Lush Science Prize 2020
Background Paper

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1. Executive Summary

1.1 What is the Lush Science Prize?

The Lush Prize is a major initiative to stimulate worldwide research in animal-free 21st century toxicity testing of consumer products and ingredients. The global award - a fund of £250,000, which is the biggest prize in the non-animal testing sector - rewards the most effective groups and individuals working across science and campaigning towards the goal of replacing animals in product or ingredient safety testing, particularly in the area of toxicology research.

Now in its eighth prize cycle, the Lush Prize has given over £2 million to 110 winners in 28 countries since its inception in 2012. Originally held annually, since 2019 the Lush Prize has become a biennial event.

Prizes are awarded for developments in five strategic areas: science; lobbying; training; public awareness; and young researchers, thereby complementing the many projects already addressing the use of animals in medical testing.

In any prize cycle where there is 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 of up to the entire £250,000 fund can be awarded to the individual or team 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), in the preceding prize cycle, to the replacement of animal testing. In 2019, the Lush Prize team refocussed its science prize selection criteria towards rewarding those projects most likely to lead to practical non-animal tests which could be accepted by regulators. Of particular interest were investigations of adverse outcome pathways (AOPs), organs on chips (OoCs), and computational, or *in silico*, toxicology (aka computox). The Lush Prize team is also particularly interested in human-relevant adverse outcome pathways for systemic toxicology or developmental toxicology.

This 2019 Science Background paper identifies 19 pieces of work carried out by researchers whom we believe constitute potential candidates for the Judges' shortlist.

1.2 Methodology

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

In our search for candidate prize winners, we identified conferences focussing on animal replacement in toxicity testing that have been held in the preceding 18 months. For this year these were the 2019 Society of Toxicology (SoT) annual conference, the 2018 and 2019 EUSAAT conferences, and the 2019 EuroTox conference. There was a total of 3,121 abstracts from oral and poster presentations from these four conferences, but only 941 were relevant to the Lush Science prize. We then performed literature searches using PubMed to identify projects describing recent advances in toxicity testing research. Three further relevant abstracts were identified directly from

institutional websites. In all, searches yielded over 1,700 potentially relevant projects which we assessed as described in Section 3. Relevant abstracts were scored using a modification of the system derived in previous years. Now 3 points are awarded for projects identifying new AOPs, OoCs, or computox tools; 2 points for reporting new knowledge or tools for existing AOPs, OoCs, or computox tools; between 0 and 4 points are awarded for the apparent level of technology readiness; and 1 additional point is available for abstracts which stand out in some other way.

Overall, from 99 abstracts which scored 1 or more: 1 scored 1; 15 scored 2; 34 scored 3; 30 scored 4; 15 scored 5; and 4 scored 6. The full abstracts of those projects scoring 5 or 6 are provided in Section 6.4. All of the other abstracts which received a score are shown in the Appendix.

1.3 Projects recommended for the shortlist

There were 19 projects which received scores of at least 5 against the new scoring criteria for their potential to make major contributions towards providing practical non-animal tests which could be accepted by regulators. The full abstracts are given in Section 6.4. We consider all to be worthy for consideration by the judges as potential prize winners.

2. Background

The Lush Prize was established to support a range of initiatives to bring about the end of the use of animals in experiments[1]. One key element of project is the Science Prize, which aims to stimulate research using 21st century toxicology techniques to replace animal use[2]. 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.

For 2020 the Lush Prize has chosen to refocus its criteria for the Science Prize on projects that are most likely to lead to practical solutions that can replace animal tests as soon as possible and be accepted by regulators. The Lush Prize aims to focus attention on toxicity testing for consumer products and ingredients, in a way which complements those projects which address the use of animals in medical testing. The Lush Science Prize seeks to reward those researchers making 'outstanding contributions' to tt21c research. The most promising approaches which might achieve this are considered to be:

- Research aimed at elucidating adverse outcome pathways (AOPs) that describe the mechanistic steps by which a toxicant induces an adverse effect in a human;
- Developing new *in vitro* tools, known as organs-on-a-chip (OoCs, aka microphysiological systems (MPS)) that can fully replace animals in laboratory research and testing, and;
- The use of computational tools that can predict the likely hazard potential of chemicals without using animal tests.

The Lush Prize panel is particularly interested in supporting work that elucidates AOPs for the complex areas of systemic toxicology and developmental toxicology.

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

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

3. Methodology

The main aim of this paper is to assist the Lush Prize judging panel by identifying key projects that are making major contributions to the field of animal-free toxicology research. From these projects, the panel may choose to encourage nominations for the 2020 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 advance in the fields of interest (AOP, OoC, or *in silico* assay). 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 18 months preceding the preparation of this Background Paper (i.e. May 2018 – Nov 2019).

In the identification of key developments in the area of toxicology research, and in the search for candidate prize winners, we followed three separate strands of investigation. We started firstly by reviewing the recent research of some key institutions and collaborative projects working in the area of animal replacement in toxicity pathway research. These included the OECD; CAAT; ECVAM; UK NC3Rs; US Tox21 Programme; the ToxCast programme; the Human Toxicology Project Consortium; ICCVAM, the NIH, the EPA, the FDA, ESTIV, Cosmetics Europe, and EU-ToxRisk (see section 4 for highlights).

Secondly, we identified relevant conferences held in the preceding 18 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 2018 – 2019, for which abstracts were available, were the SoT 58th Annual Meeting[3] held in March 2019, in Baltimore, Maryland, the 2018[4] and 2019[5] EUSAAT conferences held in Linz, and the 2019 EuroTox[6] meeting held in Helsinki.

Of the 2,313 abstracts presented at the SoT meeting, only the 133 unique abstracts indexed under relevant headings in the Keyword Index were considered. The relevant Keywords from the SoT abstract book were: Adverse Outcome Pathways; AOP; Body-on-a-chip; Computational Toxicology; *In Silico*; Organ-Chip; and Predictive Toxicology. For the other conferences abstracts were not indexed by keyword or subject, so all abstracts were viewed and those relevant to the selection criteria were considered for scoring. For EUSAAT 2018 there were 270 abstracts, for EUSAAT 2019 there were

[3] https://www.toxicology.org/pubs/docs/Tox/2019Tox.pdf

[4] http://www.altex.ch/altex-proceedings/2-18-linz-eusaat

[5] http://www.altex.ch/altex-proceedings/1-19-linz-eusaat

[6] http://www.eurotox-congress.com/2019/_medien/_content/files/Vol_314S1_final3.pdf

229, and for EuroTox 2019 there were 309. Thus, there was a total of 941 conference abstracts considered for scoring.

Thirdly, we conducted a review of the recent literature. For this we used two separate sources. Firstly, we searched PubMed for research published from 01/05/2018 to 4/11/2019, combining search terms “Adverse Outcome Pathways,” “AOP”, “Organ on a Chip”, “Microphysiological System(s)”, “Computational Toxicology” and “*In Silico* Toxicology”. We restricted the subject matter to “humans” and excluded any review articles and clinical trials, and any papers for which abstracts were either not available or were not written in English. As a second literature source, we specifically reviewed all articles published in the ALTEX journal.

For published papers, our selection procedure was a three-stage process. At each stage of our search, research projects were carefully excluded based on our selection criteria, in order to achieve a manageable shortlist of excellent work which fully met the prize brief. In the first stage, we reviewed the title of the work, and rejected any which were clearly reviews or which were obviously unsuitable either through using animal models or through being overly focussed on a particular disease. 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 focussing on disease research and environmental pollutants (unless we felt that they additionally described a new AOP, OoC, or computox assay), and all research that included animal subjects. In the third stage, projects identified as potentially relevant based on the abstract were scored using a newly devised system which awarded points as described below. As for the previous year, because the conferences yielded a limited number of relevant abstracts and the conference abstract books presented titles and abstracts simultaneously, there was no merit to reviewing abstracts in the three stages. Thus, abstracts were either accepted or rejected for scoring and then scored in a single sweep.

