NorCal Society of Toxicology Spring Symposium

Wednesday, April 13th, 2011

Berkeley City Club, 2315 Durant Avenue, Berkeley, CA 94704-1606

Registration and Continental Breakfast 8:30-9:15

Morning Session: Current Topics in the Field

9:15-9: 30 **Welcome and Chapter Business Updates**
Tao Wang, MD, PhD, DABT, President for NorCal SOT

9:30-10:15 **Anesthetic-Induced Developmental Neurotoxicity and Pathways to Prevention**
William Slikker, Jr., PhD. National Center for Toxicological Research/FDA and Toxicologic Pathology Associates; Center for Drug Evaluation and Research/FDA

10:15-11 **Application of New Technologies and the Future of Toxicity Assessment for the 21st Century**
James S. Bus, PhD, DABT, ATS, Director of External Technology, The Dow Chemical Company

11:00-11:15 Break

11:15-12:15 **Putting Health Risks into Perspective**
Bruce N. Ames, PhD, Senior Scientist, Nutrition and Metabolism Center Children's Hospital Oakland Research Institute, Oakland, California

Afternoon Session: Mitochondrial Dysfunction and Related Issues

2:00-2:45 **Mitochondrial Homeostasis in Acute Kidney Injury**
Rick G. Schnellmann, PhD, Professor and Chair of the Department of Pharmaceutical and Biomedical Sciences, Medical University of South Carolina

2:45-3:30 **Mitochondrial Disease and Degeneration in Aging**
Gino Cortopassi, PhD, Professor, Department of Molecular Biosciences, UC Davis

3:30-4:00 Break

4:00-4:45 **Handling Mitochondrial Dysfunction and Related Toxicity in Drug Development-Case Studies**
Jeff Lawrence, PhD, Director, Biochemical Toxicology and Biomarkers, Amgen

Wine and Cheese / Poster Viewing

Registration

Pre-registration will facilitate planning for security and parking purposes. Registration is $35 for NorCal SOT members ($45 on-site registration), $45 for nonmembers ($55 on –site), and FREE for students/post-docs. Students are encouraged to bring posters documenting their research and may have their posters printed by contacting Steve Dizio at sdizio@dtsc.ca.gov. All attendees will have the opportunity to participate in a Lunch with an Expert program.

