Lush Science Prize 2018
Background Paper

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1. Executive Summary

1.1 What is the Lush Science Prize?

The Lush Prize supports animal-free testing by awarding money prizes of up to £250,000 per year to the most effective projects and individuals who have been working towards the goal of replacing animals in product or ingredient safety testing. Since its inception in 2012, the Lush Prize has distributed almost £2 million.

Prizes are awarded for developments in five strategic areas: science; lobbying; training; public awareness; and young researchers. In 2015, the judges also awarded a Black Box prize for the development of the skin sensitisation AOP and associated, approved, *in vitro* assays.

The Science Prize is awarded to the group (or groups) whose work the judging panel deem to have made the most significant contribution(s), in the preceding year, to the replacement of animal testing. This 2018 Science Background paper identifies 25 pieces of work carried out by researchers who we believe constitute potential candidates for the Judges' shortlist.

1.2 Methodology

In order to obtain an overview of developments in the field of animal replacement in toxicity research, we firstly reviewed the recent work of the relevant scientific institutions and projects in this area, including the OECD; CAAT; ECVAM; UK NC3Rs; US Tox21 Programme; the ToxCast programme; and EU-ToxRisk (see section 4). We also assessed recent developments in toxicity testing research by reviewing the relevant literature (see section 5 for some highlights).

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. For this year these were the 2017 10th World Congress on Alternatives and Animals in the Life Sciences (WC10), and the 2018 Society of Toxicology (SoT) annual conference. There were a total of 2,958 abstracts from oral and poster presentations from these two conferences, but only 782 were relevant to the Lush Science prize. We then performed literature searches using PubMed, and Google Scholar, to identify projects describing recent advances in toxicity testing research. One further relevant abstract was identified directly from the EURL-ECVAM website. In all, searches yielded over 4,100 projects which we assessed as described in Section 3. Relevant abstracts were scored using the system derived in previous years 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.

Overall, from 68 abstracts which scored 1 or more: 2 scored 1; 41 scored 2; 10 scored 2 + 1 (i.e. total 3); and 12 scored 3. Finally, 3 projects scored the maximum possible marks of 3 for reporting a new toxicity assay, pathway, or biomarker, plus an additional mark for also standing out in

some other way. 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

There were 25 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. We consider all to be worthy for consideration by the judges as potential prize winners.

2. Background

The Lush Science Prize was established to support the development and adoption of both 21st Century Toxicity Testing (tt21c) and adverse outcome pathways (AOPs)[1]. These two alternative approaches offer new and better ways for safety testing of chemicals. They achieve this using more relevant and predictive human models, and through 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 Prizes aim to focus attention on toxicity testing for consumer products and ingredients, in a way which complements those projects which address the use of animals in medical testing. The Lush Science Prize seeks to reward those researchers making 'outstanding contributions' to tt21c research. The focus of the award is on:

- Research aimed at elucidating key pathways in which perturbation results in toxicity;
- Developing 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[2], 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.

1 https://lushprize.org/background/supporting-alternatives/

2 https://lushprize.org/awards/science-prize/

3. Methodology

The main aim of this paper is to assist the Lush Prize judging panel by identifying key projects that are making major contributions to the field of animal-free toxicology research. From these projects, the panel may choose to select the winners for the 2018 Lush Science Prize. 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's interest focuses exclusively 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. Since the focus of the Lush prize is on general pathways of compound safety testing, we excluded research that focuses on specific diseases, including cancer, unless we felt that the work identified a new assay or pathway of toxicity. We also excluded those investigating the effects of environmental toxins. We considered projects based anywhere in the world, but only considered work written in the English language. As far as possible, we restricted the search to work reported in the year preceding the award (i.e. May 2017 – June 2018).

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 the OECD; CAAT; ECVAM; UK NC3Rs; US Tox21 Programme; the ToxCast programme; the Human Toxicology Project Consortium; ICCVAM, the NIH, the EPA, the FDA, ESTIV, Cosmetics Europe, and EU-ToxRisk (see section 4 for highlights).

Secondly, we identified relevant conferences held in the preceding 12 months and assessed abstracts, where available, for oral and poster 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 2017 – 2018, for which abstracts were available, were the WC10, held in Seattle, August 2017, and the SoT 57th Annual Meeting held in March 2018, in San Antonio, Texas. Another conference, for which the abstract book could not be obtained, was the 53rd EuroTox meeting (Slovakia, September 2017). Work presented at this conference could not be considered for this paper.

From the 639 abstracts which comprised the WC10 conference presentation and poster proceedings, we reviewed only the 399 that were presented within conference themes relevant to scientific research. The selected themes were: Plenary Lectures; Lessons Learned; Innovative Models; Sustainability; Systems Biology and Big Data; Translation; and Late-breaking Abstracts. We excluded abstracts presented in the Ethics, 3Rs in Academia, Refinement and Animal Welfare, and Global Co-operation

themes. Of the 2,319 abstracts presented at the SoT meeting, only the 383 indexed under relevant headings in the Keyword Index were considered. The relevant Keywords from the SoT abstract book were: Alternatives to Animal Testing; Biomarkers; Computational Toxicology, and *In Vitro* and Alternatives.

Thirdly, we conducted a review of the recent literature. For this we used three separate sources. Firstly, we searched PubMed for research published from 01/05/2017 to 31/05/2018, combining search terms "toxicity pathway," "toxicity assay" and "toxicity biomarker", excluding any review articles and clinical trials, and restricting the subject matter to "humans". We excluded any abstracts not written in English. As a second literature source, we searched Google Scholar for relevant papers published in the period 2017 to 2018, combining search terms "toxicity pathway," "toxicity assay" and "toxicity biomarker", and restricting the subject matter to "humans". We excluded any articles already identified by our PubMed searches. We did not search Terkko Feed Navigator this year since previous years' searches here have failed to yield any further relevant papers. Finally, we specifically reviewed all articles published in the ALTEX journal.

For published papers, our selection procedure was a three stage process. 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. In the first stage, we reviewed the title of the work, and rejected any which were clearly reviews or which were obviously unsuitable either through using animal models or through being overly focused on disease. We also rejected any which were not written in the English language, and those for which abstracts were unavailable. In the second stage, we assessed the abstracts of projects which passed the initial filter and further eliminated those which reported findings from clinical trials and population studies, those focusing on disease research and environmental pollutants (unless we felt that they additionally identified a new pathway or biomarker), and all research that included animal subjects. In the third stage, projects identified as potentially relevant based on the abstract were scored using a system devised and successfully applied in previous years. As for the previous year, because the conferences yielded a limited number of relevant abstracts and the conference abstract books presented titles and abstracts simultaneously, there was no merit to reviewing abstracts in the three stages. Thus abstracts were either accepted or rejected for scoring and then scored in a single sweep.

In our abstract scoring 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.

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

Using NCATS' state-of-the-art robotic screening system, Tox21 scientists have produced more than 120 million data points on approximately 8,500 chemicals since 2008. These data help researchers understand the potential effects of exposure to a substance based on how it interacts with biological molecules during screening.

Challenges remain in improving the reliability, efficiency and predictive capability of toxicity testing using in vitro high-throughput screening. To address these obstacles Tox21 program partners from NCATS, the National Toxicology Program at the National Institute of Environmental Health Sciences, the Environmental Protection Agency, and the Food and Drug Administration published a new strategic and operational plan[3], on March 8 2018, to broaden the scope of their research activities and to address new challenges. The five new areas of focus were designed to:

; . Develop alternative test systems that are predictive of human toxicity and dose response.

< Address key technical limitations of current *in vitro* test systems.

= Curate and characterize legacy in *in vivo* toxicity studies.

> Establish scientific confidence in *in vitro* test systems and integrated assay batteries.

[. Refine and deploy *in vitro* methods for characterizing pharmacokinetics and *in vitro* dispositions.

4.2 Other US Institutions

The National Toxicology Programme's ICCVAM has now published its Strategic Roadmap for Establishing New Approaches to Evaluate the Safety of Chemicals and Medical Products in the United States (January 2018)[4]. The roadmap aims to offer guidance to US federal agencies and stakeholders adopting new approach methods (NAMs) for safety assessment of chemicals that improve human relevance and replace (or reduce) animal use.

The National Institutes of Health announced 13 two-year awards totalling about $15 million per year, with financial year 2018 funds subject to availability, to develop 3-D microphysiological system platforms that model human disease. The funding is for the first phase of a five-year programme. These platforms, called tissue chips, support living cells and human tissues

3 Thomas *et al* 2018 *ALTEX* **35**, 163-168. doi.org/10.14573/altex.1803011

4 https://ntp.niehs.nih.gov/pubhealth/evalatm/natl-strategy/index.html

to mimic the complex biological functions of human organs and systems, and provide a new way to test potential drug efficacy.

The Environmental Protection Agency has released for public comment a draft strategy[5] to reduce the use of vertebrate animals in chemical testing, as required by the Toxic Substances Control Act (2016). The final version is due by June 22, 2018.

The Food and Drug Administration has also published a strategy roadmap[6] to identify and adopt suitable NAMs that expand the predictive toxicology capabilities of the FDA, and reduce animal use in testing.

4.3 EURL-ECVAM

We mentioned last year that the EU Commission held a 2-day scientific conference on non-animal alternatives, in Brussels, in December 2016. This was in part to report results of EURL-ECVAM's public survey launched in spring 2016. The conference report is now available[7]. The conclusions of the conference acknowledged "that historically there has been some value in animal tests and the information they have provided – particularly in areas such as the identification and treatment of diseases and the understanding of biological processes. There is also an appreciation that, whilst considerable progress has been made, many believe that there are not adequate replacement methods and technologies in all areas yet to conduct completely animal-free science at this time." Further, "the European Commission is keen to implement science-based policy, including animal-free science that is responsive to Citizens' demands. It is clear, however, that there are differences in opinion over the expected pace for change, some NGOs and citizens' groups call for an immediate ban on the use of animals, whilst industry (in particular the pharmaceutical industry) and regulators potentially have a longer term view. Government agencies and regulators are seen as vital to stimulate and lead the change to animal-free science rapidly through recognition of the urgent need for a revised, flexible regulatory framework."