In our new abstract scoring system, points are awarded according to the following criteria:

Does the work report a new AOP, OoC or computox method or assay with a clear and practical application ?	*Score 3*
If it is working with an apparently previously understood AOP, OoC or computox tool, does it offer significant development in the form of new knowledge or tools?	*Score 2*
How useful practically is the work? This will be dependent on its level of technology readiness	*Score 0-4*
Does the work stand out in some other way?	*Score 1*

For the 2020 Lush Science Prize there is a new focus on the practical application of reported work for replacing animal use in testing. With this in mind we have adapted the scoring system to include points for the level of practical usefulness that a piece of work appears to have reached. In awarding research grants to academics and industry, the EU uses the concept of ‘technology readiness level’ (TRL) to assess how well developed a particular idea is. The TRL system was originally developed by NASA to assess technology for its space programmes. Normally TRL scales have 9 levels, but we have simplified the concept to 5 levels, as depicted in the table below:

TRL assessment

TRL	NASA definition	Equivalence	Lush score
9	Proven in successful mission operation	Approved	4
8	Complete system tested successfully	External validation	3
7	System prototype demonstration		
6	System/subsystem prototype demonstration	In-house validation	2
5	Component/breadboard validation in field		
4	Component/breadboard validation in lab	Proof-of-principle	1
3	Function/characteristic proof-of-concept		
2	Technical concept	Pilot study	0
1	Basic principles observed/reported		

The maximum score a piece of work could possibly achieve is 8 points:

3 points for a new advance

4 points for practical usefulness (ie approved for use by regulators)

1 point for standing out in some other way.

8 points maximum.

In reality, a piece of work in this report is unlikely to be able to score 3 points for a new advance and 4 points for practical usefulness, because a new advance needs to be validated before regulatory approval and this takes some time. So the realistic maximum likely score is 7.

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 since the last Lush Prize Science Background paper was prepared.

4.1 National Toxicology Program

The NTP is a US Government inter-agency programme responsible for evaluating and reporting on toxicology activities within US public agencies. It co-ordinates several committees and programmes, some of which are highlighted here.

4.1.1 Tox21

Now in its Phase III, the Tox21 is a collaboration between the NTP, the FDA (Food and Drug Administration), and the National Centers for Computational Toxicology (part of the Environmental protection Agency (EPA)) and for Advancing Translational Sciences (NCATS).

Tox21 Phase III includes a focus on mid- to high-throughput gene expression screens using a variety of normal human cells and cell lines. These screens capture information from the whole transcriptome (i.e., the entirety of all expressed RNA molecules in a cell or biological sample) to gain insight into how biological systems respond to substance exposures. The transcriptome of a biological system is dynamic, changing in composition in response to various factors. As such, the transcriptome can be considered a read-out of the physiological or pathophysiological status of a biological system.

One approach in this project is referred to as the "S1500+ Gene Set High-Throughput Transcriptomics Project"[7]. Under the S1500+ Gene Set Strategy, the Tox21 Working Group developed and used a hybrid approach comprising five sequential modules to identify the optimal set of genes that best represents biological diversity, addresses gene-gene co-expression relationships, and represents known pathways adequately. This hybrid approach accurately balances data-driven and knowledge-based evidence while allowing for performance assessment of the selected genes with respect to the gene set's ability to extrapolate whole transcriptome changes, both at the individual gene level and at the pathway level.

4.1.2 NICEATM

The NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) is an NTP office focussed on the development and evaluation of alternatives to animal use for chemical safety testing. In 2018, NICEATM ran a workshop to encourage the development of predictive models for acute oral toxicity. The output of the workshop was published in late 2018 and is discussed in Section 5 (see footnote 16).

4.1.3 ICCVAM

The Inter-agency Co-ordinating Committee on Validation of Alternative Methods (ICCVAM) is also co-ordinated by the NTP. ICCVAM has presented information and a

[7] https://ntp.niehs.nih.gov/whatwestudy/tox21/s1500/index.html

webinar on “Non-animal Approaches for Inhalation Toxicity Testing” in early 2019[8], and recently hosted a workshop with the aim of designing strategies to predict toxicity while avoiding animal tests.

4.2 NCATS

NCATS’ Tissue Chips programme continues, but now with a focus on disease modelling & efficacy testing (rather than drug safety). Funding is not at the high levels of 2012-2017 but is still several $millions per year. Current and future projects for funding include: clinical trial on a chip; blood-brain barrier on a chip; pain on a chip; and immune system on a chip.

4.3 FDA

The FDA is actively implementing the Predictive Toxicology Roadmap described in the previous Lush Prize Science Paper and it is now working to co-ordinate development of MPS to replace animals in drug testing. These activities include in-house research, as well as collaborations with commercial entities.

4.4 EPA

The Administrator of the EPA recently signed a directive to prioritize efforts to reduce animal testing. The EPA also announced $4.25 million in funding to five universities to research the development and use of alternative test methods and strategies that reduce, refine, and/or replace vertebrate animal testing.

In June 2018 the EPA published its final version of the Strategic Plan to Promote the Development and Implementation of Alternative Test Methods, as required under the Lautenberg Chemical Safety Act[9,10].

4.5 EURL-ECVAM

The EC Joint Research Centre (JRC, of which ECVAM is a part) launched an online survey in August 2019, to gather views on non-animal mechanistic approaches, the AOP framework, and the AOP Knowledgebase[11].

EURL ECVAM scientists contributed to special issue of Toxicology & Applied Pharmacology on developmental neurotoxicity. The paper on ‘Strategies to approve the regulatory assessment of developmental neurotoxicity using *in vitro* methods’, Bal-Price *et al* (2018) is discussed in Section 5 (see footnote 19).

Since November 2018 JRC scientists have been part of an international initiative developing principles and protocols for the consistent use of computational models in

[8] https://ntp.niehs.nih.gov/whatwestudy/niceatm/3rs-meetings/past-meetings/commprac-2019/commprac-2019.html?utm_source=direct&utm_medium=prod&utm_campaign=ntpgolinks&utm_term=commprac-2019

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

[10] https://www.epa.gov/sites/production/files/2018-06/documents/epa_alt_strat_plan_6-20-18_clean_final.pdf

[11] https://ec.europa.eu/jrc/en/science-update/adverse-outcome-pathways-have-your-say-and-shape-future-chemical-risk-assessment

chemical safety assessment to promote greater acceptance in regulatory applications. The *In Silico* Toxicology (IST) Protocol initiative is focussing on how results from computational methods should be generated, interpreted, assessed and documented with a view to increasing confidence in their use. In its first publication, the IST consortium describes the general framework of IST Protocols and how they can guide the integration of computational predictions with experimental data to support the assessment of a chemical for adverse health effects. The paper, by Myatt *et al,* is discussed in Section 5 (see footnote 15).

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

The OECD published its 'Guidance Document on Good *In Vitro* Method Practices (GIVIMP)' in December 2018[12]. In the past several decades, there has been a substantial increase in the availability of *in vitro* test methods for evaluating chemical safety in an international regulatory context. To foster confidence in *in vitro* alternatives to animal testing, the test methods and conditions under which data are generated must adhere to defined standards to ensure resulting data are rigorous and reproducible. The Guidance Document on GIVIMP for the development and implementation of *in vitro* methods for regulatory use in human safety assessment aims to help reduce the uncertainties in cell and tissue-based *in vitro* method-derived chemical safety predictions. GIVIMP provides guidance for test method developers and end users of resulting data on key elements of *in vitro* methods. GIVIMP tackles ten important aspects related to *in vitro* work: (1) Roles and responsibilities, (2) Quality considerations, (3) Facilities (4) Apparatus, material and reagents, (5) Test systems, (6) Test and reference/control items, (7) Standard operating procedures (SOPs), (8) Performance of the method, (9) Reporting of results, (10) Storage and retention of records and materials. This document should be essential reading for practitioners of *in vitro* cell culture, and an integral part of all cell biology training programmes.

A new *in vitro* Test Guideline (TG 494) for identifying compounds as not requiring classification and labelling for eye irritation or serious eye damage, using a human corneal epithelia model, was published in June 2019.

[12] https://www.oecd-ilibrary.org/environment/guidance-document-on-good-in-vitro-method-practices-givimp_9789264304796-en

5. Literature Highlights

Some of the work that we reviewed in our search for potential Lush Science Prize nominees was not eligible for consideration for an award, 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 the 60th anniversary year of the publication of Russell & Burch's seminal book "The Principles of Humane Experimental Technique", Herrmann & colleagues[13] have made a call for the replacement of all animals in biomedical research. This exciting paper calls for the advancement of a 1R, replacement, approach, and its implementation not just in safety testing, but in all biomedical research, to improve its relevance to human biology. They propose the pursuit of a three-pronged strategy that focuses on (1) advancing non-animal methods as replacements of animal experiments, (2) applying them to biomedical research, and (3) improving their relevance to human biology. They observe that "as academics and scientists, we feel that educational efforts targeted at young scientists in training will be an effective and sustainable way to advance this vision". They go on to say, "Our strategy may not promise an imminent end to the use of animals in science, but it will bring us closer to an era in which the 3Rs are increasingly perceived as a solution to a receding problem".

