Toxicological alternatives and translational toxicology: opportunities and challenges

Shuangqing Peng
(Evaluation and Research Center for Toxicology, Institute of Disease Control and Prevention, Academy of Military Medical Sciences, Beijing)

Toxicity testing is an important approach for safety assessment of chemicals such as environmental agents, drugs, food-additives and cosmetics, provides evidence and protection for human health and environmental safety. Mainly relying on use of laboratory animals, traditional toxicological testing methods are too exhausting and expensive to meet the needs of rapid increase of chemical toxicity evaluation. Moreover, the traditional methods may be uncertain resulting from difference of doses and species of animals. It becomes pivotal to choose appropriate strategies and methods of toxicity testing for environmental agents, drugs and so on. It's imperative now to develop alternative testing methods and translational toxicology research of human related markers and toxicological testing approaches. A series of alternative testing methods have been applied in safety evaluation in the past few decades, relating with general toxicity, local toxicity, target toxicity, genotoxicity and reproductive and developmental toxicity. With the release of the landmark report Toxicity Testing in the 21st Century: A Vision and a Strategy, the U.S. National Academy of Sciences, in 2007, precipitated a major change in the way toxicity testing is conducted. It envisions increased efficiency in toxicity testing and decreased animal usage by transforming from current expensive and lengthy in vivo testing to in vitro toxicity pathway assays on human cells, cell lines or cell components using advanced techniques of toxicogenomics, bioinformatics and computational toxicology. Several countries have taken active measures in the face of new challenges. The U.S. EPA ToxCast was started to optimize toxicity testing of thousands of chemicals through predicting potential toxicity of chemicals using computational toxicology methods and developing inexpensive and efficient approaches. Associated projects were funded by NIH, FDA and SNSFC. A series of major schemes were executed by European, such as SEURAT project, AXL88 program, to advance the development and application of T2IC and establish in vitro testing methods with multiple endpoints.

Bio: Professor Shuangqing Peng is the director of Evaluation and Research Centre for Toxicology, Institute of Disease Control and Prevention, Academy of Military Medical Sciences. He has over 20 years experience in toxicology and pharmacology research. His research interest covers pre-clinical toxicology study in compliance with GLP, Ion channel pharmacology, and toxicological alternatives and translational toxicology. He has published over 170 papers in scientific journals. Dr. Peng actively serves in several important scientific societies such as Chinese Society of Toxicology, Chinese Society of
Environmental Mutagen, Chinese Society of Environmental Life Elements and Health, America Society of Toxicology. He won the IUTOX/SOT AstraZeneca Travel fellowship from the American Society of Toxicology in 2006, and was recognized as a national outstanding talent. More recently, Dr. Shuangping Peng is leading many key programs focusing on applying alternatives and TT21C in the risk assessment of new chemicals, drugs and food additives, and environmental pollutants.

**TT21C/AOPs: Putting mechanism and toxicity pathways at the heart of new safety assessments**

Paul Carmichael

(Safety and Environmental Assurance Centre (SEAC), Unilever Colworth, UK)