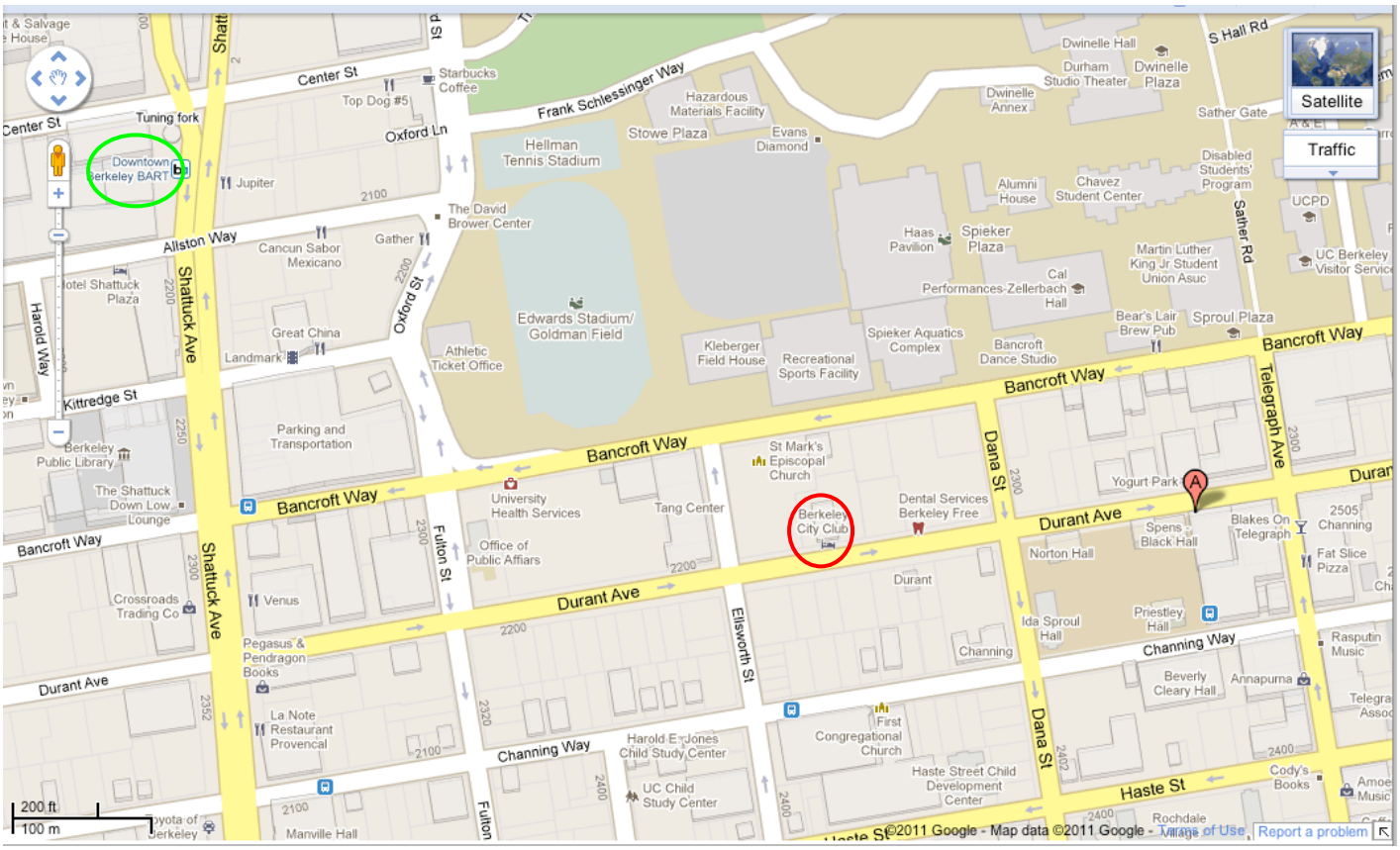
Parking

Parking for the meeting will be at the City of Berkeley Telegraph Channing Garage (formerly known as the City of Berkeley Sather Gate Parking Lot) (http://www.ci.berkeley.ca.us/ContentDisplay.aspx?id=16058), which is located at 2450 Durant Avenue, 1 block from the City Club (the City Club website is http://berkeleyhistorichotel.com/).

Parking with validation will cost $15 for the whole day ($20 unvalidated). Be sure to collect a validation coupon at the meeting registration desk when you arrive.

Map

A= parking, red=Berkeley City Club, green=BART station



Abstracts and Biographical sketches

Anesthetic-Induced Developmental Neurotoxicity and Pathways to Prevention

William Slikker, Jr., PhD.National Center for Toxicological Research/FDA and Toxicologic Pathology Associates, Jefferson, AR, USA; Center for Drug Evaluation and Research/FDA, Rockville, MD, USA

In several animal models it has been shown that the developing brain is susceptible to anesthetic-induced injury. The window of vulnerability to these neuronal effects of anesthetics is restricted to the period of rapid synaptogenesis, also known as the brain growth spurt. Similar dependencies on dose/duration of exposure and developmental stage are observed in both the non-human primate and rodent models for NMDA receptor-dependent anesthetics (e.g., ketamine). The duration of anesthesia needed to induce cell death as measured by minimal exposure requirements is similar (~ 4-6 hrs) for nonhuman primate and rodent brain cells in culture, and also *in vivo* in rodent and nonhuman primate models. The susceptible stage or period of development has not been completely described, but in the nonhuman primate it begins somewhere before the last quarter of pregnancy and continues until shortly after birth. Behavioral studies in developing primates have confirmed functional deficits following neonatal ketamine-induced anesthesia as assessed by the NCTR Operant Test Battery. In rats previously exposed to ketamine, microPET imaging data have indicated enhanced ^{18}F Annexin-V retention, a non-invasive marker of apoptosis. It has been postulated that up-regulation of the NR1 subunit of the N-methyl-D-aspartate receptor (NMDAR), a calcium channel regulator, may be an important first step in the pathway to anesthetic-induced neurotoxicity following exposure to NMDA antagonists. Both gene expression studies and NR1 antisense experiments have provided supportive evidence for NMDA receptor involvement in the neurotoxic pathway. Several recent studies have indicated that reduction of oxidative stress may protect the developing animal from anesthetic-induced brain cell death. Evidence for the role of oxidative stress in anesthetic-induced neurotoxicity has been generated in studies that apply oxidative stress blockers, including L-carnitine (mitochondrial protector) and melatonin *in vivo* and specific antioxidants *in vitro* including the superoxide dismutase mimetic, M40403 and the NOS inhibitor, 7-nitroindazole. Recent gene expression assessments indicate that genes in the oxidative stress pathway are altered by anesthetic treatment of developing animals. Together, the application of omics approaches along with traditional toxicological endpoints indicates that oxidative stress plays a critical role in the susceptibility of the developing brain to anesthetics
Supported by NICHD, NTP/NIEHS and CDER and NCTR/FDA