Scientists from the EC JRC collected and analysed mechanistic information on the effects of chemicals on eight organs identified as relevant for acute systemic toxicity in humans[14]. This knowledge is expected to support the development and application of AOPs and mechanistically relevant new approach methodologies. Currently, there is an incomplete mechanistic understanding of the key acute toxicity pathways in humans, some of which are specific for different cell types (e.g. neuronal, cardiac, liver or kidney), while others are widely applicable (e.g. general cytotoxicity). Therefore, improving the knowledge of the numerous mechanisms involved would be useful to developers of test methods and other predictive tools, as well as to validation and regulatory bodies. The authors analysed the relevant literature and confirmed that general cytotoxicity is an important determinant of acute systemic toxicity. While the nervous and the cardiovascular systems are the most frequent targets of toxic chemicals, no clear pattern was found for which specific mechanisms of target organ toxicity might be representative of compounds in different potency classes.

Myatt et al report a major collaborative effort (more than 50 participants)[15] to survey applications of *in silico* toxicology approaches across several industries. They highlighted the need to develop standardised protocols for toxicity-related predictions. The article describes the information needed for protocols to support *in silico* predictions for major toxicological endpoints of concern.

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[13] Herrmann *et al* (2019). Beyond the 3Rs: Expanding the use of human-relevant replacement methods in biomedical research. ALTEX **36**(3), 343-352. doi.org/10.14573/altex.1907031

[14] Prieto *et al* (2019). Investigating cell type specific mechanisms contributing to acute oral toxicity. ALTEX **36**(1), 39-64. doi:10.14573/altex.1805181

[15] Myatt *et al* (2018). In silico toxicology protocols. Reg. Toxicol. Pharmacol, **96**, 1-17. doi: 10.1016/j.yrtph.2018.04.014

Other projects in the *in silico* toxicology field include a workshop on predictive methods for acute oral toxicity, reported by Kleinstreuer et al[16]. This international workshop was organised by NICEATM and included representatives from industry, academia, and regulatory authorities. Prior to the workshop, interested parties were supplied with a comprehensive set of training chemicals from which to build their predictive models, and a test set of chemicals for evaluation of each models' predictive performance. The organising committee of the workshop evaluated the modelled predictions against each models' performance in ranking the test chemicals, using OECD Validation Principles. At the workshop, computational modelers and regulatory decision makers met to discuss the feasibility of using predictive model outputs for regulatory use in lieu of acute oral systemic toxicity testing. The models were combined to yield consensus predictions which demonstrated excellent performance when compared with the animal data. Workshop outcomes and follow-up activities to make these tools available and put them into practice were discussed. The specific performance outcomes of the predictive models were to be published separately.

In 2019, a joint German/Dutch-organised international workshop on the validation and regulatory acceptance of new approach methods[17] added to this discussion. Representatives from governmental institutes, regulatory agencies, industry, academia and animal welfare organizations discussed and provided recommendations for the development, validation, and implementation of innovative 3R approaches in regulatory toxicology. A more comprehensive evaluation of biological relevance, scientific validity, and regulatory purpose of new test methods and assessment strategies, together with case studies that provide practical experience with new approaches, were discussed as essential steps to build up the necessary confidence to facilitate regulatory acceptance.

In September 2018, a whole issue of Toxicology & Applied Pharmacology was dedicated to "Alternative Approaches to Developmental Neurotoxicity (DNT) Evaluation". The first article, by Fritsche et al[18], forms a consensus statement voicing the agreement of scientific stakeholders from regulatory agencies, academia, and industry that a new framework needs adopting for assessment of chemicals with the potential to disrupt brain development. An increased prevalence of neuro-developmental disorders in children has been observed that cannot solely be explained by genetics. Recently, pre- and postnatal exposure to environmental chemicals has been suspected as a causal factor. There is only very limited information on neurodevelopmental toxicity, leaving thousands of chemicals that are present in the environment with high uncertainty concerning their DNT potential. Closing this data gap with the current test guideline approach is not feasible, because the *in vivo* bioassays are far too resource-intensive in respect of time, money and animal use. A variety of *in vitro* methods is now available, that has the potential to close this data gap by permitting mode-of-action-based DNT testing, employing human stem cells-derived neuronal/glial models. *In vitro* DNT data, together with *in silico* approaches, will in the

[16] Kleinstreuer *et a*l (2018). Predictive models for acute oral systemic toxicity: A workshop to bridge the gap from research to regulation. Comput. Toxicol.*,* **8***,* 21-24. doi.org/10.1016/j.comtox.2018.08.002

[17] Burgdorf et al (2019). Workshop on the validation and regulatory acceptance of innovative 3R approaches in regulatory toxicology - Evolution versus revolution. Toxicol In Vitro. 2019 Sep;59:1-11. doi: 10.1016/j.tiv.2019.03.039

[18] Fritsche *et al* (2018) Consensus statement on the need for innovation, transition and implementation of developmental neurotoxicity (DNT) testing for regulatory purposes. *Tox Appl Pharmacol* **354**, 3-6. doi.org/10.1016/j.taap.2018.02.004

future allow development of predictive models for DNT effects. The ultimate application goals of these new approach methods for DNT testing are their usage for different regulatory purposes.

This theme is further advanced by a joint publication from the EC JRC and the OECD[19]. Anna Bal-Price and colleagues describe how currently, the identification of chemicals that have the potential to induce developmental neurotoxicity (DNT) is based solely on animal testing. Furthermore, for many chemicals there is no assessment on DNT potential, because testing is only triggered by either chemical structure-activity relationships, or by evidence of neurotoxicity in systemic acute or repeated dose toxicity studies. However, these triggers are rarely used and, in addition, do not always serve as reliable indicators of DNT, as they are generally based on observations in adult rodents. They conclude that there is a pressing need for developing alternative methodologies that can reliably support identification of DNT triggers, and more rapidly and cost-effectively support the identification and characterization of chemicals with DNT potential. They suggest that currently available cellular neuronal/glial models derived from human induced pluripotent stem cells (hiPSCs) should be used, as they allow evaluation of chemical impacts on key neuro-developmental processes by reproducing different windows of exposure during human brain development. A battery of DNT in vitro test methods derived from hiPSCs could generate valuable mechanistic data, speeding up the evaluation of thousands of compounds present in industrial, agricultural, and consumer products that lack safety data on DNT potential.

A t4 Workshop report on “Optimising drug discovery by Investigative Toxicology” was published in early 2019[20]. While this workshop focussed on drug discovery, its observations and outcomes were acknowledged to be relevant also to risk assessment in other chemical industries. The term ‘investigative toxicology’ was coined to describe non-regulatory toxicology research that is free to embrace new technologies, enhancing translational steps from *in silico* and *in vitro* to *in vivo* mechanistic understanding, to eventually predict human response. One major goal of Investigative Toxicology is improving preclinical decisions, which coincides with the concept of animal-free safety testing. The report represents a position paper for investigative toxicology based on the topics of - and discussions during - the workshop. It starts with a gap analysis, followed by a critical assessment of new technologies, and finishes by summarizing challenges, and presenting perspectives and recommendations.

[19] Bal-Price *et al* (2018). Strategies to improve the regulatory assessment of developmental neurotoxicity (DNT) using in vitro methods. Toxicol Appl Pharmacol. 2018 Sep 1;354:7-18. doi: 10.1016/j.taap.2018.02.008

[20] Beilmann et al (2019). Optimizing drug discovery by Investigative Toxicology: Current and future trends. ALTEX **36**(2), 289-313. doi: 10.14573/altex.1808181

6. Candidate Toxicity Abstracts Identified for the Judges

6.1 Conference Abstract Selection

As described in the Methodology, we reviewed abstracts from the SoT 58th 2019 Annual Meeting, the EUSAAT 20018 and 2019 meetings, and the EuroTox 2018 meeting.

From the total of 2313 abstracts presented at the Society of Toxicology's 2019 meeting, 133 were identified as potentially relevant based on the Abstract book keyword index: 8 were identified by the keywords "Adverse Outcome Pathway" or "AOP', of which 3 were scored; 2 were identified by the keywords "Body-on-a-chip" or "Organ-chip", of which 1 was scored; 68 were identified by the keywords "computational toxicology" or "*In Silico*", of which 8 were scored; and 55 were identified by the keywords "Predictive toxicology", of which 5 were scored. There were 21 abstracts which were identified by more than one keyword search; these duplicates were counted just once. A total of 17 abstracts scored 1 or more.

