NorCal SOT Spring Symposium

April 29, 2014

Venue: David Brower Center
2150 Allston Way, Berkeley, CA

Morning Session	
8:00 am – 8:30 am	Registration
8:30 am - 8:45 am	*Opening message from The President* **Dr. Sushmita Chanda, PhD, DABT** Alios Biopharma
8:45 am – 9:30 am	*Physiologically Relevant Organs on Chips* **Luke P. Lee, Ph. D.**, Arnold and Barbara Silverman Distinguished Professor Bioengineering, Electrical Engineering & Computer Science, and Biophysics, UC Berkeley, Co-Director, Berkeley Sensor & Actuator Center
9:30 am - 10:15 am	*The Changing Role of EEG in the Assessment of Pre-Clinical Seizure Risk* **Joseph Arezzo, Ph.D.,** Professor of Neuroscience and Neurology Albert Einstein College of Medicine
10:15am - 10:45 am	Coffee Break & Poster Session
10:45 am - 11:00 am	*Dioxin-Like and Non-Dioxin-Like Polychlorinated Biphenyls (PCBs) Modulate Basal and Activity-Dependent Dendritic Arborization in Primary Neuronal Cell Cultures* NorCal Graduate Student Award Winner: Christopher Barnhart, UC Davis
11:00 am - 11:45 am	*Key Note: Screening for Developmental Neurotoxicity: History, Progress and Challenges* **Kevin Crofton, Ph.D**., Acting Deputy Director, National Center for Computational Toxicology, USEPA
11:45 pm - 1:15 pm	Lunch Break, Lunch with experts, Posters, Plaques

Afternoon Session	
1:15 pm- 2:00 pm	*Abuse Liability Assessment and Current Drug Development Guidelines* **Dinah Misner, Ph.D.**, Senior Scientist and Investigative Toxicology Laboratory Group Leader, Genentech Inc.
2:00 pm – 2:15 pm	*Functional Genetic Screen in Human Haploid Cells to Identify Genes Involved in Susceptibility to Chemical Exposure* **NorCal Postdoctoral Fellow Award Winner: Hua Shen, Ph.D., UC Berkeley**
2:15 pm – 2:45 pm	Coffee Break & Poster Session
2:45 pm – 3:30 pm	*Why Profile: The Role of In Vitro Pharmacological Profiling in the Drug Discovery Process.* **Jacques Migeon, Ph.D.**, Technical Director, Pharmacology, Eurofins Cerep Panlabs
3:30 pm -4:15 pm	*Harnessing the Power of Zebrafish to Advance Environmental Health Sciences: High Content Data for 21st Century Toxicology* **Robert Tanguay, Ph.D.**, Distinguished Professor, Sinnhuber Aquatic Research Laboratory, Oregon State University
4:15 pm- 4:30 pm	**Closing Remarks**

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Abstracts:

Physiologically Relevant Organs on Chips

Luke P. Lee, Ph.D.
Arnold and Barbara Silverman Distinguished Professor
Berkeley Sensor and Actuator Center, Institute of Quantitative Biosciences,
UC Berkeley

Recent advances in integrating microengineering, life sciences, and medicine have generated promising microphysiological models for experimental medicine and pharmaceutical research. Here I will discuss the recent development of microengineered physiological systems, or also known as "organs-on-chips", that reconstitute the physiologically critical features of specific human tissues and organs and their interactions. This technology uses microengineering approaches to construct organ-specific microenvironments, reconstituting tissue structures, tissue-tissue interactions and interfaces, and dynamic mechanical and biochemical stimuli found in specific organs, to direct cells to assemble into functional 3D cell cultures. First, the demonstrations of microengineering approaches to reproduce the key elements of physiologically important, dynamic mechanical microenvironments, biochemical microenvironments, and microarchitectures of specific tissues and organs in microfluidic cell culture systems will be discussed. This is followed by examples of microengineered individual organ models that incorporate the key elements of physiological microenvironments into single microfluidic cell culture systems to reproduce organ-level functions. Finally, microengineered multiple organ systems that simulate multiple organ interactions to better represent human physiology, including human responses to drugs, is covered in this talk. As an exiting example for personalized medicine, I will discuss the progress on patient-specific iPSCs-based Integrative Microphysiological Analysis Platforms (iMAPs). This emerging organs-on-chips technology has the potential to become an alternative to 2D and 3D cell culture and animal models for experimental medicine, human disease modeling, drug development, and toxicology.

