



Welcome

Dear Colleagues and friends,

It is our pleasure to welcome you to the SEURAT-1 Symposium here at the Square meeting centre.

The research strategy termed "Safety Evaluation Ultimately Replacing Animal Testing" has given the name to SEURAT-1 indicating that this initiative is the first in a long-term strategy where more steps have to be taken before the ultimate target can be reached. The title of the Symposium, "Painting the future animal-free safety assessment of chemical ingredients: Achievements of SEURAT-1", was chosen on purpose to indicate that the acronym for SEURAT-1 goes also back to the French post-impressionist painter George Seurat (1859-1891) who is well-known for his innovative painting technique called "pointillism". As the pictures of George Seurat develop from numerous small coloured dots, which can only be meaningfully interpreted from some distance, the SEURAT-1 research has delivered new techniques and elements of scientific knowledge which form the individual cobblestones which, as a whole, pave the way towards the ultimate goal of a better science that does not need to use experimental animals. The Symposium will report on the achievements of SEURAT-1 in this direction.

The Symposium is a great opportunity to learn about the recent achievements and their impact in the field of alternative testing strategies; it allows you to network with renowned experts and get acquainted with the activities of other on-going and future initiatives. High-level presentations will showcase the SEURAT-1 success stories in a practical and accessible manner; an exhibition will then allow for deeper discussions. You will learn how the extensive research efforts during the last 5 years can be translated into solutions for safety assessment ultimately replacing animal testing. Other related on-going and future initiatives from the EU and the US will also contribute, with showcases of progress in the field, thus stimulating exchange and networking.

Sincerely,

Ian Cotgreave and Derek Knight, Co-Chairs of the Scientific Expert Panel of SEURAT-1

On behalf of the Symposium Organising Committee, composed of:

- Rob Taalman, Cosmetics Europe
- Elisabet Berggren, European Commission Joint Research Centre
- Ian Cotgreave, Swetox
- Christian Desaintes, European Commission
- Derek Knight, European Chemicals Agency
- Russell Thomas, US Environmental Protection Agency
- The COACH project team



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Note: The Conference organisers will take pictures and film the event. These pictures/films will be used for the sole purpose of documenting the event in reports and possibly in articles reporting the event publicly, for example on the SEURAT-1 project public website. Please contact the registration desk in case of objections.



ADDRESS

Square Brussels Meeting Centre

Website: <http://www.square-brussels.com/>

Glass entrance

rue Mont des Arts

B-1000 Brussels

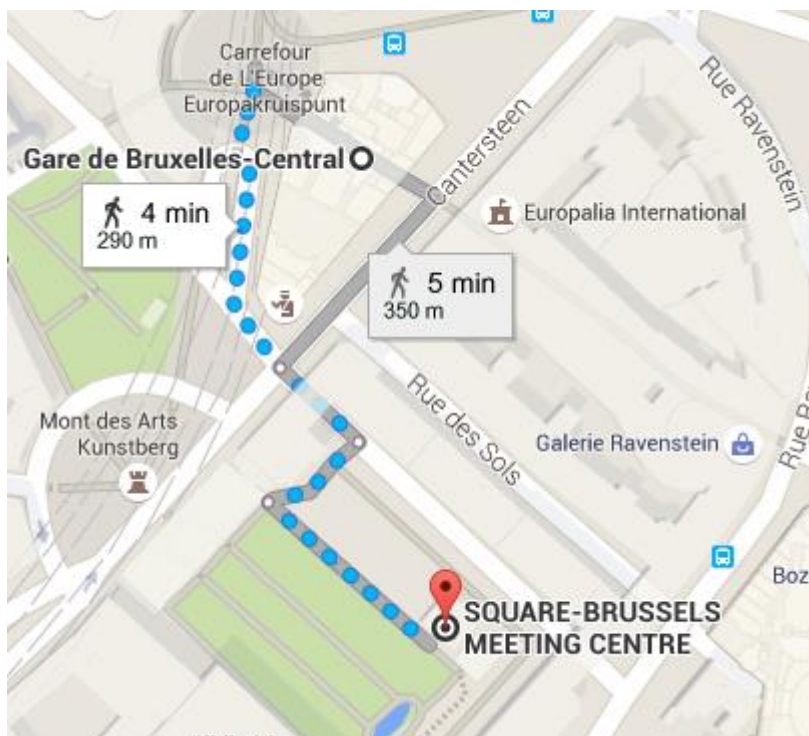
Phone number: +32 (0)2 515 13 00

info@square-brussels.com

HOW TO GET THERE ?

By train

SQUARE is just across the road from Brussels Central railway station.



National trains

Get off at Brussels Central and follow the exit signs to "Mont des Arts - SQUARE".

International trains (Eurostar, Thalys)

International trains arrive into Brussels Midi. From there, it's a simple two-minute train journey to Brussels Central. Just take any train heading north (to Antwerpen, Brussels International Airport, Dendermonde, Essen, Eupen, Genk, Hoei, Jemelle, Landen, Leuven, Liège, Louvain-la-Neuve, Luxembourg (L), Maastricht (NL), Schaarbeek, Sint-Niklaas, Tongeren, Turnhout), get off at the first stop (Brussels Central) and follow exit signs to "Mont des Arts - SQUARE".



By plane

At Brussels International Airport (Zaventem), make your way from the main arrival hall to level -1, and take a direct train for the city centre. After 17 minutes, get off at Brussels Central, and follow exit signs to "Mont des Arts - SQUARE".

By car

There are 660 parking spaces right underneath SQUARE. You can get in via Place de la Justice - Gerechtsplein, and Stuijversstraat - rue des Sols, and then walk straight into the building.

By public transport

There are numerous metro links, bus and tram stops close by: www.mivb.be. You also can hire a city bike from two nearby terminals: www.villo.be, or hail a licenced taxi from Central Station.

By taxi

You can contact one of the following companies:

- "Taxis bleus": <http://www.taxisbleus.be/en/> - Phone : +32 (0)2 268 00 00

- "Taxis verts" : <http://www.taxisverts.be/en/> - Phone : +32 (0)2 349 49 4

WIFI CONNEXION DETAILS

To get WiFi access proceed as follows:

*SSID (Network Name) : **square-guest***

If not automatically asked by your computer, open a web browser ; try to connect to any website and then you'll be automatically redirected to the authentication page.

Enter the login & password:

*Login: **Seurat-1!***

*Password: **Seurat2015!***

NB: password & login are case sensitive.

- 8:30 – 10:00 **Registration and coffee**
- 9:00 – 9:45 **Media Event** (restricted to journalists and SEURAT-1 representatives)

PLENARY SESSIONS (THE SQUARE CONFERENCE CENTRE - SILVER HALL)

Chair: Ian Cotgreave, Swetox

- 10:00 – 10:30 **Opening addresses** from the European Commission and Cosmetics Europe
*Speakers: Ruxandra Draghia-Akli, European Commission, Director of the Health Directorate at the DG RTD
John Chave, Cosmetics Europe, Director General
Lowri Evans, European Commission, Director General of the DG GROW*
- 10:30–11:40 **High-level presentation on major achievements in SEURAT-1**
What is SEURAT-1?
Speaker: Michael Schwarz, University of Tübingen
What has SEURAT-1 achieved?
Speaker: Mark Cronin, Liverpool John Moores University
What is the impact of SEURAT-1 in the international context?
Speaker: Rusty Thomas, US Environmental Protection Agency
- 11:40-12:10 **Consequences and next steps**
EU-ToxRisk: a new safety sciences flagship program on the horizon
Speaker: Bob van de Water, Leiden University
SEURAT-1 - Continuing the Successful Journey
Horst Wenck, Beiersdorf, Cosmetics Europe
- 12:10–12:30 **Concluding remarks**
*Speakers: Ian Cotgreave, Swetox
Derek Knight, European Chemical Agency
Arnd Hoeveler, European Commission, Head of Unit Innovative tools, technologies and concepts in health research at the DG RTD*

EXHIBITION (THE SQUARE CONFERENCE CENTRE - SILVER & MAGRITTE FOYERS)

12:30–14:00 Finger lunch & Exhibition

14:00–16:00 Exhibition composed of two parts

Guided educational tour: The visitors will be introduced into the field of non-animal-based toxicity testing, including its present limitations. They will be guided through a series of stations aligned in a certain logical sequence. The order of stations is given by the “SEURAT-1 conceptual framework” (see pages 8-9).

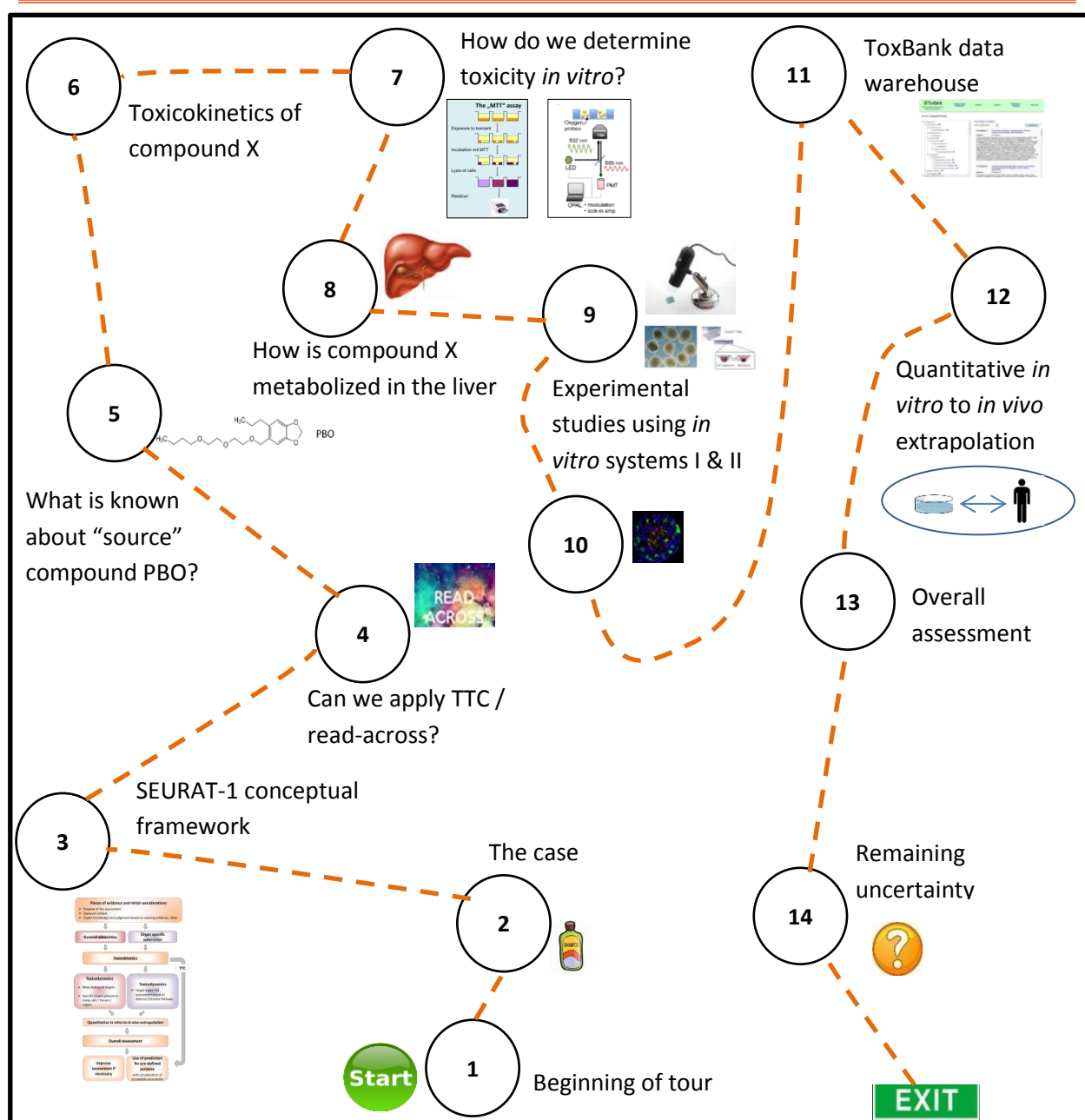
Scientific exhibition: The scientific results will be displayed on posters and hands-on demonstrations presented by scientists from the SEURAT-1 cluster projects and other EU and US initiatives (see page 10).

