Systems Toxicology 2013 - From Basic Research to Human Risk Assessment

Centro Stefano Franscini, Monte Verità, Ascona Switzerland, 28 April - 1 May 2013

Conference programme and abstracts book

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Programme and abstracts of the

Systems Toxicology 2013 Conference
From Basic Research to Human Risk Assessment

Centro Stefano Franscini, Monte Verità, Ascona, Switzerland, 28 April - 1 May 2013

Conference objectives:

(1) To evaluate how state-of-the-art systems biology tools are or can be used to elucidate toxic modes of action and provide realistic exposure and biological impact assessments.
(2) To establish a framework for interpreting and applying Systems Toxicology data that will inform risk assessment policy and regulation of chemicals.

Organizing Committee:

- Shana Sturla, ETH Zürich, Department of Health Science and Technology, Laboratory of Food and Nutrition Toxicology, Zürich, Switzerland

- Manual Peitsch, Biological Systems Research at Philip Morris International & Swiss Institute of Bioinformatics (SIB), Lausanne, Switzerland

- Martin Wilks, Swiss Centre for Applied Human Toxicology (SCAHT), Basel, Switzerland

- Rex FitzGerald, Swiss Centre for Applied Human Toxicology (SCAHT), Basel, Switzerland

Programme Overview

Day 1: Sunday 28 April 2013	
16:00	Registration open
17:30	Welcome apéro (Bar Roccia)
18:30	Buffet dinner (Sala Luce)
20:00	Opening keynote lectures (Auditorium)
Day 2: Monday 29 April 2013	
08:45	Centro Stefano Franscini (CSF) welcome (Auditorium)
09:00	Systems toxicology platforms
10:30	Coffee break (Bar Roccia)
11:00	Systems toxicology platforms (cont'd)
12:00	Lunch (Sala Luce)
13:30	Experimental enablers of systems toxicology
15:00	Coffee break (Bar Roccia)
15:30	Computational enablers of systems toxicology
17:30	Current research in Systems Toxicology: Poster session with refreshments (room Balint)
19:00	Dinner (Sala Luce)
Day 3: Tuesday 30 April 2013	
09:00	ACS / Chemical Research in Toxicology keynote lecture
09:45	Breakout sessions on current applications
10:15	Coffee break (Bar Roccia)
10:45	Breakout session work (rooms Eranos and Pioda plus main auditorium)
12:15	Lunch (Sala Luce)
13:45	Current research in systems toxicology (poster talks)
14:30	Systems Toxicology in risk assessment and management
16:00	Coffee break (Bar Roccia)
16:30	Panel discussion
18:45	Conference dinner (Sala Luce)
Day 4: Wednesday 1 May 2013	
09:00	CSF Award presentation
09:15	Breakout session reports
10:15	Closing remarks
11:00	Conference closes

Systems Toxicology 2013 Conference
From Basic Research to Human Risk Assessment

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Contents

PROGRAMME

SUNDAY 28 APRIL 2013

16:00 – 19:00 REGISTRATION

17:30 WELCOME APERO

18:30 BUFFET DINNER

20:00 – 21:00 **Opening keynote lectures**

Transforming Toxicology: The Case for Change

Bob Kavlock, EPA Office of Research & Development, Washington, USA

- Drivers for change in toxicological studies (pharmaceuticals vs environmental chemicals; US vs Europe)
- Key international research endeavours
- What success depends on (e.g. multi-disciplinary cooperation, transparency, confidence in public health protectiveness, regulatory acceptance)
- Indicators of progress and expectations for the future

Maurice Whelan, EU JRC Systems Toxicology Unit + ECVAM, Ispra, Italy

- The need to fundamentally change the way we assess the toxicological hazard of new and existing molecules to address socio-economic issues such as the cost of failure in drug development (pre and post marketing), the risk management of commodity chemicals where traditional data is lacking, while reducing our reliance on animal testing.
- Global challenges that require collaboration at an international level to deliver comprehensive and credible solutions applicable across sectors in different regulatory domains and jurisdictions.
- Calls for change in Europe emanating from top-down regulatory needs (Cosmetics, REACH) in contrast to a bottom-up science-driven focus (NAS Tox21c vision) in the USA.
- Shift towards knowledge based approaches that rely on mechanistic understanding to facilitate the rational design of integrated assessment and testing strategies.
- The role of systems biology and the positioning of modelling and computational methods at the heart of research and development.
- How to address international regulatory acceptance and end-user uptake of next generation methodology developed to support decision makers.

PROGRAMME

MONDAY 29 APRIL 2013

08:45 – 09:00 Centro Stefano Franscini (CSF) welcome
Dr Chiara Cometta, Centro Stefano Franscini
Lorenzo Sonognini, Director of Monte Verità

Systems toxicology platforms
Session Chair: Shana Sturla

09:00 – 09:30 **Toxome and –Omics in Systems Toxicology**
Thomas Hartung, Johns Hopkins Bloomberg School of Public Health, Baltimore, USA

- Mapping of pathways of toxicity in the Human Toxome project
- Integrated Testing Strategies
- Evidence-based Medicine approaches
- Mechanistic Validation

09:30 – 10:00 **EPA's Computational Toxicology Program: Predicting Chemical Toxicity, Exposure and Disease**
Kevin Crofton, EPA National Center for Computational Toxicology, Research Triangle Park, USA

- History and update on EPA's ToxCast programme, including how regulatory drivers in both the US and EU are impacting research.
- Using new science tools to understand how human body processes are affected by exposures to chemicals
- Use of ToxCast data in developing predictive computational tools for endocrine activity
- Challenges and future directions in implementation of computational tools in decision making

10:00 – 10:30 **IMI eTOX**
François Pognan, Novartis Institutes for BioMedical Research, Basel, Switzerland

- What is eTox and the Innovative Medical Initiative (IMI) framework
- Current status of *in silico* pre-clinical toxicity prediction
- eTox specifics (organisation, goals, vision)
- Current achievements and future plans

10:30 – 11:00 COFFEE BREAK

Systems toxicology platforms (continued)

11:00 – 11:20 **Systems toxicology in pharmaceutical development**

Thomas Singer, F. Hoffman-La Roche Pharmaceuticals, Basel, Switzerland

- Predicting risk in early development
- Holistic approach towards a greater mechanistic understanding of health, disease and treatment
- Improved human safety prediction using model-based approaches to reduce attrition

11:20 – 11:40 **Metabolomics *in vivo* – importance of reference data to predict toxicity**

Hennicke Kamp, BASF SE, Ludwigshafen, Germany

- MetaMap®Tox: a metabolomics reference data base for more than 500 compounds with well-known toxicological profile
- *in vivo*-based metabolomics tool to identify systemic toxicity in preclinical studies
- Potential for early detection of systemic toxicity and (rare) adverse events indicative for clinical DILI
- Case studies based on a FDA assessment for DILI and non-DILI compounds (Chen et al 2011) to show the importance of robust reference data

11:40 – 12:00 **Systems toxicology: gaining mechanistic insight through a more integrative omics approach**

Nigel Skinner, Agilent Technologies, Wokingham, UK

- Use of multi-omics approaches to gain mechanistic insights
- Software tools for conducting multi-omics studies
- Value of multi-omics to systems toxicology

12:00 – 13:30 LUNCH BREAK

Experimental enablers of systems toxicology
Session Chair: Manuel Peitsch

13:30 – 14:00 **Enzyme Response Profiling: Integrating proteomics and genomics with xenobiotic metabolism and cytotoxicity**

Shana Sturla, ETH Zürich, Department of Health Science and Technology, Laboratory of Food and Nutrition Toxicology, Switzerland

- Impact of dietary compounds on cellular enzyme activity and cytotoxicity of DNA-alkylating drugs
- Discovery-oriented and targeted quantitative measurement strategies for evaluating xenobiotic-metabolizing enzymes in human colon cells
- Integration of information at the level of chemical reactivity, protein levels, and gene expression toward developing a quantitative network model
- Cell lines as models of disease progression and measurement of markers in primary colon epithelial cells

MONDAY 29 APRIL 2013

Experimental enablers of systems toxicology (continued)

14:00 – 14:30 **Adductomics: DNA biomarkers in exposure, risk assessment and cancer prevention**

Robert Turesky, NY State Department of Health, Albany, USA

- Methods of DNA adduct measurements in human populations
- DNA adducts as biomarkers of exposure and risk assessment to carcinogens
- DNA adducts and mutational spectra in tumour suppressor genes may provide clues to the origin of human cancers for which an environmental cause is suspected
- DNA adducts in intervention studies to assess efficacy of chemopreventive agents that modulate expression/activity of phase I or II enzymes involved carcinogen metabolism

14:30 – 15:00 **Toxicogenomics in Hazard Assessment**

Joost van Delft, Maastricht University, The Netherlands

- Toxicogenomics in Hazard Assessment of carcinogens and hepatotoxicants
- Integration analyses of transcriptomics, microRNAs and metabonomics
- Integration of omics and chemoinformatics
- Hepatic *in vitro* models

15:00 – 15:30 COFFEE BREAK

Computational enablers of systems toxicology
Session Chair: Manuel Peitsch

15:30 – 16:00 **Data Management, Normalization and Integration**

Ioannis Xenarios, *Swiss Institute of Bioinformatics, Lausanne, Switzerland*

- Swiss-Prot and Vital-IT groups provide competency and resource knowledge for the scientific community.
- The role of UniProt-KB/Swiss-Prot is to represent and structure the knowledge around the functions of proteins in all organisms.
- This effort aims at maintaining a high level annotation of protein functions but more and more it encompasses systems level annotation bridging the gap between biology and chemistry.
- Rather than integrating all the data within UniProt-KB we approach our effort by coordination annotation with several international and national resources such as NeXtprot, chEMBL, chEBI, IMEX and other.

16:00 – 16:30 **Reverse Engineering Disease Pathways**

Gustavo Stolovitzky, IBM Computational Biology Center, Yorktown Heights, USA

- Crowdsourcing as a strategy for the analysis of high-throughput, high complexity data
- Example of the wisdom of crowds at work in network inference
- Lessons for a collaborative competition of disease diagnostics
- Leverage the community wisdom to translate biology between species.

MONDAY 29 APRIL 2013

Computational enablers of systems toxicology (continued)

16:30 – 17:00 **Computational Systems Toxicology: Network-based Biological Impact Assessment**

Manuel Peitsch & Julia Hoeng, PMI R&D, Neuchatel, Switzerland

- Description of a methodology for the Quantification of the Amplitude of Biological Network Perturbations and deriving Biological Impact Factors.
- Showcasing how this method can be applied in both *in vivo* and *in vitro* studies.
- Description of sbv IMPROVER, a challenge based community-focused project aimed at the Verification of Systems Biology methods and outcomes.
- Description of the second IMPROVER Challenge focused on Species Translation.

17:00 – 17:30 **Integrating Genomic Data with Virtual Populations via Hypothesis Management: Broad Applications in Systems Toxicology**

Thomas Paterson, Entelos, San Mateo, USA

- Recent collaborative efforts between Entelos and The Institute for Systems Biology (ISB) have demonstrated efficient workflows for integrating gene network analyses with large-scale physiologic modelling.
- These workflows leverage on Entelos' Hypothesis Management process that systematically explores many thousands of alternative mechanistic hypotheses (virtual populations) to explain variability in clinical phenotype.
- By enforcing gene network correlations upon a hypothesis space initially bounded by epidemiological and clinical data on endpoints and biomarkers, the set of feasible mechanistic hypotheses is vastly reduced.
- This presentation will review a case study of this process and discuss applications in systems toxicology for managing the hypothesis space of toxin-response phenotypes, determining toxic modes of action, and assessing physiologic impact and risk in diverse human populations.

