Lush Science Prize 2017
Background Paper

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1. Executive Summary

1.1 What is the Lush Science Prize?

Now in its sixth year, the Lush Prize supports animal-free testing by awarding money prizes of up to £250,000 to the most effective projects and individuals who have been working towards the goal of replacing animals in product or ingredient safety testing.

Prizes are awarded for developments in five strategic areas: science; lobbying; training; public awareness; and young researchers. Should there be a major breakthrough in 21st Century Toxicology - the area which holds out most hope for a 'Eureka' moment leading to the replacement of animal tests – a Black Box Prize equivalent to the entire annual fund will be awarded to the individual(s) or team(s) responsible. In 2015, the judges 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) to the replacement of animal testing in the preceding year. This 2017 Science Background paper identifies 44 pieces of work that 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; Human Toxome Project; ECVAM; UK NC3Rs; US Tox21 Programme; the ToxCast programme; the Human Toxicology Project Consortium; 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. These included the 2016 EUSAAT-Linz conference and the 2017 Society of Toxicology annual conference. There were a total of 2,488 abstracts from oral and poster presentations from these two conferences, but many were not relevant to the Lush Science prize. We then performed literature searches using PubMed, Google Scholar and Terkko Feed Navigator to identify projects describing recent advances in toxicity testing research. Two further relevant abstracts were identified directly from the ALTEX journal. In all, searches yielded almost 3,500 projects which we assessed as described in Section 3. Relevant abstracts were scored using the system derived in previous years (see section 3 for details) 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 95 abstracts which scored 1 or more: 4 scored 1; 47 scored 2; 9 scored 2 + 1 (i.e. total 3); and 33 scored 3 because they appeared to be reporting a new toxicity pathway, assay, or biomarker. Finally, 2 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 44 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

There are two compelling reasons for ending toxicity testing in animals: it is cruel and it is ineffective[1]. The objectives of 21st Century Toxicity Testing (tt21c) research can be summarised as: improving safety testing of chemicals and contaminants by using more relevant and predictive human models; and simplifying and automating tests so that many more chemicals can be tested for safety. The Science background papers for the 2012, 2013, and 2014 Lush Prizes provide an overview, and links to further resources, describing the concept of 21st Century Toxicology.

The Lush 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. In previous years, the focus of the award has been on research aimed at elucidating key pathways in which perturbation results in toxicity. This approach was continued in 2015, when it was further extended to cover additional aspects of tt21c beyond toxicity pathways. In particular, in 2015, we also sought to identify significant contributions, both to the development of new *in vitro* assays that advance tt21c and to the discovery of biomarkers that signal early activation of toxicity pathways. Here, for the 2017 Science Prize paper, we have repeated this approach to identify projects that are candidates for the 2017 Lush Science Prize.

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

Science Prize

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

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

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

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

1 http://www.lushprize.org/background/animal-testing/

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 select potential nominees for the 2017 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 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. June 2016 – June 2017).

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; Human Toxome Project; ECVAM; UK NC3Rs; US Tox21 Programme; the ToxCast programme; the Human Toxicology Project Consortium; 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 2016 – 2017, for which abstracts were available, were: the 17th annual congress of EUSAAT (the European Society for Alternatives to Animal Testing), held in August 2016 in Linz; and The Society of Toxicology 56th Annual Meeting held in March 2017, in Baltimore, Maryland. Of the 2,226 abstracts presented at the SoT meeting, only those indexed under relevant headings in the Keyword Index were considered. The relevant Keywords from the the SoT abstract book were: Alternatives to Animal Testing; Biomarkers; and *In Vitro* and Alternatives. Other conferences, for which the abstract books could not be obtained, included: the International Congress of Toxicology (Mexico, October 2016); the Euro Soc Tox in vitro (France, October 2016); and the 52nd EuroTox meeting (Spain, September 2016). Work presented at these conferences could not be considered for this paper.

Thirdly, we conducted a review of the recent literature. For this we used four separate sources. Firstly, we searched PubMed for research published from 01/06/2016 to 26/06/2017, 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 2016 to 2017, 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. As a third literature source, we searched Terkko Feed Navigator, combining search terms "toxicity pathway," "toxicity assay" and "toxicity biomarker", and once again eliminating already identified studies. 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 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. In a change from previous years, 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

Tox21 is a collaborative effort among the National Institutes of Health (NIH), the Environmental Protection Agency (EPA), and the Food and Drug Administration (FDA). NIH partners include NCATS (National Center for Advancing Translational Sciences) and the National Toxicology Program (NTP), administered by the National Institute of Environmental Health Sciences. The Tox21 initiative is designed to improve current toxicity assessment methods, which are slow and costly, by utilising robotic systems and high-throughput technologies to screen thousands of compounds.

A Society of Toxicology conference abstract (no. 1589, Ramaiahgari *et al*. High-dimensional toxicological assessment of chemical exposure on physiologically-relevant 3D *in vitro* models) from the Tox21 project was awarded a score of 2 + 1 for demonstrating the utility of 3D cultured HepaRG cells as a model for hepatotoxicity, and for the use of a Tox21 "sentinel" gene panel in a HTS assay. A complementary abstract (no. 1590, Ferguson *et al.*) used transcriptomics to further demonstrate the importance of 3D culture systems in modelling human liver function with cultured cell lines.

4.2 ToxCast

The US EPA's ToxCast (Toxicity Forecaster) programme aims to use high-throughput screening tools to test many thousands of chemicals in *in vitro* assays. ToxCast is now in its Phase III, and is screening a diverse library of more than 3800 chemicals, 96% of which are also undergoing screening in the Tox21 project. Most projects focus on using HTS data to build biologically-based models for predicting *in vivo* toxicity endpoints. ToxCast is also working to develop its context-focused database of descriptive ToxCast assay annotations to comply with OECD guidance for the use of novel *in vitro* test methods. No new data sets have been made available via the ToxCast website in the last year.

4.3 Other US Institutions

The National Toxicology Programme's ICCVAM has outlined a roadmap for the replacement of animals for the six most commonly used acute toxicity tests: ocular and dermal irritation; dermal sensitization; and acute oral, dermal, and inhalation toxicity. ICCVAM is also seeking input for developing a strategy to implement human-based predictive approaches for complex toxicological endpoints such as developmental toxicity and carcinogenicity.

The NTP's Interagency Center for the Evaluation of Alternative Toxicological Methods and the PETA International Science Consortium (PISC) organized an expert meeting on "Alternative Approaches for Acute Inhalation Toxicity Testing to Address Global Regulatory and Non-regulatory Data Requirements". Participants in the meeting, held September 22-23, 2016, in Bethesda, MD, identified four key activities needed to reduce and replace animal use for required inhalation toxicity testing:

- Establishing a database of existing toxicity data
- Preparing a state-of-the-science review on how chemicals cause inhalation toxicity, and identifying the non-animal methods that are available to identify such hazards
- Developing computer models to help identify testing needs
- Designing a proof-of-concept study for a non-animal testing approach

4.4 EU

REACH registrants should use alternative test methods for skin sensitization

The amended REACH annexes concerning skin sensitization came into force in autumn 2016. The information needed for the classification or risk assessment of a substance must now be obtained through non-animal methods as a first step. *In vivo* methods can only be used if the *in chemico* or *in vitro* test methods are not adequate for the substance or cannot be used for classification and risk assessment.

With the amended requirements, if a substance is predicted to be a skin sensitizer based on the available data, skin sensitization potency should also be assessed. There is currently no standardized way to assess potency with the *in vitro* methods and therefore the *in vivo* test may still be necessary.

However, estimating potency is not necessary if an existing *in vivo* study does not allow potency estimation and the study has been performed according to internationally-adopted test methods and good laboratory practice.

EURL-ECVAM

The EU Commission held a 2-day scientific conference on non-animal alternatives, in Brussels, in December 2016 – see here for agenda. This was in part to report results of EURL-ECVAM's public survey launched in spring 2016. A conference report does not seem to be available.

4.5 EU-ToxRisk

See http://www.eu-toxrisk.eu/

From the EU-ToxRisk website: The vision of EU-ToxRisk is to drive the required paradigm shift in toxicological testing away from 'black box' animal testing towards a toxicological assessment based on human cell responses and a comprehensive mechanistic understanding of cause-consequence relationships of chemical adverse effects. EU-ToxRisk will integrate advancements 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 consortium will provide proof-of-concept for such a mechanism-based chemical safety testing strategy. The focus of this project is on two areas: repeated dose systemic toxicity, using the lung, kidney, liver and nervous system as examples of potential target organs; and developmental and reproductive toxicity. It will also provide guidance for its universal application, allowing to push the entire field forward in an integrated manner. The ultimate goal is to deliver testing strategies to enable reliable, animal-free hazard- and risk- assessment of chemicals.

EU-ToxRisk published a summary of its first year in the ALTEX Journal (vol 34, issue 1, 2017). Key points were:

- Work in the first year has resulted in the setup of a methods database, a definition of data format files and their link to the methods database, and in the setup of an internal validation group for cell-based assays.
- The consortium started the main objective: the case studies.
- The global interaction with interested stakeholders is demonstrated by the project's activities during 2016. In a series of workshops and training modules, the consortium involved relevant external scientists and entities. These included EURL-ECVAM representatives as trainers during the first summer school, renowned international scientists from academia as speakers during the Good Read-Across Practice forum in Brussels, and OECD representatives as instructors during the EU-ToxRisk AOP workshop in Vienna. A collaboration with the US Tox21 consortium was initiated ,and the first workshop meeting took place in September in Mainz, to ensure alignment of research activities and collaboration on specific case studies and technologies.
- The successful outreach and visibility of the project in this first year is underlined by over 15 publications, as well as key-notes, satellites and symposia at relevant scientific meetings.

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

Animal-free Test Guidelines published by the OECD since the 2016 Lush Science Prize research paper was prepared include:

- TG 442E: *In Vitro* Skin Sensitisation *(hCLAT, published 29 July 2016)*

- TG 458: Stably Transfected Human Androgen Receptor Transcriptional Activation Assay for Detection of Androgenic Agonist and Antagonist Activity of Chemicals.

OECD releases Guidance Document on IATA

In December 2016 the OECD released a "Guidance Document for the Use of Adverse Outcome Pathways in Developing Integrated Approaches for Testing and Assessment (IATA)". This guidance document is intended to:

- Provide a framework for developing and using IATA
- Describe how IATA can be based on the adverse outcome pathway concept
- Provide examples on how AOPs can be used in the development of IATA

IATA provide a means for combining the data from different testing methods, with the objective of predicting chemical toxicity more accurately than with any single test method.

OECD Series on AOPs

In summer 2016 the OECD published a User's Handbook to support the Guidance Document for developing and assessing AOPs, along with several new AOPs, including:

- Protein alkylation leading to liver fibrosis
- Alkylation of DNA in male pre-meiotic germ cells leading to heritable mutations
- Chronic binding of antagonist to NMDA receptors during brain development inducing impairment of learning and memory abilities
- Excitotoxicity via agonist binding to ionotropic glutamate receptors in adult brain that mediates cell death, contributing to learning and memory impairment

5. Literature Highlights

Some of the work that we reviewed to identify 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.

In January 2017 a report of the Society of Toxicology's FutureTox III workshop was published[2]. The workshop was held in November 2015 and was attended by more than 300 scientists from government research and regulatory agencies, research institutes, academia, and the chemical and pharmaceutical industries in Europe, Canada, and the US. Participants reviewed and discussed the state of the science in toxicology and human risk and exposure assessment with a focus on moving tt21c science into the arena of regulatory decision-making. Great optimism was expressed that tt21c results can be used to help inform regulatory decision-making; the report describes two case studies that demonstrate this. The first was the EPA's Endocrine Disruptor Screening Programme, and the second was the FDA-endorsed Comprehensive *In Vitro* Pro-arrhythmia Assay for testing candidate drugs for the potential to cause adverse cardiac events. Contributors to the workshop also expressed the opinion that tt21c is emerging beyond the 'tipping point' whereby its concepts become the norm, and that there is no going back to the old way of performing toxicity testing.

This optimism is in some contrast to the ambivalence to alternative testing strategies (ATS) exposed by a comprehensive survey on attitudes held by toxicologists to such strategies, undertaken by Patrick Allard's group at UCLA[3]. Allard's survey asked participants about their opinions on the viability and acceptance of ATS, and their perceptions of the barriers and drivers for the adoption of ATS for a range of applications. Nearly 1400 respondents, from government, industry, and academia, in North America and Europe, provided opinions, and data were subjected to ranking, hierarchical clustering, and correlation analyses. While ATS were thought to be viable for some applications such as screening or prioritising chemicals (70% and 86% acceptance, respectively), their acceptance for quantitative risk assessments were lower (QSARs 43%, and HTS assays 25%). A majority of respondents thought that ATS were viable for the comparative evaluation of alternative chemicals (53% to 69%, depending on the ATS approach). Other results suggest that many toxicologists do not see ATS as viable, or even potentially viable, for quantitative risk assessment – 25% of respondents viewed QSARs as unfeasible for quantitive risk assessment, while a third thought this was true of HTS assays. The principal technical barriers to adoption of ATS were: concerns about interpretation and extrapolation of data; the difficulty in developing dose-response relationships; and concerns with false positive and false negative results.

2 Juberg *et al*, FutureToxIII: Bridges for Translation. Toxicol Sci. 2017;155(1):22-31. doi: 10.1093/toxsci/kfw194

3 Zaunbrecher *et al*, Has Toxicity Testing moved into the 21st Century? A Survey and Analysis of Perceptions in the Field of Toxicology. Environ Health Perspect. 2017 Under review.

There was a wide range of other concerns. By far the most significant social/legal/institutional barrier to adoption of ATS was concern about regulatory acceptance of ATS data in submissions and decision-making. Concerns about the validation process of ATS were also common, including the complexity and pace of validation, and the availability of validated methods. The respondents' sector of employment explained the highest proportion of differences in perception of barriers to acceptance, with all sectors perceiving fewer barriers when compared with national governments. By contrast, the top drivers (expedited information, reduced costs, and regulatory demand) were nearly universal.

Two SoT 2017 meeting abstracts addressed issues about the acceptance of ATS for skin sensitisation. Firstly, Jacobsen *et al*[4] highlights that some *in vitro* methods "miss" potential skin sensitisers, and that there is potential for discordance between "old methods" and "new methods". In contrast, Kleinstreuer *et al*[5] describe a workshop of the International Co-operation on Alternative Test Methods (ICATM), held in October 2016, on acceptance of non-animal test methods for assessing skin sensitisation potential. An outcome of the workshop was the development of an assessment framework for integrated non-animal approaches that could serve as replacements for the LLNA: "Multiple non-animal testing strategies incorporating *in vitro*, *in chemico*, and *in silico* inputs demonstrated comparable or superior performance to the LLNA, fulfilled the criteria in the ICATM assessment framework, and should be considered as acceptable replacements for regulatory purposes." These contrasting reports raise the question of whether society is willing to risk some chemicals being found to be hazardous after non-animal testing. One very obvious response is that some chemicals are found to be hazardous when they have been tested on animals. However, for those seeking to promote non-animal testing of chemicals, this does raise the possibility of a potential future public acceptance barrier, in the event of a high profile safety issue with a chemical that has been approved solely following non-animal testing.

The global antibody industry is valued at $80 billion[6]. This industry uses millions of unaccounted-for animals, thereby raising important animal welfare implications, despite a wide range of technological alternatives. Gray and her co-authors made a further call for regulators to publish statistics on the number of animals used for antibody production and, ultimately, to end the production and importation of antibodies within and into the EU.

4 Jacobson *et al,* Discordance between *In Vitro* and *In Vivo* Skin Classifications for 2-Ethylhexyl Acrylate. Toxicol. Sci. 'The Toxicologist' Suppl:2017;156(1) Abstr. 2736

5 Kleinstruer *et al,* Evaluating Defined Approaches to Testing and Assessment of Skin Sensitisation Potential. Toxicol. Sci. 'The Toxicologist' Suppl:2017;156(1) Abstr. 3160

6 Gray *et al,* Animal Friendly Affinity Reagents (AFAs): making animal immunisation obsolete. ALTEX Proc:5(1):EUSAAT 2016:Abstr 205, p69

We are pleased to note that a project that was recommended as a potential nominee for the 2016 Lush Science Prize[7], describing an *in vitro* assay for the determination of botulinum neurotoxin potencies, has been recognised with the award of the Ursula M Händel Animal Protection Prize. Drs Birgit Kegel and Beate Krämer from the Paul Ehrlich Institut in Langen, together with four further members of their team, were awarded the €100,000 prize from the *Deutsche Forschungsgemeinschaft.* Additional work, describing the further development of these assays, has been identified this year as a potential nominee for the Lush Science Prize (see Behrensdorf-Nicol *et al*. 'The "BINACLE" (binding and cleavage) assay allows *in vitro* determination of botulinum and tetanus neurontoxin activity.' in Section 6.4 Nominated Abstracts of this document).

