Lush Science Prize 2024
Background Paper

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December 2023
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1. Introduction and Overview

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

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.

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 animals in product testing.

From 2020 the Lush Prize organisation has chosen to refocus its criteria for eligibility 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 aim of the Lush Science Prize is to reward those researchers making 'outstanding contributions' to twenty-first century toxicology 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, or *in* silico, toxicology tools that can predict the likely hazard potential of chemicals without using animal tests.

The principal aim of this paper is to assist the Lush Prize Team by identifying key projects that are making major contributions to the field of animal-free toxicology research. From these projects, the Team may choose to invite applications for the 2024 Lush Prize for Science. Lush Prize is particularly interested in supporting work that elucidates AOPs for the complex areas of systemic toxicology and developmental toxicology.

This 2024 Science Background paper identifies 43 pieces of work carried out by researchers, which we believe constitute potential candidates for the Judges' shortlist. These projects received scores of 5* or 6 against our scoring criteria for their potential to make major contributions towards providing practical, non-animal, tests which could be accepted by regulators. The full abstracts for these pieces of work are given in the Appendix.

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; FDA; ECVAM; UK NC3Rs; US Tox21 Programme; the ToxCast programme; and EU projects (see section 3). We also assessed recent developments in toxicity testing research by reviewing the relevant literature (see section 4 for some highlights).

In our search for candidate prize winners, we identified conferences which focussed on the replacement of animals in toxicity testing and which have been held in the 17

months since the last Lush Science Prize Background paper was prepared. For this year these were the 2023 Society of Toxicology (SoT) annual conference, the International Congress of Toxicology in 2022, the EUSAAT Linz conference 2022, the ESTIV conference 2022, the 12th World Congress on Alternatives and Animal Use in the Life Sciences in 2023, and the 1st and 2nd World Summits on MPS, held in 2022 and 2023. There was a total of around 6,800 abstracts from oral and poster presentations from these seven conferences, and around 2,450 were potentially relevant to the Lush Science prize. We then performed literature searches using PubMed to identify projects describing recent advances in toxicity testing research. In all, these searches yielded about 1,450 potentially relevant projects which we assessed as described in Section 2. In the scoring system, 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 awarded to abstracts which stand out in some other way.

Overall, from 109 abstracts which scored 1 or more, 90% scored 4 or more points, with 12% (13 abstracts) scoring 6 points. No abstracts scored 7 points. Abstracts scoring 5 points were reassessed to determine if any merited highlighting to the judges. A further 30 abstracts were then given a score of 5* and those, along with the 13 abstracts scoring 6, were recommended to be invited to apply for the Lush Science Prize. The full abstracts of those projects scoring 5* or 6 are provided in the Appendix. Details of the other abstracts which received a score are shown in the table in Section 5.4.

2. Methodology

In this section we describe how we identified projects that might be worthy of consideration as potential prize winners, and then how we scored each project to create a shortlist for the panel's consideration.

Of the "3 Rs", Lush Prize's interest focuses 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 and COVID-19, unless we felt that the work identified a new advance, or significant development, in the fields of interest (AOP, OoC, or *in silico* assay). Work describing environmental toxicity was also excluded unless it was evidently of wider relevance and applicability. We considered projects based anywhere in the world, but only considered work for which an abstract was available and written in the English language. As far as possible, we restricted the search to work reported since the last Lush Prize award (i.e. June 2022 – October 2023 inclusive).

In the identification of key developments in 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 around animal replacement in toxicity pathway research. These included the OECD; CAAT; ECVAM; UK NC3Rs; US Tox21 Programme; the ToxCast programme; ICCVAM, the NIH, the EPA, the FDA, ESTIV, and Cosmetics Europe (see section 4 for highlights).

Secondly, we identified relevant conferences held during the research time frame and assessed abstracts, where available, for their 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 seven relevant conferences for 2022 – 2023, for which abstracts were available, were the 62nd meeting of the Society of Toxicology, held in Nashville, Tennessee in March 2022[1]; the International Congress of Toxicology, held in Maastricht in September 2022[2], the 20th EUSAAT Congress in Linz, Austria in September 2022[3], the ESTIV 2022 conference in Barcelona, Spain in November 2022[4], the WC12 conference in Niagara Falls, Canada in August 2023[5], and the 1st and 2nd Microphysiological Systems World Summits[6],[7] in New Orleans, Louisiana (May 2022) and Berlin, Germany (June 2023), respectively.

[1] https://www.toxicology.org/pubs/docs/Tox/2023Tox.pdf

[2] https://ict-2022.elsevierdigitaledition.com/IV/

[3] https://eusaat.eu/wp-content/uploads/Altex_2022_-Linz.pdf

[4] https://www.estiv.org/content/uploads/2022/11/full-abstract-book-estiv-2022-final-corr.pdf

[5] https://proceedings.altex.org/data/2023-02/altex_WC12.pdf

[6] https://mpsworldsummit.com/wp-content/uploads/2022/05/altex_MPS1.pdf

[7] https://proceedings.altex.org/data/2023-01/altex_MPS2.pdf

For SoT 2023 there were approximately 3,760 abstracts in total, of which 125 were indexed under relevant Keyword Index headings. The relevant Keywords from the SoT 2023 abstract book were: Adverse Outcome Pathway; AOP; Computational Toxicology; In Silico (Models); Microphysiological Systems: MPS; Organ-on-a-chip; Organ-chip; and Predictive Toxicology.

From the more than 600 abstracts which comprised the ICT conference presentation and poster proceedings, we reviewed only those that were presented within conference sessions relevant to the Lush Science Prize.

For EUSAAT 2022, abstracts were not grouped by theme, so we reviewed all 219 abstracts.

From the 500 or so abstracts presented at the ESTIV congress presentation and poster proceedings, we reviewed only those that were presented within conference sessions relevant to the Lush Science Prize.

For WC12, the 680 or so abstracts were not grouped by theme, so we reviewed them all. Likewise, for the World Summits on MPS, we reviewed all abstracts.

Thirdly, we conducted a review of the recent literature. Firstly, we searched PubMed for research published from 01/06/2022 to 01/11/2023, combining search terms “adverse outcome pathway,” “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.

For published papers, our selection procedure was a three-stage process. At each stage, 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 system which awarded points as described below.

As for previous years, because 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.

The abstract scoring system awards points 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*

We have continued to use a scoring system which includes 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, plus 4 points for practical usefulness (ie approved for use by regulators), plus 1 point for standing out in some other way.

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. Thus, the realistic maximum likely score is 7.

In applying this regime for this Lush Prize cycle we found that many abstracts scored 5 points, with few scoring 6 and none scoring 7. We therefore modified the scoring system and reviewed all abstracts that had scored 5 points to identify those which we judged to be the most noteworthy/relevant to the Lush Prize for Science criteria. These abstracts were given a 5* score and, along with the abstracts scoring 6, have been recommended to the Lush Prize team.

3. Significant Institutional and Project Developments

This section summarises some significant events or news relating to 21st century toxicology testing from selected institutions and major collaborative projects that have been reported since the last Lush Prize Science Background paper was prepared in 2022.

3.1. USA

3.1.1. National Toxicology Programme

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, including Tox21, ICCVAM, and the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM). ICCVAM has recently published (August 2023) a draft document on the "Validation, Qualification, and Regulatory Acceptance of New Approach Methodologies"[8].

3.1.2. FDA

Although not the work of the FDA, the most significant development for the FDA in this prize cycle is the FDA Modernisation Act 2.0 which authorizes the use of certain alternatives to animal testing, including cell-based assays and computer models, to test the safety and effectiveness of new drugs. The Act lifts the mandate to test new drugs on animals.