In conclusion, the Scientific Conference provided a positive outlook for significant progress towards animal-free science, requiring many stakeholders and disciplines to work together to gain acceptance for more ethical and relevant methods for experimental science in medicine and safety assessment.

EURL-ECVAM has just completed (31/5/2018) a survey on issues influencing end-user confidence in complex *in vitro* models for use in research and testing. The results will be available later this year.

5 https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/alternative-test-methods-and-strategies-reduce

6 https://blogs.fda.gov/fdavoice/index.php/2017/12/fda-launches-predictive-toxicology-roadmap-to-enable-advances-in-toxicity-testing/

7 http://ec.europa.eu/environment/chemicals/lab_animals/3r/pdf/scientific_conference/non_animal_approaches_conference_report.pdf

EURL-ECVAM has also recently launched a review of how alternative methods and models are being used for research on respiratory tract diseases and neurodegenerative disorders. The study aims to identify and describe specific contexts where animal models have been put aside in favour of novel non-animal techniques. The expectation is that, by sharing information on the successful adoption of non-animal methods, the transition towards their wider adoption can be accelerated.

EURL-ECVAM hosted a workshop in September 2017, on how the complexities of inflammatory responses to chemicals could be integrated into AOP frameworks. The workshop brought together experts from around the world to discuss and identify the important events of inflammation that are common to many organs and tissues and which could inform AOP networks. Recommendations and guidance will be published in due course.

4.4 ESTIV

The European Society of Toxicology *In Vitro* conducted a survey of its members, on the use of alternative methods, during the summer of 2017. The results of the survey[8] indicated that all respondents thought that the use of alternative methods could be improved in both research and regulatory applications. The main obstacles to improved use in research were:

- Too conservative an approach from regulators, journal reviewers, and many scientists, who consider animal studies to be a 'gold standard'
- A continuing lack of availability of methods/models to study physiology
- Relative lack of funding and personnel for the expansion of research using alternative methods

The main obstacles to wider use of alternative methods in regulatory approval were:

- A lack of acceptance/familiarity from regulators
- Long validation and implementation processes
- Complexities and costs of using multiple tests/testing strategies

4.5 EU-ToxRisk

EU-ToxRisk aims to shift toxicological testing towards assessment based on human cells, with a comprehensive mechanistic understanding of causal relationships for chemical adverse effects. The programme integrates advances in cell biology, 'omics technologies, systems biology, and computational modelling to define the complex chains of events that link chemical exposure to toxic outcome. The two main areas of focus are: repeated dose systemic toxicity (using lung, kidney, liver, and the nervous system as target organs) and developmental and reproductive toxicity.

8 http://www.estiv.org/docs/ESTIV_Survey_AltMeth_summer2017_final.pdf

The first two years of the project have seen the development of ten case studies to assess NAMs as a viable approach for safety testing. EU-ToxRisk is putting a particular emphasis on the regulatory applications of these new methods. To this end, the case studies are designed to use the AOP concept in regulatory applications. Significantly, EU-ToxRisk has established a Regulatory Advisory Board, to operate alongside its Scientific Advisory Board, with the aim of ensuring that EU-ToxRisk develops the best science that fits regulatory needs.

4.6 Cosmetics Europe

Cosmetics Europe's Long-range Science Strategy aims to determine the safety of cosmetics ingredients without the use of animals. The strategy programme was launched in 2016, and collaborative research continues on the three pillars of the programme: developing non-animal methods, testing strategies, and alternative approaches; implementing alternative approaches in a risk assessment paradigm to demonstrate that safety assessments are possible on a broad spectrum of effects, and; support for the regulatory acceptance of these approaches.

Cosmetics Europe is also a member of the EU-ToxRisk project.

4.7 Organisation for Economic Co-operation and Development (OECD)

OECD Test Guideline 442E *In Vitro* Skin Sensitisation has been modified to include two further tests that can be used instead of the hCLAT test. These are the U-SENS (U937 cell line activation test) and the IL-8 Luc (interleukin-8 reporter gene test) assays.

5. Literature Highlights

Some of the work that we reviewed in our search for potential Lush Science Prize nominees was not eligible for consideration, but nevertheless was relevant or noteworthy in the broader context of tt21c. Those articles or news items which seem most relevant to the Lush Science Prize are summarised here.

The Cosmetics Europe Skin Tolerance Taskforce hosted an industry workshop in June 2016, the outcomes of which were published in ALTEX in April 2018[9]. The workshop addressed issues around the lack of safety assessment methods for the fourth key event of the skin sensitisation AOP – i.e. the immunological response in the draining lymph node, where activated dendritic cells present MHC-bound peptide fragments to naïve T cells, so priming the proliferation of antigen-specific T cells. Workshop participants came from academia, industry, and regulatory authorities to review the scientific status of T cell-based assays and to develop new options for assessing T cell activation. It was agreed that, while significant progress has been made in developing T cell assays, more work is required to simplify assays, to optimise their sensitivity, to define better the human donor-to-donor variability, and to evaluate the assays' value to predict sensitiser potency. It was also acknowledged that experience from case studies will be required before T cell assay data can be used for safety assessment.

There have been many claims regarding the value, or lack thereof, of animal tests in predicting human safety, especially for drug discovery. A 2017 paper[10] by a consortium of pharmaceutical companies claims that its data support the use of animal experiments in drug safety testing before human trials. They created a database of industry-wide preclinical to clinical translational data for 182 molecules, to determine how safety assessments in animal models translated to human risk. The authors declare that animal tests are an effective means of testing compound safety in humans, with specificity of 84% and negative predictive value of 86%, and sensitivity of 48% and positive predictive value of 43%. However, the clinical data were only from phase I clinical trials that are conducted on very small numbers of individuals, and many drug withdrawals on safety grounds come after several tens of deaths following some years of market use. In the context of the mortal risk to a very small proportion of users of unsafe drugs, the data presented in this work would be more convincing if they considered all of the clinical data available for each compound and set the threshold for acceptable predictive values much higher.

Pisani *et al*[11] highlighted the importance of using human-derived serum in cell culture, rather than serum from animal sources, by showing that the toxicity of silica nanoparticles is affected by the source of the protein corona that forms around them. Coronas preformed in foetal bovine serum (FBS) resulted in nanoparticles that were up to 4-fold more toxic in HepG2 cells

9 Van Vliet *et al* 2018 *ALTEX* **35**, 179-192 doi.org/10.14573/altex.1709011

10 Monticello *et al* 2017 *Toxicol Appl Pharmacol* **334**, 100-109 doi.org/10.1016/j.taap.2017.09.006

11 Pisani *et al* 2017 *PloS One* **12** doi.org/10.1371/journal.pone.0182906

than those preformed in human serum. They surmise that "markers of self" are present in the serum and are recognized by human cell receptors.

In a similar vein, Lam and Ohayon advocate the production of xenobiotic-free iPSCs (induced pluripotent stem cells) for research and testing[12]. They argue that the use of animal products in the production and maintenance of iPSCs has grave scientific, clinical, and ethical implications. They then describe the development of an online database (the Xeno-Free Stem Cell (XFSC) Toolkit Initiative) to help researchers develop standards for XFSC protocols, certify XF reagents, and create an online community to foster collaboration.

A Consensus Report on the 3rd Workshop on the replacement of FBS[13] in the biosciences made a series of recommendations, ranging from funding to promote the use of human-derived alternatives to FBS and chemically-defined media, to the full and effective regulation of collection of FBS, including killing or stunning foetuses prior to cardiac puncture.

The Society of Environmental Toxicology and Chemistry undertook a formal, global, "horizon scanning" approach[14] to identify barriers to the advance of AOP frameworks for safety testing. Key questions and challenges were identified regarding the applicability and readiness of the AOP framework to address current regulatory and scientific needs. It also identified frequently asked questions that uncovered common misconceptions about AOPs.

Piersma *et al*[15] reported on a 2017 workshop on accelerating the validation and regulatory acceptance of alternative methods. Representatives from academia, industry, and regulatory agencies across Europe took part. The workshop noted that classical validation of alternative methods usually involves one-to-one comparison with the 'gold standard' animal test. This comparison suffers from the reductionist nature of the alternative method, and from the problems associated with the animal test's being considered a gold standard. Modern approaches combine individual alternatives into testing strategies, for which integrated and combined approaches are emerging at OECD level. The workshop concluded that the transition to a mechanistically-based, human-focussed, hazard and risk assessment of chemicals requires an open mind towards moving away from the animal study as a gold standard, and defining human-based regulatory requirements for safety testing.

Fay *et al*[16] have used ToxCast data to identify AOP molecular targets that correspond to ToxCast assays as a means of detecting pathway-specific effects. Previous work had detected a "cytotoxic burst" (CTB) phenomenon whereby large numbers of ToxCast assays begin to respond at or near

12 Lam & Ohayon 2017 WC10 Abstract VII-3-708. Promise & pitfalls of induced pluripotent stem cells: Learning from past mistakes.

13 Van der Valk *et al* 2018 *ALTEX* **35**, 99-118. doi.org/10.14573/altex.1705101

14 LaLone *et al Environ. Toxicol. Chem.* **36**, 1411-1421 doi.org/10.1002/etc.3805

15 Piersma *et al* 2018 *Toxicol In Vitro* **48**, 53-70 doi.org/10.1016/j.tiv.2018.02.018

16 Fay *et al* 2018 *Toxicol Sci* **163**, 500-515 doi.org/10.1093/toxsci/kfy049

cytotoxic concentrations of chemicals. They used a statistical approach to undertake a meta-analysis of assays to identify those that most frequently respond to concentrations below the CTB concentration, and might be considered pathway-specific. A diagnostic-odds-ratio approach was used to refine this list of assays, and the two approaches identified several novel targets to prioritize for AOP development, as well as affirming the importance of a number of existing AOPs.

6. Candidate Toxicity Abstracts Identified for the Judges

6.1 Conference Abstract Selection

As described in the Methodology, we reviewed abstracts from the SoT 57th Annual Meeting, held in March 2018 in San Antonio, Texas, and the WC10, held in August 2017 in Seattle, Washington.