Two papers from Paul Anastas' group at Yale, describing approaches to the design of safer chemicals[8,9], highlight an interesting benefit of the elucidation of AOPs for toxicity, and the development of *in vitro* assays to measure the activation of key events in an AOP. Their work builds a predictive model for cytotoxicity, based on ToxCast data, that provides probabilistic design rules to help synthetic chemists minimize the chance that a newly synthesised chemical will be cytotoxic. They then tested the model to distinguish between NRF2 -active and -inactive chemicals, based on ToxCast HTS assay data, and showed excellent predictive power to distinguish active and inactive chemicals. *De novo* design of chemicals that are likely to be nontoxic reduces the likelihood that such chemicals would require animal testing during a safety assessment.

Finally, a noteworthy t4 (Transatlantic Think Tank for Toxicology) workshop report on microphysiological systems (MPS, aka organs-on-a-chip & humans-on-a-chip) for substance testing was published in ALTEX[10] in 2016. As well as providing a comprehensive overview of the different approaches and technologies for MPS, the workshop also presented a roadmap for the impact of adoption of MPS for both drug discovery and hazard assessment over the next 20 years. The roadmap envisages the first validated regulatory safety assays using MPS during the 2020s. The longer-term vision is of 'organisms-on-a-chip' for chemical and candidate drug testing, personalised medicine ('you-on-a-chip'), and on-chip modelling of clinical trials, obviating the need for any pre-clinical animal testing. The roadmap suggests that these developments will begin to appear in the late 2020s and into the 2030s.

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7 Wild *et al*. *In vitro* Assays for the Potency Determination of Botulinum Neurotoxin serotypes A and B. ALTEX Proc:4(2):EUSAAT 2015:Abstr 17, p256

8 Shen *et al*. Probabilistic diagram for designing chemicals with reduced potency to incur cytotoxicity. Green Chem:18:2016:4461-4467 DOI: 10.1039/C6GC01058J

9 Shen *et al*. Coupled molecular design diagrams to guide safer chemical design with reduced liklihood of perturbing the NRF2-ARE antioxidant pathway and inducing cytotoxicity. Green Chem:18:2016:6387-6394 doi.org/10.1039/C6GC02073A

10 Marx *et al*. Biology-Inspired Microphysiological System Approaches to Solve the Prediction Dilemma of Substance Testing. ALTEX 33(3):2016:272-321.

6. Toxicity Pathway Abstracts

6.1 Conference Abstract Selection

As described in the Methodology, we reviewed abstracts from the 17th annual congress of EUSAAT (the European Society for Alternatives to Animal Testing), held in August 2016 in Linz; and The Society of Toxicology 56th Annual Meeting, held in March 2017 in Baltimore, Maryland.

From the 262 abstracts which comprised the EUSAAT 2016 conference presentation and poster proceedings, we identified 14 abstracts which scored 1 or more.

From the total of 2226 abstracts presented at the Society of Toxicology's 2017 meeting and identified as potentially relevant based on the Abstract book keyword index: 69 were identified by the keyword "biomarker", of which 2 were scored; 105 were identified by the keywords "*In vitro* and alternatives", of which 15 were scored; and 125 were identified by the keywords "alternatives to animal testing," of which 14 were scored. There were 18 abstracts which we identified by more than one keyword search, so duplicates were eliminated. Thus a total of 31 abstracts scored 1 or more.

6.2 Published Abstract Selection

From the PubMed search: we identified 2396 relevant titles from the "Toxicity assay" search; a further 316 relevant projects from the "Toxicity biomarker" search; and finally an additional 242 titles from the "Toxicity pathway" search: a combined total of 2954 articles.

Stages 1 and 2 of the selection process (review of titles 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 research or other disease) reduced these 2954 titles by around 70%. Of these, after review of abstracts in stage 3, only 46 abstracts scored 1 or more (33 from the Toxicity assay search, 8 from the Toxicity biomarker search, and 5 from the Toxicity pathway search).

The Google Scholar search for the period 2016 to 2017 identified 481 possibly relevant abstracts additional to those identified by PubMed described above. Of these: 439 were identified by the Toxicity assay search; a further 31 from the Toxicity biomarker search; and 11 from the Toxicity pathway search. Of the 481, only 21 survived selection stages 1 and 2 (16 from the Toxicity assay search and 5 from the Toxicity biomarker search; none from the pathway search) and were passed on to the scoring stage. Of these 21, only a final 2 scored 1 or more.

As in the previous year, the Terkko FeedNavigator search yielded no further relevant papers beyond those already identified by the other literature searches.

We also identified two further relevant projects, directly from the ALTEX Journal, which scored 1 or more.

6.3 Scores

From the three separate sources of potential shortlisted projects, we identified a total of 95 abstracts describing work which scored at least one point according to our given criteria. Of these, 4 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"); 47 scored 2 for bringing new knowledge or tools to a previously identified pathway, assay, or biomarker of toxicity; 9 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 33 scored 3 because they appeared to be reporting a new toxicity pathway, assay, or biomarker. Only 2 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 (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, ALTEX, SoT and EUSAAT. All of the 44 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 identified in the PubMed searches, the abstract title in the Table is a hyperlink to the PubMed index for that paper.

Title	Authors	Category	Source	Score
Integrative analysis of mRNA and microRNA expression of a human alveolar epithelial cell(A549) exposed to water and organic-soluble extract from particulate matter (PM)2.5.	Jeong SC et al	Biomarker	PubMed	3
para-Phenylenediamine induces apoptosis through activation of reactive oxygen species-mediated mitochondrial pathway, and inhibition of the NF-κB, mTOR, and Wnt pathways in human urothelial cells.	Reena K et al	Pathway	PubMed	3
Multiparametric luminescent cell viability assay in toxicology models: A critical evaluation.	Sali N et al	Assay	PubMed	2
Discrimination of skin sensitizers from non-sensitizers by interleukin-1α and interleukin-6 production on cultured human keratinocytes.	Jung D et al	Assay	PubMed	2
Inter-laboratory study of human in vitro toxicogenomics-based tests as alternative methods for evaluating chemical carcinogenicity: a bioinformatics perspective	Herwig R et al	Assay	PubMed	3
Development of novel in vitro photosafety assays focused on the Keap1-Nrf2-ARE pathway.	Tsujita-Inoue K et al	Assay	PubMed	2
Complementarity of phosphorylated histones H2AX and H3 quantification in different cell lines for genotoxicity screening.	Khoury L et al	Assay	PubMed	3
An in vitro human skin test for assessing sensitization potential	Ahmed SS et al	Assay	PubMed	2
High content analysis assay for prediction of human hepatotoxicity in HepaRG and HepG2 cells.	Saito J et al	Assay	PubMed	2

Title	Authors	Category	Source	Score
Computational Models for Human and Animal Hepatotoxicity with a Global Application Scope.	Mulliner D et al	Assay	PubMed	2
Assessment of cosmetic ingredients in the in vitro reconstructed human epidermis test method EpiSkin™ using HPLC/UPLC-spectrophotometry in the MTT-reduction assay.	Alépée N	Assay	PubMed	2
Low cost quantitative digital imaging as an alternative to qualitative in vivo bioassays for analysis of active aflatoxin B1.	Rasooly R et al	Assay	PubMed	2 + 1
Integrated decision strategies for skin sensitization hazard.	Strickland J et al	Assay	PubMed	3
MicroRNAs as early toxicity signatures of doxorubicin in human-induced pluripotent stem cell-derived cardiomyocytes.	Chaudhary U et al	Biomarker	PubMed	3
Metabolomic network analysis of estrogen-stimulated MCF-7 cells: a comparison of overrepresentation analysis, quantitative enrichment analysis and pathway analysis versus metabolite network analysis.	Maertens A et al	Pathway	PubMed	3
Prototype Systems Containing Human Cytochrome P450 for High-Throughput Real-Time Detection of DNA Damage by Compounds That Form DNA-Reactive Metabolites.	Brito-Palma B et al	Assay	PubMed	3
Systematically evaluating read-across prediction and performance using a local validity approach characterized by chemical structure and bioactivity information.	Shah I et al	Assay	PubMed	2
The Vitotox and ToxTracker assays: A two-test combination for quick and reliable assessment of genotoxic hazards	Ates G et al	Assay	PubMed	2

Title	Authors	Category	Source	Score
A novel method to generate monocyte-derived dendritic cells during coculture with HaCaT facilitates detection of weak contact allergens in cosmetics.	Frombach J et al	Assay	PubMed	3
High-content imaging-based BAC-GFP toxicity pathway reporters to assess chemical adversity liabilities.	Wink S et al	Assay	PubMed	3
ROS mediated crosstalk between endoplasmic reticulum and mitochondria by Phloxine B under environmental UV irradiation.	Goyal S et al	Pathway	PubMed	2
Improving drug safety with a systems pharmacology approach.	Schotland P et al	Assay	PubMed	3
Estimation of bisphenol A-Human toxicity by 3D cell culture arrays, high throughput alternatives to animal tests.	Lee DW et al	Assay	PubMed	1
Design of a high-throughput human neural crest cell migration assay to indicate potential developmental toxicants	Nyffeler J et al	Assay	PubMed	3 + 1
Evaluation of fibrin-based dermal-epidermal organotypic cultures for in vitro skin corrosion and irritation testing of chemicals according to OECD TG 431 and 439.	Morales M et al	Assay	PubMed	2
From genome-wide arrays to tailor-made biomarker readout - Progress towards routine analysis of skin sensitizing chemicals with GARD.	Forreryd A et al	Assay	PubMed	2
Identification of time-dependent biomarkers and effects of exposure to volatile organic compounds using high-throughput analysis.	Hong JY et al	Biomarker	PubMed	2

Title	Authors	Category	Source	Score
Neutrophil gelatinase-associated lipocalin production negatively correlates with HK-2 cell impairment: Evaluation of NGAL as a marker of toxicity in HK-2 cells.	Hauschke M et al	Biomarker	PubMed	3
Prevalidation trial for a novel in vitro eye irritation test using the reconstructed human cornea-like epithelial model, MCTT HCE™.	Yang H et al	Assay	PubMed	3
Editor's Highlight: Development of an In vitro Assay Measuring Uterine-Specific Estrogenic Responses for Use in Chemical Safety Assessment.	Miller MM et al	Assay	PubMed	3
Evaluation of in vitro assays for the assessment of the skin sensitization hazard of functional polysiloxanes and silanes.	Petry T et al	Assay	PubMed	2
Stem Cell Transcriptome Responses and Corresponding Biomarkers That Indicate the Transition from Adaptive Responses to Cytotoxicity.	Waldmann T et al	Biomarker	PubMed	3
Development of an in vitro method to estimate the sensitization induction level of contact allergens.	Galbiati V et al	Assay	PubMed	2
Comparative study of human neuronal and glial cell sensitivity for in vitro neurogenotoxicity testing.	Laffon B et al	Assay	PubMed	2
A chemical-biological similarity-based grouping of complex substances as a prototype approach for evaluating chemical alternatives.	Grimm FA et al	Assay	PubMed	3
Human BJ Fibroblasts is an Alternative to Mouse BALB/c 3T3 Cells in In Vitro Neutral Red Uptake Assay.	Mannerström M et al	Assay	PubMed	1

Title	Authors	Category	Source	Score
Copper oxide nanoparticle toxicity profiling using untargeted metabolomics.	Boyles MS et al	Biomarker	PubMed	2 + 1
Predictive performance of the Vitrigel-eye irritancy test method using 118 chemicals.	Yamaguchi H et al	Assay	PubMed	3
Can currently available non-animal methods detect pre and pro-haptens relevant for skin sensitization?	Patlewicz G et al	Assay	PubMed	2
Comprehensive Landscape of Nrf2 and p53 Pathway Activation Dynamics by Oxidative Stress and DNA Damage.	Hiemstra S et al	Pathway	PubMed	3
Functional Toxicogenomic Assessment of Triclosan in Human HepG2 Cells Using Genome-Wide CRISPR-Cas9 Screening.	Xia P et al	Assay	PubMed	3
A combined proteomics and metabolomics approach to assess the effects of gold nanoparticles in vitro.	Gloria S et al	Assay	PubMed	2
Protein-based nanotoxicology assessment strategy.	Elnegaard MP et al	Assay	PubMed	2
Modeling Exposure in the Tox21 in Vitro Bioassays.	Fischer FC et al	Assay	PubMed	2
In vitro assessment of skin irritation potential of surfactant-based formulations by using a 3-D skin reconstructed tissue model and cytokine response.	Walters RM et al	Assay	PubMed	2
Long-term exposure of A549 cells to titanium dioxide nanoparticles induces DNA damage and sensitizes cells towards genotoxic agents.	Armand L et al	Assay	PubMed	2
Adenosine diphosphate involvement in THP-1 maturation triggered by the contact allergen 1-fluoro-2, 4-dinitrobenzene	Martins JD et al.	Pathway	Google scholar	2

Title	Authors	Category	Source	Score
Contact dermatitis: in pursuit of sensitizer's molecular targets through proteomics	Guedes S et al	Pathway	Google scholar	2
Is the Time Right for In Vitro Neurotoxicity Testing Using Human iPSC-Derived Neurons?	Tukker AM et al	Assay	ALTEX	2
Keratinocytes improve prediction of sensitization potential and potency of chemicals with THP-1 cells	Hennen J & Blömeke B	Assay	ALTEX	2
An In Vitro Testicular Cells Co-Culture Model for Assessing Testicular Toxicities of Bisphenol A and Its Analogues Using High-Content Analysis	Liang S et al	Assay	SoT 2017 abstract 1190	1
Do Some Autism Spectral Disorder Patients Have Inefficient Mitochondria or Poor Responses to DNA Damaging Agents?	Mansfield C et al	Biomarker	SoT 2017 abstract 1239	3
Kinetic Assessment of Acute Toxicological Risk with a Bioluminescent Annexin V Reagent	Niles A et al	Assay	SoT 2017 abstract 1437	2
Discovery of Toxic Metabolites by High-Content Imaging in the SciFlow 1000 96-Well Microfluidic Culture System	Sloan D et al	Assay	SoT 2017 abstract 1440	2+1
A Multidimensional High-Throughput Approach for Toxicity Evaluation of Environmental Chemicals in Induced Pluripotent Stem Cell- Derived Endothelial Cells	Iwata Y et al	Assay	SoT 2017 abstract 1457	3
Investigating Drug-Induced Cardiomyocyte Dysfunction through Combined Analysis of Beating, Metabolic Flux, and Cellular Oxygenation	Carey C et al	Assay	SoT 2017 abstract 1458	2+1
Toxicological Response of Healthy and Asthmatic 3D In Vitro Airway Model to CuO Nanoparticles at the Air-Liquid Interface	Kooter IM et al	Assay	SoT 2017 abstract 1514	2+1

Title	Authors	Category	Source	Score
Amorphous Silica Coating Potentially Protects against Iron Homeostasis Disruption Induced by Nano-Iron Oxide in Acute and Sub-Chronic In Vitro Exposure Models	Kornberg T et al	Assay	SoT 2017 abstract 1521	2
Assessment of the Phototoxicity of Three Different TiO2 Nano-Forms Using Reconstructed Human Tissue Model Epiderm	Liskova A et al	Assay	SoT 2017 abstract 1526	3
Mechanism-Based Genotoxicity Screening of Nanomaterials Using the Toxtracker Panel of Reporter Cell Lines	Hendriks G et al.	Assay	SoT 2017 abstract 1532	2
Genotoxicity Evaluation of Nanomaterials: What Is the Most Suitable Option of In Vitro Cytokinesis-Block Micronucleus Cytome Assay?	Brand F et al	Assay	SoT 2017 abstract 1540	3
Toxicokinetic Study on Retinoic Acid in a Multi- Organ Chip Comprising Epiderm Skin Models and Liver Organoids	Maschmeyer I et al	Assay	SoT 2017 abstract 1588	2 + 1
High-Dimensional Toxicological Assessment of Chemical Exposure on Physiologically Relevant 3D In Vitro Models	Ramaiahgari SC et al	Assay	SoT 2017 abstract 1589	2 + 1
Modification of the ICH Accepted Dye-Based Reactive Oxygen Species Assay Improves Photoreactivity Assessment	Hu M et al	Assay	SoT 2017 abstract 1674	2
Development of a Targeted Mass Spectrometry Protein Assay to Identify Early Stages of Pulmonary Response to Carbon Nanotube Exposure in a 3D Lung Model	Hilton GM et al	Assay	SoT 2017 abstract 2001	2
Gene Expression Analysis to Elucidate the Cross- Talk between Antigen-Presenting Cells and Epithelial Cells In Vitro	Schellenberger MT et al	Pathway	SoT 2017 abstract 2188	2