17:30 – 19:00 **Current research in Systems Toxicology:**
Poster session *with refreshments*

19:00 **Dinner**

TUESDAY 30 APRIL 2013

ACS / Chemical Research in Toxicology Keynote Lecture
Session Chair: Rex FitzGerald

09:00 – 09:45 **Exposing the Exposome**

Stephen M. Rappaport, University of California, Berkeley, USA

- Although chronic diseases are primarily caused by environmental (i.e. non-genetic) factors, aside from smoking and infections, the major etiologic exposures are unknown.
- The exposome, representing all exposures received by an individual, opens avenues for data-driven research to discover important causes of disease.
- The exposome can be functionalized as the universe of circulating molecules (including the metabolome, proteome and adductome) in human serum.
- Exposome-wide association studies (EWAS) compare serum profiles between healthy and diseased subjects to pinpoint discriminating molecules. These molecules represent candidate biomarkers of either exposure or disease progression.
- Candidate biomarkers of exposure motivate knowledge-driven applications to confirm causality, to provide mechanistic understanding, to identify and quantify sources of exposure, and to suggest mitigation strategies.

09:45 – 10:15 **Breakout Sessions on Current Applications**

Introduction, allocation of participants, moderator and rapporteur nomination

Genotoxins in food
Systems toxicology in drug safety
Thyroid endocrine disruptors

10:15 – 10:45 COFFEE BREAK

10:45 – 12:15 **Breakout session work**

- What is the basis of the current relevance/importance of the topic?
- Assess and critique systems-oriented efforts to address the topic: who are leaders in this area and what has been accomplished?
- What information or tools are missing?
- How and to what degree can systems-oriented strategies impact this topic?

12:15 – 13:45 LUNCH BREAK

13:45 – 14:30 **Current research in Systems Toxicology (poster talks)**
Session Chair: Thomas Hartung

Oral presentation of three selected posters

TUESDAY 30 APRIL 2013

Systems Toxicology in risk assessment and management
Session Chair: Martin Wilks

14:30 – 15:00 **What does Risk Assessment need from Systems Toxicology?**
Alan Boobis, Imperial College London, UK

- What systems toxicology is and what it is not, from a risk assessment perspective
- Emergent properties of the system
- Potential contributions of systems toxicology in risk assessment, not otherwise achievable, e.g.
  - Mode of action in humans
  - Nature of the dose-response relationship at human relevant exposures
  - Use of *in vitro* and *in silico* approaches for accurate prediction of effects
  - Effects of combined exposures to similarly- and dissimilar-acting compounds
  - Impact of non-chemical stressors on effects of chemicals
  - Human variability and sub-population susceptibility
- Establishing confidence in value of systems toxicology in risk assessment.

15:00 – 15:30 **Advancing the Next Generation of Risk Assessment**
Ila Cote, EPA National Center for Environmental Assessment, Washington, USA

- Proof of concept case studies
  - hazards
  - exposure-response
- Decision rules necessary for use of new data to characterize potential risks
- Limitations in current data and needed improvements

Please also refer to abstract on page 15

15:30 – 16:00 **Translating Systems Toxicology-based Assessment into Risk Management**
Marcel Leist, University of Konstanz, Germany

- Roadmap/stages to new regulatory toxicity testing
- Validation of Systems Toxicology approaches for regulatory use
- Probabilistic risk assessment

16:00 – 16:30 COFFEE BREAK

16:30 – 17:30 **Panel Discussion:**
What data from biological systems-wide technologies will be needed in risk assessment and what can facilitate their interpretation?

18:45 **Conference dinner**

PROGRAMME

WEDNESDAY 1 MAY 2013

09:00	**CSF Award presentation** Established in 2009 to commemorate the 20th anniversary of the Centro Stefano Franscini, the CSF award is given to the best presentation at the conference by a young scientist.
09:15 – 10:15	**Breakout session reports** Presentations and Q&A (preparation for publication)
10:15 – 11:00	**Closing remarks**: **Transforming Toxicology: The Way Forward** *Bob Kavlock, EPA Office of Research & Development, Washington, USA and* *Maurice Whelan, EU JRC Systems Toxicology Unit + ECVAM, Ispra, Italy*

Advancing the Next Generation of Risk Assessment

Ila Cote, EPA National Center for Environmental Assessment, Washington, USA

This report describes the results of the Next Generation of Risk Assessment (NexGen) Program, a multiyear, multi-organization effort to develop and evaluate new molecular, computational and systems biology informed approaches to risk assessment. The goal of this effort is to facilitate faster, less expensive and more robust assessments of potential public health and ecologic effects by EPA's National Center for Environmental Assessment (NCEA). The specific aims of the program are to: (1) to demonstrate proof of concept that recent advances in biology can better inform risk assessment, (2) to understand what information is most useful for particular purposes (valued of information), and (3) to articulate decision rules for use of new types of data and methods to inform risk assessment.

To help achieve this goal and specific aims, prototypes or case studies were developed. These prototypes are intended : 1) to move molecularly informed risk assessment from concept to practical application; and 2) to provide concrete examples to the risk assessment and management communities, and the general public, in order to facilitate further discussion, understanding, and refinement of new risk assessment tools. The prototypes illustrate broad categories of "fit" for purpose" assessments aimed at differing risk management needs or context. Primary drivers for risk managers are the number of chemicals that must be addressed and the confidence needed in the scientific data to support a specific type of decision. The types of assessments illustrated by the prototypes are grouped into three categories or tiers for simplicity, but data and methods are in fact arrayed as a continuum of approaches that can be deployed in various situations. Among the chemicals evaluated that are used to illustrate different approaches to risk assessment are: benzene, polycyclic aromatic hydrocarbons, ozone, chemicals that cause metabolic disorder syndrome (e.g. diabetes and obesity), and endocrine disruptors. Among the methods highlighted are: quantitative structure activity modelling, bioinformatics, a wide variety of high throughput *in vitro* assays, *in vivo* and *in vitro* high content assays using mammalian and nonmammalian species and molecular clinical studies and epidemiology.

An integrated approach to risk assessment is proposed based on the current state of knowledge, with full awareness that the pace of discovery is such that any such approach must evolve over the near timeframe. Among the conclusions of the report are that causal molecular patterns associated with specific diseases can be identified. These patterns are also exposure-dose dependent. These two features can enable the use of molecular biology data to estimate public health risks, including screening and ranking of large numbers of chemical in an unprecedented fashion, and more robust consideration of human variability and mixtures exposures. However, correct interpretation of the data is fraught with challenges. For examples, portions of a specific molecular pattern can be causally related to multiple diseases, and substantial variability in health outcomes are often observed as a result of exposure/dose, exposure duration, time post exposure, species, tissue, lifestage and interindividual variability. As a result, sometimes we can estimate the relative potency of a chemical to cause important biologic disruptions, but not be able to predict the specific health outcome that may occur in humans. In addition, measurement error is high with many current methods. As a consequence, specious associations among phenomena are a substantial concern. Hence, systematic review of data and methods are critical to effectively deploying new risk assessment method.

01

Anchoring the molecular responses, transcriptome and proteome, to physiological phenotype in *Chlamydomonas reinhardtii* exposed to silver

Smitha Pillai[1], *Renata Behra*[1], *Marc Suter*[1], *Holger Nestler*[1], *Laura Sigg*[1,2], *and Kristin Schirmer*[1,2]

1 Department of Environmental Toxicology, Eawag (Swiss Federal Institute for Aquatic Science and Technology) Ueberlandstrasse 133, 8600 Duebendorf, Switzerland

2 Department of Environmental Sciences, ETH Zürich, Switzerland

Background:

Silver in aquatic bodies is of concern due to the high toxicity of free silver ions. A major problem is the increasing use of silver nanoparticles (AgNP) in several consumer products, exploiting the antibacterial properties of Ag+ released from the N. *Chlamydomonas reinhardtii*, a unicellular alga, is sensitive to low concentrations of silver which cause the inhibition of photosynthesis and production of reactive oxygen species. These effects do not necessarily reflect changes at the molecular level or functional changes at the organism or population level. The aim of our study was to obtain a global view of the molecular responses in *C. reinhardtii* to exposure to silver and to link them to physiological changes at the organism and population levels.

Methodology/Principal Findings:

Differential gene transcription was studied using whole nuclear genome oligonucleotide microarray and protein expression by multidimensional protein identification technology (MudPIT). In addition we measured commonly used physiological endpoints and also developed new endpoints related to the transcriptome and proteome response. To obtain time- and dose-dependent responses, *C. reinhardtii* cultures in biological replicates were exposed to silver in concentrations varying from low environmentally relevant to high for durations from 15min to 16h. The key findings of our study are as follows:

i) The molecular responses at the transcriptome and proteome level are highly sensitive and are silver-specific even at very low concentrations where no physiological changes were observed

ii) Silver not only inhibits the functions of chloroplast and mitochondria, both at the molecular and physiological levels, but also inhibits the transport of functional proteins to these organelles. We hypothesize that these effects are specifically on these organelles, probably due to their prokaryotic origin.

iii) We could follow the effects of silver on the various metabolic pathways and thereby identify the mechanism of action in *C. reinhardtii*

Conclusion/Significance:

In this manuscript we show the importance of using molecular endpoints to estimate the toxicity of silver. The transcriptome and proteome data showed that toxicants induce changes at much lower concentrations that estimated via classical toxicological endpoints.

02

Characterization of gene expression patterns caused by developmental exposure to different mixtures of anti-androgenic and estrogenic endocrine disrupters in developing rat brain regions

***Walter Lichtensteiger*[1]**, *Catherine Bassetti-Gaille[1], Oliver Faass[1], Julie Boberg[2], Sofie Christiansen[2], Ulla Hass[2], Andreas Kortenkamp[3], Margret Schlumpf[1]*

[1]GREEN Tox and Institute of Anatomy, University of Zurich, Zurich, Switzerland, [2]National Food Institute, Technical University of Denmark, Søborg, Denmark, [3]Institute for the Environment, Brunel University, Uxbridge, UK

Objective:

Brain sexual differentiation is a potential target of endocrine disrupters (EDCs). Recent data indicate that male brain differentiation is achieved by a combination of estrogen receptor- and androgen receptor-mediated effects. Female brain development is dependent on estrogens. It is not known how these processes are affected by real-world mixtures of endocrine disruptors (EDCs). In order to identify developmental target processes at the gene expression level, we used a combination of transcriptomics and quantitation of individual mRNAs.

Methods:

Three different mixtures of EDCs, Amix (anti-androgenic chemicals: DBP, DEHP, pp'-DDE, vinclozolin, prochloraz, procymidone, linuron, epoxyconazole), Emix (estrogenic chemicals: Bisphenol A, EHMC, 4-MBC, butyl-paraben) and Tmix (chemicals of Amix + Emix + paracetamol (anti-androgenic activity)) were administered by oral gavage to rat dams from gestational day 7 until weaning (postnatal day 21), at doses corresponding to 450x, 200x and 100x the high-end human intakes (S. Christiansen et al., 2012). At postnatal day 6, during the last part of sexual brain differentiation, exon microarray analyses were performed in medial preoptic area (MPO) of both sexes in the highest dose group, and real time reverse transcription polymerase chain reaction (RT PCR) of selected mRNA species in MPO and ventromedial hypothalamus (VMH) of all dose groups

Results:

Microarray analyses in MPO revealed treatment- and sex-specific effects on gene expression patterns. The majority of genes affected by individual mixtures were selective for that mixture. Real time RT PCR of individual mRNA species demonstrated treatment- and sex-dependent differences between MPO and VMH. Effects were dose-dependent. Prominent are effects of all three EDC mixtures on the expression of genes encoding for proteins involved in excitatory glutamatergic synapse formation and function.

