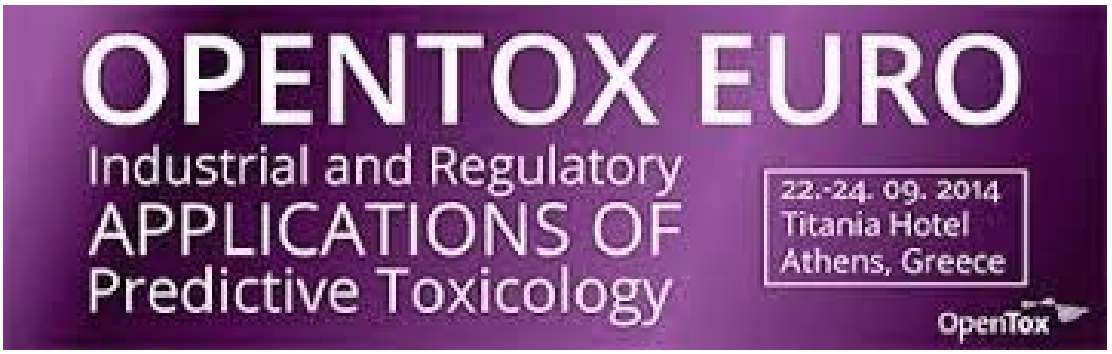
OpenTox



Scientific program & abstracts

MONDAY, SEPTEMBER 22

8:30-8.45 **Chair's Introduction**

8:45-10:15 **Session 1 Modeling Metabolism and Reactivity**

Chaired by Philip Judson, Lhasa Limited

8:45-9:15 **Refining Concrete Cascade Networks to Explain Acetaminophen Hepatotoxicity Phenomena Within and Across Multiple Scales**
Anthony Hunt[1], Andrew Smith[1], Glen Ropella[2]
[1]Bioengineering and Therapeutic Sciences, University of California, San Francisco [2]Tempus Dictum, Inc.

9:15-9:45 **Assessing the performance of systems that predict metabolism**
Philip Judson, Lhasa Limited

9:45-10:15 **PBTK/TD modelling for predicting mammalian toxicokinetics and toxicodynamics**
Mohammed I. Atari, Prakash Patel and Simon Thomas, Cyprotex Discovery Limited

10:15-10:45 ***Coffee Break***

10.45-12:30 **Session 2 (Part I) Assessing the Safety of Nanotechnology**

Chaired by David Carlander, Nanotechnology Industries Association (NIA)

10:45-11:00 **Session introduction and NANoREG presentation**
David Carlander, Nanotechnology Industries Association (NIA)

11:00-11:30 **Nanomaterials: Lessons learned and testing the regulatory approach**
Keld Alstrup Jensen [1] and Steffi Friedrichs [2]
[1] National Research Centre for the Working Environment, Denmark
[2] Nanotechnology Industries Association

11:30-12:00 **NM Series of Representative Nanomaterials for harmonized Safety Assessment**
Kerstin Hund-Rinke, Karlheinz Weinfurtner
Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Schmallenberg, Germany

12:00-12:30 **Towards a harmonized Safety Assessment of Nanomaterials**
Carol Aristimuño, Felipe Goñi de Cerio, Pedro Heredia, Blanca Suarez-Merino
GAIKER Technology Center, Spain.

12:30-13:30 **Lunch at Olive Garden restaurant**

13:30-15:00 **Session 2 (Part II) Assessing the Safety of Nanotechnology**
Chaired by David Carlander, Nanotechnology Industries Association (NIA)

13:30-14:00 **Standardized In vitro high-throughput and high-content analyses serve efficiently to broadly assess nanomaterials safety and influences of different dispersion and testing protocols**
Roland Grafström[1,2] and Vesa Hongisto[1]
[1]Health R&D, Knowledge Intensive Products and Services, VTT Technical Research Centre of Finland, Turku, Finland [2]Institute of Environmental Medicine, Karolinska Institutet, Stockholm,Sweden

14:00-14:30 **Nanoinformatics Study in South Korea for the developments of Safe and Sustainable Nanotechnology**
Tae Hyun Yoon, Hanyang University

14:30- 15:00 **Concluding panel discussion**
David Carlander, Nanotechnology Industries Association (NIA)

15:00-15:30 ***Coffee Break***

15:30-17:00 **Knowledge Café**

17:00-18:00 **Poster session**

- **An in silico model for the identification of small molecules for the treatment of thalassaemia**

Antreas Afantitis
Georgia Melagraki *NovaMechanics Ltd*

- **Comparison Analysis Of Toxicity Evaluation Algorithms Using Frequently Used Drugs**

Yu Ri An [1]
Jae Young Kim [1] Seung Hun Baek [2] Yang-Seok Kim[2,3]
[1] Daewoong Co.LTD, Korea
[2] Bio-age Co.LTD., Korea
[3] College of Oriental Medicine, Seoul, Korea

▪ **Identification Of Molecular Signatures Of Inflammation-mediated Organ Toxicity**

Yu Ri An [1]
Jae Young Kim [1] Seung Hun Baek [2] Yang-Seok Kim[2,3]

[1] Daewoong Co.LTD, Korea
[2] Bio-age Co.LTD., Korea
[3] College of Oriental Medicine, Seoul, Korea

▪ **A web-based software tool for individualized drug dosing using PBPK models and control theory**

Sotiris Douskas [1], Pantelis Sopasakis [1,2], Haralambos Sarimveis[1]

[1] National Technical University of Athens
[2] IMT Lucca

▪ **Predictive Toxicology Tools Application in Contaminated Sites Health Risk Assessment and Remediation**

Barry Hardy[1]
Asish Mohapatra[2]

[1]Douglas Connect
[2]Health Canada

▪ **A ToxBank Integrated Data Analysis of SEURAT-1 Reference Compounds**

Barry Hardy [1], Glenn Myatt [2], Pekka Kohonen [3], Roland Grafström [3]

[1] Douglas Connect
[2] Leadscope
[3] Karolinska Institutet

▪ **Evaluation Of Toxicological Biomarkers In Multi-organs Including Liver, Kidney And Aorta Of Rats Exposed To Thioacetamide**

Yoonjin Kim [1], Ji-Youn Lim [1], Kyoung -Sik Moon [2], Yong-Bum Kim [2], Donggeun Sul [1]

[1] Graduate School of Medicine, Korea
[2] Department of Toxicological Evaluation and Research, Korea Institute of Toxicology

▪ **A Set of Predictive Models for Ames Mutagenicity**

Nikolay Kochev [1], Nina Jeliazkova [2], Veselina Paskaleva [1]

[1]University of Plovdiv
[2]Ideaconsult Ltd.

▪ **Response of human bronchial organotypic culture to mainstream cigarette smoke exposure**

R. Kostadinova, C. Mathis, S. Frentzel, D. Kuehn, S. Majeed, C. Merg, A. Elamin, E. Guedj, R. Dulize, Y. Xiang, P. Leroy, M.C. Peitsch, J. Hoeng

Philip Morris International Research and Development

▪ **Comprehensive Gene Expression Analysis Of Mice Liver Treated With Diclofenac**

Eun-Hee Lee, Heeyoung Yang, Jung-Hwa Oh, Se-Myo Park, Mi-Sun Choi, A-young Choi, Soojin Kim, and Seokjoo Yoon

Molecular Toxicology Research Center, Department of Predictive Toxicology, Korea Institute of Toxicology (KIT)

- **Computer aided nanoparticles design through Enalos InSilicoNano Platform**

Georgia Melagraki
Antreas Afantitis — *NovaMechanics Ltd*

- **Evaluation Of In Vitro Toxicity Of Nanoparticles In Inverted 3D Culture Systems**

Soojin Kim, Jung-Hwa Oh, Se-Myo Park, Mi-Sun Choi, A-young Choi, Eun-Hee Lee, Heeyoung Yang and Seokjoo Yoon — *Molecular Toxicology Research Center, Department of Predictive Toxicology, Korea Institute of Toxicology*

- **Human Pluripotent Stem Cell-Derived Hepatocyte as a Useful Model for Detecting Toxic Chemicals Triggering AHR Signaling**

Han-Jin Park
Hye-Min Kim
Seokjoo Yoon — *Korea Institute of Toxicology*

- **Simultaneous Transcriptomic Analysis Of Nephrotoxic Compounds In Different Target Organs**

Se-Myo Park, Mi-Sun Choi, Eun-Hee Lee, Soojin Kim, A-young Choi, Heeyoung Yang, Seokjoo Yoon and Jung-Hwa Oh — *Korea Institute of Toxicology*

- **Fullerenes potentially aggravate atherosclerosis onset by disregulating lipid homeostasis in vascular endothelial cells**

Bart Smeets
Lars Eijssen — *Maastricht University*
Egon Willighagen

- **nanoQSAR modelling using protein corona fingerprints**

Georgia Tsiliki
Haralambos Sarimveis — *National Technical University of Athens*

TUESDAY, SEPTEMBER 23

8:25-10:30 **Session 3 Data Resources and Analysis**

Chaired by Ola Spjuth, Uppsala Universitet

8:25-8:50 **e-Science infrastructures for enabling large-scale predictive modelling in toxicology and pharmacology.**
Ola Spjuth
Department of Pharmaceutical Biosciences and Science for Life Laboratory, Uppsala University, Sweden

8:50- 9:15 **On chemical structures, substances, nanomaterials and data exploration.**
Nina Jeliazkova, Vedrin Jeliazkov
Ideaconsult Ltd.