The Changing Role of EEG in the Assessment of Pre-Clinical Seizure Risk

Joseph Arezzo, Ph.D.
Professor of Neuroscience and Neurology
Albert Einstein College of Medicine

In standard pre-clinical studies, seizures are detected by visual observation. This practice is extremely insensitive; seizures may be missed due to the limited periods of observation (often 1-2 hours over a 24-hour period), the brief duration of some seizures (1-3 minutes in most cases) and to the fact that many types of seizure (e.g., absence seizures, partial seizure) may not be associated with "convulsions." EEG provides a sensitive and validated biomarker for frank seizure or altered seizure thresholds, and it has been the "gold standard" for the clinical detection of seizures for the past 100 years. It is now feasible to use implanted electrodes coupled with telemetry or a series of non-invasive, removable subcutaneous electrodes to monitor EEG from multiple brain regions in awake, unanaesthetized mice, rats, dogs or monkeys. Driven in large part by changes in technology and the guidance of the FDA, the use of pre-clinical EEG procedures has been greatly expanded. We present examples of the use of pre-clinical EEG to: a) identity the onset, pattern, location and time course of frank seizures, b) to detect more subtle pre-seizure patterns (e.g. repetitive, organized sharp waves) c) to document that behaviors often thought to reflect possible seizures (e.g., ataxia, tremors, repetitive movements) can be dissociated from any seizure or pre-seizure activity and d) to pre-screen dogs that may have an identified seizure risk prior to dosing (approximately 4% of beagles).

Dioxin-Like and Non-Dioxin-Like Polychlorinated Biphenyls (PCBs) Modulate Basal and Activity-Dependent Dendritic Arborization in Primary Neuronal Cell Cultures

Christopher Barnhart
Ph.D. Graduate Student in Pharmacology and Toxicology
UC Davis

PCBs are ubiquitous environmental contaminants that have been linked to cognitive and behavioral deficits in children and experimental animals. We previously demonstrated that exposure to the non-dioxin-like (NDL) PCB congener 95 increases dendritic arborization of primary hippocampal neurons *in vitro* via activation of the ryanodine receptor (RyR) calcium channel. However, whether other PCBs similarly trigger dendritic growth via RyR-dependent mechanisms is unknown. To address this question, we measured the effects of NDL PCB congeners 95 and 52 and dioxin-like (DL) PCB congener 77 on RyR activation and on dendritic arbor complexity. Radioligand-receptor binding analysis with tritiated ryanodine (^{3}H-Ry) showed that PCBs 95 and 52 increased specific receptor occupancy, indicating RyR sensitization; however, the concentration-effect relationships differed. In contrast, PCB 77 did not affect ^{3}H-Ry binding. To determine whether RyR sensitization predicted effects on dendritic growth patterns, primary cultures of perinatal rat hippocampal neurons were exposed to varying concentrations of each congener for 48 h, and the number of dendritic termini was quantified as a measure of dendritic arbor complexity. Each congener enhanced dendritic growth, but exhibited a unique concentration-effect relationship that did not correlate with potency of RyR activation. PCB exposure reduced arbor complexity in neurons treated with bicuculline, a GABA receptor antagonist that stimulates dendritic arborization, indicating an antagonistic interaction between PCBs and activity on dendritic growth. These data demonstrate that dendritic arborization may be a convergent cellular outcome in PCB developmental neurotoxicity, but that NDL and DL PCBs may stimulate dendritic growth via RyR-dependent and/or –independent pathways. This work was supported by NIH (grants R01 ES014901, R01 ES017425, T32 ES007059), the ARCS Foundation, and the Superfund Research Program (P42 ES04699).

