National Capital Area Chapter
Society of Toxicology Newsletter
Electronic Edition

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M. Biggs, Editor

CHAPTER MISSION STATEMENT

The National Capital Area Chapter of the Society of Toxicology (NCAC-SOT) was established to provide a regional focus for scientists of all disciplines interested in toxicology. The Chapter acts to:

- Sponsor and co-sponsor symposia on current issues in toxicology
- Provide an annual award to an outstanding student in toxicology to assist in attending the annual meeting of the SOT
- Maintain communication with the National SOT regarding current toxicology and regulatory concerns
- Sponsor Regional Chapter events at the SOT Annual Meetings

PRESIDENT'S MESSAGE

Thank you for the opportunity to serve as your President for the next year. I would like to begin by thanking the outgoing officers who have done a great job over the past several years leading NCAC-SOT: Dr. Pam Chamberlain, Dr. Robert Mitkus, Dr. Jessica Ryman-Rasmussen

(who recently relocated to North Carolina), and Colleen McLoughlin (Graduate Student Representative). We wish them well in their "retirement"!

Also, please join us in congratulating Dr. McLoughlin in completing her Ph.D. at Virginia Commonwealth University. It is always exciting to see our student members advance!

In addition, I want to welcome back the officers continuing their terms of service, Past President Dr. Laurie Roszell; Secretary Erik Janus, and Councilors Dr. Rosemary Schuh and Dr. Syril Pettit. Anna Schlappal has stepped up into the role of Graduate Student Representative, and Linnzi Wright continues as Postdoctoral Representative.

There are four new officers within the NCAC-SOT leadership. Dr. Bruce Fowler is now Vice-President/President-Elect, Dr. Christopher Sheth is stepping in as Treasurer, Dr. Melanie Biggs is Councilor (and the newsletter editor), and Abhishruti Saitu Parihar is the new Graduate Student Vice-Representative.

Our agenda for the coming year includes continued enhancements to outreach and communication through ToXchange, Facebook and Twitter, and the NCAC-SOT website. Our most ambitious goal is to increase student and post-doc involvement. We will also be forming an advisory committee to help devise a more effective communication strategy. If you are interested in participating, please contact me.

In other events, Dr. Bruce Fowler is planning a Fall Symposium focused on NexGen Risk Assessment, which will be held in late September or early October. Stay tuned for more information on this, which will be posted on our website and distributed via social media!

Until then, I wish everyone a happy, healthy, and cool summer.

Cal Baier-Anderson, Ph.D.
NCAC-SOT President

GRADUATE STUDENT REPRESENTATIVE'S MESSAGE

Greetings Students,

We recently held our Annual Spring Symposium, *Systems Toxicology,* at the NIH campus in Bethesda, MD. Speakers addressed a variety of topics from the historical basis of systems toxicology to identifying biomarkers, to risk assessment. The symposium finished with a question and answer panel and a social happy hour.

Students also participated in a poster competition, and the winners included:

1st Place, Colleen McLoughlin, Title: *In vivo* immunotoxicological evaluation of electrospun polycaprolactone (EPCL) and investigation of EPCL as a drug delivery system for immunomodulatory compounds.

2nd Place, Raju Khatri, Title: Increase in Nrf2 Contributes to Reduced Apoptosis and Resistance to Aromatase Inhibitors.

Joining NCAC-SOT as a student only costs $10.00! It gives you the opportunity to apply for NCAC-SOT awards as well as network with professionals from EPA, FDA, academia, and industry. We highly encourage student participation. Please get in contact with us if you have additional questions or want to get involved!

You can also contact us online! Find us on ToXchange, and follow us on Facebook (SOT NCAC Group) and Twitter (SOTNCAC) to keep up with current events within the local chapter!

Anna E. Schlappal, Student Representative
Abhishruti Saitu Parihar, Student Vice-Representative

POST-DOCTORAL REPRESENTATIVE'S MESSAGE

Dear NCAC-SOT Postdocs,

I hope that you're all having an enjoyable summer! The Postdoctoral Assembly (PDA) has been hard at work since the national meeting in San Francisco, and I wanted to provide you with a quick update. First, an informational pamphlet entitled, "Employee Benefits: A Primer for Postdocs," was put together by Natalie Johnson, Sky Pike, Marie Bourgeois, and Anne Loccisano. It's a must read for everyone in the market for a new job (http://www.toxicology.org/ai/spd/PDA_Docs/2012EmployBenef-Primer-Postdocs.pdf).

