Plenary Sessions

Lecture PL1:

The U.S. "Tox21 community" and the future of toxicology testing

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In early 2008, the National Institute of Environmental Health Sciences/National Toxicology Program, the NIH Chemical Genomics Center, and the Environmental Protection Agency's National Center for Computational Toxicology entered into a Memorandum of Understanding to collaborate on the research, development, validation and translation of new and innovative test methods that characterize key steps in toxicity pathways.

A central component is the exploration of high throughput screening assays and tests using phylogenetically lower animal species (e.g. fish, worms), as well as high throughput whole genome analytical methods, to evaluate mechanisms of toxicity. The goals of the "Tox21 Community" are to investigate the use of these new tools to (1) prioritize substances for further in-depth toxicological evaluation, (2) identify mechanisms of action for further investigation, and (3) develop predictive models for *in vivo* biological response. Success is expected to result in test methods for toxicity testing that are more mechanistically based and economically efficient; as a consequence, a reduction or replacement of animals in regulatory testing is anticipated to occur in parallel with an increased ability to evaluate the large numbers of chemicals that currently lack adequate toxicological evaluation. The initial focus of this collaboration has been on identifying toxicity-related pathways (and assays for those pathways), establishing a Tox21 library of ~10,000 compounds, and developing the databases and bioinformatic tools needed to mine the resulting data. This presentation will summarize the coordinated approaches being taken to achieve our goals, the lessons learned, and expectations for the future.

Lecture PL2:

Animal health and welfare in the food chain: food for thought

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Basically we need food only for two reasons: (i) to provide building blocks necessary for the development, growth and repair of our body and allowing it to replace worn-out cells and tissues; and (ii) to provide our body with the energy necessary for it to perform adequately. With respect to the latter we are all well aware that while people starve to death in a greater part of the world, in our 'western societies our energy intake has reached levels which can be considered as severely hazardous. With respect to the former, today's major protein source is animal meat, a very inefficient source which, with a growing glo-

bal shortage of food, may not be sustainable. Intensive farming was thought to be the solution but the consequent poor animal health and welfare conditions seem to meet with increasing societal criticism these days. Fish farming, while still in its early days, apparently has not learned from the mistakes made by the bio-industry, and thus high fish density in fish farms has again already resulted in antimicrobial resistance in fish exposed to 'preventive' high levels of antimicrobials.

Next to the use of animals as a source of food, animals are used in considerable numbers to assess the safety of food and feed. Extensive legislation in the EU and elsewhere require at least some form of animal testing of food ingredients prior to its marketing. In addition, the call for more animal testing of food is sometimes used for political reasons, rather than being based on scientific concerns (i.e. for Genetically Modified Organisms – GMO's). On the other hand, the huge numbers of food addditives, enzymes, flavours and food contact materials to be assessed for safety, have forced regulatory scientists to develop more pragmatic and yet scientifically sound alternative approaches requiring substantially less experimental animals. Such approaches include: the use of QSARS (quantitative or qualitative structure-activity relationships), TTC (threshold of toxicological concern), and QPS (qualified presumption of safety).

The lecture will address the health and welfare of both food producing and experimental animals. In addition to science based arguments and suggestions the lecture will touch on some more philosophical aspects with an eye to the future of 2040-2050.

Lecture PL3:

Brueghel's two monkeys: passing the final exam in the history of mankind

I. Newkirk

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As society's ethical values expand over time, we understand that we must have consideration for more than just ourselves, our race, our gender, and our species. This speech will confront our biases and provide food for thought in moving beyond our current understanding of human-animal relations. It will help enable us to say what needs to be said about the use of animals, their suffering, and the appropriateness of the behavior of those around us.

History provides a lens through which our current norms can be viewed. It allows us to discern how our behavior might be perceived by future generations and this perspective can help us understand how to improve our behavior. While it is easy to be appalled by what has been done in the past, it is more challenging to uncover the actions taking place today that will be regarded with horror in the future and most important by far to be a part of the necessary change. By modifying one's perspective to include a more empathetic view of other animals, our obligations and potential become clear.

Examples will be provided of both resistance to change and the growing understanding that scientific knowledge does not have to be based on animal experimentation. Recent developments in science that allow – indeed demand – non-animal approaches to chemical testing are being embraced and developed even by institutions that have long been great promoters of animal testing. The new advances are building momentum to replace the use of animals and reinforce the animal rights community's long-standing contention that, where there is a will, there is a way to obtain information without the use of animals.

Lecture PL4:

Metabonomics-driven top-down systems biology: techniques and applications

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There has been a greater understanding of "druggable" targets through their characterisation involving measurement of gene expression (transcriptomics) and protein expression changes (proteomics). Metabonomics is a crucial and integrating component of a systems biology view of an organism because it allows inclusion of environmental factors such as diet, age ethnicity, life-style and gut microfloral populations can have a large influence.