Title	Authors	Category	Source	Score
Utility of the GADD45a Genotoxicity Screening Assays–A Review of 10 Years of Published Data	Tate M et al	Assay	SoT 2017 abstract 2243	2
In Vitro Micronucleus and CometChip With Metabolically Competent HepaRG Cells	Swartz C et al	Assay	SoT 2017 abstract 2267	1
Optimization of Direct Double-Strand Break Labeling Assay for Genotoxicity Assessment	Dunnick KM et al	Assay	SoT 2017 abstract 2270	3
Dose-Dependent Increase of Triglyceride Levels in Human HepaRG Liver Cells Exposed to the Fungicide Cyproconazole	Peijnenburg A et al	Pathway	SoT 2017 abstract 2532	3
High-Throughput Screening for Acute and Developmental Neurotoxicity Using Human Embryonic Stem Cell-Derived Neuronal Cells	Oh J-H et al	Assay	SoT 2017 abstract 2639	3
Development of Risk Assessment Models for Skin Sensitization Based on an Artificial Neural Network Analysis of Human Cell Line Activation Test (h-CLAT), Direct Peptide Reactivity Assay (DPRA), KeratinoSens, and In Silico Parameter	Hirota M et al	Pathway	SoT 2017 abstract 2735	2
Skin Sensitization Testing Strategy and In-House Fit-for-Purpose Validations at Charles River Laboratories	Roper CS et al	Pathway	SoT 2017 abstract 2737	2
Assessment Strategy of Skin Sensitization for Botanical Extracts Using In Vitro Test Methods and Acceptable Exposure Level	Nishijo T et al	Pathway	SoT 2017 abstract 2741	2
In Vitro Testing Strategy for Assessing the Skin- Sensitizing Potential of Cosmetic Ingredients with “Particular Physicochemical Properties”	Bergal M et al	Pathway	SoT 2017 abstract 2743	2

Title	Authors	Category	Source	Score
In Vitro Skin Sensitization: Adapted Prediction Model of the U937 Cell Line Activation-Validated (U-SENS) Test Method	Piroird C et al	Assay	SoT 2017 abstract 2744	3
Comparative Study of Ocular Irritancy between SIRC and HCE-T Using Short Time Exposure Test (STE) and the Effect of Polyol on Corneal Cells	Park S et al	Assay	SoT 2017 abstract 2751	3
Protocol Modification for Highly Volatile Substances in the Short Time Exposure (STE) Test	Abo T et al	Assay	SoT 2017 abstract 2755	2
A Mechanistic Approach Using Adverse Outcome Pathways (AOPs) to Aid Design of In Vitro Inhalation Testing	Clippinger AJ et al	Pathway	SoT 2017 abstract 2826	3
Human Mesenchymal Stem Cell Cultures to Model Teratogen-Induced Limb Malformations	Alexander PG et al	Assay	SoT 2017 abstract 3150	3
Diversity in a Dish: A Population-Based Organotypic Human In Vitro Model for cardiotoxicity testing	Grimm FA et al	Pathway	SoT 2017 abstract 3223	2
The use of human (non-3D equivalent) skin assays (Skimune™) for the detection of adverse reactions, potency and efficacy	Ribeiro A et a l	Assay	EUSAAT, Linz, 2016 abstract 8	2 + 1
A new validated in vitro skin sensitization test: SENS-IS	Brée F et al	Assay	EUSAAT, Linz, 2016 abstract 49	2 + 1
The “BINACLE” (binding and cleavage) assay allows in vitro determination of botulinum and tetanus neurotoxin activity	Behrensdorf-Nicol H et al	Assay	EUSAAT, Linz, 2016 abstract 102	3 + 1

Title	Authors	Category	Source	Score
LUCS (Light-Up Cell System), a novel live cell assay for acute and other regulatory toxicity applications	Derick S et al	Assay	EUSAAT, Linz, 2016 abstract 185	2
Complex skin models and impedance spectroscopy as new tools for hazard identification and efficacy testing	Groeber F et al	Assay	EUSAAT, Linz, 2016 abstract 21	2
GARD - the future of sensitization testing using a genomics-based platform	Johansson H et al	Assay	EUSAAT, Linz, 2016 abstract 91	3
CON4EI: EpiOcular Eye Irritation Test (EIT)	Kandarova H et al	Assay	EUSAAT, Linz, 2016 abstract 127	3
Use of ECVAM validated epiderm skin corrosion test (EpiDerm SCT) for sub-categorization according to the UN-GHS	Letasiova S et al	Assay	EUSAAT, Linz, 2016 abstract 136	2
Development of an in vitro inhalation toxicity test with potential regulatory applicability	Maione A et al	Assay	EUSAAT, Linz, 2016 abstract 199	3
ITS for skin sensitization potential – 1 out of 2 or 2 out of 3?	Roberts D & Patlewicz G	Assay	EUSAAT, Linz, 2016 Abstract 250	2
Automation-compatible EST assay	Ströbel S	Assay	EUSAAT, Linz, 2016 Abstract 165	2

Title	Authors	Category	Source	Score
Non-animal testing for skin sensitization: Replacement or mere supplement?	Urbisch D et al	Assay	EUSAAT, Linz, 2016 Abstract 38	2
Assessment of pre- and pro-haptens using non-animal test methods for skin sensitization	Wareing B et al	Assay	EUSAAT, Linz, 2016 abstract 35	2
3D networks of iPSC-derived neurons and glia for high-throughput neurotoxicity screening	Wevers N et al	Assay	EUSAAT, Linz, 2016 abstract 246	2

6.4 Recommended Abstracts

This year 44 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 44 abstracts are given below. For abstracts from published work, we provide the digital object identifier (DOI) which allows the original document to be located online. For conference abstracts, we give the abstract or poster number for identification.

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

Integrative analysis of mRNA and microRNA expression of a human alveolar epithelial cell(A549) exposed to water and organic-soluble extract from particulate matter (PM) 2.5.

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Environ Toxicol. 2017 Jan;32(1):302-310. doi: 10.1002/tox.22236. Epub 2016 Jan 20.

Score 3 (Potential biomarkers for respiratory toxins)

Abstract

MicroRNA (miRNA) is now attracting attention as a powerful negative regulator of messenger RNA(mRNA) levels, and is implicated in the modulation of important mRNA networks involved in toxicity. In this study, we assessed the effects of particulate matter 2.5 (PM2.5), one of the most significant air pollutants, on miRNA and target gene expression. We exposed human alveolar epithelial cell (A549) to two types of PM2.5 [water (W-PM2.5) and organic (O-PM2.5) soluble extracts] and performed miRNA microarray analysis. A total of 37 miRNAs and 62 miRNAs were altered 1.3-fold in W-PM2.5 and O-PM2.5 , respectively. Integrated analyses of miRNA and mRNA expression profiles identified negative correlations between miRNA and mRNA in both W-PM2.5 and O-PM2.5 exposure groups. Gene ontology and Kyoto encyclopedia of genes and genomes (KEGG) pathway analyses showed that the 35 W-PM2.5 target genes are involved in responses to nutrients, positive regulation of biosynthetic processes, positive regulation of nucleobase, nucleoside, and nucleotide, and nucleic acid metabolic processes; while the 69 O-PM2.5 target genes are involved in DNA replication, cell cycle processes, the M phase, and the cell cycle check point. We suggest that these target genes may play important roles in PM2.5 -induced respiratory toxicity by miRNA regulation. These results demonstrate an integrated miRNA-mRNA approach for identifying molecular events induced by environmental pollutants in an in vitro human model.

para-Phenylenediamine induces apoptosis through activation of reactive oxygen species-mediated mitochondrial pathway, and inhibition of the NF-κB, mTOR, and Wnt pathways in human urothelial cells.

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Environ Toxicol. 2017 Jan;32(1):265-277. doi: 10.1002/tox.22233. Epub 2016 Jan 19.

Score 3 (Potential pathway for hair dye component that causes bladder cancer)

Abstract

para-Phenylenediamine (PPD) has long been used in two-thirds of permanent oxidative hair dye formulations. Epidemiological studies and in vivo studies have shown that hair dye is a suspected carcinogen of bladder cancer. However, the toxicity effects of PPD to human bladder remains elusive. In this study, the effects of PPD and its involvement in the apoptosis pathways in human urothelial cells (UROtsa) was investigated. It was demonstrated that PPD decreased cell viability and increased the number of sub-G1 hypodiploid cells in UROtsa cells. Cell death due to apoptosis was detected using Annexin V binding assay. Further analysis showed PPD generated reactive oxygen species (ROS), induced mitochondrial dysfunction through the loss of mitochondrial membrane potential and increased caspase-3 level in UROtsa cells. Western blot analysis of PPD-treated UROtsa cells showed down-regulation of phosphorylated proteins from NF-κB, mTOR, and Wnt pathways. In conclusion, PPD induced apoptosis via activation of ROS-mediated mitochondrial pathway, and possibly through inhibition of NF-κB, mTOR, and Wnt pathways.

Inter-laboratory study of human in vitro toxicogenomics-based tests as alternative methods for evaluating chemical carcinogenicity: a bioinformatics perspective.

R Herwig[1], H Gmuender[2], R Corvi[3], KM Bloch[4], A Brandenburg[2], J Castell[5], L Ceelen[6], C Chesne[7], TY Doktorova[6], D Jennen[8], P Jennings[9], A Limonciel[9], EA Lock[4], T McMorrow[10], P Phrakonkham[3], R Radford[10], C Slattery[10], R Stierum[11], M Vilardell[12], T Wittenberger[2], R Yildirimman[12], M Ryan[10], V Rogiers[6], J Kleinjans[8].

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Arch Toxicol. 2016 Sep;90(9):2215-29. doi: 10.1007/s00204-015-1617-3. Epub 2015 Nov 2.

Score 3 (Integrated genomics/bioinformatics assay for carcinogenicity)

Abstract

The assessment of the carcinogenic potential of chemicals with alternative, human-based in vitro systems has become a major goal of toxicogenomics. The central read-out of these assays is the transcriptome, and while many studies exist that explored the gene expression responses of such systems, reports on robustness and reproducibility, when testing them independently in different laboratories, are still uncommon. Furthermore, there is limited knowledge about variability induced by the data analysis protocols. We have conducted an inter-laboratory study for testing chemical carcinogenicity evaluating two human in vitro assays: hepatoma-derived cells and hTERT-immortalized renal proximal tubule epithelial cells,

representing liver and kidney as major target organs. Cellular systems were initially challenged with thirty compounds, genome-wide gene expression was measured with microarrays, and hazard classifiers were built from this training set. Subsequently, each system was independently established in three different laboratories, and gene expression measurements were conducted using anonymized compounds. Data analysis was performed independently by two separate groups applying different protocols for the assessment of inter-laboratory reproducibility and for the prediction of carcinogenic hazard. As a result, both workflows came to very similar conclusions with respect to (1) identification of experimental outliers, (2) overall assessment of robustness and inter-laboratory reproducibility and (3) re-classification of the unknown compounds to the respective toxicity classes. In summary, the developed bioinformatics workflows deliver accurate measures for inter-laboratory comparison studies, and the study can be used as guidance for validation of future carcinogenicity assays in order to implement testing of human in vitro alternatives to animal testing.

Functional Toxicogenomic Assessment of Triclosan in Human HepG2 Cells Using Genome-Wide CRISPR-Cas9 Screening.

Xia P[1], Zhang X[1], Xie Y[1], Guan M[1], Villeneuve DL[2], Yu H[1].

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Environ Sci Technol. 2016 Oct 4;50(19):10682-10692.
DOI:10.1021/acs.est.6b02328 Epub 2016 Aug 13.

Score 3 (Innovative screening assay for chemical toxicity)

Abstract

There are thousands of chemicals used by humans and detected in the environment for which limited or no toxicological data are available. Rapid and cost-effective approaches for assessing the toxicological properties of chemicals are needed. We used CRISPR-Cas9 functional genomic screening to identify the potential molecular mechanism of a widely used antimicrobial triclosan (TCS) in HepG2 cells. Resistant genes at IC50 (the concentration causing a 50% reduction in cell viability) were significantly enriched in the adherens junction pathway, MAPK signaling pathway, and PPAR signaling pathway, suggesting a potential role in the molecular mechanism of TCS-induced cytotoxicity. Evaluation of the top-ranked resistant genes, FTO (encoding an mRNA demethylase) and MAP2K3 (a MAP kinase kinase family gene), revealed that their loss conferred resistance to TCS. In contrast, sensitive genes at IC10 and IC20 were specifically enriched in pathways involved with immune responses, which was concordant with transcriptomic profiling of TCS at concentrations of <IC10. It is suggested that the CRISPR-Cas9 fingerprint may reveal the patterns of TCS toxicity at low concentration levels. Moreover, we retrieved the potential connection between CRISPR-Cas9 fingerprint and disease terms, obesity, and breast cancer from an existing chemical-gene-disease database. Overall, CRISPR-Cas9 functional genomic screening offers an alternative approach for chemical toxicity testing.

Complementarity of phosphorylated histones H2AX and H3 quantification in different cell lines for genotoxicity screening.

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Arch Toxicol. 2016 Aug;90(8):1983-95. doi: 10.1007/s00204-015-1599-1. Epub 2015 Sep 24.

Score 3 (New assay approach for genotoxicity screening)

Abstract

The in vitro micronucleus assay is broadly used, but is not per se able to discriminate aneugenic from clastogenic compounds, and cytotoxicity can be a confounding factor. In vitro genotoxicity assays generally rely on cell lines with limited metabolic capabilities. Recently, the use of histone H2AX and H3 phosphorylation markers (γH2AX and p-H3) was proposed to discriminate aneugenic from clastogenic chemicals. The aim of the present study was to develop a new genotoxic screening strategy based on the use of the γH2AX and p-H3 biomarkers in combination with cell lines with distinct biotransformation properties. First, we tested a training set of 20 model chemicals comprised of 10 aneugens, five clastogens and five cytotoxics on three human cell lines (HepG2, LS-174T and ACHN). Our data confirm the robustness of these two biomarkers to discriminate efficiently clastogens, aneugens and misleading cytotoxic chemicals in HepG2 cells. Aneugenic compounds induced either an increase or a decrease in p-H3 depending on their mode of action. Clastogens induced γH2AX, and cytotoxic compounds generated a marked decrease in these two biomarkers. Moreover, the use of different cell lines permits to discriminate direct from bioactivated genotoxins without the need of an exogenous metabolic activation system. Finally, we further evaluated this strategy using a test set of 13 chemicals with controversial genotoxic potential. The resulting data demonstrate that the combined analysis of γH2AX and p-H3 is an efficient strategy. Notably, we demonstrated that three compounds (fisetin, hydroquinone and okadaic acid) display both aneugenic and clastogenic properties.

Low cost quantitative digital imaging as an alternative to qualitative in vivo bioassays for analysis of active aflatoxin B1.

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[2] Western Regional Research Center, Foodborne Contaminants Research Unit, Agricultural Research Service, USDA, Albany, CA, United States.

Biosens Bioelectron. 2016 Jun 15;80:405-10. doi: 10.1016/j.bios.2016.01.087. Epub 2016 Feb 4.