SEURAT-1 film: During the exhibition the SEURAT-1 film will be projected in the auditorium

16:00–18:00 Coffee & networking

The Guided Educational Tour - **one of the highlights** of the SEURAT-1 symposium and a unique opportunity to learn about alternative safety assessment methods explained in layman's terms. The Guided Educational Tour will take the audience through a typical case where the safety of a chemical ingredient is assessed using non-animal test methods and tools that were developed in SEURAT-1.

MAP OF THE GUIDED EDUCATIONAL TOUR STATIONS



MAP LEGEND AND MORE INFORMATION

	Station	More information
1	Beginning of Tour	
2	The case	
3	Seurat-1 conceptual framework	
4	Can we apply TTC / read-across?	<i>See guided tour poster: What is TTC/read-across? See also Case study stand in scientific exhibition</i>
5	What is known about “source” PBO?	<i>See also ToxCast stand in scientific exhibition</i>
6	Toxicokinetics of compound X	
7	How do we determine toxicity in vitro?	<i>See guided tour poster: What is omics? See guided tour poster: What is an AOP?</i>
8	How is compound X metabolised in the liver?	<i>See guided tour poster: What biological models do we have to simulate liver function?</i>
9	Experimental studies using <i>in vitro</i> systems I	<i>See guided tour poster: What is an iPS cell? See also Scr&Tox, Notox, DETECTIVE and HeMiBio stands in scientific exhibition</i>
10	Experimental studies using <i>in vitro</i> systems II	<i>See also Scr&Tox, Notox, DETECTIVE and HeMiBio stands in scientific exhibition</i>
11	ToxBank data warehouse	<i>See also ToxBank stand in scientific exhibition</i>
12	Quantitative <i>in vitro</i> to <i>in vivo</i> extrapolation	<i>See also Case study stand in scientific exhibition</i>
13	Overall assessment	
14	Remaining uncertainty	



Scientific exhibition

Silver Foyer

The **scientific exhibition** (Silver Foyer) includes posters and demonstrations of highlights of SEURAT-1 initiative and US related initiative, ToxCast.

It includes height stands as follows:

- **The six SEURAT-1 research projects:**
 - o **NOTOX** (*Predicting long-term toxic effects using computer models based on systems characterization of organotypic cultures*)
 - o **HeMibio** (*Hepatic Microfluidic Bioreactor*)
 - o **Detective** (*Detection of endpoints and biomarkers for repeated dose toxicity using in vitro systems*)
 - o **SCR&Tox** (*Stem cells for relevant efficient extended and normalized toxicology*)
 - o **COSMOS** (*Integrated In Silico Models for the Prediction of Human Repeated Dose Toxicity of Cosmetics to Optimise Safety*)
 - o **ToxBank** (*Supporting Integrated Data Analysis and Servicing of Alternative Testing Methods in Toxicology*)
- **Case Studies** set up to achieve cluster-level objectives
- **ToxCast** (US EPA's Toxicity Forecaster): a US research initiative which generates data and predictive models on thousands of chemicals of interest to the EPA.

Abstracts of each stand are presented in the following section.

NOTOX STAND

Abstract n°1	Development of 3D-hepatic model using HepaRG cells for repeated dose toxicity assessment	p.12
Abstract n°2	In silico modeling for the prediction of dose and pathway related adverse effects in humans from <i>in vitro</i> repeated-dose studies	p.13
Abstract n°3	ToxicoProteomics as a way to gain insights into molecular responses to toxicants: liver toxicity investigation using <i>in vitro</i> HepaRG cells	p.14
Abstract n°4	Multi-level spatially resolved modeling of toxic damage and its consequence: APAP & ammonia detoxification	p.15
Abstract n°5	A 3D <i>in vitro</i> HepaRG model for the identification and study of compounds with cholestatic liability	p.16
Abstract n°6	Coupled Modeling of PBPK and Mechanisms of Action of Valproic Acid Toxicity in Liver	p.17

Abstract n°1

Development of 3D-hepatic model using HepaRG cells for repeated dose toxicity assessment

Daniel Mueller¹, Patrina Gunness², Lisa Krämer¹, Valery Schevchenko³, Elmar Heinzle¹, Magnus Ingelman-Sundberg² and Fozia Noor¹

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Due to lack of adequate *in-vitro* models or methods of limited application, repeated-dose toxicity is still heavily dependent on animal studies in the assessment of long-term safety of compounds such as drugs and chemicals. This is mainly due to the fact that liver cultures rapidly lose their functions in 2D cultures and cannot be maintained viable and functional longer than a few days. 3D environment is essential for maintaining cell-cell communication and cell to matrix contacts which in turn is necessary for viability and functions. We established hanging drop 3D spheroid cultures of differentiated HepaRG cells and maintained them in culture for several weeks. The viability was constant for 4 weeks and longer. These hepatic cultures were functional during the tested period of 3 weeks as was shown by constant albumin production, transporter and CYP 450 activity. We tested several compounds in repeated dose toxicity studies which show that 3D HepaRG cultures are more sensitive to acetaminophen, aflatoxin B1, troglitazone and rosiglitazone toxicity. Toxicity studies show that the organotypic cultures are more sensitive to acetaminophen- and rosiglitazone induced toxicity but less sensitive to troglitazone-induced toxicity than the 2D cultures. These 3D cultures are amenable to high throughput screening of compounds. 3D organotypic HepaRG cultures are a promising preclinical tool in the study of human relevant long-term repeated-effects and in the assessment of chronic drug-induced hepatotoxicity.

References:

Gunness P, Mueller E, Shevchenko V, Heinzle E, Ingelman-Sundberg M, Noor F (2013) 3D organotypic cultures of human HepaRG cells: a tool for in vitro toxicity studies *Tox Sci*, 133:67-78. doi:10.1093/toxsci/kft021.

Mueller D, Krämer L, Hoffmann E, Klein S, Noor F. (2014) 3D organotypic HepaRG cultures as in vitro model for acute and repeated dose toxicity studies. *Toxicol In Vitro*. **28**:104-12. doi: 10.1016/j.tiv.2013.06.024.

Abstract n°2

***In silico* modeling for the prediction of dose and pathway related adverse effects in humans from *in vitro* repeated-dose studies**

Sebastian Klein¹, Silvia Maggioni², Joachim Bucher³, Daniel Mueller¹, Jens Niklas³, Valery Shevchenko⁴, Klaus Mauch³, Elmar Heinzle¹, Fozia Noor¹

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For the reduction and ultimately replacement of animal studies in preclinical long-term toxicity assessment, a combined strategy of using advanced *in vitro* cell culture methods based on functional human cell cultures and computational modeling is expected to play an essential role. HepaRG cultures remain viable and functional for long periods and have been applied in toxicity studies. We have previously established a serum free culture medium for HepaRG cells in which they retain viability, transporter and metabolic activity. We used these cultures to assess long term toxicity of valproic acid and bosentan over a period of 28 days. The metabolic competence of the cultures and the transporter activity of the multidrug resistance pump 2 (MRP2) was monitored over this time under serum free conditions. *In vitro* biokinetics were also assessed for the consideration of plastic binding and compound degradation. The long-term dose response data was used to calculate oral equivalent doses for both valproic acid and bosentan. Using a simple PBPK model with a virtual population of 100, *in vitro* to *in vivo* extrapolation (IVIVE) is possible. The model predicts that bosentan is safe under the assumed conditions upon 4 weeks of exposure. However, valproic acid is predicted to be hepatotoxic in 4 and 47% of the virtual population at the maximum daily recommended dose after 3 and 4 weeks of exposure, respectively. The analysis of central carbon metabolism shows that there are significant changes in the glucose metabolism and urea production. These metabolic changes may have a pronounced impact in susceptible patients such as those with compromised liver function and urea cycle deficiency leading to idiosyncratic toxicity. The combination of modeling based on long-term *in vitro* repeated-dose data and metabolic changes allows the prediction of human relevant *in vivo* toxicity with mechanistic insights.

Reference:

Klein S, Maggioni S, Bucher J, Mueller D, Niklas J, Shevchenko V, Mauch K, Heinzle E, Noor F (2015) *In silico* modeling for the prediction of dose and pathway related adverse effects in humans from *in vitro* repeated-dose studies. *Tox Sci*, in press, doi: 10.1093/toxsci/kfv218.

Abstract n°3

ToxicoProteomics as a way to gain insights into molecular responses to toxicants: liver toxicity investigation using *in vitro* HepaRG cells

Georg Tascher¹, Daniel Müller², Sebastian Klein², Lisa Fredriksson³, Inger Johansson³, Valery Shevchenko⁴, Christiane Guguen-Guillouzo⁵, Christophe Chesné⁴, Magnus Ingelman-Sundberg³, Elmar Heinzle², Fozia Noor³, Alain van Dorsselaer¹, and Fabrice Bertile¹

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The emerging field of toxicoproteomics has been boosted by recent advances in quantitative and qualitative proteomic technologies and its increasing applications in toxicology testing and research. Therefore, in an attempt to develop *in vitro* toxicological approaches, *in vitro* screening tests for toxicity evaluation should nowadays take advantage of the wide diversity of proteomic platforms.

The NOTOX project (www.notox-sb.eu/; from the initiative “FP7-HEALTH-2010-Alternative-Testing-Strategies”) develops systems biology approaches for *in vitro* toxicity studies. Within that framework, the human hepatic cell line HepaRG, which has been shown to be suitable for functional cultivation up to several weeks, appeared as advantageous surrogate for primary human hepatocytes because of a better availability and no restriction because of donor-to-donor variances.

In a first set of experiments, we characterized the proteome of monolayer cultures of HepaRG cells using GeLC-MS/MS and 2D-gel electrophoresis combined with MS. Based on approximately 3800 identified proteins in total, HepaRG cells were shown to exhibit a phenotype very similar to that of functional liver cells. Subsequently, we showed good batch to batch reproducibility of the production/differentiation process of the cell line, by comparing three different HepaRG-batches using 2D-DIGE and label-free quantitative proteomics. Because secreted proteins are a valuable source for biomarkers, we also examined the secretome of HepaRG cells using shotgun proteomics, and identified 240 secreted proteins.

