# Lush Training Prize 2014

# Background Paper

**Prepared by TJM Consultancy July 2014**

# 1. Executive Summary

## 1.1 What is the Lush Science Prize?

Now in its third year, the Lush Prize supports animal-free testing by awarding money prizes totalling £250,000 to the most effective projects and individuals who have been working towards the goal of replacing animals in product or ingredient safety testing. Prizes are awarded for developments in five strategic areas: science; lobbying; training; public awareness; and Young Researcher Awards. Should there be a major breakthrough in 21st Century Toxicology - the area which holds out most hope for a 'Eureka' moment leading to the replacement of animal tests – a Black Box Prize equivalent to the entire annual fund of £250,000 will be awarded to the individual or team responsible.

## 1.2 Methodology

In order to obtain an overview of developments in the field of animal replacement in toxicity pathway research, we firstly reviewed the recent work of the relevant scientific institutions and projects in this area, including AXLR8; OECD; CAAT; Hamner Institutes; Human Toxome Project; ECVAM; ICCVAM; US Tox21 Programme, the ToxCast programme; and the Human Toxicology Project Consortium (see section 4). We also assessed recent developments in toxicity pathway research by reviewing the relevant literature (see section 5).

In our search for candidate prize winners, we identified conferences focusing on animal replacement in toxicity testing that have been held in the preceding 12 months. These included EUSAAT 2013 and SEURAT -1. From these we obtained 170 abstracts for oral and poster presentations. These we scored using the system derived in previous years (see section 3) in which 3 points are awarded for projects identifying new toxicity pathways, 2 points for reporting new knowledge or tools, and 1 point for abstracts which stand out in some other way. We then performed literature searches using Pub Med, Google Scholar and Terkko Feed Navigator to identify projects describing recent advances in toxicity pathway research. A further two relevant projects were identified directly from the Tox21 and ToxCast programme websites. In all, searches yielded in excess of 1000 projects which we assessed as described (see Section 3).

Overall, from all sources combined, from 68 abstracts which scored 1 or more, 7 scored 1; 39 scored 2; 14 scored 2 +1 (i.e. total 3); and 7 scored 3 because they appeared to be reporting a new toxicity pathway. A single project scored the maximum possible marks of 3 for reporting a new toxicity pathway plus an additional mark for combining methodologies and for using human non-embryonic iP stem cells. The titles and authors of those abstracts scoring 1 or more are shown in section 6, whilst full abstracts of those projects scoring a total of 3 (either as 3 or as 2+1) or more are given in Appendix 1.

## 1.3 Projects recommended for the shortlist

There were 8 projects which received the highest scores of either 3 or 3+1 for reporting new pathways of toxicity. The full abstracts are given in Section 6.4. We consider all worthy of being considered by the judges as potential prize winners. We have not included those scoring 2+1 because the emphasis of the award was on new pathways,

rather than on bringing new knowledge or tools to previously understood toxicity pathways.

## 2. Background

The objectives of 21st Century Toxicology research can be summarised as: improving safety testing of chemicals and contaminants by using more relevant and predictive human models; and simplifying and automating tests so that many more chemicals can be tested for safety. The Science background papers for the 2012 and 2013 Lush Prizes provide an overview, and links to further resources, describing the concept of 21st Century Toxicology.

The 2014 Lush Science Prize seeks to reward those researchers making 'outstanding contributions' to 21st Century Toxicology research. In previous years, the Science prize has been £50,000 which, in 2013, was divided equally between two winning groups. The focus of the award has been on research aimed at elucidating key pathways in which perturbation results in toxicity. For 2014, we have continued this approach, and further extended it to endeavours to build up a map of all known substances and their effects on the human organism. The vision is that, when new substances become available, their molecular similarity to other substances on the map, and the pathways they take, will enable computer simulations to give greater predictive accuracy of toxicity than any animal model, with extremely rapid results. Once this map is built and working, animal testing should become a thing of the past.

The Brief for prize applicants, taken from the Lush prize website http://www.lushprize.org/ was as follows:

*Science Prize*

*For individuals, research teams or institutions for work conducted on relevant toxicity pathways. Outstanding research producing an effective non-animal safety test based on an approach other than toxicity pathways, where none existed before, may also be considered.*

*There is a £50,000 prize fund shared between all the winners of the Science Prize.*

*21st Century Toxicology is a new approach to safety testing which is exciting regulators, toxicologists, campaigners and companies around the world. It has become possible because of advances in biology, genetics, computer science and robotics.*

*It offers better relevance to humans (rather than using mice, rats and rabbits), and will explain the underlying causes of toxicity. Unlike animal methods, the new tests will help predict human variability and differential effects on embryos, children and adults. And as the superior scientific basis of the new approach is recognised, outdated animal tests will be replaced.*

## 3. Methodology

A primary purpose of this report is to identify key projects making major contributions to the field of animal-free toxicology research from which the Lush Prize judging panel will select the winners.

Of the "3 Rs", Lush is interested primarily in Replacement, so our search for potential prize winners focused on projects working towards the replacement of animals in product testing. We did not include research aimed at either Refining or Reducing the use of animals in experimentation, and we excluded research that was linked to animal testing in other ways. Whilst we gave consideration to work taking place anywhere in the world, as far as possible we only included work reported in the year preceding the award (i.e. July 2013 – July 2014).

At each stage of our search, research projects were excluded based on careful selection criteria, in order to achieve a manageable shortlist of excellent work which fully met the prize brief. Our selection procedure was a three stage process, starting by reviewing the project title and rejecting any articles which were clearly reviews or which were obviously unsuitable through using animal models or being overly focused on disease. In the second stage, assessment of the abstracts which passed the initial filter permitted further elimination of those reporting findings from clinical trials, those focusing on cancer research, all research including animal subjects, or work conducted on non-human cell lines. In the third stage, projects were scored using a system devised and successfully applied in previous years. In this system, points are awarded according to the following criteria:

| | |
|---|---|
| Does the work appear to be reporting a new pathway discovery? | *Score 3* |
| If it is working with apparently previously understood pathway research, does it bring new knowledge or tools? | *Score 2* |
| Does it stand out in any other way? | *Score 1* |

As source material from which to identify key developments in the area of toxicology research, and begin the search for candidate prize winners, we started by reviewing the recent research of some key institutions and collaborative projects working in the area of animal replacement in toxicity pathway research. These included AXLR8; OECD; CAAT; Hamner Institutes; Human Toxome Project; ECVAM; ICCVAM; US Tox21 Programme, ToxCast Programme; and the Human Toxicology Project Consortium.

Secondly, we identified relevant conferences held in the preceding 12 months and assessed abstracts, where available, for oral and poster conference presentations. Scientific conferences provide the forum in which the most up-to-date science is shared, reporting on recent developments and work-in-progress, without the lag time required for formal presentation as a journal publication. The relevant conferences for 2013-2014 included the European Society for Alternatives to Animal Testing (EUSAAT) 2013 (Linz, September 2013) and the EU SEURAT-1 Project meeting 2014 (Barcelona, February 2014). None of these abstracts was currently available online (mid-July 2014). Abstracts for the EUSAAT 2013 conference were made available to us in advance of their public dissemination through the kind assistance of Dr. Ursula G. Sauer, Secretary General of EUSAAT. Proceedings from the SEURAT-1 2014 meeting were kindly provided by Sara Vinklatova, Administrator, COACH SEURAT-1. Due to a confidentiality issue, one of the 31 SEURAT abstracts was not available for our review. The 9th World Congress on Alternatives & Animal Use in the Life Sciences (9WC)

meeting will be held in Prague in August of this year: clearly proceedings were not available so far ahead of time and so they should be included in the 2015 Lush Prize Science Review.

Thirdly, we conducted a review of the recent literature. For this we used three sources. Firstly, we searched PubMed for research published from 01/07/2013 to date, with search term “toxicity pathways” and restricting the subject matter to “humans”. As a second literature source, we searched Google Scholar for relevant papers published in the period 01/07/2014 to date, using the term “toxicology pathway” and excluding review articles. As a final literature source, we searched Terkko Feed Navigator using the terms “toxicology” and “pathway.”

## 4. Significant Institutional and Project Developments

This section summarises significant events or news focussing on 21st century toxicology from selected Institutions and major collaborative projects, reported within the last year.

### 4.1 Tox21

Tox 21 is a collaborative effort among NIH, the Environmental Protection Agency (EPA), and the Food and Drug Administration. NIH partners include NCATS (National Center for Advancing Translational Sciences) and the National Toxicology Program, administered by the National Institute of Environmental Health Sciences. The Tox21 initiative is designed to improve current toxicity assessment methods, which are slow and costly.

Tox21 scientists are currently testing a library of more than 10,000 chemical compounds (Tox21 10K) in NCATS' high-throughput (large-scale) robotic screening system. To date, the team has produced nearly 50 million data points from screening the Tox21 10K library against cell-based assays. Data generated from 12 of these assays form the basis of the 2014 challenge described below.

Tox21 partner NCATS has recently launched a Chemical Toxicity Data Model Competition. NCATS describes the Toxicology in the 21st Century (Tox21) Data Challenge 2014 as "a crowdsourcing competition to develop computational models that can better predict chemical toxicity". Participants who submit the winning models, as judged by the Tox21 Data Challenge Committee, will have the opportunity to submit a paper for publication in a special thematic issue of *Frontiers in Environmental Science*. NCATS will also recognize winning submissions in national communications, including on the NCATS website and in social media channels. Selected models will become part of the Tox21 programme's arsenal of tools which help researchers assess how various chemicals might disrupt biological processes in the human body and lead to negative health effects.

Tox21 has come under some criticism because critics have claimed that its assays do not necessarily predict human or animal toxicity very accurately. Marchan *et al* [1] summarise some of these criticisms, but observe that the Tox21 approach is an elegant one and that future projects may benefit from a combination of Tox21's high-throughput technology and Europe's '*in vivo* relevant' *in vitro* systems and systems biology approaches.

### 4.2 ToxCast

The US Environmental Protection Agency (EPA)'s ToxCast (Toxicity Forecaster) programme aims to use high-throughput screening tools to test many thousands of chemicals in in vitro assays.

Completed in 2013, the second phase of ToxCast evaluated over 2,000 chemicals from a broad range of sources, including industrial and consumer products, food additives, and potentially "green" chemicals that could be safer alternatives to existing chemicals. These chemicals were evaluated in more than 700 high-throughput assays covering a range of high-level cell responses and approximately 300 signaling pathways. ToxCast

[1] R Marchan, C van Thriel, & HM Bolt. Recent developments in *in vitro* toxicology: perspectives of European research and Tox21. (2013). Arch. Toxicol **87**, 2043-2046

research is ongoing to determine which assays, and under what conditions, may lead to toxicological responses. The results of this research then can be used to propose the context in which decision makers can use the data. The EPA's Endocrine Disruptor Screening Program has already begun the scientific review process necessary in order that ToxCast data may be used to prioritize the thousands of chemicals that need testing for potential endocrine-related activity.

### 4.3 AXLR8

The EU AXLR8 Project's mission was to "accelerate the transition to a toxicity pathway-based paradigm for chemical safety assessment through internationally co-ordinated research and technology development". It published its final report in December 2013.