Score 2 + 1 (Development of assay for aflatoxin contamination for simplicity & cost reduction which increases scope of use)

Abstract

Aflatoxin B1 (AFB1) producing fungi contaminate food and feed and are a major health concern. To minimize the sources and incidence of AFB1 illness there is a need to develop affordable, sensitive mobile devices for detection of active AFB1. In the present study we used a low cost fluorescence detector and describe two quantitative assays for detection of detoxified and active AFB1 demonstrating that AFB1 concentration can be measured as intensity of fluorescence. When the assay plate containing increasing concentrations of AFB1 is illuminated with a 366 nm ultraviolet lamp, AFB1 molecules absorb photons and emit blue light with peak wavelength of 432 nm. The fluorescence intensity increased in dose dependent manner. However, this method cannot distinguish between active AFB1 which poses a threat to health, and the detoxified AFB1 which exhibits no toxicity. To measure the toxin activity, we used a cell based assay that makes quantification more robust and is capable of detecting multiple samples simultaneously. It is an alternative to the qualitative duckling bioassay which is the "gold-standard" assay currently being used for quantitative analysis of active AFB1. AFB1 was incubated with transduced Vero cells expressing the green fluorescence protein (GFP) gene. After excitation with blue light at 475 nm, cells emitted green light with emission peak at 509 nm. The result shows that AFB1 inhibits protein expression in a concentration dependent manner resulting in proportionately less GFP fluorescence in cells exposed to AFB1. The result also indicates strong positive linear relationship with R(2)=0.90 between the low cost CCD camera and a fluorometer, which costs 100 times more than a CCD camera. This new analytical method for measuring active AFB1 is low in cost and combined with in vitro assay, is quantitative. It also does not require the use of animals and may be useful especially for laboratories in regions with limited resources.

Integrated decision strategies for skin sensitization hazard.

J Strickland[1], Q Zang[1], N Kleinstreuer[1], M Paris[1], DM Lehmann[2], N Choksi[1], J Matheson[3], A Jacobs[4], A Lowit[5], D Allen[1], W Casey[6].

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J Appl Toxicol. 2016 Sep;36(9):1150-62. doi: 10.1002/jat.3281. Epub 2016 Feb 6.

Score 3 (ITS for skin sensitisation – in vitro assays and computational analysis to replace LLNA)

Abstract

One of the top priorities of the Interagency Coordinating Committee for the Validation of Alternative Methods (ICCVAM) is the identification and evaluation of non-animal alternatives for skin sensitization testing. Although skin sensitization is a complex process, the key biological events of the process have been well characterized in an adverse outcome pathway (AOP) proposed by the Organisation for Economic Co-operation and Development (OECD). Accordingly, ICCVAM is working to develop integrated decision strategies based on the AOP using in vitro, in chemico and in silico information. Data were compiled for 120 substances tested in the murine local lymph node assay (LLNA), direct peptide reactivity assay (DPRA), human cell line activation test (h-CLAT) and KeratinoSens assay. Data for six physicochemical properties, which may affect skin penetration, were also collected, and skin sensitization read-across predictions were performed using OECD QSAR Toolbox. All data were combined into a variety of potential integrated decision strategies to predict LLNA outcomes using a training set of 94 substances and an external test set of 26 substances. Fifty-four models were built using multiple combinations of machine learning approaches and predictor variables. The seven models with the highest accuracy (89-96% for the test set and 96-99% for the training set) for predicting LLNA outcomes used a support vector machine (SVM) approach with different combinations of predictor variables. The performance statistics of the SVM models were higher than any of the non-animal tests alone and higher than simple test battery approaches using these methods. These data suggest that computational approaches are promising tools to effectively integrate data sources to identify potential skin sensitizers without animal testing.

MicroRNAs as early toxicity signatures of doxorubicin in human-induced pluripotent stem cell-derived cardiomyocytes.

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Arch Toxicol. 2016 Dec;90(12):3087-3098. Epub 2016 Feb 3.

Score 3 (New biomarker for cardiotoxicity in hi-PSCs)

Abstract

An in depth investigation at the genomic level is needed to identify early human-relevant cardiotoxicity biomarkers that are induced by drugs and environmental toxicants. The main objective of this study was to investigate the role of microRNAs (miRNAs) as cardiotoxicity biomarkers using human-induced pluripotent stem cell (hiPSC)-derived cardiomyocytes (CMs) that were exposed to doxorubicin (DOX) as a "gold standard" cardiotoxicant. hiPSC-CMs were exposed to 156 nM DOX for 2 days or for 6 days of repeated exposure, followed by drug washout and incubation in drug-free culture medium up to day 14 after the onset of exposure. The induced miRNAs were profiled using miRNA microarrays, and the analysis of the data was performed using the miRWalk 2.0 and DAVID bioinformatics tools. DOX induced early deregulation of 14 miRNAs (10 up-regulated and 4 down-regulated) and

persistent up-regulation of 5 miRNAs during drug washout. Computational miRNA gene target predictions suggested that several DOX-responsive miRNAs might regulate the mRNA expression of genes involved in cardiac contractile function. The hiPSC-CMs exposed to DOX in a range from 39 to 156 nM did not show a significant release of the cytotoxicity marker lactate dehydrogenase (LDH) compared to controls. Quantitative real-time PCR analyses confirmed the early deregulation of miR-187-3p, miR-182-5p, miR-486-3p, miR-486-5p, miR-34a-3p, miR-4423-3p, miR-34c-3p, miR-34c-5p and miR-1303, and also the prolonged up-regulation of miR-182-5p, miR-4423-3p and miR-34c-5p. Thus, we identified and validated miRNAs showing differential DOX-responsive expression before the occurrence of cytotoxicity markers such as LDH, and these miRNAs also demonstrated the significant involvement in heart failure in patients and animal models. These results suggest that the DOX-induced deregulated miRNAs in human CMs may be used as early sensitive cardiotoxicity biomarkers for screening potential drugs and environmental cardiotoxicants with a similar mechanism of action.

Metabolomic network analysis of estrogen-stimulated MCF-7 cells: a comparison of overrepresentation analysis, quantitative enrichment analysis and pathway analysis versus metabolite network analysis.

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Arch Toxicol. 2017 Jan;91(1):217-230. doi: 10.1007/s00204-016-1695-x. Epub 2016 Apr 2.

Score 3 (Bioinformatics to deduce new toxicity pathways)

Abstract

In the context of the Human Toxome project, mass spectroscopy-based metabolomics characterization of estrogen-stimulated MCF-7 cells was studied in order to support the untargeted deduction of pathways of toxicity. A targeted and untargeted approach using overrepresentation analysis (ORA), quantitative enrichment analysis (QEA) and pathway analysis (PA) and a metabolite network approach were compared. Any untargeted approach necessarily has some noise in the data owing to artifacts, outliers and misidentified metabolites. Depending on the chemical analytical choices (sample extraction, chromatography, instrument and settings, etc.), only a partial representation of all metabolites will be achieved, biased by both the analytical methods and the database used to identify the

metabolites. Here, we show on the one hand that using a data analysis approach based exclusively on pathway annotations has the potential to miss much that is of interest and, in the case of misidentified metabolites, can produce perturbed pathways that are statistically significant yet uninformative for the biological sample at hand. On the other hand, a targeted approach, by narrowing its focus and minimizing (but not eliminating) misidentifications, renders the likelihood of a spurious pathway much smaller, but the limited number of metabolites also makes statistical significance harder to achieve. To avoid an analysis dependent on pathways, we built a de novo network using all metabolites that were different at 24 h with and without estrogen with a p value <0.01 (53) in the STITCH database, which links metabolites based on known reactions in the main metabolic network pathways but also based on experimental evidence and text mining. The resulting network contained a "connected component" of 43 metabolites and helped identify non-endogenous metabolites as well as pathways not visible by annotation-based approaches. Moreover, the most highly connected metabolites (energy metabolites such as pyruvate and alpha-ketoglutarate, as well as amino acids) showed only a modest change between proliferation with and without estrogen. Here, we demonstrate that estrogen has subtle but potentially phenotypically important alterations in the acyl-carnitine fatty acids, acetyl-putrescine and succinoadenosine, in addition to likely subtle changes in key energy metabolites that, however, could not be verified consistently given the technical limitations of this approach. Finally, we show that a network-based approach combined with text mining identifies pathways that would otherwise neither be considered statistically significant on their own nor be identified via ORA, QEA, or PA.

Prototype Systems Containing Human Cytochrome P450 for High-Throughput Real-Time Detection of DNA Damage by Compounds That Form DNA-Reactive Metabolites.

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Chem Res Toxicol. 2016 May 16;29(5):747-56. doi: 10.1021/acs.chemrestox.5b00455. Epub 2016 Apr 14.

Score 3 (New assay for detecting compounds/metabolites that damage DNA)

Abstract

The formation of reactive metabolites through biotransformation is the suspected cause of many adverse drug reactions. Testing for the propensity of a drug to form reactive metabolites has increasingly become an integral part of lead-optimization strategy in drug discovery. DNA reactivity is one undesirable facet of a drug or its metabolites and can lead to increased risk of cancer and reproductive toxicity. Many drugs are metabolized by cytochromes P450 in the liver and other tissues, and these reactions can generate hard electrophiles. These hard electrophilic reactive metabolites may react with DNA and may be detected in standard in vitro genotoxicity assays; however, the majority of these assays fall short due to the use of animal-derived organ extracts that inadequately represent human metabolism. The current study describes the development of bacterial systems that efficiently

detect DNA-damaging electrophilic reactive metabolites generated by human P450 biotransformation. These assays use a GFP reporter system that detects DNA damage through induction of the SOS response and a GFP reporter to control for cytotoxicity. Two human CYP1A2-competent prototypes presented here have appropriate characteristics for the detection of DNA-damaging reactive metabolites in a high-throughput manner. The advantages of this approach include a short assay time (120-180 min) with real-time measurement, sensitivity to small amounts of compound, and adaptability to a microplate format. These systems are suitable for high-throughput assays and can serve as prototypes for the development of future enhanced versions.

A novel method to generate monocyte-derived dendritic cells during coculture with HaCaT facilitates detection of weak contact allergens in cosmetics.

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Arch Toxicol. 2017 Jan;91(1):339-350. doi: 10.1007/s00204-016-1722-y. Epub 2016 Apr 30.

Score 3 (New assay system to detect weak skin sensitisers *in vitro*)

Abstract

The in vitro sensitization assay LCSA (Loose-fit Coculture-based Sensitization Assay) has proved reliable for the detection of contact sensitizers in the past. However, the coculture of human monocyte-derived dendritic cells (DCs) with primary human keratinocytes (KCs) in serum-free medium is relatively complex compared to other sensitization assays which use continuous cell lines. To facilitate high-throughput screening of chemicals, we replaced KCs with the HaCaT cell line under various culture conditions. Coculture of HaCaT with peripheral blood mononuclear cells in serum-supplemented medium leads to generation of CD1a+/CD1c+ DCs after addition of GM-CSF, IL-4, and TGF-β1 (as opposed to CD1a-/CD1c- DCs which arise in the "classic" LCSA coculture). These cells resemble monocyte-derived DCs generated in monoculture, but, unlike those, they show a marked upregulation CD86 after treatment with contact allergens. All of the nine sensitizers in this study were correctly identified by CD1a+/CD1c+ DCs in coculture with HaCaT. Among the substances were weak contact allergens such as propylparaben (which is false negative in the local lymph node assay in mice) and resorcinol (which was not detected by CD1a-/CD1c- DCs in the "classic" LCSA). The level of CD86 upregulation on CD1a+/CD1c+ DCs was higher for most allergens compared to CD1a-/CD1c- DCs, thus improving the assay's discriminatory power. Three out of four non-sensitizers were also correctly assessed by the coculture

assay. A false-positive reaction to caprylic (octanoic) acid confirms earlier results that some fatty acids are able to induce CD86 on DC in vitro. In conclusion, change of the LCSA protocol led to reduction of time and cost while even increasing the assay's sensitivity and discriminatory power.

High-content imaging-based BAC-GFP toxicity pathway reporters to assess chemical adversity liabilities.

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Arch Toxicol. 2017 Mar;91(3):1367-1383. doi: 10.1007/s00204-016-1781-0. Epub 2016 Jun 29.

Score 3 (New battery of GFP reporter genes in generalised assay for hepatotoxicity)

Abstract

Adaptive cellular stress responses are paramount in the healthy control of cell and tissue homeostasis and generally activated during toxicity in a chemical-specific manner. Here, we established a platform containing a panel of distinct adaptive stress response reporter cell lines based on BAC-transgenomics GFP tagging in HepG2 cells. Our current panel of eleven BAC-GFP HepG2 reporters together contains (1) upstream sensors, (2) downstream transcription factors and (3) their respective target genes, representing the oxidative stress response pathway (Keap1/Nrf2/Srxn1), the unfolded protein response in the endoplasmic reticulum (Xbp1/Atf4/BiP/Chop) and the DNA damage response (53bp1/p53/p21). Using automated confocal imaging and quantitative single-cell image analysis, we established that all reporters allowed the time-resolved, sensitive and mode-of-action-specific activation of the individual BAC-GFP reporter cell lines as defined by a panel of pathway-specific training compounds. Implementing the temporal pathway activity information increased the discrimination of training compounds. For a set of >30 hepatotoxicants, the induction of Srxn1, BiP, Chop and p21 BAC-GFP reporters correlated strongly with the transcriptional responses observed in cryopreserved primary human hepatocytes. Together, our data indicate that a phenotypic adaptive stress response profiling platform will allow a high throughput and time-resolved classification of chemical-induced stress responses, thus assisting in the future mechanism-based safety assessment of chemicals.

Improving drug safety with a systems pharmacology approach.

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Eur J Pharm Sci. 2016 Oct 30;94:84-92. doi: 10.1016/j.ejps.2016.06.009. Epub 2016 Jun 7.

Score 3 (New bioinformatics approach to predicting adverse drug affects, with potential applicability to toxicology)

Abstract

Systems pharmacology is used to mechanistically analyze drug-adverse drug reaction (ADRs) pairs and is a promising solution to the complex problem of understanding mechanisms of toxicity. In this research, we have explored the feasibility of retrospectively mapping population-level adverse events from the FDA Adverse Event Reporting System (FAERS) to chemical and biological databases to identify drug safety signals and the underlying molecular mechanisms. We used an analytic platform - Molecular Analysis of Side Effects (MASE™). For this purpose, we selected the adverse event of severe and potentially fatal cutaneous reactions (SCARs) that are associated with acetaminophen (APAP). SCARs encompass the continuum between Stevens-Johnson Syndrome (SJS) and Toxic Epidermal Necrolysis (TEN). We found a statistically significant association between APAP and TEN, the most severe form of SCARs. We also explored the influence of APAP on other classes of drugs commonly associated with SCARs. We found that APAP significantly reduced the risk of SCARs commonly associated with carbamazepine (CBZ). We used molecular docking simulations to propose a mechanism for APAP's reduction in CBZ-induced SCARs which is competitive inhibition of the binding of CBZ to HLA-B*15:02. We conclude that systems pharmacology can complement established surveillance methodologies by providing a means to undertake an independent investigation and review of the mechanisms by which drugs cause adverse events.

Design of a high-throughput human neural crest cell migration assay to indicate potential developmental toxicants.

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ALTEX. 2017;34(1):75-94. doi: 10.14573/altex.1605031. Epub 2016 Jul 27.

Score 3 + 1 (New assay for detecting developmental toxicants plus 1 for use of hiPSCs & HCS)

Abstract

Migration of neural crest cells (NCCs) is one of the pivotal processes of human fetal development. Malformations arise if NCC migration and differentiation are impaired genetically or by toxicants. In the currently available test systems for migration inhibition of NCC (MINC), the manual generation of a cell-free space results in extreme operator dependencies, and limits throughput. Here a new test format was established. The assay avoids scratching by plating cells around a commercially available circular stopper. Removal of the stopper barrier after cell attachment initiates migration. This microwell-based circular migration zone NCC function assay (cMINC) was further optimized for toxicological testing of human pluripotent stem cell (hPSC)-derived NCCs. The challenge of obtaining data on viability and migration by automated image processing was addressed by developing a freeware. Data on cell proliferation were obtained by labelling replicating cells, and by careful assessment of cell viability for each experimental sample. The role of cell proliferation as an experimental confounder was tested experimentally by performing the cMINC in the presence of the proliferation-inhibiting drug cytosine arabinoside (AraC), and by a careful evaluation of mitotic events over time. Data from these studies led to an adaptation of the test protocol, so that toxicant exposure was limited to 24 h. Under these conditions, a prediction model was developed that allows classification of toxicants as either inactive, leading to unspecific cytotoxicity, or specifically inhibiting NC migration at non-cytotoxic concentrations.

Neutrophil gelatinase-associated lipocalin production negatively correlates with HK-2 cell impairment: Evaluation of NGAL as a marker of toxicity in HK-2 cells.

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Toxicol In Vitro. 2017 Mar;39:52-57. doi: 10.1016/j.tiv.2016.11.012. Epub 2016 Nov 22.