The bill also removes a requirement to use animal studies as part of the process to obtain a license for a biological product that is biosimilar or interchangeable with another biological product.

Passed in the senate in September 2022 and signed into law by President Biden in December 2022, the potential impact of the Act on the use of animals in research is discussed in an article on *MedScape*[9].

In a related development, the FDA has also proposed a new NAMs programme to actively implement 3Rs principles in the work of the FDA[10]. This shift is to take place across all of the FDA's centres. No timeline is proposed for the shift, but the FDA said that the programme is a priority.

3.1.3. EPA

The EPA-sponsored Committee on Variability and Relevance of Current Laboratory Mammalian Toxicity Tests and Expectations for New Approach Methods (NAMs) for Use in Human Health Risk Assessment has now published a consensus report on 'Building Confidence in New Evidence Streams for Human Health Risk Assessment:

[8] https://ntp.niehs.nih.gov/sites/default/files/2023-08/VWG%20Report%20Draft_for%20public%20comment_08Aug2023.pdf

[9] https://www.medscape.com/viewarticle/989738?form=fpf#vp_1

[10] https://doi.org/10.1038/d41586-022-03569-9

Lessons learned from Laboratory Mammalian Toxicity Tests'[11]. The report aims to bridge the gap between the potential for NAMs and their practical application in human health risk assessment.

3.1.4. COLAAB

The Physicians Committee for Responsible Medicine has initiated the Coalition to Illuminate and Address Animal Methods Bias (COLAAB)[12], following an initial workshop in April 2022. COLAAB has identified that, "despite the availability of more reliable, effective, and ethical nonanimal experimental systems in many areas of biomedical research and testing, animal use remains the "gold standard" due to institutional inertia, financial interests, and other barriers. While systemic in nature, these barriers are carried out at an individual level, such as through biased manuscript peer reviews, often involving reviewers requesting that authors perform animal experiments to validate their findings". COLAAB works with stakeholders, including publishers, academia, industry, and funding bodies to highlight these issues and work to mitigate their impact.

3.2. EU & UK

3.2.1. EURL-ECVAM

The EURL-ECVAM Status Report 2022 describes the range of work undertaken during that year[13]. No significant advances were reported.

3.2.2. ECHA & REACH

ECHA has recently published an overview[14] of its "key areas of regulatory challenges", one of which is identified as the shift away from animal testing. ECHA also continues to emphasize that safety testing of chemicals on animals is only permitted as a "last resort"[15] and seems to be feeling some pressure from previous criticism of its approach to allowing animal testing (see the 2022 Lush Prize Science Background paper for a brief summary[16]) and footnote 17. ECHA conducted a preliminary market research exercise in late 2022 on the use of NAMs for hazard identification and classification of industrial chemicals.

3.2.3. RISK-HUNT3R

RISK-HUNT3R[18] is ongoing and has begun to publish research papers.

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[11] https://nap.nationalacademies.org/catalog/26906/building-confidence-in-new-evidence-streams-for-human-health-risk-assessment

[12] https://www.pcrm.org/ethical-science/animalmethodsbias

[13] https://publications.jrc.ec.europa.eu/repository/handle/JRC132525

[14] https://echa.europa.eu/documents/10162/17228/key_areas_regulatory_challenge_en.pdf/fbaa76cf-acd0-0c8a-5dd7-3195379946aa

[15] https://echa.europa.eu/animal-testing-under-reach

[16] https://lushprize.org/wp-content/uploads/Lush-Science-Prize-2022-background-paper.pdf

[17] https://doi.org/10.1016/j.yrtph.2022.105278

[18] https://www.risk-hunt3r.eu

3.2.4. UK NC3Rs

The NC3Rs works with the global research community to replace, refine, and reduce the use of animals in research and testing. Among their programmes are several funding rounds including the CRACK IT challenges. For 2023 these included SensOoChip (to increase the reproducibility and predictive power of organ-on-chips through multiparametric real-time monitoring and data modelling) and CrossDART (to develop *in vitro* developmental toxicity testing across multiple species).

An off-shoot of CRACK IT is the Mega-CRACK IT challenge, the first of which was funded in 2023 to develop a "virtual dog" to replace the use of dogs in assessing the potential toxicity of new medicines. "Working with seven pharmaceutical industry sponsors from the UK, Europe and North America, and partnering with the IMI2 consortium eTRANSAFE, a team at esqLABs GmbH will develop a machine learning-aided multi-scale modelling framework for toxicological endpoint prediction for new chemical entities in the dog".

In October 2022 NC3Rs announced a new programme to accelerate the adoption of non-animal derived antibodies. This work will focus on bridging the gap between the recommendations of a 2020 ECVAM report to use non-animal antibodies and continued use of traditional antibodies by researchers, supported by funding bodies.

3.3. Global

3.3.1. Organisation for Economic Co-operation and Development (OECD)

The OECD has published 13 new AOPs during the most recent Lush Prize cycle, including for lung cancer, developmental defects, infant leukaemia, and learning and memory impairment, among others[19].

The Working Party of the National Coordinators of the Test Guidelines Programme (WNT) held a workshop in December 2022 on how to prepare the Programme for emerging science and technologies[20]. The workshop addressed issues such as evaluating test method readiness for emerging technologies, evolving the concept of performance standards, incentivising validation, and better reporting of validation studies.

3.3.2. International Collaboration on Cosmetic Safety (ICCS)

The ICCS[21] was launched in February 2023. It is a global initiative focussed on advancing the adoption of animal-free assessments of cosmetics, and their ingredients, for human health and environmental safety. The ICCS brings together cosmetics manufacturers and supplies, industry and research associations, and animal welfare organizations to drive the adoption of animal-free approaches.

[19] https://doi.org/10.1787/2415170X

[20] https://one.oecd.org/document/ENV/CBC/MONO(2023)14/en/pdf

[21] https://www.iccs-cosmetics.org

4. Literature Highlights

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

Pereira *et al* (2022, footnote 17) published a comprehensive analysis of the failures of the REACH regulations to achieve some of their key objectives, including the reduction of animal use in regulatory safety testing. This group, from the Humane Society International/Europe, describe in detail the limitations of REACH, and offer concrete steps to improve REACH and the work of the European Chemicals Agency in implementing REACH. Although this work is referenced in Section 3.2.2, the importance and potential impact of these recommendations is sufficiently high that it requires highlighting at every opportunity.

Related to this, Schmeisser *et al* (2023)[22] published a summary of a symposium and workshop organised by the German Federal Institute for Risk Assessment. A distinguished group of nearly thirty leading toxicologists describe a compelling consensus on how to implement NAMs in human regulatory toxicology systems. Among their recommendations are the reframing of legislation towards human-relevant apical endpoints, avoidance of prescribing fixed assays in order to allow the use of more flexible toolboxes of test methods and approaches, and abstaining from making existing assays a gold standard to which new developments and technologies must adhere.

Jan Lauwereyns presented an illuminating analysis of the validity of animal models for biomedical research at the 2022 EUSAAT conference[23]. He points out that the issue of external validity (ie how relevant the animal model is) is often ignored, with animal models chosen out of habit, or on the basis of untested assumptions. The issue of internal validity (how reliable or reproducible the findings are) has often been a cause of loss of confidence in science. Lauwereyns offers compelling examples of primate research on COVID19 infection as evidence of poor external and internal validation of animal models, and suggests concrete action that can be taken by researchers, reviewers, publishers, and funding bodies to question the rationale behind animal experiments.

Also at EUSAAT, Merel Ritskes-Hoitinga highlighted the opportunity to demonstrate the validity of an evidence-based transition to animal-free testing[24]. She uses an analysis of the marketing approval of the Pfizer/BioNTech mRNA vaccine to illustrate how allowing fewer animal tests and permitting more alternatives can speed up the process of approving new medical products – from 10 years to 10 months!