The report highlighted a number of overarching recommendations that have arisen from AXLR8 workshops as suggestions for optimising future EU research under the Horizon 2020 funding programme, including:

- A need for effective, early, co-ordination in order to
- maximise potential synergies within and between projects
- define relationships, milestones, deliverables, and responsibilities clearly
- undertake "horizon-scanning" and long-range planning
- ensure accountability of all participants.
- Use a hybrid of European and international funding models. Europe tends to fund research on the basis of individual scientific merit, independently of a wider strategy, whereas programmes in other regions have adopted a science-driven 'top-down' approach. AXLR8 recommends a novel framework that reconciles these approaches in the future.
- Maintain a policy focus on key health-related societal challenges, while incorporating '21st century' tools as core building blocks of the research strategy.
- Recognise that classical 'integrated projects' may not provide the best return on investment, since funds are divided in too many directions. Alternative funding models should be considered under Horizon 2020.
- Dedicate funding to support '21st century' research and testing infrastructures throughout EU member states.
- Encourage 'value-added' collaborations among established research teams in other parts of the world, to share the workload and develop synergies without duplication.

AXLR8 recommends that the EU invest €250 million over the seven years of the Horizon 2020 funding round, focussing on three pillars of work:

- Adverse Outcome Pathway (AOP) discovery and informatics
- Enabling technologies and infrastructures
- Implementation

This work should be co-ordinated by a strong, central, multiagency Steering Group. AXLR8 goes on to suggest a number of priority targets for Horizon 2020 funding, grouped under the three “pillar” headings. They hope these targets will provide a foundation upon which the Steering Group can build in developing calls for proposals under Horizon 2020. They will allow capitalisation on already ongoing innovations, while also offering perspectives for longer term research and development, with opportunities to exploit synergies within and across different pillars.

Source: The AXLR8 Project: Final Report & Recommendations. December 2013 http://axlr8.eu/

### 4.4 The Organisation for Economic Co-operation and Development (OECD)

As noted in the 2013 Lush Prize Science paper, the OECD launched a programme to develop AOPs for the testing of chemicals. The AOP concept has been broadly adopted within the field of toxicity sciences. The OECD reports that its AOP development programme has 18 projects completed or in progress – 3 of these are based on animal models (2 rat, 1 fish). In addition, 3 AOP case studies are being developed, one of which uses animals (aquatic organisms).

Source: www.oecd.org/env/ehs/testing/listsofprojectsontheaopdevelopmentprogrammeworkplan.htm

### 4.5 Hamner Institutes

Based in North Carolina, USA, The Hamner Institutes for Health Sciences brings together safety assessments for chemicals and drugs, world-class education and training, and accelerated technology development.

In December 2013, The Hamner Institutes and the European Union Reference Laboratory for alternatives to animal testing (EURL EVCAM) published a report on the outcome of a joint workshop (held in September 2011) on how Toxicity Pathways concepts are being applied. The Hamner press release said “The US National Research Council's report (2007), "Toxicity Testing in the 21st Century: A Vision and Strategy”, represented a step-change in thinking surrounding the safety assessment of environmental chemicals. The take-home message was that, by understanding toxicity in terms of Toxicity Pathways, we'll be able to use intelligent combinations of computational and in vitro methods to assess the potential hazard and risk that a chemical may pose to humans and the environment, without resorting to animal testing. Shifting to this new paradigm promises more efficient, comprehensive and cost effective testing strategies for every chemical in commerce and will provide a convincing scientific basis to move away from animal testing. But 7 years on from the publication of the NRC landmark report, much still needs to be done to translate Toxicity Pathway concepts into safety assessment practice.”

Dr. Melvin Andersen, Chief Science Officer at The Hamner Institutes, commented, “Our report remains timely as international risk assessment and toxicology communities debate new ideas regarding definitions and use of toxicity pathway and network biology concepts for making regulatory and public health decisions about chemical safety. Adoption of cell-based, high throughput tools would signal a new era for responsible regulation and more efficient use of resources in supporting regulation. These meetings of experts, allowing opportunity for wide-ranging discussions, will help gain clarity and consensus on the value of these tools”.

Source: www.thehamner.org/news-events/entry/the-hamner-and-eurl-ecvam-publish-report-on-how-toxicity-pathway-concepts-c

### 4.6 European Union Reference Laboratory for alternatives to animal testing (EURL EVCAM)

The role of EURL EVCAM is the validation of methods which reduce, refine or replace the use of animals for safety testing and efficacy/potency testing of chemicals, biologicals and vaccines.

In June 2014, EURL EVCAM announced formation of the EU Network of Laboratories for the Validation of Alternative Methods (EU-NETVAL) to improve the validation process of alternative methods.

In March 2014 EURL EVCAM hosted a Workshop on "Advancing Adverse Outcome Pathways for Integrated Toxicology and Regulatory Applications", with the Joint Research Centre (JRC), Environment Canada, US EPA, US Education Research & Data Center (ERDC), and US, Swiss, & Norwegian academic institutes.

“The workshop brought more than 50 experts from regulatory agencies, academia and industry together to discuss the Adverse Outcome Pathway concept as a framework to characterise, organise, and define predictive relationships between measurable key events that reflect the progression from a chemical-induced perturbation to an adverse outcome considered relevant to regulatory decision-making. The workshop progressed ongoing efforts (e.g. at the level of the OECD) and provided consideration and expert opinion on the critical next steps required to advance the use and acceptance of the AOP framework to support integrated toxicology and regulatory decision-making. In addition, discussions fed into further refinements of the AOP wiki – an IT system jointly led by the EC Joint Research Centre and the US Environmental Protection Agency for the purpose of the OECD programme on the development of AOPs, which was first released in August 2013. The AOP wiki is intended to facilitate the development and evaluation of Adverse Outcome Pathways, providing developers with a structured wiki type environment to capture the scientific information in a user-friendly manner in accordance with the elements of an AOP.”

Source: http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam/workshop-on-advancing-adverse-outcome-pathways-for-integrated-toxicology-and-regulatory-applications

### 4.7 HSI-Human Toxicology Project Consortium (HTPC)

The HSI-Human Toxicology Project Consortium is a group of stakeholders currently drawn from the corporate and public interest communities that share the objective of accelerating implementation of a biological pathway-based approach to toxicology as

described in the National Research Council's 2007 report on "Toxicity Testing in the 21st Century."

The HTPC is co-sponsoring training in non-animal cosmetics testing in China. China is preparing to end mandatory animal testing for domestically-produced cosmetics.

## 5. Literature Highlights

We have undertaken an extensive literature search to identify potential Lush Prize nominees. Details of the search and the results are given elsewhere in this document (see Sections 3 and 6). In the course of the research for this Science Prize paper two particular reports that did not specifically meet the criteria of the prize stood out. The first was a workshop report from the transatlantic think tank for toxicology ($t^4$) entitled "Consensus Report on the Future of Animal-Free Systemic Toxicity Testing" by Leist et al[2]. The second was an article in 'The Biochemist' (the magazine of the UK Biochemical Society) in which Unilever scientists describe several case studies showing how they are actively implementing alternative methods in safety testing of consumer products and their constituents[3].

The landmark $t^4$ report is the consensus of three years of international collaboration and discussion by 200+ experts in the field. The report makes several important recommendations on how alternative testing methodologies can be improved, and provides a scientific roadmap for future risk assessment approaches. It builds on the Basketter paper mentioned in the 2013 Lush Science paper, and makes several key recommendations:

- focus on improving existing methods rather than favouring *de novo* design
- combine hazard testing with toxicokinetics predictions
- develop integrated test strategies
- incorporate new high-content endpoints to classical assays
- evolve test validation procedures
- promote collaboration and data-sharing of different industrial sectors
- integrate new disciplines, such as systems biology and high-throughput screening
- involve regulators early on in the test development process.

The Leist *et al*. report emphasised the importance of focussing on data quality and the scientific background of test methods.

The Unilever article is an example of how a major multinational consumer goods manufacturer is implementing animal replacement strategies in its safety testing work, utilising the concepts and tools of 21st century toxicology, toxicity pathways, and AOPs.

One other significant report in the ALTEX journal was a $t^4$ Workshop on Pathways of Toxicity, held in October 2012[4]. More than 30 front-line researchers and experts spent three days outlining the benefits of mapping Pathways of Toxicity (PoT, aka AOP), and clarifying the meaning and definition of PoT. The workshop came up with a preliminary

---

2 Leist *et* al 2014, ALTEX **31**, 3/14, p341-356

3 Reynolds F, Westmoreland C and Fentem J (2014). Non-animal approaches and safety science. Biochemist, 36, 19-25

4 t4 workshop report: Pathways of Toxicity. A Kleensang, A Maertens et al. Altex **31**, 1/14, 53-612014 DOI: 10.14573/altex.1309261

definition of PoT as “A molecular definition of cellular processes shown to mediate adverse outcomes of toxicants”. It further recognized that normal physiological pathways exist that maintain homeostasis and these, if sufficiently perturbed, can become PoT. Secondly, the workshop sought to define the adequate public and commercial resources for PoT information, including data, visualization, analyses, tools, and use-cases, as well as the types of efforts that will be necessary to enable the creation of such a resource. Thirdly, the workshop explored ways in which systems biology approaches could inform pathway annotation, and which resources are needed and available that can provide relevant PoT information to the diverse user communities.

# 6. Toxicity Pathway Abstracts

## 6.1 Conference Abstract Selection

As described in the Methodology, we reviewed abstracts from the EUSAAT 2013 conference and the SEURAT-1 2014 conference. From the 140 abstracts which comprised the EUSAAT 2013 conference presentation and poster proceedings, we identified 59 abstracts which we passed through our selection process to the scoring (3rd) stage. Of these, 37 scored 1 or more. From the 30 SEURAT-1 abstracts, 10 scored 1 or more.

## 6.2 Published Abstract Selection

The PubMed search identified a total of 470 articles, and these were further narrowed to 112 by reviewing the titles and rejecting reviews and articles which were obviously unsuitable through using animal models or being overly focused on disease. These 112 were then further whittled down through review of the abstracts in the second stage of the selection process as described above. The final 53 abstracts were passed to the third assessment stage, to be scored as potential prize winners. Of these, 12 scored 1 or more. The Google Scholar search yielded a further 24 relevant abstracts, of which 7 were passed to the scoring stage, a final 3 scoring 1 or more. The Terkko FeedNavigator search yielded 504 papers of which we identified 10 novel papers to pass through to the scoring stage, of which 4 scored 1 or more. We also scored two papers identified via the Tox21 and ToxCast websites.

## 6.3 Scores

Of the total 68 abstracts scoring 1 or more, 7 scored 1 for standing out in some way (for example for providing opportunities for data sharing, or for combining methodologies to give “added value”); 39 scored 2 for bringing new knowledge or tools to a previously identified pathway of toxicity; 14 scored 2 for bringing new knowledge or tools to a previously identified pathway of toxicity but with an additional 1 (i.e. total 3) because they stood out in some way; and 7 scored 3 because they appeared to be reporting a new toxicity pathway. One scored the maximum possible marks of 3 for reporting a new toxicity pathway with an additional mark for standing out by combining methodologies and for using human non-embryonic iP stem cells.