Bio

Dr. William Slikker, Jr. is the Director of the FDA's National Center for Toxicological Research (NCTR). Bill received his Ph.D. in Pharmacology and Toxicology from the University of California at Davis in 1978. Dr. Slikker holds Adjunct Professorships in the Departments of Pediatrics, and Pharmacology and Toxicology at the University of Arkansas for Medical Sciences. He has held committee chairmanships or elected offices in several scientific societies including the Teratology Society (serving as President) and the American Society for Pharmacology and Experimental Therapeutics (chair, Developmental Pharmacology Section and member of the Program Committee) and co-founder and past President of the MidSouth Computational Biology and Bioinformatics Society. He is currently Associate Editor for NeuroToxicology and Toxicological Sciences and past Treasurer, SOT and past President of The Academy of Toxicological Sciences. Dr. Slikker is currently the Vice President-elect of the Society of Toxicology. Dr. Slikker has authored or co-authored over 300 publications in the areas of transplacental pharmacokinetics, developmental neurotoxicology, neuroprotection, systems biology, and risk assessment. He has also served on several National/International advisory panels for HESI/ILSI, CIIT Centers for Health Research, EPA, NIEHS, NAS, NIH and WHO.

Application of New Technologies and the Future of Toxicity Assessment for the 21st Century

James S. Bus, PhD, DABT, ATS, Director of External Technology, The Dow Chemical Company, Midland, MI

The advent of a variety of exciting new technologies such as toxicogenomics, bioinformatics, and high-throughput mechanism-based screening tests affords an opportunity to dramatically reframe the existing toxicity testing and risk assessment paradigm that is burdened with inarguable concerns of cost, speed, use of animals, ability to inform risk, and others. Nonetheless, implementation of these technologies into mainstream use in, or even replacement of, conventional toxicity evaluations must be done thoughtfully and with great caution. Decades of experience with modern toxicology has shown that effective prediction of *in vivo* toxicity, much less estimation of actual human health risk, from high-throughput technologies is certain to be complicated by the complexity of whole-animal reactions to chemical exposures that drive toxicity expression. For example, the central role of complex ADME phenomena in mediating target-directed toxicity expression will prove challenging to replicate in any single or combination of high-throughput methods. Despite these concerns, however, the new technologies, effectively implemented, will indeed open doors of opportunity to dramatically and rapidly improve risk-based assessments of drugs and chemicals. Thus, these methods can significantly impact many of the vexing issues currently facing conventional toxicity testing, such as: improving rapid identification and understanding of mode-of-action; cross-species extrapolations; dose-dependent transitions; individual and population-level susceptibilities; implications of complex mixture exposures; and building better understanding of true risks of real-world, low-dose exposures.