9:15-9:40 **Open PHACTS: Solutions and the Foundation**
Egon L. Willighagen [1,2], Open PHACTS Consortium[2]
[1] Dept. Bioinformatics - BiGCaT, NUTRIM, Maastricht University

9:40-10:05 **Toxygates - a hybrid linked data and microarray platform for user-friendly toxicogenomics**
Johan Nyström-Persson [1,2], Yoshinobu Igarashi [1], Maori Ito [1], Mizuki Morita [1], Noriyuki Nakatsu [1], Hiroshi Yamada [1], Kazuyoshi Ikeda [2] and Kenji Mizuguchi [1]
[1]National Institute of Biomedical Innovation, Osaka, Japan
[2]Level Five Co. Ltd, Tokyo, Japan

10:05-10:30 **Toxicogenomics-considering applications to predictive toxicology**
Roland C Grafström[1,2], Rebecca Ceder[2] and Pekka Kohonen[2]
[1]Health R&D, Knowledge Intensive Products and Services, VTT Technical Research Centre of Finland, Turku, Finland
[2]Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden

10:30-11:00 ***Coffee Break***

11.00-12:30 **Session 4** **Systems Biology-based Weight of Evidence for Predicting Toxicity**

Chaired by Jürgen Borlak, Hannover Medical School, Germany

11:00-11:30 **Predictive toxicology models based on the multi-organ interaction**

Seokjoo Yoon, Korea Institute of Toxicology

11:30-12:00 **A systems approach for drug safety evaluation by integrating toxicogenomics and heterogeneous data with bioinformatics**

Yuji Morikawa, Shionogi & Co., Ltd., Tokyo, Japan

12:30-13:00 **In vivo extrapolation of in vitro drug-induced hepatocyte accumulation of triglycerides to predict steatosis in rodents and humans**

Prakash Patel, Mohammed Atari, Heather Woodhouse, Samantha Bevan, Caroline Bauch, Paul Walker and Simon Thomas, Cyprotex Discovery Limited

12:30-13:30 **Lunch at Olive Garden restaurant**

13:30-16:30 **Workshops**

Chaired by Philip Doganis, National Technical University of Athens, Greece

- **The role for physiologically-based pharmacokinetic (PBPK) modelling in predicting toxicity**

  Mohammed Atari, Cyprotex Discovery Limited

- **Carrying out a Meta-Analysis across Multiple Heterogenous Sources of Evidence**

  Barry Hardy, Douglas Connect

- **Chemical substances, nanomaterials and endpoint data in AMBIT**

  Nina Jeliazkova, Ideaconsult Ltd.

- **Development of predictive NanoQSAR models using OpenTox infrastructure and the R language**

  Georgia Tsiliki, Philip Doganis, National Technical University of Athens, Greece

- **Open Science Pathway Analysis**

  Egon Willighagen, Maastricht University

17:30-23:00 **Acropolis museum and dinner at Strofi restaurant**

WEDNESDAY, SEPTEMBER 24

8:30-10:30 **Session 5 Integrated Testing Strategies**
Chaired by Gladys Ouedraogo, L'Oréal Research & Innovation

8:30-9:00 **Integrated Testing Strategies: The Benefits of Quantifying Uncertainty in Toxicity and Exposure Assessment**
Dr John E Doe, Parker Doe Partnership

9:00-9:30 **The ChemScreen project to design a pragmatic alternative approach to predict reproductive toxicity of chemicals**
Bart Van Der Burg, BioDetection Systems

9:30-10:00 **Integrating cosmetic industrial needs in skin sensitization Integrated Approaches to Testing and Assessement (IATA)**
Silvia Teissier, L'Oréal Research & Innovation

10:00-10:30 **An integrated approach on testing and assessment for skin irritation and corrosion**
Michael Oelgeschlaeger, Bfr

10:30-11:00 ***Coffee Break***

11.00-13:00 **Session 6 Read Across**
Chaired by Barry Hardy, Douglas Connect

11:00-11:30 **Metabolomics as tool for read-across**
Dr V. Strauss[1], H. Kamp[1], G. Montoya[1], E. Fabian[1], M. Herold[2], G. Krennrich[1], R. Looser[2], W. Mellert[1], E. Peter[2], T. Walk[2], B. van Ravenzwaay[1]
[1]BASF SE, Ludwigshafen [2]metanomics GmbH, Berlin, Germany

11:30-12:00 **Use of alternative methods to support read across- experiences form the Detective project**
Sylvia Escher [1], Jan Hengstler [2]
[1]Fraunhofer Institute of Toxicology and Experimental Medicine (ITEM)
[2]IFADO- Leibniz Research Centre for Working Environment and Human Factors

12:00-12:30 **Read Across Use of Alternative Evidence in Replacement Research and Safety Assessment supported by OpenTox and ToxBank**

Barry Hardy [1], Glenn Myatt [2], Micha Rautenburg [3], Pekka Kohonen P. [4],Grafström R. [4]

[1] Douglas Connect, [2] Leadscope, [3] in silico toxicology, [4]Karolinska Institutet

12:30-13:00 **Cascaded Classification and Regression for the Integrated Prediction of Activity/Non-Activity and Degree of Activity**

Layal Kazma, Andreas Karwath, Stefan Kramer

Johannes Gutenberg Universitat Mainz

13:00-13:30 **Concluding Session**

Chaired by Barry Hardy and Haralambos Sarimveis

Abstracts

Oral Presentations

Session 1 Modeling Metabolism and Reactivity

Refining Concrete Cascade Networks to Explain Acetaminophen Hepatotoxicity Phenomena Within and Across Multiple Scales

Anthony Hunt[1], Andrew Smith[1], Glen Ropella[2]
[1]*Bioengineering and Therapeutic Sciences, University of California, San Francisco*
[2]*Tempus Dictum, Inc.*

The challenge for this work has been discovering and iteratively improving minimal in silico component interactions that stand as challengeable mechanistic hypotheses capable of explaining characteristic features of APAP hepatotoxicity. To insure broader usefulness within the larger pharm/tox context, we adhere to delineated near and long-term requirements [DOI:10.1002/wsbm.1222] along with best software engineering, simulation, and scientific practices.

METHODS: We use discrete event, agent-based analogs comprised of nested modular spaces and components assembled into biomimetic mechanisms. Our validation targets are drawn from a diverse set of phenomena that we wish to explain eventually. Each phenomenon is a targeted attribute (TA). We cycle through a five-stage Iterative (mechanism and component) Refinement Protocol.

1) Specify the subset of TAs to be validation targets during the current work cycle along and quantitative similarity criteria (SC). A validation target is achieved when an analog phenomenon attains the SC prespecified for a TA.

2) Formulate minimal computational mechanisms intended to mimic essential features of referent mechanisms. Phenomena generated during execution are products of component interactions.

3) Instantiate those mechanisms by refactoring and reparameterizing components and mechanisms from already validated analogs. Design and execute experiments.

4) Use SC to compare corresponding simulated and wet-lab measurements. When SC for several TAs are achieved, the analog has attained a degree of validation: it stands as a plausible, concrete, explanation of the targeted phenomena.

5) Challenge/falsify Stage 4 mechanisms in the next work cycle by including new TAs at Stage 1.

RESULTS: I will describe achieving multiple validation targets, including dose dependent hepatocyte necrosis occurring first adjacent to lobule central veins. At stage 5 in the most recent cycle, we added TAs characterizing SP600125 inhibition of hepatocyte necrosis following a single dose given two hours after a toxic APAP dose. Simulating inhibition required: 1) separate SP600125 objects that, simultaneous with APAP objects, percolate through and interact with components; 2) giving analog components the ability to distinguish between APAP and SP600125 and interact appropriately; and 3) achieving SP600125 absorption, distribution, and clearance validation targets. I will describe a coarse grain interaction mechanism that enabled achieving time-dependent validation targets for inhibition of necrosis.

CONCLUSION: The evidence presented strengthens the case that biomimetic synthetic analogs using concrete parsimonious mechanistic hypotheses provide an explanation for how APAP toxicity emerges within and across biological scales, and that enhances prediction credibility. The methods facilitate in silico experimentation to falsify (or not) previously validated mechanisms.

Analogs and mechanisms are intended for reuse in studying any number of xenobiotics, alone and in combination. They make it straightforward to refine components iteratively to establish new, more explanatory mechanisms.

Keywords: *Acetaminophen, hepatotoxicity, biomimetic, explanatory mechanisms, agent-based, simulation, validation*

Assessing the performance of systems that predict metabolism

Philip Judson
Lhasa Limited

There are problems with assessing the performance of systems that predict metabolism. Measures such as the Cooper statistics, selectivity, sensitivity and concordance, and receiver operating characteristic (ROC) curves are primarily suited to binary predictions. Some metabolism prediction systems attach varying confidence to predictions – i.e. they do not deliver simple binary predictions. Standard metrics require figures for false positive predictions. Failing to observe, or record, the presence of a metabolite is not the same as confirming its absence and so there is uncertainty about false positive predictions. Possible approaches to improving the assessment of performance of metabolism predictions will be discussed, including the use of a new metric, "veracity", that takes into account how well systems judge levels of confidence in their predictions.

PBTK/TD modelling for predicting mammalian toxicokinetics and toxicodynamics

Mohammed I. Atari, Prakash Patel and Simon Thomas
Cyprotex Discovery Ltd., United Kingdom

Physiologically-based toxicokinetic/toxicodynamic (PBTK/TD) modelling is a mathematical modelling approach which aims at integrating a priori knowledge of physiological processes with other known/observed information to mimic the fates and effects of compounds in the bodies of humans, preclinical species and/or other organisms. Recently, PBTK/TD models have been widely used by regulatory agencies and consortia including the FDA and ITC (International Transporter Consortium).

Understanding the toxicokinetics (TK) and toxicodynamics (TD) of xenobiotics is vital in the pharmaceutical, agrochemical, chemical, cosmetic and other industries; these industries generate numerous compounds to which living organisms are exposed, and that can have a significant influence on their health. In the pharmaceutical industry it is well documented that toxicity is a leading cause of late-stage attrition during the development process, and of post-approval withdrawals. The associated costs and difficulties with developing new chemicals with acceptably low toxicity is becoming challenging. Therefore, the traditional approach for risk assessment is moving towards in silico predictive toxicology techniques that provide fast and cost effective replacements of (or supplements to) in vivo experiments to identify toxic effects at the different stages of the R&D process

To investigate the fates and effects of typical drugs and other chemicals, a novel PBTK/TD model for the in vivo TK of compounds has been developed. This model predicts the distribution of chemical substances in the body. The model has been optimised using xenobiotic plasma concentration data following intravenous and oral dosing. Before estimating the unknown model parameters from the experimental, data it is essential to determine parameter uniqueness (or otherwise) from the imposed output structure. This is formally performed as a structural identifiability analysis, which demonstrates that all of the unknown model parameters are uniquely determined by the output structure corresponding to the experiment. The model has been used to study a number of examples relating to in vivo toxicity, such as cytochrome P450 induction, steatosis and acute lethality. Such a coupled TK/TD model, has the potential to fulfil several roles in novel compound discovery, including: identification of compounds that are likely to have unacceptable in vivo toxicity; ranking compounds on expected toxicity; optimising the design of pharmaceutical dosing regimes to minimise side effects, whilst maintaining desired therapeutic efficacy.