Second, the PDA will be hosting its first Gordon Research Seminar on Cellular and Molecular Mechanisms of Toxicology in August 2013. This will be a great opportunity for everyone to present their research so please save the date (August 10-12, 2013). Finally, applications for the Best Postdoctoral Paper Award are currently being accepted. If you've had a paper published in the last year, please consider applying (http://www.toxicology.org/ai/spd/PD-PubAwardAnn.asp).

If you have any questions or suggestions for how I might better serve you, please don't hesitate to contact me (linnzi.k.wright.ctr@us.army.mil).

Sincerely,

Linnzi Wright
NCAC-SOT Postdoc Representative

NCAC-SOT EXECUTIVE BOARD MEMBERS

President:	Cal Baier-Anderson (2012-2013) US Environmental Protection Agency 202-564-1933 baier-anderson.caroline@epamail.epa.gov
Vice-President/ President-Elect:	Bruce Fowler (2012-2013) ICF International 703-934-3324 bfowler@icfi.com
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Graduate Student Representative:	Anna Schlappal (2012-2014) University of Maryland College Park 301-405-7989 aschlapp@umd.edu
Graduate Student Vice-Representative:	Abhishruti Saitu Parihar University of Maryland School of Medicine 410-706-7307 Asparihar@umaryland.edu

2012 FALL SYMPOSIUM AND ADDITIONAL FALL EVENT

NCAC-SOT is in the process of planning the annual fall symposium on NexGen Risk Assessment (e.g., see http://www.epa.gov/risk/nexgen/). This will be held in late September or early October. In addition to finalizing the date and lining up speakers, we are exploring alternative locations. If you have suggestions, please pass them on to Bruce Fowler at Bruce.Fowler@icfi.com.

In addition, NCAC-SOT is exploring a joint event with the local chapters of the Society of Risk Analysis and Society for Environmental Toxicity & Chemistry to be held later in October. We're hoping to arrange a short panel discussion of topics of mutual interest, building on the theme of NexGen risk assessment. In the meanwhile, watch for the meeting announcements.

2012 SPRING SYMPOSIUM

Title: Systems Toxicology
Date: May 14, 2012
Location: NIH Lister Hill Center Auditorium, Bethesda, MD

Speaker Abstracts

Keynote Address: The Evolution of Systems Toxicology: A Historical Perspective

Bruce Fowler, Ph.D., A.T.S., ICF International

Abstract: The science of toxicology has evolved rapidly towards understanding mechanistic relationships between exposure to toxic chemicals and events initiating processes of cell injury, cell death and carcinogenesis by looking at alterations of inter-related cellular systems and the molecular factors which regulate them. In order to understand how "systems toxicology" arrived at its current state, it may be useful to examine contributions from other disciplines which provided conceptual and technical foundations for advances in toxicology over the last 40 years. Among these, the advent of cell biology in the 1960's-1970's which combined electron microscopy with biochemistry to examine physical relationships between intracellular organelle structure and biochemical function provided useful insights into how biochemical

systems were localized in specific organelles and that organelle systems interacted with each other (eg. proteins made in the endoplasmic reticulum were transferred into the mitochondria and heme produced in the mitochondria was transferred into the endoplasmic reticulum for production of cytochrome-based enzymes). It was clear that chemical- induced disruption of the structural/functional relationships between cooperating intracellular systems could ultimately lead to cellular dysfunction unless other regulatory biological processes were able to maintain cellular homeostasis and viability. Chemical-specific alterations of gene/protein expression patterns, including stress proteins, are one such general manifestation of an early cellular response to chemical insult. These data also suggested that this information, validated with other indicators of toxicity, could be used to develop new classes of biochemical tests (eg. "omic " biomarkers) for early detection of toxicity. Further, an appreciation that receptor molecules play a role in mediating the activity of some chemicals at low dose levels added another level of complexity to understanding and interpreting systems toxicology data since multiple regulatory layers of inter-related biological systems are involved. The development of computational bio-information systems has proven very useful for interpreting such complex toxicology data through development of knowledge maps. At present, major challenges for the toxicology community are how to apply the above molecular information tools for "evidence-based risk assessments "and to develop risk communication approaches to translate systems toxicology information into "plain" English for persons who may not have extensive technical backgrounds.