Development of novel ingredients that can provide new functional benefits is the life blood of several industries, including the pharmaceutical and consumer product sectors. It is essential however that robust scientific approaches are used to assure the safety of these new ingredients for the patients and consumers who will use them, the workers who manufacture them, and the environment into which they may ultimately be disposed. Traditionally, toxicology data generated in mammals, fish and invertebrates, together with information on levels of human and environmental species exposure, have been used to enable informed safety decisions to be taken. The current reliance on animal data in these safety assessments is reflected in the majority of global regulations concerned with drug, consumer product and environmental safety. In 2007 the United States National Academies of Science report “Toxicity Testing in the 21st Century (TT21C): a Vision and a Strategy” (www.nap.edu) challenged Toxicologists to think differently. Since then, the OECD Adverse Outcome Pathway (AOP) program (www.oecd.org/env/ehs/testing/) has strengthened the growing trend to frame new safety assessments in terms of human- (or environmental species-) relevant mechanism(s) of action. In Europe the SEURAT research program (www.seurat-1.eu), along with the US multi-agency ToxCast and Tox21 programs (www.epa.gov/ncct/toxcast and www.epa.gov/ncct/Tox21) reflect the growing body of work aiming to modernize and improve this field; seeking to provide greater human health and/or environmental relevance and more efficient tools for safety assessments. Case study examples that utilize high content, high throughput in vitro systems, complemented by computational modeling linking exposure biokinetics with cellular biodynamics, are being put in place to illustrate the value of these novel safety assessments. Mechanisms of mitochondrial toxicity can be considered a key molecular target in such pathways-based safety assessments. The opportunities presented to Industry by these new approaches and their place in a framework for exposure- and pathways-based safety assessments will be discussed. Such in vitro tools combined with mechanistic chemistry information on ingredients allow the identification of potential biological targets, toxicological liabilities and mechanistic information for elucidation of adverse outcome pathways.
Bio: Professor Paul Carmichael works in the Safety & Assurance Centre of Unilever in the UK. He is responsible for thought leadership in non-animal approaches for human health (consumer safety), and has over twenty years experience in toxicology and cancer research, largely in the academic arena, before joining Unilever from Imperial College in 2003. He has published around one hundred research papers in peer-reviewed scientific journals and has served or served on several external committees or fora in the UK, EU and China. His passion is the development and implementation of new safety assessment approaches using the inspiration of ‘Toxicity Testing in the 21st Century’. A Surrey graduate with a PhD from King’s College, he is an Honorary Professor at both Lancaster University in the UK and Peking University in China.

US Tox21 Program: application of qHTS approach for assessment of mitochondrial toxicity

Menghang Xia
(Division of Preclinical Innovation, National Center for Advancing Translational Sciences, National Institutes of Health, USA)

To meet the needs of toxicity testing in the 21st century, the National Toxicology Program (NTP), the NIH Chemical Genomics Center (NCGC, now is part of the National Center for Advancing Translational Sciences, NCATS), the U.S. Environmental Protection Agency (EPA), and the U.S. Food and Drug Administration (FDA) formed the Tox21 partnership. The goals of Tox21 are to identify mechanisms of compound action at the cellular level, prioritize chemicals for further toxicological evaluation, and develop useful predictive models of in vivo biological response. As part of the Tox21 program, we have developed the quantitative high-throughput screening (qHTS) paradigm that has provided a cost-effective alternative to traditional tests for profiling the toxicity of environmental chemicals. In this program we have screened a library of approximately ten thousand environmental chemicals in three independent runs against a group of stress response pathway assays and nuclear receptor assays in a qHTS format. In the seminar, I will describe the Tox21 program, selection of qHTS-based assays and the various Tox21 screening assays including mitochondrial membrane potential assay that have been validated and screened at the NCATS.

Bio: Dr. Menghang Xia has been a group leader of the Systems Toxicology section within the Division of Preclinical Innovation, National Center for Advancing Translational Sciences (NCATS), NIH. Since joining the NIH Chemical Genomics Center (NCGC, now is part of the NCATS) in 2005, Dr. Xia plays a key leadership role in multiple projects, including the Tox21 program. Serving as a co-chair in the Assays/Pathways working group of the Tox21 program, Dr. Xia led the major effort to develop and validate a battery of in vitro toxicological assays in a quantitative high through screening (qHTS) platform. She and her associates have developed and screened more than 100 assays, and profiled
environmental chemicals on various pathways and targets, such as hypoxia/HIF-1, p53 and ARE signaling, and nuclear receptors. Dr. Xia is interested in studying the mechanism of action of drugs/chemicals in multiple cellular signaling pathways including hypoxia/HIF-1, CREB and NFkB. She has also developed and implemented numerous assays into a qHTS format using advanced technologies, such as bioluminescent cytotoxicity assay for assessment of membrane integrity, several beta-lactamase reporter gene assays for cellular pathways and nuclear receptor signaling, and membrane potential assay for mitochondrial function assessment. Dr. Xia received her bachelor of medicine in Shanghai Medical University (now is part of Fudan University) in China and her Ph.D in pharmacology and toxicology from the State University of New York at Buffalo. She did her postdoctoral training at the University of California at San Francisco. Prior to joining NCGC, Dr. Xia identified and validated several targets for drug development at Merck Research Lab.