Session 2 Assessing the Safety of Nanotechnology

(Part I)

Nanomaterials: Lessons learned and testing the regulatory approach

Keld Alstrup Jensen [1] and Steffi Friedrichs [2]
[1] *National Research Centre for the Working Environment, Denmark*
[2] *Nanotechnology Industries Association*

There is an urgent need to identify suitable methods and standard operation procedures for reliable characterization of manufactured nanomaterials (MN) and the exposure characteristics in toxicological testing. Specific need of methods for MN size-distribution of the primary nano-objects and specific surface area (SSA) measurement has been sparked by the EU regulatory definition of a MN, which has moved the measurement paradigm from mass- to number-weighed size-distribution of the minimum dimensions of nano-objects and the volume-SSA. At the same time, the variability in toxicological test results increases with the growing number of toxicological studies. This may in part be caused by the many different procedures for nanomaterial dispersion in both dry and liquid state, use of different exposure media, and lack of harmonized exposure characterization. Numerous data has been generated on a specific suite of OECD test materials in several national and EU-funded research projects. These projects included a.o. the EU-FP7 projects ENPRA, the EAHC-project NANOGENOTOX, and the DEFRA-project PROsPECT besides the so-called sponsorship program in the OECD Working Party on Manufactured Nanomaterials. So far the results have shown that sometimes specific characterization end-points considered straight-forward such as quantification of chemical composition and surface coatings cause the greatest challenges and vice-versa where a method for determination of the number-weighed size-distribution of pure NM appears within reach already. The presentation will include a discussion on the lessons learned from these previous projects and the attempt to test and characterize NM following a highly harmonized top-down designed approach in the EU FP7 project NANoREG.

NM Series of Representative Nanomaterials for harmonized Safety Assessment

Kerstin Hund-Rinke, Karlheinz Weinfurtner
Fraunhofer Institute for Molecular Biology and Applied Ecology IME,
Schmallenberg, Germany.

OECD's Working Party on Manufactured Nanomaterials (WPMN) launched a Sponsorship Programme in November 2007. This collaborative programme supports the development and collection of data on the characterization, on the toxicological and ecotoxicological testing as well as on the risk assessment and safety evaluation of nanomaterials. The WPMN agreed on a priority list of 13 nanomaterials to be tested considering materials which are in or close to commerce. The aim of the project is to provide a basis for the measurement, toxicology testing and risk assessment of nanomaterials. Various nanomaterials were selected from the following substance groups: Ag, Fe, TiO_2, Al_2O_3, CeO_2, ZnO, SiO_2, Au, fullerenes (C60), nanoclays, dendrimers, single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs).
Nanomaterials are characterized by a particle size distribution, therefore standardized sub-samples are required for scientific testing. For a scientifically sound comparison of results obtained in world-wide testing comparable sub-samples are a prerequisite. Sub-sampling of the nanomaterials under Good Laboratory Practice is carried out by Fraunhofer IME. The sub-samples are prepared and homogenized using an air-cleaned and solvent-free laboratory. As the nanomaterials behave differently and are available in different forms as dispersion or in powder form, sub-sampling is performed individually. The prepared

sub-samples are fumigated with Argon to ensure long-term stability and sealed with Teflon stopper and crimped caps. Since the sub-samples are intended for single use, they consist of small amounts (250 – 2000 mg) of the nanomaterials. Regardless of the material the characterization of the final material as well as homogeneity and stability testing are performed by the European Commission Joint Research Centre (JRC). The nanomaterials are made available for every test purpose independent of the Sponsorship Programme. They are supplied by Fraunhofer IME and JRC. Also reports on the results of the TEM characterization performed by Coda-Cerva and financially supported by Fraunhofer IME are available. The nanomaterials were dispersed in three different media (double distilled water, buffer with respect to human toxicity, mineral medium with respect to ecotoxicity). Furthermore, comprehensive reports on the characterization, the stability and homogeneity of the nanomaterials are already available for Zn, CeO_2 and for one Ag-nanomaterial under the series title 'NM-Series of Representative Manufactured Nanomaterials'. Reports on further types of materials are planned.

Towards a harmonized Safety Assessment of Nanomaterials

Carol Aristimuño, Felipe Goñi de Cerio, Pedro Heredia, Blanca Suarez-Merino
GAIKER Technology Center, Zamudio, Vizcaya, Spain.

Traditional toxicology´s main concern is to study the adverse effects of chemicals on a given set of known cytological, physiological and morphological parameters. However, these set of well defined studies do not take into account the special nature of nanomaterials, such as their small size, aggregation capacity and reactivity. These new properties may be able to alter the absorption and transport capacity of nanomaterials across membranes. There is also a potential for nanomaterials to accumulate in organs, enter into blood circulation or even cross the placental-foetal barrier.
One main objective of the European Project “NANoREG” is to produce a set of high quality toxicity data from a selection of industrially relevant nanomaterials with the aim to speed nanomaterial toxicity assessment. To achieve this aim the proposed strategy is two-fold; on one hand a set of standard in vitro toxicity protocols will be duly reviewed and adapted to the particulars of nanomaterials if necessary based on previous experiences from other initiatives and the Pharma industry. On the other hand, in vitro technologies will be challenged with a direct and unique comparison to in vivo data. In this regard, complex inhalation toxicity models have been included as a way to simulate inhalation toxicity in vitro. The final aim of this exercise being to assess the potential of in vitro models to predict nanotoxicity, speeding, in this way, nanomaterial toxicity assessment. Currently, an initial harmonization step has taken place focusing mainly on cell lines, dosage, incubation times, exposure mechanisms and detection techniques and covering mainly intestine, liver, lung and the immune system.
Our strategy aims at the core of engineered nanomaterial development, assisting in nanomaterial design by providing a fast and reliable evaluation of nanomaterial toxicity in the same fashion as any other conventional drugs are assessed for toxicity at their preclinical stage.

(Part II)

Standardized In vitro high-throughput and high-content analyses serve efficiently to broadly assess nanomaterials safety and influences of different dispersion and testing protocols

Roland Grafström[1,2] and Vesa Hongisto[1]
[1]Health R&D, Knowledge Intensive Products and Services, VTT Technical Research Centre of Finland, Turku, Finland
[2]Institute of Environmental Medicine, Karolinska Institutet, Stockholm,Sweden

There is global concern that known engineered nanomaterials (ENMs) in use and those to be synthesized in coming years will adversely influence environmental and health safety. Future ENM production is expected to be substantial, and the application of high--- throughput screening (HTS) technologies would potentially permit for human cell---based synchronous safety evaluations of the ENMs to be generated. Complicating the application of this technology, diverse nanomaterial dispersion and cell testing protocols generates variables that might influence the safety interpretation of the results. We report now on a standardized protocol for HTS---based safety analysis of ENMs in vitro. It applies the BEAS---2B human lung epithelial cell line, a common model system for studying effects of ENMs, and the Promega's CellTiter---Glo assay for cellular ATP content as a surrogate measure for alterations in cell numbers and viability. Assessed under a 384---well HTS format, established reference ENMs, including oxide forms of iron, titanium, zinc and copper, and so far untested, customer---based ENMs, demonstrated dose---dependent toxicity over a wide range of concentrations, indicating manifold differences in potency among different ENMs. Many factors, including the ENM dispersion protocol and volume, storage time of the dispersions, the cell density, the exposure time, as well as the length of growth and exposure of cells with or without serum, were found to influence the testing results. Thousands of experiments together indicated variable sensitivity of the ENMs to the protocol parameters, e.g., a particular dispersion protocol could variably act to increase, decrease or not affect the ENM toxicity effect relative another dispersion protocol. Importantly, it could be demonstrated that the ENMs as such would not influence the toxicity assay readout. Quantification under the HTS format of cell surface areas by microscopic imaging under time lapse demonstrated the feasibility of high---content screening for morphological toxicity. Such data can potentially validate effects indicated by the ATP assay. Interestingly, a modified, yet still fully functional, form of an original customer---produced ENM was found to generate significantly lower toxicity relative to the parent ENM. Taken together, we demonstrate standardized, multi---assay HTS protocols for ENM safety testing. The technology permits rapid sorting of possible influences of testing variables. The results argue for the implementation of HTS methods for proactive ENM safety evaluation, applicable generally to support novel ENM production under a safe--- by---design concept. Our planned continued collection of thousands of HTS results under a standardized ISA---Tab---nano format will ultimately lead to a valuable resource for ENMs ranking.

Nanoinformatics Study in South Korea for the developments of Safe and Sustainable Nanotechnology

Tae Hyun Yoon
Hanyang University, South Korea

Nanomaterials are often found to have very unique physicochemical properties and biological toxicities. However, due to the ever-increasing numbers of novel nanoparticles (NPs) and related consumer products, toxicity assessment of all these novel NPs using currently available in vitro/ in vivo testing methods seems impractical. Therefore, systematic categorizing (or grouping) of NPs as well as computational nanoinformatics approaches are considered as promising alternatives for the efficient risk assessment of NPs and screening those NPs with higher potential hazards for further in vitro and in vivo tests. In this presentation, current status of nanoinformatics studies in Korea will be introduced. Particularly, I will present current progress and future direction of nanoinformatics project in Korea (a.k.a. S2NANO:QNTR, funded by Korean MOTIE), which is recently launched for the development of an "User-friendly Nanosafety Prediction System" that can be used in the development stages of Engineered Nanoparticles and Nano-Products.

Keywords: Nanoinformatics, Engineered Nanoparticles, Predictive Nanotoxicology, QNTR

Session 3 Data Resources and Analysis

e-Science infrastructures for enabling large-scale predictive modelling in toxicology and pharmacology.

Ola Spjuth
Department of Pharmaceutical Biosciences and Science for Life Laboratory, Uppsala University, Sweden

Data sets in toxicology and pharmacology have recently increased greatly in size due to new high-throughput molecular technologies and large data resources generated and made available by international consortia. Taking advantage of such large data in many cases requires access to e-Science infrastructure of large-scale storage and high-performance computing (HPC) for storage and analysis. This presentation aims to highlight the challenges and opportunities for working with e-Science infrastructures, and showcases some of our latest developments for large-scale predictive modeling in toxicology and pharmacology including workflows, cloud computing and Hadoop.

On chemical structures, substances, nanomaterials and data exploration.

Nina Jeliazkova, Vedrin Jeliazkov
Ideaconsult Ltd.