Use of New Approaches to Identify Biomarkers of Liver Injury

Donna Mendrick, Ph.D., US Food and Drug Administration

Abstract: Although animal models identify many toxic compounds, they do not serve as a complete safety net. Unfortunately, some drugs are approved for human use only to find out postmarket that they cause severe liver toxicity in some individuals. Such findings can lead to restrictions on the drug's use or even its removal from the market because of the lack of tools to recognize susceptible individuals early. New approaches are being employed to identify adverse events earlier in the drug development pipeline and in the practice of medicine to enable safer use of valuable drugs.

Systems Toxicology of Chemicals of Military Interest

Jennifer Sekowski, Ph.D., US Army Edgewood Chemical Biological Center

Abstract: Characterization and defense against the toxic effects of traditional threat agents, toxic industrial chemicals (TICs), and explosive compounds pose serious challenges to both military and civilian defense communities worldwide. While much is known about the most serious and acute effects of these agents and general mechanisms of action, there is also a growing community of investigators examining the toxicology of sub-acute and lower level exposures. This research is examines the complex interplay of responses at the organism, tissue, cellular, and molecular levels. To bridge and incorporate these data, from the genome to the whole animal, a systems approach employing multiple levels of computational biology is being initiated. This presentation focuses on a few examples of how the systems approach is

currently being used to examine the molecular targets of TICs, such as pesticides, explosive compounds, such as RDX and TNT, and chemical threat agents, such as nerve and blistering agents. There will also be a brief discussion regarding how these data will be used to move toward building robust in silico models to predict toxicology and aid in the more rapid development of medical chemical countermeasures.

Neurotoxicology from Proteins to Cells to Tissues to Behavior

Marion Ehrich, Ph.D. Virginia Tech

Abstract: With recent emphasis on integrated studies of biological systems, an example can be provided using organophosphate neurotoxicants that have acute or delayed effects. Comparisons at protein, cellular, tissue and whole body levels show differences as well as similarities. Research questions need to address the value of less delineated perturbations at the subcellular level and what this means to whole organisms and extrapolations across species.

The Role of Thyroid Hormones in Neurodevelopment: Using the Adverse Outcome Pathway Concept to Focus Research Strategies

Kevin Crofton, Ph.D., US Environmental Protection Agency

Abstract: Epidemiological and laboratory data have demonstrated that disruption of maternal thyroid hormones during fetal developmental may result in irreversible neurological consequences. Coupled with increasing evidence that many environmental chemicals may pose a risk to thyroid hormone signaling pathways during development, yields a priority to better characterize potential hazards of exposure. An impediment in using a majority of peer reviewed research to inform regulatory action is that many of these studies are not designed to provide research targeted at known uncertainties in hazard and exposure assessments. The adverse outcome pathway concept provides a framework to document and test linkages between chemical-induced the molecular initiating events and adverse outcomes. Development of AOPs can provide insight into the uncertainties in linking chemical use, exposure and outcome, thereby focusing research on critical data needs for hazard assessments (Watanabe et al, 2011). The AOP framework was used to target research projects on the potential health outcomes associated with exposure to triclosan, a bacteriosat used in a wide variety of commercial products. The focused research allowed a better understanding of the uncertainties associated with developmental exposures and extrapolation of animal data to human and thereby improved the hazard assessment of this environmental contaminant.