Score 3 (Biomarker for nephrotoxicity *in vitro*)

Abstract

Neutrophil gelatinase-associated lipocalin is an extracellular protein produced mostly in kidney. Recently, it has become a promising biomarker of renal damage in vivo. On the other hand, the validation of NGAL as a biomarker for nephrotoxicity estimation in vitro has not been characterized in detail yet. Since the HK-2 cells are frequently used human kidney cell line, we aimed to characterize the production of NGAL in these cells and to evaluate NGAL as a possible marker of cell impairment. We used heavy metals (mercury, cadmium), peroxide, drugs (acetaminophen, gentamicin) and cisplatin to mimic nephrotoxicity. HK-2 cells were incubated with selected compounds for 1-24h and cell viability was measured together with extracellular NGAL production. We proved that HK-2 cells possess a capacity to

produce NGAL in amount of 2pg/ml/h. We found a change in cell viability after 24h incubation with all tested toxic compounds. The largest decrease of the viability was detected in mercury, acetaminophen, cisplatin and gentamicin. Unexpectedly, we found also a significant decrease in NGAL production in HK-2 cells treated with these toxins for 24h: to 11±5%, 54±5%, 57±6% and 76±9% respectively, compared with controls (=100%). Our results were followed with qPCR analysis when we found no significant increase in LCN2 gene expression after 24h incubation. We conclude that extracellular NGAL production negatively correlates with HK-2 cell impairment.

Prevalidation trial for a novel in vitro eye irritation test using the reconstructed human cornea-like epithelial model, MCTT HCE™.

H Yang[1], DE Kim[1], WH Jang[2], S An[2], SA Cho[2], MS Jung[3], JE Lee[3], KW Yeo[4], SB Koh[4], TC Jeong[5], MJ Kang[5], YJ Chun[6], SHE Lee[7], KM Lim[8], S Bae[9].

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Toxicol In Vitro. 2017 Mar;39:58-67. doi: 10.1016/j.tiv.2016.11.010. Epub 2016 Nov 19.

Score 3 (Development of new in vitro eye irritation assay - validation)

Abstract

Here, we report the results of a prevalidation trial for an in vitro eye irritation test (EIT) using the reconstructed human cornea-like epithelium, MCTT HCE™. The optimal cutoff to determine irritation in the prediction model was established at 35% with the receiver operation characteristics(ROC) curve for 126 substances. Within-lab(WL) and between-lab(BL) reproducibility was tested for 20 reference substances by 3 participating laboratories. Viability data described by mean±SD or ±1/2 difference between duplicate wells, and scatter plots, demonstrated the WL/BL consistency. WL/BL concordance with the binary decision, whether non-irritant or irritant was estimated to be 85-95% and 95%, respectively. WL/BL reproducibility of viability data was further supported by a strong correlation(ICC, r>0.9). WL/BL agreement of binary decisions was also examined by Fleiss' Kappa statistics, which showed a strong level of agreement (>0.78), nevertheless weaker than the reproducibility of the viability. The EIT with MCTT HCE™ exhibited a sensitivity of 82.2% (60/73), a specificity of 81.1% (43/53), and an accuracy of 81.8% (103/126) for 126 reference substances (for liquids; a sensitivity of 100%

(47/47), a specificity of 70.6% (24/34), and an accuracy of 87.7% (71/81), and for solids, a sensitivity of 50% (13/26), a specificity of 100% (19/19), and an accuracy of 71.1% (32/45), suggesting that the accuracy is satisfactory but the sensitivity needs improvement, which shall be addressed through correcting the poor sensitivity for solid substances in future full validation trials.

Editor's Highlight: Development of an In vitro Assay Measuring Uterine-Specific Estrogenic Responses for Use in Chemical Safety Assessment.

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Toxicol Sci. 2016 Nov;154(1):162-173. DOI: 10.1093/toxsci/kfw152 Epub 2016 Aug 7.

Score 3 (New assay)

Abstract

A toxicity pathway approach was taken to develop an in vitro assay using human uterine epithelial adenocarcinoma (Ishikawa) cells as a replacement for measuring an in vivo uterotrophic response to estrogens. The Ishikawa cell was determined to be fit for the purpose of recapitulating in vivo uterine response by verifying fidelity of the biological pathway components and the dose-response predictions to women of child-bearing age. Expression of the suite of estrogen receptors that control uterine proliferation (ERα66, ERα46, ERα36, ERβ, G-protein coupled estrogen receptor (GPER)) were confirmed across passages and treatment conditions. Phenotypic responses to ethinyl estradiol (EE) from transcriptional activation of ER-mediated genes, to ALP enzyme induction and cellular proliferation occurred at concentrations consistent with estrogenic activity in adult women (low picomolar). To confirm utility of this model to predict concentration-response for uterine proliferation with xenobiotics, we tested the concentration-response for compounds with known uterine estrogenic activity in humans and compared the results to assays from the ToxCast and Tox21 suite of estrogen assays. The Ishikawa proliferation assay was consistent with in vivo responses and was a more sensitive measure of uterine response. Because this assay was constructed by first mapping the key molecular events for cellular response, and then ensuring that the assay incorporated these events, the resulting cellular assay should be a reliable tool for identifying estrogenic compounds and may provide improved quantitation of chemical concentration response for in vitro-based safety assessments.

Stem Cell Transcriptome Responses and Corresponding Biomarkers That Indicate the Transition from Adaptive Responses to Cytotoxicity.

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Chem Res Toxicol. 2017 Apr 17;30(4):905-922. doi: 10.1021/acs.chemrestox.6b00259. Epub 2016 Dec 21.

Score 3 (Analysis of mechanisms of transcriptional change highlights potential biomarkers of cytotoxicity)

Abstract

Analysis of transcriptome changes has become an established method to characterize the reaction of cells to toxicants. Such experiments are mostly performed at compound concentrations close to the cytotoxicity threshold. At present, little information is available on concentration-dependent features of transcriptome changes, in particular, at the transition from noncytotoxic concentrations to conditions that are associated with cell death. Thus, it is unclear in how far cell death confounds the results of transcriptome studies. To explore this gap of knowledge, we treated pluripotent stem cells differentiating to human neuroepithelial cells (UKN1 assay) for short periods (48 h) with increasing concentrations of valproic acid (VPA) and methyl mercury (MeHg), two compounds with vastly different modes of action. We developed various visualization tools to describe cellular responses, and the overall response was classified as "tolerance" (minor transcriptome changes), "functional adaptation" (moderate/strong transcriptome responses, but no cytotoxicity), and "degeneration". The latter two conditions were compared, using various statistical approaches. We identified (i) genes regulated at cytotoxic, but not at noncytotoxic, concentrations and (ii) KEGG pathways, gene ontology term groups, and superordinate biological processes that were only regulated at cytotoxic concentrations. The consensus markers and processes found after 48 h treatment were then overlaid with those found after prolonged (6 days) treatment. The study highlights the importance of careful concentration selection and of controlling viability for transcriptome studies. Moreover, it allowed identification of 39 candidate "biomarkers of cytotoxicity". These could serve to provide alerts that data sets of interest may have been affected by cell death in the model system studied.

A chemical-biological similarity-based grouping of complex substances as a prototype approach for evaluating chemical alternatives.

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Green Chem. 2016 Aug 21;18(16):4407-4419. doi: 10.1039/c6gc01147k. Epub 2016 May 16.

Score 3 (Prototypical screening system for evaluating toxicity of complex substances)

Abstract

Comparative assessment of potential human health impacts is a critical step in evaluating both chemical alternatives and existing products on the market. Most alternatives assessments are conducted on a chemical-by-chemical basis and it is seldom acknowledged that humans are exposed to complex products, not individual substances. Indeed, substances of Unknown or Variable composition, Complex reaction products, and Biological materials (UVCBs) are ubiquitous in commerce yet they present a major challenge for registration and health assessments. Here, we present a comprehensive experimental and computational approach to categorize UVCBs according to global similarities in their bioactivity using a suite of in vitro models. We used petroleum substances, an important group of UVCBs which are grouped for regulatory approval and read-across primarily on physico-chemical properties and the manufacturing process, and only partially based on toxicity data, as a case study. We exposed induced pluripotent stem cell-derived cardiomyocytes and hepatocytes to DMSO-soluble extracts of 21 petroleum substances from five product groups. Concentration-response data from high-content imaging in cardiomyocytes and hepatocytes, as well as targeted high-throughput transcriptomic analysis of the hepatocytes, revealed distinct groups of petroleum substances. Data integration showed that bioactivity profiling affords clustering of petroleum substances in a manner similar to the manufacturing process-based categories. Moreover, we observed a high degree of correlation between bioactivity profiles and physico-chemical properties, as well as improved groupings when chemical and biological data were combined. Altogether, we demonstrate how novel in vitro screening approaches can be effectively utilized in combination with physico-chemical characteristics to group complex substances and enable read-across. This approach allows for rapid and scientifically-informed evaluation of health impacts of both existing substances and their chemical alternatives.

Copper oxide nanoparticle toxicity profiling using untargeted metabolomics.

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Part Fibre Toxicol. 2016 Sep 8;13(1):49. doi: 10.1186/s12989-016-0160-6.

Score 2 + 1 (Biomarker of apoptosis plus 1 for metabolomics approach)

Abstract

BACKGROUND:

The rapidly increasing number of engineered nanoparticles (NPs), and products containing NPs, raises concerns for human exposure and safety. With this increasing, and ever changing, catalogue of NPs it is becoming more difficult to adequately assess the toxic potential of new materials in a timely fashion. It is therefore important to develop methods which can provide high-throughput screening of biological responses. The use of omics technologies, including metabolomics, can play a vital role in this process by providing relatively fast, comprehensive, and cost-effective assessment of cellular responses. These techniques thus provide the opportunity to identify specific toxicity pathways and to generate hypotheses on how to reduce or abolish toxicity.

RESULTS:

We have used untargeted metabolome analysis to determine differentially expressed metabolites in human lung epithelial cells (A549) exposed to copper oxide nanoparticles (CuO NPs). Toxicity hypotheses were then generated based on the affected pathways, and critically tested using more conventional biochemical and cellular assays. CuO NPs induced regulation of metabolites involved in oxidative stress, hypertonic stress, and apoptosis. The involvement of oxidative stress was clarified more easily than apoptosis, which involved control experiments to confirm specific metabolites that could be used as standard markers for apoptosis; based on this we tentatively propose methylnicotinamide as a generic metabolic marker for apoptosis.

CONCLUSIONS:

Our findings are well aligned with the current literature on CuO NP toxicity. We thus believe that untargeted metabolomics profiling is a suitable tool for NP toxicity screening and hypothesis generation.

Predictive performance of the Vitrigel-eye irritancy test method using 118 chemicals.

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J Appl Toxicol. 2016 Aug;36(8):1025-37. doi: 10.1002/jat.3254. Epub 2015 Oct 15.

Score 3 (Development of in vitro eye irritancy test)

Abstract

We recently developed a novel Vitrigel-eye irritancy test (EIT) method. The Vitrigel-EIT method is composed of two parts, i.e., the construction of a human corneal epithelium (HCE) model in a collagen vitrigel membrane chamber and the prediction of eye irritancy by analyzing the time-dependent profile of transepithelial electrical resistance values for 3 min after exposing a chemical to the HCE model. In this study, we estimated the predictive performance of Vitrigel-EIT method by testing a total of 118 chemicals. The category determined by the Vitrigel-EIT method in comparison to the globally harmonized system classification revealed that the sensitivity, specificity and accuracy were 90.1%, 65.9% and 80.5%, respectively. Here, five of seven false-negative chemicals were acidic chemicals inducing the irregular rising of transepithelial electrical resistance values. In case of eliminating the test chemical solutions showing pH 5 or lower, the sensitivity, specificity and accuracy were improved to 96.8%, 67.4% and 84.4%, respectively. Meanwhile, nine of 16 false-positive chemicals were classified irritant by the US Environmental Protection Agency. In addition, the disappearance of ZO-1, a tight junction-associated protein and MUC1, a cell membrane-spanning mucin was immunohistologically confirmed in the HCE models after exposing not only eye irritant chemicals but also false-positive chemicals, suggesting that such false-positive chemicals have an eye irritant potential. These data demonstrated that the Vitrigel-EIT method could provide excellent predictive performance to judge the widespread eye irritancy, including very mild irritant chemicals. We hope that the Vitrigel-EIT method contributes to the development of safe commodity chemicals.

Comprehensive Landscape of Nrf2 and p53 Pathway Activation Dynamics by Oxidative Stress and DNA Damage.

S Hiemstra[1], M Niemeijer[1], E Koedoot[1], S Wink[1], JE Klip[1], M Vlasveld[1], E de Zeeuw[1], V van Os[1], A White[2], BV Water[1].

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Chem Res Toxicol. 2017 Apr 17;30(4):923-933. doi: 10.1021/acs.chemrestox.6b00322. Epub 2016 Dec 16.

Score 3 but check not animal experiments (Pathway map of oxidative stress and DNA pathways for toxicity)

Abstract

A quantitative dynamics pathway map of the Nrf2-mediated oxidative stress response and p53-related DNA damage response pathways as well as the cross-talk between these pathways has not systematically been defined. To allow the dynamic single cell evaluation of these pathways, we have used BAC-GFP recombineering to tag for each pathway's three key components: for the oxidative stress response, Keap1-GFP, Nrf2-GFP, and Srxn1-GFP; for the DNA damage response, 53bp1-GFP, p53-GFP, and p21-GFP. The dynamic activation of these individual components was assessed using quantitative high throughput confocal microscopy after treatment with a broad concentration range of diethyl maleate (DEM; to induce oxidative stress) and etoposide (to induce DNA damage). DEM caused a rapid activation of Nrf2, which returned to baseline levels at low concentrations but remained sustained at high concentrations. Srxn1-GFP induction and Keap1-GFP translocation to autophagosomes followed later, with upper boundaries reached at high concentrations, close to the onset of cell death. Etoposide caused rapid accumulation of 53bp1-GFP in DNA damage foci, which was later followed by the concentration dependent nuclear accumulation of p53-GFP and subsequent induction of p21-GFP. While etoposide caused activation of Srxn1-GFP, a modest activation of DNA damage reporters was observed for DEM at high concentrations. Interestingly, Nrf2 knockdown caused an inhibition of the DNA damage response at high concentrations of etoposide, while Keap1 knockdown caused an enhancement of the DNA damage response already at low concentrations of etoposide. Knockdown of p53 did not affect the oxidative stress response. Altogether, the current stress response landscapes provide insight in the time course responses of and cross-talk between oxidative stress and DNA-damage and defines the tipping points where cell injury may switch from adaptation to injury.

Do Some Autism Spectral Disorder Patients Have Inefficient Mitochondria or Poor Responses to DNA Damaging Agents?

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[2] Greenwood Genetics Institute, Greenwood, SC.

Abstract 1239, SoT 2017

Score 3 (Biomarker for tox-induced ASD; score 3 for potential new biomarkers)

Abstract

Autism spectrum disorders (ASDs) represent a heterogeneous collection of neurodevelopmental disorders; indicating different disorders with similar symptoms such as impaired social interactions, communication issues, and repetitive behaviors. Over the past decade, there has been a 10X rise in the prevalence of ASDs from 0.2% to 2% of the US population of school age children. The

Organization for Economic Co-operation and Development (OECD) lists ASD/ADHD as one of the three diseases and syndromes of contemporary toxicology concern along with testicular dysgenesis and metabolic disorders. With this rise in ASD has come an interest in identifying both the causation and biomarkers for ASDs. We developed qPCR assays for measuring mtDNA damage and number to assess whether a subset of ASD patients show mitochondrial inefficiency or a susceptibility to DNA damage either through increased damage or poor repair. MtDNA is much more sensitive to disruption than nuclear DNA and recent research indicates a putative role for perturbations in tryptophan and fatty acid metabolism make mitochondria a likely target for ASD. Fibroblast cell lines from ASD+ and ASD- patients were treated with hydrogen peroxide, docosahexaenoic acid (DHA; n-3 fatty acid), or both for 24 hours and mtDNA number and damage response assessed and compared to untreated controls. 5/6 ASD- cell lines showed no significant increase in mtDNA number and all ASD- cell lines showed no signs of mtDNA damage or inefficient repair. In contrast, two ASD+ cell lines show a 5X increase in mtDNA number, indicating mitochondrial inefficiency. While two others show perturbed damage responses over 24 hours. One of these, AK10, demonstrated both mitochondrial inefficiency and a significantly increased DNA damage compared to both ASD+/- populations. We are currently measuring the initial mtDNA damage caused by hydrogen peroxide alone or in combination with DHA to determine if our ASD patients are more susceptible to mtDNA damage due to poor damage response or metabolic inefficiency. Taken together, 0/6 ASD- cell lines show significant mitochondrial deficiencies, while 4/9 of the ASD+ cell lines show mitochondrial deficiencies either in efficiency or in response to a toxic stressor.