The status-quo of chemical and bioinformatics databases is changing fast; an increasing number of online databases offer programmatic access (mostly via REST API), along with GUI. This brings both opportunities and challenges towards integrating the information, originating from diverse systems, as these interfaces are unique and incompatible, reflecting the underlying incompatible data models. A short summary, highlighting the pros and cons of the existing integration approaches is presented. We argue the structure-centric approach, adopted by the majority of chemical databases is biased towards the modelling community and fails to properly represent the complexity of the chemical substances and materials as produced, which itself is of substantial regulatory and scientific interest. Adopting a data model, describing the materials and measurements instead, provides a common ground for integration. Besides retaining the data provenance the focus on measurements provides insights how to extend chemical structures and address the challenges of representing the identity of chemical substances and nanomaterials. We illustrate the approach with the latest developments of AMBIT web services [1] and OpenTox API, in the context of supporting read across for mono- and multiconstituent substances with impurities and additives; as well as in the context of integrating nanomaterials characterization and safety data.
An exercise of data integration is only useful if it allows better insight about the subjects studied, rather than inspecting the data points alone.
While OpenTox API had been designed with the goal of supporting wide variety of machine learning methods, the emphasis of this talk is on interactive data exploration and visualization. Different web browser data views will be presented, with the help of a set of newly developed and embeddable JavaScripwidgets, acting as clients of AMBIT REST services. One use case is the chemical landscape analysis and visualisation of diverse datasets, based on a recently proposed a new and efficient method for identifying activity cliffs and visualization of activity landscapes [2]. The method is applicable large datasets, as it does not require the storage of the pairwise similarity matrix. The techniques of detecting discontinuities in property-activity landscapes are potentially useful as supporting tool for read-across, in modelling of any chemical property, as well as in guiding compound selection based on both structural and biological similarity, as we demonstrate with examples.

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Keywords: *data integration, chemical substances, nanomaterials, chemical landscapes, read across*

Open PHACTS: Solutions and the Foundation

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Open PHACTS is a five year project of the Innovative Medicines Initiative (IMI), ending in February 2016. It aims to reduce the barriers to drug discovery in industry, academia and for small businesses[1]. The Open PHACTS consortium is building a freely available platform, integrating data from a variety of information resources, and providing tools and services to query these integrated data to support life sciences research[2].

Currently, pharmaceutical companies expend significant and often duplicated efforts aligning and integrating internal information with public data sources. This process is difficult and inefficient and the vast majority of data sources cannot easily interoperate, often requiring additional steps to map identifiers or manually curate and correct the content. Open PHACTS is creating a precompetitive infrastructure to make these data integration approaches available both to industry and to academia and smaller companies, who have historically not had access to large-scale integrated data resources.
Here we give an overview of the resulting Open PHACTS Discovery Platform, the semantic web solutions used in this, and describe the integration of the data into dedicated and generic data analysis tools. The platform consists of components that communicate with each other using open standards and cover the full data lifecycle, from data loading to data sharing. Solutions underlying the platform include those for data provenance, data normalization, data standardization, and data access. In particular, we have developed minimal reporting standards for provenance, technologies to express the level of equivalence of entities from different databases, a database identifier mapping infrastructure based on semantic web technologies, unit and end point normalization, as well as chemical structure normalization. For this we use open ontologies (BioAssay Ontology, QUDT, CHEMINF, etc), standards (RDF, SPARQL, REST, etc), and proposed solutions as outlined in published specifications.
On top of these approaches, user oriented solutions have been developed based on a number of research questions selected by the pharmaceutical industry[3]. Example questions include: “Give me all oxidoreductase inhibitors active <100 nm in human and mouse” and “Compounds that agonize targets in pathway X assayed in only functional assays with a potency <1 µm”. The OPS platform provides a uniform route by which these questions can be addressed, exposed to the user by a novel pharmaceutical web service platform, called the Linked Data API (LDA).
As well as provided an API and web-portal to access integrated data, the Open PHACTS platform also supports an ecosystem of third-party applications addressing specialised needs such as polypharmacology, hit-selection, target validation and knowledge discovery. Additionally, more generic integrations have been developed too, like client libraries to the LDA in various programming languages, such as JavaScript, Scala, and Java, resulting in integration in generic data analysis platforms like PipeLine Pilot, KNIME, R, and Bioclipse.
Finally, results will be presented based on the sustainability track of the platform, indicating the future of Open PHACTS beyond the initial project. This future is partly covered by an Open PHACTS Foundation which will ensure maintenance and continued development.

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Toxygates - a hybrid linked data and microarray platform for user-friendly toxicogenomics

Johan Nyström-Persson [1,2], Yoshinobu Igarashi [1], Maori Ito [1], Mizuki Morita [1], Noriyuki Nakatsu [1], Hiroshi Yamada [1], Kazuyoshi Ikeda [2] and Kenji Mizuguchi [1]
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Open TG-Gates is a large, systematically collected dataset created by the Japanese Toxicogenomics Project. In 2013, the first user-friendly interface for this data, called Toxygates, was released. We show briefly how to use Toxygates to interrogate the transcriptome's response to toxic stimuli, and discuss the data integration challenges faced during the development process and how we addressed them, as well as our efforts to generalise the technology to easily create similar applications in the future.

Toxicogenomics-considering applications to predictive toxicology

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Toxicogenomics aims at interdependently analyzing toxicity data and gene expression profiling results for understanding and predicting chemicals toxicity. This field has advanced surprisingly slowly due to limited comprehensive data collections and lack of innovative approaches to address existing data.
We studied formaldehyde toxicity, as this agent represents an information-rich chemical for which extrapolation of in vitro data to human and animal data is possible. Formaldehyde toxicity and transformation potency in vitrowas analyzed in human epithelial cell lines, including by transcript profiling. We show new evidences for that toxicogenomics results serves better than traditional toxicity data for elucidating modes of action and for linking exposures in vitro to exposures shown to exert toxicity effects in vivo.

Session 4 Systems Biology-based Weight of Evidence for Predicting Toxicity

Predictive toxicology models based on the multi-organ interaction

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This project is the construction of the toxicity prediction model based on multi-organ interaction. Multi-organ interaction system is consisting of liver, kidney and blood vessel. Major concern is metabolic activation in the primary target organ and consequent damage in the secondary target organ.
Integrated discrete Multiple Organ Culture (IdMOC) system was applied for in vitro model (human primary hepatocytes or HepG2, HK-2, HUVEC) for multi-organ interaction. At first, transient transfection tools were applied for CYP overexpression in HepG2 and stably co-expressed cell lines for major four CYP enzymes, CYP3A4, CYP1A2, CYP2B6, and CYP2E1 was established. In vitro co-culture system using the CYP-expressing HepG2 was introduced to evaluate toxicity based on metabolic activation and subsequent cellular interaction.
We are collaborating with highly competitive research groups to achieve following subjects: 1) in vivo validation study for toxicity evaluation based on multi-organ interaction, 2) Development of in vitro cell based assay system for evaluating the chemical toxicity induced by multi-organ interaction, 3) Discovery of biomarkers for multi-organ interaction toxicity by Omics approaches, 4) Integrated analysis of associated toxicities in multiple organ and the construction of predictive model.
This research was supported by a grant (13182MFDS988) from Ministry of Food and Drug Safety in 2014.

A systems approach for drug safety evaluation by integrating toxicogenomics and heterogeneous data with bioinformatics

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Shionogi & Co., Ltd., Tokyo, Japan

Toxicogenomics is an effective mean for investigating adverse effects and mode-of-action of compounds based on microarray techniques for mRNA. Predictive model, using transcript signatures and machine learning techniques, enables the efficient selection of drug candidates at an early stage of drug development, resulting in a significant reduction in the time and cost associated with development of new molecular entities. In Japan, the Toxicogenomics Project (TGP; Uehara et al, 2010) has constructed a large-scale database called TG-GATEs. The open accessible version, Open TG-GATEs, is available for free public download. In recent years, biomarkers to predict for several toxicity endpoints (e.g. nongenotoxic hepatocarcinogenicity, nephrotoxicity) have been successfully identified by using this database. Furthermore, development of other high throughput techniques, such as microarrays for microRNA, NGS, metabolomics, leads to the emergence of promising new fields of toxicogenomics. In this session, we will share our experiences on exploration of biomarkers and challenges in bioinformatics analysis in dealing with integrated 'omics' data.
Keywords: *toxicogenomics, omics, bioinformatics, data integration*

In vivo extrapolation of in vitro drug-induced hepatocyte accumulation of triglycerides to predict steatosis in rodents and humans

Prakash Patel, Mohammed Atari, Heather Woodhouse, Samantha Bevan, Caroline Bauch, Paul Walker and Simon Thomas
Cyprotex Discovery Ltd., UK

Drug induced liver injury (DILI) is a major cause of late stage attrition in drug development. Although hepatic steatosis (fatty accumulation of the liver) is regarded as a relatively mild effect, it may lead to more serious DILI and may be indicative of serious underlying causes such as the inhibition of mitochondrial respiration.
The aim of our study was to predict in vivo steatosis in rodents and humans from in vitro high-content screening (HCS) data and estimated in vivo pharmacokinetics (PK), by constructing a phenomenological model of cellular triglyceride response to drug challenge. Fitting the model to the HCS data for individual drugs generated parameters for each drug. These were used with estimated in vivo plasma concentrations to drive in vivo steatotic response, and compared with in vivo observations.
Steatosis in mouse, rat and human on exposure to tetracycline was modelled using HCS triglyceride accumulation data from HepG2 cells. The model was also used to predict triglyceride accumulation on exposure to cyclosporin A and tamoxifen.
Using this model, in vitro HCS data and physiologically-based pharmacokinetic (PBPK) modelling could be used to predict the steatotic potential of drug candidates, or be used to aid optimization of dosing regimens to minimise steatosis of existing drugs whilst maintaining efficacy.
Keywords: *DILI, steatosis, HCS, IVIVE, modelling*

Session 5 Integrated Testing Strategies

Integrated Testing Strategies: The Benefits of Quantifying Uncertainty in Toxicity and Exposure Assessment

Dr John E Doe
Parker Doe Partnership

The quest to replace the use of animals in toxicity assessment has been arduous but recent advances are showing promise. It has been recognised that study for study replacement will not be successful and an integration of in silico and in vitro methodology will be required. Some of the currently available methods have been shown to give reasonable estimations of toxicity but they have lower precision than the conventional battery of in vivo assays which has limited their acceptability. However the precision provided by the conventional assays is not always needed to carry out a risk assessment.
Quantifying the uncertainty in both the estimate of toxicity and in the estimate of exposure allows a judgment to be made on whether safety can be assured or whether more precision is required. The HESI Risk 21 project has codified this concept into a risk assessment matrix. The use of the matrix will be exemplified and opportunities for the near future will be identified.
Keywords: *Integrated testing strategy, Risk Assessment, Toxicity assessment*

The ChemScreen project to design a pragmatic alternative approach to predict reproductive toxicity of chemicals

Bart Van Der Burg
BioDetection Systems BV Netherlands

There is a great need for rapid testing strategies for reproductive toxicity testing, avoiding animal use. The EU Framework program 7 project ChemScreen aimed to fill this gap in a pragmatic manner preferably using validated existing tools and place them in an innovative alternative testing strategy. In our approach we combined knowledge on critical processes affected by reproductive toxicants with knowledge on the mechanistic basis of such effects. We used in silico methods for prescreening chemicals for relevant toxic effects aiming at reduced testing needs. For those chemicals that need testing we have set up an in vitro screening panel that includes mechanistic high throughput methods and lower throughput assays with apical endpoints. Developed modules for rapid exposure predictions via diverse exposure routes greatly improved predictivity of the in vitro tests. As a further step, we have generated examples how to predict reproductive toxicity of chemicals using available data. We are actively engaged in promoting regulatory acceptance of the tools developed as an essential step towards practical application. With this, a significant saving in animal use and associated costs seems very feasible.
Keywords: *Chemical risk assessment, REACH, High throughput screening, integrated testing, reproductive toxicology*

Integrating cosmetic industrial needs in skin sensitization Integrated Approaches to Testing and Assessement (IATA)

Silvia Teissier
L'Oréal Research & Innovation

Skin sensitization remains a major environmental and occupational health hazard. If different mechanical approaches, including structure-activity, skin metabolism determination and biological comprehension of contact sensitization have been developed over the last decades, till recently, only animal tests were

accepted by regulations. Since March 2013, the 7th Amendment of the Cosmetics Directive prohibits in Europe the marketing of cosmetic products containing ingredients which were tested on animal-based assays and prompted the implementation of Integrated Approaches to Testing and Assessment (IATA) for Skin Sensitization.