Conclusion:

Effects of complex mixtures of EDCs on developing brain can be characterized by mixture-, sex- and region-specific gene expression patterns. Excitatory synapse development emerged as a potentially relevant focus. Supported by EU 7th Framework (CONTAMED).

03

Neuroinflammation induced by the mycotoxin Ochratoxin A – a downstream key event in an adverse outcome pathway (AOP)

Jenny Sandström von Tobel*[+], *Paola Antinori**[&], *Marie-Gabrielle Zurich**[+], *Robin Rosset*[+], *Florent Gluck**[&], *Alex Scherl**[&], *and Florianne Monnet-Tschudi*[+]**

** Swiss Centre for Applied Human Toxicology (SCAHT)*

[+] Department of Physiology, University of Lausanne, CH-1005 Lausanne, Switzerland

[&] Genetical Medicine, University Hospital, CH-1200 Geneva, Switzerland

Objective:

Adverse effects of the environmentally abundant mycotoxin Ochratoxin A (OTA) were further investigated and organized according to an Adverse Outcome Pathway (AOP) [1].

Methods:

Neurotoxic effects of OTA were assessed using 3D rat brain cell cultures – comprising all brain cell types. Cultures were exposed to OTA (2 or 10 nM) for 48 hours and 10 days. Adverse effects were examined by measuring changes in gene and protein expression using real-time reverse transcription polymerase chain reaction (PCR), mass spectrometry (MS), western blot and immunocytochemistry.

Results:

OTA disrupted the axonal cytoskeleton, as evidenced by a decrease in NF-H content, already visible after 48 hours, and reduced expression of several oligodendrocyte markers consistent with demyelination. Astrocyte cytoskeletal disruption was evidenced by reduced intracellular glial fibrillary acidic protein (GFAP) expression, and a concurrent increase in GFAP in the culture medium. Furthermore, diminished content of astrocytic metallothionein 1 and 2, a regulator of inflammatory response, was observed. After 10 days of OTA exposure, a neuroinflammatory response could be observed in terms of an expansion of isolectin B4 labelled cells, accompanied by an increase of pro-inflammatory cytokine mRNA expression. The presence of macrophagic ED1 positive cells and upregulation of marker genes for microglial M1 degenerative phenotype suggests that OTA-induced neuroinflammation could aggravate the neurodegenerative process.

Conclusion:

OTA-induced neuroinflammation may be considered secondary to adverse effects on neurons, oligodendrocytes and astrocytes. A partial recovery of OTA adverse effects on oligodendrocytes was obtained by co-treatment with sonic hedgehog (SHH). We therefore propose, using the AOP framework terminology, that interaction with SHH represents one molecular initiating event, and that the adverse effects on neurons, oligodendrocytes and astrocytes are early key events which lead to neuroinflammation as a downstream key event. OTA-induced neuroinflammation should be considered as a stress response pathway that may lead to neurodegeneration as the adverse outcome.

[1] OECD 2012. www.oecd.org/chemicalsafety/testingofchemicals/49963554.pdf

04

Identification and Prioritization for Risk Management of Carcinogenic Substances in Food

***Alessia Stornetta*[1]**, *Barbara E. Engeli*[2], *Jürg A. Zarn*[2], *Gérard Gremaud*[3], *and Shana J. Sturla*[1],
[1]*Department of Health Science and Technology, Swiss Federal Institute of Technology Zurich, Switzerland;*
[2]*Nutritional and Toxicological Risk Section, Swiss Federal Office of Public Health, Zurich, Switzerland,* [3]*Federal Department of Home Affairs, Federal Office of Public Health, Bern, Switzerland*

Objective:

To rank the relative risks of foodborne carcinogens on the basis of their carcinogenic potencies and estimated exposure levels in Switzerland.

Methods:

Data on toxicity in humans or animals and estimates of exposure in Switzerland are combined in order to create a ranked list of selected foodborne carcinogens. Toxicity reference values are gathered from published scientific reports and databases. The estimates of exposure in the Swiss population are derived from analyses of carcinogenic contaminants in food by Swiss public and industry laboratories, and from the estimated consumption of food in the Swiss population. The different sources and variability of the carcinogens in food, as well as susceptibility and intake in different population groups (adult, child) are considered.

Results:

The output of this study is a ranked list of the most relevant foodborne carcinogens on the basis of estimated exposure levels in the Swiss population. We aim at generating a list of about 50 foodborne carcinogens, including mycotoxins, polycyclic aromatic hydrocarbons (PAHs), metals, and carcinogenic substances formed during the cooking process such as acrylamide.

Conclusion:

The compiled results can guide more precise selection of foods for surveillance, as well as enable researchers and industries to identify the most relevant carcinogens in food for further study.

05

How well does RNA-seq quantification really work?

Matthias Roesslein
Materials-Biology Interactions, Empa, St. Gallen, Switzerland

Objective:

System biological modelling strongly depends on the reliability and on the quality of the available data. Quantitative RNA transcriptome sequencing (RNA-seq) provides a detailed view of the up and down regulation of genes on an isoform level. So far most developers of quantification algorithms have depended solely on *in silico* simulation to test them. We have now thoroughly evaluated the most popular algorithms using real data from the Illumina body map data 2.0.

Methods:

First expression levels of genes and their known isoforms were investigated in two different tissues to identify the genes expressed solely in one tissue. Approximately 3,000 to 4,000 isoforms and the corresponding genes were selected, depending on the tissue combination. Then different ratios of reads of the two tissues were used to perform the RNA-seq analysis followed by an in-depth statistical analysis comparing the observed and expected ratios.

Results:

The statistical evaluation of the different quantification approaches revealed that on average the expected ratios were found in the selected genes and their isoforms. However, the spread of results is considerable and is strongly dependent on the algorithms employed. The de facto standard Cufflinks algorithms [1] performed especially poorly with a very wide spread. The new version 2.0 [2] showed some improvements.

Conclusion:

There is considerable need for improvement, since some systematic and random effects are not incorporated into the different algorithms. Isoform-specific external RNA spike-in standards, following the concept of the ERCC standards [3], would allow a more systematic approach to improve algorithms.

References:

[1] Trapnell C, Williams BA, Pertea G, Mortazavi A, Kwan G, van Baren MJ, Salzberg SL, Wold BJ, Pachter L. Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Nat Biotechnol. 2010 May, 28(5), 511-5.
[2] Trapnell, C. et al., Differential analysis of gene regulation at transcript resolution with RNA-seq. Nature Biotechnol. 2012 Dec 9, 31(1), 46-53.
[3] Baker, S.C. et al., The external RNA Controls Consortium: a progress report. Nature Meth. 2005, 2, 731-734

06

Mapping Reductases in Human Colon Cells – Influence of Bioactive Food Components

***Melanie M. Erzinger**, Cédric Bovet, Katrin M. Hecht, Paul van Midwoud, Shana J. Sturla*
Institute of Food, Nutrition and Health, ETH Zürich, Switzerland

Objective:

The purpose of this study was to investigate the influence of two bioactive food components (BFCs), namely sulforaphane and sodium selenite, on reductase enzymes that are involved in drug metabolism and to determine if BFC pre-treatment alters the susceptibility of human colon-derived cells toward bioreductive anticancer drugs.

Methods:

Protein levels of reductase enzymes were quantified by targeted proteomics and corresponding enzyme activities were determined with standard UV/Vis-spectroscopy assays. Cellular uptake of BFCs was measured with ICP-MS (selenium) or HPLC (sulforaphane). The functional influence of BFC pre-conditioning on cellular susceptibility to chemotherapy was assessed on the basis of cytotoxicity assays and mass spectrometric analyses of cellular DNA adduct burdens.

Results:

Alterations in protein levels or enzyme activities of selected reductase enzymes, as a result of BFC treatment, were determined for various colon cancer cells and cells derived from healthy colon epithelial. Corresponding fold changes were compared on the basis of transcriptional regulation of the enzymes and the cellular uptake of the BFCs. Further, the influence of BFC pre-treatment on drug cytotoxicity and drug DNA adduct formation was linked to changes in reductase protein levels and activities.

Conclusion:

Depending on their structure and biological activity, BFCs are taken up differently by distinct colon cell types. Entering the cell, they can change enzyme levels and activities of selected reductases involved in cancer drug metabolism. These changes depend strongly on the cell type and are responsible for changes in drug cytotoxicity and drug DNA adduct formation of bioreductive anticancer drugs.

07

Systems approach to improve mechanistic understanding of benzo(a)pyrene action in a liver cell model

***Danielle Jannuzzi Madureira*[1,2]*; Paul Van Midwoud*[3]*; Frederik Weiss*[1]*; Damian Helbling*[1]*; Saskia Trump*[4]*; Irina Lehmann*[4]*; Shana Sturla*[3]*; Kristin Schirmer*[1,2,5]

[1] Eawag, Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland [2] ETH Zürich, Institute of Biogeochemistry and Pollutant Dynamics, Department of Environmental Systems Science, Zürich, Switzerland, [3] ETH Zürich, Institute of Food, Nutrition and Health, [4] UFZ-Helmholtz Centre for Environmental Research, Leipzig, Germany, [5] EPF Lausanne, School of Architecture, Civil and Environmental Engineering, Lausanne, Switzerland

Objective:

The overall aim of this work was to investigate the molecular response of the murine hepatoma cell line, Hepa1c1c7, to benzo(a)pyrene (BaP) exposure, combining high throughput technique, bioinformatics, phenotype characterization and chemical analysis in a time and concentration dependent manner. BaP was chosen as a relevant environmental contaminant because it is widely distributed and is classified as carcinogenic to humans by the International Agency for Research on Cancer.

Methods:

Cellular responses to BaP were evaluated at different levels of cell organization, considering both time (up to 24 h) and low and high exposure concentration (50 nM and 5 μM).

Results:

Transcriptome analysis indicated that BaP entered the cells rapidly. Quantification of cell internal concentrations confirmed rapid uptake; maximum cell internal concentrations were achieved within one hour of exposure. Gene regulation did not differ between the low and high BaP concentrations for the first hours but then greatly diverged with cells returning to control levels for the low BaP exposure whereas cell death resulted at the high BaP exposure concentration. Based on Cyp1A activity, quantification of adducts, and reduction of parent BaP amount, metabolism was induced between 4 and 8h and more than 90% of BaP was metabolized at both concentrations within 24h. DNA adducts were first detected at 8h for both BaP concentrations; complete DNA-adduct repair was seen only at the low concentration. With the conditions used, it seems that biotransformation, not uptake, was the rate limiting step.

Conclusion:

The work presented here adds more detailed understanding of the relationship between the molecular initiating events and the cell response. Furthermore, the time resolved information and rate associated constants can support building of computational models to predict cellular responses in the Hepa1c1c7 cell line and can contribute to increasing the predictive power of hazard characterization and exposure assessment for risk assessment.