From the 639 abstracts which comprised the WC10 conference presentation and poster proceedings, we reviewed only those that were presented within conference themes relevant to scientific research. The selected themes were: Plenary Lectures; Lessons Learned; Innovative Models; Sustainability; Systems Biology and Big Data; Translation; and Late-breaking Abstracts. We excluded abstracts presented in the Ethics, 3Rs in Academia, Refinement and Animal Welfare, and Global Co-operation themes. From the 399 potentially relevant abstracts that we reviewed, we identified 17 abstracts which scored 1 or more.

From the total of 2319 abstracts presented at the Society of Toxicology's 2018 meeting, 383 were identified as potentially relevant based on the Abstract book keyword index: 81 were identified by the keyword "biomarker", of which 1 was scored; 66 were identified by the keywords "computational toxicology", of which 3 were scored; 114 were identified by the keywords "*In vitro* and alternatives", of which 5 were scored; and 122 were identified by the keywords "alternatives to animal testing," of which 16 were scored. There were 34 abstracts which we identified by more than one keyword search, these duplicates were counted just once. A total of 25 abstracts scored 1 or more.

6.2 Published Abstract Selection

From the PubMed search we identified a combined total of 2669 articles: 2245 relevant titles from the "Toxicity assay" search; a further 135 relevant projects from the "Toxicity biomarker" search; and finally an additional 289 titles from the "Toxicity pathway" search.

Stages 1 and 2 of the selection process (review of titles, and then abstracts, to reject review articles, articles not written in English, results of clinical trials, articles reporting use of animal subjects, or those overly focused on cancer, or other disease, research, or environmental pollution) reduced these 2669 titles by around 80%. Of the remainder, after review of abstracts in stage 3, only 18 abstracts scored 1 or more (14 from the Toxicity assay search, 2 from the Toxicity biomarker search, and 2 from the Toxicity pathway search).

The Google Scholar search for the period 2017 to 2018 identified 689 possibly relevant abstracts. Of these: 634 were identified by the Toxicity assay search; a further 13 from the Toxicity biomarker search; and 42 from the Toxicity pathway search. Of the 689, only 83 survived selection stages 1 and 2 (43 from the Toxicity assay search, 5 from the Toxicity biomarker search; and 35 from the pathway search) and were passed on to the scoring stage. Of these 83, only a final 7 scored 1 or more.

We also identified one additional project, from the EURL-ECVAM website, which scored 1 or more.

6.3 Scores

From the three separate sources of potential shortlisted projects, we identified a total of 68 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 by providing an alternative test to those using animal cell lines, opportunities for data sharing, or for combining methodologies to give "added value"); 41 scored 2 for bringing new knowledge or tools to a previously identified pathway, assay, or biomarker of toxicity; 10 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 other way; and 12 scored 3 because they appeared to be reporting a new toxicity pathway, assay, or biomarker. Only 3 scored the maximum possible 4 marks, with 3 marks awarded for reporting a new toxicity pathway, assay, or biomarker, plus an additional mark for standing out in some other way.

The Table lists details (Title, Authors, contribution category (pathway, assay, or biomarker), source, and score) of all the abstracts scoring 1 or more. The Table is ordered by source of abstract – PubMed, Google Scholar, EURL-ECVAM, SoT and WC10. All of the 25 abstracts for those projects scoring a total of 3 or more (as 2+1, 3 or 3+1) are shown in full in Section 6.4. For abstracts of published papers, the abstract title in the Table is a hyperlink to the DOI (digital object identifier) for that paper. For conference abstracts, we give the abstract or poster number for identification.

Title	Authors	Category	Source	Score
XPF plays an indispensable role in relieving silver nanoparticle induced DNA damage stress in human cells	Wang D et al	Pathway	PubMed	2
Metabolomics profiling of steatosis progression in HepaRG® cells using sodium valproate	Cuykx M et al	Biomarker	PubMed	2
Development of Decision Forest Models for Prediction of Drug-Induced Liver Injury in Humans Using A Large Set of FDA-approved Drugs	Hong H et al	Assay	PubMed	2
The species origin of the serum in the culture medium influences the in vitro toxicity of silica nanoparticles to HepG2 cells	Pisani C et al	Assay	PubMed	1

Title	Authors	Category	Source	Score
The TGx-28.65 biomarker online application for analysis of transcriptomics data to identify DNA damage-inducing chemicals in human cell cultures	Jackson MA et al	Assay	PubMed	2+1
Customised in vitro model to detect human metabolism-dependent idiosyncratic drug-induced liver injury	Tolosa L et al	Assay	PubMed	2
The development and validation of EpiComet-Chip, a modified high-throughput comet assay for the assessment of DNA methylation status	Townsend TA et al	Assay	PubMed	2
Assessment of the DNA damaging potential of environmental chemicals using a quantitative high-throughput screening approach to measure p53 activation	Witt KL et al	Assay	PubMed	2
Enzymatic reactive oxygen species assay to evaluate phototoxic risk of metabolites	Kato M et al	Assay	PubMed	2
Induced pluripotent stem cell-derived limbal epithelial cells (LiPSC) as a cellular alternative for in vitro ocular toxicity testing	Aberdam E et al	Assays	PubMed	1
Serum microRNA signatures as "liquid biopsies" for interrogating hepatotoxic mechanisms and liver pathogenesis in human	Krauskopf J et al	Biomarker	PubMed	2
Real-time cell toxicity profiling of Tox21 10K compounds reveals cytotoxicity dependent toxicity pathway linkage	Hsieh JH et al	Pathway	PubMed	3
Prediction of skin sensitization potency using machine learning approaches	Zang Q et al	Assay	PubMed	3+1
DTNI: a novel toxicogenomics data analysis tool for identifying the molecular mechanisms underlying the adverse effects of toxic compounds	Hendrickx DM et al	Pathway	PubMed	2+1
Integrative omics data analyses of repeated dose toxicity of valproic acid in vitro reveal new mechanisms of steatosis induction	van Breda SGJ et al	Pathway	PubMed	2
Mechanisms of Skin Toxicity Associated with Metabotropic Glutamate Receptor 5 Negative Allosteric Modulators	Shah F	Pathway	PubMed	3+1

Title	Authors	Category	Source	Score
Human liver-kidney model elucidates the mechanisms of aristolochic acid nephrotoxicity	Chang SY et al	Pathway	PubMed	2+1
Metabolomics profiling of steatosis progression in HepaRG® cells using sodium valproate	Cuykx M et al	Biomarker	PubMed	2
Alternative Integrated Testing for Skin Sensitization: Assuring Consumer Safety	Del Bufalo A et al	Assay	Google Scholar	2
An Adverse Outcome Pathway for Sensitization of the Respiratory Tract by Low-Molecular-Weight Chemicals: Building Evidence to Support the Utility of In Vitro and In Silico Methods in a Regulatory Context	Sullivan KM et al	Pathway	Google Scholar	2
The Toxmatrix: Chemo-Genomic Profiling Identifies Interactions That Reveal Mechanisms of Toxicity	Zhi-Bin Tong et al	Pathway	Google Scholar	2
Intra- and inter-laboratory reproducibility and predictivity of the HaCaSens assay: A skin sensitization test using human keratinocytes, HaCaT	Chung H et al	Assay	Google Scholar	2
In Vitro Micropatterned Human Pluripotent Stem Cell Test (μP- hPST) for Morphometric-Based Teratogen Screening	Xing J et al	Assay	Google Scholar	3
Building predictive in vitro pulmonary toxicity assays using high-throughput imaging and artificial intelligence	Lee, JY.J. et al	Assay	Google Scholar	2+1
Developmental toxicity assessment of common excipients using a stem cell-based in vitro morphogenesis model	CJ Yuan & Y Marikawa	Assay	Google Scholar	2
Nrf2 pathway activation upon rotenone treatment in human iPSC-derived neural stem cells undergoing differentiation towards neurons and astrocytes	Pistollato N et al	Assay	EURL-ECVAM	2
Use of a Chronic Multiplatform Assay to Evaluate Cardiotoxicity of BMS-986094 in iPSC-Derived Human Cardiomyocytes	Strock CJ et al	Assay	SoT 2018 abstract 1138	3+1

Title	Authors	Category	Source	Score
Development of an In Vitro Assay to Predict Cardiotoxicity Potential Using Targeted Metabolomics and Human-Induced Pluripotent Stem Cell-Derived Cardiomyocytes	Palmer JA et al	Biomarker	SoT 2018 abstract 1139	3
Phenotypic Profiling in Human-Based Phenotypic Assays Supports an AOP for CV Toxicity	Berg EL & Folias AE	Pathway	SoT 2018 abstract 1152	3
Effect of 2-Acetylaminofluorene and Its Genotoxic Metabolites on DNA Adduct Formation and DNA Damage in 3D-Reconstructed Human Skin Models	Downs TR et al	Assay	SoT 2018 abstract 1516	2
Validation of the In Vitro- Reconstructed Skin Micronucleus Assay as a Potential Animal Alternative for Fragrance Materials	Wahler J et al	Assay	SoT 2018 abstract 1521	2
Organotypic Human Epidermal Skin Models and Genotoxicity: Safety Assessment for Dermal Applications	Pfitzner I et al	Assay	SoT 2018 abstract 1523	2
Validation of a Co-Culture Cell Model for Identifying Angiogenesis Inhibitors on a High-Content and High-Throughput Screening Platform	Li S et al	Assay	SoT 2018 abstract 2348	2
In Vitro Model for the Prediction of Respiratory Sensitization of Inhaled Chemicals and Protein Allergens	Chary A et al	Assay	SoT 2018 abstract 2364	2
Detection of Reactive Chemicals and Oxidants Using an Organotypic Human Airway Model with Nrf2 Reporter Activity: Application to Evaluation of Tobacco Products	Maione A et al	Assay	SoT 2018 abstract 2372	2
Integrating Exposure, Pharmacokinetics, and Dosimetry with In Vitro Dose-Response Data to Evaluate Chemical Risk	Leonard J et al	Assay	SoT 2018 abstract 2477	2
A Defined Approach to Skin Sensitization Using Derek Nexus and Non-Animal Assays	Canipa SJ et al	Assay	SoT 2018 abstract 2505	2