**Using metabolically-modified in vitro models to examine the role of mitochondrial dysfunction in adverse drug reactions**

**Amy Chadwick**
(Institute of Translational Medicine, the University of Liverpool, UK)

The talk will discuss recent work to evaluate the use of metabolic modulation to generate improved pre-clinical models for the mechanistic investigation of mitochondrial-induced toxicity using a panel of hepatotoxins. In most standard preclinical models the tumour-derived cells have undergone a change in energy production in order to provide sufficient ATP for continued growth; by switching energy metabolism to glycolysis alongside oxidative phosphorylation thus reducing their sensitivity to mitotoxicants. In addition these models have been used to uncover novel mechanisms of mitochondrial dysfunction. The importance of this data, specifically the role played by mitochondrial dysfunction in the initiation and progression of adverse-drug reactions and its translation to man will be discussed.

**Bio: Dr. Amy Mercer Chadwick** is a tenure track fellow and group leader based at the MRC Centre for Drug Safety Science within the Institute of Translational Medicine at the University of Liverpool, UK. Dr Mercer’s research focus is elucidating the fundamental chemical and molecular mechanisms of drug-induced cell death (in vitro and in vivo), in particular delineating the causative role of mitochondrial dysfunction in drug-induced liver injury and kidney injury. Dr Mercer had previously presented her work describing the use of metabolically modified in vitro models in the study of drug-induced toxicity at the 1st World Congress on Targeting Mitochondria (2010, Berlin, Germany) and the CHI Targeting Mitochondrial Dysfunction & Toxicity conference (2014, Boston, USA).
Computational system biological modelling and its role in pathway-based mitochondrial toxicity assessment

Qiang Zhang

(Center for Dose Response Modeling, Institute for Chemical Safety Sciences, the Hamner Institutes for Health Sciences, USA)

Perturbation of existing biochemical pathways operating in cells is a key event underlying the adverse health effects of environmental and therapeutic chemicals. As chemical toxicity testing and safety assessment makes the necessary turn toward a cell-based in vitro approach for efficiency, relevancy, and accuracy, understanding how toxicity pathways function and behave under perturbations is fundamentally important for mechanistic data interpretation and prediction across chemical dose and time. Such efforts require computer-aided simulation of relevant well-mapped pathways and studying them as dynamic systems. This in turn requires knowledge of recurring network motifs, their structures, and signalling properties. Large pathways underlying integrated cellular functions such as homeostasis, proliferation, differentiation, and apoptosis are organized assembly of network motifs. The departure from their physiological function baselines can be better studied through mathematical modelling of the systems-level behaviours of these complex pathways. Mitochondrial function is highly regulated through a network of interconnected metabolic and transcriptional pathways. Cardiovascular toxicity by drugs such as doxorubicin is believed to involve perturbations of such a network comprising coupled feedback and feedforward network motifs that function to maintain energy and redox homeostasis. Here we present a simple mathematical model of mitochondrial toxicity pathway and illustrate how it is used for mechanistic interpretation of in vitro toxicity data and evaluation of point of departure.

Bio: Dr. Qiang Zhang is Director of the Center for Dose Response Modeling at The Hamner Institutes for Health Sciences (formerly CIIT Centers for Health Research), a preeminent toxicological research institution located in North Carolina, USA. He develops, as a key component in 21st century toxicity testing, mechanistically-based predictive computational models that allow quantitative understanding of low-dose chemical health risk. Working closely with experimental toxicologists, Dr. Zhang has used computer simulations of molecular toxicity pathways to probe the nature of many nonlinear, including non-monotonic, responses induced by oxidative, immunotoxic, and genotoxic chemicals and their health consequences. He is particularly interested in pathways that underpin cellular homeostasis and adaptation and the underlying network motifs which often involve feedback and feedforward regulations. Dr. Zhang has contributed considerably to educating biologists and toxicologists on the emerging computational tools for pathway and dose response modeling by organizing and teaching workshops. He has authored and co-authored many peer-reviewed publications and book chapters. He earned the Outstanding New Investigator Award by The International Dose-Response Society in 2010 for his original contributions to research in nonlinear cellular responses. Dr. Zhang received education and training in biomedical sciences. He earned his M.D. degree from Harbin Medical University in China and Ph.D. degree in endocrinology from the University of
Connecticut in the United States. Recognizing the emerging importance of quantitative approaches in biological research, Dr. Zhang received his computational biology training as a postdoctoral fellow at The Hamner Institutes.