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System-level response of colon cancer cell lines after exposure to bioactive food components: integrative data analysis

***Simona Constantinescu**, Department of Biosystems Science and Engineering, ETH Zurich, Basel, Switzerland;* Cedric Bovet, Institute of Food, Nutrition and Health, ETH Zurich, Zurich, Switzerland; Melanie M. Erzinger, Institute of Food, Nutrition and Health, ETH Zurich, Zurich, Switzerland; Bernd Wollscheid, Institute of Molecular Systems Biology, ETH Zurich, Zurich, Switzerland; Giancarlo Marra, Institute of Molecular Cancer Research, The University of Zurich, Zurich, Switzerland, Shana J. Sturla, Institute of Food, Nutrition and Health, ETH Zurich, Zurich, Switzerland; Niko Beerenwinkel, Department of Biosystems Science and Engineering, ETH Zurich, Basel, Switzerland.

Objective:

Studying the effect of sulforaphane and selenite exposure on HT29 colon cancer cells, as compared to HCEC epithelial cells: data analysis and integration

Methods:

Even though previously investigated, the influence of a diet enriched with various bioactive food components on outcome in colon cancer patients remains poorly understood. Here, we address this question by comparing the response of the colon cancer cell line HT29 and the epithelial cell line HCEC to two widely encountered bioactive food components: sulforaphane and selenite. We conducted this comparison by statistically analyzing and integrating different types of transcriptomics (microarray gene expression profiling), proteomics (SILAC and SRM), and enzyme activity data. In the SRM and enzyme activity experiments, the following proteins have been targeted: PTGR1, TrxR, and NQ01.

Results:

Most of the variation in the data is explained by the cell lines, followed by the food component. For both cell lines and both food components, the induced fold changes in protein concentration and enzyme activity level for the three targeted enzymes are highly significant and in high agreement. On the transcriptome level, sulforaphane induces higher fold-changes and targets more genes in the HT29 colon cancer cells than in the HCEC cells, while sulforaphane has the opposite effect. Moreover, there is no overlap between the differentially regulated genes in the two cell lines and the two food components. Members of the aldo-kedo reductase family 1 are clustering as an upregulated gene group after sulforaphane exposure, in both the transcriptomics and the SILAC proteomics experiment.

Conclusion:

The two bioactive food components sulforaphane and selenite trigger very different responses in the HT29 colon cancer cell line, as compared to the HCEC epithelial cell line, on both the transcriptomics and the proteomics level.

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A systems biology approach to unravel mechanisms of cyclosporin A induced hepatotoxicity

Wim F.P.M. Van den Hof[A,D], *Ainhoa Ruiz-Aracama*[B,D], *Anke Van Summeren*[B,D], *Danyel G.J. Jennen*[A,D], *Stan Gaj*[A,D], *Maarten Coonen*[A,D], *Karen Brauers*[A], *Will K.W.H. Wodzig*[C,D], *Joost H.M. van Delft*[A,D], *Jos C.S. Kleinjans*[A,D];
[A] *Department of Toxicogenomics, Maastricht University, Maastricht, the Netherlands,* [B] *RIKILT – Institute of Food Safety, Wageningen University and Research Centre, Wageningen, the Netherlands,* [C] *Department of Clinical Chemistry, Maastricht University, Maastricht, the Netherlands,* [D] *Netherlands Toxicogenomics Centre, Maastricht, the Netherlands*

Objective:

There is a need for a better understanding of the mechanisms behind drug-induced hepatotoxicity to improve preclinical drug testing and diminish the number of hepatotoxic compounds going into animal experiments and clinical trials. Although transcriptomics and metabonomics both give valuable information about toxicological responses, separately they do not give a complete overview. By combining these different techniques in one toxicogenomic analysis, we aim to better characterize the system-wide response to a toxic compound. Therefore, we combined transcriptomic and metabonomic profiling of HepG2 cells exposed to Cyclosporin A. Cyclosporin A is a calcineurin inhibitor and is widely used as immunosuppressant drug in transplant patients. A side effect of Cyclosporin A is that it can cause cholestasis which will lead to hepatocellular damage.

Methods:

HepG2 cells were exposed to two concentrations of Cyclosporin A, 3 µM and 20 µM, and were investigated at several time points. The mRNA and miRNA expression levels were investigated using array technology at 12, 24, 48 and 72 hours. A metabonomic investigation was done using 1H-Nuclear Magnetic Resonance spectroscopy at 24 and 72 hours and Liquid Chromatography coupled to Mass Spectrometry at 24 hours.

Results:

The transcriptomics and metabonomics analyses demonstrated many dose- and time-dependent changes in Cyclosporin A-exposed HepG2 cells. Integrated analyses showed that Cyclosporin A induces endoplasmic reticulum and mitochondrial stress and impairment of cytochrome P450 enzymes. Bile acid synthesis is inhibited resulting in intracellular cholesterol accumulation.

Conclusion:

Our findings indicate that a systems wide approach combining metabonomics and transcriptomics with bioinformatics may lead to a better understanding of the underlying mechanisms of drug-induced hepatotoxicity and may help to improve the *in vitro* assessment of novel compounds for hepatotoxic properties at an early stage in drug discovery.

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In vitro systems toxicology approach to investigate the effects of repeated cigarette smoke exposure on respiratory tract tissue cultures

Carole Mathis*, *Radina Kostadinova**, *Sandra Wagner, Stefan Frentzel, Fabio Talamo, Nikolai Ivanov, Yang Xiang, Florian Martin, Manuel C. Peitsch, Julia Hoeng*
Quai Jeanrenaud 5, 2000 Neuchâtel, Philip Morris Product SA, Switzerland

Objective:

The aim of this study is to develop assay systems that **model *in* *vitro*** the impact of cigarette smoke (CS) on different human organotypic respiratory tract tissue cultures (bronchial, nasal, buccal and gingival) which are in contact with CS upon inhalation *in vivo*. Such assay systems may have a role in the assessment of potential Modified Risk Tobacco Products.

Methods:

We intended to mimic the smoking behavior of a moderate smoker during one day by exposing repeatedly and in parallel four tissue cultures (bronchial, nasal, buccal and gingival) directly at the air/liquid interface (Vitrocell® System) to machine-generated mainstream smoke from four cigarettes with one hour intervals between each cigarette. These organotypic cultures are generated from primary cells derived from non-smoking donors and contain fibroblasts and epithelial cells in order to reproduce as closely as possible the *in vivo* situation.

Results:

Firstly, we simultaneously exposed all tissues to various CS concentrations ranging from 8% to 35% (diluted vol/vol with humidified air) or to humidified air (control). We found that gingival tissue was more sensitive to CS exposure than the other tissues based on cell viability assay. Secondly, all tissues were exposed in parallel to two doses of whole CS (10% and 16% - doses inducing less than 20% cell death) or to humidified air and various endpoints (e.g., gene and microRNA expression, CYP activity, pro-inflammatory markers release, differential cell counts, cytotoxicity measurement) were captured at different post-exposure times.

Conclusion:

The systems biology endpoints obtained were analyzed using computational approaches. This allowed the identification of biological perturbations which are expected to be similar to those occurring *in vivo* in smokers. This suggests that these four human *in vitro* tissue cultures when exposed to whole CS may be valuable models which could be used to investigate the impact of CS on the respiratory tract.

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NICEdrug as an integrated computational approach in drug metabolism: an application on antiplatelet drugs

***Aiketerini Zisaki**, Noushin Hadadi, Keng Cher Soh, Marianne Seijo, Vassily Hatzimanikatis*
Laboratory of Computational Systems Biotechnology, EPFL | École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

Objective:

We present the development of NICEdrug (Network Integrated Explorer for Drug Metabolism), a computational framework for prediction of drug biodegradation and metabolism. In this report we applied the NICEdrug framework on the metabolism of antiplatelet drugs (clopidrogel and prasugrel) in order to: (i) identify how differential metabolism of these drugs can affect their therapeutic efficacy, (ii) identify possible toxic intermediates in their metabolism.

Methods:

NICEdrug is based on concepts from the BNICE (Biochemical Network Integrated Computational Explorer) methodology. BNICE is a computational network generation method based on a set of enzyme reaction rules and a set of starting compounds to generate every possible biochemical reaction. In the NICEdrug project, we developed biochemical reaction rules based on all known enzymes participating in Phase I and Phase II drug metabolism. By using a pathway search algorithm we were able to derive novel metabolic routes based on the individual reactions, and also performed thermodynamic analysis to establish the feasibility of all derived pathways. The framework also identified all the possible metabolites that are expected based on the Phase I and Phase II biotransformation reaction rules. These metabolites can be then evaluated for potential adverse effects according to their chemical structure, based on existing chemoinformatics and computational methods.

Results:

NICEdrug was able to reproduce the metabolism of both antiplatelet drugs and moreover it also predicted the participation of alternative enzymes that were not part of the “traditional” hypothesis regarding their metabolism. The analysis of all possible and thermodynamically feasible derived pathways allowed us to confirm these results with novel experimental and clinical data for clopidogrel and prasugrel metabolism. As NICEdrug reproduced all the possible metabolites through all the possible metabolic pathways, it allows us to estimate their potential toxic effects.

Conclusion:

In this project we describe the NICEdrug as a powerful method for the study of drug metabolism. The method can have broad use for research in translational medicine and Systems Toxicology.

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Discovering biomarkers of acute dioxin poisoning from metabolomic data: the outstanding case of Victor Yushchenko

Fabienne Jeanneret[1,2,3], *Julien Boccard*[3], *Olivier Sorg*[1], *Jean-Hilaire Saurat*[1], *Denis Hochstrasser*[2,4], *Serge Rudaz*[1,3]
[1]*Swiss Centre for Applied Human Toxicology, Switzerland,* [2]*Biomedical Proteomics Research Group, University of Geneva, Switzerland,* [3]*School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Switzerland,* [4]*Geneva University Hospitals, Geneva, Switzerland.*

Objective:

As there is currently no reliable human biomarker of dioxin intoxication, a metabolomic strategy was carried out for the investigation of human urine samples. The extreme phenotype of Victor Yushchenko (VY) was studied to allow an easier detection of the characteristic metabolic pattern of acute dioxin poisoning.

Methods:

Urine samples collected from VY at different time points after intoxication were analyzed by ultra-high pressure liquid chromatography coupled to quadrupole time-of-flight mass spectrometry and compared to healthy volunteers [1, 2].

Results:

Initial analyses highlighted changes related to the various drug therapies which VY received to treat clinical complications during the years following poisoning. Because previous clinical analyses had indicated abnormal steroid levels, a filtering strategy was performed to detect metabolic alterations within this specific class of compounds. This approach removed the interferences of exogenous structures, and the resulting models still differentiated VY urine samples from healthy controls. Modified metabolic pattern of several steroids and putative bile acids were revealed. Metabolites such as DHEAS and androsterone-glucuronide were confirmed with authentic standards, but the majority remain under investigation for unambiguous identification.

Conclusion:

Metabolomics and data analysis including chemical and phenotypic information were combined to highlight relevant dioxin toxicity biomarkers. This methodology will be applied to other cases of dioxin exposure for a better understanding of the effects of this toxicant.

[1] Badoud, F. et al. *Quantification of glucuronidated and sulfated steroids in human urine by ultra-high pressure liquid chromatography quadrupole time-of-flight mass spectrometry.* Anal Bioanal Chem. 400, 503-516 (2011).
[2] Boccard, J. et al. *A steroidomic approach for biomarkers discovery in doping control.* Forensic Sci Int. 213, 85-94 (2011).