In a second set of experiments, we showed that acute acetaminophen (APAP) exposure alters cell's viability and triggers proteome changes. In particular, using a targeted proteomics strategy (LC-SRM), we found molecular regulations in line with the fact that APAP is predominately detoxified by glucuronidation rather than sulfation. Using a spectral-count-based proteomics strategy, we identified 189 proteins exhibiting differential abundance out of ~1500 analysed. Differences were either due to culture conditions or treatment with APAP with energy metabolism being the main affected biological process. We finally designed two different experiments to evaluate the impact of chronic valproic acid (VPA) exposure on monolayer and 3D-spheroid cultures of HepaRG cells. Using a label-free proteomics approach (XICs), ~2000 proteins could be quantified of which ~170 were found differentially expressed as a function of time and/or VPA dose. Pathway analysis of

the differential proteins indicated that, among other processes, lipid metabolism was affected by VPA-treatment, which is in accordance with the expected steatotic effects of VPA. Furthermore, the effects on the proteome were visible already when commonly applied endpoint assays like e.g. cell viability or ATP content did not yet indicate toxicity.

In a systems biology perspective, proteomics results were integrated with the transcriptomics and metabolomics data obtained by the NOTOX consortium. Simultaneous visualization of different omics data using home-developed bioinformatics tools was helpful to better understand these data.

In summary, HepaRG cells represent a good model system for *in vitro* toxicity studies, provided that culture conditions are sufficiently optimized. Applied to HepaRG cells, toxicoproteomics is powerful enough to produce new data of importance for gaining insights into adverse outcome pathways.

Contact: Dr. Fabrice BERTILE, IPHC-LSMBO, CNRS-Université de Strasbourg, fbertile@unistra.fr

Abstract n°4

Multi-level spatially resolved modeling of toxic damage and its consequence: APAP & ammonia detoxification

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On this poster we will present a multi-scale model permitting to mimic the impact of drugs and toxins on tissue architecture and composition, as well as its consequences on cell metabolism. As an example, we consider acetaminophen (APAP) overdose induced of damage in a liver lobule within a model displaying the lobular architecture. Using the same model, we consider the ammonia detoxification after CCl₄-induced peri-central tissue damage. CCl₄ generates a similar damage pattern as APAP. The model integrates ODE models of chemical reactions after APAP administration, and of ammonia detoxification (Schliess et al., Hepatology, 2014; Ghallab et al., J. Hepatol. (accepted)) into a spatial temporal agent-based model, in which each hepatocyte is represented as individual unit (Hoehme et al., PNAS, 2010; Drasdo et al., J. Hepat. 2014). The lobular architecture has been constructed from confocal laser micrographs using the recently established software TiQuant (Hammad et al., Arch. Toxicol. 2014; Friebel et al., Bioinformatics, 2015).

Blood and bile flow, as well as molecular transport with blood and bile are included in the model. Cells can grow and divide or undergo apoptosis or necrosis. The model is integrated in a novel software (TiSim) that should be made available upon publication of results generated with it. The tissue architecture can readily be parameterized from the image analysis software TiQuant. The advantage of this approach is that it permits to represent the precise experimental setting (Fig. below; e.g. bioreactors with the precise transport and/or supply with nutrients, growth factors or drugs as well as the distribution of cells and composition of cell types) and cell-to-cell variability representing each individual cell.

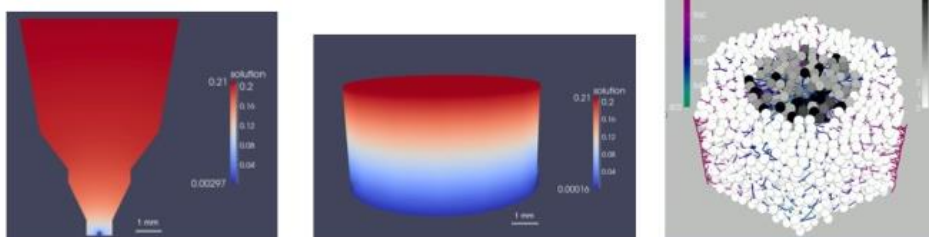


Fig.: Settings in modeling multi-level cell testing *in vitro* (left, middle) and *in vivo* (right). Left: Oxygen simulation in a well with a multicellular spheroid in the bottom. Middle: equivalent simulation in a monolayer, with cells located on the bottom of the culture disk. Right: Multiscale simulation of necrosis after APAP overdose in a hepatic lobule.

Abstract n°5

A 3D *in vitro* HepaRG model for the identification and study of compounds with cholestatic liability

Lisa Fredriksson Puigvert*, Delilah Hendriks* and Magnus Ingelman-Sundberg

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Drug-induced cholestasis (DIC) is one of the leading causes of drug-induced liver injury and often only manifests weeks/months after the start of drug treatment. Preclinical detection of DIC is still often limited to measuring the compound's potential to inhibit the bile salt export pump (BSEP). Yet, recent studies emphasize the importance to consider other mechanisms by which drugs can induce cholestasis and it is clear that there is a need for novel *in vitro* models which allows for a comprehensive analysis of the cholestatic risk of compounds. HepaRG cells and spheroids have previously been described as appropriate for long-term toxicity testing and here we show that the spheroids accurately express two main bile acid (BA) transporters, bile salt export pump (BSEP) and multidrug resistance-associated protein 2 (MRP2) at the site of the bile canaliculi (Fig 1A); this makes them suitable for long-term toxicity testing of cholestatic drugs. By co-exposing the HepaRG spheroids to a non-toxic dose of a bile acid mix and compounds known to cause cholestasis (bosentan, chlorpromazine and troglitazone) we show that the bile acid co-exposure led to enhanced toxicity after 14 days of repeated dosing (Fig 1B). Importantly, this effect was not observed with the hepatocellular toxicant paracetamol or steatosis-inducing tetracycline (Fig 1B). Furthermore, in accordance with the definition of cholestasis, chlorpromazine induced accumulation of bile acids after 8 days of repeated dosing in the spheroids, accompanied by decreased BSEP mRNA expression (Fig 1C). Finally, when further investigating the mechanism behind the synergistic toxicity between bile acids and chlorpromazine, we found a selective increase in oxidative stress dependent sulfiredoxin 1 (SRXN1) expression as well as an increase in death receptor 5 (DR5) RNA (Fig 1D). In summary the use of HepaRG spheroids allows for distinction of compounds with cholestatic liability, as well as in depth mechanistic studies of cholestatic liver injury.

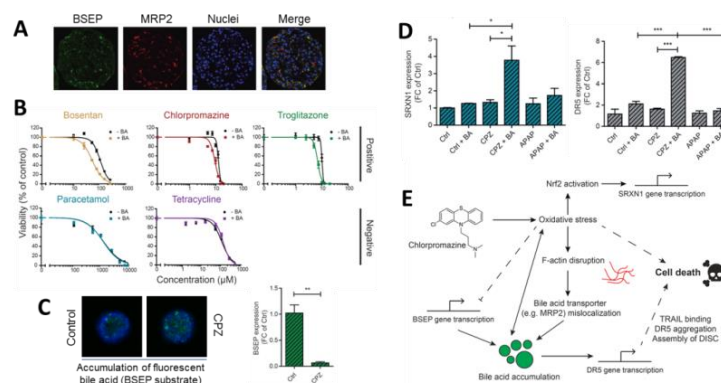


Figure 1. HepaRG spheroids as a model for the detection of compounds with cholestatic liability and for mechanistic studies. A – Immunofluorescent staining of fixed and cryosectioned HepaRG

spheroids; B – The viability (ATP levels) after repeated dosing of compounds for 14 days with and without the presence of bile acids (BA); C – Live confocal imaging of fluorescent bile acid and RNA expression of BSEP in spheroids exposed to chlorpromazine (CPZ) for 8 days; D – CPZ+BA selectively induces the oxidative stress Nrf2 target gene SRXN1 as well as the death receptor 5 (DR5) RNA; E – Proposed mechanism of chlorpromazine induced bile acid accumulation (cholestasis) and their combined toxicity.

Abstract n°6

Coupled Modeling of PBPK and Mechanisms of Action of Valproic Acid Toxicity in Liver

Joachim Bucher¹, Juan Diaz¹, Sebastian Klein², Georg Tascher³, Inger Johansson⁴, Silvia Maggioni⁵, Fabrice Bertile³, Magnus Ingelman-Sundberg⁴, Alain van Dorsselaer³, Emilio Benfenati⁵, Fozia Noor², Elmar Heinzle², Lothar Terfloth¹, Klaus Mauch¹

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Cellular metabolic and toxicity-related mechanism of action models coupled to pharmacokinetic or physiologically-based pharmacokinetic (PBPK) models are advantageous because they allow simulating the connection of route and dose of administration to local effective toxic concentrations. Here, we present a PBPK model of valproic acid (VPA) distribution connected to models of hepatic VPA metabolism and mechanisms/modes of toxic action. This coupled pharmacokinetic/toxicokinetic model (PK/TK) comprises VPA metabolism as well as mechanistic mode of action steps attenuating cell viability via toxic metabolites and by disturbance of lipid metabolism. The cellular model was further identified with measured metabolite, transcript and protein data from the second case study VPA experiment on HepaRG culture.

To consider the zoned heterogeneity in liver lobules, which influences both substance clearance and toxic response, structured tissue and vascular flow models have to be implemented. Previous modeling studies demonstrated that PBPK models can be coupled to simple structured liver lobule and cellular network models (Ochoa et al., 2013). Here, we present a simple liver tubular flow model which captures essential periportal, midlobular and perivenous characteristics, coupled to PBPK. The advantage of a less granular model is its simplified definition, which still captures the essentials of the liver zonation and simultaneously increases the calculation performance in connection to the cellular networks, and its integration into a workflow that can be implemented with the KNIME software.

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This work is result of a cross-cluster cooperation between COSMOS and NOTOX.

Reference:

Diaz Ochoa JG, Bucher J, Péry AR, Zaldivar Comenges JM, Niklas J, Mauch K 2013. A multi-scale modeling framework for individualized, spatiotemporal prediction of drug effects and toxicological risk. *Front Pharmacol.* 3:204. doi: 10.3389/fphar.2012.00204.

HeMiBio STAND

<i>Abstract n°1</i>	Long-term culture and expansion of primary human hepatocytes	p.21
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Abstract n°1

Long-term culture and expansion of primary human hepatocytes

Gahl Levy¹, David Bomze¹, Stefan Heinz², Sarada Devi Ramachandran³, Astrid Noerenberg², Merav Cohen^{1,6}, Oren Shibolet⁴, Ella Sklan⁵, Joris Braspenning^{3,7} & Yaakov Nahmias^{1,6}

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Hepatocytes have a critical role in metabolism, but their study is limited by the inability to expand primary hepatocytes *in vitro* while maintaining proliferative capacity and metabolic function. Here we describe the oncostatin M (OSM)-dependent expansion of primary human hepatocytes by low expression of the human papilloma virus (HPV) genes *E6* and *E7* coupled with inhibition of epithelial-to-mesenchymal transition. We show that *E6* and *E7* expression upregulates the OSM receptor gp130 and that OSM stimulation induces hepatocytes to expand for up to 40 population doublings, producing 10¹³ to 10¹⁶ cells from a single human hepatocyte isolate. OSM removal induces differentiation into metabolically functional, polarized hepatocytes with functional bile canaliculi. Differentiated hepatocytes show transcriptional and toxicity profiles and cytochrome P450 induction similar to those of primary human hepatocytes. Replication and infectivity of hepatitis C virus (HCV) in differentiated hepatocytes are similar to those of Huh7.5.1 human hepatoma cells. These results offer a means of expanding human hepatocytes of different genetic backgrounds for research, clinical applications and pharmaceutical development.