Further to the two papers discussing the ethical issues around foetal bovine blood collection to produce foetal bovine serum (FBS) that were published in ALTEX in 2021(see Section 4 of footnote 16), McCann and Treasure[25] elaborated on these

[22] https://doi.org/10.1016/j.envint.2023.108082

[23] https://eusaat.eu/wp-content/uploads/Altex_2022_-Linz.pdf Abstract #28

[24] https://eusaat.eu/wp-content/uploads/Altex_2022_-Linz.pdf Abstract #72

[25] https://doi.org/10.1177/02611929221117992

themes, including the lack of evidence that calves are unconscious while their dam is slaughtered, and highlighted the European Food Safety Authority's most cautious advice that calves should be humanely euthanised as soon as they are detected in a recently slaughtered cow.

5. Outcome: Candidate Abstracts Identified

5.1. Conference Abstract Selection

As described in the Methodology, we reviewed abstracts from the Society of Toxicology 62nd Annual Meetings in 2023, the International Congress of Toxicology meeting in 2022, the 2022 EUSAAT Linz meeting, the European Society of Toxicology *In Vitro* 2022, the 12th World Congress on Alternatives and Animal Use in the Life Sciences, 2023 (WC12,) and the two World Summits on MPS, held in 2022 and 2023.

From the 125 SoT abstracts identified as relevant from the keyword index, 16 were scored. For the ICT meeting we reviewed abstracts based on scientific session titles, excluding those that were clearly outside the scope of the Lush Science Prize. We reviewed a total of 330 abstracts and scored 22 of them. We scored 8 of the 219 abstracts presented at the EUSAAT meeting. Similarly, we reviewed ESTIV abstracts based on session titles, and scored 6 of 47 relevant abstracts.

The 680 abstracts which comprised the WC12 conference presentation and poster proceedings were not grouped by theme, so we reviewed them all and 15 were selected for scoring.

The World Summit on MPS is a new meeting first held in 2022, but it is already a notable forum in which progress on MPS technologies and applications are presented, although much of the work is disease focussed. From the 2022 meeting we scored 6 of 310 abstracts and, from the 2023 meeting, 15 from 750 abstracts.

Overall, from a total of 2,461 potentially interesting abstracts identified at the seven relevant conferences since the last Lush Prize cycle, 88 (3.7%) were selected as being suitable for scoring.

5.2. Published Paper Abstract Selection

From the PubMed search we identified a total of 1,453 articles published since the 2022 Lush Prize Science paper was prepared that were of potential interest to Lush Prize. This represents a decrease in publications compared with the 2,254 papers identified in the two years preceding the 2022 Lush Prize report. Of these 1,453 articles, 674 (46%) relevant titles were from the “Adverse Outcome Pathways” and “AOP” searches; a further 65
(4%) relevant projects from the “Organ on a chip” search, 104 (7%) from the “Microphysiological system(s)” search; and finally, an additional 612 titles (42%) 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, COVID-19 or other disease, or environmental pollution) reduced the 2,254 titles by around 80%. Of the remainder, after review of abstracts in stage 3, just 21 abstracts were scored (6 from the AOP searches, 3 from the OoC searches, 2 from the Microphysiological system(s) search, and 10 from the Computox searches). This is a decrease in the number of scoring published abstracts compared with those considered in the 2022 Lush report; however, a large proportion of this year’s abstracts were ‘high-scoring’ (i.e. scored 5 or more).

Our review of key institutions and projects identified no additional articles for scoring, beyond those already identified through review of conference and published abstracts.

5.3. Scores

From the two separate sources of potential shortlisted projects, we identified a total of 109 abstracts, from an overall total of 3,914, describing work which scored at least one point according to our given criteria. This is a slightly smaller number of scoring abstracts than in the 2022 Lush report.

The distribution of scores was as follows: no abstracts scored just 1 or 2 points; 10 (9%) scored 3 points; 42 (39%) scored 4; 44 (40%) scored 5; and 13 abstracts (12%) scored 6 points. Unlike in the 2022 report, no abstracts scored the realistic maximum likely score of 7 points.

The distribution of scores meant that an unmanageably large number of abstracts scored the highest rankings of 5 or 6 points – a total of 58. To restrict the number of recommended abstracts to a more workable number, we re-examined the 44 abstracts scoring 5, and identified 30 of these that made the most significant contribution to the goals of the Lush Science Prize. These abstracts were given a score of 5* and, along with the 13 abstracts scoring 6 points, were recommended as potential Science Prize nominees. All 43 of these high-scoring abstracts are shown in full in the Appendix. 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 2. All the scored abstracts are listed in the Table in Section 5.4.

5.4. All Scored Abstracts

The Table lists details (title, authors, source, and score) of all 109 abstracts scoring 1 or more. The Table is ordered by score (from 6 – 3) and by source of abstract – PubMed, SoT, ICT, EUSAAT, ESTIV, WC12, World Summit on MPS22, and World Summit on MPS23. For abstracts of published papers, the abstract title in the Table is a hyperlink to the DOI (digital object identifier) for that paper.

Title	Authors	Source	Score
Integrating Concentration-Dependent Toxicity Data and Toxicokinetics To Inform Hepatotoxicity Response Pathways	Russo *et al*	PubMed	6
Development of a network of carcinogenicity adverse outcome pathways and its employment as an evidence framework for safety assessment	Cayley *et al*	PubMed	6
CTD tetramers: a new online tool that computationally links curated chemicals, genes, phenotypes, and diseases to inform molecular mechanisms for environmental health	Davis *et al*	PubMed	6
In vitro to in vivo extrapolation and high-content imaging for simultaneous characterization of chemically induced liver steatosis and markers of hepatotoxicity	Müller *et al*	PubMed	6

Title	Authors	Source	Score
Toxicogenomics scoring system: TGSS, a novel integrated risk assessment model for chemical carcinogenicity prediction	Lu *et al*	PubMed	6
TIRESIA: An eXplainable Artificial Intelligence Platform for Predicting Developmental Toxicity	Togo *et al*	PubMed	6
Prioritization of mixtures of neurotoxic chemicals for biomonitoring using high-throughput toxicokinetics and mixture toxicity modeling	Braun & Escher	PubMed	6
Innovative toxicology approaches to predict safety of inhaled candidate drugs	Ollerstam *et al*	ICT	6
SkinEthic™ HCE Time-to-Toxicity: on the way of being the first OECD adopted new approach methodology allowing the identification on its own of substances and mixtures for eye hazard identification	Alépée *et al*	ICT	6
Human test methods for developmental neurotoxicity (DNT) evaluation: Set-up, scientific validation and statistical analyses	Fritsche *et al*	EUSAAT	6
Tissue-specific network analysis to predict the hepatotoxicity of chemicals	Scott-Boyer *et al*	WC12	6
Acutox: An animal product-free assay for predicting acute oral toxicity	Goldsby *et al*	WC12	6
Scalable application of RosetteArray™ technology for modeling the complex etiology of human neural tube defects and screening for risk factors	Lundin *et al*	MPS23	6
Quantitative in vitro to in vivo extrapolation for developmental toxicity potency of valproic acid analogues	Chang *et al*	PubMed	5*
In vitro circulation model driven by tissue-engineered dome-shaped cardiac tissue	Kikuchi *et al*	PubMed	5*
Toxicogenomics Data for Chemical Safety Assessment and Development of New Approach Methodologies: An Adverse Outcome Pathway–Based Approach	Saarimäki *et al*	SoT	5*
Toward a Virtual Cornea: An Agent-Based Model to Study Interactions between the Cells and Layers of the Cornea under Homeostasis and following Chemical Exposure	Vanin *et al*	SoT	5*