**Stress induced cardiovascular injury: from protein changes to mitochondrial dysfunction**

_Lingjia Qian_

(Key laboratory of Stress Medicines, Institute of Basic Medical Sciences, Academy of Military Medical Sciences, Beijing)

Stress is an important interface of interaction between the environment and human body. Stress over-loading causes a wide variety of damage, in which the cardiovascular system is a key target. During the development of stress induced cardiac injury, it was observed that stress resulted in mitochondrial swelling, cristae rupture and significantly increase of mitochondrial membrane permeability in cardiomyocytes, which in turn caused not only mitochondrial oxidative phosphorylation uncoupling, and also cellular homeostasis imbalance. The mitochondrial damage start-up the mitochondrial pathway of cell death, finally lead to cardiac function injury. Study of mitochondrial proteomics on the stressed cardiomyocytes showed that the distribution of a variety of proteins, including TR3, PHA and ARC, changed obviously. The translocation of proteins in the mitochondria significantly affected the structure and function of mitochondria, which were closely related to cell survival and death. As molecular chaperone, HSP70 play an important role in the regulating protein-translocation in stressed cardiomyocytes. These findings provide important scientific evidence for the new interpretation of the molecular mechanisms of stress induced myocardial injury, and also give us the scientific enlightenment for exploring new diagnostic markers and drug targets in prevention and treatment of stress related diseases.

_Bio: Dr. Lingjia Qian_ is professor in Beijing Institute of Basic Medical Sciences, the Academy of Military Medical Sciences. She graduated from Duesseldorf University of Germany and got Doctor Degree. As an expert in the field of environmental medicine and stress medicine, she has long engaged in research of pathophysiological mechanisms underlying cardiovascular diseases induced by extreme environmental factors, especially focus on mitochondrial mechanisms stress induced cardiomyocyte damage. She has published more than 100 papers about environment and stress in *Proteomics, Stress* and so on. She proposed that the changes in mitochondrial proteomics play an important role in stressed cardiomyocyte injury and regulating of mitochondrial proteomics maybe a novel pathway to control stress induced cardiovascular diseases.
The role of 21st century toxicology in refining environmental risk assessment

Steve Gutsell

(Safety and Environmental Assurance Centre (SEAC), Unilever Colworth, UK)

Safety risk assessment science is currently undergoing one of the largest paradigm shifts in recent history. This is partly being catalysed by the need to assess increasing numbers of chemicals with fewer resources and the increasing public and political concerns regarding the use of animal studies for assuring the safety of new chemicals. In addition there is acknowledgement that the current methods of Environmental Risk Assessment (ERA) do not address several key areas; including the uncertainty inherent in extrapolation of effects between species and the poor ability to predict impacts on the structure and function of natural ecosystems. Here we discuss the use of a Source to Outcome Pathway (S2OP) approach to help identify some of the deficiencies in the current methods of ERA and the work planned to begin to address these. Using two narcotic chemicals as case studies, existing methods such as QSAR, chemical activity and critical body burdens can be placed into context more clearly than previously. Mapping current knowledge of narcosis on a pathway framework helps highlight clear gaps in our understanding of mechanistic links at several levels of biological organisation. One of the key future challenges is the translation of measured sub-individual responses into population-relevant endpoints (e.g. reproduction and growth) across species and further into ecologically relevant impacts. In addition, we demonstrate that the use of apical endpoint data generated under current test guidelines is often incompatible with the types of models required to extrapolate individual effects to populations and communities. We discuss the relevance of various elements of the S2OP in addressing different risk assessment challenges and how additional individual and sub-individual data may improve extrapolations to meaningful protection goals. However considerable work is needed before such methods are fully applicable to supporting more ecologically relevant risk assessment decisions.