While there is a common understanding of the Adverse Outcome Pathways (AOP's) leading to skin sensitization, as well as a wide appropriation of a core battery of assays addressing these AOP key events, the ways of integrating such data to allow risk assessment of new ingredients is still in its early experimental phase. Like others, we have developed our own integrated testing strategies. Based on this experience on cosmetic case studies and through a comparative review of different approaches that were published, we will present the opportunities and remaining challenges to support the ongoing OECD IATA initiative to reach the final goal of safety evaluation and risk assessment of new ingredients.

Keywords: *Skin sensitization, Integrated testing strategies, Adverse Outcome Pathways*

An integrated approach on testing and assessment for skin irritation and corrosion

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During the last years, several in vitro methods for skin corrosion or irritation have received regulatory acceptance that might allow the classification of substances according to UN GHS, including OECD TG 439 (2013), OECD TGs 430 (2013), 431 (2013) and 435 (2006).. However, the OECD TG 404 on in vivo acute dermal irritation and corrosion testing contains a supplement describing a sequential testing and evaluation strategy that still requires confirmatory in vivo testing in case of negative in vitro tests. In addition, the experience gained by regulatory authorities revealed a need for guidance for users as well as regulatory agencies on the appropriate use of in vitro methods as well as the evaluation of the results. Therefore, the Federal Institute for Risk Assessment (BfR) initiated an OECD project with the aim to develop a Guidance Document (GD). This document now describes an "Integrated Approach on Testing and Assessment" (IATA) for skin corrosion and irritation to replace the "Testing and Evaluation Strategy" currently provided in the supplement to OECD TG 404. The GD provides information on key performance characteristics of the individual information sources and guidance on how to integrate the information for classification and labeling.

The IATA is composed of "Modules" according to the type of information.

The eight modules of the IATA are integrated into three main parts that describe the evaluation of existing data in a Weight of Evidence approach as well as the use of in vitro methods, if additional testing is required.

Animal testing is foreseen only as a last resort. Thus, this document might help to minimize the use of animals to the extent possible, while ensuring human safety.

Keywords: *IATA, skin irritation corrosion, in vitro, in vivo*

Session 6 Read Across

Metabolomics as tool for read-across

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BASF SE and metanomics GmbH have developed a metabolomics data base (MetaMap®Tox) containing plasma metabolome changes in Wistar rats induced by more than 500 data rich chemicals, agrochemicals and active pharmaceutical ingredients derived from 28 day repeated dose toxicity studies. This database is used as tool to identify toxicological modes of action and to compare the toxicity of new compounds with reference substances.
In addition the MetaMap®Tox database could very well serve as tool for (biology based) grouping of chemicals and for subsequent read-across according to the definition of EChA: "Substances that are structurally similar with physicochemical, toxicological, ecotoxicological and/or environmental fate properties that are likely to be similar or to follow a regular pattern may be considered as a group of substances. Within this group of substances, a data gap might be filled by read-across (Ref.: ECHA-13-R-02-EN, April 2013)".
In the presentation, various examples for the successful use of metabolomics for read-across will be discussed:

- Structure related similarities regarding receptor-binding capacities
- Similarities regarding enzyme inhibiting potentials
- Structure related quantitative toxicological effects
- Structure related versus biology-based comparison of compounds: from QSAR to QBAR

Use of alternative methods to support read across- experiences form the Detective project

Sylvia Escher [1], Jan Hengstler [2]
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Read across is a well know approach in regulatory risk assessment, where toxicity data from one/many data rich source compound(s) is/are used to predict the toxicity of one/many data poor target compound(s). Quantitative predictions such as no observed adverse effect level (NOAEL), as well a qualitative predictions e.g. mutagenic potential are possible.
For read across the chemical as well as biological similarity between the source and target compounds has to be clearly demonstrated. Chemical similarity is often based on structural similarity and the evaluation of relevant physico-chemical properties (being similar or following a consistent trend). Biological similarity comprises same mode of action in vivo, same toxikokinetics e.g. absorption, distribution, metabolism and excretion. Very often these data on mode of action are not available.
This presentation will introduce the Detective read across case study. In the Detective project (http://www.detect-iv-e.eu/), we aim to evaluate, whether and how mechanistic data e.g. from in vivo /in vitro omic experiments can be used to support read across. Detective is one out of the six project of the SEURAT cluster (http://www.seurat-1.eu), which evaluates new strategies to replace in vivo repeated dose toxicity testing.
Valproic acid (VPA), one of the gold compounds in the Detective project, was selected for this pilot read across case study. It is known to be of concern to induce microvesicular steatosis in the liver. The strategy

of the read across use case will be presented, the selection of source and target compounds as well as promising results from the first in vitro experiments.
Keywords: *read across, mechnistic knowledge, alternative methods, QSAR, repeated dose toxicity, systemic toxicity, biomarker*

Read Across Use of Alternative Evidence in Replacement Research and Safety Assessment supported by OpenTox and ToxBank

Barry Hardy [1], Glenn Myatt [2], Micha Rautenburg [3], Pekka Kohonen P. [4],Grafström R. [4]
[1] *Douglas Connect*
[2] *Leadscope*
[3] *in silico toxicology*
[4] *Karolinska Institutet*

Data on SEURAT-1 Gold Compounds (SGCs) were obtained from the literature and organised and made available through the ToxBank wiki and data warehouse. The data integration included transcriptomics data from TG-Gates, assay data from PubChem, toxicokinetics data and parameters from the literature, and in vivo data resources. Interoperability between ToxBank components and other resources was based on service implementation of OpenTox standards. Analysis methods for Read Across, enriched meta analysis of multiple omics and functional data, background knowledge from GO ontologies and Kegg pathways, and pathway visualisation were developed and applied to the SGCs. We present an analysis case study for Doxorubicin based on publically available data examining the variation of pathway interactions as a function of dose and time. Using SGC examples, we discuss the differing information requirements and solutions for computational and expert-based components to Read Across and Weight of Evidence for the following contexts with consideration of the value and current limitations in Alternative-based evidence:

a) Hypothesis-driven mechanistic-based research;
b) Predictive goal of an integrated testing strategy;
c) Incorporation of evidence in safety assessment decision making.

The role of current studies and methods in providing guidance supporting Read Across practice and application development will be discussed.

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Keywords: *Read Across, OpenTox, ToxBank, SEURAT-1, Gold Compounds*

Cascaded Classification and Regression for the Integrated Prediction of Activity/Non-Activity and Degree of Activity

Layal Kazma, Andreas Karwath, Stefan Kramer
Johannes Gutenberg Universität Mainz

I will present new approaches to predicting the activity/non-activity of a chemical and the degree its activity in an integrated fashion, without having to define a combined objective function for classification and regression. For the task of classification, the approach first learns a regression model for predicting the degree of activity and then uses the predicted degree of activity as a feature for the classification problem. We call this approach Regression Classification Cascade (RCC). If the task is to improve the quality of a regression model instead, the opposite approach, called Classification Regression Cascade (CRC), is possible. In this approach, a classification model is first learned for discriminating active from inactive compounds. The predicted classification is then used as an additional feature for improving the regression. In this setting, the inactive compounds serve as extra information besides the compounds along with their known activities. In comprehensive experiments, we show that both approaches can be beneficial across a number of different endpoints and data sets. When used together with k-Nearest Neighbor approaches, it can also be useful in a read-across like context. Finally, we will discuss the potential of both approaches for elucidating mechanistic hypotheses.

Workshops

The role for physiologically-based pharmacokinetic (PBPK) modelling in predicting toxicity

Mohammed I. Atari, Prakash Patel and Simon Thomas
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Physiologically based pharmacokinetic (PBPK) modelling provides a powerful means of integrating ADME and physicochemical data to predict in vivo pharmacokinetics in humans and pre-clinical animals. Predictions of pharmacokinetics (PK) from ADME data can enhance the ability to select compounds that are most likely to have appropriate PK in vivo. The determination of physicochemical and ADME properties during early drug discovery ('early ADME data') enables PK prediction to be performed at any stage from lead identification onwards. PK prediction thus serves to integrate the data from various ADME/physicochemical screens – whether in vitro or in silico – greatly increasing their value over and above that of the raw data alone. In particular the role of sensitivity analysis – in which the effect of uncertainty in an input property on the value of an output (predicted) property is quantified – is a powerful tool for informing, and helping to direct – chemistry during lead optimization.
In this workshop, the focus will be on understanding the fundamentals of PBPK modeling, the use of appropriate ADME and physicochemical data as inputs, and the utilization of results during early drug discovery. For case study investigation of various aspects of PK prediction, participants will have access to Cloe® PK software. This is a powerful, yet intuitive, web-based program using a PBPK model for PK prediction. Its simple input data and comprehensive reporting make it suitable, not only for ADME/PK scientists, but also for toxicologists, medicinal chemists and biologists. In addition to the prediction of PK properties, the use of univariate and multivariate sensitivity analyses as an aid to directing chemistry optimization will be investigated. Such models have the potential to fulfill several roles in novel compound discovery, including: identification of compounds that are likely to have unacceptable in vivo toxicity; ranking compounds on expected toxicity; optimising the design of pharmaceutical dosing regimes to minimise side effects, whilst maintaining desired therapeutic efficacy.
Keywords: *Physiologically based pharmacokinetic (PBPK) modeling, ADME data, physicochemical data, in vivo toxicity*

Carrying out a Meta-Analysis across Multiple Heterogenous Sources of Evidence

Barry Hardy, Markus Hegi
Douglas Connect

In this workshop we will examine the meta analysis of evidence based upon data on SEURAT-1 Gold Compounds (SGCs) used as mechanistic reference compounds. We will examine data which were obtained from the literature and organised and made available through the ToxBank wiki and data warehouse.
The data analysis will include transcriptomics data from TG-Gates, assay data from PubChem and ToxCast, toxicokinetics data and parameters from the literature, and in vivo data resources. Analysis methods for Read Across, enriched meta analysis of multiple omics and functional data, background knowledge from GO ontologies and Kegg pathways, and pathway visualisation will be applied to the SGCs. We will examine the variation of pathway interactions as a function of dose and time.
SGCs within ToxBank available for study will include reactive compounds (e.g., acetaminophen, CCl4), mitochondrial disruptors (e.g., Rotenone), promiscuous binders (e.g., valproic acid, amiadarone), nuclear hormone receptor ligands (e.g., tamoxifen, WY14643), selective binders (e.g. fluoxetine) and cardiotoxins

(e.g., Doxorubicin, Nifedipine). Adverse events of interest that are represented include cytotoxicity, fibrosis, steatosis, cholestasis and phospholipidosis.