The EUSAAT 2018 meeting had 270 abstracts, of which 8 received a score, while the 2019 meeting presented 229 abstracts, of which we scored 10. The EuroTox 2019 meeting had 309 abstracts and 7 were scored.

Thus, from a total of 941 reviewed abstracts, 42 received a score of 1 or more.

6.2 Published Abstract Selection

With the change in focus of the Lush Science Prize we identified many fewer articles of interest than in previous years (about 29% of the 2018 total). However, a much higher proportion of these abstracts described work worthy of receiving a score according to our criteria. From the PubMed search we identified a combined total of 772 articles: 368 relevant titles from the "Adverse Outcome Pathways" and "AOP" searches; a further 114 relevant projects from the "Organ on a chip" and "microphysiological system(s)" searches; and finally an additional 290 titles from the "Computational toxicity" and "*In Silico* toxicology" searches.

Stages 1 and 2 of the selection process (review of titles, and then abstracts, to reject review articles, articles not written in English, results of clinical trials, articles reporting use of animal subjects, or those overly focussed on cancer, or other disease, research, or environmental pollution) reduced the 772 titles by around 75%. Of the remainder, after review of abstracts in stage 3, 56 abstracts scored 1 or more (17 from the AOP searches, 27 from the OoC searches, and 12 from the Computox searches). This is three times the number of scoring abstracts from the PubMed searches in 2018.

We also identified 3 additional articles, which scored 1 or more, from our review of key institutions and projects.

6.3 Scores

From the three separate sources of potential shortlisted projects, we identified a total of 99 abstracts describing work which scored at least one point according to our given criteria. This is an increase of 50% in scoring abstracts from 2018.

As was intended, the change in the scoring system has provided us with a broader spread of scores. The distribution of scores was: 1 scored 1 for standing out in some way; 15 scored 2 for bringing new knowledge or tools to a previously identified AOP,

OoC model, or computox tool; 34 scored 3; 30 scored 4; 15 scored 5; and 4 abstracts scored 6 points. Six points were the most awarded for any one abstract.

The distribution of scores allowed us to select the top 20% (those scoring 5 or 6 points) for recommendation as potential Science Prize nominees. All 19 of these high-scoring abstracts are shown in full in Section 6.4. For abstracts of published papers, the DOI (digital object identifier) for that paper is provided. For conference abstracts, we give the abstract or poster number for identification – the conference abstract books can be obtained from the links provided in Section 3. The abstracts scoring up to 4 points are fully listed in the Appendix.

6.4 High Scoring Abstracts

This year 19 projects received the highest scores of either 5 or 6 for reporting new or significantly improved AOPs, *in silico* assays, or organ-on-a-chip models and demonstrating their practical potential with some level of validation. The 19 abstracts are given below.

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

Machine Learning of Toxicological Big Data Enables Read-Across Structure Activity Relationships (RASAR) Outperforming Animal Test Reproducibility.

T Luechtefeld*†, D Marsh†, C Rowlands‡, T Hartung*§.

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Total score 6; **Score 3** for new computational models for read-across + **2** for in-house validation + **1** for standing out for utilising large scale public databases as a source of information.

ABSTRACT

Earlier we created a chemical hazard database via natural language processing of dossiers submitted to the European Chemical Agency with approximately 10 000 chemicals. We identified repeat OECD guideline tests to establish reproducibility of acute oral and dermal toxicity, eye and skin irritation, mutagenicity and skin sensitization. Based on 350–700+ chemicals each, the probability that an OECD guideline animal test would output the same result in a repeat test was 78%–96% (sensitivity 50%–87%). An expanded database with more than 866 000 chemical properties/hazards was used as training data and to model health hazards and chemical properties. The constructed models automate and extend the read-across method of chemical classification. The novel models called RASARs (read-across structure activity relationship) use binary fingerprints and Jaccard distance to define chemical similarity. A large chemical similarity adjacency matrix is constructed from this similarity metric and is used to derive feature vectors for supervised learning. We show results on 9 health hazards from 2 kinds of RASARs—"Simple" and "Data Fusion". The "Simple" RASAR seeks to duplicate the traditional read-across method, predicting hazard from chemical analogs with known hazard data. The "Data Fusion" RASAR extends this concept by creating large feature vectors from all available property data rather than only the modeled hazard. Simple RASAR models tested in cross-validation achieve 70%–80% balanced accuracies with constraints on tested compounds. Cross validation of data fusion RASARs show balanced accuracies in the 80%–95% range across 9 health hazards with no constraints on tested compounds.

Differentiating Pathway-Specific From Nonspecific Effects in High-Throughput Toxicity Data: A Foundation for Prioritizing Adverse Outcome Pathway Development.

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***Total score 5*; Score 3** for accelerating the development of AOPs using unbiased analyses of ToxCast assay data, + **1** for proof of principle, + **1** for standing out by combining in silico tools with ToxCast data to develop new AOPs.

Abstract

The U.S. Environmental Protection Agency's ToxCast program has screened thousands of chemicals for biological activity, primarily using high-throughput in vitro bioassays. Adverse outcome pathways (AOPs) offer a means to link pathway-specific biological activities with potential apical effects relevant to risk assessors. Thus, efforts are underway to develop AOPs relevant to pathway-specific perturbations detected in ToxCast assays. Previous work identified a "cytotoxic burst" (CTB) phenomenon wherein large numbers of the ToxCast assays begin to respond at or near test chemical concentrations that elicit cytotoxicity, and a statistical approach to defining the bounds of the CTB was developed. To focus AOP development on the molecular targets corresponding to ToxCast assays indicating pathway-specific effects, we conducted a meta-analysis to identify which assays most frequently respond at concentrations below the CTB. A preliminary list of potentially important, target-specific assays was determined by ranking assays by the fraction of chemical hits below the CTB compared with the number of chemicals tested. Additional priority assays were identified using a diagnostic-odds-ratio approach which gives greater ranking to assays with high specificity but low responsivity. Combined, the two prioritization methods identified several novel targets (e.g., peripheral benzodiazepine and progesterone receptors) to prioritize for AOP development, and affirmed the importance of a number of existing AOPs aligned with ToxCast targets (e.g., thyroperoxidase, estrogen receptor, aromatase). The prioritization approaches did not appear to be influenced by inter-assay differences in chemical bioavailability. Furthermore, the outcomes were robust based on a variety of different parameters used to define the CTB.

Using 2D Structural Alerts to Define Chemical Categories for Molecular Initiating Events.

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Toxicol Sci. 2018 Sep 1;165(1):213-223. doi: 10.1093/toxsci/kfy144.

Total score 5; **Score 2** for developing tool to predict MIEs for chemical structure motifs, + **2** for in-house validation, + **1** for standing out by linking chemical structures to toxicity endpoints.

Abstract

Molecular initiating events (MIEs) are important concepts for in silico predictions. They can be used to link chemical characteristics to biological activity through an adverse outcome pathway (AOP). In this work, we capture chemical characteristics in 2D structural alerts, which are then used as models to predict MIEs. An automated procedure has been used to identify these alerts, and the chemical categories they define have been used to provide quantitative predictions for the activity of molecules that contain them. This has been done across a diverse group of 39 important pharmacological human targets using open source data. The alerts for each target combine into a model for that target, and these models are joined into a tool for MIE prediction with high average model performance (sensitivity = 82%, specificity = 93%, overall quality = 93%, Matthews correlation coefficient = 0.57). The result is substantially improved from our previous study (Allen, T. E. H., Goodman, J. M., Gutsell, S., and Russell, P. J. 2016. A history of the molecular initiating event. Chem. Res. Toxicol. 29, 2060-2070) for which the mean sensitivity for each target was only 58%. This tool provides the first step in an AOP-based risk assessment, linking chemical structure to toxicity endpoint.

Evaluation of Chemical Effects on Network Formation in Cortical Neurons Grown on Microelectrode Arrays.

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Toxicol Sci. 2019 Jun 1;169(2):436-455. doi: 10.1093/toxsci/kfz052.