Title	Authors	Source	Score
Combining In Silico and In Vitro Information to Avoid Acute Oral Toxicity Testing in Animals	Nelms *et al*	SoT	5*
3D Nephroscreen: High-Throughput Drug-Induced Nephrotoxicity Screening on a Microfluidic Proximal Tubule Model	Gijzen *et al*	SoT	5*
Allergic respiratory diseases linked with AOP caused by chemicals in the workplace	Cho & Cha	ICT	5*
AOP-based in vitro assay development for assessment of inhalational toxicants — oxidative stress leading to decreased lung function	Goralczyk *et al*	ICT	5*
Identify chemicals potentially able to interfere with the endocrine system using a suite of complementary in silico models employing semi-automation	Fioravanzo & Russel	ICT	5*
Performance of a new defined approach for surfactants for eye hazard assessment based on in vitro test methods	Giusti *et al*	ICT	5*
Integration of human-stem-cell-based embryoid bodies into a microfluidic multi-tissue platform for systemic embryotoxicity testing	Boos *et al*	ESTIV	5*
Physiological map to study kidney toxicity in the ONTOX project	Gamba *et al*	ESTIV	5*
Designing physiological maps as a tool to study liver toxicology	Maia Ladeira *et al*	ESTIV	5*
Building virtual cohorts via the integration of public data	Matos-Felipe *et al*	EUSAAT	5*
cellasys #8: A microphysiometric test to identify serum-free cell culture media	Eggert *et al*	EUSAAT	5*
Development of physiologically relevant in vitro inhalation model to predict acute respiratory toxicity of mists and volatile liquids	Kaluzhny *et al*	EUSAAT	5*
Development of a microphysiological skin-liver-thyroid Chip3 and its application to evaluate the effects on thyroid hormones of topically applied cosmetic ingredients under consumer-relevant conditions	Tao *et al*	WC12	5*
OECD validation of the ToxTracker assay for genotoxic mode of action assessment	Hendriks	WC12	5*

Title	Authors	Source	Score
Development of a 3D genotoxicity model for assessment of cosmetic formulations	Jacobs *et al*	WC12	5*
Development and execution of an occupational next generation risk assessment (NGRA) on an exclusive use cosmetic ingredient under EU REACH: A case study on C12-15 alkyl benzoate	Dawick *et al*	WC12	5*
Insights from profiling transcription factor transactivation with CYP450 metabolism integration	Karmaus *et al*	WC12	5*
Developmental neurotoxicity in vitro assays applied for molecular initiation and key event identification to create an AOP network related to cognitive function defects	Klose *et al*	WC12	5*
A PBPK-compliant human intestine-liver-brain-kidney chip for QIVIVE in drug development	Horland *et al*	MPS22	5*
InterOrgan multi-tissue chip system for linking matured tissue niches by vascular flow	Ronaldson-Bouchard *et al*	MPS22	5*
Perfused Organ Panel™ microphysiological system with synthetic hemoglobin, blood substitute, builds confidence in mitochondrial and xenobiotic metabolism of 3D liver models	Shoemaker *et al*	MPS22	5*
A multiscale computational framework for modeling microphysiological systems	German *et al*	MPS22	5*
A novel microfluid liver-on-chip model: Application in regulated genotoxicity testing	Hamel	MPS23	5*
Automation of multi-organ-chip assays	Erfurth *et al*	MPS23	5*
On the way to a digital twin in preclinical studies – how automation and continuous data acquisition enable AI-based in silico models	Huber *et al*	MPS23	5*
Development of a microphysiological skin-liver-thyroid Chip3 and its application to evaluate the effects on thyroid hormones of topically applied cosmetic ingredients under consumer-relevant conditions	Tao *et al*	MPS23	5*
Development, Validation, and Application of a Human Reproductive Toxicity Prediction Model Based on Adverse Outcome Pathway	Tan *et al*	PubMed	5

Title	Authors	Source	Score
Evaluation of an imaging-based in vitro screening platform for estrogenic activity with OECD reference chemicals	Duijndam *et al*	PubMed	5
Computational Analysis of Discontinued Neurological Drugs without Defined Primary Target Pharmacology	Rao & Sachs	SoT	5
Utility of an In Vitro 3D Kidney Microphysiological System to Assess Drug-Induced Nephrotoxicity	Tseng *et al*	SoT	5
Cell-Free DNA Derived from Cardiac Organoids as a Potential Indicator of Toxicity and Tissue-Level Events	Silver *et al*	SoT	5
Development of 3D functional platforms to study peripheral nervous system toxicity	Turner	ICT	5
Validation of the ToxProfiler reporter assay and its application in chemical read across	ter Braak *et al*	ICT	5
An all iPSC liver model for advanced risk assessment	Niemeijer	ICT	5
Assessment of human neural network formation and function using 2D and 3D hiPSC-derived cell systems	Bartmann *et al*	WC12	5
Automation and validation of the OrganoPlate LiverTox for hepatotoxicity detection	Bircsak *et al*	MPS23	5
Combining organ-on-a-chip and TK/TD modeling	Amiri *et al*	MPS23	5
REVskin, a skin-on-chip equivalent with advanced blood-flow mimicry, represents a significant improvement in 3D culture models for woundhealing and skin-ageing studies	Teissier *et al*	MPS23	5
Human-induced pluripotent stem cell reporters for high-content screening of stress response activation identifying target organ-specific toxicities	Niemeijer *et al*	MPS23	5
Application of a human in vitro testing battery for endocrine disruptor (ED)-induced developmental neurotoxicity (DNT) to refine EDC risk assessment	Koch *et al*	MPS23	5
Application of a new approach method (NAM) for inhalation risk assessment	Ramanarayanan *et al*	PubMed	4

Title	Authors	Source	Score
Development of oxidative stress-associated disease models using feto-maternal interface organ-on-a-chip	Richardson *et al*	PubMed	4
A glomerulus and proximal tubule microphysiological system simulating renal filtration, reabsorption, secretion, and toxicity	Zhang & Mahler	PubMed	4
Augmenting Expert Knowledge-Based Toxicity Alerts by Statistically Mined Molecular Fragments	Chakravarti	PubMed	4
Probabilistic Points of Departure and Reference Doses for Characterizing Human Noncancer and Developmental/Reproductive Effects for 10,145 Chemicals	Aurisano *et al*	PubMed	4
A KNIME Workflow to Assist the Analogue Identification for Read-Across, Applied to Aromatase Activity	Caballero Alfonso *et al*	PubMed	4
A multi-label learning model for predicting drug-induced pathology in multi-organ based on toxicogenomics data	Su *et al*	PubMed	4
3D Inkjet-Bioprinted Lung-on-a-Chip	Kim *et al*	PubMed	4
A hiPSC-derived lineage-specific vascular smooth muscle cell-on-a-chip identifies aortic heterogeneity across segments	Liu *et al*	PubMed	4
Machine Learning and AI Applications for Assessing Chemicals and Drugs	Wallqvist *et al*	SoT	4
Development of a Quantitative Systems Toxicology Model of Multidrug Resistance Protein 3 (MDR3) Inhibition to Predict Bile Acid–Mediated Cholestatic Drug–Induced Liver Injury	Beaudoin *et al*	SoT	4
Development of Novel Organ-on-a-Chip Platforms for 3D Liver In Vitro Models and Preclinical Drug Screening	Llabjani *et al*	SoT	4
ToxiOmics: A Transcriptome-Based Database and Web Tool to Query and Understand the Associations between Environmental Toxicants and Human Diseases	Kowal-Safron *et al*	SoT	4
Microphysiological Systems Detect Hepatotoxicity More Sensitively Than Current Safety Assessment Models	Schmidlin *et al*	SoT	4

