THE 2015 LUSH PRIZE

SUPPORTING ANIMAL-FREE TESTING

Lush Prize for Science - 2015

Research Paper

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1. Executive Summary

1.1 What is the Lush Science Prize?

Now in its fourth 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 Researchers. 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(s) or team(s) 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; Hamner Institutes; Human Toxome Project; ECVAM; US Tox21 Programme, the ToxCast programme; and the Human Toxicology Project Consortium (see section 4). We also assessed recent developments in toxicity testing 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 WC9, the Society of Toxicology annual conference, and the SEURAT-1 annual conference. There were a total of 3370 abstracts from oral and poster presentations from these conferences, but many were not relevant to the Lush Science prize. Relevant abstracts were scored using the system derived in previous years (see section 3) in which 3 points are awarded for projects identifying new toxicity pathways, assays, or biomarkers; 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 PubMed, Google Scholar and Terkko Feed Navigator to identify projects describing recent advances in toxicity testing research. One additional relevant project was identified directly from the Hamner Institutes website. In all, searches yielded in excess of 2200 projects which we assessed as described (see Section 3).

Overall, from all sources combined, from 50 abstracts which scored 1 or more, 2 scored 1; 32 scored 2; 11 scored 2 +1 (i.e. total 3); and 4 scored 3 because they appeared to be reporting a new toxicity pathway, assay, or biomarker. A single project scored the maximum possible marks of 3 for reporting a new toxicity pathway feature plus an additional mark for standing out as well (use of high-throughput techniques). The titles and authors of those abstracts scoring 1 or more are shown in section 6.3, whilst full abstracts of those projects scoring at least 3 (either as 2+1, 3 or 3+1) are given in Section 6.4.

1.3 Projects recommended for the shortlist

Research papers for each Prize category identify projects that merit particular consideration because of their outstanding work in the field. There were 16 projects which received scores of at least 3 for reporting new pathways, assays, or biomarkers of toxicity. The full abstracts are given in Section 6.4.

2. Background

The objectives of 21st Century Toxicity Testing (tt21c) 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, 2013, and 2014 Lush Prizes provide an overview, and links to further resources, describing the concept of 21st Century Toxicology.

The Lush Science Prize seeks to reward those researchers making 'outstanding contributions' to tt21c research. In previous years, the focus of the award has been on research aimed at elucidating key pathways in which perturbation results in toxicity. For 2015, we have continued this approach, and further extended it to cover additional aspects of tt21c, beyond toxicity pathways. In particular, we have also sought to identify significant contributions to the development of new *in vitro* assays that advance tt21c, and the discovery of biomarkers that signal early activation of toxicity pathways.

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

The primary purpose of this report is to assist the Lush Prize judging panel by describing significant advances in animal-free toxicity testing that have taken place over the preceding year, and by identifying key projects that are making major contributions to the field of animal-free toxicology research. From these, along with additional nominations received directly via the Lush Prize website, the panel may select the winners of this year's Lush Prize for Science. In this section we describe how we identified projects that might be worthy of consideration as potential prize winners, and then how we scored each project to create a shortlist for the panel's consideration.

Of the "3 Rs", Lush Prize's interest focuses primarily on Replacement, so our search for potential prize winners targeted projects working towards the replacement of animals in product testing, and we excluded research aimed at either Refining or Reducing the use of animals in experimentation. We also excluded research that was linked to animal testing in other ways. We considered projects based anywhere in the world, but restricted the search as far as possible to work reported in the year preceding the award (i.e. June 2014 – July 2015).

In the identification of key developments in the area of toxicology research, and in the search for candidate prize winners, we followed three separate strands of investigation. We started firstly 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 OECD; Hamner Institutes; Human Toxome Project; ECVAM; US Tox21 Programme, the ToxCast programme; and EU Horizon 2020 (see section 4).

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 2014 - 2015 included the 9th World Congress on Alternatives & Animal Use in the Life Sciences (WC9), held in Prague in August 2014, the EU SEURAT-1 Project's 5th Annual Meeting, held in Barcelona in January 2015, and finally the Society of Toxicology's (SoT's) 54th Annual Meeting, held in San Diego in March 2015. The European Society for Alternatives to Animal Testing (EUSAAT) did not hold a meeting in 2014 – the next EUSAAT meeting is in September 2015. Proceedings from the SEURAT-1 2015 meeting were kindly provided by Sara Vinklatova, Administrator, COACH SEURAT-1. Due to confidentiality issues, several SEURAT abstracts were not available for our review.

Thirdly, we conducted a review of the recent literature. For this we used three separate sources. Firstly, we searched PubMed for research published from 01/06/2014 to date, combining search terms "toxicity pathway," "toxicity assay" and "toxicity biomarker", excluding any review articles and clinical trials, and restricting the subject matter to "humans". As a second literature source, we searched Google Scholar for relevant papers published in the period 2014 to 2015, combining search terms "toxicity pathway," "toxicity assay" and "toxicity biomarker", and restricting the

subject matter to “humans”. As a final literature source, we searched Terkko Feed Navigator combining search terms “toxicity pathway,” “toxicity assay” and “toxicity biomarker” and considered only those papers that were published in 2014 or 2015. As per last year, our selection procedure was a three stage process. In the first stage, we reviewed the project title and rejected any articles which were clearly reviews or which were obviously unsuitable through using animal models or being overly focussed on disease. In the second stage, we assessed the abstracts which passed the initial filter and further eliminated those which reported findings from clinical trials, those focusing on cancer research, all research that included animal subjects, and any 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: discovery of a new pathway; a significant advance in assay technology or approach; or a new biomarker for early activation of toxicity?	*Score 3*
If it is working with an apparently previously understood pathway, assay technology, or biomarker, does it bring new knowledge or tools?	*Score 2*
Does it stand out in any other way?	*Score 1*

Projects awarded a score of 2 or 3 could also receive an extra 1 point if they also stood out in some other way, so the maximum possible score is 4.

At each stage of our search, research projects were carefully excluded based on our selection criteria, in order to achieve a manageable shortlist of excellent work which fully met the prize brief.

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

Tox21 is a collaborative effort among the National Institutes of Health (NIH), the Environmental Protection Agency (EPA), and the Food and Drug Administration (FDA). 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, by utilising robotic systems and high-throughput technologies to screen thousands of compounds.

In addition to testing thousands of chemicals for their effects on a few human cell lines, the Tox21 programme has recently used its technology to look at differences in human sensitivity to compounds[1]. NCATS' screening capabilities have been used to screen the effect of 179 chemicals on more than 1000 individuals, using lymphoblastoid cells from 1,086 people from the 1,000 Genomes Project[2]. The study found that the toxicodynamic factor for risk assessment of chemicals varied much more than previously thought, with half of the chemicals having a much larger factor than usually applied.