Abstract n°2

Transforming predictive toxicology: Real-time monitoring of metabolic function in liver-on-chip microdevices using tissue-embedded biosensors

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The development of prescription drugs and cosmetics requires the assessment of long-term toxicity. Current methods rely on daily drug exposure, and dozens of end-point assays, resulting in limited kinetic information and prognostic value. Here, we present a liver-on-chip device capable of maintaining metabolically active liver organoids for over a month *in vitro* under oxygen gradients mimicking the native microenvironment. Mitochondrial respiration was monitored using two-frequency phase modulation of phosphorescent microprobes embedded in the tissue. Phase modulation is focus independent and unaffected by cell death or migration providing *real-time* measurement of cellular respiration. A computer-controlled microfluidic switchboard allowed for contiguous electrochemical measurements of glucose and lactate concentrations providing unparalleled insight into minute shifts from mitochondrial respiration to anaerobic glycolysis, early indication of mitochondrial stress. We show a hereto-unknown mechanism of acetaminophen (Tylenol®) toxicity that is independent of CYP450-metabolism, and thus might be responsible for clinically observed nephrotoxicity and dermatitis. We also demonstrate sub-threshold effects of the anti-diabetic drug Troglitazone (Rezulin®) and anti-arrhythmic medication Amiodarone (Cordarone®) on mitochondrial respiration. Taken together, our work marks the importance of tracing toxicity effects in real-time, demonstrating specific advantages of human-on-chip technology in predictive toxicology.

Abstract n°3

Integrative analysis of genome-wide DNA-methylation, microRNA and gene expression patterns in purified, uncultured human liver cells and activated hepatic stellate cells

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Fibrosis, or scarring of the liver, is a chronic wound-healing response that recruits a range of cell types and mediators to intercept the injury caused by virtually all types of chronic liver injury such as viral infections, auto-immune, cholestatic and metabolic diseases as well as drugs or alcoholic-induced injury. This pathologic condition is characterized by the excessive accumulation of extracellular matrix proteins that causes stiffening of the liver. Fibrosis is a dynamic process which can, if left unmanaged, progress to worse forms of liver disease such as cirrhosis and cancer. A main event during fibrogenesis is the activation of hepatic stellate cells (HSCs) during which they transdifferentiate from cells with a quiescent phenotype into cells with a fibrogenic-myofibroblast-like phenotype, the most downstream cellular effectors of liver fibrosis.

In HeMiBio we proposed to generate a liver-simulating device reproducing the heterotypic interactions between hepatocytes (HEPs) and non-parenchymal cells (HSCs, Liver sinusoidal endothelial cells (LSECs)) of the liver that could serve to test the effects of chronic exposure to cosmetic ingredients. Ideally, this system would also allow for the reliable detection of potential pro-fibrogenic compounds.

A handful of studies in rodent cells and models of fibrosis suggest that the activation of HSCs is under tight control of a number of epigenetic mechanisms, including DNA methylation, histone modifications and the activities of non-coding RNAs. A role for these mechanisms in

the control of human HSC activation however remains to be demonstrated and gaining more insight into the nature of these changes yields the great potential to discover novel biomarkers for disease stage and progression that are of potential interest to HeMiBio.

Using a two-step collagenase perfusion technique and fluorescence-activated cell sorting, we isolated HEPs, HSCs ($UV^+ CD32^- CD45^-$) and LSECs ($CD32^+ CD45^-$) from healthy cadaveric liver tissue. The purity of the different liver cell populations was evaluated by differential expression of distinct liver cell type marker genes. A collaborative effort between different HEMIBIO partners resulted in the first comprehensive and integrative analysis of the transcriptome and genome-wide promoter DNA methylome, microRNA expression profile and locus-specific changes in histone modifications that underpin the differentiated phenotype of these liver cell populations. By mapping the changes in the epigenetic blueprint of qHSCs following culture activation, we identified a set of potential relevant markers that could be used to monitor the activation state of HSCs.

Abstract n°4

Hepatocyte differentiation from pluripotent stem cells: remaining hurdles

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Although many studies have demonstrated that cells with hepatocyte-like features (hepatocyte-like cells, HLCs) can be generated from pluripotent stem cells (PSCs), they still do not have all functions of mature hepatocytes. By combining transcriptome, epigenetic and metabolic profiling, we demonstrate here that the HLCs continue to have features of fetal hepatocytes. HLCs do not express a complement of hepatocyte transcription factors (TFs), and fail to express a number of phase I- and II-detoxifying enzymes. In addition, the HLCs incorrectly express TFs for non-hepatocyte lineage cells. Furthermore, promoters and enhancers of key hepatocyte genes remain marked by inactivating epigenetic modifications. Finally we show that the metabolic profile of HLCs is more akin to PSCs than mature hepatocytes, i.e. high expression of glycolytic enzymes and low expression of mitochondrial associated genes, including the key mitochondrial biogenesis gene, PGC-1 α . These insights should enable to systematically address each of these bottlenecks in the hepatocyte differentiation protocol to create mature hepatocytes with drug metabolism capabilities similar to those of primary hepatocytes to fully model hepatocyte toxicity.

Abstract n°5

Efficient recombinase mediated cassette exchange in hPSC to study the hepatocyte lineage

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Tools for rapid and efficient transgenesis in a “safe harbor” locus in an isogenic context are of importance to exploit the use of human pluripotent stem cells (hPSC) in for instance toxicology studies. We created hPSC master cell lines that allow very fast and efficient FLPe recombinase-mediated cassette exchange (RMCE) by incorporating a FRT-flanked cassette in the *AAVS1* that allows positive and negative selection of the incoming cassette. Using RMCE, we successfully incorporated several transgenes useful for lineage identification, cell toxicity studies, and gene over-expression, and this within 10-15 days, with 100% efficiency and without random integrations. Even though we observed unexpected and variable inhibited expression of some transgenes in the *AAVS1* locus *in vitro*, due to DNA methylation and other unknown mechanisms, both in undifferentiated hESC and differentiating hepatocytes, the created cell lines are very useful for incorporation of toxicity sensing cassettes, and / or for induced overexpression of for instance transcription factors to improve hepatocyte differentiation.

Abstract n°6

Human hepatic organoid model for drug-induced liver fibrosis

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Repeated liver injury (by drugs, alcohol or viruses) leads to the development of chronic liver diseases that degenerate into fibrosis and subsequent cirrhosis. In Europe, liver cirrhosis accounts for around 170,000 deaths a year according to WHO.

At the organ level, liver fibrosis results from the interplay of different cell types which can be summarized as a response of the hepatic stellate cells (HSCs) to hepatocyte injury. This response results in the accumulation of collagen and consequent formation of scar tissue in the liver. Even though liver fibrosis is reversible, there is still no clinically efficient treatment. Drug development is hampered by the poor predictivity of the *in vitro* and *in vivo* (normally rodent) models to test liver fibrosis. While *in vitro* models have the limitation of being too simple or incomplete, *in vivo* ones have not only ethical limitations, but also low relevance to human liver disease.

Within HeMiBio we developed a 3D human hepatic co-culture system consisting of HSCs and hepatocytes which mimics drug-induced liver fibrosis. We test it by using three different compounds that are expected to induce toxicity and/or a fibrosis outcome. Cells were exposed to the compounds once or repeatedly over a 14 day period. Outcomes were evaluated at the toxicological level, gene expression level and increased production of collagen, a hallmark of liver fibrosis.

We detected different toxicity profiles for each compound which altered upon repeated exposure, and the fibrosis outcomes were independent of the toxicity observed, but was always present after repeated exposure with these compounds.

In conclusion, we developed an *in vitro* co-culture model that mimics drug-induced liver fibrosis. Further development of such co-culture models is of high relevance for early identification of potentially pro-fibrotic compounds in the pharma industry and could also be used to test novel anti-fibrotic drugs.

DETECTIVE STAND

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Abstract n°1

A High Content Imaging (HCI) BAC-GFP Toxicity Pathway Reporter Platform for Mechanism-based Assessment of Drug-Induced Liver Injury (DILI)

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DILI remains a major concern for drug development and in clinical practice. At the moment primary human hepatocytes are regarded as the golden standard for DILI toxicity testing. However, problems with the availability, inter-donor variability and stability (dedifferentiation of hepatocytes and thus loss of CYP/drug metabolizing capacity) remain a critical issue. Here we present a BAC-GFP reporter platform in which we monitor the activation of mal-adaptive stress pathways that are typically activated by chemical-induced cellular injury. We have established and characterized reporters for oxidative-stress (KEAP1/Nrf2 pathway), ER-stress (UPR pathway), and DNA-damage (p53 pathway), allowing single cell time-resolved and quantitative analysis of the toxicity pathway activation. We have exposed these individual reporters to a library of >150 DILI compound and mapped the dynamic activation of these toxicity pathways in a 24 hr time period at 1, 5, 10, 50 and 100 Cmax concentrations. We applied bioinformatics tools to cluster the entire time-concentration HepG2-BAC-GFP reporter response profiles of all compounds. Our approach allows the clustering of similar mode-of-action compounds. Moreover, using our screening strategy we do enrich for compounds with severe DILI drug labeling. We anticipate that our cellular stress response reporters in combination with HCI may play a key role in future safety assessment of DILI as well as other toxicity liabilities.

Abstract n°2

***In vitro* verification of an adverse outcome pathway of cholestatic liver injury using “omics” technologies**

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Cholestasis is a liver disorder caused by the abnormal accumulation of bile in the liver. It accounts for about half of the cases of drug-induced liver injury and is characterized by bilirubinuria and hyperbilirubinemia that subsequently lead to icterus and pruritus. An adverse outcome pathway (AOP) construct has been introduced to pinpoint the mechanisms involved in the development of cholestasis. Hereby, the inhibition of the bile salt export pump (BSEP) is considered as the main molecular initiating event. The key events which are subsequently triggered include bile accumulation, induction of oxidative stress and inflammation, and the activation of specific nuclear receptors. The present study evaluates the reliability and predictive capacity of the established AOP for cholestatic liver injury. For this purpose, human hepatoma-derived HepaRG cells were exposed to sub cytotoxic concentrations of bosentan, a potent BSEP inhibitor and a clinically relevant cholestasis inducer. The cellular response to the inflicted toxicity was evaluated by a series of ‘omics’ read-outs, namely transcriptomics, proteomics, metabolomics and epigenomics. Transcriptomics analysis showed that half of the predicted gene changes related to the activation of nuclear receptors were correctly modulated. Pathway analysis further identified cholestasis as a major toxicological event induced by bosentan. Furthermore, 37 genes could be selected as potential novel transcriptional biomarkers of bosentan-induced

cholestasis based on the discrepancy in expression between treated cells and respective controls. In parallel, proteomics analysis showed 145 proteins with significantly higher expression in HepaRG cells exposed to bosentan. Some of these proteins could be mechanistically linked to cholestasis and may represent unidentified key events and thus toxicity biomarkers. Metabolomics analysis indicated the presence of specific metabolites that collectively point to mitochondrial impairment, another key event in cholestasis development. Epigenomics analysis identified several differentially methylated genes following exposure of HepaRG cells to bosentan, yet the mechanistic relevance of these findings remains to be elucidated. Overall, the results of this study underscore the scientific soundness of the established AOP and demonstrate that AOPs are flexible tools that may be continuously optimized by feeding in new experimental data.