Bio: Dr Steve Gutsell has been with Unilever's Safety and Environmental Assurance Centre for over 9 years. With a background in Organic Chemistry and expertise in the area of Computational Chemistry, specifically using predictive methods such as (Quantitative) Structure–Activity Relationships ((Q)SAR), Read Across and other techniques to predict both toxicological and ecotoxicological endpoints from chemical structure. He has published several papers in this area and presented at numerous international scientific and regulatory meetings. Recent areas of interest include how pathways-based approaches can be used to create novel risk assessments for consumer products.
High content-based cellular phenotypic assay platform and its application in drug mitochondrial toxicity research

Lili Wang

(Institute of Pharmacology and Toxicology, Academy of Military Medical Sciences, Beijing)

High cost, high risk and low output are substantial challenges for drug research and development. Recently, drug-induced mitochondrial toxicity (MT) is rapidly gaining recognition within the pharmaceutical industry as an important etiological factor of organ toxicity, which contributes to compound attrition, post-market drug withdrawals, and Black Box warnings from the FDA. To avoid mitochondrial liabilities, early routine screens and predictions need to be set up within the drug-development process. However, drug-induced mitochondrial dysfunction cause more subtle effects, develop slowly and may be confounded by multiple complicating interactions. In addition, most of assessments of drug-induced MT have been retrospective, examining drugs with organ toxicity and known adverse effects for potential mitochondrial liabilities. Even more, some clinic validated drug with MT could not be detected in preclinical tests both in vitro and in vivo. Therefore, the effective strategy and methods for assessment MT of drug in preclinical phase is absent. Toxicity pathways and targeted testing is the core component of Tox21. Systematically testing and integrating the MIE and AOP of different drugs induced MT by high/medium-through approaches using cells or cell lines, could help the establishment of the effective strategy and approach for screening and prediction the MT of lead or candidate compounds. High Content-based cellular phenotypic assay allows HTS and quantitative measure of both ‘classical’ (such as cell cycle perturbations, differentiation or apoptosis) and molecular events (such as changes in protein localization and intracellular phosphorylation) cellular phenotypes, acquires a highly detailed and unbiased multiparametric feature profile, which provide the enabling technologies for detecting various cellular responds relevance to MT. The report introduce the potential applications of HCA in MT research.

Bio: Dr. Lili Wang is a professor in the Institute of Pharmacology and Toxicology, the Academy of Military Medical Sciences (AMMS). She got her Ph.D from the Fourth Military Medical University in 1998 and finished her postdoc training in AMMS. Her research interests include 1) Cellular phenotypic assay technology, have established the first level HCA drug screening platform in China; 2) Insulin sensitizer regulation mechanism and metabolic disease drug evaluation; 3) Cancer drug combination screening and molecular mechanism research; 4) Pathway based drug toxicity prediction and molecular mechanism research. She presided over a number of scientific projects including: “National Science and Technology Major Project of the Ministry of Science and Technology of China”, “973” and “863” et al. she has published 42 SCI Papers and acquired more than 30 patents authorized by China, USA and others countries.
In vitro approaches to assess mitochondrial toxicity and mitochondria-mediated drug toxicity: A decade of learning

Yvonne Will
(Compound Safety Prediction- WWMC, Cell Based Assays and Mitochondrial Biology, Pfizer R&D, USA)

Drug Induced Mitochondrial Toxicity has contributed to market withdrawals and black box warning and can lead to a variety of organ toxicities, most noticeable drug induced liver injury, but also cardiac, muscle and CNS toxicity. Animal models used in preclinical safety assessment fail to reveal mitochondrial toxicity. The withdrawal of the two blockbusters troglitazone and cerivastatin prompted the pharmaceutical industry about implementing a screening paradigm, as in both cases, mitochondrial toxicity was thought to at least in part contribute to the observed liver toxicity of troglitazone and the rhabdomyelisis of cerivastatin. Whereas mitochondrial toxicity had been studied in academic setting for over 25 years, the lack of high throughput applicable assays provided a challenge. In this lecture I will introduce currently available technologies that can help to elucidate potential mitochondrial toxicity. This includes screens using isolated mitochondria and cell models that truly reveal mitochondrial toxicity. In addition, several methodologies will be discussed that can elucidate the target(s) of such toxicity. In addition, we will discuss the contribution of physic-chemical drug properties to mitochondrial toxicity and the importance of drug plasma concentrations when projecting toxicity.