Metabonomics is defined as "the quantitative measurement of the time-related multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification" and involves the generation of metabolic databases, based on tissue or biofluid samples, for control animals and humans, diseased patients, animals used in drug safety testing, etc., allowing the simultaneous acquisition of multiple biochemical parameters on biological samples. It is also possible to use cell culture supernatants, tissue extracts and similar preparations and is hence directly implicated for animal replacement. Because animals an act as their own controls in many studies, it is particularly useful when considering animal reduction and refinement. Metabonomics now impacts many areas including animal models of disease, preclinical evaluation of drug safety, assessment of safety in clinical trials, improved understanding of idiosyncratic toxicity, improved differential diagnosis and prognosis of clinical diseases, better understanding of environmental population effects through epidemiological studies, patient stratification and the effects of interactions between drugs, and between drugs and diet.

The two most information-rich analytical techniques are mass spectrometry and nuclear magnetic resonance spectroscopy and the metabolic response of an organism is then extracted from the complex data sets by application of appropriate multivariate statistical analyses. Metabonomics also allows time-dependent patterns of change in response (metabolic trajectories) to stimuli to be measured.

This talk will cover the concept of systems biology and put into context some recently developed metabonomics data analysis methodologies and show how the information is integrated. The effects of symbiotic gut microflora on metabolic profiles will be covered. Some pharmaceutical applications of the approach will be illustrated, including the COMET project for evaluating drug adverse effects, and the prediction of an individual's response to therapy before drug administration. These examples will serve to show the usefulness of the approach in animal sparing and indicate how metabonomics is an integral part of systems biology.

Lecture PL5:

Predictive testing strategies: R&D achievements and perspectives

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Since the last world congress, a sustained and massive investment in the R&D for Alternative Safety can be acknowledged from academics, industries, trade associations and regulatory bodies in Europe and worldwide. The resulting scientific progresses and promises remain nevertheless driven in an industry perspective by realism and the only objective of perpetuating and insuring the Consumer Safety in the regulatory context of the 7th amendment to the Cosmetic Directive.

In a first part, mostly based on large number of data and case studies, an overview of the Research achievements for the development and the implementation of useful and usable batteries of alternative assays will be illustrated. The ongoing refinement and improvement of the currently existing or validated methods in cutaneous irritancy and genotoxicity will be discussed in the double perspective of the applicability domains expansion and/or the continuous improvement of specificity-sensitivity perform-

ances. The progresses in the development of rapid, efficient and cost effective skin bioavailability assays will be also presented as an another example of the optimization/refinement of the already implemented OECD TG428 *in vitro* skin bioavailability guideline. This effort was seen as a necessity since the availability of exposure estimates is indeed key for the implementation of new alternatives strategies for Safety assessment. The ocular irritancy and the acute toxicity endpoints will then be analyzed as study cases of the progresses and difficulties in the development and implementation of integrated testing strategy in these fields.

In a second part the lessons in terms of research policy will be drawn from the concrete study cases discussed previously. One major output of the R&D progresses and efforts reside in the work (done and ongoing) for development and integration of *In Silico* tools in the processes of safety evaluation. The R&D of *in silico* tools and strategies can thus certainly be seen as a paradigm of what is needed for an effective R&D in alternative toxicology: a close R&D collaboration effort between academic, regulatory bodies and industry, sharing the objectives, some data, and understanding of the needs of each parties.

Naturally mirroring the emergence of *in silico* technologies will also be discussed the place and role of new promising (bio) technologies in that quest for a new way of assessing the human safety of chemicals. Many trans-national and trans-organizational initiatives such as the US-EPA ToxCast program, research projects funded by the European Partnership for Alternative Approaches to Animal Testing (EPAA) and/or EU, rely on these new technologies marrying biology, micro electronic, microfluidic and data-mining. Illustrations of the promises and anticipated difficulties raised by this essential and necessary trend for the post 2009 challenges and their remaining scientific gaps will be presented and discussed. The skin-sensitization and systemic toxicity endpoints are domains in which such technologies could facilitate the emergence of new possibilities.

Finally the question of how the R&D efforts developed these last years, the amount of data generated on chemicals, the weight of evidence and the best industry practices could be used to support and accelerate the scientific validation and regulatory acceptance of the resulting Alternative Integrated Testing Strategies (ITS) will be discussed. The ocular irritancy, where hundreds of ingredients from a wide range of chemical classes have served for the optimization and development of such an ITS, will be here again used as a study case. What could be the place of the weight of evidence in the future, how to validate a battery of in silico plus *in vitro* assays, how to use these progresses in the frame of the European REACh regulation will be questioned.

To conclude a call will be made for a coordinated and sustained worldwide R&D effort for the raise of this new era of the alternative toxicology.

Lecture PL6:

Is forefront science considered and applied in regulatory risk assessment?

V. Silano

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Modern societies are confronted with major uncertainties and complexities when making decisions, particularly in the public health sector. Achieving specific health targets requires the ability to predict possible interactions of many factors determining the end result of each decision. To this end, the approach known as risk assessment has been developed to identify the likelihood of unwanted end results.

Science is an absolutely necessary tool to understand the impact of health determinants and their complex interactions and to increase the likelihood of success of specific policies or proposals under consideration for possible adoption by decision makers. Science is also necessary to follow up the results of any decisions adopted, to assess the causes of success or failure and to understand what improvements may be needed on a case by case basis. Therefore, science and derived evidence-based knowledge are the key factors to ensure the success of public health policies and interventions.