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Screening for Developmental Neurotoxicity: History, Progress and Challenges

Kevin Crofton, Ph.D., Acting Deputy Director
Timothy J. Shafer, Ph.D. and William R. Mundy, Ph.D.
National Center for Computational Toxicology
United States Environmental Protection Agency

Adverse effects on the nervous system following exposure to environmental contaminants during development have been well documented. Research efforts over the past decade have endeavored to develop alternative methods/models to the time, animal and resource intensive EPA and OECD testing guideline methods. These new efforts include a wide variety of in vitro methods and some alternative species models. There are major challenges to both the acceptance and use of data from alternative methods in health decisions. Major scientific challenges for DNT include development and validation of new methods and models, development of tiered decision logic, and regulatory acceptance of the methods. Use of data from high-throughput screening (HTS), high-content imaging (HTI), and alternative species will be driven mainly by the regulatory problems being addressed. Some problems may be addressed with limited datasets, while others may require extensive data and biological modeling efforts. An integrated tiered testing strategy has been developed that informs both the development of new methods and use of data from such methods. This framework includes four method/data tiers: 1) data based entirely on the inherent properties of chemicals derived from 'read-across' and QSAR models which rely on existing biological data, 2) generation of new data for large number of chemicals using HTS and HCI that will allow prioritization of chemicals for further in depth testing, 3) targeted testing of chemicals using alternative animal models or 'refined' rodent models to generate data that better inform adverse outcomes and dose, and 4) system models providing detailed characterization of the links between exposures, molecular toxicity pathways and adverse outcomes that allow more accurate extrapolation across species, genders, and life stages. In this strategy, testing is driven by decision-making needs, and the amount of resource utilization is adjusted to provide efficient and timely data to address the needs. As the potential impact of the decision increases, data needs increase, resource use increases, and the need increases for reduced scientific uncertainty in estimates of risk and impact. Recent advances in testing methods and models hold great promise the development and use of efficient tiered testing strategies for DNT that are capable of initial screening, hazard characterization, and hazard prediction. *This abstract does not necessarily reflect U.S. EPA policy.*

Abuse Liability Assessment and Current Drug Development Guidelines

Dinah Misner, Ph.D., DABT
Senior Scientist and Group Leader
Investigative Toxicology, Genentech Inc.

In March 2006, the "Guideline on the Nonclinical Investigation of the Dependence Potential of Medicinal Products" was released by the CHMP. The guideline utilizes a tiered approach to investigate the dependence potential of new CNS active substances, including the potential for withdrawal. The guidelines will be discussed, along with discussion around current in vitro and in vivo assessment methods. Lastly, case studies of molecules, both in the development phase and approved drugs, will be presented

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Functional Genetic Screen in Human Haploid Cells to Identify Genes Involved in Susceptibility to Chemical Exposure

Hua Shen, Ph.D.
UC Berkeley

Functional genetic screening systems have been successfully applied to study susceptibility to chemical toxicity. However, some approaches have certain limitations, such as lack of relevance to humans of yeast mutant screen findings and incomplete gene knock-out and off-target effects of RNA interference. Human haploid cell models are advantageous as an induced gene mutation can result in a clear phenotype due to the absence of a second gene copy. We recently developed a more efficient semi-solid medium based screening platform that employs a human haploid cell mutant library (KBM7-Mu) to identify genes that modulate sensitivity to chemical exposures. Compared to the liquid medium-based approach, our method allows for simultaneously screening and generating mutant colonies from cells resistant to the chemical of interest. This shortens the entire screening process by approximately 3 weeks and decreases the rate of false positives. Using this new approach, we identified eleven human genes that confer the resistance to formaldehyde (FA), a known human leukemogen. Among these genes, *LPR5* (Low-density lipoprotein receptor-related protein 5), *GOT1* (Glutamic-oxaloacetic transaminase 1) and *M1AP* (Meiosis 1 Associated Protein) were confirmed in two independent screening experiments. *LRP5*, *GOT1* and *M1AP* mutant KBM7 cells showed significant resistance to FA-induced toxicity compared to wild type cells (KBM7-Wt). Further studies on *LPR5* mutant KBM7 cells using quantitative RT-PCR and western blotting confirmed the knockdown of transcription and knockout translation of the *LRP5* gene. These findings suggest that *LPR5, GOT, M1AP* and other genes are involved in susceptibility to FA toxicity. They further demonstrate the broad applicability of this optimized approach to screen genetic susceptibility to toxic chemicals, identify novel susceptibility genes, and gain insight into potential mechanisms of toxicity of chemical exposures. (Supported by NIEHS P42ES004705 to MTS and R01ES017452 to LZ)

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Why Profile: The Role of In Vitro Pharmacological Profiling in the Drug Discovery Process.