Systems Biology and Risk Assessment: Promises, Challenges and a Proposed Integrative Path Forward

Lynne Haber, Ph.D., TERA; John Reichard, Ph.D., TERA; and John R. “Jack” Fowle III, Independent consultant, US EPA, ret

Abstract: Systems biology and related new testing approaches have substantial potential to aid in risk assessment, both in hazard characterization and dose-response assessment. Multiple

research initiatives are ongoing to develop and validate high- and medium-throughput tests with the goals of enhancing the predictive accuracy of traditional toxicology testing methods and reducing reliance on animal models. A congruent effort is required in risk assessment to develop methods to meaningfully apply such data. Near-term applications include identification of target biological pathways, improved mode of action determination and target organ identification. Dose-response assessment from new toxicology approaches will also be enhanced by the use of human cells for testing, decreasing the need for extrapolation to doses well below the data. To reach these goals requires new risk assessment tools and improved incorporation of decision analytic methods. Key challenges to implementing such applications include the need to "anchor" the results of *in vitro* testing to classical toxicity tests, distinguishing homeostatic changes from adverse effects, and compensating for the inability of in vitro tests to model changes that may occur at the intercellular, organ and organism level in vivo. Addressing these issues requires a hierarchical suite of approaches for modeling data (e.g., linked exposure-effect modeling). Furthermore, building collaborative and open alliance among governmental, non-governmental and international organizations with a stake in next generation toxicology testing will facilitate the connection of research to risk assessment paradigms. We propose a multi-stakeholder research effort to use data on pesticides, as data-rich chemicals with a well-understood MOA, as the basis for developing the needed tools to integrate systems biology with risk assessment.

Student Poster Abstracts

Increase in Nrf2 Contributes to Reduced Apoptosis and Resistance to Aromatase Inhibitors

Raju Khatri, Angela Brodie and Anil K. Jaiswal. Department of Pharmacology and Experimental Therapeutic. University of Maryland School of Medicine, Baltimore, MD

Breast Cancer is the leading cause of death in women. Excellent chemotherapeutic drugs including aromatase inhibitors (AIs) are available to reduce or eliminate breast cancer. However, there is often the problem of drug resistance. INrf2 (Keap1):Nrf2 serve as sensors of drugs and radiation-induced oxidative/electrophilic stress. INrf2 constitutively suppresses Nrf2 in the absence of stress by functioning as an adapter protein for Cul3/Rbx1 mediated ubiquitination/degradation of Nrf2. Upon exposure to stress, Nrf2 is dissociated from INrf2, stabilized and translocates to the nucleus and coordinately induce 200+ cytoprotective gene expressions. Studies showed that AI letrozole resistant breast cancer LTLT cells contain lower INrf2 and higher Nrf2 levels, as compared to drug sensitive MCF-7Ca cells. The removal of letrozole from LTLT cells led to increase in INrf2, decrease in Nrf2 and increased sensitivity to letrozole-induced death. Higher levels of Nrf2 were also observed in anastrozole resistant breast cancer AC1AnaR cells, as compared to sensitive AC1 cells. Further studies revealed that higher Nrf2-mediated activation of biotransformation enzymes, drug-transporters and anti-apoptotic proteins contributed to reduced efficacy of drugs and prevention of apoptosis that led to drug resistance. Current studies are investigating the mechanism of AI-mediated decrease in INrf2 gene expression. These together suggest that breast cancer cells during persistent treatment with AI drugs generate ROS and electrophiles that signal INrf2 down regulation and Nrf2 activation leading to reduced cell death and increased cell survival/drug resistance.

In vivo immunotoxicological evaluation of electrospun polycaprolactone (EPCL) and investigation of EPCL as a drug delivery system for immunomodulatory compounds