Bio: Dr. Yvonne Will pursued her graduate studies at Oregon State University in the lab of Dr. Donald Reed where she focused on the relationships between glutathione deficiency and cellular and mitochondrial function/dysfunction. During her years at MitoKor, San Diego (2000-2003) she was involved in drug discovery aimed on improving mitochondrial function or preventing mitochondrial dysfunction in obesity, diabetes, and CNS related diseases. From 2003 until 2007, Dr Will was a group leader in Drug Safety at Pfizer La Jolla, pioneering a screening paradigm for drug induced mitochondrial toxicity, supporting many therapeutic areas. This platform has been adapted throughout all major pharmaceutical companies. During that time she also held an adjunct faculty position at San Diego State University in the Toxicology program where she conducted lectures, taught laboratory courses and mentored MS students. In the fall of 2007 Dr Will transferred to Pfizer Groton to lead a group of scientists in the Compound Safety Prediction Group within Medicinal Chemistry. This group is set out to conduct in vitro safety assessment as early as possible within the drug discovery process to reduce late stage attrition. Dr Will’s group has pioneered many new technologies throughout the years. Dr Will has published a book on drug induced mitochondrial toxicity and has contributed to more than 10 book chapters. Dr Will has given many national and international lectures, conducted workshops and seminars and continues to publish numerous papers each year in peer reviewed journals. In 2012, Dr Will was honored with the Connecticut Technology Council’s Woman Research Innovation and Leadership Award. Dr Will’s passion is to develop young scientists through external influence such as publications and participations in national meetings.
Release of Cyt c from mitochondria by methylated arsenic occurs through interaction with voltage dependent anion channel in vitro

Hua Naranmandura
(School of Medicine and Public Health, Zhejiang University)

Arsenic is known to be a human carcinogen as well as one of the most effective drugs for treatment of patients with acute promyelocytic leukemia (APL). Although it has been reported that arsenic intermediate metabolites monomethylarsonous acid (MMAIII) and dimethylarsinous acid (DMAIII), which transformed by methylation reactions are more reactive and toxic than that of inorganic precursor arsenite (iAsIII), however, the detailed mechanism is poorly understood. Here, we studied the effects of three arsenic compounds on mitochondrial permeability transition pore (mPTP) as well as the release of apoptotic cytochrome c after incubating with rat liver mitochondria. Inorganic iAsIII had no effect on mitochondrial swelling even at higher concentration i.e. up to 100 μM, but it was significantly induced in the presence of Ca2+. Additionally, mitochondrial swelling was strongly induced by exposure to the methylated MMAIII and DMAIII in dose dependent manner without Ca2+, suggesting the methylated forms may have potent effect on cellular mitochondria. Although mitochondrial swelling was completely inhibited in the presence of Cyclosporin A (a inhibitor of mitochondrial permeability transition) or Ruthenium Red (a inhibitor of Ca2+ uniporter) following exposure to methylated arsenicals, the release of apoptotic Cytochrome c (Cyt c) form mitochondria were not inhibited, indicating the release of Cyt c is probably not dependent the mPTP opening. In addition, inhibitor of Bax (e.g., Bax-inhibiting peptide) did not reduce the Cyt c release from mitochondria by formation of Bax-VDAC complex process, while the recombinant Bcl-xL proteins significantly reduced the release of Cyt c with exposure to DMAIII, suggesting the dimethylated DMAIII directly interacted with voltage dependent anion channel (VDAC) in mitochondria and resulted in the release of Cyt c from mitochondria.

Bio: Dr. Naranmandura (mongolian) is professor in Department of Toxicology, School of Medicine and Public Health at Zhejiang University, China. He obtained undergraduate training from inner mongolian mongolian medical college and received his Ms and Ph.D degrees in pharmacology and toxicology from Chiba University (Japan), where he studied molecular toxicity of drug and arsenic. Then, he completed postdoctoral work at the University of Alberta (Canada), where he studied the cellular and molecular mechanisms of acquired tolerance to metal toxicity and metal speciation. He is currently focusing on several topics, including: (1) Identification of the arsenic-binding proteins in animals’ organs and body fluids (blood); (2) Molecular mechanisms of carcinogenic effect of the thio-arsenic species; (3) The role of arsenic trioxide and its intermediate metabolites in patients with acute promyelocytic leukemia (APL); (4) Molecular mechanisms of toxicity of marine toxin in cells and animals; (5) Screening of marine toxin for anticancer drugs and development of aptamer-marine toxin Conjugates (AMCs). He is a Guest Professor at Chiba University, and Showa Pharmaceutical University, Japan. He currently serves on the Editorial Boards for《Chemical Research in Toxicology》 (IF 4.2) ,
MIES and mitochondrial toxicity – the applied view

Paul Russell
(Safety and Environmental Assurance Centre (SEAC), Unilever Colworth, UK)

Consumer and environmental safety decisions are based on exposure and hazard data interpreted using risk assessment approaches. The Adverse Outcome Pathway (AOP) conceptual framework has been presented as a logical sequence of events and processes within biological systems which can be used to understand adverse effects and refine the current risk assessment practice. Through this framework, current risk assessment practice can be refined through developing sound scientific and mechanistic understanding, shifting focus away from traditional toxicological apical endpoints to an increased understanding of a chemical’s interactions and effects at a molecular level.