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Metabolomics applied to dioxin intoxication: biologically-driven strategy for the discovery of biomarkers in human urine

*Julien Boccard[1,2], **Fabienne Jeanneret**[1,3,4], Olivier Sorg[3], Jean-Hilaire Saurat[3], Daniela Pelclova[5], Douglas N. Rutledge[2], Denis Hochstrasser[4,6], Serge Rudaz[1,3]*

[1]School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Switzerland, [2]Laboratoire de Chimie Analytique, AgroParisTech, France, [3]Swiss Centre for Applied Human Toxicology, Switzerland, [4]Biomedical Proteomics Research Group, University of Geneva, Switzerland, [5]Charles University Prague, First Faculty of Medicine and General University Hospital, Czech, [6]Geneva University Hospitals, Geneva, Switzeland

Objective:

Untargeted metabolomic approaches offer new opportunities for a deeper understanding of the molecular events related to toxic exposure. This study was a metabolomic investigation of biochemical alterations in urine as a result of dioxin toxicity.

Methods:

Urine samples were collected from Czech workers with substantial dioxin occupational exposure in the late 1960's. Experiments were carried out with ultra-high pressure liquid chromatography coupled to quadrupole time-of-flight mass spectrometry, followed by a biologically-driven feature selection focusing on steroid-related metabolites [1].

Results:

Supervised multivariate data analysis allowed biomarkers, mainly related to bile acids, to be highlighted in the Czech cohort. These results supported the hypothesis of liver damage and oxidative stress from long-term dioxin toxicity. Additionally, the urine analysis of Victor Yushchenko (extreme poisoning phenotype [2]) resulted in a subset of 24 relevant urinary markers of acute dioxin toxicity including glucuro- and sulfoconjugated endogenous steroid metabolites and bile acids. This subset of biomarkers was further used to characterize the Czech cohort. No false negative were found and a specificity of 81.8% was obtained. Extreme phenotype analysis was demonstrated as a very effective approach for biologically-driven data dimensionality reduction and toxicity biomarkers discovery.

Conclusion:

The metabolomic strategy presented in this work investigated urinary metabolic patterns related to dioxin intoxication and allowed the discovery of a highly predictive subset of biologically relevant compounds, providing valuable information for the detection of dioxin toxicity.

[1] Boccard, J. et al. A steroidomic approach for biomarkers discovery in doping control. Forensic Sci Int. 213, 85-94 (2011).
[2] Sorg, O. et al. 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) poisoning in Victor Yushchenko: identification and measurement of TCDD metabolites. Lancet 374, 1179-1185 (2009).

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diXa — Data infrastructure for chemical safety

Hans Gmuender, *Timo Wittenberger**,** Scientific Consulting, Genedata AG, Basel, Switzerland; Vera Matser, Stathis A. Kanterakis, EBI, Hinxton, Cambridge, UK; Jos Kleinjans, University of Maastricht, Netherlands;*

Objective:

The diXa project aims to support the Toxicogenomics Research Community in replacing animal-based test models by robust, non-animal assays *in vitro/in silico* which better predict human toxicity *in vivo*, are less costly, and are socially better acceptable. The general objective of the project is to develop and adopt a robust, sustainable and openly accessible data infrastructure for data sets produced by past, current and future EU research projects on developing non-animal tests for predicting chemical safety.

Methods:

Data warehousing relates to retrieving all raw and processed data with their contextual information (sample characteristics, technologies used, type of measurements, etc) from relevant EU toxicogenomics projects, to centrally store these data in a standardized and sustainable way, to assess the quality of the data, and to perform data normalization procedures. The chemicals data base portal will create a portal for accessing existing chemical/toxicological data bases, deemed relevant and of sufficient quality. Using human diseases data bases a common portal will be created for holding molecular signatures of human diseases, thus allowing for meta-analyses connecting 'omics responses induced by exposures to toxic chemicals to molecular data on human diseases. Integrative statistical analyses will be performed at various levels of complexity exploiting data from different studies, different cellular models, and different analytical platforms. Computational modeling will exploit molecular pathways of liver toxicity to develop computational models for repeated dose liver toxicity.

Results:

Genedata, as diXa partner, will perform cross-platform integrative uni- and multivariate statistical analyses and cross-study meta-analyses, and will deliver commonly agreed core service support, by providing SOPs for seamless data sharing, and by offering quality assessments and newly developed tools and techniques for data management.

Conclusion:

The diXa project will thus create a scientific infrastructure for researchers in the domain of finding alternatives to current animal-based test models for chemical safety.

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Mapping Pathways of Toxicity using Weighted Gene Correlation Networks

Alexandra Maertens, Thomas Luechtefeld, André Kleensang, Thomas Hartung
Johns Hopkins Bloomberg School of Public Health, Centre for Alternatives to Animal Testing, Baltimore MD, USA

Objective:

In order to detect signatures of toxicity and extract networks from -omics data, typically many data sets from different studies are combined. Combining studies has the potential to increase power and improve robustness, detecting network connections not obvious from a single study - a problem that is especially critical in toxicity studies when the changes in gene expression are subtle if the dose used is reflective of human exposures. Microarrays are intrinsically noisy and often show poor reproducibility - a recent meta-analysis of data sets that involved highly similar protocols showed no overlap in significantly differentially expressed genes between the two independent data sets [1]. Here we present a method that provides both a metric of comparability of data-sets and a way to combine datasets that looks for conserved modules without relying on lists of differentially expressed genes.

Methods:

Microarray data were selected based on inclusion in meta-analysis studies. We used both ranked expression levels and ranked connectivity as metrics to determine the similarity of the data-sets. Networks were built with using a Weighted Gene Correlation Network (WGCN), which uses strength of the correlation between any two genes to determine the strength of the network connections [2].

Results:

We show that studies with similar protocols have different networks owing to factors that are not obvious from looking at the differentially regulated genes. Furthermore, neither a gene-by-gene approach nor a simple correlation network is likely to be useful when combining such studies, but a WGCN uncovers conserved modules between two data-sets even when the connectivity of the genes showed minimal similarity.

Conclusion:

Data-sets should be analyzed for comparability before being combined into a meta-analysis. Furthermore, using a WGCN will uncover conserved modules between data sets that will not be apparent from other methods.

[1] Ochsner, S., et al. "GEMS (Gene Expression MetaSignatures), a Web resource for querying meta-analysis of expression microarray datasets: 17β-estradiol in MCF-7 cells." Cancer Research 69.1 : 23-26 (2009).
[2] Langfelder, H. et al. "WGCNA: an R package for weighted correlation network anlysis.“ BMC Bioinformatics 9.1: 559 (2008).

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MicroRNA expression as a tool to elucidate the effect of cellular exposure to developmental neurotoxicants during differentiation.

***Georgina Harris**, Helena T Hogberg, Suzanne Martos, Tyna Dao, David Pamies, Thomas Hartung, Lena Smirnova*
Center for Alternatives to Animal Testing (CAAT), Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA

This work is aimed at identifying changes in microRNA (miRNA) expression after exposure to neurotoxicants using a human neuronal progenitor mesencephalic cell line (LUHMES). miRNAs are small non-coding RNAs, post-transcriptional regulators of gene expression which play a significant role in developmental timing, cell proliferation and differentiation. LUHMES cells were cultured and differentiated into dopaminergic neurons according to an established protocol [1] with some modifications. Throughout the differentiation process cells were exposed to sub-toxic concentrations of the developmental neurotoxicants Lead Chloride and Valproate. Neuronal marker genes B-TubIII, DAT and TH were used to characterize the differentiation upon exposure using qPCR. Neural specific/enriched miRNAs (mir-124, mir-7, mir-9, mir-132, mir-133b, mir-137 and mir-153) expression was quantified on day 6 and 9 of exposure during differentiation. In parallel, a 3D LUHMES model for neuronal toxicity testing has been developed and characterized to better mimic the *in vivo* response. Both neuronal markers (β-III-Tubulin, DAT and TH), as well as neural specific miRNAs (mir-124, mir-132, mir-133b and mir-137) were strongly induced during neuronal differentiation in 2D and 3D culture conditions. After exposure to neurotoxicants, substance-specific effects were observed. Exposure to Valproate but not Lead Chloride reduced mir-133b while both down-regulated mir-153 and mir-124. For selected neuronal marker genes, VPA significantly down-regulated TH expression as well as slightly induced expression of β-III-Tubulin while Lead Chloride exposure decreased β-III-Tubulin and DAT expression and induced TH expression. We conclude that LUHMES cells are a suitable *in vitro* model to investigate the effect of exposure to developmental neurotoxicants during neuronal differentiation. MicroRNA expression is a promising tool to determine substance-specific effects on neuronal differentiation. Further experiments with more compounds and analysis of miRNAs-mRNA target interactions will facilitate determining the mechanisms of developmental neurotoxicity.

[1] Scholz, D. et al. Rapid, complete and large-scale generation of post-mitotic neurons from the human LUHMES cell line. J Neurochem. 119, 957-71 (2011).

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Toxicity testing in the 21st Century: away from classification and labelling towards a probabilistic risk assessment using integrated testing strategies derived from complex probabilistic models

Thomas Luechtefeld, *Alexandra Maertens, Andre Kleensang, Thomas Hartung*
Johns Hopkins Bloomberg School of Public Health, Center for Alternatives to Animal Testing, Baltimore, MD, USA

Objective:

Modern toxicology is in the process of undergoing a profound paradigm change: away from animal-based black-box models towards a systems toxicology approach based on high-throughput, *in vitro* testing on human tissue. However, since no single *in vitro* test can provide sufficient information about an adverse effect, this new approach requires using several information sources in a more sophisticated manner than existing test batteries (e.g. genotoxicity), where every positive result is a liability.

The use of Integrated Testing Strategies (ITS) have been proposed to effectively make use of the wide variety of existing data (QSAR, ToxCast, etc) and incorporate new data that can streamline toxicity testing including a fundamental change away from classification and labeling towards probabilistic approaches.

Here we will present two approaches for deriving ITS from flexible complex probabilistic models. Both algorithms aim to make the output of complex models human readable and applicable by regulators and other stakeholders.

Methods:

We developed two algorithms in a framework that can reduce complex probabilistic models such as Random Forests and Bayesian Networks into human readable ITS. We describe a static method where tests are ordered by their independent value of information, and a conditional approach wherein a generative model is used to condition testing on prior test values.

Results:

We use cholinesterase inhibition as an example endpoint and show that the balanced accuracy of both testing methods converge asymptotically to the underlying model's balanced accuracy.

These methods achieve comparable accuracy with fewer tests than the underlying model. We show that conditional testing performs best in terms of accuracy and number of tests.

Conclusion:

Our framework derives ITS from reference datasets to assess a probability of toxicity in a consistent and iterative way, including measures of model robustness and confidence intervals.

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Drug induced protein adducts

Paola Antinori Malaspina[1, 2], Markus Muller[3]; Adelina Acosta Martin[2, 4]; Youssef Daali[4]; Denis Hochstrasser[4]; Pierre Lescuyer[2, 4]; **Alexander Scherl[1, 2]**

[1]Swiss Center of Applied Human Toxicology, Geneva, Switzerland;

[2]Biomedical Proteomics Research Group, Geneva, Switzerland

Objective:

Chemicals from our environment and their reactive metabolites can form adducts on proteins. Common examples are glycated hemoglobin, used as a marker for average blood glucose level, or the acetaminophen (paracetamol) metabolite NAPQI, which forms stable adduct on cysteine residues. Today, targeted assays exist to measure specific adducts created by some of these chemicals. They generally rely on hydrolysis of the adduct itself, or of the amino acids containing the modification. Thus, the nature of the modified protein and the position of adducts are unknown. We propose to analyze adducts on full length peptides from abundant proteins while keeping the information on the amount of modifications and exact sequence localization by tandem mass spectrometry. Thus, we have to develop a toolbox to identify such adducts.