4.2 ToxCast

The US EPA's ToxCast (Toxicity Forecaster) programme aims to use high-throughput screening tools to test many thousands of chemicals in in vitro assays. ToxCast has published an expanded model of endocrine profiling using its data on 1858 chemicals. It should be noted, however, that the EPA ToxCast programme continues to use animal models for some of its work (see http://pubs.acs.org/doi/abs/10.1021/tx500501h for an example).

4.3 AXLR8/Horizon 2020

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". As reported in the 2014 Science background paper, AXLR8 published its final report in December 2013 and recommended that the EU invest €250 million over the seven years of the Horizon 2020 funding round, focussing on three pillars of work:

1 http://ehp.niehs.nih.gov/1408775/
2 http://www.1000genomes.org/

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

The EU's call for proposals under the Horizon 2020 'Personalising health and care (PHC)' programme opened on July 30th 2014. One of the topics for proposals is 'New approaches to improve predictive human safety testing' (PHC-33-2015). The call closed on February 24th 2015, and 28 proposals were submitted. These proposals are competing with 54 others for a share of €88 million of PHC-allocated funds. Clearly this is considerably short of AXLR8's recommendations so far.

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

The OECD launched its AOP development programme in 2012. Within this programme is a 'Knowledge Base project (AOP-KB)' – co-led by the European Commission's Joint Research Centre and the US Environmental Protection Agency. In September 2014, the first element of the AOP-KB was released – the AOP Wiki (https://aopkb.org). The AOP Wiki is an interactive and virtual encyclopaedia for AOP development, structured in accordance with the OECD's original guidance document. Other modules of the AOP-KB are under development, and it is expected that the next module released will be Effectopedia – a knowledge aggregation and modelling platform designed for collaborative development and utilisation of AOPs. The other significant development this year is the publication of two new Test Guidelines (442c and 442d) for non-animal tests for skin sensitivity, which occurred in February 2015. Guideline 442c is an *in chemico* test (Direct Peptide Reactivity Assay, DRPA) for discriminating between skin sensitizing chemicals and non-sensitizers. Guideline 442d is an *in vitro* assay using human keratinocytes (KeratinoSens™) for the same purpose.

4.5 Hamner Institutes

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

The Hamner is focussing on case studies that show a pathway for the implementation of new methods for safety assessment. The oestrogen pathway case study emphasises the need to develop more comprehensive assessments of the various oestrogen receptors and to focus on the 'adverse' responses, rather than relying on more simple pathway perturbations such as binding, transactivation etc. The p53-DNA damage pathway case study has indicated that DNA-reactive compounds have threshold responses at low doses: this work is due to be published this year.

The Hamner has announced the formation of the Comprehensive Center for Liver and Metabolic Disease Research (CCLMDR), a pre-competitive, collaborative partnership between The Hamner and a diverse set of stakeholders across industry and academia. The Center aims to pool resources and co-develop platforms

focussed on liver and metabolic disease research. The CCLMDR's vision is to be the recognized global leader and ultimate collaborative research resource for the development of strategies and next-generation technologies aimed at conquering liver and liver-related metabolic diseases. The Steering Committee for the CCLMDR is headed by Dr. Paul Watkins, director of the Hamner-University of North Carolina Institute for Drug Safety Sciences, and Dr. Edward LeCluyse, associate investigator at The Hamner.

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 in safety testing and in efficacy/potency testing of chemicals, biologicals and vaccines.

In March 2015, EURL EVCAM announced the validation of the human Cell Line Activation Test (h-CLAT) for skin sensitization. The h-CLAT test identifies chemicals that can trigger skin allergies – estimated to affect 20% of the population in Europe. Having validated h-CLAT, ECVAM recommends its use as part of an integrated approach to chemical testing and assessment, along with other non-animal methods and *in silico* predictions. The ECVAM validation and recommendation of h-CLAT should help in the development of an OECD Test Guideline for h-CLAT, applicable globally.

In April 2015, ECVAM scientists published work (in collaboration with CAAT) which shows gender differences in drug sensitivity and hepatotoxicity between male- and female- derived primary human hepatocytes. This is the first time that gender differences in drug-induced liver injury have been demonstrated at the cellular level – see Section 5 for more details.

The Joint Research Centre (JRC), which hosts EURL ECVAM, has stated its intention to collaborate with those consortia that are awarded funding under the EU Horizon 2020 programme call for 'New approaches to improve predictive human safety testing'.

Although not a complete move to eradicate animal use, EURL ECVAM has released a strategy document for the replacement, reduction, and refinement of the use of animals in the assessment of acute mammalian systemic toxicity[3]. EURL ECVAM has also published a state-of-the-art review on 'Alternative methods for regulatory toxicology'[4].

4.7 HSI-Human Toxicology Project Consortium (HTPC)

3 https://eurl-ecvam.jrc.ec.europa.eu/news/acute-mammalian-systemic-toxicity-testing-eurl-ecvam-releases-its-strategy?utm_medium=twitter&utm_term=%23animalfreetests&utm_source=twitterfeed

4 http://publications.jrc.ec.europa.eu/repository/bitstream/111111111/32662/1/echa_jrc_sla_report_public_05-09-14_withcover%20ipo.pdf

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 the 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." Its work in the 2014/2015 period has focussed mainly on dissemination of advances in assays and technologies for tt21c, such as organs-on-a-chip.

4.8 Human Toxome Project

The Human Toxome Project is a five-year collaborative project, funded by the NIH NIEHS, that aims to map comprehensively the pathways of endocrine disruption in humans, as a first step to mapping all of the toxicity pathways that make up the human 'toxome'. The institutional collaborators are: CAAT; Bloomberg School of Public Health; EPA; Agilent Technologies; Hamner Institute for Chemical Safety Sciences; Brown University; and Georgetown University Medical Center. Progress to date is summarised in a recent ALTEX article[5]. The focus of the project is to develop methods for deducing and validating molecular pathways of toxicity, using oestrogen endocrine disruption as a model.

5 http://altweb.jhsph.edu/altex/32_2/Bouhifd.pdf

5. Literature Highlights

We have undertaken an extensive literature search to identify potential Lush Prize for Science nominees. Details of the search and our results are given elsewhere in this document (see Sections 3 and 6). In the course of the research for this Science Prize paper, three particular reports that did not specifically meet the criteria of the prize stood out. One report that does meet the criteria for consideration for the science prize is also discussed here, because although it does not describe a new pathway or assay *per* se, its impact is highly significant.

The first was a roundtable discussion between leading figures in the field on the subject "A Vision of Toxicity Testing in the 21st Century", reported in the first edition of the new journal *Applied In Vitro Toxicology*[6]. Two particular points stand out, both made by Mel Andersen, one of the authors of the seminal 2007 EPA policy document 'Toxicity Testing in the 21st Century'. The first is that disappointingly little progress has been made on establishing the global consensus that *in vitro* testing, rather than high-dose animal studies, are the preferred way to approach tt21c. Andersen observes that, in the EU, animal testing is preferred, but this has had to change because of pressure to implement the 3Rs, while in the US he suggests that animal testing is perceived to be better, but that it is too slow and costly. Andersen believes that more effort needs to be made to build the consensus that testing must focus on human-relevant biology. Secondly, Andersen highlights the perception that AOPs may not be as helpful as hoped in persuading regulators to adopt tt21c: there is a risk that they may reinforce a focus on high-dose animal effects as the adverse outcome of interest.