Title	Authors	Category	Source	Score
The Validation of GARDskin	Johansson A et al	Assay	SoT 2018 abstract 3066	3
A Multi-Centre Validation Study of Amino Acid Derivative Reactivity Assay (ADRA): A Novel In Chemico Alternative Test Method for Skin Sensitization	Ono A et al	Assay	SoT 2018 abstract 3070	3
Assessment of Potential Photosensitization Via In Chemico Direct Peptide Reactivity Assay	Mishra PK et al	Assay	SoT 2018 abstract 3144	2
Performance of the OptiSafe Ocular Irritation Assay in a Three-Laboratory Validation Study	Choksi N et al	Assay	SoT 2018 abstract 3160	3
Exploring a New Toolbox for Repeated Dose Systemic Toxicity Assessment: First Application to a Cosmetic Ingredient	Riu A et al	Assay	SoT 2018 abstract 3165	2
Use of a Reliable Metabolically Competent Human RGHep+ Hepatocyte Model Engineered with Biological Tracers for In Vitro Micronucleus Test	Chesne C et al	Assay	SoT 2018 abstract 1511	2
Assessment of the Accuracy of OECD Toolbox Profilers to Identify Reactive Chemicals Associated with Skin Sensitization	Settivari RS et al	Assay	SoT 2018 abstract 1108	2
Differential Gene Expression and Concentration-Response Modelling Workflow for High-Throughput Transcriptomic (HTTr) Data: Results from MCF7 Cells	Harrill JA et al	Assay	SoT 2018 abstract 2116	2+1
Use of ToxCast High-Throughput In Vitro Data to Develop a Computational Model to Identify Compounds That Interact with Neurological Receptors	Marty MS et al	Assay	SoT 2018 abstract 2535	2
Utilization of Human-Focused 3D Liver Models in Conjunction with Multi-Parametric Cell Health Approaches for the Prediction of Human Drug-Induced Liver Injury	Dilworth C	Assay	SoT 2018 abstract 1355	2

Title	Authors	Category	Source	Score
Characterization of the Novel Direct Double Strand Break Labeling Assay following Genotoxic Chemical Exposure	Dunnick KM et al	Assay	SoT 2018 abstract 1513	2
Applying Concepts from Adverse Outcome Pathways to Assessment of Airway Irritants	Haber LT et al	Pathway	SoT 2018 abstract 2362	3
Predicting Chemical Mechanisms of Action Using High-Throughput Transcriptomic Data	Shah I et al	Assay	SoT 2018 abstract 2507	2+1
Integrated Analysis of Transcriptomics Data and the Adverse Outcome Pathway Framework for Risk Assessment of Chemicals: An Exploratory Case Study Using Piperonyl Butoxide and Liver Models	Oki NO et al	Assay	SoT 2018 abstract 2510	2+1
Development of the in vitro assay for evaluating reversible eye irritation	Yamamoto N et al	Assay	WC10 2017 abstract II-4-369	3
Developing the next generation of organ on chip technology	Nahmias Y	Assay	WC10 2017 abstract II-6-341	3
Construction of mechanism-based hepatotoxicity prediction system by combining in silico and in vitro technology	Sone M et al	Assay	WC10 2017 abstract II-6-291	2+1
Non-animal testing strategy for skin sensitization assessment of hydrophilic and lipophilic chemicals	Mizumachi H et al	Assay	WC10 2017 abstract II-94	2
Development of an animal product free acute toxicity screen	Longmore C et al	Assay	WC10 2017 abstract II-251	2
The controlled formation of perfused vascularized 3D neural constructs and their utilization in neurodevelopmental disease modelling and toxin screening	Daly W et al	Assay	WC10 2017 abstract III-2-673	2+1

Title	Authors	Category	Source	Score
Development of a suite of assays to screen for developmental neurotoxicity *Note: Check species of cells*	Mundy WR	Assay	WC10 2017 abstract III-3-842	2
The C-DILI assay: A new in vitro method to predict chemically-induced liver toxicity	McKim J et al	Assay	WC10 2017 abstract III-7-285	2
In vitro vasculogenesis to interconnect organoids in a multiorgan-chip platform	Hübner J et al	Assay	WC10 2017 abstract III-14-303	3
A human heart-liver-skin microfluidic platform to assess systemic toxicity of compounds absorbed through the skin	Carmona-Moran CA et al	Assay	WC10 2017 abstract III-14-596	3
Cross-laboratory testing of the human proximal tubule tissue chip	Sakolish C et al	Assay	WC10 2017 abstract III-14-506	2
Molecular mechanisms of corneal oxidative stress: In vitro reconstructed human corneal tissue model (EpiCorneal™)	Kaluzhny Y et al	Pathway	WC10 2017 abstract III-654	2
Use of 3 animal product free methods as part of a novel integrated approach to skin sensitisation testing	Edwards A et al	Assay	WC10 2017 abstract III-253	2
Assessing the allergenic potential of proteins from their sequence and 3D structure using novel computational approaches	Gerberick GF et al	Assay	WC10 2017 abstract III-457	2
Human-based phenotypic profiling uncovers mechanisms of toxicity	Berg E et al	Pathway	WC10 2017 abstract III-735	2+1

Title	Authors	Category	Source	Score
Use of transcriptional profiling in in vitro systems to determine the biological activity of chemicals of interest	Naciff J et al	Pathway	WC10 2017 abstract V-1-342	2
Adaptation of the human Cell Line Activation Test (h-CLAT) to animal product-free conditions	Edwards A et al	Assay	WC10 2017 abstract VII-4-250	2

6.4 Recommended Abstracts

This year 25 projects received the highest scores of either 3 (as 2+1 or as 3) or 3+1 for reporting new pathways, assays, or biomarkers of toxicity. The 25 abstracts are given below.

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

The TGx-28.65 biomarker online application for analysis of transcriptomics data to identify DNA damage-inducing chemicals in human cell cultures.

MA Jackson[1], L Yang[1], I Lea[2], A Rashid[2], B Kuo[3], A Williams[3], C Lyn Yauk[3], J Fostel[4].

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[2] ASRC Federal - Vistronix, Morrisville, North Carolina.

[3] Environmental Health Science & Research Bureau, Health Canada, Ottawa, ON, Canada.

[4] National Institute of Environmental Health Sciences/National Institutes of Health, National Toxicology Program, Research Triangle Park, North Carolina.

Environ Mol Mutagen. 2017 Aug;58(7):529-535. doi: 10.1002/em.22114. Epub 2017 Aug 2.

Score 2 + 1 (ASSAY for genotoxicity + 1 for online predictive bioinformatics tool)

Abstract

The TGx-28.65 biomarker is a 65-gene expression profile generated from testing 28 model chemicals (13 that cause DNA damage and 15 that do not) in human TK6 cells. It is used to predict whether a chemical induces DNA damage or not. We expanded availability to the biomarker by developing the online TGx-28.65 biomarker application for predicting the DNA damage inducing (DDI) potential of suspect toxicants tested in p53-proficient human cells and assessing putative mode(s) of action (MOA). Applications like this that analyse gene expression data to predict the hazard potential of test chemicals hold great promise for risk assessment paradigms. The TGx-28.65 biomarker interfaces with an analytical tool to predict the probability that a test chemical can directly or indirectly induce DNA damage. User submitted in vitro microarray data are compared to the 28-chemical x 65-gene signature profile and the probability that the data fit the profile for a DDI or a non-DDI (NDDI) chemical is calculated. The results are displayed in the

Results Table, which includes the classification probability and hyperlinks to view heatmaps, hierarchical clustering, and principal component analyses of user-input data in the context of the reference profile. The heatmaps and cluster plots, along with the corresponding text data files of fold changes in gene expression and Euclidean distances can be downloaded. Review of the test chemical data in relationship to the biomarker allows rapid identification of key gene alterations associated with DNA damage as well as chemicals in the reference set that produced a similar response.

Real-time cell toxicity profiling of Tox21 10K compounds reveals cytotoxicity dependent toxicity pathway linkage.

JH Hsieh[1], R Huang[2], JA Lin[3], A Sedykh[4], J Zhao[2,] RR Ti[5], RS Paules[5], M Xia[2], SS Auerbach [5].

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[2] National Center for Advancing Translational Sciences, National Institutes of Health, Rockville, Maryland, United States of America.

[3] US Food and Drug Administration, Silver Spring, Maryland, United States of America.

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[5] Division of the National Toxicology Program, National Institute of Environmental Health Sciences, National Institutes of Health, Durham, North Carolina, United States of America.

PLoS One. 2017 May 22;12(5):e0177902. doi: 10.1371/journal.pone.0177902. eCollection 2017.

Erratum in PLoS One. 2017 Jul 7;12 (7):e0181291. (Figures 2 & 3 shown in wrong order)

Score 3 (Cytotoxicity PATHWAY identification based on analysis of 10k Tox21 chemicals)

Abstract

Cytotoxicity is a commonly used in vitro endpoint for evaluating chemical toxicity. In support of the U.S. Tox21 screening program, the cytotoxicity of ~10K chemicals was interrogated at 0, 8, 16, 24, 32, & 40 hours of exposure in a concentration dependent fashion in two cell lines (HEK293, HepG2) using two multiplexed, real-time assay technologies. One technology measures the metabolic activity of cells (i.e., cell viability, glo) while the other evaluates cell membrane integrity (i.e., cell death, flor). Using glo technology, more actives and greater temporal variations were seen in HEK293 cells, while results for the flor technology were more similar across the two cell types. Chemicals were grouped into classes based on their cytotoxicity kinetics profiles and these classes were evaluated for their associations with activity in the Tox21 nuclear receptor and stress response pathway assays. Some pathways, such as the activation of H2AX, were associated with the fast-responding cytotoxicity classes, while others, such as activation of TP53, were associated with the slow-responding cytotoxicity classes. By clustering pathways based on their degree of association to the different cytotoxicity kinetics labels, we identified clusters of pathways where active chemicals presented similar kinetics of cytotoxicity. Such linkages could be due to shared underlying biological processes between pathways, for example, activation of H2AX and heat shock factor. Others involving nuclear receptor activity are likely due to shared chemical structures

rather than pathway level interactions. Based on the linkage between androgen receptor antagonism and Nrf2 activity, we surmise that a subclass of androgen receptor antagonists cause cytotoxicity via oxidative stress that is associated with Nrf2 activation. In summary, the real-time cytotoxicity screen provides informative chemical cytotoxicity kinetics data related to their cytotoxicity mechanisms, and with our analysis, it is possible to formulate mechanism-based hypotheses on the cytotoxic properties of the tested chemicals.