Considering the nature of science and its development as a prerogative of human development, attributing an adequate role to science and ensuring regular use of science (and particularly of forefront science) in decision making could be seen an indicator of the social and technological development reached in a given country. Such an objective can be achieved only if an adequate regulatory framework is established to recognize such a need and clear procedures are adopted to ensure science independence. The answer to the question "is forefront science considered and applied in regulatory risk assessment?" depends, therefore, largely on the specific sector and country it applies.

While the current situation cannot be seen as satisfactory in all cases, an outstanding successful approach is represented by the EU Food Law, adopted in 2002 and in the following years mainly to overcome the consequences of the "mad cow disease". Such a regulation and its current implementation will be used to highlight key issues in addressing the above question.

Lectures PL7:

The principles of humane experimental technique: timeless insights and unheeded warnings

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In The Principles of Humane Experimental Technique, Russell & Burch said that "the central problem is that of determining what is and what is not humane, and how humanity can be promoted without prejudice to scientific and medical aims". They then explained how the Three Rs can be used to diminish or remove direct inhumanity ("the infliction of distress as an unavoidable consequence of the procedure employed") and contingent inhumanity ("the infliction of distress as an incidental and inadvertent by-product of the use of a procedure"). They concluded that "Replacement is always a satisfactory answer, but Reduction and Refinement should, whenever possible, be used in combination".

Many of the commonsense insights in The Principles are no less relevant today than they were in 1959. However, their warnings about the limited value of models and, in particular, the danger of succumbing to the high-fidelity fallacy (whereby it is assumed that the best models for humans are always placental mammals, because they are more like humans than other animals), appear to have largely gone unheeded. Of particular importance is their discussion on toxicity testing, which they saw as one use of laboratory animals "which is an urgent humanitarian problem, for it regularly involves considerable and sometimes acute distress". How, then, can it be that mammalian models are still routinely used in attempts to detect chemical carcinogens and reproductive toxins, despite the fact that the relevance to humans of the data they provide has not been, and perhaps could never be, satisfactorily established?

However, as Alan Goldberg points out, there are signs that some significant changes in attitude are taking place, which could be more in line with the thrust of The Principles, that good science and human technique inextricably go hand in hand. As Russell & Burch put it, "If we are to use a criterion for choosing experiments, that of humanity is the best we could possibly invent. The greatest scientific experiments have always been the most humane and attractive, conveying that sense of beauty and elegance which is the essence of science at its most successful." If we are to live up to that ideal in the 21st century, all concerned should take to heart the insights and heed the warnings.

The principles of humane experimental technique – is it relevant today?

A. M. Goldberg

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In the 1959 publication on "The Principles of Humane Experimental Technique", Bill Russell and Rex Burch stated, at the end of the chapter on Replacement, that "As new fields of biology open in the future, it may become a matter of routine to apply the lessons of the past and turn as soon as possible to the techniques of replacement." They recognized that *in vitro* techniques, in their infancy at that time, would become the science of the future.

Today, in the US, the National Academy of Sciences publication of "Toxicity Testing in the 21st Century – a Vision and a Strategy", proves their point. The recognition in this publication that the future of toxicity testing lies in the use of human cells in culture and methods that Bill Russell and Rex Burch could not have possibly conceived of in 1959 but identified generically as the future.

To truly establish the approach will now require very specific training in translational toxicology (the use of clinical observations to develop *in vitro* methods to understand pathways and systems biology) , the development of transnational programs, and ways to evaluate the accuracy, validity and importance of new and/or traditional studies (these evaluations are known as evidence based toxicology (EBT).

Science is the "Art of the Question". The concepts identified above are the tools to answer these questions. The principles that Bill Russell and Rex Burch developed during the 1954-59 writing of The Book are possibly more important today than they have been in the last 50 years. Their concept that the newest science and the most humane science is the very best science is being proven as each of us contribute to the worlds body of knowledge.

Lecture PL8:

Calling on science: making alternatives the new toxicity-testing gold standard

M. Andersen

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All of life's great journeys start with a goal in mind. The 2007 NAS report, "Toxicity Testing in the 21st Century - A Vision and A Strategy", has proposed a clear goal. This report envisions a not too-distant future where routine toxicity testing will be done in human cells *in vitro*, by evaluating perturbations of cellular responses in a suite of toxicity pathway assays. Dose response modeling would comprise computational systems biology models of the circuitry underlying each toxicity pathway; *in vitro* to *in vivo* extrapolations would use pharmacokinetic models, ideally physiologically based pharmacokinetic models, to predict human blood and tissue concentrations under specific exposure conditions. These toxicity assays and dose response tools would become the new gold standard for chemical risk assessment rather than high dose studies in animals. This talk focuses on the scientific challenges required to make this vision a reality, including characteristics of assay design, prospects for mapping and modeling toxicity pathways, concepts of assay validation, and biokinetic modeling. All of these tools are either available or in advanced development. Science must lead this transformation. However, the scientific community, regulatory agencies, and funding organizations will also have to muster resolve to quickly make this vision a reality.