Jacques Migeon, Ph.D.,
Technical Director, Pharmacology
Eurofins Cerep Panlabs

In vitro pharmacological profiling is performed at different times in the drug discovery process to different ends. We will discuss what different types of profiles are used when moving from hit to lead work to lead optimization and finally to the pre IND phase. Based on our long history of performing this work for diverse clients in the pharmaceutical industry, we shall try to elucidate the logic behind the use of in vitro pharmacology profiling and the information that can be gained from performing these studies. Finally we shall comment on some the things to look out for when interpreting this data.

Harnessing the Power of Zebrafish to Advance Environmental Health Sciences: High Content Data for 21st Century Toxicology

Robert Tanguay, Ph.D.
Distinguished Professor
Sinnhuber Aquatic Research Laboratory
Oregon State University, Corvallis, OR

Early developmental life stages are often uniquely sensitive to environmental insults, due in part to the enormous changes in cellular differentiation, proliferation and migration required to form the required cell types, tissues and organs. Molecular signaling underlies all of these processes. Thus, most toxic responses result from disruption of proper molecular signaling; making early developmental life stages the ideal life stage to determine if a chemical can perturb the expression or activity of essential molecular pathways. A central goal of our group is to identify adversely bioactive compounds, and to identify their molecular targets that are acted upon to disrupt vertebrate development. We developed an efficient in vivo phenotypic screening process using embryonic zebrafish to assess chemical effects on behavior, morphology and gene expression. As a proof of concept, we obtained the EPA phase I and II Toxcast chemicals that consist of 1,078 compounds made up of pesticides, drugs, "green" chemicals, chemicals in cosmetics and other consumer products. A static non-renewal exposure paradigm was initiated beginning at 6 hours post fertilization (hpf) using a wide range of concentrations in 96-well plates. A total of 32 individual animals were assessed at each concentration. We also kept exposed embryos completely in the dark until 24 hpf, and assessed a simple photo-motor response using the Photo-motor Response Assessment Tool (PRAT) that we developed. PRAT quantifies individual embryonic photo-motor response following two pulses of bright light. The initial pulse normally results in pronounced movement, and the second light pulse usually produces no activity. At 120 hpf we also assessed photo-induced larval locomotor activity using Viewpoint Zebrabox to determine if chemical exposure impacted CNS-dependent motor responses. Finally, each larva was assessed for changes in a suite of 20 morphological endpoints at 120 hpf and RNA was collected from each exposure group for future gene expression analysis. We have successfully conducted the phenotypic screening procedure on all 1,078 compounds, and a summary of the results will be discussed.

Biographies:

Joseph Arezzo, Ph.D.

Joseph is a Professor of Neuroscience and Neurology and the Director of the Laboratory for Behavioral Neurophysiology at Albert Einstein College of Medicine. His research focuses on structural and functional correlates of neurotoxic insult, the identification of sensitive biomarkers for the onset and progression of neurologic diseases and the pre-clinical assessment of drug-induced seizure risk. His work spans genetic models in rodents, cortical physiology in monkeys and human multicenter clinical trials. He has published more than 130 peer-reviewed articles and more than 25 chapters. Dr. Arezzo has served as a consultant for several government agencies, including the CDC, the EPA, the WHO, NIOSH and the US Air force and he has worked with more than 50 pharmaceutical or biotechnology companies.

Christopher Barnhart, Ph.D. candidate

Chris Barnhart grew up in the eastern Oregon town of Pendleton and attended Oregon State University (OSU) where he earned a B.S. in Bioengineering. Chris made honor roll five times and graduated *cum laude* while participating in extracurricular activities including mentoring underrepresented elementary and middle school students in the sciences. After graduation, Chris found an internship in a traumatic brain injury laboratory in Portland and consequently an entry-level tech position in at Oregon Health & Science University. After a year in the lab he applied to several PhD programs in Toxicology and selected the UC Davis PhD program in Pharmacology and Toxicology. He joined the laboratory of Dr. Pamela Lein to study the molecular and cellular mechanisms by which polychlorinated biphenyls (PCBs) cause developmental neurotoxicity. He is co-author on 3 publications and has 3 pending manuscripts directly relevant to his predoctoral thesis research.

Kevin Crofton, Ph.D.