The second was a workshop report from the transatlantic think tank for toxicology (t^4) on the state-of-the-art of 3D cell culture and 'organs-on-a-chip' in safety testing[7]. The review summarises the current technology for creating organotypic models, and offers consensus recommendations for the successful implementation of 3D models in routine screening.

Thirdly, Mennecozzi *et al*[8] report for the first time gender differences, in terms of susceptibility to drug-induced liver effects, at the cellular level. While no new pathways were described, the study highlighted the value of using human-derived cells (in this case cryo-preserved human hepatocytes from pre-menopausal and post-menopausal women, and from men) to show subtle but significant differences between populations.

Finally, Yang *et al*[9] have impressively demonstrated the power of *in silico* modelling for drug safety analysis. The drug troglitazone was introduced in 1997 as a treatment for type 2 diabetes. It was withdrawn from the market in 2000 after 63 people died from liver failure after taking it. Preclinical studies had shown no danger to the liver in rats, and trials had not highlighted a significant risk to humans. It was only when the drug was used in a large enough population that individuals appeared who were unable to process the drug safely. The Yang paper, published last year,

6 http://online.liebertpub.com/doi/pdf/10.1089/aivt.2014.1501
7 http://www.altex.ch/All-issues/Issue.50.html?iid=151&aid=6
8 http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0122786
9 http://onlinelibrary.wiley.com/doi/10.1038/clpt.2014.158/abstract

has used the Hamner Institutes DILIsym software model of drug-induced liver injury to model the effects of troglitazone in rat and human populations. The findings were that the DILIsym model successfully predicted that troglitazone would, very rarely, cause life-threatening liver injury in humans, and that the accumulation of bile acids would be the likely cause of such liver injury. The model also predicted that rats would respond differently to the drug than humans. This study powerfully illustrates that animal-based toxicity testing cannot be relied upon to predict effectively the risks to humans, and also demonstrates that modern tt21c methods, such as *in silico* modelling, can successfully predict potentially life-threatening toxicities.

6. Toxicity Pathway Abstracts

6.1 Conference Abstract Selection

As described in the Methodology, we reviewed abstracts from the WC9 2014 meeting, from the SoT's 2015 meeting, and also from the EU SEURAT-1 2015 meeting.

From the 891 abstracts which comprised the WC9 2014 conference presentation and poster proceedings, we identified 37 abstracts which passed through our selection process to the final (3rd) scoring stage. Of these, 11 scored 1 or more. From the 2468 SoT abstracts, and based on the Abstract book keyword index, 53 were passed to the scoring stage, of which 3 scored 1 or more. From 11 abstracts made available to us from the SEURAT-1 meeting all were reviewed, but only 3 scored 1 or more.

6.2 Published Abstract Selection

From the PubMed search, we identified 364 relevant titles from the "Toxicity pathway" search, 1283 from the "Toxicity assay" search, and 345 from the "Toxicity biomarker" search: a combined total of 1992 articles.

Stages 1 and 2 of the selection process (review of titles and, if necessary abstracts, to reject review articles, results of clinical trials, articles reporting use of animal subjects or non-human cell lines, or those on cancer research or overly focussed on other disease), reduced these 1992 titles to just 99 abstracts (38 + 32 + 29 for the 3 separate searches, respectively). These final 99 abstracts were passed to the third assessment stage, to be scored as potential prize winners. Of these, 27 scored 1 or more.

The Google Scholar search identified 355 possibly relevant abstracts, of which only 8 survived selection stages 1 and 2 and were passed on to the scoring stage. Of these 8, a final 4 scored 1 or more.

The Terkko FeedNavigator search yielded 32 papers, of which we identified 2 novel papers to pass through to the scoring stage. Of these, 1 scored 1 or more.
We also identified one further relevant project, directly from the Hamner Institutes website, which scored 1 or more.

6.3 Scores

From the three separate sources of potential shortlisted projects, we thus identified a total of 50 abstracts describing work which scored at least one point according to our given criteria. Of these, 2 scored 1 for standing out in some way (for example for providing opportunities for data sharing, or for combining methodologies to give "added value"); 32 scored 2 for bringing new knowledge or tools to a previously identified pathway, assay, or biomarker of toxicity; 11 scored 2 for bringing new

knowledge or tools to a previously identified pathway, assay, or biomarker of toxicity but with an additional 1 (i.e. total 3) because they stood out in some way; and 4 scored 3 because they appeared to be reporting a new toxicity pathway, assay, or biomarker. Only one scored the maximum possible 4 marks with 3 awarded for reporting a new toxicity pathway plus an additional mark for standing out - in this case by using a high throughput approach.

The Table lists details (Title, Authors, contribution (pathway, assay, or biomarker), source, and score) of all the abstracts scoring 1 or more. All of the abstracts for those projects scoring a total of 3 or more are shown in full in Section 6.4.

Title	**Authors**	**Category**	**Source**	**Score**
Paired image- & FACS-based toxicity assays for HCS of spheroid-type tumor cell cultures.	Trumpi *et al*	Assay	Google Scholar	2
Certain Phase I and II enzymes as toxicity biomarker: An overview.	Hassan *et al*	Biomarker	Google Scholar	2
Toxicity mechanisms identification via gene set enrichment analysis of time-series toxicogenomics data: Impact of time and concentration.	Gao *et al*	Pathway/ assay	Google Scholar	2 + 1
Systematic proteomic approach to characterise the impacts of chemical interactions on protein and cytotoxicity responses to metal mixture exposures.	Ge *et al*	Pathway	Google Scholar	2 + 1
Profiling dose-dependent activation of p53-mediated signalling pathways by chemicals with distinct mechanisms of DNA damage.	Clewell *et al*	Pathway	Terkko Feed Navigator	3
Transciptional profiling and biological pathway analysis of human equivalence PCB exposure *in vitro*: Indicator of disease and disorder development in humans.	Ghosh *et al*	Pathway/ biomarker	PubMed	2
Potential of extracellular microRNAs as biomarkers of acetaminophen toxicity in children.	Yang *et al*	Biomarker	PubMed	2