Prediction of skin sensitization potency using machine learning approaches.

Q Zang[1], M Paris [1], DM Lehmann[2], S Bell[1], N Kleinstreuer[3], D Allen[1], J Matheson[4,] A Jacobs [5], W Casey[3], J Strickland[1].

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[4] US Consumer Product Safety Commission, Bethesda, MD, 20814, USA.

[5] FDA/CDER, Silver Spring, MD, 20993, USA.

J Appl Toxicol. 2017 Jul;37(7):792-805. doi: 10.1002/jat.3424. Epub 2017 Jan 10.

Score 3 + 1 (Prediction of skin sensitisation using in vitro ASSAYS and machine learning + 1 for demonstrating that this combined in vitro/in silico approach is superior to the LLNA)

Abstract

The replacement of animal use in testing for regulatory classification of skin sensitizers is a priority for US federal agencies that use data from such testing. Machine learning models that classify substances as sensitizers or non-sensitizers without using animal data have been developed and evaluated. Because some regulatory agencies require that sensitizers be further classified into potency categories, we developed statistical models to predict skin sensitization potency for murine local lymph node assay (LLNA) and human outcomes. Input variables for our models included six physicochemical properties and data from three non-animal test methods: direct peptide reactivity assay; human cell line activation test; and KeratinoSens™ assay. Models were built to predict three potency categories using four machine learning approaches and were validated using external test sets and leave-one-out cross-validation. A one-tiered strategy modeled all three categories of response together while a two-tiered strategy modeled sensitizer/non-sensitizer responses and then classified the sensitizers as strong or weak sensitizers. The two-tiered model using the support vector machine with all assay and physicochemical data inputs provided the best performance, yielding accuracy of 88% for prediction of LLNA outcomes (120 substances) and 81% for prediction of human test outcomes (87 substances). The best one-tiered model predicted LLNA outcomes with 78% accuracy and human outcomes with 75% accuracy. By comparison, the LLNA predicts human potency categories with 69% accuracy (60 of 87 substances correctly categorized). These results suggest that computational models using non-animal methods may provide valuable information for assessing skin sensitization potency.

DTNI: a novel toxicogenomics data analysis tool for identifying the molecular mechanisms underlying the adverse effects of toxic compounds.

DM Hendrickx [1,2], T Souza[3,4,] DGJ Jennen[3,4], JCS Kleinjans[3,4].

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Biology, Maastricht University, Universiteitssingel 40, 6229 ER, Maastricht, The Netherlands.

[4] P.O. Box 616, 6200 MD, Maastricht, The Netherlands.

Arch Toxicol. 2017 Jun;91(6):2343-2352. doi: 10.1007/s00204-016-1922-5. Epub 2016 Dec 28.

Score 2 + 1 (Novel genomics analysis tool for identifying toxicant PATHWAYS + 1 for using TG-GATES data to show potential identification of molecular initiating events, and key events, in AOPs. Potential conflict as the TG-GATES database (created between 2002 & 2011) used experiments on rats & rat hepatocytes as part of the data source)

Abstract

Unravelling gene regulatory networks (GRNs) influenced by chemicals is a major challenge in systems toxicology. Because toxicant-induced GRNs evolve over time and dose, the analysis of global gene expression data measured at multiple time points and doses will provide insight in the adverse effects of compounds. Therefore, there is a need for mathematical methods for GRN identification from time-over-dose-dependent data. One of the current approaches for GRN inference is Time Series Network Identification (TSNI). TSNI is based on ordinary differential equations (ODE), describing the time evolution of the expression of each gene, which is assumed to be dependent on the expression of other genes and an external perturbation (i.e. chemical exposure). Here, we present Dose-Time Network Identification (DTNI), a method extending TSNI by including ODE describing how the expression of each gene evolves with dose, which is supposed to depend on the expression of other genes and the exposure time. We also adapted TSNI in order to enable inclusion of time-over-dose-dependent data from multiple compounds. Here, we show that DTNI outperforms TSNI in inferring a toxicant-induced GRN. Moreover, we show that DTNI is a suitable method to infer a GRN dose- and time-dependently induced by a group of compounds influencing a common biological process. Applying DTNI on experimental data from TG-GATEs, we demonstrate that DTNI provides in-depth information on the mode of action of compounds, in particular key events and potential molecular initiating events. Furthermore, DTNI also discloses several unknown interactions which have to be verified experimentally.

Mechanisms of Skin Toxicity Associated with Metabotropic Glutamate Receptor 5 Negative Allosteric Modulators.

F Shah[1], AF Stepan [2], A O'Mahony [3,] S Velichko [3], AE Folias [3], C Houle [4], CL Shaffer [5], J Marcek [4], J Whritenour [4], R Stanton [2], EL Berg [6].

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[6] Bioseek Inc., Division of DiscoverX, 310 Utah Avenue, South San Francisco, CA 94080, USA. Email eberg@discoverx.com.

Cell Chem Biol. 2017 Jul 20;24(7):858-869.e5. doi: 10.1016/j.chembiol.2017.06.003. Epub 2017 Jun 29. Comment in Cell Chem Biol. 2017 Jul 20;24(7):781-782.

***Score* 3 + 1** (Identification of new PATHWAY of drug-induced skin toxicity + 1 for highlighting that a human model can predict toxicity making testing in non-human primates unnecessary)

Abstract

Cutaneous reactions represent one of the most common adverse drug effects observed in clinical trials leading to substantial compound attrition. Three negative allosteric modulators (NAMs) of metabotropic glutamate receptors (mGluRs), which represent an important target for neurological diseases, developed by Pfizer, were recently failed in preclinical development due to delayed type IV skin hypersensitivity observed in non-human primates (NHPs). Here we employed large-scale phenotypic profiling in standardized panels of human primary cell/co-culture systems to characterize the skin toxicity mechanism(s) of mGluR5 NAMs from two different series. Investigation of a database of chemicals tested in these systems and transcriptional profiling suggested that the mechanism of toxicity may involve modulation of nuclear receptor targets RAR/RXR, and/or VDR with AhR antagonism. The studies reported here demonstrate how phenotypic profiling of preclinical drug candidates using human primary cells can provide insights into the mechanisms of toxicity and inform early drug discovery and development campaigns.

Human liver-kidney model elucidates the mechanisms of aristolochic acid nephrotoxicity.

SY Chang[1], EJ Weber[2], VS. Sidorenko[3], A Chapron[2], CK Yeung [2,4], C Gao[2], Q Mao[2], D Shen [2], J Wang[2], TA Rosenquist[3], KG Dickman[3], T Neumann[5], AP Grollman [3,6], EJ Kelly[2], J Himmelfarb [4], DL Eaton[1].

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JCI Insight. 2017 Nov 16;2(22). pii: 95978. doi: 10.1172/jci.insight.95978.

Score 2 + 1 (PATHWAY of aristolochic acid nephrotoxicity + 1 for including organ-organ interactions in microphysiological system)

Abstract

Environmental exposures pose a significant threat to human health. However, it is often difficult to study toxicological mechanisms in human subjects due to ethical concerns. Plant-derived aristolochic acids are among the most potent nephrotoxins and carcinogens discovered to date, yet the mechanism of bioactivation in humans remains poorly understood. Microphysiological systems (organs-on-chips) provide an approach to examining the complex, species-specific toxicological effects of pharmaceutical and environmental chemicals using human cells. We microfluidically linked a kidney-on-a-chip with a liver-on-a-chip to determine the mechanisms of bioactivation and transport of aristolochic acid I (AA-I), an established nephrotoxin and human carcinogen. We demonstrate that human hepatocyte-specific metabolism of AA-I substantially increases its cytotoxicity toward human kidney proximal tubular epithelial cells, including formation of aristolactam adducts and release of kidney injury biomarkers. Hepatic biotransformation of AA-I to a nephrotoxic metabolite involves nitroreduction, followed by sulfate conjugation. Here, we identify, in a human tissue-based system, that the sulfate conjugate of the hepatic NQO1-generated aristolactam product of AA-I (AL-I-NOSO3) is the nephrotoxic form of AA-I. This conjugate can be transported out of liver via MRP membrane transporters and then actively transported into kidney tissue via one or more organic anionic membrane transporters. This integrated microphysiological system provides an ex vivo approach for investigating organ-organ interactions, whereby the metabolism of a drug or other xenobiotic by one tissue may influence its toxicity toward another, and represents an experimental approach for studying chemical toxicity related to environmental and other toxic exposures.

In Vitro Micropatterned Human Pluripotent Stem Cell Test (μP- hPST) for Morphometric-Based Teratogen Screening

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ScIeNtIfIc REPORTS 2017, 7: *8491,* DOI:10.1038/s41598-017-09178-1

Score 3 (New ASSAY technology)

Abstract

Exposure to teratogenic chemicals during pregnancy may cause severe birth defects. Due to high inter-species variation of drug responses as well as financial and ethical burdens, despite the widely use of *in vivo* animal tests, it's crucial to develop highly predictive human pluripotent stem cell (hPSC)-based *in vitro* assays

to identify potential teratogens. Previously we have shown that the morphological disruption of mesoendoderm patterns formed by geometrically-confined cell differentiation and migration using hPSCs could potentially serve as a sensitive morphological marker in teratogen detection. Here, a micropatterned human pluripotent stem cell test (μP-hPST) assay was developed using 30 pharmaceutical compounds. A simplified morphometric readout was developed to quantify the mesoendoderm pattern changes and a two-step classifcation rule was generated to identify teratogens. The optimized μP-hPST could classify the 30 compounds with 97% accuracy, 100% specificity and 93% sensitivity. Compared with metabolic biomarker-based hPSC assay by Stemina, the μP-hPST could successfully identify misclassified drugs Bosentan, Diphenylhydantoin and Lovastatin, and show a higher accuracy and sensitivity. This scalable μP-hPST may serve as either an independent assay or a complement assay for existing assays to reduce animal use, accelerate early discovery-phase drug screening and help general chemical screening of human teratogens.