The Table lists details (Title, Authors, source, and score) of all the abstracts scoring 1 or more. All of the abstracts for those projects scoring a total of 3 (either as 3 or as 2+1) or more are shown in full in Appendix 1.

| Title | Authors | Source | Score |
|---|---|---|---|
| Profiling of the Tox21 10K compound library for agonists and antagonists of the estrogen receptor alpha signaling pathway | R Huang, S Sakamuru et al | Tox21 website | 2 |

| Title | Authors | Source | Score |
|---|---|---|---|
| Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms | N C Kleinstreuer, J Yang et al. | ToxCast website | 2 |
| Genomic Allergen Rapid Detection In-House Validation—A Proof of Concept | H Johansson, F Rydnert et al. | Google Scholar | 2+1 |
| Dose response modeling of etoposide-induced DNA damage response | Z Li, B Sun et al. | Google Scholar | 2+1 |
| Assessing dose-dependent differences in DNA-damage, p53 response and genotoxicity for quercetin and curcumin | B Sun, SM Ross et al. | Google Scholar | 2 |
| Implementing Toxicity Testing in the 21st Century (TT21C): Making safety decisions using toxicity pathways, and progress in a prototype risk assessment | Y Adeleye, M Andersen et al. | Terkko Feed-Navigator | 2+1 |
| Proposal of an in silico profiler for categorisation of repeat dose toxicity data of hair dyes. | MD Nelms, G Ates et al. | Terkko Feed-Navigator | 2+1 |
| Molecular modelling study of the PPARγ receptor in relation to the mode of action/ adverse outcome pathway framework for liver steatosis. | I Tsakovska, M Al Sharif et al. | Terkko Feed-Navigator | 2+1 |
| Thimerosal induces apoptotic and fibrotic changes to kidney epithelial cells in vitro | MF Hornos Carneiro, C Morais et al. | Terkko Feed-Navigator | 3 |
| Monomethylarsonous acid inhibited endogenous cholesterol biosynthesis in human skin fibroblasts | Guo L et al | PubMed | 1 |
| Effect of myeloperoxidase inhibition on gene expression profiles in HL-60 cells [human] exposed to 1,2,4,-benzenetriol. | E Miyahara, T Nishikawa et al. | PubMed | 1 |
| DON shares a similar mode of action as the ribotoxic stress inducer anisomycin while TBTO shares ER stress patterns with the ER stress inducer thapsigargin based on comparative gene expression profiling in Jurkat T cells. | PC Schmeits, MR Katika et al. | PubMed | 2 |
| Effect of zinc oxide nanomaterials-induced oxidative stress on the p53 pathway. | MI Setyawati et al. | PubMed | 1 |
| Building predictive models for mechanism-of-action classification from phenotypic assay data sets. | EL Berg et al. | PubMed | 2 |

| Title | Authors | Source | Score |
|---|---|---|---|
| An integrated metabonomics and transcriptomics approach to understanding metabolic pathway disturbance induced by perfluorooctanoic acid. | S Peng, L Yan et al. | PubMed | 2 |
| miRNA expression profiling in a human stem cell-based model as a tool for developmental neurotoxicity testing. | G Pallocca, M Fabbri et al. | PubMed | 2 |
| Activation of Egr-1 in human lung epithelial cells exposed to silica through MAPKs signaling pathways. | L Chu, T Wang et al. | PubMed | 3 |
| Toxicogenomics-based identification of mechanisms for direct immunotoxicity. | J Shao, MR Katika et al. | PubMed | 3 |
| Evaluation of a human neurite growth assay as specific screen for developmental neurotoxicants. | AK Krug, NV Balmer et al. | PubMed | 2 |
| Glutathione metabolism modeling: a mechanism for liver drug-robustness and a new biomarker strategy. | S Geenen, FB du Preez et al. | PubMed | 2 |
| Titanium dioxide nanoparticles activate the ATM-Chk2 DNA damage response in human dermal fibroblasts. | RY Prasad, PD Chastain et al. | PubMed | 3 |
| Human 3D corneal models for a detailed quantification of the initial depth of injury as an indicator for cellular damage in the human eye | M. Bartok, D. Gabel, et al | EUSAAT 2013 | 2+1 |
| Further refinement of the reconstructed skin micronucleus genotoxicity assay (RSMN) | R. Curren, S. Pfuhler et al. | EUSAAT 2013 | 1 |
| hSKP-derived hepatocyte-like cells for toxicity testing | J. De Kock, R. Rodrigues et al. | EUSAAT 2013 | 2 |
| The way forward in case of a false positive in vitro genotoxicity result for a cosmetic substance? | T. Doktorova, G. Ates et al. | EUSAAT 2013 | 2+1 |
| Testing neurodevelopmental toxicity on differentiating human embryonic stem cells | A. C. Feutz and C. de Geyter | EUSAAT 2013 | 1 |
| Development and application of an algorithm to determine statistically-valid non-cytotoxic concentrations from imperfect in vitro cytotoxicity data sets | S. Foerster and M. Leist | EUSAAT 2013 | 2+1 |

| Title | Authors | Source | Score |
|---|---|---|---|
| Human neural progenitor cell (hNPC) aging is mimicked by 3D cultures in vitro: an “Adverse Outcome Pathway” gains importance at susceptible life stages | E. Fritsche | EUSAAT 2013 | 3 |
| Immortalized primary-like human cells as novel model systems in nephrotoxicity | J. Grillari | EUSAAT 2013 | 2 |
| Dynamic culture of human liver equivalents inside a micro-bioreactor for long-term substance testing | T. Hasenberg, E. M. Materne et al. | EUSAAT 2013 | 2 |
| Assessing the effects of repeated cigarette smoke exposure using human organotypic systems reproducing the respiratory tract in vitro | J. Hoeng, C. Mathis et al. | EUSAAT 2013 | 2 |
| In vitro phototoxicity screening assay or systemically administered pharmaceuticals using a reconstructed skin model EpiDerm | Y. Kaluzhny, M. W. Kinuthia et al. | EUSAAT 2013 | 2 |
| Optimization of the EpiOcular eye irritation test for hazard identification and labelling of chemicals in response to the requirements of the EU Cosmetic Directive and REACH Legislation | Y. Kaluzhny, H. Kandarova et al. | EUSAAT 2013 | 2 |
| Analysis of the validated Epiderm Skin Corrosion Test (EpiDerm SCT) and a prediction model for sub categorization according to the UN GHS and EU CLP | H. Kandarova, S. Letasiova et al. | EUSAAT 2013 | 1 |
| The diXa Project | J Kleinjans | EUSAAT 2013 | 2+1 |
| Alternative method in practice: postvalidation experience of the skin sensitization in vitro test strategy | S. N. Kolle, A. Mehling et al. | EUSAAT 2013 | 2 |
| Defining compound subcytotoxic effects on epigenetic determinants of human embryonic stem cell renewal and lineage commitment | E. Koutsouraki and P. De Sousa | EUSAAT 2013 | 2 |
| Integrating transcriptomics and metabolomics to identify pathways of toxicity of the parkinsonian toxin MPP+ | A. Krug et al. | EUSAAT 2013 | 2+1 |
| Use of transcriptome profiling in stem cell based test systems for reproductive toxicity | M. Leist | EUSAAT 2013 | 2+1 |

| Title | Authors | Source | Score |
|---|---|---|---|
| Modelling local and systemic toxicity: incorporation of in silico predictions in the development of adverse outcome pathways | J. Madden, M. Hewitt et al. | EUSAAT 2013 | 2 |
| The COSMOS project – developing integrated computational approaches to predict repeated dose toxicity | J. Madden, A. Richarz et al. | EUSAAT 2013 | 1 |
| Characterization of lymphoblastoid cell lines as a novel in vitro test system to predict immunotoxicity of xenobiotics | T. Markovic, M. Gobec et al. | EUSAAT 2013 | 2 |
| A multi-organ-chip platform for long-term maintenance and substance testing of human tissue co-culture | E. M. Materne, A. Lorenz et al | EUSAAT 2013 | 2 |
| Human embryonic stem cells differentiation reveals toxicity signatures for HDAC inhibitors and mercuric toxicants | K. Meganathan, S. Jagtap et al | EUSAAT 2013 | 3 |
| Enhancing the readout of the embryonic stem cell test with molecular approaches | A. Piersma | EUSAAT 2013 | 2 |
| Neurotoxicity in vitro: assessment of the predictivity of neuronal networks coped to microelectrode arrays for identification of neurotoxicants | T. Ramirez Hernandez, T. Weisschu et al | EUSAAT 2013 | 2 |
| Inter-laboratory validation of the yeast estrogen and yeast androgen screens for identification of endocrine active substances | T. Ramirez Hernandez, C. Woitkowiak et al. | EUSAAT 2013 | 2+1 |
| Identification of thalidomide-specific transcriptomics and proteomics signatures during differentiation of human embryonic stem cells | A. Sachinidis | EUSAAT 2013 | 2 |
| Construction of an impedance monitoring system for cell seeding and drug screening in a 3D cell culture model | C. Schäfer, W. Hilber et al. | EUSAAT 2013 | 2 |
| New perspectives for realistic and efficient in vitro screening for inhalable drugs | O. Schmid, A. G. Lenz et al. | EUSAAT 2013 | 2 |
| Development of a microfluidic biochip for chronic monitoring of 3D neural tissues derived from human embryonic stem cells | L. Stoppini et al. | EUSAAT 2013 | 2 |
| Cerium oxide nanoparticles air exposure: a comparison study using a human 3D airway model, A549 and BEAS-2B cell lines | F. van Acker, I. Kooter et al | EUSAAT 2013 | 2 |

| Title | Authors | Source | Score |
|---|---|---|---|
| “Skin on a Chip” – Perfused long term culture of skin tissue | I. Wagner, B. Atac et al. | EUSAAT 2013 | 2 |
| Phase I biotransformation of testosterone by human skin and reconstructed skin tissues | G. Weindl, W. Klipper et al. | EUSAAT 2013 | 2 |
| Evaluation of the sub-classification of dermal corrosives in vitro using the epiCS® (CellSystems) reconstructed human skin model | D. Weisensee, O. Engelking et al | EUSAAT 2013 | 2 |
| Monitoring of multilayer development of human 3D cornea constructs by trans-epithelial impedance measurement | J. Wiest et al | EUSAAT 2013 | 2 |
| Elucidation of perturbed pathways by using stem cell derived neural progenitors | D. Zagoura, F. Pistollato et al | EUSAAT 2013 | 2 |
| Development and characterization of a bioengineered conjunctiva model on the basis of immortalized cells | M. Zorn-Kruppa, M. Bartok et al. | EUSAAT 2013 | 2 |
| Towards a High Throughput Microscopy Pathway in Toxicity Reporter Platform for Chemical Safety Assessment. | S. Wink et al. | SEURAT-1 2014 | 2 |
| Infection of stem cell-derived hepatocytes with hepatotropic viruses. | N Helsen, J Paeshuyse et al. | SEURAT-1 2014 | 2 |
| Electrochemical Microsensors for Monitoring the Functions of Liver Cells in a Modular Based Microfluidic System. | J Bonne la Cour, S Generell et al. | SEURAT-1 2014 | 2 |
| Use of in silico tools to perform extrapolation for risk assessment of chemicals | M Gajewska, A Pain et al | SEURAT-1 2014 | 2+1 |
| Hepatic Toxicity Evaluation Using Oxygen-based Phosphorescent Particles and Microfluidics-based Glucose and Lactate sensors | D Bavli, S Prill et al. | SEURAT-1 2014 | 2 |
| TK /TD modelling to analyze real time hepatotoxicity data for cosmetics | S. Teng, S. Barcellini, R. et al | SEURAT-1 2014 | 2+1 |
| COSMOS Database: Public Availability of Repeated Dose Toxicity Data and Collaborative Interoperability with the ToxBank Data Warehouse Supporting Integrated Data Analysis | D Hristozov, N Jeliazkova et al. | SEURAT-1 2014 | 2 |

| Title | Authors | Source | Score |
|---|---|---|---|
| Doxorubicin irreversibly inhibits the hypoxia-inducible factor (HIF) pathway in human cardiomyocytes | J Louisse, M Barilari, et al. | SEURAT-1 2014 | 3+1 |
| Alerting Chemotypes for Liver Steatosis, Steatohepatitis and Fibrosis Identified by Mining COSMOS DB | A Mostrag-Szlichtyng, V Vitcheva et al. | SEURAT-1 2014 | 2 |
| ToxBank Integrated Analysis of SEURAT-1 Reference Compounds | P Kohonen, R Grafström et al. | SEURAT-1 2014 | 3 |