A critical step in the framework is the molecular initiating event (MIE) which is defined as the initial interaction between a molecule and a biomolecule or biosystem that can be causally linked to an outcome via a pathway. Fundamentally, MIEs can be considered as molecular interactions occurring in a dynamic and complex matrix system and as such the knowledge developed in one species can potentially be applied to other species in a unified risk assessment approach (e.g. human to species of environmental significance and vice-versa). MIE knowledge will be important to enable prediction of the subsequent biological pathways which may be initiated or perturbed by an exogenous chemical.

Identification of key MIEs, including those implicated in mitochondrial disruption, can be considered from either an exposure or chemical driven approach, or alternatively via the biological signature created by the effect of the chemical. For the former, predictive chemistry techniques including quantitative structural activity relationships (QSARs) can be developed based on existing data and through mapping networks of chemicals, MIE’s and subsequent pathways. To develop a retrospective response based approach, in-vitro assays can be used to provide a biological signature for a chemical. This data can then be applied to inform which pathways are required to be considered for risk assessment. Evidence of a lack of pathway perturbation provided via either approach will be just as critical to provide confidence in risk assessment of novel chemicals across a broad spectrum of potential effects.

Bio: Dr. Paul Russell is a Science Leader at Unilever’s Safety and Environmental Assurance Centre in Sharnbrook, Bedfordshire, UK. Paul gained a BSc in Chemical and Pharmaceutical Science from the University of Sunderland and has over 17 years industrial experience in analytical chemistry. Paul started his career working in the pharmaceutical industry focussing on drug metabolism and
pharmacokinetics before moving into contract research. He joined Unilever SEAC in 2004 and has subsequently completed a PhD with King’s College London developing comprehensive analytical fingerprinting techniques in support of natural product risk assessment. He is a technical specialist in liquid chromatography and mass spectrometry and has a specific scientific focus on the development of chemistry based approaches to aid the toxicological risk assessment of novel materials. Dr Russell has published a number of articles in the areas of bioanalysis, the analysis of natural products, and chemistry in risk assessment. He regularly presents at international conferences and is also Secretary of the Separation Science Group of the Royal Society of Chemistry.

TT21C and AOP: Cumulative benzo(a)pyrene exposure toxicological research

Hui Wang
(Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai)

Benzo(a)pyrene (B(a)P) is a common environmental and foodborne pollutant. Although the carcinogenicity of high-dose of B(a)P has been extensively reported, the adverse effects of long-term B(a)P exposure at lower environmental dosage on cancer development are less understood. We investigated the impact of B(a)P on human hepatocellular carcinoma (HCC) progression at various levels of exposure, determined the mechanism of action, and identified a potential intervention target. A model based on human HCC cells exposed to various concentrations of B(a)P (i.e., 0.01 nM, 1 nM, or 100 nM) for a month, was utilized to examine the effects of B(a)P on cell growth, migration, invasion, and angiogenicity. And a bioluminescent murine model was established to assess tumor metastasis in vivo. We found that chronic B(a)P exposure did not alter HCC cell growth but promoted cell migration and invasion both in vitro and in vivo. There was an association between B(a)P exposure and the survival of tumor-bearing mice. In addition, B(a)P-treated HCC cells recruited vascular endothelial cells and promoted tumor angiogenesis through elevating VEGF secretion. Furthermore, the NF-κB pathway may serve as an intervention target because it mediated the cumulative effects of B(a)P on HCC metastasis. These findings (a) showed that B(a)P, at environmental-relevant concentrations, has effects on HCC progression; (b) identify a potential mechanism and intervention target; and (c) contribute to a better understanding of the adverse effects of chronic exposure of B(a)P to human health.