Dr. Kevin M. Crofton is the Acting Deputy Director of the National Center for Computational Toxicology of the US Environmental Protection Agency in Research Triangle Park, NC. Dr. Crofton received his Ph.D. in Toxicology from the University of North Carolina, Chapel Hill. He has been a toxicologist at EPA since 1986 and is an Adjunct Assistant Professor in the Department of Environmental and Molecular Toxicology at North Carolina State University and in the Curriculum in Toxicology, University of North Carolina at Chapel Hill. His interests include adverse outcome pathways and development of alternative testing methods for developmental neurotoxicity and endocrine disruption. His current research efforts include development of in vitro and alternative methods for detecting thyroid disrupting chemicals and assessing impact on the developing nervous system. Dr. Crofton's professional activities include membership in numerous scientific societies and participation on many professional review boards. He has presented invited lectures for a variety of government agencies in Europe, Canada, and the U.S., and for numerous professional societies and universities. In addition, he has authored or coauthored over 150 peer reviewed publications

Luke P. Lee, Ph.D.
Dr. Lee is Arnold and Barbara Silverman Distinguished Professor at UC Berkeley. He is also Co-Director of Berkeley Sensor & Actuator Center. He received both his B.A. in Biophysics and Ph.D. in Applied Physics (major) & Bioengineering (minor) from UC Berkeley. Prof. Lee has authored and co-authored over 250 papers on biophotonics, single cell analysis, microfluidic quantitative cell biology, and biomedical devices. His current research interests are integrative molecular diagnostics of infectious and neurodegenerative diseases, *in vitro* neurogenesis, bioinspired neural interfaces and organs on a chip.

Jacques Migeon, Ph.D.,
Strangely enough, Jacques received bachelors (Dartmouth College) and masters (Johns Hopkins University) in medieval art history before joining the Neurobiology graduate program at the University of Washington and receiving a Ph.D. degree in Pharmacology. Jacques did a postdoctoral fellowship at the Fred Hutchinson Cancer Research Center before joining what was then Cerep Inc. in 1998. After having played many diverse roles at Cerep, Jacques is now a Technical Director for *in vitro* Pharmacology Services for Eurofins Cerep Panlabs where he is responsible for Cerep's BioPrint Database of *in vitro* and *in vivo* Drug data. Using the BioPrint platform he works on custom profile design as well as interpretation of pharmacological screening results. He shares in responsibility for custom assay development projects and is an active participant in R&D and Innovation Projects.

Dinah Misner, Ph.D., D.A.B.T.
Dinah has been in the pharmaceutical industry for approximately 13 years, of which she has spent significant time in the cardiovascular safety assessment field. She was originally trained as an electrophysiologist in neuroscience at UCSD and the Salk Institute, but transitioned over to cardiovascular assessment shortly after entering the pharmaceutical industry. She is currently leading the investigative toxicology group at Genentech, where they perform hypothesis-driven investigation of toxicity and run primarily *in vitro* toxicology safety assessment assays for both small molecules and biotherapeutics.

Hua Shen, Ph.D.
Dr. Hua Shen earned his PhD in Human Toxicology at University of Iowa in 2011. His PhD research focused on mechanism(s) of toxicity of polychlorinated biphenyls (PCBs) particularly focusing on dioxin like PCBs. He found that paraoxonase 1, an enzyme which plays key role in protection against lipid peroxidation, can modulate the oxidative stress caused by PCB126 through an AhR receptor mediated mechanism. Currently he is a postdoctoral research fellow working in the Berkeley Superfund Research Program. He has authored or co-authored over 20 peer reviewed papers on toxicology and environmental science. His research interest is to develop a new functional genetic screening platform using human haploid cells and CRISPR-Cas9 techniques to identify genes that modulate toxicity of environmental chemicals and anticancer drugs.

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Robert Tanguay, Ph.D.
Robert Tanguay is a Distinguished Professor in the Department of Environmental and Molecular Toxicology and the Director of the Sinnhuber Aquatic Research Laboratory. He received his BA in Biology from California State University-San Bernardino and his PhD in Biochemistry from the University of California-Riverside and postdoctoral training in developmental toxicology from the University of Wisconsin-Madison. He has published over 130 manuscripts and book chapters. Over the past several years he has developed automated high throughput instrumentation to accelerate phenotype discovery in zebrafish. Phenotypic anchoring coupled with the inherent molecular and genetic advantages of zebrafish are used to define the mechanisms by which chemicals, drugs and nanoparticles interact with and adversely affect vertebrate development and function.