Total score 5: **Score 3** for a new DNT in vitro assay, + **2** for in-house validation

Abstract

Thousands of chemicals to which humans are potentially exposed have not been evaluated for potential developmental neurotoxicity (DNT), driving efforts to develop a battery of in vitro screening approaches for DNT hazard. Here, 136 unique chemicals were evaluated for potential DNT hazard using a network formation assay (NFA) in cortical cells grown on microelectrode arrays. the effects of chemical exposure from 2 h postplating through 12 days in vitro (DIV) on network formation were evaluated at DIV 5, 7, 9, and 12, with cell viability assessed at DIV 12. Only 82 chemicals altered at least 1 network development parameter. Assay results were reproducible; 10 chemicals tested as biological replicates yielded qualitative results that were 100% concordant, with consistent potency values. Toxicological tipping points were determined for 58 chemicals and were similar to or lower than the lowest 50% effect concentrations (EC50) for all parameters. When EC50 and tipping point values from the NFA were compared to the range of potencies observed in ToxCast assays, the NFA EC50 values were less than the lower quartile for ToxCast assay potencies for a subset of chemicals, many of which are acutely neurotoxic in vivo. For 13 chemicals with available in vivo DNT data, estimated administered equivalent doses based on NFA results were similar to or lower than administered doses in vivo. Collectively, these results indicate that the NFA is sensitive to chemicals acting on nervous system function and will be a valuable contribution to an in vitro DNT screening battery.

Improvement of quantitative structure-activity relationship (QSAR) tools for predicting Ames mutagenicity: outcomes of the Ames/QSAR International Challenge Project.

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Total score 6; **Score 2** for improving Q(SAR) models of predicting mutagenicity via establishing a new database of chemicals, + **3** for externally validating a range of QSAR tools, + **1** for standing out by convening a competitive challenge to encourage QSAR tools developers to improve their products

Abstract

The International Conference on Harmonization (ICH) M7 guideline allows the use of in silico approaches for predicting Ames mutagenicity for the initial assessment of impurities in pharmaceuticals. This is the first international guideline that addresses the use of quantitative structure-activity relationship (QSAR) models in lieu of actual toxicological studies for human health assessment. Therefore, QSAR models for Ames mutagenicity now require higher predictive power for identifying mutagenic chemicals. To increase the predictive power of QSAR models, larger experimental datasets from reliable sources are required. The Division of Genetics and Mutagenesis, National Institute of Health Sciences (DGM/NIHS) of Japan recently established a unique proprietary Ames mutagenicity database containing 12140 new chemicals that have not been previously used for developing QSAR models. The DGM/NIHS provided this Ames database to QSAR vendors to validate and improve their QSAR tools. The Ames/QSAR International Challenge Project was initiated in 2014 with 12 QSAR vendors testing 17 QSAR tools

against these compounds in three phases. We now present the final results. All tools were considerably improved by participation in this project. Most tools achieved >50% sensitivity (positive prediction among all Ames positives) and predictive power (accuracy) was as high as 80%, almost equivalent to the inter-laboratory reproducibility of Ames tests. To further increase the predictive power of QSAR tools, accumulation of additional Ames test data is required as well as re-evaluation of some previous Ames test results. Indeed, some Ames-positive or Ames-negative chemicals may have previously been incorrectly classified because of methodological weakness, resulting in false-positive or false-negative predictions by QSAR tools. These incorrect data hamper prediction and are a source of noise in the development of QSAR models. It is thus essential to establish a large benchmark database consisting only of well-validated Ames test results to build more accurate QSAR models.

QSAR Modeling of ToxCast Assays Relevant to the Molecular Initiating Events of AOPs Leading to Hepatic Steatosis.

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J Chem Inf Model. 2018 Aug 27;58(8):1501-1517. doi: 10.1021/acs.jcim.8b00297. Epub 2018 Jul 26.

***Total score 5*; Score 3** for creation of models to predict MIEs for hepatic steatosis, + **1** for proof of principle, + **1** for standing out by using ToxCast data to predict MIEs

Abstract

Nonalcoholic hepatic steatosis is a worldwide epidemiological concern since it is among the most prominent hepatic diseases. Indeed, research in toxicology and epidemiology has gathered evidence that exposure to endocrine disruptors can perturb cellular homeostasis and cause this disease. Therefore, assessing the likelihood of a chemical to trigger hepatic steatosis is a matter of the utmost importance. However, systematic in vivo testing of all the chemicals humans are exposed to is not feasible for ethical and economical reasons. In this context, predicting the molecular initiating events (MIE) leading to hepatic steatosis by QSAR modeling is an issue of practical relevance in modern toxicology. In this article, we present QSAR models based on random forest classifiers and DRAGON molecular descriptors for the prediction of in vitro assays that are relevant to MIEs leading to hepatic steatosis. These assays were provided by the ToxCast program and proved to be predictive for the detection of chemical-induced steatosis. During the modeling process, special attention was paid to chemical and toxicological data curation. We adopted two modeling strategies (undersampling and balanced random forests) to develop robust QSAR models from unbalanced data sets. The two modeling approaches gave similar results in terms of predictivity, and most of the models satisfy a minimum percentage of correctly predicted chemicals equal to 75%. Finally, and most importantly, the developed models proved to be useful as an effective in silico screening test for hepatic steatosis.

Conference abstracts

SoT 2019

1074 **A Multi-Tissue Organotypic Human *In Vitro* Model for Rapid Hazard Identification of Environmental Chemicals and Mixtures**

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Total score 6; **Score 3** for new OoC assay, + **2** for in-house proof-of-principle, + **1** for standing out as comprehensive analysis for health hazard.

Abstract

Environmental disasters such as the flooding that impacted Houston, Texas after Hurricane Harvey may lead to the redistribution of contaminants from both sediment as well as industrial/hazardous waste sites, resulting in exposure and potential health risks to residents who live in close proximity. Traditional hazard identification methods are not suitable to determine the chemical composition and potential hazard of the exposures to hazardous mixtures after environmental emergency events. Therefore, new methods are urgently needed to enable faster responses to emergency events. This study aimed to test whether a panel of physiologically-relevant human cell-based models can serve as a rapid screening tool for evaluating potential health hazards of complex environmental mixtures. Forty-two known chemical contaminants from Superfund sites represent a diverse range of chemical classes (heavy metals, pesticides, industrial chemicals, polycyclic aromatic hydrocarbons and plasticizers) were used. In addition, 16 “designed” mixtures were prepared from these chemicals. Soil/sediment samples collected after Hurricane Harvey were also evaluated. We used human induced pluripotent stem cell (iPSC-derived) hepatocytes, neurons, endothelial cells, and cardiomyocytes, to evaluate concentration response effects on both physiological and cytotoxicity using high-content imaging. Point of departure values were derived and integrated using the Toxicological Prioritization Index (ToxPi). Our analysis revealed chemical class-based similarities among the 42 representative chemicals tested. Data integration in ToxPi further identified cell-type specific clustering patterns among different groups of individual chemicals. The data from 16 designed mixtures indicated that their effects were dose- and mixture-dependent. Environmental soil/sediment samples were also clustered into groups based on integrating bioactivity profiling with geographical distributions. In summary, we demonstrate the potential appli cability of rapid *in vitro* screening to group complex environmental mixtures for rapid hazard identification during environmental emergency events.

1325 **Development of a Neurotoxicity Assay That Is Tuned to Detect Mitochondrial Toxicants**

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Total score 5; **Score 2** for enhancing DNT assay, + **2** for in-house validation, + **1** for standing out as addressing mitochondrial toxicity.

Abstract

Mitochondrial toxicity is one of the major reasons for drug withdrawals. In particular, many neurotoxicants affect energy metabolism. Both animal models and cell-based test methods often fail to predict mitochondrial effects and neurotoxicity of chemicals reliably. Thus there is a need for improved sensitivity in this area. LUHMES cells are conditionally immortalized human neuronal precursors that can be quickly differentiated to fully mature dopaminergic neurons. They have been routinely used for the NeuriTox assay to screen and characterize neurotoxicants. Using this test method, cells have been exposed here to a panel of 30 test chemicals for 24 h. Data (cell viability and overall neurite area) were acquired, after live cell staining, by high content imaging microscopy coupled to a fully automated data processing pipeline. In parallel, metabolic data, such as ATP content, central carbon metabolite levels, mitochondrial oxygen consumption and lactate production were monitored. Experiments were run in medium, containing either 18 mM glucose or 18 mM galactose as main carbohydrate source. Mitochondrial toxicity was predicted by the ratio of the EC25 values (for neurite outgrowth) in glucose medium (cells little dependent on mitochondria) vs. galactose medium (cells highly dependent on mitochondrial function). The panel of test compounds contained at least 5 inhibitors for each mitochondrial respiratory chain (MRC) complex, and also chemicals not affecting mitochondrial respiration. We found that inhibitors of MRC complexes I, III, IV and V were detected more sensitively in

galactose medium, and their EC25 ratio (glucose/galactose) was >2. Uncouplers, complex II inhibitors and non-mitochondrial toxicants had EC25 ratios close to 1. Therefore, the modified NeuriTox assay represents a novel and more sensitive method to detect neurotoxicants. Moreover, it pinpoints chemicals that inhibit various functions of the MRC (except for complex II).