Abstract n°3

In vitro based prediction of human hepatotoxicity

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Drug induced liver injury represents one of the most critical issues during drug development and leads to failure of many drug candidates during preclinical or clinical studies. Currently, the common model for safety evaluation/human health risk assessment is repeated dose toxicity (RDT) testing in rodents. RDT studies require numerous animals and the capacity for this conventional testing is limited. However, this goal remains challenging as complex *in vivo* processes like absorption, distribution, metabolism, excretion (ADME) and different mechanisms of toxicity need to be addressed by a network of reliable test systems. *In vitro* based prediction of hepatotoxicity is challenging, because it requires an *in vitro* system, which reflects critical mechanisms of *in vivo* toxicity. In this project we evaluated –omics readouts to identify predictive biomarkers and established an *in vitro* based model to predict human hepatotoxic blood concentrations. We used publically available, genome wide expression data from 150 compounds tested in primary human hepatocytes at a slightly cytotoxic concentration. The following strategy was applied to identify potential biomarker candidate genes: (i) Identification of genes that are altered by many compounds. (ii) Identification of genes, which are as well altered in human liver diseases such as cirrhosis, hepatocellular carcinoma and non-alcoholic steatohepatitis. The overlap with human liver disease genes implies a certain role for the gene *in vivo* and makes it is less probable that the chemically induced deregulation is just an *in vitro* artifact. (iii) Exclusion of unstable baseline genes, which are altered just by the hepatocyte isolation and cultivation procedure. (iv) Selection of genes belonging to various biological motifs to cover the most relevant toxic mechanisms. From the top genes with the highest fold changes among all compounds, 7 genes were selected as biomarkers: CYP 1B1, CYP 3A7, SULT 1C2, G6PD, TUBB2B, RGCC and FBXO32. These genes cover the biological motifs metabolism of xenobiotics, energy and lipid metabolism, cell cycle and cytoskeleton as well as protein degradation. A set of hepatotoxic as well as non-hepatotoxic compounds was defined and literature search was performed to identify plasma peak concentrations at therapeutic doses. HepG2 cells as well as primary human hepatocytes were exposed for 24h and each compound was tested in a concentration range covering the plasma peak concentration but also ranging up to slightly cytotoxic concentrations. Two readouts were used to evaluate the hepatotoxic potential of the compounds: (i) the expression of the selected biomarker genes was analyzed. The *in vitro* alert concentration was defined as the lowest concentration that causes a significant increase of at least 2.5 fold induction of at least one biomarker. (ii) Cytotoxicity tests were performed to identify the lowest cytotoxic concentration, corresponding to 20 % loss of viability. Both readouts were considered to identify the lowest observed effect concentration *in vitro*, which was finally compared to the plasma peak concentration of a therapeutic dose *in vivo*. Already in HepG2 cells, the prediction model separates hepatotoxic



from non-hepatotoxic compounds. The majority of hepatotoxic compounds show alerts at concentrations *in vitro* which correspond to therapeutic doses *in vivo*. In primary human hepatocytes, the prediction sensitivity improves and hepatotoxic effects are observed at even lower concentrations. Preliminary results indicate that both systems are suitable to predict human hepatotoxic blood concentrations, at least within a certain error range. Even some idiosyncratic compounds were identified and distinguished from compounds, which are not associated with an increased risk of hepatotoxicity. The novel prediction system might provide a promising tool to identify hazard compounds during early screening processes in drug development.

Abstract n°4

Identification of genomic biomarkers for anthracycline-induced cardiotoxicity in human iPSC-derived cardiomyocytes: an *in vitro* repeated exposure toxicity approach for safety assessment

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The currently available techniques for the safety evaluation of candidate drugs are usually cost-intensive and time-consuming and are often insufficient to predict human relevant cardiotoxicity. The purpose of this study was to develop an *in vitro* repeated exposure toxicity methodology allowing the identification of predictive genomics biomarkers of functional relevance for drug-induced cardiotoxicity in human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs). The hiPSC-CMs were incubated with 156 nM doxorubicin, which is a well-characterized cardiotoxicant, for 2 or 6 days followed by washout of the test compound and further incubation in compound-free culture medium until day 14 after the onset of exposure. An xCELLigence Real-Time Cell Analyser was used to monitor doxorubicin-induced cytotoxicity while also monitoring functional alterations of cardiomyocytes by counting of the beating frequency of cardiomyocytes. Unlike single exposure, repeated doxorubicin exposure resulted in long term arrhythmic beating in hiPSC-CMs accompanied by significant cytotoxicity. Global gene expression changes were studied using microarrays and bioinformatics tools. Analysis of the transcriptomic data revealed early expression signatures of genes involved in formation of sarcomeric structures, regulation of ion homeostasis and induction of apoptosis. Eighty-four significantly deregulated genes related to cardiac functions, stress and apoptosis were validated using real-time PCR. The expression of the 84 genes was further studied by real-time PCR in hiPSC-CMs incubated with daunorubicin and mitoxantrone, further anthracycline family members that are also known to induce cardiotoxicity. A panel of 35 genes was deregulated by all three anthracycline family members and can therefore be expected to predict the cardiotoxicity of compounds acting by similar mechanisms as doxorubicin, daunorubicin or mitoxantrone. The identified gene panel can be applied in the safety assessment of novel drug candidates as well as available therapeutics to identify compounds that may cause cardiotoxicity.

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Abstract n°5

Watching the detectives: Renal injury biomarkers

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Each kidney consists of approximately 1 million nephrons at birth. *De novo* nephrogenesis does not occur after birth and there is no strong evidence suggesting that the kidney harbours resident stem cells. Thus, the kidney unlike the liver is not a highly rejuvenative organ, which is most likely an evolutionary compromise allowing the anatomical complicity required for high function and the maintenance of the very narrow margins required for whole body homeostasis. Indeed, the kidney is an extremely accomplished organ and can carry out 100 % of its duties with only a fraction of the nephrons we are born with. However, we continually lose nephrons through-out life and will, all things being equal, eventually breach the renal functional reserve and enter end-stage-renal disease. Thus, anything that contributes to chronic renal failure has the potential to seriously curtail life quality and life-span. Aging populations and associated risk factors such as diabetes and heart disease, have pushed chronic kidney disease (CKD) incidence to unprecedented levels (currently stands at 10 % of the European population). Due to the role of the kidney in elimination of waste products, renal cells, particularly the proximal tubule, will internally process the majority of drugs and chemicals, and thus often have higher concentrations of these compounds than any other cell in the body. Compounds that injure renal epithelial cells can initiate and/or accelerate CKD. Within the DETECTIVE project we have endeavoured to further understand chemical-induced renal epithelial injury, recovery from injury and to identify biomarkers thereof. To this end we exposed an immortalised non-cancerous, stable highly differentiated human proximal tubule cell line, RPTEC/TERT1 to repeat exposures of model nephrotoxins. Together with our DETECTIVE collaborators we conducted transcriptomic (Agapios Sachinidis, UKK), epigenomic (Simone van Breda, UM), proteomic (Laxmikanth Kollipara and Rene Zahedi, ISAS), metabolomic (Hector Keun, ICL) and physiology-based assays. In this poster we present highlights of our findings over the last 5 years.

In memory of Andre Schrattenholtz - a no-nonsense guy !

Abstract n°6

Valproic acid case study: Detection and verification of biomarkers by using a read across approach

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Human safety evaluation in chemical risk assessment is currently mainly based on *in vivo* data i.e repeated dose (RDT) studies. Along with the restriction of animal testing, e.g. for cosmetics, there is an increasing need to advance the integration of animal free alternative methods eventually reduce or replace animal testing. Aims of the FP7 project DETECTIVE are to gain mechanistic knowledge, define adverse outcome pathways and identify biomarkers of toxicity. Before use for safety assessment, the identified biomarkers reflecting key and/or intermediate events of a given AOP still have to be validated. We here describe the use of a read across case (RAX) study to contribute to this validation.

VPA was chosen as lead compound for this RAX case study. Valproic acid is a branched carboxylic acid, which induces microvesicular steatosis in the liver of humans as well as rodents. Steatosis is discussed to be the result of an impaired β -oxidation in the mitochondria of hepatocytes. 10 candidate biomarkers were identified using transcriptomics data from the TG-Gates database. These 10 biomarker were strongly upregulated after VPA treatment and cover relevant toxic mechanisms related to liver steatosis e.g. changes associated with lipid and energy metabolism, metabolism of xenobiotics, ER and general stress responses. Further a causal relationship could be drawn to apical findings in rodent RDT studies, in which VPA induced histopathological alterations such as steatosis, fatty degeneration or vacuolization of hepatocytes. To test the afore mentioned 10 genes as possible biomarkers of VPA-like hepatotoxicity, 11 structurally similar analogues to VPA, consisting of branched and unbranched carboxylic acids were identified. RDT studies for each of them were extracted from the databases RepDose, IMI eTOX, ECHA CHEM, Cosmos, Leadscope, Nedo as well as peer reviewed publications. Six VPA analogues induced alterations such as fatty degeneration in the *in vivo* studies and were classified as 'in vivo positive'. Five analogues did not cause any adverse effects in the livers of rats up to the highest tested doses and were classified as 'in vivo negative'. Further, 2 negative controls were included, namely melantonin and buspiron, which are structurally dissimilar and do not cause any liver effects.

The present poster shows the result of the biomarker testing by quantitative RT-PCR and by BAC-GFP toxicity pathway reporter HepG2 cell line activation using high content microscopy. Three candidate biomarkers were identified being predictive for the “*in vivo* positive” analogues, which do not need metabolic activation. These biomarkers are Chop, Atf3 and Pcmt1. Three biomarkers related to β -oxidation were, however, positive for all tested RAX-analogues, including VPA. Chop-GFP and Srxn1-GFP toxicity pathway reporter cell lines gave similar results. Overall these results are in good agreement to previous findings that VPA and 4-ene VPA inhibit mitochondrial β -oxidation only partly. A concept, how to integrate the resulting biomarker data qualitatively in the context of a read across approach and/or quantitatively into risk assessment is discussed

Scr&Tox STAND

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Abstract n°1

Considerations for criteria for reference cells for standardisation of *in vitro* human cell-based assays for toxicology

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SCR&TOX is a research project (www.scrtox.eu) co-funded by the European Commission and Cosmetics Europe as part of the SEURAT-1 cluster (www.seurat-1.eu), aiming at the reduction and replacement of animal models with *in vitro* stem cell models, for use in predictive systemic toxicology. The role of the UK Stem Cell Bank SCR&Tox project is to develop induced pluripotent stem cells (iPSCs) as candidate cells for development of reference preparations to assist in standardisation and control of acute and chronic toxicity assays.