Methods:

To validate the method, we choose the reactive NAPQI metabolite from acetaminophen on albumin and hemoglobin proteins. Briefly, NAPQI was incubated with the proteins prior to digestion with trypsin. Liquid chromatography hyphenated with tandem mass spectrometry was used to acquire tandem mass spectra on modified and non-modified peptides. A library search approach was used to search for similarities between modified and non-modified spectra and thus to identify peptides carrying the NAPQI adduct.

Results:

This approach permitted the identification of several peptides from albumin and hemoglobin modified by NAPQI. Interestingly, with our *in vitro* conditions, adducts were observed as expected on cysteine, but also on tyrosine residues. The approach was thus validated.

Conclusion:

This method will now be used on human blood samples after paracetamol ingestion/intoxication as well as other compounds known to react with proteins such as the anti-cancer drug Busulfan.

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DNA adductomics methodology utilizing high resolution accurate mass MSn technology for analysis of human DNA samples

Peter W. Villalta, Silvia Balbo, and Stephen S. Hecht
Masonic Cancer Center, University of Minnesota, Minneapolis, Minnesota, USA

Objective:

To develop a methodology for the screening of DNA adducts for development of exposure biomarkers and cancer risk markers.

Methods:

DNA hydrolysis produces deoxynucleosides (dR) which are analyzed by data dependent nanospray LC-HR/AM-MSn analysis. Deoxynucleosides universally fragment with loss of the deoxyribose moiety ([M + H – 116.0474]+). Observation of this neutral loss at a mass tolerance of ± 5 ppm indicates DNA adduct identity and triggers MS3 fragmentation which provides structural information. The final output spectra has sufficient mass accuracy for unambiguous adduct molecular formula determinations and extensive fragmentation data identifying the nucleobases and potentially sufficient structural information to determine adduct structure.

Results:

We assembled a test mixture of 18 DNA adducts with modifications of all four nucleobases and at various reactive sites and differing polarities. This mixture was spiked into DNA samples and used to optimize the methodology. Parameters adjusted include isolation width, CID energy, activation Q, activation time, and number of ions fragmented for each full scan analysis with a “Top 3” approach found to be optimal. Processing of the DNA hydrolysates by reverse phase HPLC fractionation was shown to be superior to solid phase extraction. The methodology was tested by analysis of DNA from the liver of a mouse treated with two potent tobacco carcinogens, benzo(a)pyrene and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). MS3 spectra for all known DNA adducts were observed as well as for two isotopically labelled internal standards added prior to sample preparation. Several previously uncharacterized DNA adducts were also observed. Efforts are ongoing to identify the observed uncharacterized adducts as well as to further refine the methodology.

Conclusion:

A method has been developed for observation and identification of previously unknown DNA adduct utilizing accurate mass measurement of MS1-3 spectra to reduce false positives and provide extensive adduct identity information.

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INTEGRATION of RNA-interference screens with high content imaging identifies novel regulators of drug-induced liver injury (DILI)

Suzanna Huppelschoten, Bram Herpers, Lisa Fredriksson, Gulia Benedetti, Zi Di, Hans de Bont, John Meerman, Marjo de Graauw and Bob van de Water, LACDR, Leiden University, Leiden, The Netherlands

Objective:

Identification of signalling modalities that determine the synergy of TNFα and DILI compounds with respect to target gene activation and onset of cytotoxicity.

Methods:

Fluorescent reporter HepG2 cell lines, e.g. RelA, IkBα and ICAM1 were generated to elucidate the dynamics of TNFα-induced NFκB signaling. These reporters were applied in a live-cell imaging-based NFκB nuclear translocation RNAi screen to identify kinases and ubiquitinases that critically determine TNFα/DILI drug synergy. Nuclear oscillation of GFP-tagged p65 (NFκB-subunit) of ~300 cells was simultaneously followed for 6 hrs, using automated confocal laser scanning microscopy combined with ImageJ-based image analysis [1]. The biological relevance of screen hits was evaluated in apoptosis assays.

Results:

TNFα-induced NFκB oscillation was inhibited by DILI drugs, which was associated with reduced expression of ICAM1. To unravel the mechanism of the inhibition of the NF-κB oscillatory response in relation to DILI, we performed an imaging-based functional genomics screen of in total 1571 individual gene knockdowns. This revealed 46 validated kinases and ubiquitinases that affect the NFκB oscillation response under TNFα and/or TNFα/diclofenac conditions. Inhibition of NFκB oscillation is linked to increased cytotoxicity upon TNFα/diclofenac exposure [2]. Knockdown of several validated genes protected against TNFα/diclofenac-induced cell death. Knockdown of these genes was associated with increased levels of A20. Double knockdown conditions of screen hits and A20 attenuated the cytoprotection.

Conclusion:

We identified important regulators of TNFα-induced NFκB signalling that control the outcome of cytotoxicity in the context of DILI. This effect was related to the expression of A20, an ubiquitin editing enzyme and negative feedback regulator of TNF receptor signalling. Our current RNAi-based screening method demonstrates its power in modern systems toxicology.

[1] Di, Z. et al. *Automated analysis of NF-kappaB nuclear translocation kinetics in high-throughput screening*. PLoS One 7, e52337, doi:10.1371/journal.pone.0052337 (2012).

[2] Fredriksson, L. et al. *Diclofenac inhibits tumor necrosis factor-alpha-induced nuclear factor-kappaB activation causing synergistic hepatocyte apoptosis*. Hepatology 53, 2027-2041, doi:10.1002/hep.24314 (2011).

Robert J Kavlock

Robert J. Kavlock, PhD, is the Deputy Assistant Administrator in the U.S. Environmental Protection Agency's (EPA) Office of Research and Development (ORD). In this capacity he is responsible for strategic research planning and product delivery for a research enterprise with 1800 employees and a budget of approximately $600M. He has over 33 years of scientific experience with the EPA and was previously the Director of the National Center for Computational Toxicology (NCCT) within ORD, a post he occupied since its founding in 2005. The ToxCast program within the NCCT is on the leading edge of the state of the science in computational toxicology.

Dr. Kavlock has published more than 200 scientific papers, 16 book chapters, edited three books, including co-editor of the Global Assessment of the State-of-the-Science of Endocrine Disruptors (WHO, 2002) and serves on a number of international scientific advisory committees. He is the co-recipient of the US Human Society North American Alternative Award (2008), ORD's Statesmen of the Year Award (2007), ORDs Science Achievement Award (2012), the Warkany Lecturer at the Teratology Society (2013) and is past president of the Teratology Society.

Dr. Kavlock graduated from the University of Miami in 1973 with a B.S. in Biology. In 1977, he earned a PhD in Biology also from the University of Miami.

Maurice Whelan

Maurice Whelan is head of the Systems Toxicology Unit of the Institute for Health and Consumer Protection (IHCP) of the European Commission's Joint Research Centre (JRC), based in Ispra, Italy. He is also head of the European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM), hosted by the IHCP-JRC. The focus of his Unit and ECVAM is on the development, evaluation and promotion of new integrated methods for the safety assessment of chemicals and nanomaterials that do not require testing on animals. The premise underpinning his approach is that advancement of safety assessment science requires a shift to a knowledge-based paradigm built on the understanding of toxicological mode-of-action to guide systems biology based solutions. Whelan is co-chair of the OECD Advisory Group on Molecular Screening and Toxicogenomics that is responsible for the OECD programme on Adverse Outcome Pathways and is a member of the Steering Committee of the European Partnership for Alternative Approaches to Animal Testing (EPAA – see http://ec.europa.eu/enterprise/epaa/). He is also involved in the scientific coordination of the major EU programme on alternative approaches to repeat does systemic toxicity – SEURAT-1 (www.seurat-1.eu).

Thomas Hartung

Thomas Hartung, MD PhD, is Professor of Toxicology (Chair for Evidence-based Toxicology), Pharmacology, Molecular Microbiology and Immunology at Johns Hopkins Bloomberg School of Public Health, Baltimore, and University of Konstanz, Germany; he also is Director of their Centers for Alternatives to Animal Testing with the portal AltWeb. CAAT hosts the secretariat of the Evidence-based Toxicology Collaboration and the industry refinement working group. As PI, he heads the Human Toxome project funded as an NIH Transformative Research Grant. He is the former Head of the European Center for the Validation of Alternative Methods (ECVAM), Ispra, Italy. He has authored more than 370 scientific publications.

Kevin Crofton

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 PhD 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 developmental neurotoxicity, adverse outcome pathways, cumulative risk of thyroid disruptors, and development of

alternative testing methods. His current research efforts include development of *in vitro* and alternative methods for detecting thyroid disrupting chemicals. 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 130 peer reviewed publications.

Hennicke Kamp

Dr. rer. nat. Hennicke G. Kamp (food Chemist and certified toxicologist DGPT, ERT) was born in 1974 and studied food chemistry from 1995 to 2000. After preparing a PhD thesis in food toxicology on the genotoxic/carcinogenic action of the mycotoxin Ochratoxin under supervision of Prof. G. Eisenbrand, he joined BASF SE as a toxicologist in 2004. He was responsible for setting up a laboratory for the development of alternative methods and thereafter became head of the laboratory for mechanistic toxicology and project leader for the BASF internal metabolomics in toxicology project, who he still is. From 2008 to 2010 he was consulting toxicologist at metanomics GmbH, Berlin. Since 2010, he is leading a group in the experimental toxicology and ecology department of BASF SE, responsible for the management of contract work including monitoring, controlling and logistic functions within the department. In 2012, he and his team were awarded the BASF innovation award.

François Pognan

Since 1993, François Pognan's entire focus has been the understanding of drug-induced toxicity mechanisms using a variety of tools, ranging from *in vitro* biochemistry to *in vivo* investigative studies. He has a PhD in Molecular Oncogenesis from University Pierre and Marie Curie in Paris, and after a post-doc in Rhone-Poulenc-Rorer, he has held various mechanistic toxicology positions in Pfizer-France, AstraZeneca UK & USA, and is now based in Novartis-Pharma in Switzerland as Executive Director in Pre-Clinical Safety. Currently, he is also coordinating one of the European IMI consortia: 'eTox – Expert Systems for *in silico* Drug Toxicity Prediction', regrouping 25 public and private institutions.

Thomas Singer

Thomas Singer is the Head of Non-Clinical Safety and Translational Technologies and Bioinformatics for Pharma Research and Early Development at F. Hoffmann-La Roche and responsible for the scientific integrity of decision-making, regulatory documentation submissions and the on-market support of Roche compounds. Thomas is strongly committed to optimizing knowledge exchange and cooperation to create, and foster, a strong global identity of the company's various safety departments. Thomas is a great protagonist of animal replacement assays using human tissue to markedly lower attrition which underlines his concern for the 3Rs and his dedicated commitment to animal welfare. Thomas is a Doctor of Veterinary Medicine, and holds board certifications in pharmacology, toxicology, animal welfare science and clinical pathology and thus possesses a profound professional experience in non-clinical safety assessment and a comprehensive knowledge of experimental toxicology; he received the status of a Diplomat of the American Board of Toxicology (DABT) and served this organization for many years as its European Liaison Officer. In addition to these responsibilities, Thomas is heavily engaged in cooperation of the pharmaceutical industry with the European Commission and is a lecturer in pharmacology and toxicology at the Medical School Frankfurt University, Germany.

