of Fish Consumption. *Comments Toxicol.* 8(4-6): 335-536.
6. NAS (2009), Science and Decisions: Advance Risk Assessment. National Research Council. Washington, D.C.
7. EPA (2003), Framework for Cumulative Risk Assessment.
8. Meek et al. (2011), Risk assessment of combined exposure to multiple chemicals: A WHO/IPCS framework. *Regul Toxicol Pharm* 60(2): S1-S14.
9. Mumtaz. (2011), Principles and Practice of Mixtures Toxicology. Wiley
10. Rider et al. (in press) Incorporating Nonchemical Stressors into Cumulative Risk Assessment. *Tox Sci*
11. Sexton et al. (in press) Cumulative Risk Assessment: An Overview of Methodological Approaches for Evaluating Combined Health. *IJERPH*

Webinars with RASS

In trying to bring more mixtures-related science to our members, MixSS has partnered with the Risk Assessment Specialty Section (RASS) to co-sponsor two webinars per year through their established monthly webinar series. This past year, we invited two very exciting speakers with broad appeal for both RASS and MixSS (see below). We plan to continue this offering in the coming year. If you would like to suggest a mixtures-related speaker for future webinars contact Michael Dourson (dourson@tera.org) or Ken Wallace (kwallace@d.umn.edu).

Paul Price (Dow Chemical Company)
Date: May 11, 2011
Title: The Maximum Cumulative Ratio (MCR): A Tool that Uses Both Exposure and Toxicity Data to Determine when Cumulative Assessments are Most Necessary
Abstract: Individuals are exposed to vast numbers of combinations of chemicals and there is a need to determine when cumulative assessments are most required. The maximum cumulative ratio (MCR) is a quantitative measure of how much toxicity is underestimated by not performing a cumulative risk assessment. Case studies of the application of MCR for human and ecological effects and for the evaluation of biomonitoring data are provided.

Christopher J. Portier (NCEH/ATSDR)
Date: November 9, 2011
Title: Risk from Multiple Chemicals in Polluted Communities
Abstract: Evaluating the effects of a mixture of chemical agents affecting a community is an important issue for the Agency for Toxic Substances and Disease Registry. In this talk, I will detail an experimental process that ATSDR is using to evaluate multiple hazards at the same site. The method, based on relative risks rather than dose, allows us to combine risks across multiple routes and for multiple chemicals. Mechanistic information is used to decide how to combine the risks.

Access pdf slides and recordings of the webinars from the MixSS website (http://www.toxicology.org/isot/ss/Mix/#downloads)

Thanks for Your Support!

At the 50th Anniversary SOT Meeting in Washington DC, we had the chance to thank Past President, **Dr. Kannan Krishnan** (Université de Montréal), for his wonderful service to the MixSS. Under his dedicated leadership, this relatively young Specialty Section took another important step in its development. Now, we would like to thank him again for his continued support. Kannan provided a generous donation to the MixSS to help sustain its progress and activities. The MixSS sincerely thanks you, Kannan!

If members are interested in contributing financially to support the MixSS, please contact Kimberly von Brook (kimberly@toxicology.org).

Mixtures Events – updates

In 2011, there were many excellent meetings on mixtures. If you were unable to attend, it is not too late to reap the benefits of shared knowledge.

The NAS Emerging Science for Environmental Health Decisions Workshop titled: **Mixtures and Cumulative Risk Assessment: New Approaches Using the Latest Science and Thinking about Pathways** took place July 27-28, 2011. You can find slides and recordings from the meeting, as well as an informative reading list at the following website: http://nas-sites.org/emergingscience/workshops/mixtures/

The NIEHS held a workshop titled: **Advancing Research on Mixtures: New Perspectives and Approaches for Predicting Adverse Human Health Effects** on Sept. 26-27, 2011 (see picture from the workshop below). Presentation slides can be found at: http://tools.niehs.nih.gov/conferences/dert/mixtures/

A report from the meeting is currently in progress and will be released later this year.

Dr. Ray Yang drops some knowledge on fellow workshop participants

A Special Issue of the Elsevier journal *Toxicology* will result from the **International Conference on the Toxicology of Mixtures**, held October, 2011 in Arlington, Virginia. Jane Ellen Simmons and Ken Wallace who Co-Chaired the Conference will serve, respectively as Editor and Co-Editor. Ken is currently Vice-President of the MixSS, becoming President for 2012-2013 and has long held the position of Editor of the journal *Toxicology*. Papers have been solicited from all speakers at the Conference (keynote speakers and oral presenters) as well as from selected poster presentations. Authors are busy writing as Jane Ellen, Ken, and Elsevier have made a commitment to publish the Special Issue within one year of the date of the meeting. Look for the Special Issue to hit the stands in fall, 2012!

If you would like to provide an opinion piece for the MixSS Newsletter (800 words or less) or get the word out about mixtures-related events, contact Cynthia Rider (cynthia.rider@nih.gov)