Practical criteria for suitable cell lines intended for use in high throughput toxicology assays are 1) adequate growth rate of cells, to provide rapid scale up 2) genetic stability of cells to avoid variability in data derived from cell batches over time. In addition, in differentiated cultures, the biochemical pathways need to be representative of typical differentiated *in vivo* cell types being modelled in the toxicology assay. From a fundamental cell biology perspective it is important that the test cell line used is unperturbed by transformation associated changes in the cell, which could influence cellular responses i.e. acceptable cells should retain their full pluripotent potential. However, chromosomally aberrant (non-diploid) cell lines have some advantages in that they are often associated with increased growth rate and stable expansion. Here we investigate diploid hPSC lines in parallel with a number which are karyologically abnormal, to consider their suitability for investigation of cellular toxicology.

All 3 iPSC cell lines displayed an abnormal karyotype and retained their ability to differentiate into all 3 germ layers via directed differentiation, thus demonstrating their potential as pluripotent cells. The iPSC lines were compared to the hESC line H9 cell line, commonly used in toxicology studies. These lines are now being examined for their expression of *nrf-2* (a known indicator of oxidative stress) and other related downstream toxicology responsive markers such as 'Ho-1, NQO-1, GST'. We describe how these aberrant and diploid cells compare as potential test substrates for toxicological studies and provide a justification for the utilisation of karyotypically abnormal cells under controlled conditions and the scientific interpretation of toxicology data generated from such systems. We suggest that the use of cells with abnormal karyology may be useful tools in the study of certain toxicological activities and provide reliable readouts for compounds of unknown biological impact.

Abstract n°2

Towards Safety Pharmacology in the Human Brain – A Hybrid Impedance / Field Potential Platform for Neurophysiology and –Pathology Analysis on Biochip

Diana Seidel, Heinz-Georg Jahnke, Delphine Laustriat, Mathilde Girard, Vesselina Semkova, Simone Haupt, Oliver Brüstle and Andrea A. Robitzki

When developing active pharmaceutical ingredients in health care or cosmetic, a major aim is to identify toxic substances at early stage. In the field of neuronal disorder therapy and toxicity assessment, human induced pluripotent stem cell (hiPS)-derived neuronal networks are at present the only *in vitro* cell model combining standardized, high-throughput (HT) generation with a primary human neuronal phenotype. However, for meaningful neurophysiological and –pathological assay development, the on-line monitoring of the neuronal maturation process is a critical issue. Therefore, an analysis platform is necessary that enables the sensitive and non-invasive real-time identification of neuronal differentiation and maturation characteristics as well as the subsequent monitoring of drug-induced degenerative and regenerative processes.

Here we describe for the first time a bioelectronics impedance/field potential platform, which allows the high-content (HC) monitoring of complex cellular properties that accompany neuronal differentiation of hiPS-derived neurons. The observed distinct relative impedance progression and impedance spectrum shape as well as frequency shift could be specifically associated with the quality and state of neuronal differentiation.

In addition to electrophysiological activity, neuronal networks fulfilling the above mentioned criteria were subsequently used in acrylamide neurotoxicity screening. In this process, we were able to detect molecular and structural changes causing neurotoxicity much faster and with increased sensitivity using impedance/electrophysiology recording compared to traditional molecular-based methods.

Combining a mature human neuronal cell model and a highly efficient hybrid impedance/electrophysiology read-out system, we established a HC/HT analysis platform for label-free neuronal differentiation and toxicity monitoring that is suitable for short and, much more important, long-term studies.

Abstract n°3

A novel preclinical *in vitro* biosensor system for monitoring drug-induced cardiotoxicity in a human 3D-cardiomyocytes culture model

Fleischer S., Jahnke H.-G., Steel D., Sartipy P., Robitzki A.A.

Up to now, unexpected negative side effects on the human heart are the major reasons for withdrawal of an approved drugs from the market. In this context, there is the need for improved cardiac safety assays. Especially for chronic or repeated dose toxicity, there are no *in vitro* tests available. Therefore, state of the art tests are animal studies that are questionable because of the ethical issue as well as the critical extrapolation of the results to human.

In contrast to current cell models based on 2D cultures, we used human embryonic stem cell derived cardiomyocytes clusters (hCMCs) for chronic toxicity monitoring that provide an intact human *in vivo* like cellular environment for improved cardiac safety assays. Monitoring of drug-induced adverse side effects on hCMCs was performed by a new developed unique microcavity array (MCA) based screening technology. Our hybrid biosensor allows to measure cellular alterations by electrochemical impedance spectroscopy (EIS) as well electrophysiological changes by field potential recording (FPR) in one system. In contrast to other methods like manual patch clamp our array allows easy long-term monitoring of 3D-cultures in a non-invasive, real-time, label-free and quantitative manner.

To demonstrate the performance of our novel *in vitro* long-term drug safety assessment platform we used the compound doxorubicin, a widely used chemotherapeutic agent that is known for long-term cardiotoxic side effects in human. We applied sub-acute toxic concentrations of doxorubicin to the hCMC over a period of 4 weeks. First structural changes and cellular damages measured by impedance spectroscopy were detected after seven days and increased over the next weeks. According to the morphological changes we also observed a decrease of the contraction rate at highest concentration but also changes in the signal strength at lower concentrations. So we could demonstrate for the first time, the bioelectronic monitoring of doxorubicin-induced chronic cardiotoxicity in a human *in vitro* 3D-cardiomyocyte model after application of concentrations that are in the range of the clinical relevant plasma level.

In conclusion, hES derived CMCs in combination with the MCA technology are a promising and feasible tool for *in vitro* pharmaceutical safety testing to detect drug-induced adverse side effects.

Abstract n°4

Development of an hPSC-based neurotoxicity screening platform

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Toxicity is mediated by a small number of stress-response pathways, which are activated in response to chemical stimuli to maintain homeostasis, or to evoke organ/cell type-specific adversity. Human pluripotent stem cells (hPSC) can be triggered to differentiate into any cell type of the body and are thus ideally suited to develop cellular models for *in vitro* high-throughput testing of tissue-specific toxicity. Previously, we have developed hPSC-derived longterm neuroepithelial stem cells (lt-NES; Koch et al. PNAS, 2009), a stable intermediate population which retains its neuro- and gliogenic potential even after long-term proliferation (>70 passages) and thus represents a suitable source for *in vitro* neurotoxicity assay applications. In order to meet the challenges of industrial-scale neurotoxicity screens we have set up a semi-automated, standardized, and scalable method for the generation of mature lt-NES neurons in 96-well plates. Batches of up to 1x10⁹ cryopreserved lt-NES neurons can be produced and thawed on multi-well plate formats. We developed a fully automated walk-away process for the cultivation and neuronal differentiation of lt-NES neurons on our robotic system (CellHOST, Hamilton). The process yields highly standardized neural cultures in ready-to-use 96-well microtiter plate formats for neurotoxicity assay development. Diverse neurotoxic and neuropathological events lead to excess formation of free radicals, which trigger an oxidative stress response. The Nrf2 pathway is a powerful sensor for cellular redox state and is activated directly by oxidative stress and/or indirectly by stress response protein kinases. Exploitation of the Nrf2 pathway as an indicator of cell stress-associated events could thus provide a valuable readout for *in vitro* toxicity assay development. To assess the toxicological relevance of the Nrf2 pathway in our cellular model, we evaluated its basal and inducible activity in lt-NES cells and lt-NES-derived neurons at different maturation stages. QRT-PCR analysis of the Nrf2 downstream targets SRXN1, NQO1 and HMOX1 revealed increased expression levels with the progression of neuronal maturation. In accordance with the qRT-PCR results, dose-response curves for repeated-dose toxicity revealed an increased resistance of more mature neurons to Rotenone as determined by AlamarBlue assay. Removal of antioxidants from the cell culture medium revealed that Nrf2 pathway activation is strongly increased in response to Rotenone treatment. Our data depict lt-NES cells as a robust and versatile system for toxicity testing at various stages of neural differentiation.



COSMOS STAND

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Abstract n°1

Biokinetic Modelling Approaches Contributing to Assessing Cosmetic Ingredients Safety

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The ban of animal testing for cosmetic ingredients and finished products in the European Union has generated a strong momentum for the development of *in silico* and *in vitro* alternatives for the assessment of their safety. One focus of the COSMOS project - funded by the European Commission under the 7th Framework Programme - was the prediction of kinetics and toxic effects through multiscale pharmacokinetic modelling and *in vitro* data integration. Mathematical or computer modelling and *in vitro* experiments are seen to be complementary.

A summary of the models and results obtained within the framework of the project is presented: 1. The Virtual Cell Based Assay, to simulate the intracellular concentration of a chemical perturbing cell populations; 2. Simple physiologically-based kinetic (PBK) effect models to perform route to route extrapolation; 3. Multiscale modelling, developed as a computational approach to explore toxic effects in a living organism, to establish an interface between different biological levels of data: cells, organ and organism level. Their coupling facilitates *in vitro* to *in vivo* extrapolation producing an estimated effect close to observed values.

We believe that the current batch of models developed could help to obtain quantitative risk assessment for humans at realistic low dose levels and for repeated exposure.

Abstract n°2

COSMOS DB: A Database of Toxicological Information to Support Knowledge Discovery and Safety Assessment

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COSMOS Database (DB) is a high quality web-based database which links chemical structures to repeated dose toxicity, skin permeability and other endpoint data. In total, COSMOS DB v1.0 contains more than 12,000 toxicity studies across 27 endpoints for over 1,600 compounds and more than 80,000 chemical records with more than 40,000 unique structures. A subset of the data is the COSMOS oral repeated dose toxicity database (oRepeatToxDB) of 230 cosmetics-related chemicals, describing dose-level phenotypic effects with a controlled vocabulary. The data have been collected from multiple sources, curated, quality-controlled, stored and managed in a flexible and sustainable manner to support predictive modelling tasks. The chemistry content includes a cosmetics inventory compiled from the EU CosIng database and the US PCPC list for cosmetics-related chemicals, serving as a reference. The Database is flexibly searchable by name, CAS number, graphical representation, SMILES strings as well as toxicological effects.

COSMOS DB version 1.0 was made publicly available in December 2013 from the URL <http://cosmosdb.cosmostox.eu>. The COSMOS DB data model has been revisited and updated to host the COSMOS No Observable Adverse Effect Levels (NOAEL) and Threshold of Toxicological Concern (TTC) databases. The updates allow the storage and display of No/Lowest Observable Adverse Effect Levels (N/LOAEL) associated with a chemical and/or toxicological study as well as the extraction of TTC datasets from the COSMOS NOAEL DB. N/LOAEL values are stored together with all relevant metadata including, but not limited to, the source of the value and the regulatory body which has determined that endpoint particular value.