Shana Sturla

Shana Sturla is Professor of Food & Nutrition Toxicology in the Department of Health Science and Technology at the ETH Zurich. She earned a degree in Chemistry from the University of California at Berkeley in 1996 and a PhD in Organic Chemistry from the Massachusetts Institute of Technology in 2001. Following postdoctoral research in Chemical Carcinogenesis and Chemoprevention at the University of Minnesota Cancer Center under the mentorship of Professor Stephen Hecht, she joined

the faculty of the University of Minnesota. Professor Sturla is the recipient of a European Research Council Starting Independent Researcher Grant. The goal of her research is to understand how chemicals such as diet-derived natural products impact disease incidence and treatment. Research strategies involve investigating relationships between chemical structure, biotransformation and cellular responses. Professor Sturla leads an ongoing Swiss National Science Foundation Sinergia project regarding systems wide responses of colon cells to food components and the impact on cancer drug action that integrates research in chemical toxicology, molecular biology, systems biology and computational biology.

Robert Turesky

Rob Turesky received a B.S. degree in Biochemistry from the University of Massachusetts, Amherst, in 1978. He earned a PhD in the Department of Nutrition and Food Science at the Massachusetts Institute of Technology, under the mentorship of Steven Tannenbaum. Thereafter, he worked for 14 years (1986 - 2000) at the Nestlé Research Center, vers-chez-les-Blanc, in Switzerland, conducting research on the biochemistry of dietary genotoxicants, and advising the company of the safety of food processing methods. Thereafter, he served as Division Director of Chemistry at the National Center for Toxicological Research, in Arkansas. Since 2004, he has been a principal research investigator at the Wadsworth Center, NYS Department of Health, Albany, NY. His research interest is focused on the biotransformation of dietary and environmental genotoxicants into reactive electrophiles, which cause DNA damage and protein adduct formation. A long-term goal is developing bioanalytical tools, primarily by mass spectrometric techniques, to measure biomarkers of dietary and environmental genotoxicants in human population studies for assessing cancer risk.

Joost van Delft

After an MSc in biology and a PhD in Biochemistry at the University of Leiden, Dr. van Delft joined the Toxicology Division of TNO Nutrition and Food Research Institute in 1988. His research focused on the analyses of DNA adducts and their mutagenic effects, combined with regulatory genotoxicity testing. From 1998 Dr. van Delft is a registered toxicologist for the Dutch and European Societies of Toxicology. Since 1999 he is Associate Professor at the Department of Toxicogenomics of Maastricht University, The Netherlands. His research interests are in unraveling environment-gene interactions involved in chemical (anti)carcinogenesis, thereby focusing on regulations at the genome level. Currently, his main focus is on modulation of omics profiles by chemicals in order to unravel their mechanisms of action, to apply omics technologies for risk assessment and classification of chemical carcinogens, and to develop new biomarkers for molecular epidemiology studies of human populations. Within various multicenter (inter)national projects (from the Netherlands Toxicogenomics Centre, and the EU FP6/7 Integrated Projects carcinoGENOMICS, diXa and Exposomics, and CEFIC-LRI project DECO) he leads activities on toxicogenomics in cancer hazard and hepatotoxicity assessment. Currently, he leads a team of 4 PostDocs, 3 PhD students and 4 technicians. He is frequently invited as speaker at (inter)national scientific meetings and is author and co-author of over 110 peer-reviewed papers in international journals.

Nigel Skinner

Dr Nigel Skinner holds the position of Global Director of Segment Marketing, Life Science Group, at Agilent Technologies, a leading supplier of analytical instruments and consumables for life science and clinical research. In this role, Nigel is the driving force behind the company's marketing of integrated biology solutions for disease research and toxicology in academic, government and pharmaceutical industry labs worldwide. He has extensive senior management experience in the United States, Switzerland and Britain at a number of respected corporations, including General Electric and Invitrogen, in addition to a senior executive position at CSEM in Neuchatel, Switzerland. Nigel holds both a PhD in Biotechnology from Cambridge University, and an MBA focusing in Technology Management from Open Business School. He previously obtained his M.Eng. from McMaster University, Hamilton, Canada. Nigel has published the DMD Market Report High Density

Multiplexed Assays, is the author of over 30 publications in peer-reviewed journals, and holds eight patents.

Ioannis Xenarios
Ioannis Xenarios is the Director of Vital-IT Group in Lausanne as well as the Swiss-Prot Group in Geneva. He received a Ph.D in immunology at the Ludwig Institute of Cancer Research and the Institute of Biochemistry. He worked on the development of the Database of Interacting Proteins (DIP) under the supervision of Prof. David Eisenberg at the University of California Los Angeles. He then became the head of Translational Bioinformatics at Serono (now Merck Serono) where his group developed computational methodologies in the area of proteomics, microarray and genetics. He is one of the Principal investigators of the ENFIN project aiming at providing methods in dynamical systems modeling. Ioannis Xenarios is UNIL full Professor *ad personam*, affiliated with the CIG, since August 2010

Gustavo Stolovitzky
Gustavo Stolovitzky received his MSc in Physics from the University of Buenos Aires (1987) and his PhD in Mechanical Engineering from Yale University (1994). In 1998 he joined the IBM Computational Biology center at IBM Research where he is the manager of the IBM Functional Genomics & System Biology Group. He holds a position as an adjunct Associate Professor at Columbia University. His most recent scientific interests are in the field of high-throughput biological-data analysis, reverse engineering biological circuits, the mathematical modeling of biological processes and new generation technologies for DNA sequencing.

Manuel Peitsch
Manuel Peitsch is Vice President for Biological Systems Research with Philip Morris International R&D. Manuel joined PMI R&D from Novartis where he spent seven years and successively led "Informatics and Knowledge Management" and "Systems Biology". Prior to joining Novartis in 2001, Manuel held several leadership positions in bioinformatics, scientific computing and knowledge management with GlaxoWellcome and GlaxoSmithKline.
Manuel is a co-founder of the Swiss Institute of Bioinformatics and later played a key role in extending this institute to Basel. In 2003 he co-founded the SwissBioGRID.
Manuel obtained his PhD in biochemistry from the University of Lausanne (Switzerland) and spent his post-doctoral years at the National Cancer Institute (Dr. J.V. Maizel Jr.) and the University of Lausanne (Prof. J. Tschopp). Since 2002 he is Professor for Bioinformatics at the University of Basel.

Julia Hoeng
Julia Hoeng PhD: Julia Hoeng is Manager of Computational Disease Biology at PMI Research & Development where she leads the Systems Biology Program, covering a portfolio of projects from *in vitro*, *in vivo* and *in silico* research for product testing. Julia has established her team with systems biology excellence at PMI Research & Development over the last five years and she is the PMI project leader of the Systems Biology Verification project. She holds a PhD and Post-doc from Cambridge University and a MS in Bioinformatics from the Georgia Institute of Technology, Atlanta, Georgia, USA. Julia has published numerous articles and book chapters highlighting the use of systems biology approaches for toxicology.

Thomas Paterson
Thomas Paterson, a co-founder of Entelos, leads research methodologies and product development for Entelos's core predictive biosimulation systems. He has pioneered the concepts of Hypothesis Quality and Hypothesis Management to bridge the domains of biomedical research, risk assessment, and business decision making, and is the lead inventor on 14 related patents. Mr. Paterson has been the lead architect on many of Entelos' PhysioLab® platforms in such diverse areas as cholesterol metabolism, atherosclerosis, erythropoiesis, asthma, viral infection, adaptive immunity, and drug-

induced liver injury. He has worked in the pharmaceutical industry for 18 years and has over 24 years of experience in the development and application of simulation-based decision support systems. Mr. Paterson has held technical and management positions at Strategic Decisions Group, GTE Government Systems, and the Institute for Defense Analyses. He received a Bachelors Degree in Aeronautics and Astronautics from the Massachusetts Institute of Technology, and an M.S. in Decision Analysis from Stanford University.

Stephen Rappaport

Dr. Stephen M. Rappaport is Professor of Environmental Health at the University of California, Berkeley. He is a pioneer in the emerging field of 'Exposure Biology' and a prominent advocate of the concept of the 'Exposome' as a new paradigm for environmental health. Much of his current research involves the development and application of blood protein adducts as biomarkers of exposure to toxic chemicals arising from inhalation, ingestion, and endogenous processes. This has led to the concept of the 'Protein Adductome', representing signatures of exposures to toxic chemicals. By comparing adductomes across populations, Prof. Rappaport hopes to identify important biomarkers of chronic diseases. He has also used environmental measurements and biomarkers to elucidate the human metabolism of several toxic chemicals, notably benzene, and to quantify interindividual variability in biomarker levels due to genetic, environmental and lifestyle factors.

Alan Boobis

Alan Boobis obtained his PhD at the University of Glasgow in 1974 and was then a post-doctoral fellow at NIH, USA. In 1976 he moved to what became part of Imperial College London, where he is professor of Biochemical Pharmacology and director of the Health Protection Agency Toxicology Unit. His research interests include drug metabolism, chemical carcinogenesis and mechanisms of toxicity. He has published over 220 original papers. He is a member of several national and international expert advisory committees on chemical risk assessment. He obtained an OBE in 2003 for his work on the risk assessment of pesticides.

Ila Cote

Dr. Ila Cote is currently the Senior Science Advisor in EPA's National Center for Environmental Assessment, and the director of the Next Generation of Risk Assessment Program (NexGen). NexGen is aimed at improving environmental risk assessments by incorporating recent advances in molecular biology. Her expertise is in environmental risk assessment, and the interface of science and public policy. She is a board certified toxicologist and has just completed a sabbatical at the University of Colorado Department of Molecular, Cellular and Developmental Biology.

Marcel Leist

Marcel Leist, PhD, is Director of the Center for Alternatives to Animal Testing in Europe (CAAT-Europe) and full Professor at the University of Konstanz (D). He holds an MSc in Toxicology from the University of Surrey (UK) and a PhD in Biochemical Pharmacology. After an assignment as assistant/associate professor of Toxicology at the University of Konstanz (Konstanz, D) from 1995-2000, he worked on a broad range of toxicological, immunological and pharmacological projects in the mid-size (2 billion annual turnover) pharmaceutical company H. Lundbeck A/S (Copenhagen, DK) as Head of Department/Senior Director. Since 2006, he has held the endowed Doerenkamp-Zbinden chair for *in vitro* toxicology and biomedicine at the University of Konstanz (D). The research concentrates on neurotoxicity, and on *in vitro* models of developmental neurotoxicity toxicity. Moreover, he is a specialist for the use of human stem cells in toxicology, for high content screens and for genome-wide analysis of toxicity pathways. His work, cited more than 11,000 times, was published in 150 peer-reviewed papers, and he is a member of several editorial and scientific advisory boards.