Colleen McLoughlin. Virginia Commonwealth University, Richmond, VA

Electrospun materials have potential use in many biomedical applications such as soft tissue replacements or as scaffolds to target drug delivery to local sites. Electrospinning is a polymerprocessing technique that can be used to create materials composed of fibers with diameters ranging from the micron to the nanoscale. We investigated the effects of microfibrous and nanofibrous electrospun polycaprolactone demonstrated that in both young (12 week) and old (6 month) mice, EPCL had no effect on various immune parameters. With its lack of immunotoxicity, EPCL presents an excellent polymer scaffold for use in delivering drugs to local sites. Drug delivery studies focused on using EPCL nanofiber scaffolds with the known immunosuppressive compound dexamethasone (DEX) incorporated within the matrix. The ability of the EPCL-DEX scaffold to suppress cell-mediated immunity (CMI) was evaluated using the delayed-type hypersensitivity (DTH) response to *Candida albicans.* Preliminary studies were conducted following subcutaneous implantation of a single disk (6-mm or 3-mm diameter) with 3, 10, 30, or 100 % w/w DEX in EPCL in the thigh region. Based on footpad swelling, dose - responsive suppression of the DTH was observed based on DEX equivalent units (DEU) at all but the lowest dose. The animals that received the high dose (100% in 6-mm) had decreased spleen weights, however no change in spleen weight was observed at the lower doses. Thymus weights were only affected at the four highest doses. These preliminary results suggest that implantation of a drug-containing electrospun scaffold may achieve local immunosuppression without systemic toxicity. Finally, we evaluated the EPCL-DEX scaffold in an acute inflammatory model (keyhole limpet hemocyanin) and a mouse model of rheumatoid arthritis (collagen induced arthritis). While similar trends were observed in other the models, the EPCL-DEX system achieved greatest success in the DTH model.

Chronic Pain after TBI: Thalamic abnormalities and prophylaxis with glyburide

Abhishruti Saitu Parihar[1,2], Kaspar Keledjian[2], Volodymyr Gerzanich[2], J. Marc Simard[2], and Asaf Keller[1]. [1]Department of Anatomy and Neurobiology, [2]Department of Surgery. University of Maryland, Baltimore, MD

In the US alone, about 1.4 million people suffer from traumatic brain injury (TBI) every year. TBI is an important health issue in both military and civilian life, and is very common among war-fighters. About one half of these individuals suffer not only from cognitive and motor deficits, but also from excruciating, unrelenting and chronic pain. In the current study, we aim to understand the mechanisms that lead from brain trauma to the development of chronic pain. We also explore the prophylactic potential of glyburide (GLY) to prevent the development of this devastating condition. The mechanisms of chronic headaches in general, and post-TBI pain in particular, are largely unknown. Considerable evidence, from both human and animal studies, indicates that this is the result of "central sensitization", a process by which brain structures undergo maladaptive plasticity, resulting in abnormal activity of brain neurons. We have recently demonstrated that after spinal cord injury (SCI), such central sensitization occurs

in the posterior thalamus (PO). Here, we test the hypothesis that TBI-Pain results in changes in PO similar to those after SCI. Testing for mechanical hyperalgesia revealed that TBI-Pain rats have a significantly lower tolerance to pain compared to the control group, and to TBI-Pain rats that received GLY. GLY (infusion of 200 ng/h of GLY yields plasma concentrations ~ 5 ng/ml, this has a minimal effect on serum glucose) was administered through mini-osmotic pumps implanted subcutaneously immediately after TBI surgery. Consistent with the behavioral data, single unit electrophysiological recordings from the PO showed an increase in the spontaneous firing rate of neurons from TBI-Pain rats, compared to sham. Furthermore, histology from TBI-Pain rats revealed upregulation of SUR-1-regulated NCCA-ATP channel (molecular target for GLY), GFAP (astrocytic marker), and Iba1 (marker for microglial activation) in the thalamus of TBI-Pain rats. These data support the hypothesis that TBI-Pain is associated with maladaptive plasticity in the PO thalamus and that prophylactic treatment with GLY prevents TBI-Pain.

Anti-oxidant-activated PI3K/Akt Blocks GSK3□ from Regulating Nrf2 Export and Degradation that Allows Unimpeded Nrf2 Activation of Cytoprotective Gene Expression

Phillip M. Shelton and Anil K. Jaiswal. Department of Pharmacology and Experimental Therapeutics. University of Maryland School of Medicine, Baltimore, MD