Title	Authors	Category	Source	Score
Identification of novel biomarkers for doxorubicin-induced toxicity in human cardiomycytes derived from pluripotent stem cells.	Holmgren *et al*	Biomarker	PubMed	2 + 1
Identification of lipidomic biomarkers for coexposure to subtoxic doses of benzo[a]pyrene and cadmium: The toxicological cascade approach.	Jungnickel *et al*	Biomarker/ assay	PubMed	3
OxLDL induces injury and defenestration of human liver sinusoidal endothelial cells via LOX1.	Zhang *et al*	Pathway	PubMed	2
How useful are clinical liver function tests in in vitro human hepatotoxicity assays?	Borlak *et al*	Assay	PubMed	2
Molecular mechanisms of 3'3-dichlorobenzidine-mediated toxicity in HepG2 cells.	Chen *et al*	Pathway	PubMed	2
Development of a pluripotent stem cell-derived neuronal model to identify chemically induced pathway perturbations in relation to neurotoxicity: Effects of CREB pathway inhibition.	Pistollato *et al*	Pathway/ model	PubMed	1
Toxicity of silver nanoparticles to human dermal fibroblasts on microRNA level.	Huang *et al*	Pathway	PubMed	2
More than just hormones: H295R cells as predictors of reproductive toxicity.	Maglich *et al*	Assay	PubMed	3
Performance of the N/TERT epidermal model for skin sensitizer identification via Nrf2-Keap1_ARE pathway activation.	Alloul-Ramdhani *et al*	Assay/ model	PubMed	2
Systems biology modelling of omics data: effect of cyclosporinA on the Nrf2 pathway in human renal cells.	Hamon *et al*	Modelling/ predictive	PubMed	2 + 1
Silica nanoparticles induce cytokine activation responses in lung epithelial cells through activation of a p38/TACE/TGFa/EGFR-pathway and NfkB signalling.	Skuland *et al*	Pathway	PubMed	2

Title	Authors	Category	Source	Score
Evaluation of cytotoxicity and DNA damage response with analysis of intracellular ATM signalling pathways.	Bandi *et al*	Assay	PubMed	2
The acquisition of cancer stem cell-like properties and neoplastic transformation of human keratinocytes induced by arsenite involves epigenetic silencing of let-7c via Ras/NFkB.	Jiang *et al*	Pathway	PubMed	2
Replication stress by Py-Im polyamides induces a non-canonical ATR-dependent checkpoint response.	Martinez *et al*	Pathway	PubMed	2
Modulation of the DNA repair system and ATR-p53-mediated apoptosis is relevant for tributyltin-induced genotoxic effects in human hepatoma G2 cells.	Li *et al*	Pathway	PubMed	2
Induction of glutathione synthesis in human hepatocytes by acute and chronic arsenic exposure: Differential roles of mitogen-activated protein kinases.	Hou *et al*	Pathway	PubMed	2
AP-1 activation attenuates the arsenite-induced apoptotic response in human bronchial epithelial cells by up-regulating HO-1 expression.	Aodengqim-uge *et al*	Pathway	PubMed	2
MicroRNA regulation of DNA repair gene expression in 4-aminobiphenyl-treated HepG2 cells.	Huan *et al*	Pathway	PubMed	2
Topological, functional, and dynamic properties of the protein interaction networks rewired by benzo(a)pyrene.	Ba *et al*	Pathway	PubMed	2+1
Heavy metal-induced metallothionein expression is regulated by specific protein phosphatase 2A complexes.	Chen *et al*	Pathway	PubMed	2
Evaluation of the Multi-ImmunoToxAssay composed of 3 human cytokine reporter cells by examining immunological effects of drugs.	Kimura *et al*	Assay	PubMed	3

Title	Authors	Category	Source	Score
The low molecular weight DNA diffusion assay as an indicator of cytotoxicity for the in vitro comet assay.	Speit *et al*	Assay	PubMed	2
A cell-based biosensor system HepG2CDKN1A-DsRed for rapid and simple detection of genotoxic agents.	Blagus *et al*	Assay	PubMed	2
Identification of microRNAs involved in growth arrest and cell death in hydrogen peroxide-treated human dermal papilla cells.	Kim *et al*	Pathway	PubMed	2
Body-on-a-chip simulation with gastrointestinal tract and liver tissues suggests that ingested nanoparticles have the potential to cause liver injury.	Esch *et al*	Assay	PubMed	2+1
Systems pharmacology modelling predicts delayed presentation and species differences in bile acid-mediated troglitazone hepatotoxicity.	Yang *et al*	Pathway	Hamner Institutes website	2+1
Novel computational approaches for high content image analyses (HCA) of organoid 3D neurosphere cultures in vitro.	Fritsche *et al*	Assay	WC9	2
Human beating heart-on-a-chip for cardiotoxicity testing as an example case of the Dutch organ-on-a-chip initiative.	Van de Stolpe & van den Eijnden	Assay	WC9	2
Neurotoxicity *in vitro*: Assessment of the predictivity of neuronal networks coped [*sic*] to microelectrode arrays for identification of neurotoxicants.	Ramirez *et al*	Assay	WC9	2+1
LUHMES 3D neuronal model for (developmental) neurotoxicity testing.	Smirnova *et al*	Assay	WC9	2+1
Using high-content imaging to analyze cell-state trajectories and biological tipping points for chemical exposures.	Shah	Assay/ pathway	WC9	3+1

Title	Authors	Category	Source	Score
Identification of cornifelin and early gene response-1 as new biomarkers for in vitro eye irritation using a 3D reconstructed human cornea model, MCTT HCE™.	Choi *et al*	Biomarker	WC9	2
C24:1-ceramide may be a novel lipid biomarker for eye irritation in 3D human corneal epithelial model, MCTT HCE™.	Lee *et al*	Biomarker	WC9	2
SDF-1 induced migration of THP-1 cells through a monolayer of human coronary artery endothelial cells promotes differentiation of monocytes to an adhesive phenotype.	Van der Toorn *et al*	Assay	WC9	2
Development of EYEIRR-IS®, an toxicogenomic assay using the Skinethic HCE model for evaluating chemical ocular irritation potency.	Cottrez *et al*	Assay/ biomarker	WC9	2
New biomarkers for endocrine disruption evaluation on microplate using a human placental cell line.	Wakx *et al*	Assay/ biomarker	WC9	2
Nano aerosol chamber for realistic in vitro toxicity studies – NACIVT.	Jeannet *et al*	Assay	WC9	2
Prediction of Chemical Respiratory Sensitizers Using GARD, a Novel In Vitro Assay Based on a Genomic Biomarker Signature.	Forreryd *et al*	Assay	SoT 2015	2
Organelle Imaging Toxicology: Novel Analysis of the Sandwich High-Content Screening Project.	Irwin & Liccione	Assay	SoT 2015	2
Activation of the NLRP3 Inflammasome As a Potential Biomarker of Idiosyncratic Drug Reactions.	Weston & Uetrecht	Biomarker	SoT 2015	2
A HepaRG 3D system for studying cholestatic drug hepatotoxicity.	Fredriksson *et al*	Pathway	SEURAT-1 2015	2+1
Repeated dose toxicity of valproic acid in a HepaRG 3D system.	Fredriksson *et al*	Model/ assay	SEURAT-1 2015	1

Title	Authors	Category	Source	Score
Hepatic toxicity evaluation using oxygen-based phosphorescent particles and microfluidics-based glucose and lactate sensors.	Bavli *et al*	Pathway/ model	SEURAT-1 2015	2+1

6.4 Nominated Abstracts

This year there were only 5 projects which received the highest scores of either 3 or 3+1 for reporting new pathways, assays, or biomarkers of toxicity. A further 11 abstracts received a score of 2+1. Since the scope of the selection process has been expanded this year, it seems appropriate to include those abstracts that scored 2+1 as they do stand out as making significant contributions and advances to the adoption of non-animal testing protocols. The 16 abstracts are given below. Due to space constraints, we have given the affiliations of the first author only, although many of these projects were collaborative efforts between several partners. For abstracts from published work, we provide the digital object identifier (DOI) which allows the original document to be located online. For conference abstracts, we give the abstract or poster number for identification.