Participants List

Name	Organisation	Country
Lothar Aicher	Swiss Centre for Applied Human Toxicology (SCAHT)	Switzerland
Paola Antinori Malaspina	University of Geneva	Switzerland
Alan Boobis	Imperial College London	UK
Beat Brüschweiler	Swiss Federal Office of Public Health	Switzerland
Timo Buetler	University of Zurich	Switzerland
Doris Bühler	Swiss Federal Office for Agriculture	Switzerland
Simona Costantinescu	ETH Zurich	Switzerland
Kevin Crofton	US Environmental Protection Agency	USA
Mardas Daneshian	University of Konstanz	Germany
Barbara Engeli	Swiss Federal Office of Public Health	Switzerland
Melanie Erzinger	ETH Zurich	Switzerland
Rex FitzGerald	Swiss Centre for Applied Human Toxicology (SCAHT)	Switzerland
Hans Gmuender	Genedata AG	Switzerland
Georgina Harris	Johns Hopkins Bloomberg School of Public Health	USA
Thomas Hartung	Johns Hopkins Bloomberg School of Public Health	USA
A. Wallace Hayes	Harvard University School of Public Health	USA
Katrin Hecht	ETH Zurich	Switzerland
Julia Hoeng	Philip Morris International	Switzerland
Suzanna Huppelschoten	Leiden University	The Netherlands
Danielle Jannuzzi Madureira	Eawag / ETH Zurich	Switzerland
Fabienne Jeanneret	University of Geneva	Switzerland
Hennicke Kamp	BASF SE	Germany
Robert Kavlock	US Environmental Protection Agency	USA
Marcel Leist	University of Konstanz	Germany
Walter Lichtensteiger	University of Zurich	Switzerland
Thomas Luechtefeld	Johns Hopkins Bloomberg School of Public Health	USA
Alexandra Maertens	Johns Hopkins Bloomberg School of Public Health	USA
Petros Malitas	IPQ Analytics	Switzerland
Carole Mathis	Philip Morris International	Switzerland
Thomas Paterson	Entelos	USA
Manuel Peitsch	SIB Swiss Institute of Bioinformatics /Philip Morris International	Switzerland
Smitha Pillai	Eawag	Switzerland
Francois Pognan	Novartis	Switzerland
Ans Punt	Wageningen University	The Netherlands
Stephen Rappaport	University of California, Berkeley	USA
Matthias Roesslein	Empa	Switzerland
Natalia Root	Sigma Aldrich	Switzerland
Nicolas Roth	University of Basel	Switzerland
Serge Rudaz	University of Geneva	Switzerland
Jenny Sandström von Tobel	University of Lausanne	Switzerland
Benoit Schilter	Nestlé Research Center	Switzerland
Kristin Schirmer	Eawag	Switzerland
Margret Schlumpf	University of Zurich	Switzerland
Thomas Singer	F. Hoffmann-La Roche Ltd	Switzerland
Nigel Skinner	Agilent Technologies	UK
Holger Stalz	Agilent Technologies	Switzerland
Gustavo Stolovitzky	IBM Research	USA
Luc Stoppini	Hepia University of Applied Sciences	Switzerland
Alessia Stornetta	ETH Zurich	Switzerland
Shana J. Sturla	ETH Zurich	Switzerland
Anette Thiel	DSM Nutritional Products AG	Switzerland

Participants List (continued)

Name	Organisation	Country
Florianne Tschudi-Monnet	University of Lausanne	Switzerland
Robert Turesky	Wadsworth Center	USA
Joost van Delft	Maastricht University	The Netherlands
Wim Van den Hof	Maastricht University	The Netherlands
Peter Villalta	University of Minnesota	USA
Maurice Whelan	European Commission Joint Research Centre	Italy
Martin Wilks	Swiss Centre for Applied Human Toxicology (SCAHT)	Switzerland
Ioannis Xenarios	SIB Swiss Institute of Bioinformatics /University of Lausanne	Switzerland
Aikaterini Zisaki	EPF Lausanne	Switzerland

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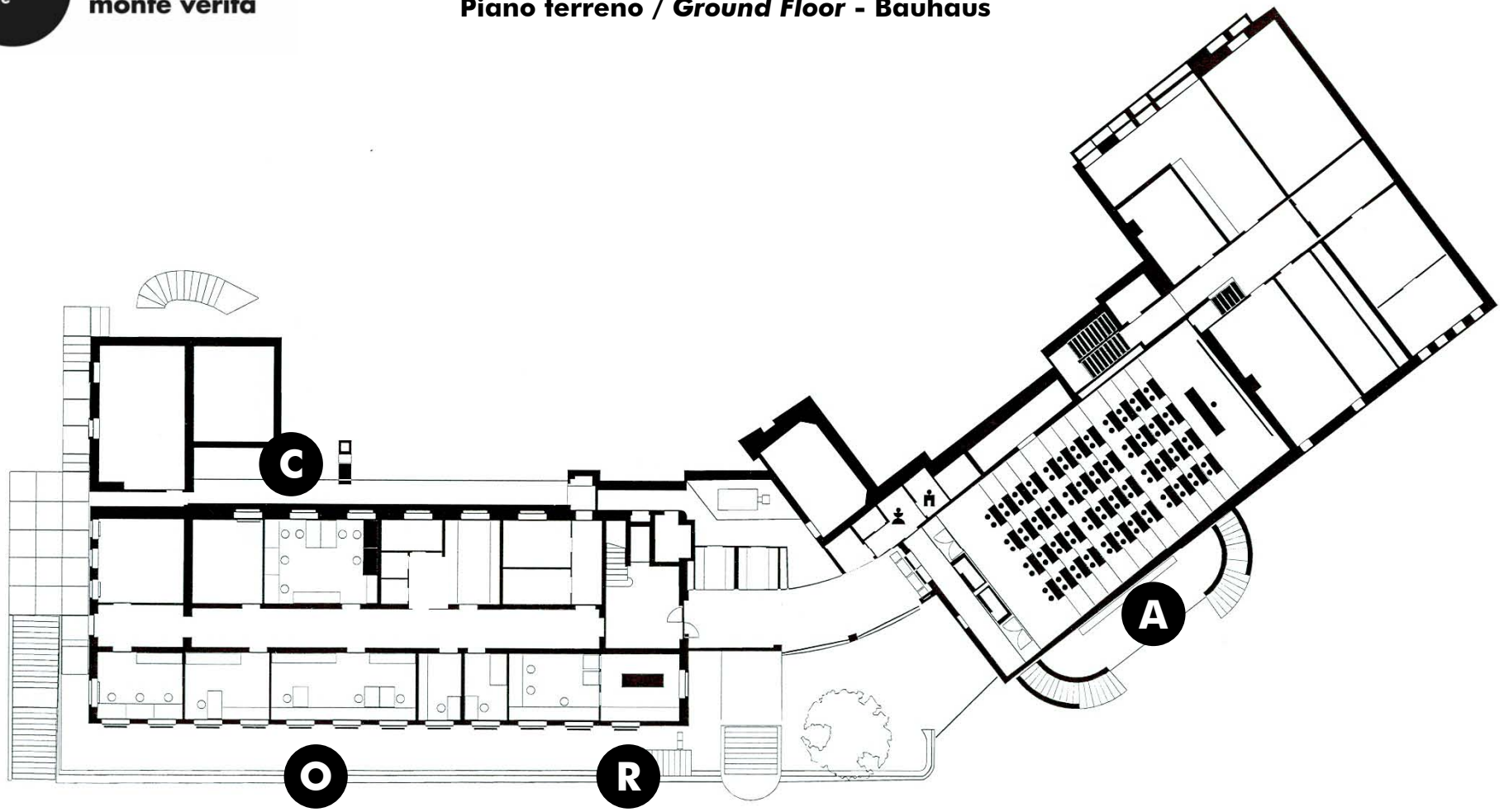

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C Computer-Room

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O Ufficio congressi / *Conference Office*

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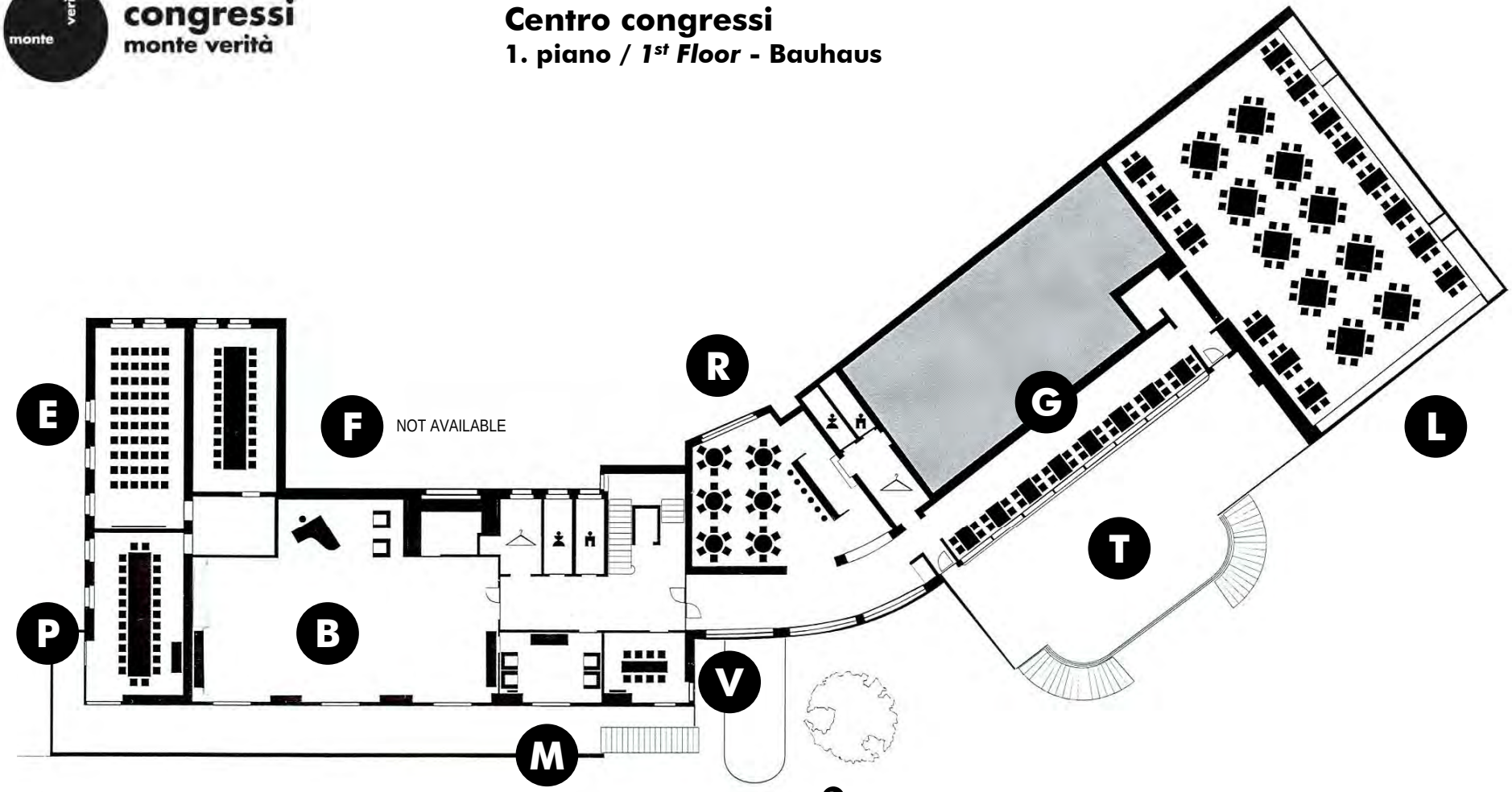

- B Sala Balint
- P Sala Pioda
- E Sala Eranos
- F Sala Fahrenkamp NOT AVAILABLE
- M Sala Mandala
- V Sala Von der Heydt

- L Sala Luce
- G Galleria del Barone
- R Bar Roccia
- T Terrazza panoramica
  Panoramic Terrace