Discovery of Toxic Metabolites by High-Content Imaging in the SciFlow 1000 96-Well Microfluidic Culture System

D. Sloan, T. Jensen, M. McCartney, S. Klose, and R. McClelland.

SciKon Innovation, Inc., Chapel Hill, NC.

Abstract 1440 SoT 2017

Score 2 + 1 (Application of microfluidics to allow toxicity assessment of drugs and metabolites in one system, using HCS assay)

Abstract

Drug induced liver injury (DILI) is the leading cause of acute liver failure in the US, and is the most common reason cited for the withdrawal of an approved drug. DILI can result from toxicity caused by a parent compound or metabolites of that compound. The ability to identify DILI inducing compounds or metabolites early in the drug discovery process results in significant savings to pharmaceutical companies. This requires a system that can both produce and retain drug metabolites, parent compounds, and measure the toxic effects on cells. The SciFlow 1000 is a unidirectional gravity driven microfluidic tissue culture system based on a 96-well plate footprint, with all wells in a row linked through microchannels. To demonstrate metabolic capacity and observe the pattern of metabolite production in SciFlow, substrates specific for 5 different CYP enzymes (1A2, 2B6, 2C9, 2D6 and 3A4) were introduced into SciFlow wells containing hepatocytes, and metabolite production was monitored over a 72-hour period. The products of CYP metabolism begin to appear at the 24-hour time point and by 72-hours significant concentrations of these metabolites are measured in the downstream fluidic wells.

Using these metabolite profiles, we set out to quantify the effects of metabolism on cellular toxicity. In a second experiment, a panel of 10 drugs with varying degrees of toxicity were selected, and their effects on liver cell viability was monitored. HepaRG cells were plated in all wells of the SciFlow 1000 and drug was added 3 times over 72 hours to the source well. This method allowed the drug to flow over the hepatocytes (left-to-right), for metabolism to occur, and for metabolites to travel downstream. Cell viability was monitored in real time via high content imaging using CellTox Green and Hoechst stain. We identified drugs that were hepatotoxic, and based on the pattern of cell death, those drugs whose toxicity was mediated through toxic metabolites. Additionally, we calculated the total drug exposure and determined an IC50 based on cell viability. This powerful methodology, using the combination of metabolically active liver cells, the SciFlow 1000 Fluidic Culture System, and high content imaging demonstrates the ability to identify cellular toxicity and distinguish between parent compound vs. metabolite effects without any prior knowledge of the compound's metabolite profile, allowing earlier identification of a compound's mechanism of toxicity.

A Multidimensional High-Throughput Approach for Toxicity Evaluation of Environmental Chemicals in Induced Pluripotent Stem Cell- Derived Endothelial Cells

Y. Iwata, W. Klaren, F. Grimm, and I. Rusyn.

Texas A&M University, College Station, TX.

Abstract 1457 SoT 2017

Score 3 (New vascularisation assay for detecting environmental toxins)

Abstract

Endothelial cells (ECs) play a major role in blood vessel formation and function. While chemical exposures have been shown to adversely affect EC function and blood vessel development, resulting in cardiovascular and developmental effects, large numbers of environmentally relevant chemicals have yet to be evaluated for their potential to exert vascular effects. Recent advancements in the development of stem cell technologies have resulted in the availability of human induced pluripotent stem cell (iPSC)-derived ECs that may provide a physiologically-relevant, high-throughput applicable in vitro model for toxicity screening of environmental hazards and open a possibility of testing effects across a population of individuals. In this work we aimed to develop a highthroughput screening approach, encompassing functional vascularization and cytotoxicity assays for the multidimensional evaluation of chemical effects on endothelial cell using iPSC-derived ECs and human umbilical vein endothelial cells (HUVECs). Cells were exposed to selected angiogenesis inhibitors, cytotoxic agents and vehicle in concentration- response for either 16 or 24 hours in high-throughput compatible 384-well plate format. Chemical inhibition of vascularization was quantified using EC tube formation on biological (Geltrex.) and synthetic (SP-103 Angiogenesis Hydrogel, Stem Pharm) extracellular matrices.

Bioactivity was assessed by high-content live cell imaging of EC monolayers cultured on fibronectin-coated plates. Assay performance validation indicated good to excellent assay robustness, sensitivity, and replicability for both cell types based on evaluation of inter-day and -plate replicability, coefficients of variation (%CV) of vehicle controls, and Z'-factor calculation using positive and negative chemical controls. Our data demonstrate that both iPSC-derived ECs and HUVECs formed

tubes on Geltrex. and Angiogenesis Hydrogel that could be affected in a concentration- dependent manner by various angiogenesis inhibitors and cytotoxic agents. In conclusion, in vitro vascularization and cytotoxicity assays were successfully established in a high-throughput compatible format that will enable evaluation of a large number of environmentally relevant chemicals for their potential effects on angiogenesis.

Investigating Drug-Induced Cardiomyocyte Dysfunction through Combined Analysis of Beating, Metabolic Flux, and Cellular Oxygenation

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[2] Roche Innovation Center, Basel, Switzerland.

Abstract 1458 SoT 2017

Score 2 + 1 (Use of metabolic/bioenergetic probes plus 1 for combination with microelectrode plates to look at drug-induced cardiotoxicity)

Abstract

Stem cell derived cardiomyocytes are finding increased use as an in vitro model for the assessment of drug-induced cardiotoxicity, prompting a parallel development in multi-parametric high-throughput assays to measure cardiomyocyte function. Metabolic perturbations are of particular importance in this regard due to the aerobic poise of cardiac tissue and the implication of mitochondrial dysfunction in the etiology of drug-induced cardiotoxicity. Here we combine measurement of cardiomyocyte contractility, metabolism and oxygenation on a single plate to better characterise cellular responses to drug treatment. This is achieved by integrating microelectrode-based contractility measurements (E-plates) with a multiplexed fluorescence-based bioenergetics assessment, measuring O2 consumption (MitoXpress.-Xtra), glycolytic flux (pH-Xtra) and cellular oxygenation (MitoXpress.-Intra). This combination facilitates an interrogation of the relationship between metabolism and contractility, allowing a delineation of altered beat rate and concomitant metabolic perturbation. Using a panel of classical mitochondrial modulators (incl. antimycin, rotenone and FCCP), data are presented demonstrating that iPS-derived cardiomyocytes can circumvent mitochondrial impairment and maintain beating by increasing glycolysis- derived ATP supply. This effect is less pronounced when glucose is replaced by long chain fatty acids (oleate) as respiratory substrate, important as fatty acid oxidation can be responsible for up to 90% of cardiac ATP requirements. Data is also presented demonstrating that pharmacological beat rate modulation (Isoproterenol, Amiodarone, Nifedipine) impacts metabolic flux with increasing beat rate increasing ATP demand causing increased OxPhos. Beat rate is also seen to significantly impact cellular oxygenation with elevated rates causing oxygenation to drop from 21% to 6% O2, a drop that can impact related signalling pathways. Again these differences are modulated by metabolic substrate. This combined analysis of critical cardiomyocyte functions facilities the design of more physiologically relevant in vitro assays and provides a more holistic cardiotoxicity screen.

Toxicological Response of Healthy and Asthmatic 3D In Vitro Airway Model to CuO Nanoparticles at the Air-Liquid Interface

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[3] Triskelion, Zeist, Netherlands. Sponsor: A. Dankers.

Abstract 1514 SoT 2017

Score 2 + 1 (New tool and noteworthy for the use, and comparison, 3D models of healthy & asthmatic lung epithelia)

Abstract

In this study we investigated the biological effects of three concentrations of CuO and carboxylated CuO nanoparticles on human 3D in vitro airway model of healthy and asthmatic origin. The human 3D in vitro airway model consist of human stem cells and fully differentiated human ciliated respiratory and goblet cells. The epithelial lining is covered by a mucus layer that is produced by goblet cells and moved by ciliary beating. The cells form a layer with an intact integrity (at least in the healthy inserts), and are apically exposed to air, allowing a realistic exposure to a test atmosphere. CuO was tested at low, mid, and high concentrations of 23, 120, 470 mg/m3 for CuO and of 32, 128, 495 mg/m3 for CuO-COOH respectively. Results were obtained on the exposure characteristics (SEM, deposition, APS/SMPS). Exposure of cells from healthy (n=3) and asthmatic origin (n=5) resulted in visual observable particle deposition. Cells of asthmatic origin showed a less homogenous cell layer compared to the cells of healthy origin. Aerosol exposures to CuO showed 5% cytotoxicity (LDH) at the highest concentration in the cells of healthy origin. The cytotoxicity response of the cells of asthmatic origin shows a dose response which reaches around 15% toxicity at the highest concentration. Cytokines (IL-8, IL-6 and MCP-1) showed a dose response in both cells of healthy and asthmatic origin, after exposure to CuO. In the highest concentration applied the response of the healthy cells is higher, which might be due to more cells (due to less cytotoxicity) compared to the asthmatic cells. Aerosol exposures to the carboxylated CuO (CuO-COOH) resulted in 5% cytotoxicity (LDH) at the highest concentration in the cells of healthy origin. The cytotoxicity response of the cells of asthmatic origin shows a dose response wich reaches around 10% toxicity at the highest concentration. Cytokines (IL-8, IL-6 and MCP-1) showed a dose response in both cells of healthy and asthmatic origin, after exposure to CuO-COOH. In the highest concentration applied, the response of the healthy cells is lower for CuO-COOH compared to CuO exposure. We conclude that human 3D in vitro airway models of healthy and asthmatic origin can be used to study the biological effects of different forms of CuO nanoparticles.

Assessment of the Phototoxicity of Three Different TiO2 Nano-Forms Using Reconstructed Human Tissue Model Epiderm

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Abstract 1526 SoT 2017

Score 3 (Development of assay for phototoxicity on human skin)

Abstract

Absorption of the solar light by photo-sensitive substances and consequent formation of reactive oxygen species (ROS) and other photo- products may lead to the cellular damage as well as to responses of the immune system. Taken that into the consideration, the determination of phototoxicity of substances absorbing UV and visible spectra of the solar light (VIS) belongs therefore to the basic toxicology tests. One of the methods used for the determination of phototoxicity is a test based on the use of 3D in vitro reconstructed human skin tissue model-EpiDermTM, the EpiDerm H3D-PT. This test was developed and pre-validated by organization ZEBET already in 1997. The main objective of this work was to verify and determine the phototoxicity and phototoxic potential of the selected reference substances and three different types of TiO2 nanoparticles using the EpiDermTM H3D-PT. We firstly evaluated and standardized the measurement conditions of the sunlight simulator SOL-500 and verified the sensitivity of the EpiDermTM tissues towards UV/VIS light. Next, we evaluated correct prediction of phototoxicity of the EpiDermTM H3D-PT using six reference substances, of which four were known phototoxins (chlorpromazine hydrochloride, two types of bergamot oil and anthracene) and two compounds were UV-absorbing, but without phototoxic potential (cinnamaldehyde, p-aminobenzoic acid). Finally, we have used this method to predict the phototoxicity of three different types of titanium dioxide (P25 AEROXID, Eusolex T-2000 , TIG-115). Based on the results obtained in this work, we conclude that the EpiDermTM H3D-PT is a reliable test for the detection of phototoxicity and prediction of the phototoxic potential of selected substances. This conclusion is supported by the fact that during the measurements we obtained the same or better results as published by Liebsch et al. (1997). Phototoxicity of TiO2 has not been demonstrated in any of the three samples tested. This is likely because TiO2 nanoparticles do not penetrate deep enough into the epidermis to cause cytotoxicity by irradiation with UVA/VIS.

Genotoxicity Evaluation of Nanomaterials: What Is the Most Suitable Option of In Vitro Cytokinesis-Block Micronucleus Cytome Assay?

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Abstract 1540 SoT 2017

Score 3 (Development of OECD genotox test for use with nanomaterials)

Abstract

Previous studies showed that the unique physicochemical features presented by nanomaterials (NMs) are responsible for unexpected interactions with toxicity assay components, indicating that standardized toxicity tests used for hazard assessment of chemicals might not be fully suitable for assessing the safety of NMs. The in vitro cytokinesis-block micronucleus cytome assay (CBMN) is one of the OECD-recommended tests for genotoxicity. However, it has been shown that Cytochalasin-B (Cyt-B) used in this test to inhibit the cytokinesis, may affect the uptake of NMs due to inhibition of filaments formation in endocytosis. An official report of OECD

Working Party on Manufactured Nanomaterials presented two possible options to prevent interference between NMs and Cyt-B: i) post-treatment with Cyt-B (NM application, then removal of the NM and replacement with medium containing Cyt-B) and ii) delayed co-treatment (NM application followed by the addition of Cyt-B for the remaining time of treatment). This study aims to evaluate the efficiency of these alternatives and which of them is preferable. Based on literature, A549 cell line and TiO2 nanoparticles were selected to carry out this study. Cytotoxicity was evaluated using MTT to guarantee that at the selected concentrations (10-200 µg/mL), cells showed a viability above 60%. Genotoxicity of TiO2 nanoparticles was then analyzed by CBMN after exposure (co-exposure, post-treatment and delayed co-treatment) for 3h and 29h. In addition, micronuclei analysis and NM internalization in cells were also assessed using flow cytometry (in the presence and absence of Cyt-B during NM exposure). Results obtained contribute to the clarification of which procedure is the most suitable to the genotoxicity evaluation of NMs using the CBMN and to its standardization for NMs hazard assessment. Acknowledgments F. Brand.o is supported by the grant SFRH/BD/101060/2014, funded by FCT (subsidized by national fund of MCTES) and V. Valdiglesias by a Xunta de Galicia postdoctoral fellowship (reference ED481B 2016/190-0).

Toxicokinetic Study on Retinoic Acid in a Multi- Organ Chip Comprising Epiderm Skin Models and Liver Organoids

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Abstract 1588 SoT 2017

Score 2 + 1 (Develops organ-on-a-chip approach for evaluation of bioavailability & metabolism + 1 for addressing absence of in vitro repeated dose assay)

Abstract

The understanding of the bioavailability and metabolism of a chemical, either locally or systemically, is a key aspect in safety assessment. For european cosmetics industry, the current lack of in vitro assays for repeated subacute toxicity is a bottleneck for innovation, since safety assessment relies exclusively on alternatives to animal approaches. Dynamic organ-on-chip cell culture technologies are expected to provide future in vitro testing options. We aimed to evaluate the ability of TissUseÅLs two-organ-chip technology to provide relevant information on compound metabolism and effects on gene expression after repeated, long-term, dosing regimens. To this end, we performed a case study with all-trans retinoic acid (ATRA) in an integrated system comprising EpiDerm skin models and 3D liver organoids. Gene Expression data and mass spectromety analyses show that I) repeated topical application is feasible for seven days. II) Topical application onto the skin model partially emulates the in vivo time course of ATRA bioavailability III) Timing and extent of gene induction in liver organoids are affected by the route of application. IV) ATRA exposure alters the expression of genes pertaining to ATRA metabolism, being potentially involved in the observed stabilization of ATRA concentration after repeated application. V) Repeated application results in elevated concentrations of phase I and phase II ATRA metabolites and an altered metabolite profile, the latter revealing the potential to induce (and to identify) new detox/ metabolic options in the chip. VI) ATRA exposure results in a decreased albumin

concentration and an increase in LDH release over a time course of seven days, reflecting adverse effects on functionality and viability of tissues. Hence, multi-organ-chip-based toxicokinetic approaches may add relevant information to safety assessment of chemicals.

High-Dimensional Toxicological Assessment of Chemical Exposure on Physiologically Relevant 3D In Vitro Models

S. C. Ramaiahgari, S. Waidyanatha, D. Dixon, M. J. DeVito, R. S. Paules, S. S. Ferguson

NIEHS, Durham, NC.