Building predictive in vitro pulmonary toxicity assays using high-throughput imaging and artificial intelligence

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Arch Toxicol (2018). https://doi.org/10.1007/s00204-018-2213-0

Score 2 + 1 (Improved ASSAY approach, + 1 for transferability of phenotypic screening)

Abstract

Human lungs are susceptible to the toxicity induced by soluble xenobiotics. However, the direct cellular effects of many pulmonotoxic chemicals are not always clear, and thus, a general in vitro assay for testing pulmonotoxicity applicable to a wide variety of chemicals is not currently available. Here, we report a study that uses high-throughput imaging and artificial intelligence to build an in vitro pulmonotoxicity assay by automatically comparing and selecting human lung-cell lines and their associated quantitative phenotypic features most predictive of in vivo pulmonotoxicity. This approach is called "High-throughput In vitro Phenotypic Profiling for Toxicity Prediction" (HIPPTox). We found that the resulting assay based on two phenotypic features of a human bronchial epithelial cell line, BEAS-2B, can accurately classify 33 reference chemicals with human pulmonotoxicity information (88.8% balance accuracy, 84.6% sensitivity, and 93.0% specificity). In comparison, the predictivity of a standard cell-viability assay on the same set of chemicals is much lower (77.1% balanced accuracy, 84.6% sensitivity, and 69.5% specificity). We also used the assay to evaluate 17 additional test chemicals with unknown/unclear human pulmonotoxicity, and experimentally confirmed that many of the pulmonotoxic reference and predicted-positive test chemicals induce DNA strand breaks and/or activation of the DNA-damage response (DDR) pathway. Therefore, HIPPTox helps us to uncover these common modes-of-action of pulmonotoxic chemicals. HIPPTox may also be applied to other cell types or models, and accelerate the development of predictive in vitro assays for other cell-type- or organ-specific toxicities.

Conferences – SoT

1138

Use of a Chronic Multiplatform Assay to Evaluate Cardiotoxicity of BMS-986094 in iPSC-Derived Human Cardiomyocytes

C. J. Strock, J. Bradley, S. Qin.

Cyprotex US, LLC, Watertown, MA.

Score 3 + 1 (Advance in ASSAY technology/approach + 1 for demonstrating hiPSC better than animal model)

Abstract

BMS-986094 (INX-08189) was developed as a prodrug of a guanosine nucleotide analogue developed to treat Hepatitis C virus (HCV). It was discontinued in Phase 3 clinical trials due to cardiac toxicity with 1 death and 8 patients hospitalized with significantly reduced left ventricular ejection fraction (LVEF). Further analysis has shown cardiotoxic effects in 14 of 34 patients, where evaluation of the ECGs of patients with LV dysfunction showed ST depressions, T-wave inversions, or loss of T-wave amplitude. Mitochondrial effects through inhibition of the mitochondrial RNA polymerase have been reported to be the mechanism for the toxicity. Retrospective animal studies with high doses of compound have been shown to recapitulate some of the LVEF and ECG effects. Here, we exposed hiPSC-derived cardiomyocytes to BMS-986094 for 14 days and assessed their electrophysiologic function using an MEAN assay. In addition, we compared these results to those obtained from a cardiac mitochondrial protein biogenesis assay, calcium flux assay, and a cardiac cytotoxicity assay. We show that the MEA assay is more sensitive at lower concentrations, with a complete loss of electrical activity at greater than 80nM and significant electrophysiologic functional effects at 80nM. These effects were time dependent, with many of the progressive effects taking place after 10 days of treatment. At the 80nM concentration, BMS-986094 caused a 20% increase in Na+ peak amplitude and a doubling of the beat rate. There was also a decrease in the T-wave amplitude at earlier time points before a loss in beating was observed. Notably, the MEA trace mimics the T-wave amplitude effects consistent with observations in patients who had cardiotoxicity in the clinical trial. We also show that the calcium flux effects mimic the effects seen in the MEA assay. Interestingly, although it has been suggested that mitochondrial biogenesis was blocked with this compound, we observe an increase in mitochondrial-coded protein using a high-content assay with immunofluorescent staining. These results suggest that the cardiotoxicity from BMS-986094 is not related to the mitochondrial toxicityand likely related to a yet undetermined cumulative mechanism. This study shows that the use of stem cell-derived cardiomyocytes in longterm physiological-based assays can improve the prediction of cardiac liabilities.

1139

Development of an In Vitro Assay to Predict Cardiotoxicity Potential Using Targeted Metabolomics and Human- Induced Pluripotent Stem Cell-Derived Cardiomyocytes

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Sponsor: N. Kleinstreuer

Score 3 (Advance in BIOMARKER & ASSAY)

Abstract

Cardiac safety is one of the leading causes of late-stage compound attrition in the pharmaceutical industry and accounts for 28% of the safety- related withdrawals of FDA-approved drugs from the market. Current cardiac safety preclinical evaluations are heavily focused on approximately 3-7 main ion channels involved in maintaining the cardiac action potential; however, over 70 different types of ion channels are expressed in the heart and participate in the overall cardiac current. These safety testing methods overemphasize electrophysiologicalassessment of cardiotoxicity and fail to evaluate cardiomyopathyand other forms of structural cardiotoxicity. Metabolic perturbations areone of the primary mechanisms underlying the cardiotoxicity elicitedby pharmaceuticals. Stemina has developed a small-molecule biomarker-based assay for evaluating the cardiotoxicity potential of compoundsbased on changes in the metabolism and viability of human-inducedpluripotent stem cell (hiPSC)-derived cardiomyocytes. In this study, weexposed hiPSC-derived cardiomyocytes to a training set of 57 compounds (37 positive, 20 negative) and blinded test set of 12 compounds (6 positive, 6 negative). The cardiotoxic compounds were broken into three categories: structural, functional, and general (compounds that cause both). Metabolomic analysis of spent media identified a set of predictive biomarkers. The biomarker-based model classified the test set with 92% accuracy and the training set with 86% accuracy, based on comparing the concentration where metabolism was perturbed to the therapeutic Cmax. This assay is an attractive new model that can identify both structural and functional cardiotoxic compounds that could be used in conjunction with CiPA and other endpoints to provide a more comprehensive evaluation of a compound's cardiotoxicity potential.

1152

Phenotypic Profiling in Human-Based Phenotypic Assays Supports an AOP for CV Toxicity

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Score 3 (Data supporting new PATHWAY for cardiovascular toxicity)

Abstract

Data mining of a large reference database containing drugs, experimental chemicals, and other agents profiled in a panel of human primary cell-based systems has been used to discover novel associations and mechanisms of toxicity associated with certain adverse events. These include acute toxicity, organ toxicity, liver toxicity, skin rash, skin sensitization, and thrombosis-related side effects. Using this approach, anin vitro signature for cardiovascular toxicity comprised of increased cell surface levels of serum amyloid A (SAA) measured in a coronary artery smooth muscle cell-based model of vascular inflammation (BioMAP CASM3C system) was found and was associated with MEK, HDAC, GR/ MR, IL-6 pathway, and SIRT1. Since SAA is a clinical biomarker associated with risk of cardiovascular disease in humans, to further understand the regulation of SAA, we performed data mining in the same assay system to identify those agents that reduce the level of

SAA. Agents were selected if treatment with that agent for 24 hours at two or more concentrations decrease the cell surface level of SAA without causing overt cytotoxicity. Fewer than 1% of agents in the database were found to decrease level of SAA relative to vehicle control at two or more testedconcentrations and with an effect size of ≥ 20% (33/3,800). Notable agents that met these criteria include GLP-1, an endogenous peptide developed as a drug used for treatment of diabetes, roflumilast, a PDE IV inhibitor used for the treatment of chronic obstructive pulmonary disorder, the BCR-Abl inhibitor and oncology drug, imatinib, and a mimetic of ApoA-1, the major lipoprotein of HDL. These represent agents that have been shown to have cardiovascular protective effects in clinical or in vivo studies (some within their class). These results, showing that decreased cell surface levels of SAA in this human primary cell-based phenotypic model are associated with beneficial cardiovascular outcomes, along with the previous finding that increased levels of SAA are associated with cardiovascular toxicity, support the construction of an adverse outcome pathway for cardiovascular toxicity.

3066

The Validation of GARDskin

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Score 3 (Validation of ASSAY for skin sensitisation)

Abstract

Chemical handling and chemicals in consumer products may cause severe health effects including allergic contact dermatitis (ACD). Since ACD is a widespread disease estimated to effect at least 20% of the western population there is high interest to protect people from sensitizing chemicals not only for the patient itself, but for the entirely society. To minimize exposure chemicals must be safety tested. Traditional testing strategies as the murine local lymph node assay (LLNA) comprise animals, but the regulatory authorities, public and economic interests require animal-free models. The Genomic Allergen Rapid Detection skin (GARDskin) is a state of the art in vitro assay that addresses this question. For validity of the assay we here present the results of the GARDskin ring trial (OECD TGP 4.106). The initiation of skin sensitization in vivo includes activation of epidermal dendritic cells. The GARDskin assay relies on test substance stimulation in vitro of SenzaCells; a human myeloid cell line similar dendritic cells, and gene expression analysis of 200 predictive biomarkers. The biomarker panel is derived from whole genome expression data analyzed during the development of GARDskin, but for stream lined work the NanoString platform has been adopted. A machine learning algorithm is employed to analyze the high dimensional data and to perform the final binary classification. For the validation of the GARDskin three independent laboratories were selected: two external CROs and the in-house development laboratory. The structure of the study was designed to cover training and transferability of GARDskin to the two external test laboratories. The final study includes reproducibility of the assay within and between the three laboratories. The two external test laboratories tested eleven (11) chemicals by GARDskin three times and all substances (11/11) were classified correct (100% accuracy),

confirming the transferability of the assay. The final validation confirming the reproducibility of the assay was confirmed by testing 28 blinded substances in all three test laboratories. This manifests GARDskin as a valid test for assessment of skin sensitizers.