### 6.4 Nominated Abstracts

There were 8 projects which received the highest scores of either 3 or 3+1 for reporting new pathways of toxicity. The abstracts are given below. We consider all worthy of being considered by the judges as potential prize winners. We have not included those scoring 2+1 because the emphasis of the award was on new pathways, rather than on bringing new knowledge or tools to previously understood toxicity pathways. Due to space constraints, we have given the affiliations of the first author only, although many of these projects were collaborative efforts between international partners. For abstracts from published work, we provide the digital object identifier (DOD) which allows the original document to be located online.

**Doxorubicin irreversibly inhibits the hypoxia-inducible factor (HIF) pathway in human cardiomyocytes**

J Louisse, M Barilari, U Chaudhari, J Antonydas Gaspar, J Hengstler, A Sachinidis, S Hoffmann- Bremer. Systems Toxicology Unit / EURL- ECVAM, Institute for Health and Consumer Protection, Joint Research Centre, European Commission, Ispra, Italy

SEURAT-1 2014

*Score 3+1*

Abstract

We aimed to identify mechanisms underlying doxorubicin-induced delayed cardiotoxicity. To this end we used transcriptomic and high content imaging (HCI) data of iPSC-derived human cardiomyocytes that had been exposed to doxorubicin at a low, clinically relevant, concentration of 150 nM using both single and repetitive dosing regimen, including washout (recovery) studies. Analysis of the transcriptomic data indicates that besides well-known pathways related to doxorubicin's DNA-damaging effect; also genes controlled by the hypoxia- inducible factor (HIF) are affected. Interestingly, modulations of the HIF pathway were not seen in doxorubicin exposed primary liver cells. Currently, HCI analyses are being performed in order to assess the deregulation of the HIF-pathway in cardiomyocytes at the protein level. The findings indicate that the observed delayed cardiotoxicity of doxorubicin may result from the down-regulation of the HIF pathway that leads to an impairment of blood vessel formation. Therefore, the selection of biomarkers for predictive cardiotoxicity testing should include those related to an impaired hypoxia response.

**Thimerosal induces apoptotic and fibrotic changes to kidney epithelial cells in vitro**

M F Hornos Carneiro, C Morais, D M. Small, D A. Vesey, F Barbosa Jr. Aand G C. Gobe. Environmental Toxicology. Article first published online: 18 JUN 2014 DOI: 10.1002/tox.22012. Centre for Kidney Disease Research, School of Medicine, University of Queensland, Translational Research Institute, Kent Street, Woolloongabba, Brisbane, Queensland, Australia

*Score 3*

Abstrac*t*

Thimerosal is an ethyl mercury-containing compound used mainly in vaccines as a bactericide. Although the kidney is a key target for mercury toxicity, thimerosal nephrotoxicity has not received the same attention as other mercury species. The aim of this study was to determine the potential cytotoxic mechanisms of thimerosal on human kidney cells. Human kidney proximal tubular epithelial (HK2) cells were exposed for 24 h to thimerosal (0–2 μM), and assessed for cell viability, apoptosis, and cell proliferation; expression of proteins Bax, nuclear factor-κB subunits, and transforming growth factor-β1 (TGFβ1); mitochondrial health (JC-1, MitoTracker Red CMXRos); and fibronectin levels (enzyme-linked immunosorbent assay). Thimerosal diminished HK2 cell viability and mitosis, promoted apoptosis, impaired the mitochondrial permeability transition, enhanced Bax and TGFβ1 expression, and augmented fibronectin secretion. This is the first report about kidney cell death and pro-fibrotic mechanisms promoted by thimerosal. Collectively, these in vitro results demonstrate that (1) thimerosal induces kidney epithelial cell apoptosis via upregulating Bax and the mitochondrial apoptotic pathway, and (2) thimerosal is a potential pro-fibrotic agent in human kidney cells. We suggest that new evidence on toxicity as well as continuous surveillance in terms of fibrogenesis is required concerning thimerosal use. © 2014 Wiley Periodicals, Inc. Environ Toxicol, 2014.

**Activation of Egr-1 in human lung epithelial cells exposed to silica through MAPKs signaling pathways.**

Chu L, Wang T, Hu Y, Gu Y, Su Z, Jiang H. PLoS One. 2013 Jul 18;8(7):e68943. doi: 10.1371/journal.pone.0068943. Department of Pathology, Third Xiangya hospital, Central South University, Changsha, Hunan, PR China

*Score 3*

Abstract

The alveolar type II epithelial cell, regarded historically as a key target cell in initial injury by silica, now appears to be important in both defense from lung damage as well as elaboration of chemokines and cytokines. The molecular basis for silica-induced epithelial cell injury is poorly understood. In this study we explored the activation of nuclear factor Egr-1 and related signal pathway. Human II alveolar epithelial line A549 cells were exposed to silica for indicated time to assay the expression and activation of Egr-1 and upstream MAPKs. Immunofluorescence, western-blot techniques, RT-PCR, Electrophoretic mobility shift assay (EMSA), transient transfection assay, kinase inhibitor experiments were performed. It was found that the expression of Egr-1 at mRNA and protein level was significantly increased in A549 cells after administration

with silica and the activity of Egr-1 peaked by silica treatment for 60 minutes. Furthermore, phosphorylated-ERK1/2, P38 MAPKs (the upstream kinase of Egr-1) ballooned during 15-30minutes, 30-60minutes respectively after silica exposure in A549 cells. By administration of ERK1/2, P38 inhibitor, the expression and transcription of Egr-1 were both markedly decreased. But PKC inhibitor did not prevent the increase of Egr-1. These results indicated Egr-1 played a critical role in silica-induced pulmonary fibrosis in an ERK1/2, P38 MAPKs-dependent manner, which suggests Egr-1 is an essential regulator in silicosis, and underlines a new molecular mechanism for fibrosis induced by silica.

**Toxicogenomics-based identification of mechanisms for direct immunotoxicity.**

Shao J, Katika MR, Schmeits PC, Hendriksen PJ, van Loveren H, Peijnenburg AA, Volger OL. Toxicol Sci. 2013 Oct;135(2):328-46. doi:10.1093/toxsci/kft151. RIKILT-Institute of Food Safety, Wageningen University and Research Centre, 6700 AE Wageningen, The Netherlands

*Score 3*

Abstract.

Compounds with direct immunotoxic properties, including metals, mycotoxins, agricultural pesticides, and industrial chemicals, form potential human health risks due to exposure through food, drinking water, and the environment. Insights into the mechanisms of action are currently lacking for the majority of these direct immunotoxicants. Therefore, the present work aimed to gain insights into the molecular mechanisms underlying direct immunotoxicity. To this end, we assessed in vitro the effects of 31 test compounds on the transcriptome of the human Jurkat T-cell line. These compounds included direct immunotoxicants, immunosuppressive drugs with different mode of actions, and nonimmunotoxic control chemicals. Pathway analysis of the microarray data allowed us to identify canonical pathways and Gene Ontology processes that were transcriptionally regulated in common by immunotoxicants (1) with structural similarities, such as tributyltin chloride and tributyltin oxide that activated the retinoic acid/X receptor signaling pathway and (2) without structural similarities, such as As2O3, dibutyltin chloride, diazinon, MeHg, ochratoxin A (OTA), S9 treated OTA, S9-treated cyclophosphamide, and S9-treated benzo[a]pyrene, which activated unfolded protein response, and FTY720, lindane, and propanil, which activated the cholesterol biosynthesis pathway. In addition, processes uniquely affected by individual immunotoxicants were identified, such as the induction of Notch receptor signalling and the downregulation of acute-phase response genes by OTA. These findings were validated by quantitative real-time PCR analysis of genes involved in these processes. Our study indicated that diverse modes of action are involved in direct immunotoxicity and that a set of pathways or genes, rather than one single gene, can be used to screen compounds for direct immunotoxicity.

**Titanium dioxide nanoparticles activate the ATM-Chk2 DNA damage response in human dermal fibroblasts.**

Prasad RY, Chastain PD, Nikolaishvili-Feinberg N, Smeester L, Kaufmann WK, Fry RC. Nanotoxicology. 2013 Sep;7(6):1111-9. doi: 10.3109/17435390.2012.710659. Department of Environmental Sciences and Engineering, Gillings School of Global Public Health, University of North Carolina, Chapel Hill, NC, USA

*Score 3*

Abstract

The use of nanoparticles in consumer products increases their prevalence in the environment and the potential risk to human health. Although recent studies have shown in vivo and in vitro toxicity of titanium dioxide nanoparticles (nano-TiO2), a more detailed view of the underlying mechanisms of this response needs to be established. Here, the effects of nano-TiO2 on the DNA damage response and DNA replication dynamics were investigated in human dermal fibroblasts. Specifically, the relationship between nano-TiO2 and the DNA damage response pathways regulated by ATM/Chk2 and ATR/Chk1 was examined. The results show increased phosphorylation of H2AX, ATM, and Chk2 after exposure. In addition, nano-TiO2 inhibited the overall rate of DNA synthesis and frequency of replicon initiation events in DNA-combed fibres. Taken together, these results demonstrate that exposure to nano-TiO2 activates the ATM/Chk2 DNA damage response pathway.

**Human neural progenitor cell (hNPC) aging is mimicked by 3D cultures in vitro: an "Adverse Outcome Pathway" gains importance at susceptible life stages.**

E. Fritsche. Leibniz Research Institute of Environmental Medicine, Duesseldorf, Germany

EUSAAT 2013.