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Keywords: *Meta-Analysis, Evidence, ToxBank, OpenTox, SEURAT-1, Gold Compounds*

Chemical substances, nanomaterials and endpoint data in AMBIT

Nina Jeliazkova, Vedrin Jeliazkov
Ideaconsult Ltd.

The hands-on workshop will introduce AMBIT (http://ambit.sf.net) approach towards representing the substance identity and composition, and support for integrating and visualizing data from various sources, including IUCLID5 data files and nanomaterials related data. The participants will primarily use the new Web browser user interface of AMBIT via web browser, including structure searching, substance search, endpoint search and web interface to Toxtree predictions. A set of embeddable JavaScript widgets with the above functionality will be presented for developers, which allows providing the same user experience in remote web sites. Finally, the participants will be kindly asked to provide feedback on the upcoming read across support in AMBIT.

Keywords: *AMBIT, substance identity, nanomaterials, endpoints, web browser , read across, IUCLID5*

Development of predictive NanoQSAR models using OpenTox infrastructure and the R language

Georgia Tsiliki[1], Philip Doganis[1], Nikolaos Lampovas[1], Pantelis Sopasakis[2], Haralambos Sarimveis[1]
NTUA
IMT Lucca

This workshop will offer hands-on work on the development of NanoQSAR models, focusing on the use case of predicting cell association of Gold and Silver Nanoparticles, based on experimental data, publically available in the publication of Walkey et. al. (1). The workshop will include the preparation of a data-file properly structured, so that they can be explored using the OpenTox infrastructure (2), the development of a PMML (Predictive Model Markup Language) XML-based file for defining transformation of descriptors, and the creation of predictive models using statistical and machine learning algorithms. We will also explore how descriptors can be derived from nanoparticle images, using the Fiji/ImageJ open source software. Finally, R tools combined with protein corona data and Gene Ontology will be used to derive useful information on the mechanisms of action.

OpenTox web services are built on the RESTful API, a contemporary web technology characterized by flexibility and modularity, together with implementation and development independence. Popular statistical and machine learning methodologies (such as Multiple Linear Regression and Support Vector Machines) as well as algorithms developed in-house (like Fast Training RBF Neural Networks) have been implemented in our group and are deployed as REST web services within the OpenTox framework. Security of services is controlled with the design and implementation of an SSO-based authorization and authentication API.

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Keywords: *Predictive Toxicology, Web services, Nanoparticle, Image processing*

Open Science Pathway Analysis

Egon Willighagen, Bart Smeets
Maastricht University

This workshop will focus on the use of Open Science tools to support pathway analysis of experimental data to explore biological response to toxicants. It will explore WikiPathways [1], PathVisio [2], and BridgeDb [3]. These tools will be discussed as standalone tools, and we will examine approaches how the can be combined.
During the workshop you will first learn how to install and use PathVisio to draw custom pathways, how you can extend existing and upload new pathways to WikiPathways.org, and how to use BridgeDb for identifier matching. Second, you will use PathVisio to map experimental data available from the FP7 DiXA database onto WikiPathways and how to visualize the results.
The workshop is based on previous workshops given by members the Department of Bioinformatics of Maastricht University. Previous example material can be found for pathway analysis [http://projects.bigcat.unimaas.nl/ebi-roadshow/pathway-analysis/instructions/]
and for pathway drawing
[http://www.wikipathways.org/index.php/Help:Tutorial].
Keywords: *pathway analysis, WikiPathways, PathVisio, BridgeDb, Open Science*
Notes*: The abstract and exact details of the workshop content will be coordinated with Barry Hardy.*

Posters

Poster 1. An in silico model for the identification of small molecules for the treatment of thalassaemia

Antreas Afantitis ,Georgia Melagraki
NovaMechanics Ltd

Pharmacological reactivation of the γ-globin gene for the production of fetal haemoglobin (HbF) is a very promising therapeutic avenue for β-thalassaemia. Increased production of γ-globin can ameliorate the symptoms of the disease by partly substituting the non-functional β-globin gene, and restoring the balance between α and non-α globin chains. Drugs currently available for this purpose have limited application due to moderate therapeutic properties, variable patient response and potential cytotoxic effects. The main aim of this work is to discover agents which have higher efficacy and are safer than existing drugs through the development of a novel pathway for drug discovery and design involving the use of molecular modeling and data mining.

Towards this goal we have for long been involved in the in silico exploration of various molecular patterns for the identification of novel HbF inducers.

For this purpose we have combined data mining, machine learning with similarity search and virtual screening techniques to understand the structural characteristics that affect the reactivation of HbF from different molecular patterns. We have developed KNIME workflows (analyzing data and automatically reporting) to support customized needs of this work. We used existing and developed new custom-made KNIME nodes to develop a customized processing, analysis, and exploration platform. A model has been built using in vitro screening data in K562 human erythroleukaemia cultures from different molecular structures in order to study their ability to activate the γ-globin gene. K562 human erythroleukaemic cell line has the potential to highly express the embryo-fetal globin genes such as ζ, ε, and γ-globin genes. This characteristic makes K562 cells a useful model cell line for the study of compounds that are potential γ-globin inducers. The produced model was subsequently tested for accurate class predictions with an independent set of samples, and agreement of results was assessed.

Keywords: *thalassaemia, fetal haemoglobin (HbF), QSAR, K562 human erythroleukaemia, KNIME, Enalos KNIME nodes*

Notes: *This work is supported by funding under the Seven Research Framework Programme of the European Union. Project THALAMOSS (HEALTH.2012.1.2-1 Grant agreement no: 306201)*

Poster 2. Comparison analysis of toxicity evaluation algorithms using frequently used drugs

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Recently, in silico evaluation of drug toxicity has been issued because of its economic feasibility and speed. Up to now, many different types of algorithms have been applied to construct precise evaluation of toxicity by analyzing physic-chemical properties of drugs. However, there is no benchmarking analysis of applied algorithms by comparing performance using same data set. We had performed benchmark analysis of widely used 4 classification algorithms, KNN, LDA, SVM, and ANN. For benchmark analysis, we have extracted molecular signatures of 155 frequently used drugs using Mold2.

For training, the toxicity information of 155 drugs is also retrieved from The Comparative Toxicogenomics Database (CTD) and each drug is sub-grouped by their organ toxicity. The performance of applied algorithms was evaluated using LOOCV. From the benchmark analysis, we have found LDA and SVM are better than other algorithms for structure-based toxicity evaluation.

Keywords: *In silico prediction, Molecular structure, Risk assessment*

Poster 3. Identification Of Molecular Signatures Of Inflammation-mediated Organ Toxicity

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Toxicities of drug in specific organ are mediated by direct drug-organ interaction and/or mediated by other lesion which caused by drug. The inflammation is one of the key lesions which can cause multi-organ toxicity.

However, there is no comprehensive study to investigate biological characteristics of toxicity mediated by inflammation. In the present study, we have investigated the characteristics of biological network involved in inflammation-mediated organ toxicity. As a first step, we extracted the toxicity pathways of 155 frequently used drugs in each organs, liver, kidney, and blood vessel. And at the same time, inflammation related pathways were also extracted and compared with organ toxicity pathways. Signature pathways of inflammation mediated pathways were selected by BSS/WSS and fisher's exact test. From this analysis, we have found 13 pathways which were related to inflammation-mediated toxicity in the liver and kidney. For example, Hepatitis C pathway (KEGG:05160) was liver related inflammation pathway and they can cause hepatitis. These results can be applied to understand molecular mechanism of multi-organ toxicity cause by inflammation.

Keywords: Toxicity-pathway, Inflammation, Inflammation-mediated organ toxicity

Poster 4. A web-based software tool for individualized drug dosing using PBPK models and control theory

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Drug administration is a key element in medicine which is traditionally based on average population pharmacokinetic and/or pharmacodynamic profiles. This consideration is likely to exhibit adverse effects due to violation of toxicity constraints or may fail to retain therapeutic levels. Due to the lack of feedback, there is also the assumption of zero disturbances, which contributes even more to the probability of adverse effects.

Control theory can be used in the field of drug administration in order to face these challenges and design drug dosing strategies, which are individualized for each particular patient. In this work, the Model Predictive Control (MPC) methodology is used for optimal individualized continuous-time drug administration, by exploiting the advantages of this popular control technique: MPC leads to a stable and robust closed-loop system, and it takes into account the state and the actuator constraints, which in some cases are patient-specific. These constraints result from safety considerations against adverse effects and are qualified by means of tissue-specific minimum toxic concentration (MTC) values. Bounds are also imposed on the influx rate when the administration is intravenous.

Physiologically-Based Pharmacokinetic (PBPK) models are used for predicting the dynamic behavior of drug concentrations in plasma, tissue and tumors following drug delivery, using knowledge of the physiology and anatomy of the individual patient. PBPK models rely on fundamental principles such as mass balance equations and reactions kinetics and are mathematically quantified as systems of ordinary differential equations (ODEs). Coupled with a state observer, the overall system can control drug concentration at any organ using only blood measurements.

The overall setting allows the treating physician to modify the desired concentration at each target organ at any time during the therapeutical treatment. Furthermore, there is ability of achieving the desired concentration as well as monitoring in real time the overall distribution of the drug concentration in patient's organism.

A web application is currently under development for implementing the proposed methodology and make it available to physicians and other possible users. The user may use a pre-existing PBPK structure or create his own to describe as accurate as possible a patient and save it for future use. He can also define toxicological bounds or other state and input constraints, having the choice to use default values. Next, he sets the MPC parameters, like a target concentration to a specific organ and the control horizon. The result of the application is the optimal drug dose for the individual patient, as well as the dynamic simulated prediction drug concentration in the organs of interest.