Title	Authors	Source	Score
How to Get Physiological 3D Peristalsis Motion into In Vitro Models? A Novel Gut-on-Chip Model	Stucki *et al*	SoT	4
ALS-on-a-Chip: Toward Patient-Derived Models for Personalized Therapy Development	Spijkers *et al*	SoT	4
ToxGAN: an AI approach alternative to animal studies	Tong	ICT	4
Fabrication of a lung-on-a-chip device using porous silicon, a novel biomaterial, to quantify cell-mediated dissolution and extracellular matrix deposition	Blake *et al*	ICT	4
Towards an ADME-competent human 4-organ chip for risk assessment	Atac	ICT	4
Development of quantitative adverse outcome pathways to address the effects of PFAS on cholesterol metabolism. Benchmarking with human epidemiological data and comparison with threshold values	Westerhout *et al*	ICT	4
Embryonic stem cell-derived human lung organoids for nanomaterial testing	Issa *et al*	ICT	4
New approaches for evaluating kidney toxicity using physiological maps	Gamba *et al*	ICT	4
Development of chemical and toxicological domains to support a chemoinformatics tool to identify chemicals promoting cholestatic liver injury	Cronin *et al*	ICT	4
ReproTracker: Next generation in vitro developmental toxicity testing	Flatt *et al*	ICT	4
Adaptation of the E-Morph Assay to serum-free cell culture conditions for CellPainting-based phenotypic screening of environmental estrogens	von Coburg *et al*	ICT	4
Chronic compound analysis of hiPSC-CMs in a physiological environment for preclinical cardiac risk evaluation: defined serum-free medium and long-term culture on the FLEXcyte 96	Gossmann *et al*	ICT	4
Innovative animal-free assessment of thyroid-mediated developmental neurotoxicity based on human biology	Dierichs *et al*	ICT	4
Organotypic small intestinal model for long-term high-throughput screening toxicity studies	Puskar *et al*	ICT	4

Title	Authors	Source	Score
A new immunocompetent OOC platform for culturing 3D human tissues with capillary flow-driven immune cells and investigating their cross	Scaglione *et al*	ESTIV	4
On the Real-Time Oxygen Consumption of Hepatocytes in a Microphysiological System	Flechner *et al*	ESTIV	4
Combining gene expressions and imaged-based morphological features for chemical-phenotype profiles	Cerisier *et al*	ESTIV	4
3D multi-layered epithelial models based on animal-free fibrous scaffolds	Weigel *et al*	EUSAAT	4
Phenomics and transcriptomics applied for key event identification resulting in an AOP network for developmental neurotoxicity	Klose *et al*	EUSAAT	4
High throughput intestinal tissues on newly fabricated 96-well culture plates	Stevens *et al*	EUSAAT	4
Humanized 3D cell culture materials – The next generation of in vitro testing	Custódio *et al*	EUSAAT	4
Deep learning profile QSAR modeling to impute in vitro assay results and predict chemical carcinogenesis mechanisms	Borrel *et al*	WC12	4
A liver and testis multi-organ-chip: Towards a systemic male reprotoxicity model	Maschmayer *et al*	WC12	4
Sens-ocular model: Cell-based assay to evaluate eye stinging potential of cosmetic formulations	Barroso Brito *et al*	WC12	4
Use of a dynamic skin and liver co-culture model to investigate the effect of application route on the metabolism of the hair dye, 4-amino-2-hydroxytoluene	Tao *et al*	MPS22	4
Differential monocyte actuation in a three-organ functional innate immune system-on-a-chip	Emmnos *et al*	MPS22	4
Engineering metabolically active reconstructed human skin for organ-on-chip	Jäger *et al*	MPS23	4
Development of epidermis-on-a-chip for toxicological evaluation of nanomaterials	Costa *et al*	MPS23	4
A liver and testis multi-organ-chip: Towards a systemic male reprotoxicity model	Maschmayer *et al*	MPS23	4

Title	Authors	Source	Score
Knowledge graph aids comprehensive explanation of drug and chemical toxicity	Hao *et al*	PubMed	3
Identification of Adverse Outcome Pathway (AOP) Network Leading to Pulmonary Fibrosis and Its Application to Inhalation Toxicants Screening: Integrative Data Mining Approach Using Comparative Toxicogenomics Database and AOP Wiki	Jeong *et al*	SoT	3
An In Vitro 3D Model of the Human Renal Proximal Tubule for Nephrotoxicity Screening Studies	Pearson *et al*	SoT	3
Integrating in vitro distribution kinetics analysis in IVIVE-PBK	Kramer	ICT	3
Development of a network of adverse outcome pathways for chemically induced oxidative stress leading to (non-)genotoxic carcinogenesis	Veltman *et al*	ICT	3
Vascularization of multi-organon-chips with blood and lymphatic endothelial cells for the generation of immunocompetent skin models	Koning *et al*	WC12	3
High-throughput preclinical model of breathing human alveoli	Asadi *et al*	WC12	3
HUMIMIC-InHALES: A human-relevant aerosol test platform for systemic exposure studies	Schimek *et al*	WC12	3
Corneal toxicity screening: Successful replacement of rabbits by human in vitro corneal tissues	Dernick *et al*	MPS23	3
Integration of human stem cell-based embryoid bodies into a microfluidic multi-tissue platform for systemic embryotoxicity testing	Boos *et al*	MPS23	3

6. Conclusions

This review of 3,900 research projects described either in the major seven conferences held since the last Lush Prize cycle, or in the published literature, yielded 43 abstracts describing projects by investigators whom we believe could be nominees for the 2024 Lush Science Prize. These abstracts are presented in Appendix that follows.

While we have found more relevant papers for the current, shorter, prize cycle than last time (3,900 in 2023 (17 months) compared with nearly 3,200 in 2022 (2 years)) we believe that this is, at least in part, accounted for by the concentration of three major conferences in the late summer/autumn of 2022, just after the time cut-off for the previous analysis, and the introduction of a new conference, dedicated to MPS, which was held twice in this Prize cycle. The number of scoring abstracts was slightly lower than last time (109 in 2023 vs 129 in 2022). The modified scoring system, which aims to take account of the practical readiness of reported work, continues to create a wider spread of scores, although no abstracts met the criteria for a score of 7, leaving just 13 abstracts that scored 6 points.

We felt that including all abstracts scoring the highest two marks (5 and 6) gave too many abstracts to allow easy short-listing and winner identification. We decided to re-examine those abstracts that scored 5 to see if any stood out as more closely matching the goals for the Science Prize. From this exercise we identified a further 30 (of 44 that scored 5) abstracts that we felt worthy of consideration for the Prize.

The nominated abstracts are very diverse, and they 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, the developing of 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 2024 Science Prize.

Appendix – High-scoring Abstracts

This year 43 projects received the highest scores of either 5* or 6 for reporting new or significantly improved AOPs, *in silico* assays, validation techniques, computational modelling approaches, or organ-on-a-chip models, and for their potential to replace animals in research and testing. The 43 abstracts are given below. We consider all worthy of being considered by the Lush Prize Team.

PubMed

Environ Sci Technol. 2023 Aug 22;57(33):12291-12301. doi: 10.1021/acs.est.3c02792. Epub 2023 Aug 11.

Integrating Concentration-Dependent Toxicity Data and Toxicokinetics To Inform Hepatotoxicity Response Pathways.