We consider all worthy of being considered by the judges as potential prize winners.

6.4.1 Using high-content imaging to analyze cell-state trajectories and biological tipping points for chemical exposures

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WC9 Abstract I-6-875
Score 3+1 (3 for discovery of low concentration 'tipping points', +1 for using high throughput approach)

Abstract
Translating results obtained from high-throughput screening to risk assessment is vital for reducing dependence on animal testing. We studied the effects of 976 chemicals (ToxCast Phase I and II) in HepG2 cells using high-content imaging (HCI) to measure dose- and time-dependent perturbations in p53, JNK, oxidative stress, cytoskeleton, mitochondria, and cell cycle. A novel computational model was developed to describe the dynamic response of the system as cell-state trajectories based on multidimensional HCI datastreams. Cell-state trajectories produced by 10 concentrations (0.4 to 200 μM) of 976 chemicals showed resilience of the HepG2 system in many cases, however, we also found “tipping points” in system recovery. Further analysis of trajectories identified dose-dependent transitions, or critical points, in system recovery for 340/976 chemicals. The critical concentration was generally 5-times lower than the concentration that produced 50% cell loss. We believe that HCI can be used to reconstruct cell state trajectories, and provide insight into adaptation and resilience for in vitro systems. With additional research, cellular

tipping points could be used to define an in vitro point of departure (PoD) for risk-based prioritization of environmental chemicals.

6.4.2 Profiling Dose-Dependent Activation of p53-Mediated Signaling Pathways by Chemicals with Distinct Mechanisms of DNA Damage

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Toxicol. Sci. (November 2014) 142 (1): 56-73. doi: 10.1093/toxsci/kfu153
Score 3 (Pathway)

Abstract
As part of a larger effort to provide proof-of-concept in vitro-only risk assessments, we have developed a suite of high-throughput assays for key readouts in the p53 DNA damage response toxicity pathway: double-strand break DNA damage (p-H2AX), permanent chromosomal damage (micronuclei), p53 activation, p53 transcriptional activity, and cell fate (cell cycle arrest, apoptosis, micronuclei). Dose-response studies were performed with these protein and cell fate assays, together with whole genome transcriptomics, for three prototype chemicals: etoposide, quercetin, and methyl methanesulfonate. Data were collected in a human cell line expressing wild-type p53 (HT1080) and results were confirmed in a second p53 competent cell line (HCT 116). At chemical concentrations causing similar increases in p53 protein expression, p53-mediated protein expression and cellular processes showed substantial chemical-specific differences. These chemical-specific differences in the p53 transcriptional response appear to be determined by augmentation of the p53 response by co-regulators. More importantly, dose-response data for each of the chemicals indicate that the p53 transcriptional response does not prevent micronuclei induction at low concentrations. In fact, the no observed effect levels and benchmark doses for micronuclei induction were less than or equal to those for p53-mediated gene transcription regardless of the test chemical, indicating that p53's post-translational responses may be more important than transcriptional activation in the response to low dose DNA damage. This effort demonstrates the process of defining key assays required for a pathway-based, in vitro-only risk assessment, using the p53-mediated DNA damage response pathway as a prototype.

6.4.3 Identification of lipidomic biomarkers for coexposure to subtoxic doses of benzo[a]pyrene and cadmium: the toxicological cascade biomarker approach

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Environ Sci Technol. 2014 Sep 2;48(17):10423-31. doi: 10.1021/es502419w
Score 3 (Biomarker)

Abstract

The search for model bioassay systems indicating activation of different toxicological signaling pathways is one of the paramount goals of modern toxicology. Especially coexposure scenarios need to be investigated with respect to synergistic and interdependent effects for the activation of toxicological signaling pathways. The present study introduces an experimental in vitro model system for nontoxic and low-dose coexposures of human mammary carcinoma MCF-7 cells against polycyclic aromatic hydrocarbons (PAHs) such as benzo[a]pyrene (BP) and heavy metals such as cadmium. For the first time, a multivariate model that identifies 18 metabolic biomarkers has been shown to be sufficient to separate BP-treated cells from coexposed or control cells. A "toxicological pathway color code model" is introduced to visualize the results. Different biomarker subsets can be associated with specific HER2 signaling steps. A tiered cascade biomarker approach is proposed that could be used to identify profiles associated with tumorigenic potency of environmental toxicants in coexposure scenarios, including possible synergistic or additive effects.

6.4.4 More than just hormones: H295R cells as predictors of reproductive toxicity

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Reprod Toxicol. 2014 Jun;45:77-86. doi: 10.1016/j.reprotox.2013.12.009
Score 3 (Assay)

Abstract
Many of the commonly observed reproductive toxicities associated with therapeutic compounds can be traced to a disruption of the steroidogenic pathway. We sought to develop an in vitro assay that would predict reproductive toxicity and be high throughput in nature. H295R cells, previously validated as having an intact and functional steroidogenic pathway, were treated with 83 known-positive and 79 known-negative proprietary and public-domain compounds. The assay measured the expression of the key enzymes STAR, 3βHSD2, CYP17A1, CYP11B2, CYP19A1, CYP21A2, and CYP11A1 and the hormones DHEA, progesterone, testosterone, and cortisol. We found that a Random Forest model yielded a receiver operating characteristic area under the curve (ROC AUC) of 0.845, with sensitivity of 0.724 and specificity of 0.758 for predicting in vivo reproductive toxicity with this in vitro assay system.