The proposed methodology was tested on a hypothetical PBPK model with 7 compartments (and overall 14 sub-compartments). All constraints are satisfied so the adverse effects are minimized, since the drug doses are kept within the recommended bounds. We demonstrated also that the use of MPC performs well in the presence of modelling errors and measurement noise, which makes it suitable for medical applications.

Keywords: *Optimal drug administration, Individualized dosing, Minimum toxic concentration, PBPK models, Model predictive control*

Acknowledgement: *This work was funded by project 11ΣΥΝ_10_1152, which was co-financed by the European Union and Greece, Operational Program "Competitiveness & Entrepreneurship", NSFR 2007-2013 in the context of GSRT- National action "Cooperation".*

Poster 5. Predictive Toxicology Tools Application in Contaminated Sites Health Risk Assessment and Remediation

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Health Canada (HC) has developed a tool that lists remediation technologies and a decision matrix for evaluating chemical exposure issues. We reviewed current practices involved in the design and intended use of the tool, evaluating the use of various toxicology data resources and predictive tools in providing value to risk management decision making, including the use of human health risk characterization data and tools in remedial alternatives and option analyses. We evaluated a number of chemicals from Petroleum Hydrocarbons (PHCs), Polycyclic Aromatics Hydrocarbons (PAHs), Chlorinated Hydrocarbons (CHCs), and Perfluorinated chemicals (PFCs). The research included consideration of the physical and chemical properties of the contaminants, reactivity, fate and transport, and the spatial and temporal exposure analysis including analysis of multiple sources of in silico, in vitro, and in vivo data and consensus models under a Weight of Evidence framework. The methodology was applied to case studies by selecting specific remedial technologies (e.g., bioremediation and chemical based remediation) and prioritizing site-specific contaminants and used to support risk-based predictions and recommendations for management

of the contaminated sites. We were able to integrate relevant background knowledge, new data and predictive models on those test chemicals from publicly available toxicology databases and resources including OpenTox, ToxBank, Comparative Toxicogenomics Database, TG-GATES, SEURAT, PubChem, and Tox21 databases for risk assessment and management decision-making supported by the remediation tool for contaminated sites and identified data gaps and limitations in applications.

Keywords: *contaminated site, remediation, OpenTox, Health Canada, risk management*

Poster 6. A ToxBank Integrated Data Analysis of SEURAT-1 Reference Compounds

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The SEURAT-1 (Safety Evaluation Ultimately Replacing Animal Testing-1) research cluster is comprised of seven EU FP7 Health projects and is co-financed by Cosmetics Europe. The SEURAT-1 strategy is to adopt a mode-of-action framework to describe repeated dose toxicity to derive predictions of in vivo toxicity responses. ToxBank is the cross-cluster infrastructure project which provides a web-accessible shared repository of research data and protocols. Experiments generate dose response data over multiple timepoints using different omics platforms including transcriptomics, proteomics, metabolomics, and epigenetics over different cell lines and a common set of reference compounds (details available at wiki.toxbank.net). Data is also generated from functional assays and bioreactors and supplemented with in silico approaches. This complex and heterogeneous data is consolidated and harmonized through the ToxBank data warehouse in order to perform an integrated data analysis. We describe for 14 reference compounds the meta-analysis of currently public data including Open TG-GATEs human in vitro liver data of the reference compounds including reactive compounds (e.g., acetaminophen, CCl4), mitochondrial disruptors (e.g., Rotenone), promiscuous binders (e.g., valproic acid, amiadarone), nuclear hormone receptor ligands (e.g., tamoxifen, WY14643), selective binders (e.g. fluoxetine) and cardiotoxins (e.g., Doxorubicin, Nifedipine).

Adverse events of interest that are represented include cytotoxicity, fibrosis, steatosis, cholestasis and phospholipidosis.

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Keywords: *ToxBank, Integrated, Data Analysis, SEURAT-1, Reference Compounds*

Poster 7. Evaluation Of Toxicological Biomarkers In Multi-organs Including Liver, Kidney And Aorta Of Rats Exposed To Thioacetamide

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Proteomic analysis was conducted to determine the effects of thioacetamide on multi-organs including liver, kidney and aorta of rats. Rats were exposed to three different concentrations of thioacetamide (0, 10, 30 mg/kg bw) for 4 weeks in oral administration. AST, ALT, TBIL, and ALP increased significantly in the exposed group when compared with control group. Large size two dimensional electrophoresis using three different pI ranges (3-5, 4-7, 6-9) showed the presence of 3325, 3267, and 2801 in livers, kidney and aorta, respectively. Thirty nine (32 up-and 7 down-regulated), thirty one (4 up-and 27 down-regulated) and twenty four (18 up- and 6 down-regulated) proteins were identified as toxicological biomarkers in liver, kidney and aorta, respectively. Proteins involved in apoptosis, immune response, energy metabolism, cell cycle, signaling, transportation, and cell structure were found to be up- and down-regulated. Among these, three proteins were found in all organs and five proteins were found in liver and kidney. Only one protein was found in liver and aorta. Of these 94 proteins, 23 proteins were confirmed by western blot assay. They could used as potential toxicological biomarkers of thioacetamide.

Poster 8. A Set of Predictive Models for Ames Mutagenicity

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Ames mutagenicity test gives valuable experimental information for the drug discovery process – an estimation of the potential carcinogenicity of the drug candidates. The in-silico implementation of the Ames test is typically a QSAR model applied in the virtual screening process. We present a recent thorough study of a large set of Ames QSAR models. An exhaustive data mining was performed for a publicly available set of 6512 chemical compounds and their experimental Ames test results [1]. The original chemical structures were presented on a topological level via connections tables or/and linear notation SMILES [2]. The 3D geometry of the molecules was generated by means of OpenMopac [3]. We calculated a large set of 1D, 2D and 3D descriptors as well as more than 10 different sets of molecular fingerprints. The total number of the initial pool of molecular descriptors exceeded 15 000 descriptors. The Ames models were derived by means of various data mining
techniques: k-nearest neighbors (KNN), Support vector machines (SVM), Random Forest classification, Logistic regression, Gaussian process, Radial Basis Function classification etc. For each modeling technique, several methods for descriptor selection were applied – Principal component analysis (PCA), Correlation approach, Best First search method, Information Gain Ratio, Genetic algorithms etc. Additionally some of the descriptor selection procedures were tested with different parameter settings. Also each modeling technique was applied with various combinations of the molecular descriptors sets and fingerprint sets. More than 100 QSAR models were studied. The model validation was performed by external 5-fold cross validation where the descriptor selection was applied for each of the 5 data set resamplings (validation 'folds'). The top 10 and top 20 models were selected and used for the implementation of a consensus model. The descriptor/fingerprint calculation was performed with DRAGON [4] software version 5.4 and PaDEL [5] software as well as some custom fingerprints from Ambit2 [6] software system were used. Model building was performed by a collection of machine learning algorithms implemented in Weka [7] software version 3.7.11.

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Keywords: *Ames, Mutagenicity, Modelling, QSAR, Descriptors, fingerprints*

Poster 9. Response of human bronchial organotypic culture to mainstream cigarette smoke exposure

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The goal of our study is to combine a state-of-the art systems toxicology approaches with a relevant human *in vitro* airway model which mimics closely the *in vivo* situation (C.Mathis et al., 2013) to investigate the impact of direct cigarette smoke (CS) exposure on respiratory tract epithelium tissues. Additionally we aim to determine the robustness of our study design by analysing multiple end points of two batches of airway tissue cultures exposed in the same way to separate mainstream CS exposures.
We used differentiated human bronchial epithelial tissue model (EpiAirwayTM, MatTek®) produced by culturing human primary cells at the air-liquid interface. This tissue culture consist of basal cells, mucus secreting cells and ciliated cells and show an *in vivo*-like structure.
Bronchial organotypic epithelial tissue culture was exposed for 28 min at the air-liquid interface to fresh air or to 8%/15% (vol/vol with air) diluted mainstream CS (reference cigarette 3R4F; Health Canada smoking regimen and nicotine doses of 0.15mg/L/0.25mg/L). Different endpoints (adenylate kinase (AK) assay, cytochrome P450 CYP1A1/CYP1B1 activity, histology/immunostaining, pro-inflammatory markers release and gene expression) were then collected at 4h, 24h, 48h and 72h after exposure to assess time- and dose-dependent effect of whole CS exposure.
Our results demonstrated that 15% of CS induced cytotoxicity in the bronchial tissues measured by the release of AK in the media at 24h, 48h and 72h post-exposure times. Histological and immunohistochemical analysis showed that 3R4F induced dose- and time- dependent bronchial tissue damage characterized with reduction of the tissue thickness and decrease of the number of proliferating and ciliated cells. Furthermore CS induced time- and dose-dependent increase of the CYP1A1/1B1 activities (at 48h and 72h post-exposure time points) and of the release of pro-inflammatory mediators such as VEGF, MMP-1, MMP-9, GM-CSF, IL-1□, IL-6, IL-8, IP-10, sICAM-1, TNF-□ _and GRO at 24h, 48h and 72h post-exposure time points). Transcriptomic analyses combined with quantitative biological network modeling indentified biological processes (e.g. inflammation, proliferation and cellular stress) which were triggered by acute CS exposure in the bronchial tissue culture at various post-exposure time points. Finally, good reproducibility of the obtained results (AK assay, CYP1A1/CYP1B1 activity, pro-inflammatory markers release and gene expression) was observed in two independent experiments.

The described study and the reproducible data obtained from two independent experiments suggest that combining biological end points, large scale molecular measurements and computational method is a powerful approach to assess CS effect
using a relevant *in vitro* model such as bronchial epithelial tissue culture exposed at the air-liquid interface.

Poster 10. Comprehensive Gene Expression Analysis Of Mice Liver Treated With Diclofenac

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As a nonsteroidal anti-inflammatory drug, diclofenac is used to treat pain and reduce inflammation associated with arthritis. It is known to be associated with idiosyncratic adverse drug reaction incidence. The cause of idiosyncratic liver injury remains vague and likely to interact with multifactor. To better understand the hepatotoxic mechanism of diclofenac, we analyzed whole genome expression profiles in mouse liver administered diclofenac (30 mg/kg, i.p.) once per day for 1 day or 3 days. In total, 295 and 467 significantly regulated genes were selected, respectively, as defined by the Welch's t-test ($P < 0.05$) and threshold of fold change ≥1.5 using the GenePlex analysis program. Notably, 29 genes were commonly regulated after single and repeated treatment. The majors being closely associated with inflammatory, immune and acute phase responses. We analyzed bio-functions, canonical pathways and regulator effects prediction using the ingenuity pathway analysis software. The most distinguishable regulations in gene expression change were linked to inflammatory response, cellular movement, hematological system development/function and immune cell trafficking in both samples. Also, in the canonical pathways, acute phase response and interleukin-related signaling were significantly implicated in single and repeated treatment. Among the upstream regulator, Stat3, transcriptional factor, was significantly influenced by diclofenac. And some of inflammatory protein was increased by diclofenac. In the results of the microarray analysis, the expression of genes associated with the inflammatory, acute phase, and immune responses was significantly influenced by diclofenac.