*Score 3*

Abstract

The "Adverse Outcome Pathway" (AOP) concept is currently promoted by the OECD because it puts existing information, e.g. the linkage between a direct molecular initiating event and an adverse outcome at a biological level of organization, into a framework that is applicable for risk assessment. Here, I present an AOP for neurotoxicity, which links an excess of reactive oxygen species (ROS) to impaired neurogenesis. This exercise gathers existing information on the molecular and key events involved in ROS-induced disturbance of this process, which is fundamental during development as well as for neuronal regeneration in the adult. In the elderly, cognition declines during the process of aging, also referred to as normal brain aging. which is the adverse outcome. One reason for this loss of organ function is decline in regenerative capacities of NPCs. We have mimicked this decline of NPC function during the aging process with human neurospheres in vitro and show that the key player in adaptation against ROS, the transcription factor Nrf2, is playing a causal role in this age-related susceptibility towards oxidative stress. In summary, we describe an AOP for neurotoxicity and show that we can mimic the cellular responses of this AOP with a 3D in vitro model. Moreover, we identify a susceptibility factor for a vulnerable life stage, which is Nrf2 in NPCs of the aging brain.

**Human embryonic stem cells differentiation reveals toxicity signatures for HDAC inhibitors and mercuric toxicants.**

K. Meganathan, S. Jagtap, S. Perumal Srinivasan, V. Shindev, V. Wagh, J. Hescheler, and A. Sachinidis. Center of Physiology and Pathophysiology, Institute of Neurophysiology, Cologne, Germany

EUSAAT 2013.

*Score 3*

Abstract.

Differentiation of human embryonic stem cells (hESCs) has been aimed to use as a tool for many clinical applications, previously we have used hESC differentiation to show the mechanism of developmental toxicants. In the present study we have attempted to show the toxicity mechanisms of HDAC inhibitors (Valproic acid (VPA), SAHA, Trichostatin A (TSA)) and mercuric toxicants (Methyl mercury (MeHg), Mercury Chloride (HgCl2), thimerosal) using hESC differentiation in combination with genome wide expression profiling. Gene expression analysis showed that VPA, reduced neural tube (OTX2, DNMT3A) and forebrain (ISL1, and EMX2) related transcripts which have been proved previously with in-vivo studies. In addition VPA over expressed axonogenesis related markers such as SLIT1, SLITRK3, and SEMA3A. GO enrichment score analysis reveals dysregulation of neuronal related biological processes such as neurogenesis and neuronal differentiation with all three HDAC inhibitors. The mRNA and miRNA correlation analysis explains many miRNA signatures for VPA toxicity including miR-20a, miR-302, miR-301, miR-155, and its corresponding mRNA targets. Also we could demonstrate a set of neuronal related markers (including transcription factors) that has been dysregulated by all HDAC inhibitors and mercuric toxicants preferably MeHg and thimerosal. Together this multilineage differentiation system attempts to represent the toxicity of the positive developmental neuronal toxicants with substantiate molecular evidence.

References

Meganathan, K., Jagtap, S., Wagh, V., et al. (2012). Identification of thalidomide-specific transcriptomics and proteomics signatures during differentiation of human embryonic stem cells. PLoS One 7, e44228.

Colleoni, S., Galli, C., Gaspar, J. A., et al. (2011). Development of a neural teratogenicity test based on human embryonic stem cells: response to retinoic acid exposure. Toxicol Sci 124, 370-377.

Copp, A. J. and Greene, N. D. (2010). Genetics and development of neural tube defects. J. Pathol 220, 217-230.

Cotariu, D. and Zaidman, J. L. (1991). Developmental toxicity of valproic acid. Life Sci 48, 1341-1350.

Krug, A. K., Kolde, R., Gaspar, J. A., et al. (2013). Human embryonic stem cell-derived test systems for developmental neurotoxicity: a transcriptomics approach. Arch Toxicol 87, 123-143.

Jagtap, S., Meganathan, K., Gaspar, J., et al. (2011). Cytosine arabinoside induces ectoderm and inhibits mesoderm expression in human embryonic stem cells during multilineage differentiation. Br J Pharmacol 162, 1743-1756.

**ToxBank Integrated Analysis of SEURAT-1 Reference Compounds**

P Kohonen, R Grafström, G Myatt, and B Hardy. Karolinska Instituet, Sweden

SEURAT-1 2014

*Score 3*

Abstract

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, amiadarone), nuclear hormone receptor ligands (e.g., tamoxifen, WY14643), selective binders (e.g. fluoxetine) and cardiotoxins (e.g., Doxorubicin, Nifedipine). Adverse events of interest that are represented include cytotoxicity, fibrosis, steatosis, cholestasis and phospholipidosis. 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.

## 7. Conclusions

The review of the most recent EUSAAT and SEURAT-1 conference proceedings, and an extensive literature search, yielded eight abstracts describing projects which are potential candidates for the Lush Science Prize shortlist - see Section 6.

In the course of the research for this year's prize, three trends were noted. Firstly, there is a significant amount of research centred around the potential toxicity of nanoparticles, including pathways of toxicity. Given the ubiquity of nanoparticles in cosmetics and personal care products, it may be worth considering giving particular attention to this area of research in future years.

Secondly, we noted that research efforts are emerging which address the development and validation of toxicity assays, and the identification of biomarkers of toxin exposure. These are the key elements of Phase II of the seminal 2007 “Toxicity Testing in the 21st Century: A Vision and Strategy” paper – briefly outlined in the 2012 Lush Science Prize background paper. The 2012 Science prize identified Phase I of the 21st century toxicity roadmap – identifying toxicity pathways – as the focus of its efforts. This emphasis was also applied in 2013 and 2014. We recommend that the 2015 Science Paper expands its remit to include research that falls within Phase II of the adoption of 21st century toxicity – assay development and validation, and identification of biomarkers of toxin exposure.

Thirdly, while not directly relevant to the Lush Science prize, we came across several EUSAAT conference abstracts that highlighted the efforts of developing countries to replace animals in toxicity testing, and to educate scientists and educators on the importance of replacing animal testing in their work. Examples include introducing the RFFIT test to check for antibody development following rabies vaccinations, instead of a mouse innoculation test, in Sri Lanka; also the work of Interniche, 2012 Lush Science prize winners, who have supported humane science teaching and the replacement of animal experiments in a number of countries including Uzbekistan and Kyrgyzstan. In passing, we also noted political efforts in major world economies to ban the use of animals for cosmetics testing, including in India, China, and the USA. These developments may be suitable for consideration for other categories of the Lush Prize.

# Appendix 1. Abstracts of projects scoring 3 or more.

### Genomic Allergen Rapid Detection In-House Validation—A Proof of Concept

H Johansson, F Rydnert, J Kühnl, A Schepky… - Toxicol. Sci. (June 2014) 139 (2): 362-370. doi: 10.1093/toxsci/kfu046

*Score 2+1*

Abstract

Chemical sensitization is an adverse immunologic response to chemical substances, inducing hypersensitivity in exposed individuals. Identifying chemical sensitizers is of great importance for chemical, pharmaceutical, and cosmetic industries, in order to prevent the use of sensitizers in consumer products. Historically, chemical sensitizers have been assessed mainly by in vivo methods, however, recently enforced European legislations urge and promote the development of animal-free test methods able to predict chemical sensitizers. Recently, we presented a predictive biomarker signature in the myeloid cell line MUTZ-3, for assessment of skin sensitizers. The identified genomic biomarkers were found to be involved in immunologically relevant pathways, induced by recognition of foreign substances and regulating dendritic cell maturation and cytoprotective mechanisms. We have developed the usage of this biomarker signature into a novel in vitro assay for assessment of chemical sensitizers, called Genomic Allergen Rapid Detection (GARD). The assay is based on chemical stimulation of MUTZ-3 cultures, using the compounds to be assayed as stimulatory agents. The readout of the assay is a transcriptional quantification of the genomic predictors, collectively termed the GARD Prediction Signature (GPS), using a complete genome expression array. Compounds are predicted as either sensitizers or nonsensitizers by a Support Vector Machine model. In this report, we provide a proof of concept for the functionality of the GARD assay by describing the classification of 26 blinded and 11 nonblinded chemicals as sensitizers or nonsensitizers. Based on these classifications, the accuracy, sensitivity, and specificity of the assay were estimated to 89, 89, and 88%, respectively.

### Dose response modeling of etoposide-induced DNA damage response

Z Li, B Sun, RA Clewell, ME Andersen, Q Zhang- Toxicol. Sci. (2013) doi: 10.1093/toxsci/kft259.

*Score 2+1.*

Abstract

The 2007 NRC Report “Toxicity Testing in the 21st Century: A Vision and A Strategy”, recommended an integrated, toxicity pathway-oriented approach for chemical testing. As an integral component of the recommendation, computational dose response modeling of toxicity pathways promises to provide mechanistic interpretation and prediction of adverse cellular outcomes. Among the many toxicity pathways, the DNA damage response is better characterized and thus more suited for computational modeling. In the present study, we formulated a minimal mathematical model of this pathway to examine the dose response for etoposide (ETP), an anti-cancer drug that causes DNA double strand breaks (DSBs). In the model, DSB results from inhibition of topoisomerase by ETP and p53 is activated by a bistable switch composed of a positive

feedback loop between ATM and γH2AX. Our stochastic model recapitulated the dose response for several molecular biomarkers measured with flow cytometry in HT1080 cells, including phosphorylated p53, ATM, γH2AX, and micronuclei. Model simulations were consistent with a bimodal pattern of p53 activation and a graded population-averaged response at high ETP concentrations. The graded response was a result of heterogeneous activation of individual cells due to molecular stochasticity. This work shows the value of combining data collection on single cell responses and mechanistic, stochastic modeling to develop and test hypothesis for the circuitry of important toxicity pathways. Future studies will determine how well this initial modeling effort agrees with a broader set of experimental studies on pathway responses by examining a more diverse group of DNA-damaging compounds.

**Implementing Toxicity Testing in the 21st Century (TT21C): Making safety decisions using toxicity pathways, and progress in a prototype risk assessment.**

Yeyejide Adeleye, Melvin Andersen, Rebecca Clewell, Michael Davies, Matthew Dent, Sue Edwards, Paul Fowler, Sophie Malcomber, Beate Nicola, Andrew Scott, Sharon Scott, Bin Sun, Carl Westmoreland, Andrew White, Qiang Zhang, Paul L. Carmichael. Toxicology. In press, DOI: 10.1016/j.tox.2014.02.00

*Score 2+1*

Abstract

Risk assessment methodologies in toxicology have remained largely unchanged for decades. The default approach uses high dose animal studies, together with human exposure estimates, and conservative assessment (uncertainty) factors or linear extrapolations to determine whether a specific chemical exposure is ‘safe’ or ‘unsafe’. Although some incremental changes have appeared over the years, results from all new approaches are still judged against this process of extrapolating high-dose effects in animals to low-dose exposures in humans. The US National Research Council blueprint for change, entitled Toxicity Testing in the 21st Century: A Vision and Strategy called for a transformation of toxicity testing from a system based on high-dose studies in laboratory animals to one founded primarily on in vitro methods that evaluate changes in normal cellular signalling pathways using human-relevant cells or tissues. More recently, this concept of pathways-based approaches to risk assessment has been expanded by the description of ‘Adverse Outcome Pathways’ (AOPs). The question, however, has been how to translate this AOP/TT21C vision into the practical tools that will be useful to those expected to make safety decisions. We have sought to provide a practical example of how the TT21C vision can be implemented to facilitate a safety assessment for a commercial chemical without the use of animal testing. To this end, the key elements of the TT21C vision have been broken down to a set of actions that can be brought together to achieve such a safety assessment. Such components of a pathways-based risk assessment have been widely discussed, however to-date, no worked examples of the entire risk assessment process exist. In order to begin to test the process, we have taken the approach of examining a prototype toxicity pathway (DNA damage responses mediated by the p53 network) and constructing a strategy for the development of a pathway based risk assessment for a specific chemical in a case study mode. This contribution represents a ‘work-in-progress’ and is meant to both highlight concepts that are well-developed and identify aspects of the overall process which require additional development. To guide our understanding of what a pathways-based risk assessment could look like in practice, we chose to work on a case study

chemical (quercetin) with a defined human exposure and to bring a multidisciplinary team of chemists, biologists, modellers and risk assessors to work together towards a safety assessment. Our goal was to see if the in vitro dose response for quercetin could be sufficiently understood to construct a TT21C risk assessment without recourse to rodent carcinogenicity study data. The data presented include high throughput pathway biomarkers (p-H2AX, p-ATM, p-ATR, p-Chk2, p53, p-p53, MDM2 and Wip1) and markers of cell-cycle, apoptosis and micronuclei formation, plus gene transcription in HT1080 cells. Eighteen point dose response curves were generated using flow cytometry and imaging to determine the concentrations that resulted in significant perturbation. NOELs and BMDs were compared to the output from biokinetic modelling and the potential for in vitro to in vivo extrapolation explored. A first tier risk assessment was performed comparing the total quercetin concentration in the in vitro systems with the predicted total quercetin concentration in plasma and tissues. The shortcomings of this approach and recommendations for improvement are described. This paper therefore describes the current progress in an ongoing research effort aimed at providing a pathways-based, proof-of-concept in vitro-only safety assessment for a consumer use product.