1329 **Developing Highly Accurate Computational Models for Neuronal Targets**

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Total score 5; **Score 3** for predictive in silico model of neurotox, + **2** for in-house validation.

Abstract.

The ability to mechanistically predict whether compounds will or will not target important protein receptor(s) is a major goal of toxicology. Thus, we sought to build such models for major neuronal targets. We mined public data sources (ToxCast, CHEMBL, BindingDB and ZINC), together with the scientific literature for compounds that did or did not interact with the nicotinic and muscarinic acetylcholine receptors, acetylcholinesterase, the GABA-A and B receptors as well as the serotonin and glycine cys-loop receptors. We developed machine-learning algorithms in KNIME using structural fingerprints and two-dimensional identification of common structural motifs (scaffolds) to screen compounds for interaction with these receptors. For targets with a sufficient number of active compounds, the fingerprint and scaffold-based prediction models were able to predict a positive outcome with high sensitivity (>80%). Exquisite sensitivity (98.4-100%) and balanced accuracy (82.6-100%) statistics were observed for data-rich targets such as the cholinergic system. The high sensitivity of these models correlated with high negative prediction values (84.7-99.8%), underscoring the confidence with which novel compounds can be aligned with these neuronal targets. By iteratively building our models with 1-90% of the compiled data, we show that <20% of data are needed to yield >75% balanced accuracy, suggesting that these models will not change for the foreseeable future. In conclusion, we demonstrate the feasibility of using computerized workflows to mine public data and develop accurate positive and negative prediction models for important neuronal targets. Due to their implementation within KNIME, these models can be used to rapidly screen in vivo datasets and provide mechanistic insights into the modes of action for substances of interest.

1768 **Computational Model for Inhibition of Mitochondrial Function**

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[2] Grand Valley State University, Grand Rapids, MI.

Total score 5; **Score 3** for new in silico tool to predict mitochondrial toxicity, + **2** for in-house validation.

Abstract

In eukaryotes, mitochondria play many life-sustaining roles, with their dysfunction linked to toxicity as well as numerous diseases. We present the development of a computational model that predicts whether a xenobiotic will or will not inhibit mitochondria, with some sub-target information (e.g., inhibition of Complexes I-V or uncoupling via protonophore action). We compiled public data on compounds that target the mitochondria together with associated control compounds that do not alter mitochondrial function. We employed molecular scaffolding and fingerprinting to identify conserved structural motifs and features associated with mitochondrial inhibitors versus controls. By using these

attributes as covariates within random-forest machine-learning models, we can identify mitochondrial inhibitors with high sensitivity (83%), specificity (79.3%) and accuracy (81%). The high negative predictive value (93.7%) of our model highlights confidence with which novel compounds can be flagged as mitochondrial agents. When tested against a database of therapeutics withdrawn from the market due to liver injury, our model rapidly identified those that had subsequently been shown to target mitochondria. Furthermore, we were able to identify two compounds that were recently identified as mitochondrial toxicants only via experimental testing, highlighting the utility of our model. Given its speed and ease of use, our profiler can be used in an integrated approach that complements in vitro mechanistic screening and as a covariate in models that address the etiology of complex biological endpoints. We will make our model publicly available to allow rapid assessment of compounds under development.

1861 **Cefic LRI AIMT-8: Prediction of STOT-RE Classification by New Approach Methodologies**

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[3] Molecular Networks GmbH, Nürnberg, Germany. Sponsor: C. Dasenbrock

Total score 5; **Score 3** for a new approach to assessing chronic specific organ toxicity, + **1** for proof of principle, + **1** for standing out by creating a practical NAM approach using existing data

Abstract

The AIMT-8 project aims to assess the ability of in vitro data from the Tox21 program to predict STOT-RE categories of a range of chemicals. The application of New Approach Methodologies (NAMs) in risk assessment is an area of intensive research. AIMT-8 aims to advance the understanding of the use of NAMs by analysing a different way of predicting systemic toxicity, especially the STOT-RE classification. STOT-RE classification is based on the NOAEL of the in vivo study and does not consider the type of effect or the organ affected. If prediction of STOT-RE classification by NAMs is possible, this will contribute to a paradigm shift in risk assessment and will motivate the use of NAMs in prioritisation and labelling, and eventually in safety assessment as well. STOT-RE classifications were gathered and derived from different sources e.g. from a set of 90 day studies with repeated oral exposure (extracted from the RepDose/ToxRef/Hess and Cosmos databases) and the inventory of harmonised classifications provided by ECHA. In parallel, we analysed the AC50 values from Tox21 and considered all values occurring at sub-cytotoxic concentrations. The spare data matrix of the 43 individual assays was aggregated to seven categories representing six toxicity pathways and cytotoxicity values. The intersection of both the in vivo and in vitro data resulted in a data set of 749 compounds. For later in vitro to in vivo extrapolation (IVIVE) relevant data such as plasma protein binding, renal and hepatic clearance were identified from existing data sets and the literature. Prior to in silico profiling, the structural information was quality controlled and corrected. From a range of statistical methods, k-nearest neighbours (kNN) and random forest (RF) approaches were selected for the development of the classification model. In addition, seventeen read-across groups were defined. The grouped compounds share structural characteristics and specific/unspecific in vivo apical findings/target organs. Groups were distinguished for which a shared toxicological effect pattern might be indicative of a shared mode of action from those with unspecific toxicological effects e.g. weight changes or no toxicological effects. In these read-across groups, we analysed the mechanistic links between the in vitro results and the in vivo apical endpoint leading to STOT-RE classification. Financial support for this work was provided by the CEFIC Long-Range Research Initiative (CEFIC LRI AIMT-8).

EUSAAT 2019

316. **3D CNS model of iPSCs derived neuron and glia for high-throughput neurotoxicity screening in Mimetas' OrganoPlate**

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***Total score 5*; Score 3** for the development of a CNS 3D model, + **2** for in-house validation

Abstract.

Prediction of neurotoxicity remains challenging due to the lack of relevant models of the human brain. Current neurotoxicity assessments rely heavily on expensive and time-consuming *ex vivo* and *in vivo* animal testing. They are not always predictive for human outcome and not amenable for high-throughput toxicity screening. Here, we describe the development of a human *in vitro* 3_D_ _CNS model in the OrganoPlate® _for neurotoxicity screening. Mimetas' OrganoPlate® _are microfluidic cell culture plates that enable culturing and screening of a range of miniaturized 3D organ and tissue models.

Human iPSCs derived neuron and glia are embedded in ECM and seeded into the OrganoPlate® _allowing formation of complex 3D neuronal network in 96 individual chips. Medium in the adjacent compartment provided the cultures of nutrients and growth factors by diffusion. This model supports the assessment of various neurotoxic endpoints in 3D and is applicable for functional readouts addressing physiological and morphological criteria that leads to improved neurotoxicity screening of compounds and safety assessment in early stages of drug development.

Complex network formation of neurons and astrocytes is seen within 24 hours and immunofluorescent staining confirms the presence of mature neurons, including GABAergic and glutamatergic sub-population and supporting astrocytes. Application of neurotoxicant, methylmercury, demonstrates concentration-dependent reduction in the integrity of the neuritis, and cytotoxic effect is further identified using mitochondrial toxicity assay and cell viability assay.

Spontaneous firing of the co-culture is seen after 4 days of culture using calcium imaging assay and changed upon stimulation of inhibitory and excitatory compounds like GABA and TTX. To further demonstrate the applicability of the CNS model for assessing seizure liability, we performed compound library screening of potential convulsant compounds. The burst pattern of each single detected neuron in the culture was extracted, including the number of bursts, burst intensity and burst duration. Exposure to the compounds show increased bursting pattern and induced synchronicity in the co-cultures, while anti-seizure compounds was able to inhibit the increased activity. This CNS-on-a-chip model provides promising usability of iPSCs derived neuronal co-culture models for screening purposes. This paves the way towards the development of a predictive and relevant brain model which could be used for high-throughput neurotoxicity studies, including seizure liability testing and can contribute to diminishing the use of animal models.