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Failure of animal models to predict hepatotoxicity in humans has created a push to develop biological pathway-based alternatives, such as those that use in vitro assays. Public screening programs (e.g., ToxCast/Tox21 programs) have tested thousands of chemicals using in vitro high-throughput screening (HTS) assays. Developing pathway-based models for simple biological pathways, such as endocrine disruption, has proven successful, but development remains a challenge for complex toxicities like hepatotoxicity, due to the many biological events involved. To this goal, we aimed to develop a computational strategy for developing pathway-based models for complex toxicities. Using a database of 2171 chemicals with human hepatotoxicity classifications, we identified 157 out of 1600+ ToxCast/Tox21 HTS assays to be associated with human hepatotoxicity. Then, a computational framework was used to group these assays by biological target or mechanisms into 52 key event (KE) models of hepatotoxicity. KE model output is a KE score summarizing chemical potency against a hepatotoxicity-relevant biological target or mechanism. Grouping hepatotoxic chemicals based on the chemical structure revealed chemical classes with high KE scores plausibly informing their hepatotoxicity mechanisms. Using KE scores and supervised learning to predict in vivo hepatotoxicity, including toxicokinetic information, improved the predictive performance. This new approach can be a universal computational toxicology strategy for various chemical toxicity evaluations.
DOI: 10.1021/acs.est.3c02792
Score 6: ***3 creating new computox approach to develop hepatox AOP/KE + 2 for in-house validation + 1 for utilising ToxCast/Tox21 data to identify KEs***

ALTEX. 2023;40(1):34–52. doi: 10.14573/altex.2201311. Epub 2022 May 13.

Development of a network of carcinogenicity adverse outcome pathways and its employment as an evidence framework for safety assessment.

Cayley AN(1), Foster RS(1), Hill E(1), Kane S(1), Kocks G(1), Myden A(1), Newman D(1), Stalford SA(1), Vessey JD(1), Zarei R(1), De Oliveira AAF(1).
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The traditional paradigm for safety assessment of chemicals for their carcinogenic potential to humans relies heavily on a battery of well-established genotoxicity tests, usually followed up by long-term, high-dose rodent studies. There are a variety of problems with this approach, not least that the rodent may not always be the best model to predict toxicity in humans. Consequently, new approach methodologies (NAMs) are being developed to replace or enhance predictions coming from the existing assays. However, a combination of the data arising from NAMs is likely to be required to improve upon the current paradigm, and consequently a framework is needed to combine evidence in a meaningful way. Adverse outcome pathways (AOPs) represent an ideal construct on which to organize this evidence. In this work, a data structure outlined previously was used to capture AOPs and evidence relating to carcinogenicity. Knowledge held within the predictive system Derek Nexus was extracted, built upon, and arranged into a coherent network containing 37 AOPs. 60 assays and 351 in silico alerts were then associated with KEs in this network, and it was brought to life by associating data and contextualizing evidence and predictions for over 13,400 compounds. Initial investigations into using the network to view knowledge and reason between evidence in different ways were made. Organizing knowledge and evidence in this way provides a flexible framework on which to carry out more consistent and meaningful carcinogenicity safety assessments in many different contexts.
DOI: 10.14573/altex.2201311
Score 6: ***3 use of computox tools to develop new cancer AOPS + 2 for in-house validation + 1 for potential impact on reducing long-term rat genotox studies***

Toxicol Sci. 2023 Sep 28;195(2):155-168. doi: 10.1093/toxsci/kfad069.

CTD tetramers: a new online tool that computationally links curated chemicals, genes, phenotypes, and diseases to inform molecular mechanisms for environmental health.
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The molecular mechanisms connecting environmental exposures to adverse endpoints are often unknown, reflecting knowledge gaps. At the Comparative Toxicogenomics Database (CTD), we developed a bioinformatics approach that integrates manually curated, literature-based interactions from CTD to generate a "CGPD-tetramer": a 4-unit block of information organized as a step-wise molecular mechanism linking an initiating Chemical, an interacting Gene, a Phenotype, and a Disease outcome. Here, we describe a novel, user-friendly tool called CTD Tetramers that generates these evidence-based CGPD-tetramers for any curated chemical, gene, phenotype, or disease of interest. Tetramers offer potential solutions for the unknown underlying mechanisms and intermediary phenotypes connecting a chemical exposure to a disease. Additionally, multiple tetramers can be assembled to construct detailed modes-

of-action for chemical-induced disease pathways. As well, tetramers can help inform environmental influences on adverse outcome pathways (AOPs). We demonstrate the tool's utility with relevant use cases for a variety of environmental chemicals (eg, perfluoroalkyl substances, bisphenol A), phenotypes (eg, apoptosis, spermatogenesis, inflammatory response), and diseases (eg, asthma, obesity, male infertility). Finally, we map AOP adverse outcome terms to corresponding CTD terms, allowing users to query for tetramers that can help augment AOP pathways with additional stressors, genes, and phenotypes, as well as formulate potential AOP disease networks (eg, liver cirrhosis and prostate cancer). This novel tool, as part of the complete suite of tools offered at CTD, provides users with computational datasets and their supporting evidence to potentially fill exposure knowledge gaps and develop testable hypotheses about environmental health.
DOI: 10.1093/toxsci/kfad069
Score 6: ***3 for bioinformatics tool to build and support AOPs + 2 for in-house validation + 1 for potential impact***

Arch Toxicol. 2023 Jun;97(6):1701-1721. doi: 10.1007/s00204-023-03490-8. Epub 2023 Apr 12.

In vitro to in vivo extrapolation and high-content imaging for simultaneous characterization of chemically induced liver steatosis and markers of hepatotoxicity.
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Chemically induced steatosis is characterized by lipid accumulation associated with mitochondrial dysfunction, oxidative stress and nucleus distortion. New approach methods integrating in vitro and in silico models are needed to identify chemicals that may induce these cellular events as potential risk factors for steatosis and associated hepatotoxicity. In this study we used high-content imaging for the simultaneous quantification of four cellular markers as sentinels for hepatotoxicity and steatosis in chemically exposed human liver cells in vitro. Furthermore, we evaluated the results with a computational model for the extrapolation of human oral equivalent doses (OED). First, we tested 16 reference chemicals with known capacities to induce cellular alterations in nuclear morphology, lipid accumulation, mitochondrial membrane potential and oxidative stress. Then, using physiologically based pharmacokinetic modeling and reverse dosimetry, OEDs were extrapolated from data of any stimulated individual sentinel response. The extrapolated OEDs were confirmed to be within biologically relevant exposure ranges for the reference chemicals. Next, we tested 14 chemicals found in food, selected from thousands of putative chemicals on the basis of structure-based prediction for nuclear receptor activation. Amongst these, orotic acid had an

extrapolated OED overlapping with realistic exposure ranges. Thus, we were able to characterize known steatosis-inducing chemicals as well as data-scarce food-related chemicals, amongst which we confirmed orotic acid to induce hepatotoxicity. This strategy addresses needs of next generation risk assessment and can be used as a first chemical prioritization hazard screening step in a tiered approach to identify chemical risk factors for steatosis and hepatotoxicity-associated events.
DOI: 10.1007/s00204-023-03490-8
Score 6: ***3 for in silico PBPK modelling with in vitro hepatotox assay + 2 for in-house validation + 1 for demonstrating chemical hazard prioritization***

Ecotoxicol Environ Saf. 2023 Jan 15;250:114466. doi: 10.1016/j.ecoenv.2022.114466. Epub 2022 Dec 30.