Abstract 1589 SoT 2017

Score 2 + 1 (Demonstration of utility of 3D HepaRG cells for assay model of hepatoxicity testing + use of Tox21 sentinel gene panel & high-throughput (384-well) assay format)

Abstract

Current high-throughput in vitro assays employ two-dimensional (2D) tissue culture models that poorly predict xenobiotic exposure effects on humans. This is principally due to the poor differentiation status resulting in aberrant xenobiotic metabolism and cellular response. To overcome this, three-dimensional (3D) cell culture models are being developed that mimic and maintain biochemical aspects of a tissue to a higher extent than two-dimensional monolayer cultures. We have developed three-dimensional models of HepaRG cell line and primary human hepatocytes to compare their sensitivity/specificity and signaling pathways that are altered upon xenobiotic exposure. Primary human hepatocytes (PHHs) are considered the 'gold standard' for xenobiotic metabolism studies, but they have limited longevity in culture and exhibit substantial donor specific variability. Primary human hepatocytes cultured as spheroids could be maintained for extended periods in culture whilst maintaining their functionality. HepaRG, a bi-potent progenitor cell line, when cultured as three-dimensional spheroids show several hallmarks of polarized hepatocytes with distinct apico-basal domains and their functions. Assessment of xenobiotic metabolism competence with clinical substrates of CYP1A2, CYP2B6 and CYP3A4 produced robust levels of liver enzymes that are within the ranges produced in PHH suspensions and a 2 to 20-fold higher than median activities observed with 2D cultures of PHHs. Spheroid size, time in culture and culture media were important factors affecting basal xenobiotic metabolism and inducibility with activators of hepatic nuclear receptors AhR, CAR and PXR. Repeated exposure studies showed differential sensitivity in identifying compounds that cause metabolism-dependent liver injury. High throughput transcriptome analysis using Tox21 human sentinel 1500+ genes that reflect whole-genome transcriptome was applied on these spheroid models to understand the molecular mechanisms associated with adverse events These assays are implemented in a 384-well format for low cost and potential high throughput screening assays.

Optimization of Direct Double-Strand Break Labeling Assay for Genotoxicity Assessment

K. M. Dunnick, S. Rowley, S. M. Ross, R. A. Clewell.

ScitoVation, Research Triangle Park, NC.

Abstract 2270 SoT 2017

Score 3 (New in vitro assay for genotoxicity, utilising direct labelling of double strand breaks)

Abstract

Most quantitative in vitro genotoxicity assays rely on indirect measurements of DNA damage. While these provide valuable information on the potential for genotoxicity, and are useful for screening applications, they are not currently useful for determining points of departure for safety assessments. Interference by cytotoxicity at high doses, cell cycle, diminished repair, and the reliance on cycling cells, limit current application. Thus, current strategies continue to rely on in vivo methods for deriving points of departure for genotoxic compounds. With support from the SOT Colgate-Palmolive Grant for In Vitro Testing, we developed a novel in vitro assay for detection of double strand breaks (DSBs) in situ. The direct DSB labelling assay (DDL) labels DSBs with a fluorescent tag in intact nuclei, which reduces confounding effects of apoptosis, cytotoxicity, and damage induced during sample processing associated with traditional methods. Implementation in 96-well plates and fluorescence measurements with flow cytometry support high throughput applications. Initial studies with known DNA damaging compounds were completed in HT1080 human fibrosarcoma cells, as these represent the species of interest and recapitulate all major cellular responses to DNA damage. Preliminary data were collected with 24 hr treatments of N-ethyl-N-nitrosurea (ENU), cisplatin (CIS), and chromium (Cr(VI)). For all compounds, the LOELs were similar between the DDL and MN assays (3 vs. 3mM ENU, 3 vs. 3 μM CIS, and 0.3v vs. 1 Cr(VI) for DDL vs. MN), indicating the DDL assay is as sensitive as the commonly used MN assay. Moreover, the DDL assay showed less interference by cytotoxicity at high doses. Preliminary data indicates that the DDL method provides a novel tool to determine direct DNA damage at relevant chemical concentrations compared to traditional in vitro models. Future studies are focused on determining optimal assay conditions, including timing of chemical treatment, additional cell models, and further validation with known false positive, false negative, and true positive chemicals.

Dose-Dependent Increase of Triglyceride Levels in Human HepaRG Liver Cells Exposed to the Fungicide Cyproconazole

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Abstract 2532 SoT 2017

Score 3 (Research into identifying key events in steatosis AOP)

Abstract

Cyproconazole is a triazole antifungal which has been associated with the development of hepatic steatosis, also referred to as fatty liver disease, in rodents. An Adverse Outcome Pathway (AOP) for steatosis has recently been proposed and one important Key Event (KE) in this AOP concerns the accumulation of triglycerides. The present work is part of a larger effort that aims to explore the usefulness of the human liver cell line HepaRG as an in vitro system to address various KEs in the steatosis AOP and to test foodborne chemicals and chemical mixtures for steatogenic properties. In this study, HepaRG cells were exposed for

24 and 72 hours to increasing, non-cytotoxic, concentrations of cyproconazole. Upon exposure, cells were lysed by sonication and extracted with a mixture of iso-octane and ethylacetate (75:25) to specifically extract triglycerides. For further analysis of the fatty acid composition of the triglycerides, extracts were treated with sodium methoxide and BF3 to generate fatty acid methyl esters (FAMEs). Triglycerides and FAMEs were analysed using Gas Chromatography with Flame Ionization Detection (GC-FID). The results indicated that cyproconazole treatment of HepaRG cells results in a dose-dependent accumulation of triglycerides. the effect was strongest after 72 hours of exposure. Accumulation was most pronounced for C50, C52, and C54 triglycerides containing mainly C16 and C18 fatty acids. This outcome underlines the value of the HepaRG cell line as a model system for studying toxicant-induced liver steatosis. Acknowledgement: This work has been funded by the EU project EuroMix (Grant Agreement 633172; www.euromixproject.eu) and the Dutch Ministry of Economic Affairs.

High-Throughput Screening for Acute and Developmental Neurotoxicity Using Human Embryonic Stem Cell-Derived Neuronal Cells

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Abstract 2639 SoT 2017

Score 3 (Development of HTS assay for neurotoxicity using hESCs-derived neuronal cells)

Abstract

To assess the neurotoxicity and prioritize the hazard of chemical for further testing, there is a big demand for high-throughput screening (HTS) of neurotoxicity. However, the traditional neuronal cell models including rodent primary or cancer cells have limitation to provide the information about potential hazards on human health because of difference of species or genetic instability. Here, we used human embryonic stem cells (hESCs)-derived neuronal cells which is well qualified according to standardized protocol, to screen the neurotoxicity. Acute and developmental neurotoxicity was evaluated for the thirteen chemicals (D-sorbitol, saccharin and acetaminophen for negative compounds; methylmercury chloride, cadmium chloride, 5-fluorouracil, acrylamide, brefeldin A, colchicine, cyclosporine A, retinoic acid, and rotenone for positive compounds) using multiple read-out assays including cell viability, apoptosis, mitochondrial membrane potential, reactive oxidative stress and neurite outgrowth assay. Bench mark concentration (BMC) for each assay represented that susceptibility or sensitivity of the toxicity was different according to the mode of actions for the test chemicals. The comprehensive interpretation of potential neurotoxicity was performed by read-out combination from battery of test. By the score of read-out combination, eight compounds from ten positive chemicals were classified with neurotoxic group and the remaining two chemicals such as retinoic acid and acrylamide with low score was classified with inconclusive group and all negative chemicals were correctly classified with non-neurotoxic group. Here we provide the HTS screening platforms with predictive power for acute and developmental neurotoxicity by multiple read-out combination using hESCs-derived neuronal cell model. This work was supported by the General Research Program (NRF- 2012M3A9C7050138) from the Ministry of Science, ICT.

In Vitro Skin Sensitization: Adapted Prediction Model of the U937 Cell Line Activation-Validated (U-SENS) Test Method

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Abstract 2744 SoT 2017

Score 3 (Experimental validation of U-SENS test (approved by EURL-ECVAM))

Abstract

The currently agreed immunotoxicological view of the chemical and biological mechanisms associated with skin sensitization has been summarized in the form of an OECD Adverse Outcome Pathway (AOP), starting with the molecular initiating event through intermediate events to the adverse effect, namely allergic contact dermatitis. In this instance, the molecular initiating event (i.e. the first key event) is the covalent binding of electrophilic substances to nucleophilic centers in skin proteins. The second key event in this AOP takes place in the keratinocytes and includes inflammatory responses. The third key event is the activation of dendritic cells (DC), typically assessed by expression of specific cell surface markers, chemokines and cytokines. The U-SENS method is proposed to address the third key event of the AOP by quantifying the change in the expression of a cell surface marker associated with the process of activation of monocytes and DC (i.e. CD86), in the human histiocytic lymphoma cell line U937, following exposure to sensitizers. The measured expression level of CD86 cell surface marker in the cell line U937 was then used for supporting the discrimination between skin sensitizers and non-sensitizers. The present study presents the transferability and reliability of the U-SENS in 4 laboratories (38 tested chemicals) and the predictivity on 175 chemicals using the adapted prediction model resulting of the EURL-ECVAM independently peer review and acceptance. The results showed that the level of reproducibility in predictions is 91% and 84% within and between laboratories, respectively. For the 38 chemicals, the predictivity capacity is 92% with a sensitivity of 99% and a specificity of 88%. Evaluation of the large set of 175 chemicals indicates that, compared with human and LLNA results, the accuracy in distinguishing skin sensitizers (i.e. UN GHS Cat.1) from non-sensitizers is in order of 86% with a sensitivity of 90% and a specificity of 71%. The U-SENS test method meets all regulatory instance criteria (method recently validated by EURL ECVAM) allowing its integration in an OECD Test Guideline (under the member states commented round) and into the corresponding OECD IATA.

Comparative Study of Ocular Irritancy between SIRC and HCE-T Using Short Time Exposure Test (STE) and the Effect of Polyol on Corneal Cells

S. Park, S.-A. Cho, J. H. Lee, and S. An.

Amorepacific, Yongin, Korea, Republic of. Sponsor: S.-A. Cho.

Abstract 2751 SoT 2017

Score 3 (Development of in vitro ocular irritancy test using human-derived cell line rather than rabbit-derived cell line)

Abstract

Since March 2013, using animal for testing final cosmetics has banned by EU. Therefore, many alternative test methods for testing toxicity of cosmetic ingredients and products have been developed and announced as OECD guideline.

Short time exposure test (STE), one of the alternative eye irritations tests, is cytotoxicity test using physiological saline or mineral oil as the test solvent and has a typical exposure condition of short time (5min). Because of these properties, it is known that this test method has several advantages for providing more similar exposure situation than other methods, easy to perform and high through screening (HTS). But SIRC(Rabbit corneal cell line) cells which are not human origin, so we studied the applicability of the Short time exposure test (STE) using HCE-T (Human corneal cell line) cells that are human origin. the results using HCE-T cells showed high correlations with the results using SIRC cells. Eye irritation categories of reference chemicals were same in both of two methods. Also, we studied the effect of polyol on corneal cell using STE. In HET-CAM test(Hen's Egg Test - Chorioallantoic Membrane), polyol showed false-positive result compared to rabbit draize test. For this reason, the cosmetic product that have polyol showed the higher ocular irritancy. So we compared irritation score of products which contain polyol and products without polyol using STE. The results show that eye irritation between Develops outline of AOP for inhalation toxicity and framework for n vitro test method development Score 3

A Mechanistic Approach Using Adverse Outcome Pathways (AOPs) to Aid Design of In Vitro Inhalation Testing

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Abstract 2826 SoT 2017

Score 3 (Develops outline of AOP for inhalation toxicity and framework for in vitro test method development)

Abstract

Testing approaches that frame mechanistic data within human toxicity pathways are needed to better understand observational toxicity data, accurately estimate the probability that a chemical will cause harmful effects, and effectively design substances that are less hazardous to humans. The route of exposure is an especially important consideration when evaluating potential toxicity. The inhalation route of exposure is particularly challenging to characterize as the respiratory tract is a complex portal-of-entry for both delivery and toxicity of chemicals. Here we demonstrate an approach using the adverse outcome pathways (AOPs) framework as a mechanistic scaffold to identify critical determinants of toxicity and thereby aid the design of intelligent testing strategies for various inhaled hazardous air pollutants (HAPs). The approach is applied to two HAPs (chlorine and vinyl chloride) with dramatically different physicochemical properties and proposed AOPs. Various extant inhalation toxicity data (human, animal, ex vivo, and in vitro) were collated, curated, and categorized according to their reliability to describe critical key events (KE) within each AOP. The degree of characterization for the relevant toxicokinetic or toxicodynamic processes, which determine the concentration and duration dependencies of the observed LIKE in the AOP, were also evaluated. These characterizations combined with chemical property information helped to identify data gaps along the AOP. These data gaps, together with the coherency, concordance and strength of the KE relationships from various data sources are used to describe the relevancy of the AOP and thereby also target strategies for in vitro and in silico testing. Thus, this approach uses the AOP as a mechanistic

scaffold for generalizing and applying these observationsto testing strategies for similar HAPs. Aided by this approach, such strategies will provide human-relevant mechanistic information that can be used to better understand human health and advance the application of in vitro data to inform assessment of potential inhalation toxicity. (The views in this abstract are those of the authors and do not represent the views or policies of the US EPA). two groups is same irritancy. It means effect of polyol about eye irritation is very slight using STE test and moreover, this method could reduce the false positive irritancy of polyol in HET-CAM assay. These results suggest that STE test using HCE-T cells could be a good model for the evaluation of eye irritation of human and good method for testing products containing polyol.

Human Mesenchymal Stem Cell Cultures to Model Teratogen-Induced Limb Malformations

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Abstract 3150 SoT 2017

Score 3 (Development of human in vitro model for teratogenicity)

Abstract

Embryonic limb mesenchyme high density micromass culture is an excellent system for studying skeletal tissue development in vitro. We are developing micromass cultures with human adult bone marrow-derived mesenchymal stem cells (hBMSC) that undergo biological processes specific to different stages of limb development. In this study, we first characterize development of static chondrogenic and hypertrophic cultures as well as mechanically stimulated cultures to model joint segmentation. Second we challenge static cultures with toxicants of known and suspected teratogenicity, reporting on the effects of valproic acid and mifepristone. These two drugs were chosen to test the possibility that hBMSC-based cultures may be used to study the effects of teratogenic compounds on limb development. Static chondrogenic and hypertrophic hBMSCs micromass cultures were induced to undergo differentiation with TGFb1 or triiodothyroxin, respectively. Joint segmentation was modeled through 20 degree flexion of cultures at 1 Hz for 4 hours per day. Gene expression and matrix remodelling markers characteristic of each process were assayed by RT-PCR, immunohistochemistry and matrix elaboration and remodeling. Candidate teratogens were added throughout the culture period and the effect on stage-specific developmental markers was assayed as a function of dose. Control micromass cultures demonstrated stage specific differentiation: chondrogenic cultures showed strong Alcian Blue staining, high COLII and AGN expression; hypertrophic cultures showed robust cell hypertrophy, alkaline phosphatase activity and MMP13 and OCN expression; and joint segmentation cultures showed reduced chondrogenesis and transient indications of joint-specific gene expression such as GDF5. Valproic acid significantly reduced chondrogenesis and subsequent hypertrophy, evident at day 10 and 14, respectively. Hypertrophy was more susceptible to mifepristone exposure than chondrogenesis, consistent with mifepristone function as a glucocorticoid inhibitor. On-going experiments are focused on correlating changes in biologicalprocesses with changes in suspected mechanistic targets. We conclude that high density, 3-dimensional hBMSC-based micromass cultures may represent a reproducible and controlled model to screen for potential limb teratogens.

The use of human (non-3D equivalent) skin assays (Skimune™) for the detection of adverse reactions, potency and efficacy

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Abstract 8 EUSAAT, Linz, 2016

Score 2 + 1 (New assay for skin sensitisation and other allergic/hypersensitivity responses + 1 for use as preclinical immunomodulatory/allergenicity test of systemic drugs)

Abstract

The current most favorable method to test compounds for allergenicity is the mouse local lymph node assay (LLNA). However, recent changes in EU legislation have banned animal testing on cosmetics. A number of alternative predictive test methods for the identification of compounds with the potential to cause skin sensitization are available but are inappropriate for assessment of relative potency. Alcyomics has developed human in vitro skin explant assays (Skimune™) as an alternative to the use of animal models. The assays can be used as diagnostic tools for the pharmaceutical, cosmetic and chemical industries for the testing of drugs, novel compounds or monoclonal antibodies for potential allergic or hypersensitivity reactions. The Skimune™ assays have been evaluated against the LLNA, with 95% concordance ($P < 0.001$ sensitivity 95%, specificity 95%) and human sensitisation data ($P < 0.001$ sensitivity 96%, specificity 95%) showing that it is a reliable tool for safety, potency and toxicity testing and also successfully identifies chemicals which have been shown to be negative in the LLNA but positive in man e.g. nickel sulphate.