**Proposal of an in silico profiler for categorisation of repeat dose toxicity data of hair dyes**

Nelms MD1, Ates G, Madden JC, Vinken M, Cronin MT, Rogiers V, Enoch SJ. Arch Toxicol. 2014 Jun 3..

*Score 2+1*

Abstract.

This study outlines the analysis of 94 chemicals with repeat dose toxicity data taken from Scientific Committee on Consumer Safety opinions for commonly used hair dyes in the European Union. Structural similarity was applied to group these chemicals into categories. Subsequent mechanistic analysis suggested that toxicity to mitochondria is potentially a key driver of repeat dose toxicity for chemicals within each of the categories. The mechanistic hypothesis allowed for an in silico profiler consisting of four mechanism-based structural alerts to be proposed. These structural alerts related to a number of important chemical classes such as quinones, anthraquinones, substituted nitrobenzenes and aromatic azos. This in silico profiler is intended for grouping chemicals into mechanism-based categories within the adverse outcome pathway paradigm.

**Molecular modelling study of the PPARγ receptor in relation to the mode of action/adverse outcome pathway framework for liver steatosis**

Tsakovska I, Al Sharif M, Alov P, Diukendjieva A, Fioravanzo E, Cronin MT, Pajeva I. Int J Mol Sci. 2014 May 5;15(5):7651-66. doi: 10.3390/ijms15057651.

*Score 2+1*

Abstract.

The comprehensive understanding of the precise mode of action and/or adverse outcome pathway (MoA/AOP) of chemicals has become a key step toward the development of a new generation of predictive toxicology tools. One of the challenges of this process is to test the feasibility of the molecular modelling approaches to explore

key molecular initiating events (MIE) within the integrated strategy of MoA/AOP characterisation. The description of MoAs leading to toxicity and liver damage has been the focus of much interest. Growing evidence underlines liver PPARγ ligand-dependent activation as a key MIE in the elicitation of liver steatosis. Synthetic PPARγ full agonists are of special concern, since they may trigger a number of adverse effects not observed with partial agonists. In this study, molecular modelling was performed based on the PPARγ complexes with full agonists extracted from the Protein Data Bank. The receptor binding pocket was analysed, and the specific ligand-receptor interactions were identified for the most active ligands. A pharmacophore model was derived, and the most important pharmacophore features were outlined and characterised in relation to their specific role for PPARγ activation. The results are useful for the characterisation of the chemical space of PPARγ full agonists and could facilitate the development of preliminary filtering rules for the effective virtual ligand screening of compounds with PPARγ full agonistic activity.

**Thimerosal induces apoptotic and fibrotic changes to kidney epithelial cells in vitro**

M F Hornos Carneiro, C Morais, D M. Small, D A. Vesey, F Barbosa Jr. Aand G C. Gobe. Environmental Toxicology. Article first published online: 18 JUN 2014 DOI: 10.1002/tox.22012.

*Score 3*

Abstrac*t*

Thimerosal is an ethyl mercury-containing compound used mainly in vaccines as a bactericide. Although the kidney is a key target for mercury toxicity, thimerosal nephrotoxicity has not received the same attention as other mercury species. The aim of this study was to determine the potential cytotoxic mechanisms of thimerosal on human kidney cells. Human kidney proximal tubular epithelial (HK2) cells were exposed for 24 h to thimerosal (0–2 μM), and assessed for cell viability, apoptosis, and cell proliferation; expression of proteins Bax, nuclear factor-κB subunits, and transforming growth factor-β1 (TGFβ1); mitochondrial health (JC-1, MitoTracker Red CMXRos); and fibronectin levels (enzyme-linked immunosorbent assay). Thimerosal diminished HK2 cell viability and mitosis, promoted apoptosis, impaired the mitochondrial permeability transition, enhanced Bax and TGFβ1 expression, and augmented fibronectin secretion. This is the first report about kidney cell death and pro-fibrotic mechanisms promoted by thimerosal. Collectively, these in vitro results demonstrate that (1) thimerosal induces kidney epithelial cell apoptosis via upregulating Bax and the mitochondrial apoptotic pathway, and (2) thimerosal is a potential pro-fibrotic agent in human kidney cells. We suggest that new evidence on toxicity as well as continuous surveillance in terms of fibrogenesis is required concerning thimerosal use. © 2014 Wiley Periodicals, Inc. Environ Toxicol, 2014.

**Activation of Egr-1 in human lung epithelial cells exposed to silica through MAPKs signaling pathways.**

Chu L, Wang T, Hu Y, Gu Y, Su Z, Jiang H. PLoS One. 2013 Jul 18;8(7):e68943. doi: 10.1371/journal.pone.0068943. Print 2013.

*Score 3*

Abstract

The alveolar type II epithelial cell, regarded historically as a key target cell in initial injury by silica, now appears to be important in both defense from lung damage as well as elaboration of chemokines and cytokines. The molecular basis for silica-induced epithelial cell injury is poorly understood. In this study we explored the activation of nuclear factor Egr-1 and related signal pathway. Human II alveolar epithelial line A549 cells were exposed to silica for indicated time to assay the expression and activation of Egr-1 and upstream MAPKs. Immunofluorescence, western-blot techniques, RT-PCR, Electrophoretic mobility shift assay (EMSA), transient transfection assay, kinase inhibitor experiments were performed. It was found that the expression of Egr-1 at mRNA and protein level was significantly increased in A549 cells after administration with silica and the activity of Egr-1 peaked by silica treatment for 60 minutes. Furthermore, phosphorylated-ERK1/2, P38 MAPKs (the upstream kinase of Egr-1) ballooned during 15-30minutes, 30-60minutes respectively after silica exposure in A549 cells. By administration of ERK1/2, P38 inhibitor, the expression and transcription of Egr-1 were both markedly decreased. But PKC inhibitor did not prevent the increase of Egr-1. These results indicated Egr-1 played a critica role in silica-induced pulmonary fibrosis in an ERK1/2, P38 MAPKs-dependent manner, which suggests Egr-1 is an essential regulator in silicosis, and underlines a new molecular mechanism for fibrosis induced by silica.

**Toxicogenomics-based identification of mechanisms for direct immunotoxicity.**

Shao J, Katika MR, Schmeits PC, Hendriksen PJ, van Loveren H, Peijnenburg AA, Volger OL. Toxicol Sci. 2013 Oct;135(2):328-46. doi: 10.1093/toxsci/kft151.

*Score 3*

Abstract.

Compounds with direct immunotoxic properties, including metals, mycotoxins, agricultural pesticides, and industrial chemicals, form potential human health risks due to exposure through food, drinking water, and the environment. Insights into the mechanisms of action are currently lacking for the majority of these direct immunotoxicants. Therefore, the present work aimed to gain insights into the molecular mechanisms underlying direct immunotoxicity. To this end, we assessed in vitro the effects of 31 test compounds on the transcriptome of the human Jurkat T-cell line. These compounds included direct immunotoxicants, immunosuppressive drugs with different mode of actions, and nonimmunotoxic control chemicals. Pathway analysis of the microarray data allowed us to identify canonical pathways and Gene Ontology processes that were transcriptionally regulated in common by immunotoxicants (1) with structural similarities, such as tributyltin chloride and tributyltin oxide that activated the retinoic acid/X receptor signaling pathway and (2) without structural similarities, such as As2O3, dibutyltin chloride, diazinon, MeHg, ochratoxin A (OTA), S9 treated OTA, S9-treated cyclophosphamide, and S9-treated benzo[a]pyrene, which activated unfolded protein response, and FTY720, lindane, and propanil, which activated the cholesterol biosynthesis pathway. In addition, processes uniquely affected by individual immunotoxicants were identified, such as the induction of Notch receptor signaling and the downregulation of acute-phase response genes by OTA. These findings were

validated by quantitative real-time PCR analysis of genes involved in these processes. Our study indicated that diverse modes of action are involved in direct immunotoxicity and that a set of pathways or genes, rather than one single gene, can be used to screen compounds for direct immunotoxicity.

**Titanium dioxide nanoparticles activate the ATM-Chk2 DNA damage response in human dermal fibroblasts.**

Prasad RY, Chastain PD, Nikolaishvili-Feinberg N, Smeester L, Kaufmann WK, Fry RC. Nanotoxicology. 2013 Sep;7(6):1111-9. doi: 10.3109/17435390.2012.710659.

*Score 3*

Abstract

The use of nanoparticles in consumer products increases their prevalence in the environment and the potential risk to human health. Although recent studies have shown in vivo and in vitro toxicity of titanium dioxide nanoparticles (nano-TiO2), a more detailed view of the underlying mechanisms of this response needs to be established. Here, the effects of nano-TiO2 on the DNA damage response and DNA replication dynamics were investigated in human dermal fibroblasts. Specifically, the relationship between nano-TiO2 and the DNA damage response pathways regulated by ATM/Chk2 and ATR/Chk1 was examined. The results show increased phosphorylation of H2AX, ATM, and Chk2 after exposure. In addition, nano-TiO2 inhibited the overall rate of DNA synthesis and frequency of replicon initiation events in DNA-combed fibres. Taken together, these results demonstrate that exposure to nano-TiO2 activates the ATM/Chk2 DNA damage response pathway.

**Human 3D corneal models for a detailed quantification of the initial depth of injury as an indicator for cellular damage in the human eye.**

M. Bartok, D. Gabel, M. Engelke, M. Zorn-Kruppa3, J. M. Brandner, K. Reisinger, K. Daton, and K. Mewes EUSAAT 2013

*score 2+1*

Abstract

Currently, the definite prediction of all GHS categories for the eye-irritating potential of chemicals in one single test system relevant to the human eye is not possible. Instead, chemicals can only be classified according to the GHS in the framework of an extensive battery.