6.4.5 Evaluation of the Multi-ImmunoTox Assay composed of 3 human cytokine reporter cells by examining immunological effects of drugs

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Toxicol In Vitro. 2014 Aug;28(5):759-68. doi: 10.1016/j.tiv.2014.02.013
Score 3 (Assay)

Abstract
We established a luciferase reporter assay system, the Multi-ImmunoTox Assay (MITA), to evaluate the effects on key predictivein vitro components of the human

immune system. The system is composed of 3 stable reporter cell lines transfected with 3 luciferase genes, SLG, SLO, and SLR, under the control of 4 cytokine promoters, IL-2, IFN-γ, IL-1β, and IL-8, and the G3PDH promoter. We first compared the effects of dexamethasone, cyclosporine, and tacrolimus on these cell lines stimulated with phorbol 12-myristate 13-acetate and ionomycin, or lipopolysaccharides, with those on mRNA expression by the mother cell lines and human whole blood cells after stimulation. The results demonstrated that MITA correctly reflected the change of mRNA of the mother cell lines and whole blood cells. Next, we evaluated other immunosuppressive drugs, off-label immunosuppressive drugs, and non-immunomodulatory drugs. Although MITA did not detect immunosuppressive effects of either alkylating agents or antimetabolites, it could demonstrate those of the off-label immunosuppressive drugs, sulfasalazine, chloroquine, minocycline, and nicotinamide. Compared with the published immunological effects of the drugs, these data suggest that MITA can present a novel high-throughput approach to detect immunological effects of chemicals other than those that induce immunosuppressive effects through their inhibitory action on cell division.

6.4.6 Identification of novel biomarkers for doxorubicin-induced toxicity in human cardiomyocytes derived from pluripotent stem cells

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Toxicology. 2015 Feb 3;328:102-11. doi: 10.1016/j.tox.2014.12.018
Score 2+1 (Biomarker + use of stem cells)

Abstract
Doxorubicin is a chemotherapeutic agent indicated for the treatment of a variety of cancer types, including leukaemia, lymphomas, and many solid tumours. The use of doxorubicin is, however, associated with severe cardiotoxicity, often resulting in early discontinuation of the treatment. Importantly, the toxic symptoms can occur several years after the termination of the doxorubicin administration. In this study, the toxic effects of doxorubicin exposure have been investigated in cardiomyocytes derived from human embryonic stem cells (hESC). The cells were exposed to different concentrations of doxorubicin for up to 2 days, followed by a 12 day recovery period. Notably, the cell morphology was altered during drug treatment and the cells showed a reduced contractile ability, most prominent at the highest concentration of doxorubicin at the later time points. A general cytotoxic response measured as Lactate dehydrogenase leakage was observed after 2 days' exposure compared to the vehicle control, but this response was absent during the recovery period. A similar dose-dependant pattern was observed for the release of cardiac specific troponin T (cTnT) after 1 day and 2 days of treatment with doxorubicin. Global transcriptional profiles in the cells revealed clusters of genes that were differentially expressed during doxorubicin exposure, a pattern that in some cases was sustained even throughout the recovery period, suggesting that these genes could be used as sensitive biomarkers for doxorubicin-induced toxicity in human cardiomyocytes. The results from this study show that cTnT release can be used as a measurement of acute cardiotoxicity due to doxorubicin. However, for the late onset of doxorubicin-

induced cardiomyopathy, cTnT release might not be the most optimal biomarker. As an alternative, some of the genes that we identified as differentially expressed after doxorubicin exposure could serve as more relevant biomarkers, and may also help to explain the cellular mechanisms behind the late onset apoptosis associated with doxorubicin-induced cardiomyopathy.

6.4.7 Systems biology modeling of omics data: effect of cyclosporine a on the Nrf2 pathway in human renal cells

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BMC Syst Biol. 2014 Jun 25;8:76. doi: 10.1186/1752-0509-8-76
Score 2+1 (Pathway model + predictive modelling)

Abstract
BACKGROUND:
Incorporation of omic data streams for building improved systems biology models has great potential for improving their predictions of biological outcomes. We have recently shown that cyclosporine A (CsA) strongly activates the nuclear factor (erythroid-derived 2)-like 2 pathway (Nrf2) in renal proximal tubular epithelial cells (RPTECs) exposed in vitro. We present here a quantitative calibration of a differential equation model of the Nrf2 pathway with a subset of the omics data we collected.
RESULTS:
In vitro pharmacokinetic data on CsA exchange between cells, culture medium and vial walls, and data on the time course of omics markers in response to CsA exposure were reasonably well fitted with a coupled PK-systems biology model. Posterior statistical distributions of the model parameter values were obtained by Markov chain Monte Carlo sampling in a Bayesian framework. A complex cyclic pattern of ROS production and control emerged at 5 μM CsA repeated exposure. Plateau responses were found at 15 μM exposures. Shortly above those exposure levels, the model predicts a disproportionate increase in cellular ROS quantity which is consistent with an in vitro EC50 of about 40 μM for CsA in RPTECs.
CONCLUSIONS:
The model proposed can be used to analyze and predict cellular response to oxidative stress, provided sufficient data to set its parameters to cell-specific values. Omics data can be used to that effect in a Bayesian statistical framework which retains prior information about the likely parameter values.

6.4.8 Topological, functional, and dynamic properties of the protein interaction networks rewired by benzo(a)pyrene

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Toxicol Appl Pharmacol. 2015 Mar 1;283(2):83-91. doi: 10.1016/j.taap.2015.01.006
Score 2+1 (Pathway + novel approach)

Abstract
Benzo(a)pyrene is a common environmental and foodborne pollutant that has been identified as a human carcinogen. Although the carcinogenicity of benzo(a)pyrene has been extensively reported, its precise molecular mechanisms and the influence on system-level protein networks are not well understood. To investigate the system-level influence of benzo(a)pyrene on protein interactions and regulatory networks, a benzo(a)pyrene-rewired protein interaction network was constructed based on 769 key proteins derived from more than 500 literature reports. The protein interaction network rewired by benzo(a)pyrene was a scale-free, highly-connected biological system. Ten modules were identified, and 25 signaling pathways were enriched, most of which belong to the human diseases category, especially cancer and infectious disease. In addition, two lung-specific and two liver-specific pathways were identified. Three pathways were specific in short and medium-term networks (<48h), and five pathways were enriched only in the medium-term network (6h-48h). Finally, the expression of linker genes in the network was validated by Western blotting. These findings establish the overall, tissue- and time-specific benzo(a)pyrene-rewired protein interaction networks and provide insights into the biological effects and molecular mechanisms of action of benzo(a)pyrene.