The Skimune™ technologies can also test the efficacy of novel immunomodulatory drugs or monoclonal antibodies, as well as potential allergic responses, before use in Phase I clinical trials.

We have shown that the Skimune™ assays could have predicted and therefore prevented the TGN1412, Northwick Park incidence. The Skimune™ assays bridge the gap between animal- to-man studies and overcome interspecies barriers which often prevent detection of adverse effects during safety testing.

The assays use a human autologous system to test for sensitivity and adverse reactions, in which activity is measured as histopathological grading of skin damage, caused by induced immune responses, which correlate with T cell proliferation and IFN-γ production. The data demonstrate that the Skimune™ technologies provide novel and reliable approaches to the determination of skin sensitization, potency assessment, drug or monoclonal antibody evaluation and efficacy testing and can be used as a first step in the risk assessment process.

Reference

Ahmed, S. S., Wang, X. N., Fielding, M. et al. (2016). J Appl Toxicol 36, 669-684. http://dx.doi.org/10.1002/jat.3197

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A new validated in vitro skin sensitization test: SENS-IS

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Abstract 49 EUSAAT, Linz, 2016

Score 2 + 1 (Additional in vitro skin sensitisation assay + 1 for giving quantitative response like LLNA)

Abstract

The SENS-IS assay previously developed by Immunosearch [1] is a new approach based on are constructed human skin model (Episkin) as test system and on the RT-PCR analysis of the expression of 62 biomarkers relevant to the considered biological processes.

Briefly, the test item is applied on the skin model at 4 different concentrations (50%, 10%; 1% and 0.1%) in the appropriate vehicle. After exposure of the test item, gene expression of three groups of genes is measured: one group (REDOX group) includes a selection of 17 genes that have an antioxidant responsive element (ARE) in their promoter and monitor the redox protective signals induced through the interaction of sensitizers binding to cysteine amino acids of the Keap1-NRF2 complex [2]. The second group (SENS-IS group) includes a selection of 21 genes involved in inflammation and cell migration to address the complex cascade of events leading to activation of DCs by a sensitizing chemical. A third group is involved in irritation signals by measuring the expression level of 23 genes. This combination attempts to reproduce the human skin situation and aims to deliver a detailed analysis of the skin response to the stress induced by the exposure to a test chemical or a mixture of ingredients. To evaluate both hazard and potency, 4 dilutions (50%, 10%; 1% and 0.1%) of the test chemical are applied on the skin model in an appropriate vehicle. The prediction model is based on the number of over-expressed genes according to the following rules: if at a given test concentration a compound induces at least 7 genes in either the REDOX or SENS-IS group of genes, it is classified as a sensitizer. Potency, using the same classification as in the LLNA, is deduced through the minimal concentration needed to induce the over-expression of ≥7 genes in the REDOX or SENSIS groups. A compound is classified as an extreme, a strong, a moderate or a weak sensitizer if detected as a sensitizer at a test concentration of 0.1%, 1%, 10% or 50% respectively. While most of the currently available assays for the sensitization evaluation gives only a qualitative response, sens-is is the only test giving a quantitative response correlated with the murine local lymph node assay (LLNA; OECD TG 429). Its capacity to predict hazard is excellent [3] as demonstrated by Cooper statistics values over 95% on a large panel of chemicals

References

[1] Cottrez, F., Boitel, E., Auriault, C. et al. (2015). Toxicol In Vitro 29, 787-802.

[2] Uruno, A. and Motohashi, H. (2011). Nitric Oxide 25, 153-160.

[3] Cottrez, F., Boite, E., Ourlin, J-C. et al. (2016). Toxicol In Vitro 32, 248-260.

The "BINACLE" (binding and cleavage) assay allows in vitro determination of botulinum and tetanus neurotoxin activity

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Abstract102 EUSAAT, Linz, 2016

Score 3 + 1 (New assay for safety testing of tetanus vaccines and botox, now in validation + 1 for impact)

Abstract

The extremely potent neurotoxins produced by Clostridium botulinum and Clostridium tetani show high similarities with regard to their structure and mode of action. They consist of two disulfide-linked subunits: a heavy chain mediating the binding to specific receptors on neurons and a light chain displaying a metalloprotease activity. Following receptor binding and translocation of the toxin into the neuron, these proteases specifically cleave proteins required for the release of neurotransmitters from the synaptic vesicles. Depending on the preferred type of target neuron, the resulting inhibition of neurotransmitter release causes either severe muscle spasms (tetanus) or a flaccid paralysis (botulism).

Methods for the activity determination of these toxins are prescribed by the European Pharmacopoeia for the following applications: – Tetanus vaccines contain tetanus neurotoxin (TeNT) which has been chemically inactivated. In order to ensure an adequate detoxification, each batch of the inactivated material must be tested for residual toxicity. Up to now, this is exclusively performed by in vivo toxicity testing in guinea pigs. – Increasing amounts of the botulinum neurotoxin (BoNT) serotypes BoNT/A and BoNT/B are produced for pharmaceutical and cosmetic purposes. Due to the high toxicity of these proteins, these preparations have to be thoroughly tested for their potency. Although several animal-free methods have been described, no generally accepted method applicable to all relevant BoNT products is available to date. Accordingly, very high numbers of botulinum potency tests are still performed in vivo. In Germany, for example, almost 50000 mice were used in 2015 for this purpose [1].

Reliable alternative methods for the activity determination of these toxins should mimic as closely as possible their natural mode of action. We have developed a binding and cleavage ("BINACLE")-assay, which is based on the receptor-binding preferences and on the proteolytic activities of the respective toxins: In the first step, active toxin molecules are bound to their specific receptor molecules which were immobilized on a microtiter plate. Then the toxin's protease domain is released by chemical reduction and transferred to a second microtiter plate containing a specific substrate protein. Finally, cleaved substrate molecules are detected using antibodies. By taking into account the two most important characteristics of the toxins, this in vitro assay format offers a very high specificity combined with a good sensitivity.

The BINACLE assay for the detection of residual TeNT [2- 5] is currently being validated in an international collaborative study, which is an important step for promoting both the acceptance of the method by manufacturers and regulators and the implementation of the method in the European Pharmacopoeia. For BoNT/B, a BINACLE assay has recently been developed, and its applicability for a pharmaceutical product has been demonstrated [6]. A BINACLE assay for the potency determination of BoNT/A has also been developed and is now entering the validation phase.

References

[1] German Federal Institute for Risk Assessment, 2016. AnimalTestInfo. http://www.animaltestinfo.de . Accessed 31 May 2016.

[2] Behrensdorf-Nicol, H. A., Bonifas, U., Kegel, B. et al.(2010). Toxicol In Vitro 24, 988-994.

[3] Behrensdorf-Nicol, H. A., Bonifas, U., Hanschmann, K.-M.et al. (2013). Vaccine 31, 6247-6253.

[4] Behrensdorf-Nicol, H. A., Bonifas, U., Isbrucker, R. et al. (2014). Biologicals 42, 199-204.

[5] Behrensdorf-Nicol, H. A., Weisser, K. and Krämer, B. (2015). ALTEX 32, 137-142.

[6] Wild, E., Bonifas, U., Klimek, J. et al. (2016). Toxicol In Vitro 34, 97-104.

GARD - the future of sensitization testing using a genomics-based platform

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Abstract 91 EUSAAT, Linz, 2016

Score 3 (Early validation of assay for sensitisation testing – skin & lung)

Abstract

Chemicals able to induce sensitization in exposed individuals are commonly referred to as chemical sensitizers. Depending on the route of exposure and chemical properties, sensitizers give rise to clinical symptoms known as Allergic contact dermatitis (ACD) or Occupational asthma (OA). ACD is an inflammatory skin disease caused by immunological responses towards chemical skin sensitizers, affecting close to 20% of western population. While OA is not as prevalent, the socioeconomic effects are considered substantial, as health implications are severe.

In order to prevent adverse effects of consumer products and in occupational environments, chemicals are tested for sensitizing properties. Historically, such testing has been performed using animal-based in vivo assays, primarily the murine local lymph node assay (LLNA). Due to new legislations on the registration and use of chemicals, a ban on the use of animals within cosmetics industry, as well as efforts stimulated by public opinion and economic interests, recent years have seen a surge of development of alternative methods for assessment of chemical sensitizers. To date, three in vitro methods for assessment of skin sensitizers have been validated. However, while relevant in the perspective of an Adverse Outcome Pathway (AOP), the predictive performance is inadequate for accurate safety assessment as stand-alone tests, and an integrated testing strategy based on these methods is still to be finalized.

We have developed a novel in vitro testing platform, called Genomic Allergen Rapid Detection – GARD [1], for the prediction of sensitizing chemicals, based on differential expression of disease-associated genomic biomarkers in human myeloid dendritic cell-like cells. By using panels of reference chemicals with known properties of the biological endpoint of interest, whole genome data sets are

created with microarray technology for biomarker discovery. Multivariate statistics and computational methods are then employed to find the most powerful genomic predictors for the endpoint of interest, using data-driven approaches.

To date, applications of the GARD platform have generated GARDskin, a novel, state of the art assay for the assessment of chemical skin sensitizers, exhibiting superior predictive performance compared to both in vivo and in vitro counterparts. the assay has been transferred from microarrays to a resource effective and easy to use technological platform [2], well suited for industrial screening, and is currently being validated for regulatory use. Similarly, GARDair is an adaptation of the GARD platform for assessment of chemical respiratory sensitizers [3]. The method is unique and highly required, as predictive assays for assessment of respiratory sensitizers are greatly underdeveloped, with no validated, or even widely accepted, in vivo or in vitro method currently in use. Here, we describe the development, scientific validation, applications and the current state of the GARD platform. The scientific rationale behind the use of genomic biomarker signatures are detailed, and linked to the AOP in a biological context, and to applications made possible through multivariate computational prediction models in a technological context. In conclusion, we argue that GARD is a next generation in vitro assay ready for industrial implementation.

References

[1] Johansson, H., Albrekt, A. S., Borrebaeck, C. A. K. And Lindstedt, M. (2013). The GARD assay for assessment of chemical skin sensitizers. Toxicol In Vitro 27, 1163-1169.

[2] Forreryd, A., Johansson, H., Albrekt, A. S. and Lindstedt, M. (2014). Evaluation of high throughput gene expression platforms using GARD – an assay for prediction of skin sensitization based on a genomic biomarker signature. BMC Genomics 15, 379[3] Forreryd, A., Johansson, H., Albrekt, A. S. et al. (2015). PLoS One 10, e0118808.

CON4EI: EpiOcular Eye Irritation Test (EIT)

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Abstract 127 EUSAAT, Linz, 2016

Score 3 (Early validation testing of in vitro eye irritation test)

Abstract

Assessment of the acute eye irritation potential is part of the international regulatory requirements for testing of chemicals. The objective of the CON4EI (CONsortium for in vitro Eye Irritation testing strategy) project is to develop tiered testing strategies for eye irritation assessment for all drivers of classification. For this, a set of 80 reference chemicals (38 liquids and 42 solids) was tested with eight different alternative methods. Here, the results obtained with reconstructed human cornea-like epithelium (RHCE) EpiOcular and the EpiOcular Eye Irritation Test (EIT) – adopted as OECD TG 492 – are shown.

The primary aim of this study was an evaluation of the performance of the test method to discriminate chemicals not requiring classification for serious eye damage/eye irritancy (No Category) from chemicals requiring classification and labelling (Category 1 and 2). In addition, the predictive capacity in terms of in vivo driver of classification was investigated. In a second step, it was investigated whether the EpiOcular EIT can be used as part of a tiered-testing strategy for eye irritation assessment. The chemicals were tested in two independent runs by MatTek IVLSL.

For the EpiOcular EIT, a sensitivity of 96.9% and specificity of 86.7% with an accuracy of 95% was obtained overall and for both runs separately (100% concordance). The results of this study seem promising with regard to the evaluation of inclusion of this test method in an integrated testing strategy for eye irritation assessment

This research is funded by CEFIC-LRI. We acknowledge Cosmetics Europe for their contribution in chemical selection.

Development of an in vitro inhalation toxicity test with potential regulatory applicability

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Abstract 199 EUSAAT, Linz, 2016

Score 3 but check no animal tests performed (New in vitro test for inhalation toxicity that performs better than current in vivo OECD TG test)

Abstract

Knowledge of acute inhalation toxicity and irritation potential is important for establishing safe handling, packaging, labeling, transport and emergency response procedures for chemicals. The US EPA High Production Volume Chemical Challenge, and the EU REACH programs have further increased the need for inhalation toxicity information [1,2,3].

A UN treaty endorsed by the US, EU and others outlines a "Globally Harmonized System" (GHS) of Classification and Labeling of Chemicals [4]. The GHS specifies 5 inhalation toxicity categories. The EPA has established a separate system that uses 4 toxicity categories.

Acute inhalation toxicity tests currently accepted within the GHS and EPA systems involve in vivo 4 hr rat inhalation LC50 tests (OECD TG 403/436). In the current work, a newly developed in vitro toxicity test was evaluated in comparison to the established in vivo tests [5]. The in vitro test exposes an organotypic human airway tissue model (EpiAirway) to test chemicals for 3 hrs, followed by

measurement of tissue viability (IC75). 64 chemicals covering a broad range of toxicity classes, chemical structures and physical properties were evaluated. Results show that the in vivo and in vitro tests had 100% concordance for identifying highly toxic chemicals (GHS CAT 1-2 and EPA CAT I-II). However, the in vivo tests had only 35.3% (EPA system) or 73.1% (GHS system) sensitivity for identifying less toxic respiratory irritants. Numerous human respiratory irritants including acids, bases, aldehydes, amines and others were not classified as respiratory toxins/irritants by the in vivo tests.

The in vitro airway model was very good (sensitivity of 81.1 - 85.1%) for distinguishing respiratory toxins and irritants (corresponding to GHS 1-3, EPA, I-III) from non-toxins, non-irritants (corresponding to GHS 4-5, EPA IV). Overall accuracy of the in vitro test was 81.2 - 86.4%. There were no false negative GHS CAT 1-2 or EPA CAT I-II predictions using the in vitro test.

These data suggest that tests based on lethality in animals, while good for predicting highly toxic chemicals, produce a high percentage of false negative predictions for moderately/ slightly toxic or irritating chemicals. The in vitro test using an organotypic human airway model EpiAirway was equal to current animal tests for predicting highly toxic inhaled chemicals, and better than animal tests for predicting moderately/slightly toxic respiratory irritants. The new in vitro testing approach should provide improved protection of human health compared to the current animal tests.

References

[1] Arts, J. H., Muijser, H., Jonker, D. et al. (2008). Exp Toxicol Pathol 60, 125-133.

[2] Costa, D. L. (2008). Exp Toxicol Pathol 60, 105-109.

[3] Bakand, S., Winder, C., Khalil, C. and Hayes, A. (2005). Inhal Toxicol 17, 775-787.

[4] UN-United Nations (2005). Globally Harmonized System of Classification and Labeling of Chemicals GHS (537p, 1st revised edition). UN, New York and Geneva, ISBN 92-1-116927-5.

[5] Neilson, L. et al. (2015). Toxicol In Vitro 29, 1952-1962. http://dx.doi.org/10.1016/j.tiv.2015.05.018.

7. Conclusions

The review of the most recent EUSAAT and SoT conference proceedings, and an extensive literature search, yielded 44 abstracts describing projects which we believe the Judges should consider as potential candidates for the Lush Science Prize shortlist. These are given in Section 6.

Once again we have reviewed many more papers and abstracts than in the previous year (almost 3,500 this year compared with around 3,100 in 2016). We scored a similar number of abstracts this year as last (95 vs 85 in 2016). It was pleasing to find an even greater proportion of high scoring (3 and 4) abstracts this year; 46% compared with 34% in 2016.

The nominated abstracts are very diverse, and cover developing models for new toxicity assays, improvements in the phenotypic status of human iPSC-hepatocytes, high-throughput- and high content- screening, *in silico* analyses, and demonstrations of the utility of modern tt21d approaches. We believe that they are all worthy candidates for the 2017 Science Prize.