Bio

James S. Bus is Director of External Technology, Toxicology and Environmental Research and Consulting at The Dow Chemical Company (1989-present). He previously held positions as Associate Director of Toxicology and Director of Drug Metabolism at The Upjohn Company (1986-1989), Senior Scientist at the Chemical Industry Institute of Toxicology (CIIT, 1977-1986), and Assistant Professor of Toxicology, University of Cincinnati (1975-1977). Dr. Bus currently participates in several external institutions including the Board of Directors of The Hamner Institutes (formerly CIIT) and the National Academy of Sciences/National Research Council Board on Environmental Studies and Toxicology (BEST). He has also has served as Chair of the American Chemistry Council and International Council of Chemical Associations Long-Range Research Initiatives; the USEPA Office of Research and Development Board of Scientific Counselors (1997-2003) and Chartered Science Advisory Board (2003-2009); the National Toxicology Program Board of Scientific Counselors (1997-2000); and the FDA National Center for Toxicological Research Science Advisory Board (2004-2010). He serves as an Associate Editor of *Toxicology and Applied Pharmacology*,and on the Editorial Boards of *Environmental Health Perspectives* and *Dose Response*. Dr. Bus is a member of the Society of Toxicology (serving as President in 1996-97), the American Society for Pharmacology and Experimental Therapeutics, the American Conference of Governmental and Industrial Hygienists, and the Teratology Society. He is a Diplomate and Past-President of the American Board of Toxicology and a Fellow of the Academy of Toxicological Sciences (member of Board of Directors, 2008-present; Vice-President and President-Elect, 2010). Dr. Bus received the Society of Toxicology Achievement Award (1987) for outstanding contributions to the science of toxicology; the Society of Toxicology Founders Award (2010) for leadership fostering the role of toxicology in improving safety decisions; Rutgers University Robert A. Scala Award (1999) for exceptional work as a toxicologist in an industry laboratory; and the K.E. Moore Outstanding Alumus Award (Michigan State University, Dept. Pharmacol. And Toxicol.). He received his B.S. in Medicinal Chemistry from the University of Michigan (1971) and Ph.D in pharmacology from Michigan State University (1975) and currently is an Adjunct Professor in the Dept. Pharmacology and Toxicology at that institution. His research interests include mechanisms of oxidant toxicity, defense mechanisms to chemical toxicity, relationships of pharmacokinetics to expression of chemical toxicity, and general pesticide and industrial chemical toxicology. He has authored/co-authored over 100 publications, books, and scientific reviews.

Putting Health Risks into Perspective

Bruce N. Ames, PhD, Senior Scientist, Nutrition and Metabolism Center Children's Hospital Oakland Research Institute, Oakland, California

To effectively prevent cancer and other age-related diseases one needs to put risks in perspective. The public is terribly confused about risks to their health, with good reason. Innumerable minor or even hypothetical risks have been blown out of proportion, making real disease prevention much more difficult. Pesticides and other miniscule traces of man-made chemicals are likely to pose minimal, or no risk and no convincing evidence supports a significant health risk. The vast bulk of chemicals ingested by humans is natural. For example, 99.99% of the pesticides we eat are naturally present in plants to ward off insects and other predators. Half of these natural pesticides tested at the maximum tolerated dose (MTD) are rodent carcinogens. Animal cancer tests, which are done at the MTD, are being misinterpreted to mean that low doses of synthetic chemicals and industrial pollutants are relevant to human cancer. Half of all chemicals tested, whether synthetic or natural, are carcinogenic in rodent tests. A plausible explanation for this high frequency is that the MTD causes chronic cell killing and consequent cell replacement, a risk factor for cancer that can be limited to high doses. Ignoring this greatly exaggerates risks. Reducing our exposure to the 0.01% of dietary pesticides, that are synthetic, is very expensive and will not reduce cancer rates. On the contrary, fruits and vegetables are effective dietary cancer fighters, and making them more expensive, e.g. organic food, by reducing synthetic pesticide use will likely increase cancer.

The two big preventable risks for health are smoking and poor diets. These are lifestyle risks, which can be changed. Smoking shortens the lifespan by about 8 years on average (2 packs/day), and a poor diet is likely to have even a more significant negative impact on lifespan. A "balanced" diet is important for minimizing the diseases of aging; understanding balance could benefit almost all of us. Most of the world's population, even in developed countries, has inadequate intake of one or more micronutrients (~40 essential vitamins, minerals, fatty acids and amino acids) that a varied and balanced diet should provide. *Triage theory* (PNAS 103, 17589, 2006; AJCN 90, 889, 2009; J Nucleic Acid 2010, FASEB J 2011, in press) posits that, as a result of recurrent shortages of micronutrients during evolution, natural selection developed a metabolic rebalancing response to shortage. The rebalancing favors micronutrient-dependent proteins needed for short-term survival while starving those only required for long-term health. Triage theory predicts that the consequence of moderate shortages of even a single micronutrient, though insufficient to cause overt clinical symptoms, will impair functions essential for long-term health. This impairment will result in insidious damage (e.g. increased DNA damage) that, over time, leads to the acceleration of age-associated diseases (e.g. increased cancer). As people with modest deficiencies have no overt clinical symptoms, there has been little incentive to correct these deficiencies, though this could change if it can be shown that they are resulting in biochemical changes, e.g. chromosome breaks, that are markers of increased risk of age-related diseases, e.g. cancer. The considerable experimental and theoretical support for the triage idea will be discussed as will a strategy for determining the optimum level of each micronutrient in humans.