Abstract n°3

Alerting Chemotypes for Liver Steatosis, Steatohepatitis and Fibrosis Identified by Mining COSMOS DB

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The COSMOS oral repeated dose toxicity database (oRepeatToxDB) – available through the COSMOS Database (<http://cosmosdb.cosmostox.eu>) – includes an ontology for phenotypic effects at each dose level using controlled vocabulary. Toxicity effects observed at target organ sites have been organised hierarchically to relate organs to tissues to cells. The majority of biological/chemical processes occur at the cell/organelle level.

Mechanistic data mining can be undertaken by investigating interactions between chemicals and proteins/genes in order to associate chemical structures with phenotypic effects initiated by related toxicity mechanisms. Common structural fragments can be extracted and refined into mechanistic chemotypes representing underlying molecular initiating events (MIE).

Liver steatosis, steatohepatitis and fibrosis were chosen as a case study for data mining. Over 20% of cosmetics-related chemicals in oRepeatToxDB were associated with lipid deposition, fatty changes, cytoplasmic vacuolisation, cellular infiltration and inflammation in various hepatocytes, ultimately leading to fibrosis. Combinations of phenotypic effects and morphological changes at various sites were mapped onto chemical classes. A set of alerting chemotypes for liver steatosis, steatohepatitis and fibrosis was identified by application of the ToxPrint chemotypes (<https://toxprint.org>). They will serve as a basis for developing chemical categories to support safety assessment. This approach also provides a way to elucidate the underlying molecular pathways and mechanisms for hepatotoxicity.

Abstract n°4

Use of *In Silico* Tools to Perform Extrapolations for Chemical Risk Assessment

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One of the aims of the COSMOS Project was to predict the kinetics and toxic effects of cosmetics-relevant substances through the integrated use of biokinetic modelling and *in vitro* toxicity data. Caffeine, an alkaloid and stimulating drug also used as a cosmetic ingredient, was chosen for a modelling case study.

The aim was to couple a human physiologically based kinetic/dynamic (PBK/D) model predicting organ-level effects to the cell level, using the Virtual Cell Based Assay (VCBA) to perform *in vitro* to *in vivo* and route-to-route extrapolations. Acute exposure at the oral no-observed-effect-level (NOEL) dose derived from rat studies was simulated via oral and dermal absorption. The dermal safe limit was determined via toxicokinetic parameters. The effect site concentration was quantified to simulate cardiovascular responses. The simulated liver kinetics were linked to the VCBA to estimate the concentration inside the liver cells to extrapolate back to an external exposure dose.

We believe that this approach could be used to support quantitative risk assessments at realistic human repeat exposure levels.

Abstract n°5

Computational Models for Toxicity Prediction Implemented as User-Friendly Web-Tools in the KNIME WebPortal

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KNIME is the modular integration platform used in the COSMOS Project for the predictive toxicology methods. By means of graphical workflows, data are read from various data sources and subsequently transformed into suitable formats for model building and / or visual analysis. The KNIME technology integrates access to databases and modelling approaches into flexible computational workflows that have been made publicly accessible and provide a transparent method to support chemical safety assessment.

Several workflows have been developed within the COSMOS project to support *in silico* modelling and ADMET (absorption, distribution, metabolism, excretion, toxicity) prediction, such as

- Biokinetic simulations using physiologically-based kinetic (PBK) models for route to route extrapolation or linked to the Virtual Cell Based Assay to perform *in vitro* to *in vivo* extrapolation (IVIVE)
- Prediction of skin and gastrointestinal absorption
- Evaluation of potential binding to nuclear receptors related to steatosis
- Profiling with chemotypes, e.g. for protein/DNA binding potential and mitochondrial toxicity.

The workflows can be accessed from the KNIME Analytics Platform desktop application, where they can be flexibly adapted to the user's needs, if required, or via the COSMOS KNIME WebPortal. The latter allows the end user to execute the workflows via a web browser without software installation. Input data can be uploaded, parameters for the model can be adjusted in a step-by-step execution, and the final results can be downloaded as files and/or as graphical reports in various formats.

Descriptions of the models as well as user guidance can be found in COSMOS Space (<http://cosmosspace.cosmostox.eu>), an interactive sharing facility for predictive toxicology resources, also providing free registration to the COSMOS KNIME WebPortal (<http://knimewebportal.cosmostox.eu>). The user guidance is complemented by web tutorials, also linked from the COSMOS website <http://www.cosmostox.eu>.

Abstract n°6

Use of Molecular Modelling Approaches for the Evaluation of Potential Binding to Nuclear Receptors Involved in Liver Steatosis

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Ligand dependent dysregulation of some nuclear receptors has been identified amongst the probable molecular initiating events for liver steatosis. Thus modelling of binding to these receptors could facilitate understanding of the molecular mechanisms that trigger further downstream events and promote development of liver toxicity.

In more detail, the use of molecular modelling approaches to predict potential binding to two nuclear receptors, i.e. liver X receptor (LXR) and peroxisome proliferator-activated receptor gamma (PPAR γ), has been investigated. This case study is a proof of concept that molecular modelling methodology, traditionally used in drug discovery, can be employed in predictive toxicology as a needful part of an integrated strategy, which combines multiple methods and approaches (e.g., *in silico*, *in vitro*, mechanistic information) to support toxicity prediction in the Mode of Action/Adverse Outcome Pathway framework.

The poster summarises the results of the molecular modelling on LXR and PPAR γ dysregulation. It includes: (i) analysis of the structural and biological data collected within the study; (ii) development and validation of different models to predict binding/activation, including structure- and ligand-based (e.g. docking, pharmacophore modelling) approaches, as well as classification models; (iii) integration of the different models within a consensus strategy to improve the predictive performances.

Liver toxicity databases were screened for LXR and PPAR γ binding using the models developed to prioritise compounds of potential concern for liver toxicity, hence suggesting further testing or supporting read-across.

ToxBank STAND

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Abstract n°1

The ToxBank infrastructure project to support the replacement for repeated dose toxicity

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Assessment of toxicity in a Mode-of-Action (MOA) framework is a multi-level undertaking. ToxBank builds databases and data management solutions to aid in the systems toxicology-based risk assessment paradigm of the SEURAT-1 (Safety Evaluation Ultimately Replacing Animal Testing-1) cluster. With the aim to contribute to this overall task, the SEURAT-1 Gold Compounds were clustered by biological similarity using chemical-gene and chemical gene ontology (GO) links from the Comparative Toxicogenomics Database (CTD). Compounds with similar MOA clustered together and MOA-specific biomarker genes derived from CTD data showed in several instances enrichment for annotations to GO categories related to the MOA. Overall, these unbiased high-throughput data reflected the MOA described in earlier studies. These data therefore provide evidence for the applicability of a central part of the MOA framework established in SEURAT-1.

Abstract n°2

The ToxBank Compound Wiki

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The ToxBank Compound Wiki was created to support the organisation and presentation of information on reference compounds to SEURAT-1 scientists. The selection of a semantic media wiki to develop this resource enables both the collaborative assembly, annotation and curation of information profiles on compounds and the structured capture of metadata such as on linked toxicological data or biochemical mechanisms.

The Wiki provides an extensible high quality information resource servicing the selection and use of reference compounds by researchers during their experimental planning. Compound information pages are linked to sources of repeated-dose toxicity *in vivo* and *in vitro* data, physical and chemical property data, linked biological resources on genes and pathways, and whenever available, human adverse event and epidemiological data.

The Wiki is extensible for further development as a critical SEURAT-1 cluster resource for the support of chemical and biological reagents evaluation, selection, quality control, distribution and use.

Abstract n°3

ToxBank Database: Development of a Biomaterials Data Resource Built on Best Practices

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ToxBank established a dedicated web-based warehouse for toxicity data management and modelling, a "gold standards" compound database and repository of selected test compounds, and a reference resource for cells, cell lines and tissues of relevance for *in vitro* systemic toxicity research carried out across the SEURAT-1 cluster. The project develops infrastructure and service functions to create a sustainable predictive toxicology support resource going beyond the lifetime of the program.

Simply putting all protocols and data into a centralized database will not ensure efficient use of that data, unless it is made available to scientists in user friendly formats and further organised to support the needs of the community. An important early step is establishing a good level of understanding between database constructors and life scientists which was a major goal in the early stage of the ToxBank project. Tools for data gathering and access have progressed in particular through use of Wiki pages for compounds and biomaterials. These not only carry fundamental data on compounds' and biomaterials' characteristics and sources/suppliers, but also carry options for annotation by users as a means of capturing up-to-date experiences of SEURAT-1 users. These datasets are rapidly searchable via highly detailed ontologies (a lexicon of scientific terms). It is intended that scientists will utilize the ToxBank system to enable them to evaluate different suppliers of cell lines and reagents, and to communicate personal experiences with specific products. This will facilitate selection of the most appropriate research materials. This poster elucidates this process through a 'story board' approach to give real examples of how ToxBank can facilitate efficient and optimized research.

Abstract n°4

The ToxBank Data Warehouse: Uploading and Sharing Protocols and Experimental Results Across the SEURAT-1 Cluster

Emilio Benfenati², David Bower³, Rebecca Ceder⁷, Kevin Cross³, Roland Grafström⁷, Barry Hardy¹, Christoph Helma⁴, Luam Kidane⁶, Nina Jeliaskova⁵, Vedrin Jeliaskova⁶, Pekka Kohonen⁷, Silvia Maggioni², Scott Miller³, Glenn Myatt³, Michael Rautenberg⁴, Glyn Stacey⁶, Jeff Wiseman⁸

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The ToxBank data warehouse project has been developed to upload and share protocols and experimental data across the entire SEURAT-1 cluster. The benefits of this approach include:

- It provides immediate access to existing and new protocols and data, as well as facilities for uploading information through a simple web interface.
- The use of standardized data templates and semantic annotation supports cross-cluster experimental consistency and will enable an integrated data analysis.
- The approach supports protocol development and collaboration which is close to current work activities, especially with the current SEURAT-1 focus on experimental development.
- The approach will link public databases and in-house data.
- The use of the ISA-TAB exchange format provides options for housing all data types including omics.

Abstract n°5

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 multiple types of time-dependent dose response omics and functional data combined with *in vitro* and *in vivo* background knowledge including consideration of modeling variations in biokinetic responses. Open TG-GATES human *in vitro* liver data of the reference compounds includes reactive compounds (e.g., acetaminophen, CCl₄), mitochondrial disruptors (e.g., Rotenone), promiscuous binders (e.g., valproic acid, amiodarone), 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. Overall we obtained 31,717 differential expression results with 14 compounds from the 45 comparisons, with Doxorubicin providing over 5000 results. Pathway enrichment analysis of Doxorubicin identified a number of key pathways including mismatch repair after 24 hours treatment and TNF-signaling at high doses after 24 hours.

We evaluate the combined use of ToxCast, PubChem and TG-GATES data in the enrichment analysis, read across and interpretation of the evidence on reference compounds as mapped to biological pathways. The approach includes the use of the ISA-Tab standard to describe experimental metadata and OpenTox services supporting interoperable data integration and analysis. In addition, a proposed data harmonization of processed omics and assay data is described. Enrichment analysis is used for data reduction to understand differentially

expressed genes in the context of toxicity pathways and gene ontology (GO) categories that describe biology. We present here a workflow for pathway analysis using Open TG-GATEs, ToxCast and other data focused on the SEURAT-1 gold compounds (Doxorubicin and VPA as examples). Characterization of the SEURAT-1 reference compounds' differential expression patterns lends support to the idea that they activate highly variable biological mechanisms, which nevertheless are compound-specific and may represent viable biomarkers for toxicity.