3070

A Multi-Centre Validation Study of Amino Acid Derivative Reactivity Assay (ADRA): A Novel In Chemico Alternative Test Method for Skin Sensitization

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Score 3 (Validation of ADRA ASSAY – improved DPRA)

Abstract

ADRA has been developed as an in chemico test method for detecting chemicals with skin sensitization potential, and represents a significant technical advance on the DPRA (Direct Peptide Reactivity Assay) adopted in OECD TG-442C in 2015. DPRA is reliable test method to detect the key initiating event of the skin sensitization AOP (adverse outcome pathway), but has significant technical limitations, e.g. cysteine peptide oxidization, precipitation and co-elution of test chemical. ADRA uses the synthetic chemicals NAC (N-2(1-napthyl)acetyl)-L-cysteine) and NAL (α-N-(2-(1-naphthyl)acetyl)- L-lysine) to overcome problems inherent in the DPRA test methods. To support the adoption of ADRA in the OECD test guideline, we conducted a four-laboratory validation study in two phases. During Phase I, the four participant laboratories performed three test runs of identical sets of 10 coded test chemicals to evaluate within-laboratory reproducibility. The concordance of each laboratory was greater than 90%. In Phase II, each participant laboratory performed one run of identical sets of an additional 30 coded test chemicals. Between-laboratory reproducibility was 92% based on the results of 40 test chemicals combined Phase I and Phase II. Moreover, an analysis of predictive capacity of Phase I and Phase II of the study,

compared with the LLNA classifications, showed a sensitivity of 81%, specificity of 98%, and accuracy of 86%. Based on this validation study, the VMT (validation management team) concluded ADRA to be an in chemico method with sufficient reproducibility for both within- and between-laboratory and predictive capacity as the regulatory acceptable test method.

3160

Performance of the OptiSafe Ocular Irritation Assay in a Three-Laboratory Validation Study

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Score 3 (Validation of ocular irritation ASSAY)

Abstract

OptiSafe is an in vitro test method that assesses a test substance's potential to cause eye irritation by measuring damage caused when the substance is applied to a semi-permeable membrane. The membrane system allows the detection of substances with different mechanisms of ocular injury. NICEATM reviewed a study conducted by Lebrun Labs, which developed OptiSafe, and concluded that the study data compared favorably to other in vitro ocular toxicity testing methods. To further assess the transferability of the method to naïve laboratories and the overall performance and applicability domain of the method, NICEATM coordinated a multi-laboratory validation study to evaluate hazard identification of non-surfactants. Phase 1 testing of five chemicals in each laboratory showed that the method could be transferred to naïve laboratories. Thirty coded chemicals selected by a validation management team were then tested by all three laboratories in Phase 2. Test method performance was assessed using both the EPA and GHS eye irritation hazard classification systems. Intralaboratory reproducibility for both classification systems ranged from 93% to 99%. Intralaboratory accuracy using the EPA classification system ranged from 82% to 88%. False negative and false positive rates ranged from 0% to 7% and 23% to 39%, respectively. Intralaboratory accuracy, false negative, and false positive rates using the GHS classification system ranged from 78% to 88%, 0% to 15%, and 23% to 36%, respectively. Interlaboratory reproducibility was 91% for both classification systems. Interlaboratory accuracy and false negative rates were 89% and 0%, respectively, for both classification systems. The false positive rates were 23% for the GHS classification system and 25% for the EPA classification system. Phase 3 testing of an additional 60 substances provided a comprehensive assessment of test method accuracy and defined the applicability domain of the method. These results suggest that the OptiSafe ocular irritation assay may represent a new tool for in vitro assessment of the ocular toxicity potential of chemicals in a tiered-testing system.

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2116

Differential Gene Expression and Concentration-Response Modeling Workflow for High-Throughput Transcriptomic (HTTr) Data: Results from MCF7 Cells

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Score 2 + 1 (Application of high-throughput transcriptomics to tox ASSAYS + 1 for potential to break bottleneck of chemical testing)

Abstract

Increasing efficiency and declining cost of generating whole-transcriptome profiles has made high-throughput transcriptomics a practical option for chemical bioactivity screening. The resulting data output provides information on the expression of thousands of genes and is amenable to a variety of downstream applications. However, HTTr chemical screening presents challenges that are not inherent in traditional cell-based screening assays, which produce univariate outputs. We present a microfluidics-based laboratory workflow for HTTr screening of MCF7 cells in 384-well format and a HTTr analysis pipeline for data quality control, differential gene expression, and concentration-response modeling. MCF7 cells were plated in either DMEM+10% HI-FBS or phenol red-free DMEM+10% CS-HI-FBS and allowed a 24 h recovery period prior to exposure. A total of 44 chemicals were screened in 8-point concentration- response (0.03-100 µM) in each media at three exposure durations (6, 12, 24 h) in three independent cultures (n=1 /treatment/culture). Chemicals were applied using an acoustic dispenser. Each test plate was uniquely randomized with respect to treatment positioning. Cell lysates were analyzed using a TempO-Seq human whole-transcriptome assay to a target read depth of 3M. Cell viability and apoptosis assays were run in parallel to exclude conditions causing cytotoxicity. For count data, total and percent mapped reads were used to exclude samples with poor performance. Data were subset by chemical x media x time with matching controls. Probes were filtered using a median raw read count > 5. Count data were scaled and transformed, and differentially expressed genes (DEGs) were determined using DESeq2. Reproducibility of read counts in technical and biological replicates was high, with pairwise correlations > 0.85 and median CVs of between 20 and 40%. The correlation of log2FC values for DEGs among biological replicates was also high (median > 0.75) within each media x time condition. ANOVA results demonstrated a broad range (10s to 1,000s) in the number of concentration-responsive genes across chemicals. Concentration-response modeling demonstrated a broad range of probe and pathway level BMDs across chemicals for each media x time combination, and facilitated identification of no observable transcriptional response levels.

This abstract does not necessarily reflect US EPA policy.

2362

Applying Concepts from Adverse Outcome Pathways to Assessment of Airway Irritants

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Score 3 (Identification of MIEs in AOP PATHWAY of airway irritation)

Abstract

Airway irritant exposures in the workplace cause rhinorrhea, cough, and bronchospasm, and are associated with intrinsic asthma, non-allergic rhinitis, and related airway conditions. Occupational exposure limits (OELs) for airborne levels of workplace toxicants are a critical means to protect the workforce from these effects. Occupational use scenarios often call for toxicity data specific to airway sensory irritation, but current sensory testing methods are limited and these data are seldom available. As a result, many OELs intended to protect against airway irritation are based on oral toxicity data and may therefore not be sufficiently protective. Conventional irritation assays, such as the Draize eye and skin corrosion tests, do not capture sensory irritation responses and their use runs against widespread emphasis on reducing animal-based testing. The objective of this work is to develop a cost-effective screening system to identify chemicals likely to be airway irritants. We hypothesize that this can be done using in vitro measurement of molecular initiating events (MIEs) upstream of sensory irritation responses, similar in principle to the use of adverse outcome pathways. We reviewed the experimental literature relevant to sensory irritation responses and used a modified adverse outcome pathway method to evaluate a key event framework beginning with MIEs. Our assessment identified (1) activation of transient receptor potential and acid-sensing ion channels, (2) lipid peroxidation, and (3) pro-inflammatory changes as MIEs upstream of sensory irritation. Downstream events include depolarization of nociceptive neurons and epithelial inflammation, both of which are events mediating airway irritation responses. Based on this framework, we have identified potential endpoints that can be evaluated in cultured human airway cells. A predictive assay suite based on multiple irritant MIEs would allow for the identification of chemicals of low concern for which derivation of OELs from oral toxicity data may be allowable, and may ultimately lead to methods to reduce animal testing for sensory irritants. This would improve the efficiency of risk assessments for these chemicals of low concern (and perhaps ultimately other chemicals) without compromising health protection.

2507

Predicting Chemical Mechanisms of- Action Using High-Throughput Transcriptomic Data

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Score 2 + 1 (Application of high-throughput transcriptomics to tox ASSAYS + 1 for potential to break bottleneck of chemical testing)

Abstract

The EPA ToxCast effort has screened thousands of chemicals across hundreds of high-throughput in vitro assays. Now, the project is leveraging high-throughput transcriptomic (HTTr) technologies to substantially expand its coverage of biological pathways by measuring the expression of 19,290 genes across multiple cell types. Our objective is to deploy HTTr as an initial screen to prioritize the existing suite of high-throughput assays for additional testing. To accomplish this goal, we must

elucidate the potential mechanisms of action (MoA) for each chemical using the transcriptomic profiles. Thus far, HTTr data have been generated for 2,200 chemicals in concentration response format in MCF7 cells at a 6 hr time point. We have also developed a computational pipeline in Python/R to streamline data processing and analysis as follows: (a) translating raw RNA-Seq data to normalized transcriptomic profiles, (b) identifying statistically significant transcriptional perturbations using DESeq2, (c) finding concentration- responsive transcripts using benchmark dose modeling (BMD), and (d) predicting chemical mechanism of action (MoA). We used "connectivity mapping" to infer the MoA of a chemical based on similarity with a database of HTTr profiles from the Connectivity Map (CMap) project (Lamb et al, 2006). the CMap transcriptomic database contains 3,334 Affymetrix transcriptomic profiles (13,029 genes) for 1,176 chemicals and 482 chemicals annotated with 90 MoA classes curated from KEGG and DrugBank. Analyzing HTTr profiles for genistein (10uM), sirolimus (0.1uM) and trichostatin A (1uM) from MCF7 cells using connectivity mapping correctly identified their known targets as estrogen receptor (ESR), mechanistic target of rapamycin (mTOR) and, histone deacytelase (HDAC), respectively. However, connectivity mapping also produced high-scoring database matches that were not consistent with the known MoA for these chemicals. We are exploring machine learning techniques to mine higher-order dependencies between genes in order to make MoA predictions that are biologically meaningful, sensitive and specific. We will present MoA predictions for 2,200 chemicals using concentration-response HTTr data and show their utility as an initial screen for high-throughput toxicity testing.

This abstract does not reflect US EPA policy.