Toxicogenomics scoring system: TGSS, a novel integrated risk assessment model for chemical carcinogenicity prediction.
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BACKGROUND: Given the increasing exposure of humans to environmental chemicals and the limitations of conventional toxicity test, there is an urgent need to develop next-generation risk assessment methods.
OBJECTIVES: This study aims to establish a novel computational system named Toxicogenomics Scoring System (TGSS) to predict the carcinogenicity of chemicals coupling chemical-gene interactions with multiple cancer transcriptomic datasets.
METHODS: Chemical-related gene signatures were derived from chemical-gene interaction data from the Comparative Toxicogenomics Database (CTD). For each cancer type in TCGA, genes were ranked by their effects on tumorigenesis, which is

based on the differential expression between tumor and normal samples. Next, we developed carcinogenicity scores (C-scores) using pre-ranked GSEA to quantify the correlation between chemical-related gene signatures and ranked gene lists. Then we established TGSS by systematically evaluating the C-scores in multiple chemical-tumor pairs. Furthermore, we examined the performance of our approach by ROC curves or prognostic analyses in TCGA and multiple independent cancer cohorts.
RESULTS: Forty-six environmental chemicals were finally included in the study. C-score was calculated for each chemical-tumor pair. The C-scores of IARC Group3 chemicals were significantly lower than those of chemicals in Group 1 (P-value = 0.02) and Group 2 (P-values = 7.49 ×10-5). ROC curves analysis indicated that C-score could distinguish "high-risk chemicals" from the other compounds (AUC = 0.67) with a specificity and sensitivity of 0.86 and 0.57. The results of survival analysis were also in line with the assessed carcinogenicity in TGSS for the chemicals in Group 1. Finally, consistent results were further validated in independent cancer cohorts.
CONCLUSION: TGSS highlighted the great potential of integrating chemical-gene interactions with gene-cancer relationships to predict the carcinogenic risk of chemicals, which would be valuable for systems toxicology.
DOI: 10.1016/j.ecoenv.2022.114466
Score 6: ***3 for new method to predict carcinogenic risk of chemicals + 2 for in-house validation + 1 for potential impact***

J Chem Inf Model. 2023 Jan 9;63(1):56-66. doi: 10.1021/acs.jcim.2c01126. Epub 2022 Dec 15.

TIRESIA: An eXplainable Artificial Intelligence Platform for Predicting Developmental Toxicity.

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Herein, a robust and reproducible eXplainable Artificial Intelligence (XAI) approach is presented, which allows prediction of developmental toxicity, a challenging human-health endpoint in toxicology. The application of XAI as an alternative method is of the utmost importance with developmental toxicity being one of the most animal-intensive areas of regulatory toxicology. In this work, the established CAESAR (Computer Assisted Evaluation of industrial chemical Substances According to Regulations) training set made of 234 chemicals for model learning is employed. Two test sets, including as a whole 585 chemicals, were instead used for validation and generalization purposes. The proposed framework favorably compares with the state-of-the-art approaches in terms of accuracy, sensitivity, and specificity, thus resulting in a reliable support system for developmental toxicity ensuring informativeness, uncertainty estimation, generalization, and transparency. Based on the eXtreme Gradient Boosting (XGB) algorithm, our predictive model provides easy interpretative keys based on

specific molecular descriptors and structural alerts enabling one to distinguish toxic and nontoxic chemicals. Inspired by the Organisation for Economic Co-operation and Development (OECD) principles for the validation of Quantitative Structure-Activity Relationships (QSARs) for regulatory purposes, the results are summarized in a standard report in portable document format, enclosing also details concerned with a density-based model applicability domain and SHAP (SHapley Additive exPlanations) explainability, the latter particularly useful to better understand the effective roles played by molecular features. Notably, our model has been implemented in TIRESIA (Toxicology Intelligence and Regulatory Evaluations for Scientific and Industry Applications), a free of charge web platform available at http://tiresia.uniba.it.
DOI: 10.1021/acs.jcim.2c01126
Score 6: ***3 for new predictive tox model + 2 for in-house validation + 1 user readiness***

Environ Int. 2023 Jan;171:107680. doi: 10.1016/j.envint.2022.107680. Epub 2022 Dec 6.

Prioritization of mixtures of neurotoxic chemicals for biomonitoring using high-throughput toxicokinetics and mixture toxicity modeling.
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Modern society continues to pollute the environment with larger quantities of chemicals that have also become more structurally and functionally diverse. Risk assessment of chemicals can hardly keep up with the sheer numbers that lead to complex mixtures of increasing chemical diversity including new chemicals, substitution products on top of still abundant legacy compounds. Fortunately, over the last years computational tools have helped us to identify and prioritize chemicals of concern. These include toxicokinetic models to predict exposure to chemicals as well as new approach methodologies such as in-vitro bioassays to address toxicodynamic effects. Combined, they allow for a prediction of mixtures and their respective effects and help overcome the lack of data we face for many chemicals. In this study we propose a high-throughput approach using experimental and predicted exposure, toxicokinetic and toxicodynamic data to simulate mixtures, to which a virtual population is exposed to and predict their mixture effects. The general workflow is adaptable for any type of toxicity, but we demonstrated its applicability with a case study on neurotoxicity. If no experimental data for neurotoxicity were available, we used baseline toxicity predictions as a surrogate. Baseline toxicity is the minimal toxicity any chemical has and might underestimate the true contribution to the mixture effect but many neurotoxicants are not by orders of magnitude more potent than baseline toxicity. Therefore, including baseline-toxic effects in mixture simulations yields a more realistic picture than excluding them in mixture simulations. This workflow did not only correctly identify and prioritize known chemicals of concern like benzothiazoles, organochlorine pesticides and plasticizers but we were also able to identify new potential neurotoxicants that we recommend to include in future biomonitoring studies and if found in humans, to also include in neurotoxicity screening.
DOI: 10.1016/j.envint.2022.107680

Score 6: ***3 for new mixture toxicity model + 2 for in-house validation + 1 for applicability***

Birth Defects Res. 2022 Oct 1;114(16):1037-1055. doi: 10.1002/bdr2.2019. Epub 2022 May 9.

Quantitative in vitro to in vivo extrapolation for developmental toxicity potency of valproic acid analogues.

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BACKGROUND: The developmental toxicity potential (dTP) concentration from the devTOX quickPredict (devTOXqP) assay, a metabolomics-based human induced pluripotent stem cell assay, predicts a chemical's developmental toxicity potency. Here, in vitro to in vivo extrapolation (IVIVE) approaches were applied to address whether the devTOXqP assay could quantitatively predict in vivo developmental toxicity lowest effect levels (LELs) for the prototypical teratogen valproic acid (VPA) and a group of structural analogues.
METHODS: VPA and a series of structural analogues were tested with the devTOXqP assay to determine dTP concentration and we estimated the equivalent administered doses (EADs) that would lead to plasma concentrations equivalent to the in vitro dTP concentrations. The EADs were compared to the LELs in rat developmental toxicity studies, human clinical doses, and EADs reported using other in vitro assays. To evaluate the impact of different pharmacokinetic (PK) models on IVIVE outcomes, we compared EADs predicted using various open-sourceand commercially available PK and physiologically based PK (PBPK) models. To evaluate the effect of in vitro kinetics, an equilibrium distribution model was applied to translate dTP concentrations to free medium concentrations before subsequent IVIVE analyses.
RESULTS: The EAD estimates for the VPA analogues based on different PK/PBPK models were quantitatively similar to in vivo data from both rats and humans, where available, and the derived rank order of the chemicals was consistent with observed in vivo developmental toxicity. Different models were identified that provided accurate predictions for rat prenatal LELs and conservative estimates of human safe exposure. The impact of in vitro kinetics on EAD estimates is chemical-dependent. EADs from this study were within range of predicted doses from other in vitro and model organism data.
CONCLUSIONS: This study highlights the importance of pharmacokinetic considerations when using in vitro assays and demonstrates the utility of the devTOXqP human stem cell-based platform to quantitatively assess a chemical's developmental toxicity potency.
DOI: 10.1002/bdr2.2019

Score 5*: ***2 for enhancement of devTOXqP assay + 2 for in-house validation + 1 for quantitative assessment of devtox potential***

Biofabrication. 2022 Jun 28;14(3). doi: 10.1088/1758-5090/ac77c1.