6.4.9 Body-on-a-chip simulation with gastrointestinal tract and liver tissues suggests that ingested nanoparticles have the potential to cause liver injury

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Lab Chip. 2014 Aug 21;14(16):3081-92. doi: 10.1039/c4lc00371c
Score 2+1 (Assay + microfluidics)

Abstract
The use of nanoparticles in medical applications is highly anticipated, and at the same time little is known about how these nanoparticles affect human tissues. Here we have simulated the oral uptake of 50 nm carboxylated polystyrene nanoparticles with a microscale body-on-a-chip system (also referred to as multi-tissue microphysiological system or micro Cell Culture Analog). Using the 'GI tract-liver-other tissues' system allowed us to observe compounding effects and detect liver tissue injury at lower nanoparticle concentrations than was expected from experiments with single tissues. To construct this system, we combined in vitro models of the human intestinal epithelium, represented by a co-culture of enterocytes (Caco-2) and mucin-producing cells (TH29-MTX), and the liver, represented by HepG2/C3A cells, within one microfluidic device. The device also contained chambers that together represented the liquid portions of all other organs of the human body. Measuring the transport of 50 nm carboxylated polystyrene nanoparticles across the Caco-2/HT29-MTX co-culture, we found that this multi-cell layer presents an effective barrier to 90.5 ± 2.9% of the nanoparticles. Further, our simulation suggests that a larger fraction of the 9.5 ± 2.9% nanoparticles that travelled across the Caco-2/HT29-MTX cell layer were not large nanoparticle aggregates, but primarily single nanoparticles and small aggregates. After crossing the GI tract epithelium, nanoparticles that were administered in high doses estimated in terms of possible daily human consumption (240 and 480 × 10(11) nanoparticles mL(-1)) induced the release of aspartate aminotransferase (AST), an intracellular

enzyme of the liver that indicates liver cell injury. Our results indicate that body-on-a-chip devices are highly relevant in vitro models for evaluating nanoparticle interactions with human tissues.

6.4.10 Toxicity Mechanisms Identification via Gene Set Enrichment Analysis of Time-Series Toxicogenomics Data: Impact of Time and Concentration

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Environ. Sci. Technol., 2015, 49 (7), pp 4618–4626. DOI: 10.1021/es505199f
Score 2+1 (Pathway + approach)

Abstract
The advance in high-throughput “toxicogenomics” technologies, which allows for concurrent monitoring of cellular responses globally upon exposure to chemical toxicants, presents promises for next-generation toxicity assessment. It is recognized that cellular responses to toxicants have a highly dynamic nature, and exhibit both temporal complexity and dose-response shifts. Most current gene enrichment or pathway analysis lack the recognition of the inherent correlation within time series data, and may potentially miss important pathways or yield biased and inconsistent results that ignore dynamic patterns and time-sensitivity. In this study, we investigated the application of two score metrics for GSEA (gene set enrichment analysis) to rank the genes that consider the temporal gene expression profile. One applies a novel time series CPCA (common principal components analysis) to generate scores for genes based on their contributions to the common temporal variation among treatments for a given chemical at different concentrations. Another one employs an integrated altered gene expression quantifier-TELI (transcriptional effect level index) that integrates altered gene expression magnitude over the exposure time. By comparing the GSEA results using two different ranking metrics for examining the dynamic responses of reporter cells treated with various dose levels of three model toxicants, mitomycin C, hydrogen peroxide, and lead nitrate, the analysis identified and revealed different toxicity mechanisms of these chemicals that exhibit chemical-specific, as well as time-aware and dose-sensitive nature. The ability, advantages, and disadvantages of varying ranking metrics were discussed. These findings support the notion that toxicity bioassays should account for the cells’ complex dynamic responses, thereby implying that both data acquisition and data analysis should look beyond simple traditional end point responses.

6.4.11 Systematic Proteomic Approach to Characterize the Impacts of Chemical Interactions on Protein and Cytotoxicity Responses to Metal Mixture Exposures

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J. Proteome Res., 2015, 14 (1), pp 183–192. DOI: 10.1021/pr500795d

Score 2+1 (Pathway + proteomics)

Abstract
Chemical interactions have posed a big challenge in toxicity characterization and human health risk assessment of environmental mixtures. To characterize the impacts of chemical interactions on protein and cytotoxicity responses to environmental mixtures, we established a systems biology approach integrating proteomics, bioinformatics, statistics, and computational toxicology to measure expression or phosphorylation levels of 21 critical toxicity pathway regulators and 445 downstream proteins in human BEAS-2B cells treated with 4 concentrations of nickel, 2 concentrations each of cadmium and chromium, as well as 12 defined binary and 8 defined ternary mixtures of these metals in vitro. Multivariate statistical analysis and mathematical modeling of the metal-mediated proteomic response patterns showed a high correlation between changes in protein expression or phosphorylation and cellular toxic responses to both individual metals and metal mixtures. Of the identified correlated proteins, only a small set of proteins including HIF-1α is likely to be responsible for selective cytotoxic responses to different metals and metals mixtures. Furthermore, support vector machine learning was utilized to computationally predict protein responses to uncharacterized metal mixtures using experimentally generated protein response profiles corresponding to known metal mixtures. This study provides a novel proteomic approach for characterization and prediction of toxicities of metal and other chemical mixtures.

6.4.12 Systems Pharmacology Modeling Predicts Delayed Presentation and Species Differences in Bile Acid–Mediated Troglitazone Hepatotoxicity

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Clinical Pharmacology & Therapeutics (2014); 96 5, 589–598.
doi:10.1038/clpt.2014.158
Score 2+1 (Pathway + importance)

Abstract
Troglitazone (TGZ) causes delayed, life-threatening drug-induced liver injury in some patients but was not hepatotoxic in rats. This study investigated altered bile acid homeostasis as a mechanism of TGZ hepatotoxicity using a systems pharmacology model incorporating drug/metabolite disposition, bile acid physiology/pathophysiology, hepatocyte life cycle, and liver injury biomarkers. In the simulated human population, TGZ (200–600 mg/day × 6 months) resulted in delayed increases in serum alanine transaminase >3× the upper limit of normal in 0.3–5.1%, with concomitant bilirubin elevations >2× the upper limit of normal in 0.3–3.6%, of the population. By contrast, pioglitazone (15–45 mg/day × 6 months) did not elicit hepatotoxicity, consistent with clinical data. TGZ was not hepatotoxic in the simulated rat population. In summary, mechanistic modeling based only on bile acid effects accurately predicted the incidence, delayed presentation, and species differences in TGZ hepatotoxicity, in addition to predicting the relative liver safety of pioglitazone. Systems pharmacology models integrating physiology and

experimental data can evaluate drug-induced liver injury mechanisms and may be useful to predict the hepatotoxic potential of drug candidates.

6.4.13 Neurotoxicity in vitro: assessment of the predictivity of neuronal networks coped [sic] to microelectrode arrays for identification of neurotoxicants

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WC9 Abstract I-3-389
Score 2+1 (Assay + importance)

Abstract
A challenging aspect to assure the safety of a product is the assessment of its neurotoxic hazard potential. Currently, only in vivo methods are regulatorily accepted and so far, no in vitro model has been fully validated. With the advance in technology and the ability to maintain primary neuronal models for prolonged periods, a promising test system emerged, combining the use of microelectrode arrays (MEAs) and in vitro culture of 2D neuronal networks (NN). In the presentation, we report on the in-house validation of the NN MEA assay using a set of 58 compounds of different chemical classes with known neurotoxic and non-neurotoxic potential with the aim to use it for screening of compounds under development and in the future for its potential application in the regulatory framework. The results demonstrate that the method presents a good sensitivity of 81% and accuracy above 75%. In order to increase the rate of false predictions we also report in the new approach integrating the electrophysiological assessment with a panel of cytotoxicity assays.