Our study aims at the complete replacement of the Draize Eye Irritation Test by a new test system which is based on biotechnologically produced hemi-cornea equivalents (Engelke et al., 2013). The sophisticated structure of the hemi-cornea model comprises both an epithelium and stroma compartment. Hence, this two-compartment model offers the potential to analyze the initial depth of injury (DoI) after substance application and to discriminate between damages induced in the epithelium and/or the stroma. We developed different approaches for the analysis of the corneal DoI in the hemi-cornea model:

1. A collagen membrane is inserted between stroma and epithelium during production as an artificial Bowman's

membrane. This membrane allows the detachment of the epithelium from the stroma and the individual quantification of the cell viability in both compartments after chemical treatment.

2. A TUNEL assay is performed on cryosections of the hemicornea in order to label all cells which have undergone

apoptosis after topical treatment with the respective test substances. The TUNEL assay can be combined with the

detection of cleaved-caspase 3, another biomarker for apoptosis. The border between the area of fluorescentlabelled cells and non-labelled cells indicates the DoI.

3. In order to specifically label the viable cells within the treated tissues, the MTT viability assay is combined with image analysis on cryosections. Quantitative interpretation of the cryosection images is based on ImageJ software analysis tools. By using this method we can distinguish between substances of all three GHS categories by means of their depth of injury.

By using one single method or a combination of the abovementioned methods we aim at the quantification of the initial depth of injury within epithelium and stroma of the hemi-cornea as a stand-alone test system for the reliable prediction of the eye irritating potential of substances according to the GHS system.

**The way forward in case of a false positive in vitro genotoxicity result for a cosmetic substance?**

T. Doktorova, G. Ates, C. Chesne, T. Vanhaecke, and V. Rogiers.

EUSAAT 2013.

*Score 2+1.*

Abstract.

The currently used regulatory in vitro mutagenicity/genotoxicity test battery has a high sensitivity for detecting genotoxicants, but it suffers from a large number of irrelevant positive results (i.e., low specificity) thereby imposing the need for additional follow-up by in vitro and in vivo genotoxicity tests. This has a major impact on the cosmetic industry in Europe, seen the imposed animal testing and marketing bans on cosmetics and their ingredients. Afflicted but safe substances will be lost. Using the example of triclosan, a cosmetic preservative, proofof- principle for the potential applicability of a toxicogenomics based in vitro assay as a potential follow-up test for positive in vitro genotoxicity results, is described. Triclosan shows a positive in vitro chromosomal aberration test but is negative during in vivo follow-up tests. Toxicogenomics analysis unequivocally shows that triclosan is identified as a compound acting through non-AND reactive mechanisms. This study illustrates the potential of genome-wide transcriptomics data in combination with in vitro experimentation as a possible weightof-evidence follow-up approach for de-risking a positive outcome in a standard mutagenicity/genotoxicity battery. As such, in the future a substantial number of compounds wrongly identified as genotoxicants could be saved.

**Development and application of an algorithm to determine statistically-valid non-cytotoxic concentrations from imperfect in vitro cytotoxicity data sets.**

S. Foerster and M. Leist.

EUSAAT 2013.

*Score 2+1*

Abstract.

Many in vitro test systems for chemicals or bioactive agents measure sophisticated functional or biochemical endpoints. However, such data can be hard to interpret, or be even meaningless, when cells are dying under the chosen assay conditions. Therefore, the determination of the non-cytotoxic concentration range for each test compound is crucial. A simple and widely used approximation is to define concentrations below those that trigger a 10% reduction in cell viability as non-cytotoxic (EC10 of the full cytotoxicity curve). However, a statistically valid approach to define non cytotoxicity is based on three conditions: first, a benchmark response (BMR) level (e.g. 10% cell death) needs to be defined; second, the benchmark concentration (BMC), i.e. the concentration of the test compound at which the BMR is reached, needs to be defined. This requires selection of a mathematical model, and its adaptation to the data points of the cytotoxicity curve. Third, the lower limit of the confidence interval of the BMC (BMCL) needs to be calculated, because only concentrations smaller than the BMCL can be considered to be non-cytotoxic with a defined minimum confidence level. An automated procedure to calculate the BMCL corresponding to a BMR of 15% cell death (BMCL15) has been developed here. For many data sets of average quality this BMCL15 corresponded to the EC10, but it provided the additional advantage of taking the overall data quality into account and thereby increases the reliability of the cytotoxicity border. The concept of BMC and BMCL is an adaptation of the benchmark dose (BMD) concept developed for in vivo toxicology studies. Several software programs are available for the purpose of BMD calculations, but the adaptation to in vitro tests is difficult for bench scientists, and the concept has still not found its way into most of the toxicology laboratories. A further reason that the BMC concept is not widely implemented yet is that this statistical approach has to be adapted to real-life problems that are typical for complex cellular assays. For instance, the data may not cover the full range of toxicities, data variation may be large, assays with different test concentrations may need to be pooled and normalizations may result in imperfect baseline data. We show here that the software solution developed by us automatically copes with such types of concentration-response data from various stem cell-based assays from the ESNATS consortium. To define cytotoxicity in these test systems it was crucial to develop a reliable and reproducible method that allows interlaboratory comparisons. An interdisciplinary programming approach was chosen by combining biological assumptions with a statistical BMC estimation concept. Using this approach it was possible to determine the highest non-cytotoxic concentration for all compounds tested in the different stem cell based test systems.

**Human neural progenitor cell (hNPC) aging is mimicked by 3D cultures in vitro: an “Adverse Outcome Pathway” gains importance at susceptible life stages.**

E. Fritsche

EUSAAT 2013.

*Score 3*

Abstract

The “Adverse Outcome Pathway” (AOP) concept is currently promoted by the OECD because it puts existing information, e.g. the linkage between a direct molecular initiating event and an adverse outcome at a biological level of organization, into a framework that is applicable for risk assessment. Here, I present an AOP for neurotoxicity, which links an excess of reactive oxygen species (ROS) to impaired neurogenesis. This exercise gathers existing information on the molecular and key events involved in ROS-induced disturbance of this process, which is fundamental during development as well as for neuronal regeneration in the adult. In the elderly, cognition declines during the process of aging, also referred to as normal brain aging. which is the adverse outcome. One reason for this loss of organ function is decline in regenerative capacities of NPCs. We have mimicked this decline of NPC function during the aging process with human neurospheres in vitro and show that the key player in adaptation against ROS, the transcription factor Nrf2, is playing a causal role in this age-related susceptibility towards oxidative stress. In summary, we describe an AOP for neurotoxicity and show that we can mimic the cellular responses of this AOP with a 3D in vitro model. Moreover, we identify a susceptibility factor for a vulnerable life stage, which is Nrf2 in NPCs of the aging brain.

**The diXa Project.**

J Kleinjans.

EUSAAT 2013.

*Score 2+1.*

Abstract

The EU nowadays witnesses increasing demands with regard to chemical safety. In particular, animal-based test models need to be replaced preferably by robust, non-animal assays in vitro/ in silico which better predict human toxicity in vivo, are less costly, and are socially better acceptable. Consumer’s and patient’s health will benefit and competitiveness of EU’s chemical manufacturing industry will be increased. For developing such assays, FP6/FP7 Research Programmes are exploiting the revenues of data-dense genomics technologies. However, till date, there is no infrastructure foreseen which aims at capturing all data produced by toxicogenomics (TGX) projects, in a standardized, harmonized and sustainable manner. Data may thus evaporate. The lack of such an infrastructure also prevents innovative breakthroughs from meta-analyses of joint databases and systems modeling. Driven by these needs of the TGX research community, diXa focuses on networking activities, for building a web-based, openly accessible and sustainable e-infrastructure for capturing TGX data, and for linking this to available data bases holding chemico/physico/toxicological information, and to data bases on molecular medicine, thus crossing traditional borders between scientific disciplines and reaching out to other research communities. To advance data sharing, diXa ensures clear communication channels and delivers commonly agreed core service support to the TGX research community, by providing SOPs for seamless data sharing, and by offering quality assessments and newly developed tools and techniques for data management, all supported by hands-on training. Through its joint research initiative, by using data available from its data infrastructure, diXa will demonstrate the feasibility of its approach by performing cross-platform integrative statistical analyses, and cross-study meta-analyses, to create a systems model for predicting chemical- induced liver injury.

**Integrating transcriptomics and metabolomics to identify pathways of toxicity of the parkinsonian toxin MPP+.**

A. Krug, T. Hartung, and M. Leist.

EUSAAT 2013

*Score 2+1*

Abstract.

Chemicals targeting the nervous system may favour the risk of the development of brain disorders such as autism or Parkinson's disease (PD). Therefore test systems are needed to first of all identify neurotoxicants and in a subsequent step to classify them according to their mode of action. Individual "omics" technologies are of rising interest in the field of toxicology for classifications, but they cannot unravel all the complexities of toxicant effects on human beings. As new alternative strategy, we integrated two "omics"-technologies, transcriptomics and metabolomics, to obtain a more precise picture of all cellular processes in an established model system of basal ganglia neurotoxicity. We exposed human dopaminergic neurons to the parkinsonian toxin MPP +, to test the usefulness of this combined "omics" technology. With the combination of transcriptomics and metabolomics we wanted to answer the question, which of the events triggered by MPP+, including mitochondrial respiration deficits, oxidative stress and energy failure, was most upstream. After 24 h of treatment with 5 µM MPP+, ATP depletion was rather minor, but pronounced changes were already seen on the transcriptome level. Gene ontology comparison did not support our expectation of altered glycolysis, but revealed changes which were mainly involved in cellular amino acid and amine metabolic processes (upregulated), as well as reactive oxygen species metabolic processes (upregulated) and DNA conformation changes (downregulated). On the metabolic level changes of glutathione, homocysteine or methionine sulfoxide confirmed the role of oxidative stress as initial pathway of toxicity. Combining data of both approaches, the transsulfuration pathway seemed to be strongly implicated in MPP toxicity. This pathway contributes to the conversion of methionine to cysteine, which is the rate limiting amino acid for glutathione synthesis. Intermediates of this pathway, such as S-adenosyl-methionine and S adenosyl-homocysteine are important contributors in DNA methylation processes, possibly explaining the changes on AND conformation. Our data suggests that combined omics analysis is more sensitive for pathway identification than individual approaches and we found that effects on DNA level as well as oxidative stress are observable before strong mitochondrial respiration deficits or energy failure were detected.

References

Ramirez, T., Daneshian, M., Kamp, H., et al. (2013). Metabolomics in toxicology and preclinical research. ALTEX 30, 209 225.

Pöltl, D., Schildknecht, S., Karreman, C., and Leist, M. (2012). Uncoupling of ATP-depletion and cell death in human dopaminergic neurons. Neurotoxicology 33, 769-779.

Scholz, D., Pöltl, D., Genewsky, A. et al. (2011). Rapid, complete and large-scale generation of post-mitotic neurons from the human LUHMES cell line. J Neurochem 119, 957- 971.

**Use of transcriptome profiling in stem cell based test systems for reproductive toxicity.**

M. Leist.

EUSAAT 2013.