Too much refined food causes a shortage of micronutrients and fiber and an excess of calories (sugar, fat, and alcohol) which contributes to chronic inflammation, obesity (a huge risk to health), and associated diseases, such as diabetes. Mitochondrial decay, (a decrease in membrane potential, respiratory control ratio, cardiolipin, and cellular oxygen consumption, and an increase in mutagenic oxidant by-products) appears to be a major contributor to aging and associated degenerative diseases. Oxidative damage to DNA, RNA, proteins, and lipids in mitochondrial membranes is a major consequence of this decay, resulting in functional decline of mitochondria, cells, and organs. Feeding the mitochondrial metabolites acetyl carnitine and lipoic acid to old rats rejuvenates the mitochondria and improves brain and other function. I will discuss how, through our work and that of others, nutrition is now being put on a firmer scientific foundation than ever before. This understanding will lead to medicine that is more prevention oriented for age-related diseases, and to inexpensive nutritional and other interventions to delay the degenerative diseases accompanying aging, such as cancer, cardiovascular disease, cognitive decline, stroke, and immune dysfunction.

Bio
Dr. Ames is a Professor of Biochemistry and Molecular Biology, Emeritus, University of California, Berkeley, and a Senior Scientist at Children's Hospital Oakland Research Institute. He is a member of the National Academy of Sciences and he was on their Commission on Life Sciences. He was on the board of directors of the National Cancer Institute, the National Cancer Advisory Board, from 1976 to 1982. His awards include: the General Motors Cancer Research Foundation Prize (1983), the Tyler Environmental Prize (1985), the Gold Medal Award of the American Institute of Chemists (1991), the Glenn Foundation Award of the Gerontological Society of America (1992), the Honda Prize of the Honda Foundation, Japan (1996), the Japan Prize, (1997), the Kehoe Award, American College of Occup. and Environ. Med. (1997), the Medal of the City of Paris (1998), the U.S. National Medal of Science (1998), the Linus Pauling Institute Prize for Health Research (2001), the American Society for Microbiology Lifetime Achievement Award (2001), the Thomas Hunt Morgan Medal from the Genetics Society of America (2004), and the American Society for Nutrition/CRN M.S. Rose Award (2008). His 540+ publications have resulted in his being among the few hundred most-cited scientists (in all fields). www.bruceames.org; bames@chori.org

Mitochondrial Homeostasis in Acute Kidney Injury
Rick G. Schnellmann, PhD, Professor and Chair of the Department of Pharmaceutical and Biomedical Sciences, Medical University of South Carolina

Mitochondrial damage is a major contributor to the initiation of tubular cell injury and the progression of acute kidney injury (AKI) produced by drugs, toxicants, and ischemia. To understand the role of mitochondria in organ damage and repair, we think that mitochondria need to be examined holistically by measuring mitochondrial homeostasis. This includes changes in mitochondrial loss, fission/fusion, mitophagy, and biogenesis over time. Using this approach, temporal differences in mitochondrial loss, dynamics and biogenesis were observed with mitochondrial loss occurring early and changes in mitochondrial fission/fusion and biogenesis occurring later after AKI.

Bio
Dr. Schnellmann is Eminent Scholar, Professor and Chair of the Department of Pharmaceutical and Biomedical Sciences at Medical University of South Carolina (MUSC). He is a renal pharmacologist & toxicologist with longstanding interests in the mechanisms of renal injury and regeneration, and the treatment thereof. Much of his previous and current research has been focused on the role of mitochondrial injury in renal cell death. In particular, he has characterized the role of a mitochondrial protease (i.e. calpain 10) and a mitochondrial phospholipase ($iPLA_2g$) in mediating and protecting mitochondria and renal cells from diverse insults. More recently, he has focused on mitochondrial homeostasis and novel approaches to promote mitochondrial biogenesis to treat acute kidney injury after it has been initiated. His group has developed a high throughput screen to identify chemicals that induce mitochondrial biogenesis and provided evidence that stimulation of mitochondrial biogenesis following injury accelerates cell repair and regeneration, and return normal cellular functions.