2510

Integrated Analysis of Transcriptomics Data and the Adverse Outcome Pathway Framework for Risk Assessment of Chemicals: An Exploratory Case Study Using Piperonyl Butoxide and Liver Models

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Score 2 + 1 (Application of high-throughput transcriptomics to tox ASSAYS + 1 for potential to break bottleneck of chemical testing)

Abstract

The integration of existing knowledge to support the risk assessment of chemicals is still a challenge for scientists, risk assessors, and managers. International initiatives like the Adverse Outcome Pathways (AOP) programme have a role in supporting the integration of information from various sources and building collaborative platforms enabling the scientific community to address risk assessment issues. In this exploratory case study, gene expression data from HepaRG and HepG2 liver cell lines for piperonyl butoxide (PBO) housed in the Data Infrastructure for Chemical Safety (diXa) database were used. The differentially expressed genes were used in pathway enrichment analysis, chemical similarity associations (similar mode of action (MOA) as PBO), and disease associations using tools from the LINCS Consortium, and the Comparative Toxicogenomics Database (CTD). The resulting pathways, chemical analogs, and disease associations were combined with specific liver AOPs and key events from the AOP knowledge base to

show evidence supporting a case for PBO as a liver toxicant. There was an 8% overlap in pathways between the two cell lines in response to treatment, and a 6.1% overlap in chemicals with similar MOA to PBO across both lines (146 and 133 known chemicals for HepG2 and HepaRG, respectively). Overall, there were fewer associations for the HepaRG cells compared to the HepG2 line, highlighting the mechanistic differences that exist between the different liver models as illustrated by the low overlap in association results. The results also highlight the reference bias that may be introduced by the computational tools used in analysis and the reference data they depend on. This work shows that human in vitro transcriptomics data and modeling tools can identify potential toxicity by highlighting the biological pathways, diseases, and related chemicals based on the biological signatures. When mapped to existing AOPs, this information can be used to identify relevant AOPs for a chemical, highlight knowledge gaps where new AOPs could be defined, and assemble AOP networks relevant for the chemical. This approach could support the evidence-based risk assessment of individuals or groups of compounds by using the transcriptomic profiles to identify data gaps and eventually propose additional testing.

Conferences – WC10

II-4-369

Development of the in vitro assay for evaluating reversible eye irritation

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Score 3 (New in vitro assay for reversible eye irritation)

Abstract

Differences in reversibility of cornea damages could not be distinguished in previously developed non-animal alternative eye irritation methods. Whether the eye damage caused in reversible or irreversible is an important hazard assessment criterion such as GHS category 2A or 2B. Based on the performance standard in OECD TG 492, the protocol was developed using a three-dimensional cornea model with cloned iHCE-NY1 cell line, which was derived from corneal epithelial cells isolated from human corneal tissue and transfected with immortalized gene (SV40 Large T antigen). To evaluate reversible cytotoxicity of test substances, the MTT assay and pathological finding were examined with the cornea model on day 1, 7 and 14 of post-culture. This protocol induced reversible responses at four test chemicals in eight ones classified by GHS category 2A or 2B. These results address the post-incubation of RhCE model after applying test chemicals may be useful to evaluate reversible cornea damage.

II-6-341

Developing the next generation of organ on chip technology

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Score 3 (Novel approach to real-time ASSAY in OoC, but little detail in abstract)

Abstract

Organ-on-chip technology aims to replace animal toxicity testing, but thus far demonstrated few advantages over traditional methods. Current methods to evaluate toxicity rely on end-point assays, resulting in limited kinetic and mechanistic information. We present the Tissue Dynamics platform capable of maintaining vascularized 3D liver tissue for over a month in vitro. Tissues acquire physiological structure and show complex metabolic zonation. Tissue-embedded metabolic sensors permit the real-time assessment of cellular function. Change in metabolic function is the first indication of stress, preceding any detectable damage. We show a new CYP450-idependent mechanism of acetaminophen toxicity that may be responsible for clinically observed nephrotoxicity. We also show troglitazone-induced metabolic changes that might underlie its observed idiosyncratic toxicity. Our work marks the importance of tracing function in real time, demonstrating specific advantages in predicative toxicology.

II-6-291

Construction of mechanism-based hepatotoxicity prediction system by combining in silico and in vitro technology

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Score 2 + 1 (Developed multiparameter in vitro ASSAY for hepatotoxicity + 1 for combining with in silico to create prediction model)

Abstract

It is difficult to establish a non-animal evaluation method of systemic toxicity because of its complexity. In this study, we focused on liver which is a main target of repeated dose toxicity test, and we tried to construct the battery methods with in silico and in vitro to confirm its usefulness for prediction of hepatotoxicity potential. Three combinations of in silico models (HESS, MultiCase, and Derek Nexus) can predict the hepatotoxicity with over 95% sensitivity using 383 chemicals. We evaluated 23 chemicals to confirm a usefulness of in vitro assay, which was constructed by several indicators of liver effect, such as cell death, oxidative stress, lipid metabolism, and bile acid accumulation in HepaRG cells. We found that there was no false negative result by combining with in silico models and in vitro assay. Moreover, in vitro assay clarified the mechanisms of the hepatotoxicity and defined the toxic doses to find out the risk by comparing the internal exposure doses in vivo.

III-2-673

The controlled formation of perfused vascularized 3D neural constructs and their utilization in neurodevelopmental disease modelling and toxin screening

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Score 2 + 1 (ASSAY for developmental neurotoxicity + 1 for combining organoids with flow to mimic perfusion)

Abstract

Modelling the cellular diversity of the developing human brain is a major challenge in neural stem cell engineering and is essential in neural disease modelling and toxin screening. Here, we have generated neural progenitor cells, endothelial cells, and microglial precursors from induced pluripotent stem cells (iPSCs) for inclusion in 3D vascularised cerebral organoids. Synthetic hydrogels formed organoids with high sample uniformity and are a suitable alternative to Matrigel. We have integrated the organoids into two bioreactor systems (a pumped recirculating wellplate and a pumpless microfluidics platform) to perfuse and mature the organoids. Organoids generated in the microfluidics platform were used to screen a panel of 70 compounds for potential neurotoxins. Additionally, vascularized organoids were generated from Rett Syndrome (RTT) and MeCP2 duplication (M2) patients and differences in their phenotypical, functional and metabolic characteristics were compared. RTT and M2 organoids were further treated with BDNF, IGF, gentamycin and a HDAC inhibitor to assess their efficacy in changing a diseased organoid towards a healthy phenotype.

III-14-303

In vitro vasculogenesis to interconnect organoids in a multiorgan- chip platform

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Score 3 (A rare significant advance in 3D/organ-on-a-chip technology - self-organising microvasculature to connect organoid cultures)

Abstract

The Multi-Organ-Chip is a microphysiological system developed to evaluate toxicity of drugs, cosmetics and alike. The system comprises compartments for the co-cultivation of human 3D tissue constructs. Organoids are physically separated, yet, interconnected through perfused microfluidics. Minute volumes of medium enable crosstalk. The organoid cultures are, however, not sufficiently vascularised to overcome limitations in size and complexity. A continuous endothelium, further, is crucial for physiological-like interactions, regulation and homeostasis within organoid (co-)cultures. Three major aspects were addressed: (1) Implementing a near-physiological, pulsatile flow. It provides an in vivo-like shear stress regime. (2) Creating an endothelial lining within the chip's microfluidic system. The optimised flow promotes long-term vitality and the expression of typical endothelial markers. (3) Establishing capillary-like vessels as a direct route to the organoids.

Fibrin hydrogels containing an endothelial/stromal cell co-culture enable the self-organised formation of microcapillaries and their connection to a bone marrow model.

III-14-596

A human heart-liver-skin microfluidic platform to assess systemic toxicity of compounds absorbed through the skin

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Score 3 (ASSAY system for testing systemic effects of compounds applied to skin in vitro)

Abstract

Assessing systemic toxicity from topically absorbed chemicals is crucial for development of new cosmetic ingredients. Current in vitro skin models can't predict the long-term systemic toxicity; animal models have limited predictivity and are banned for cosmetics since 2013. "Body-on-a-chip" platforms offer a relevant, physiological model for multi-organ interactions that can be used to test systemic responses. We have developed a novel human heart-liver-skin microfluidic chip linking cardiac, hepatic and reconstructed skin modules to evaluate a potential toxicity through topical exposure. The platform was used to evaluate the effects on hepatic and cardiac functions of 4 drugs such as diclofenac, acetaminophen, ketoconazole and hydrocortisone, applied to the skin. The organ modules maintained full functionality in serum-free medium, under flow, for 14 days. This in vitro model allows elucidation of chronic topical exposure to predict potential organ toxicity.

III-735

Human-based phenotypic profiling uncovers mechanisms of toxicity

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Score 2 + 1 (Novel PATHWAY/BIOMARKER analysis of skin irritation + 1 for use of phenotypic profiling)

Abstract

Human-based phenotypic profiling is an attractive method for the discovery of chemical toxicity mechanisms. A large reference database was created in a standardized panel of human primary cell and co-culture model systems, the BioMAP® Diversity PLUS panel. Analysis of this data identified a biomarker signature common to skin irri tants, consisting of increased prostaglandin E2 (PGE2) and decreased TNFalpha in a primary human endothelial cell co-culture model with peripheral blood monocytes stimulated with lipopolysaccharide. This

signature was uncommon (30 of 3400 reference agents) and shared by 2-Chloroethyl Ethyl Sulfide, a chemical vesicant; FICZ, an Aryl Hydrocarbon Receptor (AhR) agonist; PKC, RAR/RXR, Prostaglandin EP Receptor, and Vitamin D Receptor (VDR) agonists; inhibitors of Thromboxane A2 synthetase; and lead compounds from a drug discovery program that was terminated due to skin toxicity in non-human primates.

7. Conclusions

The review of the WC10 and most recent SoT conference proceedings, and an extensive literature search, yielded 25 abstracts describing projects by investigators whom we believe the Judges should consider as potential candidates for the Lush Science Prize. These are given in Section 6.

Once again we have reviewed many more papers and abstracts than in the previous year (over 4,100 this year compared with around 3,500 in 2017). However, we scored rather fewer abstracts this year compared with last (68 vs 95 in 2017). Just over a third of these (25) were high scoring (3 and 4) abstracts; 37% of the total scored, compared with 46% in 2017.

The nominated abstracts are very diverse, and cover a wide range of topics including the development of assays for chronic toxicity testing, detecting systemic effects in *in vitro* models, the importance of avoiding xenobiotics in preparing and maintaining cells and culture media, and the implementation of new approaches in real world risk and hazard assessment. We believe that they are all worthy candidates for the 2018 Science Prize.