*Score 2+1*

Abstract

Neurodevelopmental toxicity is usually characterized by relatively subtle changes of cellular phenotype that result in altered connectivity and network function of the nervous system. One approach to characterize in a relatively comprehensive way the changes of cells exposed to toxicants, is whole genome transcript profiling. Many developmental toxicants exert effects even after their exposure has ceased. A potential explanation are epigenetic changes, left as “memory traces” in the genome. We have used here several human embryonic stem cell (hESC)-based models of early neural development to study transcriptional and epigenetic changes exerted by several different mercurial compounds and histone deacetylase inhibitors (HDACi). For instance, one system (UKN1) modelled the directed differentiation of pluripotent hESC to PAX6-positive neural precursors, and another system (UKK) modelled the same period of early tissue specification, but allowed generation of cells of all germ layers. In all systems, transcriptome profiling clearly distinguished mercurials from HDACi and negative control compounds. The transcriptome patterns were used to predict the modes of action of unknown compounds in a “blind testing” part of this study. The transcriptome patterns were also analysed for underlying biological themes. The overrepresentation of transcription factor binding sites in different transcriptome sets indicated an astonishing overlap of the damage response to different toxicants, but also indicated “pathways” triggered only by one class of toxicant, but not by another. The idea of exploring pathways of toxicity on the basis of the altered transcriptome patterns was followed further by time-series analyses and epigenetic profiling. Preliminary results suggest that “pathways-of-toxicity” may not be constant for a given toxicant, but that they are highly dependent on the model system and the time point of exposure.

Background reading of previous work Krug, A. K., Kolde, R., Gaspar, J. A., et al. (2013). Human embryonic stem cell-derived test systems for developmental neurotoxicity: a transcriptomics approach. Arch Toxicol 87, 123-143.

**Human embryonic stem cells differentiation reveals toxicity signatures for HDAC inhibitors and mercuric toxicants.**

K. Meganathan, S. Jagtap, S. Perumal Srinivasan, V. Shindev, V. Wagh, J. Hescheler, and A. Sachinidis.

EUSAAT 2013

*Score 3*

Abstract

Differentiation of human embryonic stem cells (hESCs) has been aimed to use as a tool for many clinical applications, previously we have used hESC differentiation to show the mechanism of developmental toxicants. In the present study we have attempted to show the toxicity mechanisms of HDAC inhibitors (Valproic acid (VPA), SAHA, Trichostatin A (TSA)) and mercuric toxicants (Methyl mercury (MeHg), Mercury Chloride

(HgCl2), thimerosal) using hESC differentiation in combination with genome wide expression profiling. Gene expression analysis showed that VPA, reduced neural tube (OTX2, DNMT3A) and forebrain (ISL1, and EMX2) related transcripts which have been proved previously with in-vivo studies. In addition VPA over expressed axonogenesis related markers such as SLIT1, SLITRK3, and SEMA3A. GO enrichment score analysis reveals dysregulation of neuronal related biological processes such as neurogenesis and neuronal differentiation with all three HDAC inhibitors. The mRNA and miRNA correlation analysis explains many miRNA signatures for VPA toxicity including miR-20a, miR-302, miR-301, miR-155, and its corresponding mRNA targets. Also we could demonstrate a set of neuronal related markers (including transcription factors) that has been dysregulated by all HDAC inhibitors and mercuric toxicants preferably MeHg and thimerosal. Together this multilineage differentiation system attempts to represent the toxicity of the positive developmental neuronal toxicants with substantiate molecular evidence.

References

Meganathan, K., Jagtap, S., Wagh, V., et al. (2012). Identification of thalidomide-specific transcriptomics and proteomics signatures during differentiation of human embryonic stem cells. PLoS One 7, e44228.

Colleoni, S., Galli, C., Gaspar, J. A., et al. (2011). Development of a neural teratogenicity test based on human embryonic stem cells: response to retinoic acid exposure. Toxicol Sci 124, 370-377.

Copp, A. J. and Greene, N. D. (2010). Genetics and development of neural tube defects. J. Pathol 220, 217-230.

Cotariu, D. and Zaidman, J. L. (1991). Developmental toxicity of valproic acid. Life Sci 48, 1341-1350.

Krug, A. K., Kolde, R., Gaspar, J. A., et al. (2013). Human embryonic stem cell-derived test systems for developmental neurotoxicity: a transcriptomics approach. Arch Toxicol 87, 123-143.

Jagtap, S., Meganathan, K., Gaspar, J., et al. (2011). Cytosine arabinoside induces ectoderm and inhibits mesoderm expression in human embryonic stem cells during multilineage differentiation. Br J Pharmacol 162, 1743-1756.

**Inter-laboratory validation of the yeast estrogen and yeast androgen screens for identification of endocrine active substances**.

T. Ramirez Hernandez, C. Woitkowiak, H. A. Huener, C. Schoenlau, H. Hollert, S. Broschk, O. Zierau, G. Vollmer, M. Jaeger, A. Poth, E. Higley, M. Hecker, M. Liebsch, S. Hoffmann, B. van Ravenzwaay, and R. Landsiedel.

EUSAAT 2013

*Score 2+1*

Abstract

Endocrine disruptor compounds (EDCs) are a group of natural or synthetic compounds that have the capacity to interact with the endocrine system of living organisms and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations. Due to the impact that this interaction could have on human health, there is an increasing interest in assessing the risk of the exposure to EDCs. Currently,

several in vitro and in vivo assays have been developed and few of them validated and regulatory accepted. For instance, the US EPA developed the Endocrine Disruptor Screening Program, which has been recently implemented. For the programme a large number of experimental animals will be still use used even for testing some of the in vitro assays. Herein, we performed the inter-laboratory validation of two robust models that addresses agonistic and antagonist effect at the human hormone receptor, the YES (Yeast Estrogen Screen) and the YAS (Yeast Androgen Screen). Both assays are non-animal alternatives to the estrogen and androgen receptor binding assays proposed in the EDSP and OECD Conceptual Framework. The ring trial is the final experimental part of the validation process at the European Center for Validation of Alternative Methods (ECVAM). A set of 24 compounds with estrogenic, anti-estrogenic, androgenic or anti-androgenic activity were tested in five different laboratories in a blinded fashion. The analysis of the data demonstrates a high reproducibility for both methods among the different participating laboratories. Most importantly, both assays exhibited a good predictivity. In conclusion, the methods have been successfully transferred to other laboratories and they exhibit a high accuracy to identify EDCs that interact with sex hormone receptors.

**Use of in silico tools to perform extrapolation for risk assessment of chemicals**

M Gajewska, A Paini, J Vicente, S Benito, A Worth.

SEURAT-1 2014

*Score 2+1*

Abstract

The aim of this work is to develop a computer-assisted toxicokinetic/ toxicodynamic strategy for risk assessment of cosmetic ingredients promoting alternatives to animal testing. A physiologically based toxicokinetic-toxicodynamic (PBTK-TD) model and a virtual cell-based assay (VCBA) for humans have been developed to simulate acute exposure to cosmetics via inhalation, oral and dermal absorption. The models have also been coupled to perform in vitro- to-in vivo (IVIVE) and route-to-route (RR) extrapolations. The IVIVE is a multiscale approach that joins in vitro information at cell level with in vivo organ level kinetics. RR is useful in cases when toxicity data for one exposure route are available, but others are lacking. As an example, a case study on caffeine is presented. Blood concentrations following oral and dermal exposure are simulated at the oral no-observed-effect-level (NOEL) dose derived from animal studies to predict internal systemic concentrations and extrapolated to a dermal safe limit. The difference in resulting toxicokinetic parameters (Area Under Curve, bioavailability, peak concentration, percentage of dose absorbed) is shown for two exposure routes. The coupled dynamic model at organ level links the calculated blood levels with so-called "effect" compartment to quantify the development of caffeine tolerance and VCBA calculates the estimated concentration inside the liver cell (HepaRG). The entire analysis including data access, model simulation in R, visualisation and reporting have been implemented in a KNIME workflow which is freely available for use.

**TK /TD modelling to analyze real time hepatotoxicity data for cosmetics**

S. Teng, S. Barcellini, R. Beaudouin, R. Rahmani, A. Pery

SEURAT-1 2014

*Score 2+1*

Abstract

Recent in vitro improvements permit to monitor cell responses in real time. It then becomes straightforward to assess not only to the level of effects but also to its dynamics. However, these new tools require a change of paradigm in the way the data are analyzed, i.e. moving from descriptive statistics towards the calibration of toxico-dynamic models able to account for the time-course of the responses.

Here, we modelled Cell Index (CI) values derived from the electric impedance measured on electronic sensor plates containing adherent hepatocytes (HepaRG) exposed to three hepatotoxic cosmetics (coumarin, isoeugenol and benzophenone-2). Impedance values reflected real time cyto-morphological modifications and cell viability.

We calibrated a mechanistic toxicokinetic/toxicodynamic (TK/TD) model, which incorporates different phenomena: i) cosmetics uptake and elimination by the cell ii) decrease of exposure concentration (due, for example, to evaporation, binding to well walls, or metabolism by the cells) iii) cell morphological modifications due to exposure to cosmetics iv) heterogeneity of sensitivity between cells.

For the three cosmetics tested, we could adequately fit acute data (for 48h exposure) with only a small number of parameters. We showed that equilibrium between medium and cell concentrations was quasi-instantaneous and that no difference of sensitivity was expected between the cells.

We then challenged the model with data obtained for the same substances during 4 weeks of exposure and showed that long-term effects were generally under-estimated. The calibration of our model to analyze these data is ongoing and will permit to investigate the increase of sensitivity occurring with time.

**Doxorubicin irreversibly inhibits the hypoxia-inducible factor (HIF) pathway in human cardiomyocytes**

J Louisse, M Barilari, U Chaudhari, J Antonydas Gaspar, J Hengstler, A Sachinidis, S Hoffmann- Bremer

SEURAT-1 2014

*Score 3+1*

Abstract

We aimed to identify mechanisms underlying doxorubicin-induced delayed cardiotoxicity. To this end we used transcriptomic and high content imaging (HCI) data of iPSC-derived human cardiomyocytes that had been exposed to doxorubicin at a low, clinically relevant, concentration of 150 nM using both single and repetitive dosing regimen, including washout (recovery) studies. Analysis of the transcriptomic data indicates that besides well-known pathways related to doxorubicin's DNA-damaging effect; also genes controlled by the hypoxia- inducible factor (HIF) are affected. Interestingly, modulations of the HIF pathway were not seen in doxorubicin exposed primary liver cells. Currently, HCI analyses are being performed in order to assess the deregulation of the HIF-pathway in cardiomyocytes at the protein level. The findings indicate that the observed delayed cardiotoxicity of doxorubicin may result from the down-regulation of the HIF pathway that leads to an impairment of blood vessel

formation. Therefore, the selection of biomarkers for predictive cardiotoxicity testing should include those related to an impaired hypoxia response.

**ToxBank Integrated Analysis of SEURAT-1 Reference Compounds**

P Kohonen, R Grafström, G Myatt, and B Hardy

SEURAT-1 2014

*Score 3*

Abstract

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, amiadarone), nuclear hormone receptor ligands (e.g., tamoxifen, WY14643), selective binders (e.g. fluoxetine) and cardiotoxins (e.g., Doxorubicin, Nifedipine). Adverse events of interest that are represented include cytotoxicity, fibrosis, steatosis, cholestasis and phospholipidosis. 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.