Keywords: *Diclofenac, Hepatotoxicity, Gene Expression Profiling, Inflammation, Nonsteroidal anti-inflammatory drug (NSAID)*

Notes: *Eun-Hee Lee and Heeyoung Yang contributed equally to this work; co-first authors.*

Poster 11. Computer aided nanoparticles design through Enalos InSilicoNano Platform Georgia Melagraki, Antreas Afantitis
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Engineered nanoparticles (ENPs) are being extensively used in a great variety of application with a pace that is increasingly growing. The evaluation of the biological effects of ENPs is of outmost importance and for that experimental and most recently computational methods have been suggested. In an effort to computationally explore available datasets that will lead to ready-to-use applications we have developed and validated a QNAR model for the prediction of the cellular uptake of nanoparticles in pancreatic cancer cells. In this work we have tried to address the need of robust and predictive QNAR models for the assessment of the biological profile of ENPs and on top of that the proposed model has been made available online through Enalos InSilicoNano platform. In the proposed workflow all computational steps were incorporated in the platform and this complete line of operations was made feasible with the invaluable help of our in house made Enalos KNIME nodes, namely Enalos Mold2 node, Enalos Model

Acceptability Criteria node and Enalos Domain – Similarity node. These nodes have been developed by Novamechanics Ltd and are publicly available through the KNIME Community and the company's website. The platform was used in a virtual screening framework to identify promising compounds within PubChem. Within this proposed strategy EnalosInSilicoNano platform emerges as a key component for evaluating novel nano-structures that have not been experimentally evaluated or even synthesized.

Keywords: *Nanoparticles, Quantitative Nano-structure Activity Relationship (QNAR), PubChem, MQN descriptors, Enalos InSilicoNano platform*

Acknowledgments: *"This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no 310451 (Project NanoMILE)."*

Poster 12. Evaluation Of In Vitro Toxicity Of Nanoparticles In Inverted 3D Culture Systems

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To assess the potential toxicity of manufactured or engineered nanoparticles, traditional in vitro toxicity studies have been performed using normal 2D culture system. Different outcomes of toxicity have been argued in vitro studies according to their own physicochemical properties in contrary to small chemicals. Here, we introduce the inverted 3D culture systems based on 96-well plates to examine the cytotoxic effects of nanoparticles. Silica nanoparticles with four different sizes (70, 150, 150, and 300 nm) and silver nanoparticles are exposed to 3D hydrogel droplets containing HepG2 cells on the top insert pillar. The cytotoxic effects of nanoparticles are evaluated using tetrazolium reduction and ATP assay and then compared to the results from normal 2D culture exposure. The agglomeration and total numbers of silica and silver nanoparticles depending on volumes provoke the artifacts of cytotoxicity in normal 2D culture system.

The results reveal that 3D inverted culture system shows the more accurate cytotoxic effects comparing to normal 2D culture, by reflecting nanoparticles would be exposed to more actual transporting dose in our system. This system would significantly improve the predictive power of in vitro nanotoxicity assessment.

Keywords: *Nanoparticles, in vitro Toxicity, Nanotoxicity, Inverted Culture*

Notes: *Soojin Kim and Jung-Hwa Oh contributed equally to this work; co-first authors.*

Poster 13. Human Pluripotent Stem Cell-Derived Hepatocyte as a Useful Model for Detecting Toxic Chemicals Triggering AHR Signaling

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Human pluripotent stem cell (hPSC)-derived hepatocyte has been considered to be the most promising cell model for hepatotoxicity testing.

However, it has not been well predictive because of its low expression and activity of drug metabolizing enzymes (DMEs). In this study, we measured mRNA levels of a variety of DMEs and their major regulators including AHR, CAR and PXR during in vitro hepatic differentiation. The mRNA expression levels of most DMEs regulated by CAR and PXR were considerably low in hPSC-derived hepatocytes. On the other hand, the mRNA expression levels of CYP1A1 and CYP1B1 regulated by AHR were comparable to those seen in

human adult hepatocytes. Moreover, AHR and its signaling components were active in hPSC-derived hepatocytes, whereas the expression of CAR and PXR mRNA was weak or negligible. In addition, to demonstrate the functional utility of AHR signaling in hPSC-derived hepatocyte, we measured the induction of several AHR downstream target genes in response to well-known AHR agonists.
Quantitative real-time polymerase chain reaction (qRT-CPR) analysis revealed strong induction of both CYP1A1 and CYP1B1 by benzo(a)pyrene (BaP), 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 3-methylcholanthrene (3-MC), and 2-(1'H-indole-3'-carbonyl)-thiazole-4-carboxylicacid methyl ester (ITE).
Furthermore, 6, 2', 4'-trimethoxyflavone (TMF), a known AHR antagonist, exhibited strong inhibitory effect on the transcriptional activity associated with AHR in hPSC-derived hepatocytes. These results indicated that hPSC-derived hepatocytes can be useful model for screening toxic substances triggering human AHR signaling pathway.
Keywords: *human pluripotent stem cell, hepatocyte, Aryl hydrocarbon receptor, drug metabolizing enzyme, Inverted Culture*

Poster 14. Simultaneous Transcriptomic Analysis Of Nephrotoxic Compounds In Different Target Organs

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To elucidate the molecular mechanisms associated with renal injury based on target organ interactions, we examined simultaneously the changes in expression profiles of liver, kidney, and the blood vessels after three nephrotoxicants including cyclosporin A (CsA), tacrolimus (FK506) and cyclophosphamide (CPM). Sprague-Dawley rats were treated daily with CsA, FK506, and CPM and then sacrificed at 1, 7, and 28 day after oral administration. Serum biochemistry for major injury markers and histopathological observation were also performed. Total RNAs were extracted from the liver, the kidney, and the thoracic aorta and then microarray analysis were performed. Differentially expressed genes were selected based on fold changes and significance and commonly expressed genes were compared among three target organs. The effects of three nephrotoxicants were also compared under each each target organ. Gene functions were analyzed using Ingenuity Pathways Analysis (IPA) and biological and toxicological function of significantly regulated genes showed that genes involved in metabolic activation, renal tubule injury, and endothelial cell proliferations in nephrotoxicants-treated group in the liver, the kidney, and the aorta, respectively. In CPM-treated group, inflammatory response related genes were commonly regulated in all target organs. Several genes expressed in dose dependent manner were selected the mRNA levels were validated by real time PCR. Here we suggests molecular pathways in the target organs in response to nephrotoxicants and also provide the information about candidate genes which can evaluate the toxicity induced by nephrotoxicants. These results may be helpful to elucidate underlying mechanism of target organ interaction during nephrotoxicity.
Keywords: *Nephrotoxicants, Organ interaction, Gene expression profiling*

Poster 15. Fullerenes potentially aggravate atherosclerosis onset by disregulating lipid homeostasis in vascular endothelial cells

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Fullerenes are a class of nanomaterial that fully consist of carbon atoms, taking the form of a hollow, spherical shape. Because fullerenes are hypothesized to be applied in the future clinic as drug delivery agents or as a diagnostic tool it is important to assess and understand their possible toxicological effects, especially when applied internally. Upon intravenous injection, the particles will encounter vascular endothelial cells, which provide a barrier between the blood and tissues all across the cardiovascular system. Vascular endothelial cells play a crucial role in maintaining blood vessel homeostasis and thereby are also essential to prevent atherosclerosis, which is still a leading cause of death worldwide.

Here, we aimed to unravel the toxicological effects of fullerenes on vascular endothelial cells and investigate underlying biological mechanisms, using previously published microarray data (1). Several systems biology tools were used, including PathVisio, WikiPathways and Cytoscape (2, 3). We show that in reponse to fullerenes, vascular endothelial cells alter their gene expression profile to a situation in which intracellular cholesterol production, collection and preservation seem to be the ultimate goals. As expected, this situation was suggested to be caused by the activation of SREBF1 and/or SREBF2, which are two transcription factors known to regulate intracellular lipid concentrations. How exactly fullerenes are able to activate SREBF1 and SREBF2 and subsequently upregulate cholesterol synthesis remains speculative.

Previously, Hassan et al. described that during atherosclerosis onset endothelial cells are able to remain homeostatic by shutting down intracellular cholesterol synthesis and reduction of cholesterol scavengers, in this way remaining atheroprotective (4). Our results suggest that introduction of fullerenes could lead to disruption of this process and thus worsen atherosclerotic onset. We propose that the toxicological effects of fullerenes in endothelial cells are explored in more detail and validate that cholesterol levels are indeed increased. Furthermore, potential clinical use should take caution when introducing fullerenes into the vascular system of humans prone to developing atherosclerosis.

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Keywords: *Fullerenes, Atherosclerosis, Pathway analysis*

Poster 16. nanoQSAR modelling using protein corona fingerprints

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Recent studies have shown that the presence of serum proteins within in vitro cell culture systems forms a protein adsorption layer (a.k.a. the "protein corona") on the surface of nanoparticles that affects nanoparticle-cell interactions and cell response [1, 2]. The protein corona thus encodes information about the interface formed between the nanoparticle and the cell surface within a physiological environment. Here, we analyze a number of recently published serum protein corona ‘fingerprints’ formed around a library of 105 surface-modified gold nanoparticles [3]. The models presented aggregate relative abundances of spectral counts with gene ontology information, particularly the gene ontology information specific to each protein corona are used to calculate a new set of descriptors, referred to as GO descriptors.

Additionally, parameters extracted from nanoparticle characterization assays (e.g. nanoparticle size, aggregation state, and surface charge) are considered. Our goal is to enrich the data using gene set information whlist emphasizing the importance of -omics data in modelling toxicity. Different QSAR models are compared in terms of coefficient of determination, Root mean squared error, and Akaike information criterion. To improve the prediction accuracy of the model we employ bootstrapping techniques. The final set of GO descriptors estimated by nanoQSAR models is further exploited for their biological relevance and functional similarity.

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Keywords: *protein corona, cell association, gene ontology, omics data, clustering*