Mitochondrial disease and degeneration in aging
Gino Cortopassi, PhD, Professor, Department of Molecular Biosciences, UC Davis

Mitochondria are essential to energy generation and intermediary metabolism. Mitochondrial DNA deletions accumulate with age in human tissues, and in the brain specifically in the substantia nigra. Animal models with higher rates of mitochondrial mutagenesis age rapidly. In addition, inherited mutations in mitochondrially-expressed genes cause a wide variety of neurodegenerative diseases, including Friedreich's ataxia, MERRF, MELAS, LHON, CPEO, and NARP. Pathogenic mitochondrial mutations in the human population exist at much higher concentration than previously realized, i.e. at 1/200 individuals. We observe that mitochondrial dysfunction in human patients, in cells treated with mitotoxins, and in animal models of disease triggers a nuclear 'mitochondrial stress response' program, that could potentially be used as a biomarker of human mitotoxic stress. Although the specific mechanism(s) by which mitochondrial dysfunction lead to neurodegeneration are not completely clear, in the case of Friedreich's ataxia, the pathway appears to involve an alteration of mitochondrial redox status. Mitochondrial toxins exist in the environment, such as rotenone, however because of a lack of a high-throughput screening assay of mitotoxicity, the relative density and priority of other mitochondrial toxins has not been well-defined. However novel high-throughput assays of mitochondrial function could identify potent mitochondrial toxins and also beneficial mitochondrial drugs.

Bio
Gino Cortopassi received his B.A. in Biology from Stanford University in 1981, studying DNA repair in the laboratory of Philip Hanawalt. He received his PhD in Biochemistry in 1988, studying gene expression, evolution and mitochondria in the laboratory of Allan C. Wilson. His postdoctoral work with Norman Arnheim, was complete in 1991, and focused on the design of sensitive PCR-based assays of rare somatic mutations to be used in genetic screening. It was in this period that it was demonstrated that mitochondrial DNA deletion mutations accumulate with aging in human tissues. In 1992 he started his first faculty position at USC Dept. of Molecular Pharmacology and Toxicology. In 1995 he moved to the Dept. of Molecular Biosciences at UC Davis. His work is focused on mechanisms of mitochondrial disease, biomarkers of mitochondrial stress, and mitochondrial involvement in aging.

Handling Mitochondrial Dysfunction and Related Toxicity in Drug Development-Case Studies
Jeff Lawrence, PhD, Director, Biochemical Toxicology and Biomarkers, Amgen

During drug development it is often difficult to identify potential mechanisms of organ toxicity in a timely fashion. We have built a custom isolated mitochondrial function profiling platform to help identify potential subcellular targets of toxicity. Case studies will be presented where mitochondrial functional profiling was able to identify a putative target for a compound that caused hepatic steatosis.

Bio
Jeff Lawrence is the Director of Biochemical Toxicology and Biomarkers in the Department of Investigative Toxicology at Amgen in Thousand Oaks, CA. Jeff Lawrence holds a B.Sc. degree in Toxicology from the Philadelphia College of Pharmacy and Science, and a Ph.D in Pharmacology from the University of Florida. During his dissertation, Jeff studied DNA toposiomerases and mtDNA depletion mechanisms under the direction of Dr. Tom Rowe. Jeff completed his postdoctoral training at Eli Lilly and Co, where he worked with Dr. Pat Eacho in the Hepatotoxicity laboratory in the Biochemical Toxicology group and later the Cardiovascular Discovery Research group. Following his postdoctoral training, Jeff worked in the Department of Safety Assessment at Merck and Co., Inc. for 10 years. During this time, he was involved in toxicokinetic analysis for various drug development programs and actively led and participated in Investigative Toxicology research. Jeff Joined Amgen, Inc. in 1996 as Director, Biochemical Toxicology and Safety Biomarkers. His investigative efforts use biochemical, cellular, and molecular based approaches using in vitro and in vivo systems to solve program issues jeopardizing continued development of drug candidates.