393 **Skin-on-a-chip-based skin irritation evaluation method as an alternative to animal testing**

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Total score 5; **Score 3** for the development of a skin on a chip + **2** for in-house validation

Abstract

Recently, the trend in animal testing needed for drug screening and etc. is transferring into applying alternative test methods, prohibiting animal testing throughout the world. When test substances were evaluated *in vivo*, Draize scores based on edema and erythema were used to classify irritants. With an expanding demands of non-animal tests method, many *in vitro* alternative methods have been developed and recommended to be used for classifying irritant and non-irritant substances. However, current *in vitro* models for evaluating skin irritation only measure cell viability. Thus, development of biochip was needed for mimicking *in vivo* skin irritation. After researching on both domestic and international trends in biochip, skin-on-a-chip-based irritation evaluation method was developed. The microfluidic device was fabricated using PDMS and porous membranes were placed between each layer to separate the chambers as demonstrated in previous study [1]. Skin on a chip consists of three layers for keratinocyte, fibroblast and endothelial cell culture, representing epidermal, dermal, and endothelial layers of human skin structure. Test substances were applied to the top chamber and the chip was evaluated for skin irritation hazard identification. Irritation evaluation method includes observing the cell viability and the tight junction. Cell viability was measured adapting the OECD TG439 protocol for chemical evaluation. Furthermore, cell-to-cell junctions, or tight junctions, in endothelial cells were observed and measured to assess physiological responses, such as edema, to chemicals. Total of 20 chemicals were evaluated using skin on a chip which was then compared with LLNA *in vivo* data. Comparison between *in vivo* data and skin on a chip method for evaluating chemicals resulted in 90% accuracy, 100% sensitivity, and 78% specificity for irritation evaluation. Therefore, developed skin on a chip could serve as a momentum for a cutting-edge integration of *in vitro* toxicity evaluation methods and the result of this research suggests that biochip could better represent *in vivo* or human physiology for evaluation of skin toxicity.

This research was supported by a grant (18182MFDS462) from Ministry of Food and Drug Safety in 2018 and 2019.

Reference

[1] Wufuer, M., Lee, G., Hur, W. et al. (2016). *Sci Rep 6*, 37471. doi: 10.1038/srep37471

480 **Integrating organ-on-a-chip devices on a multimodal, microfluidic platform**

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Total score 5; **Score 3** for the development of a device to allow standardisation development of chip devices, + **1** for proof of principle, + **1** for standing out by the inclusion of vasculature to connect compartments (see abstract 474 from KIT).

Abstract

Given the constant advances in chemical synthesis allowing the rapid and divers creation of new compounds and substance libraries, the research field of Tissue Engineering becomes increasingly important for drug discovery. Especially Organ-on-a-Chip devices can be regarded as a powerful tool to improve in vitro studies and reduce animal tests for high-throughput screenings. The use of human cells and extracellular matrix material and thus, reproducing organotypic functional units of the human

organism, is a major advantage of these models. Yet, due to a lack of automatization handling often is difficult and time consuming. We therefore present a multimodal microfluidic platform that was designed to cultivate the Organ-on-a-Chip device, established at KIT, the so-called vasQchips. Center of these chips is a curved, porous micro channel lined with endothelium and connected to microfluidic flow representing the blood stream. The pores of the scaffold can be adapted individually to each organ model and allow for the supply of nutrients and gases as well as for the exchange of growth factors or immune cells with the surrounding compartment. Several organ-models are being established and validated in this chip, including liver, blood-brain-barrier, skin or tumor environment and can be used for various applications. Although, the vasQchips are compatible to most standard pumps, cultivation and analysis was to be further improved by the development of the stated microfluidic platform. It will allow straightforward setups and standardized conditions for the experiments as well as a constant observation in the connected software. The platform itself consists of a micro annular gear pump, several miniature valves and is controlled via a connected touch display. This allows various microfluidic circuits and enables automated exchange of the culture medium controlled. Additionally, fluidic connections will allow sampling as well as the integration and exchange of sensors (e.g. O2, pH) via (mini) Luer locks. Having the dimensions of a multi-well plate, the platform can easily be combined with standard devices such as microscopes or fluorescence plate readers. With all these properties, we achieved to design a multimodal platform that both makes the cultivation of microfluidic tissue culture more convenient and accurate, and is also suitable for many ways of analysis to easily acquire all the data from the executed experiments.

519 **Human Artificial Lymph Node Model (HuALN) for biopharmaceutical testing and disease modelling *in vitro***

C Giese, A Lubitz

ProBioGen AG, Berlin, Germany

Total score 5; **Score 3** for the development of a lymph node/immune system 3D model, +**1** for proof-of-principle, + **1** for standing out as an early model of immune function

Abstract

As modern biopharmaceuticals show a very high degree of species-specificity, conventional animal models are inadequate for drug development and to assess efficacy and safety. The Mode of Action (MoA) is complex, so simplified cell culture models using human cells often fail. Customized animal models, e.g. immune deficient, transgenic, and humanized animals as well as xenograft models are big in business but they show limitations in reproducibility and relevance to a certain extent. In particular for the human immune system, drawbacks in the pharmaceutical arena during the last years have raised significant doubts about their value and predictability for assessing immune modulation and immunogenicity. New “humanoid” models are required for new promising pharmaceutical treatments, e.g. by immune modulators, checkpoint inhibitors, and cell- and gene therapeutics.

The dramatically increased animal consumption triggered by customized animal model technologies pushes the ethical concerns on animal testing for pharmaceutical R&D, efficacy and toxicity testing, and risk assessment.

The Human Artificial Lymph Node Model (HuALN) is a micro physiological system (MPS) mimicking immunity in a continuously perfused 3D culture system and suitable for long-term treatment (e.g. 28 d) and repeated dosing. The MPS serves as a human micro-organoid lymph node model for induction or modulation of cellular and humoral immune responses. The implementation of stromal cells improves organoid formation. The HuALN model is designed for testing immunomodulation (e.g. MoA of checkpoint modulators), to assess unwanted immunogenicity reactions (e.g. ADA formation, sensitization) or efficacy of vaccines, adjuvants and formulation. T cell responses and shifts in the

TH1/TH2 pathway are continuously monitored by cytokine secretion profiles. The induction of primary humoral responses is demonstrated by B cell activation, plasma cell formation and antibody secretion profiles for IgM and IgG. Cells can be harvested from 3D matrix at the end of the MPS culture time and used for flowcytometric analysis and functional tests, e.g. ELISPOT assays. By integration of tumour cells or tumour spheroids the HuALN platform is extended towards disease models.

The HuALN model will be introduced, selected results of biopharmaceutical testing will be presented and the opportunities using the HuALN for modelling tumour treatment will be discussed.

References

GR Langley, F Busquet. C Giese. et al. (2017). *Drug Discov Today 22*, 327-*339.*
U Marx, TB Andersson, C Giese, C. et al. (2016). *ALTEX 33*, 272-321.
C Giese, U Marx. (2014). *Adv Drug Deliv Rev 69-70*, 103- 122.

EUSAAT 2018

275 **3D models and multi-organ-chips: Scaffold-free 3D tissue models – from static to microphysiological applications**

P Guye, O Frei, R Kostadinova, B Yesildag, F Hürlimann, T Hejhal

InSphero, Schlieren, Switzerland.

***Total score 5*; Score 2** for adapting commercially available spheroids to micro-perfusion, + **2** for in-house validation (Abst 274 by the same authors details the validation), + **1** for standing out as being commercially available and easy to adopt

Abstract

Studying and understanding the etiologies of diseases, with the goal of developing novel therapeutic approaches translated into safe and efficient drugs, presents manifold challenges. In-vitro cell-based assays represent one of the key group of techniques and with the evolution of complex 3D tissue models receive more and more attention and a greater share in the drug discovery process. Routine implementation of novel in-vitro systems requires control over the sometimes complex tissue- and disease-relevant parameters and at the same time simple and robust methods for handling, experimentation and readout. This becomes particularly true when studying organ-organ interactions involving tissue models from different types in fluidic communication. Our new generation of readily available and screening-compatible 3D microtissues models are able to emulate the healthy and various diseased states of different organ models including human liver and pancreatic islets as well as a large set of tumors. Accessing the biology of the models in a reliable and reproducible way to a large extend depends on the platforms, in which the microtissues are cultured and handled in. We therefore specially engineered and matched our 96 and 384-well plates to the microtissue morphology considering easy, but highest quality optical inspection, reliable and efficient medium exchange and compound dosing preserving maximal functionality and allowing seamless integration into automation systems. Together with the uniform, functionally robust, and long-lived characteristics of the microtissues a complete screening platform for a wide set of efficacy and safety testing can be offered.