Bio: Dr. Hui Wang is a Principal Investigator, Professor, and the director of Food Safety Research Center at INS. She obtained her Ph. D. from Tianjin Medical University / University of Alabama at Birmingham (a joint PhD program) in 1999. After graduation, she continued her research at University of Alabama at Birmingham as a post-doctoral researcher. In 2001, she joined the faculty in the Department of Pharmacology and Toxicology, Division of Clinical Pharmacology as an Instructor and was promoted to Assistant Professor in 2002. She joined INS in October 2005. Her research interests cover: 1) Drug discovery and molecular pharmacology and toxicology; 2) Nutrition & environment and
disease prevention; 3) Applied Nutriceutical Sciences and Food safety (including fast detection approach and the action mechanism of noxious substances in foods).

Enhancing non-testing approaches using the AOP framework: A case study in building scientific confidence

Grace Patlewicz
(DuPont Haskell Global Centres for Health and Environmental Sciences, USA)

Non-testing approaches encompassing (Q)SARs, chemical categories and read-across have enjoyed a renaissance in recent years following changes in the global regulatory landscape, the EU’s REACH regulation and the 7th Amendment to the Cosmetics Directive being notable examples. Whilst the uptake of these non-testing approaches for regulatory purposes is very encouraging, their practical application has identified some shortcomings. Whereas some regulatory endpoints such as irritation, skin sensitisation lend themselves to robust (Q)SAR development, others such as repeated dose toxicity are too complex to be modelled in this way and despite the role that read-across can play, acceptance is still in part thwarted by the difficulty in identifying and addressing uncertainties.

Adverse Outcome Pathways (AOPs) which are useful constructs for representing existing knowledge concerning the causal linkages between initial molecular events and an adverse outcome could offer some solutions in addressing such limitations. AOPs themselves provide the mechanistic basis for generating, integrating and interpret non standard information for key events (KEs) in a manner that can be practically useful for decision making. The framework for this is otherwise known as Integrated Approaches to Testing and Assessment (IATA). Indeed an IATA focused solely on existing data and other non-testing approaches marks a significant step forward in how future read-across and QSAR approaches can be developed and applied. Instead of predicting the atypical endpoint or ultimate adverse outcome, future QSARs could be developed to model individual KEs. In addition SARs could be derived to characterise chemistry applicability domains of different KEs based on the experimental assays used in their measurement. Uptake and acceptance of AOP based IATA and their associated testing and non-testing elements will still necessitate some level of validation to demonstrate scientific confidence for specific purposes. Here we describe a scientific confidence framework for T21 approaches anchored into AOPs and illustrate how this can be used to direct the development of non-testing approaches and their application as part of IATA. Case study examples will include insights derived from mitochondrial toxicity as well as skin sensitisation and genotoxicity endpoints.

Bio: Dr. Grace Patlewicz is a chemist/toxicologist by training. She joined DuPont’s Haskell Global Centers (HGC) for Health and Environmental Sciences 6 years ago as a computational toxicologist. Previously she worked for the European Commission’s Joint Research Centre in Italy and prior to that Unilever’s Safety and Environmental Assurance Centre in the UK. At HGC, she principally
acts as a focal point for (Q)SAR, read-across questions for regulatory purposes and product stewardship. In addition to her day to day HGC activities, Grace maintains involvement in the OECD QSAR Toolbox effort. She co-chairs the American Chemistry Council (ACC) Computational Profiling & Risk Assessment and Science Workgroup, acts as the technical lead on Adverse Outcome Pathways (AOPs) within the OECD AOP work programme on behalf of ACC through BIAC, moderates for the Alttox.org alternatives forum. In 2012, she successfully chaired the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) Task Force for read-across, co-organised on behalf of the European Chemical Industry Council’s Long-range Research Initiative (Cefic LRI) a workshop on read-across in collaboration with ECHA, and was a drafting group member for the revised OECD guidance for read-across (published April 2014) as part of her ongoing chairing role for the Cefic LRI read-across team. She has authored over 75 publications, has contributed extensively in the drafting of the REACH and OECD Technical Guidance on QSARs and chemical categories, REACH guidance on endpoint specific Integrated Testing and Assessment Approaches (IATA). She has also led the development of software such as Toxmatch for chemical similarity, Toxtree (which includes modules for TTC) and other expert systems such as TIMES and OASIS Pipeline for the prediction of sensitisation and mutagenicity.