NF-E2-related factor 2 (Nrf2) is a transcription factor that regulates a battery of cytoprotective genes that are critical for the maintenance of cellular redox balance. In the presence of oxidative stress Nrf2 disassociates from the negative regulator INrf2 (Keap1) and translocates into the nucleus where it coordinately induces the transcription of a battery of defensive genes that combat reactive oxygen species and electrophiles. Subsequently, the Src-A subfamily kinase members Fyn, Src, Yes, and Fgr phosphorylate Nrf2 at tyrosine 568, which triggers Nrf2 nuclear export and degradation during the "post induction" phase. We have previously reported that activation and nuclear accumulation of the Src-A subfamily are regulated by the upstream kinase, GSK3β. In this study, we investigated the upstream factors responsible for regulating stress-activated GSK3β in human hepatocellular carcinoma, HepG2, cells. Here we demonstrate that one of the "early induction phase" responses to the anti-oxidant *tert*-Butylhydroquinone (t-BHQ) is the activation of the PI3K/Akt pathway. Within 0.5-1hr of tBHQ treatment Akt was activated upon being phosphorylated at S473 and T308, which allowed it to phosphorylate its substrate GSK3β at S9, resulting in the inhibition of GSK3β. In this inactive, closed state GSK3β is unable to interact with and phosphorylate its substrate Fyn, thereby preventing Fyn nuclear accumulation. Treatment of cells with the PI3K inhibitors, Wortmannin or LY294002, or an Akt inhibitor blocked GSK3β S9 phosphorylation, which correlated with altered levels of Fyn and disrupted Nrf2 nuclear accumulation and activation. Together, this study demonstrates that the upstream PI3K/Akt cascade inactivates GSK3β mediated Fyn localization, thereby enabling Nrf2 to enter the nucleus unimpeded, where it can up-regulate cytoprotective gene expression.

NCAC-SOT Treasurer's Report – July 06, 2012 by Chris Sheth, Treasurer				
Account activity since last report (Dated Nov 17, 2011 in November 2011 Newsletter)				
10/ 31/2011 (Closing balance)				17,792.37
Debits	**Description**	**Notes**		
	Girl Scout Nation's Capital Science Day		2,395.90	
	National Meeting – San Francisco, CA (March 2012)	Reception at Marriot	3,726.00	
	Spring Symposium (May 2012)	Registrations (1,515.00) Expenses* (2,661.49)	1,146.49	
	Total		7,268.39	
Credits				
	Dues (renewals and new members)		2,670.00	
	Misc. deposits**		3,265.98	
	Total		5,935.98	
April 30, 2012* (Closing balance)				16, 459.97
*Most recent bank statement in my possession.				

*Does not include travel claims ($) for two other speakers.

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**June ledger from SOT not available to identify sources of deposits.

Exploring Human Genomic Plasticity and Environmental Stressors: Emerging Evidence on Telomeres, Copy Number Variation, and Transposons

October 4-5, 2012
Thursday 8:30am-5:00pm, Friday 8:30am-12:00noon
Keck Center 500 5th St NW Washington, DC 20001

Register Here: http://nas-sites.org/emergingscience/workshops/genomic-plasticity/

Ready to S-T-R-E-T-C-H Your Thinking about the Human Genome?

The human genome is routinely thought of as a static component of the cell, one that is subject to just two fates; to be inherited or to mutate. However, since the completion of the sequencing of the human genome, scientists have been exploring how the genome responds to environmental stressors and chemical exposure. It turns out the genome is much more dynamic than we thought. Genomes have the characteristic of plasticity, which makes it possible to adapt quickly in order to survive changes in environmental conditions. Changes in the genome can have a big impact on human health. The Standing Committee on Emerging Science for Environmental Health Decisions has been exploring factors that influence human health in its workshop series. An earlier forum on Epigenetics (http://nas-sites.org/emergingscience/workshops/epigenetics) looked at how genes are expressed and silenced in response to environment stressors. Mobile and evolving elements such as telomeres, transposons, and copy number variants are other important factors in understanding the potential effect of our environment on human health.

The Genomic Plasticity forum will look beyond random mutation and discuss the fundamental changes in genomic alterations that can contribute to disease and ageing, as well as new technologies and tools to identify and study genome plasticity events. The forum will initiate/foster the exploration of how environmental stressors may impact the genome, by exploring the intersection of mechanisms leading to genomic changes and mechanisms targeted by environmental stressors.