The next steps toward more complex in-vitro models includes the combinations of such advanced microtissues in a microphysiological system to study their interactions. We extended our technology platform by a microfluidic plate based on SBS standards, which enables culturing of the same microtissues also under physiological flow conditions, and with the flexibility to interconnect and culture different types of microtissues multi-tissue configurations. Up to 10 same or different microtissues can be interconnected and cultured in 8 identical or different conditions in parallel per plate. Providing continuity of the microtissue models enables maximal translatability between the different pre-clinical applications and control over the increasing model complexity along the drug discovery process.

277 **Application of human multi-organ-chips to enhance safety and efficacy assessment in drug discovery**

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Total score 5; **Score 3** for creating a commercially available 4 organ MoC, + **1** proof of principle, + **1** for standing out as being commercially available and easy to adopt

Abstract

Microphysiological systems have proven to be a powerful tool for recreating human tissue- and organ-like functions at research level. This provides the basis for the establishment of qualified preclinical assays with improved predictive power. Industrial adoption of microphysiological systems and respective assays is progressing slowly due to their complexity. In the first part of the presentation examples of established single-organ chip and two-organ chip solutions are highlighted. The underlying universal microfluidic Multi-Organ-Chip (MOC) platform of a size of a microscopic slide integrating an on-chip micro-pump and capable to interconnect different organ equivalents will be presented. The second part of the presentation focusses on the challenges to translate a MOC-based combination of four human organ equivalents into a highly predictive tool for ADME profiling and toxicity testing of drug candidates. This four-organ tissue chip combines intestine, liver and kidney equivalents for adsorption, metabolism and excretion, respectively. Furthermore, it provides an additional tissue culture compartment for a fourth organ equivalent, e.g. skin or neuronal tissue for extended toxicity testing. Issues to ensure long-term performance and industrial acceptance of such complex microphysiological systems, such as design criteria, tissue supply and on chip tissue homeostasis will be discussed.

References

S Bauer, C Wennberg Huldt, KP Kanebratt. et al. (2017). Sci Rep 7, 14620.

U Marx, TB Andersson, A Bahinski. et al. (2016). ALTEX 33, 272-321.

I Maschmeyer, AK Lorenz, K Schimek. et al. (2015). Lab Chip 15, 2688-2699.

I Maschmeyer, T Hasenberg, A Jaenicke. et al. (2015). Eur J Pharm Biopharm 95, 77-87.

S Sieber, L Wirth, N Cavak. et al. (2017). J Tissue Eng Regen Med 12, 479-489.

211 **Characterization of chemical metabolism in combined skin and liver models over extended and repeated exposure in a multi-organ chip device**

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Total score 6; **Score 2** for thoroughly assessing the functionality & practicality of a 2 organ MoC for skin & liver, + **3** for external validation, + **1** for standing out as being commercially available and easy to adopt

Abstract

New in vitro methods and testing strategies for animal-free toxicity testing are important to support risk assessment of potential new cosmetic ingredients. To guarantee consumer safety, Cosmetics Europe is committed to endorse and actively contribute to the development of alternatives to animal testing. To date, no in vitro systems have been validated to assess systemic toxicity, leaving an assessment gap pertinent to compounds that are bioavailable after skin permeation, oral uptake, or inhalation. Dynamic microphysiological systems (MPS) that integrate biological 3D tissues models have emerged as potential future in vitro testing platforms for complex toxicological endpoints such as systemic toxicity. Several approaches of "organs-on-chip" and dynamic co-cultures ("multi-organ chips") aim to emulate the in vivo physiology of single organs or the interactions between organoids, respectively. To explore the use of MPS to provide information about the influence of different application routes on the bioavailability and metabolic fate of chemicals, we employed TissUse's two organ chip (MOC) to connect reconstructed human epidermis (RhE) models (i.e. EpiDerm) and liver organoids (consisting of HepaRG and stellate cells). Applying a nested testing approach in two labs to evaluate the systems reproducibility, we assessed the metabolism of topically and systematically applied, single and repeated application, of three model chemicals: retinoic acid, permethrin and hyperforin. We first determined viability and functionality of single organoids over time and identified optimal non-toxic concentrations of test chemicals for the ultimate metabolism studies in the MOC. Pretests included i) kinetics of RhE models penetration, ii) toxicity towards RhE models and liver organoids, iii) chemical stability and binding to chip material. For each application scenario, parent and metabolites were analyzed by mass spectrometry in MOCs over five days. Transcriptional qPCR analyses of relevant xenobiotic metabolizing enzyme genes were performed in liver organoids after two and five days of exposure to determine potential autoinduction and modulation of compound metabolism. Our results show that 1) metabolic capacity in RhE and liver organoids is maintained over 5 days; 2) RhE model barrier function remains intact (according to histology and Trans Epithelial Electrical Resistance measurements); 3) repeated application of compounds resulted in higher concentrations of parent chemicals and some metabolites compared to single application; 4) compound-specific gene induction e.g. induction of CYP3A4 by hyperforin is dependent on the application route and frequency; 5) different application routes impact systemic concentrations of both parents and metabolites in the chip over the course of the experiment; 6) there was excellent intra- and inter-lab reproducibility, indicating a high robustness of the MOC for metabolism investigations and its transferability. In summary, the MOC provided important information on parent and metabolite disposition that may be relevant to risk assessment.

179 **3D NephroScreen: High throughput drug-induced nephrotoxicity screening on a proximal tubule-on-a-chip model**

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Total score 5; **Score 2** for a kidney OoC, + **3** for external validation

Abstract

Renal toxicity remains a major issue in clinical trials, and stresses the need for more predictive models fit for implementation in early drug development [1]. Here, we describe a perfused, leak-tight renal proximal tubule cell (RPTEC) model cultured within a high throughput microfluidic platform (Mimetas' OrganoPlate®) [2], along with recent results from a 12-compound nephrotoxicity screen performed within the "NephroTube" CRACK IT consortium in collaboration with sponsors and the NC3Rs.

Human RPTEC (SA7K clone, Sigma) were grown against a collagen I ECM in a 3-channel OrganoPlate®, yielding access to both the apical and basal side. Drug-induced toxicity was assessed by exposing kidney tubules to 4 benchmark and 8 blinded compounds with known clinical effects supplied by the sponsors for 24 and 48 h. The tightness of the barrier was evaluated by diffusion of a dextran dye from apical to basal compartment. Parallel to this, cell viability with a WST-8 assay and the presence of LDH in the supernatant were assessed. Finally, kidney tubules were lysed, and RNA was extracted for gene expression analysis of acute kidney injury markers.

Upon perfusion flow, RPTEC form leak-tight confluent tubular structures against the collagen I ECM in the OrganoPlate®. The NephroScreen revealed significant decreased barrier tightness and cell viability in 7 out of 12 compounds. Furthermore, the release of LDH was significantly increased in 9 out of 12 compounds. An increased expression in HMOX1, TNFα and NGAL was observed in 9, 5 and 7 out of 12 compounds, respectively whereas claudin-2 showed a decrease in 6 out the 12. Overall, more effects were observed after 48 h in comparison to 24 h exposure.

The kidney-on-a-chip model in the OrganoPlate® provides a promising in vitro renal toxicity tool to answer the desire to provide a better alternative to animal studies in terms of throughput, costs and predictivity and ultimately will be commercialised after further validation.

References

[1] MJ Wilmer et al. (2016). Trends Biotechnol 34, 156-170.

[2] SJ Trietsch et al. (2013). Lab Chip 13, 3548-3554.

7. Conclusions

The review of the 2019 SoT conference, 2018 and 2019 EUSAAT conferences, and the 2019 EuroTox conference proceedings, and an extensive literature search, yielded 19 abstracts describing projects by investigators whom we believe should be nominees for the 2020 Lush Science Prize. These abstracts are presented in Section 6.

The change in focus of the Lush Science Prize has significantly reduced the number of relevant papers that we have found (from 4,100 in 2018 to 1,713 this year) but yielded 50% more scoring abstracts than in 2018 (99 vs 68). The modified scoring system, which aims to take into account the practical readiness of reported work, has created a wider spread of scores, so that just 20% of abstracts were considered high scoring (19 abstracts).

The nominated abstracts are very diverse, and cover a wide range of topics including the development of tools to accelerate the use of non-animal methods for acute toxicity and developmental neurotoxicity testing, developing new AOPs for systemic effects in humans, and the creation of new tools to allow testing in human models of hazard and disease. We believe that they are all worthy candidates for the 2020 Science Prize.