6.4.14 LUHMES 3D neuronal model for (developmental) neurotoxicity testing

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WC9 Abstract I-4-803
Score 2+1 (Assay + importance)

Abstract
Broader testing of substances is crucial to respond to surging neurodevelopmental problems in children, including autism and ADHD. Therefore, our principal goal is to develop a human relevant, quality-assured, medium- to high-throughput testing strategy to prioritize chemicals for (developmental) neurotoxicity testing. This work aims to develop a 3D brain model to identify changes in transcriptome, microRNA expression, and metabolome after exposure to neurotoxicants. We developed such model based on well characterized LUHMES dopaminergic neuroprogenitor cell line which can be used to test compounds for neuro(developmental) toxicity effects and model chemically induced neuronal disorders (Schildknecht *et al*., 2013; Scholz *et al*., 2011). The size of differentiating spheroids, penetration rate and cell death were monitored up to day 21 of differentiation, to ensure delivery of medium supplements

to the center of the spheroids. The efficiency of differentiation in 3D was characterized by qRT-PCR, flow cytometry and immunocytochemistry. To demonstrate the modelÅs suitability for neurotoxicity testing, the aggregates were treated for 24 and 48 hours on day 6 of differentiation with dopaminergic neurotoxicants, MPP+ and rotenone. Perturbations in energy metabolism and stress response were analyzed by gene expression and miRNA profiling. In conclusion, we have successfully developed a new 3D human model that can be used for (developmental) neurotoxicity testing.

References

Schildknecht, S., Karreman, C., Pöltl, D. *et al*. (2013). ALTEX 30, 427-444. Scholz, D., Pöltl, D., Genewsky, A. *et al*. (2011). J Neurochem 119, 957-971.

6.4.15 A HepaRG 3D system for studying cholestatic drug hepatotoxicity

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SEURAT-1 2015 Poster 1

Score 2+1 (Pathway + 3D model)

Abstract

Drug-induced cholestasis is the most common cause of fulminant hepatic failure and it would therefore be advantageous to identify this injury at an earlier stage of drug development. Here we present a 3 dimensional organotypic spheroid model, based on human HepaRG cells. We show a significant increase in toxicity of cholestatic compounds chlorpromazine (CPZ), troglitazone and bosentan when co-exposed to an otherwise none-toxic concentrated mix of human bile acids. Importantly, this enhanced toxicity could not be seen with the non-cholestatic, although hepatotoxic, compounds paracetamol and tetracycline. Further mechanistic investigation of the enhanced toxicity seen with CPZ and bile acids revealed a role for oxidative stress induction. This was determined by using a fluorescent probe for oxidative stress in the intact spheroid as well as by an up-regulation of the oxidative stress-responsive transcription factor nuclear factor (erythroid-derived 2)-like 2 (NFE2L2; Nrf2) and its target gene sulfiredoxin-1 (SRXN1) on RNA level. In addition, an observed down-regulation of the bile-salt export pump (BSEP) after exposure to this drug with and without bile acids can be presented as a possible mechanism to the enhanced toxicity. This was supported by the observed accumulation of a fluorescent bile acid, which transport is dependent on BSEP, after exposure to CPZ. In conclusion we here present an organotypic hepatotoxicity model, based on drug/bile acid co-exposures, which is able to distinguish cholestatic from non-cholestatic compounds and which constitute a promising basis for further mechanistic studies of this effect.

6.4.16 Hepatic Toxicity Evaluation Using Oxygen-based Phosphorescent Particles and Microfluidics-based Glucose and Lactate sensors

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SEURAT-1 2015 Poster 8

Score 2+1 (Pathway + fluidics)

Abstract
The HeMiBio consortium aims to engineer a liver-mimicking microchip, capable of functioning for over 28 days in vitro, for repeated-dose toxicity study of pharmaceutical agents and cosmetic compounds. The over goal is to replace the use of animals as an industry standard. Current methods to detect hepatotoxicity utilize large numbers of cells and measure viability at multiple time points. Our approach is to confine minute population of cells in microfluidic chips, and continuously monitor their function over time. Microfluidic liver-on-chip devices offer an alternative for animal experiments as they can mimic the native microenvironment and support long-term function under continuous perfusion. Continuous flow enables stable dose exposure of the cells over time, eliminating the rapid loss of signal due to non-specific adsorption and metabolism that characterizes both static in vitro assays and in vivo experiments. Here we report the fabrication and validation of a microfluidic liver bioreactor capable of maintaining metabolically active HepG2 cells for over 28 days in vitro. The cell display epithelial polarization and high CYP450 expression for the period. Cell viability is continuously monitored automatically in real time using a novel on-chip frequency-based luminescence-quenching oxygen probe seeded within the self-assembled tissue and integrated off-chip glucose and lactate sensors. Continuous electrochemical monitoring of glucose and lactate levels provides additional means of detecting the shift from oxidative phosphorylation to glycolysis in conjunction with oxygen consumption, permitting the unique real-time detection of minute mitochondrial damage. Using the system developed we were able to detect a new mechanism of CYP2E1-independent acetaminophen toxicity involving direct mitochondrial damage. We were also able to show the toxicity modes of action of other drugs such as the anti-diabetic drug, troglitazone, insecticide rotenone and the antiarrhythmic agent, amiodarone, caused a time-delayed dose-dependent response with a TC50 of 285 μM, 12.5 μM and 425 μM respectively. The microfluidic system provides major benefits including reduction of required cell number, enhancement of efficiency by means of multiplexed screening, and providing the ability to maintain optimum culture conditions as required for long-term studies.

7. Conclusions

The review of the most recent World Congress, SoT, and SEURAT-1 conference proceedings, and an extensive literature search, yielded 16 abstracts describing projects which we consider to be potential candidates for the Lush Prize for Science. These are given in Section 6.

This year, the scope of the search for candidates for the Lush Prize for Science has been expanded to new assays and biomarkers of toxicity, in addition to pathway discovery. Although we reviewed many more papers and abstracts than in 2014 (over 2,200 this year compared with over 1,000 in 2014), the number that received a score was slightly lower than in 2014. In particular, we were surprised that so few SoT abstracts met the Lush Prize criteria (53 out of 2468, based on the SoT Abstract book keyword index). It was also disappointing that a large proportion of these 53 abstracts described whole animal studies. Perhaps the Lush Prize organisation should consider lobbying the Society of Toxicology, and its conference attendees, at the next SoT meeting (in New Orleans in March 2016).

The nominated abstracts are very diverse, and cover 3D and microfluidics models for toxicity assays, high-throughput- and high content- screening, *in silico* analyses, and demonstrations of the utility of modern tt21d approaches. We believe that they are all worthy candidates for the 2015 Science Prize.