Abstract n°6

Risk Assessment of Piperonyl Butoxide Using Available Human *In Vitro* Data

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A recent milestone in toxicity research was the emergence of the field of toxicogenomics, resulting from the application of knowledge gained from genomics science into conventional toxicology. This field specifically tackles the complex interactions between toxic effects and the structure and activity of the genome. The question now is if the detection of chronic and systemic toxicity is possible via these alternative testing strategies to reduce and replace animal testing.

As part of the ToxBank project in the SEURAT-1 program, *ab initio* case studies have been proposed in which the toxicity of four compounds are assessed using available human *in vitro* data only. These compounds are the reference compounds doxorubicin, valproic acid and methotrexate with well-defined modes of actions as well as the test compound piperonyl butoxide (PBO). The latter was chosen as a compound relevant for the cosmetics industry. Here, an approach using the combination of omics data with information extracted from adverse outcome pathways (AOPs) to identify areas of concern and support an evidence-driven risk assessment is presented with the example PBO.

CASE STUDIES STAND

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Abstract n°1

The *Ab Initio* Case Study

Elisabet Berggren, Gladys Ouedraogo, Alicia Paini, Andrew White and Catherine Mahony

The *ab initio* case study is an attempt to structure knowledge and data in a logical workflow that would be the basis for a full risk assessment, and would be the basis for a first integrated assessment relying only on alternative methods. This will showcase that there is a feasible way forward but also pointing on weaknesses and knowledge gaps, which then will assist in shaping a more focused strategy to advance alternative assessment approaches.

The risk assessor needs to: (i) determine *a priori* the human-relevant modes-of-action of primary concern; and (ii) define a relevant quantitative point of departure for repeated dose in relation to the exposure scenario indicated.

Based on the SEURAT-1 conceptual framework, we have developed a general workflow to assist the risk assessment. We assume that the workflow starts from the same considerations regardless of the type of safety assessment. Therefore following the workflow for the *ab initio* case study we identify exit points for different tiers. Application of the TTC concept is associated with Tier 1 and the read-across assessment with Tier 2, while when neither is considered to be applicable we need to continue to Tier 3, which then is the ultimate *ab initio* assessment.

The substance selected to illustrate the case study is piperonyl butoxide (PBO) in the imagined exposure scenarios of being an new ingredient introduced in a body lotion daily applied to the skin (overall body surface). The supportive alternative data (*in vitro* and *in silico* results) were obtained from ToxCast (EPA 2014) or generated by using methods developed within the SEURAT-1 projects. Piperonyl butoxide is a known hepatotoxin, even though the mechanism of action is unknown, and was therefore considered suitable as most methods developed within SEUART-1 are focused on hepatotoxicity, and could therefore provide relevant data for the assessment.

Abstract n°2

Data Integration And Visualization For Transparent Communication Of The Category Read Across: A Glycol Ethers Case Study

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The integration and communication of diverse information into a REACH read across application is often challenging given the wide variety of data types and sources. The goal of this study was to utilize several approaches for integrating and visualizing data in support of both category and analogue read-across. We used a case study of glycol ethers to demonstrate an integrated approach to utilization and interpretation of traditional toxicity, chemical structure, and other data to offer a workflow for transparent and effective communication of the outputs to diverse stakeholders. The information available on these chemicals included physicochemical properties, environmental fate, mutagenicity/genotoxicity, as well as data from a variety of ecological and mammalian experimental model systems. We used ToxPi and Chemical-Biological Read-Across (CBRA) software to demonstrate how various types of data can be integrated allowing for differential weighting, relative ranking, and step retention to maximize working transparency. The data were transformed into a ToxPi- and CBRA-compatible format, scaled, analyzed, and rendered multiple times to compose visualizations with alternate category grouping scenarios. Several data weighing schemes were also utilized to yield comparisons that may reflect various stakeholder priorities. We found overall that the glycol ethers group together within their structure-based category. To address the need for better analytical and communication approaches, the ToxPi- and CBRA-derived grouping/analogue selection can be applied according to different stakeholder criteria and the outputs can be adjusted in transparent manner, along with the resulting visualizations, to communicate the results.

Abstract n°3

A Strategy and Template for Read-Across Predictions of Chemicals Toxicity

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The concept of category formation, chemical grouping and of read-across is used to support chemical safety assessment by filling data gaps. It is based on the inference of properties of a chemical substance, including its toxicity, from similar chemicals with known properties.

The crucial aspect is, therefore, to define a group of similar chemicals. The similarity is based, in the first instance, on chemical structural similarity. However, slight differences in the structure can make chemicals and their effects very different. Therefore more aspects of similarity have to be considered: Apart from the structural similarity and similarity of physico-chemical properties, these are biological similarity, the mechanism of toxicity, toxicodynamics, toxicokinetics and bio-modifications.

With particular reference to regulatory submissions, the category formation and read-across process has to be transparent, reproducible and clearly documented. In order to support read-across and its documentation, and especially to further regulatory acceptance, a strategy for structuring and reporting read-across toxicity predictions in a workflow has been developed (Schultz et al 2015). It helps to describe the read-across rationale in a transparent manner and evaluate chemical category membership. The focus is on two major aspects: assessing the similarity and assessing the uncertainty.

Templates are provided to guide the user systematically through the collection of data necessary to build and underpin the similar categories as well as through a systematic assessment of the uncertainty, both for the category similarity and the overall uncertainty of the read-across prediction.

Reference:

Schultz TW, Amcoff P, Berggren E, Gautier F, Klaric M, Knight DJ, Mahony C, Schwarz M, White A, Cronin MTD (2015) A strategy for structuring and reporting a read-across prediction of toxicity. *Reg. Toxicol. Pharmacol.* 72: 586–601

Abstract n°4

SEURAT-1 Read-Across Case Studies: Reducing the Uncertainty with New Approach Data

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The SEURAT-1 Proof-of-Concept Read-across case studies aim to investigate the four most likely read-across scenarios for repeated dose toxicity, focussing on liver as the endpoint for chemical safety assessment, as appropriate (Berggren et al 2015).

These four scenarios are defined by different elements of similarity:

- 1) Compounds not requiring/undergoing metabolism to exert a potential adverse human health effect
- 2) Compounds metabolised to the same/similar toxicant (metabolite)
- 3) Compounds with general low or no toxicity
- 4) Structurally similar compounds with markedly different potency or effects.

The following examples were selected for the four scenarios: (1) perfluorinated alkyl acids, (2) β -unsaturated alcohols, 3) saturated alcohols and (4) alkyl-substituted phenols.

Categories were firstly defined and described using a “classic” read-across approach, with mechanistic information, chemistry, non-test information, and where possible existing *in vivo* data, following the read-across strategy and workflow developed by Schultz et al (2015). Toxicodynamics and toxicokinetics were taken into consideration and the overall uncertainty was assessed.

In a second iteration, “new approach” data from the SEURAT-1 projects and other initiatives were added. The overall result of the read-across prediction was then evaluated again to see if the “new data” can contribute to reduce uncertainty.

In conclusion, the case studies have demonstrated the application of the strategy for reporting a read-across prediction and documenting uncertainty, as well as the decrease in uncertainty with the use of “new approach” data.

This abstract does not reflect EPA policy.

References:

Berggren E, Amcoff P, Benigni R, Blackburn K, Carney E, Cronin M, Deluyker H, Gautier F, Judson RS, Kass GEN, Keller D, Knight D, Lilienblum W, Mahony C, Rusyn I, Schultz T, Schwarz M, Schürman G, White A, Burton J, Lostia A, Munn S, Worth A (2015) Chemical safety assessment using readacross: How can novel testing methods strengthen evidence base for decision making? *Environ. Health Perspect.*, in press

Schultz TW, Amcoff P, Berggren E, Gautier F, Klaric M, Knight DJ, Mahony C, Schwarz M, White A, Cronin MTD (2015) A strategy for structuring and reporting a read-across prediction of toxicity. *Reg. Toxicol. Pharmacol.* 72: 586–601

ToxCast STAND

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Abstract n°1

EDSP21: High-throughput Screening and Prioritization Supporting the EPA Endocrine Disruptor Screening Program

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The U.S. Environmental Protection Agency (EPA) is developing multiple high-throughput *in vitro* assays and computational models to enhance the ability of the EPA to evaluate chemicals for their ability to be endocrine disruptors. Under the U.S. Endocrine Disruptor Screening Program (EDSP), there are as many as 10,000 chemicals that must be evaluated for their ability to perturb the estrogen, androgen and thyroid signalling systems. Using the current EDSP Tier 1 battery of 11 *in vitro* and *in vivo* assays, evaluating this chemical universe would take many decades. As an alternative, EPA is focusing on using combinations of *in vitro* assays, plus computer models that integrate data across the assays, to identify chemicals that are potential endocrine disruptors. The initial focus is on prioritization of chemicals to be tested in the Tier 1 battery, but in selected cases, the original low-throughput tests will be replaced with higher throughput versions. The first example of this EDSP21 approach is a model that combines data from 18 estrogen receptor (ER) assays to derive a single score for each chemical. Those with high scores are deemed to be highly likely to interact with the estrogen receptor. This model was validated against both *in vitro* literature data, and data from the guideline *in vivo* uterotrophic assay. The performance was high enough that the EPA is allowing the results of the *in vitro* model to be used in lieu of the Tier 1 *in vitro* ER assays and the uterotrophic test. A total of 1800 chemicals have been evaluated in the ER model, and 1000 more are in progress. A similar model for androgen receptor signalling is underway. There is also active research into developing similar approaches for the thyroid hormone signalling system. All of the data from the EDSP21 program is being made publicly available through the EDSP21 dashboard: <http://actor.epa.gov/edsp21>.

The views expressed in presentation are those of the author and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.

Abstract n°2

***In Silico* Dynamics: computer simulation in a Virtual Embryo**

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Evaluating and assessing impacts to development is an Agency priority (EPA's Children's Environmental Health Research Roadmap); however, the quantity of chemicals needing assessment and challenges of species extrapolation require alternative approaches to traditional animal studies. One approach is to profile the human exposure universe of chemicals with HTS assays (*in vitro*) and then build computational (*in silico*) models that integrate these data with biological knowledge representing human development. Imputing HTS data into spatially-dynamic computer models of developmental signaling networks can then be used to simulate how an embryonic system might respond to a disturbance in the maternal environment. An *in silico* strategy with virtual tissue models can yield theoretical answers to relevant questions that are not attainable experimentally. This exhibition will demonstrate a workflow to build a cell agent-based computer model, seed it with HTS data, analyze cellular response networks and emergent properties, and compare simulations to adverse outcomes. This exploratory platform may be useful to evaluate chemical effects on development, such as disruption of cardiovascular development (angiodysplasia), palatal fusion (cleft palate), limb outgrowth (ectrodactyly) and urethral fusion (hypospadias) among other systems. Simulations of AOPs for embryonic disruption in a 'Virtual Tissue Laboratory System' can be built with biological information and parameterized with *in vitro* data for chemical prioritization and early lifestage exposure considerations.

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