In vitrocirculation model driven by tissue-engineered dome-shaped cardiac tissue.

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The heart is an essential organ for animals and humans. With the increased availability of pluripotent stem cells, the use of three-dimensional cardiac tissues consisting of cultured cardiomyocytes in in vitro drug evaluation has been widely studied. Several models have been proposed for the realization of the pump function, which is the original function of the heart. However, there are no models that simulate the human circulatory system using cultured cardiac tissue. This study shows that a dome-shaped cardiac tissue fabricated using the cell sheet stacking technique can achieve a heart-like pump function and circulate culture medium, there by mimicking the human circulatory system. Firstly, human induced pluripotent stem cells were differentiated into autonomously beating cardiomyocytes, and cardiomyocyte cell sheets were created using temperature-responsive culture dishes. A cardiomyocyte sheet and a human dermal fibroblast sheet were stacked using a cell sheet manipulator. This two-layered cell sheet was then inflated to create a dome-shaped cardiac tissue with a base diameter of 8 mm. The volume of the dome-shaped cardiac tissue changed according to the autonomous beating. The stroke volume increased with the culture period and reached 21 ± 8.9μl (n= 6) on day 21. It also responded to β-stimulant and extracellular calcium concentrations. Internal pressure fluctuations were also recorded under isovolumetric conditions by dedicated culture devices. The peak heights of pulsatile pressure were 0.33 ± 0.048 mmHg (n= 3) under a basal pressure of 0.5 mmHg on day 19. When the tissue was connected to a flow path that had check valves applied, it drove a directional flow with an average flow rate of approximately 1μl s-1. Furthermore, pressure-volume (P-V) diagrams were created from the simultaneous measurement of changes in pressure and volume under three conditions of fluidic resistance. In conclusion, this cardiac model can potentially be used for biological pumps that drive multi-organ chips and for more accurate in vitro drug evaluation using P-V diagrams.
DOI: 10.1088/1758-5090/ac77c1
Score 5*: ***3 for creating heart cell-based microfluidic pump for OoCs + 1 for proof of principle + 1 for contribution to human-on-a-chip models***

SoT 2023

3073
Toxicogenomics Data for Chemical Safety Assessment and Development of New Approach Methodologies: An Adverse Outcome Pathway–Based Approach
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Mechanistic toxicology is emerging as a powerful approach for chemical safety assessment and development. It focuses on the mechanisms through which chemicals induce their effects, hence also providing valuable insight into the development of safe-by-design compounds. Omics technologies are central to disentangling these mechanisms and are widely applied in the context of toxicogenomics.
However, despite the immense potential of toxicogenomic approaches, their implementation into the regulatory framework is hindered by the lack of standardization, and uncertainties in the analysis and interpretation of omics data. At the same time, the use of mechanistic evidence in the form of Adverse Outcome Pathways (AOPs) is promoted for the development of New Approach Methodologies (NAMs) that can offer solutions to the pressing need for faster, cheaper, and more ethical safety assessment of chemicals. We hypothesized, that embedding toxicogenomics into the AOP framework would unleash the full potential of AOPs while also building regulatory confidence towards toxicogenomics. The modelling of AOPs with molecular data would support systematic development of NAMs and reduce the need for multiple testing strategies. Similarly, it would guide the translation of complex molecular signatures captured by omics technologies into meaningful biological interpretations. Hence, we developed a multi-step strategy to systematically annotate molecular events into AOPs through their Key Events (KEs) and applied it to all currently available AOPs relevant for human health risk assessment. Using the resulting associations, we successfully highlighted relevant adverse outcomes for chemical exposures from their associated gene signatures. Furthermore, we established the concept of an AOP fingerprint and showed strong in vitro to in vivo convergence with independent toxicogenomic data sets. Finally, we identified and experimentally validated a panel of AOP-derived in vitro biomarkers for pulmonary fibrosis. These results suggest that the established associations strongly support meaningful interpretation of toxicogenomic data and guide the development of data-driven computational methodologies for chemical safety assessment.
Score 5*: ***2 for the significant development of AOP generation + 2 for in-house validation + 1 for the integration of toxicogenomics into the generation and application of AOPs for safety assessment***

3079

Toward a Virtual Cornea: An Agent-Based Model to Study Interactions between the Cells and Layers of the Cornea under Homeostasis and following Chemical Exposure

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Corneal injuries following chemical exposure differ in severity and reversibility. Various in vivo, ex vivo, and in vitro experimental methods attempt to predict whether exposure will lead to severe (corrosive), moderate, mild, or no irritancy but differ in their ability to prognosticate human-relevant eye irritation outcome. A detailed computational model of corneal injury at the multi-cellular level (depicting individual cells and biochemical processes in detail) which could predict these adverse outcomes would enable limitless virtual experiments. To improve the spatial and dynamic understanding of corneal chemical hazard, we built a multicellular agent-based model in the CompuCell3D modeling environment that aims to recapitulate complex cell behaviors underlying homeostasis and wound healing of the stratified epithelial layer and the stroma. The

model represents a two-dimensional sagittal section of the limbal area with stem and transit-amplifying cells and a stratified epithelium layer keeping the same structure seen in its biological archetype, with a bilayer of superficial cells, two to three layers of wing cells, a single layer of basal cells attached to the basement membrane, and immune cells, bounded by virtual spaces to represent the tear layer and Bowman's membrane. Beneath this epithelial membrane lies an area representing the stroma with keratinocyte cells. Homeostasis in the epithelial layer implements signal information (cytokines, growth factors) and other factors can be added to more completely simulate the emergent wound-healing behavior where tear composition changes after injury, having higher levels of EGF (proliferation and migration), TGF-α (mitogen), HGF (proliferation and migration, promotes wound healing), KGF (proliferation), and IGF (proliferation), in the regulation of composite cellular behavior and multicellular interactions on proliferation and cell migration to the wounded site. These changes in the microenvironment activate quiescent limbal stem cells to proliferate and differentiate into transient-amplifying cells, which also proliferate and consequently differentiate into the other cell types present in the stratified epithelium layer. This mechanism is enough to heal mild and moderate wounds that avoid damaging the basement membrane. In cases of severe injury, other systems, including vascular and myeloid, participate in the repair of the Bowman`s membrane and the stroma. This prototype virtual corneal model aims to define a more mechanistic human-relevant classification scheme, predict the time of recovery from each of those injuries, and offer potential explanations for the corneal anomalies (erosions and corneal ulcers) after severe damage and simulated responses to bioactivity data from various in vitro models of corneal toxicity. This will help toxicologists better understand critical events in cornea-chemical exposures as well as predict human-relevant adverse outcomes.

Score 5*: ***3 for a new virtual (computox) model of human cornea + 1 for proof of principle + 1 for potential contribution to AOP development***

3694

Combining In Silico and In Vitro Information to Avoid Acute Oral Toxicity Testing in Animals

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Globally, regulatory agencies have committed to reducing/eliminating animal testing for establishing chemical safety. Adverse outcome pathways provide a mechanistic scaffold that can be used to identify appropriate non-animal methods and connect them to apical adverse outcomes, thereby facilitating the development of tiered testing strategies for acute oral toxicity testing. Previously, we demonstrated that chemical structure and bioactivity measurements could be used to identify in vitro assays for use within a tiered testing strategy to detect acutely toxic chemicals. In this study we expanded on this work by 1) incorporating additional datasets into our original workflow to increase the number of mechanisms covered, 2) investigating how cytotoxicity information can be refined to better reflect on-target effects, and 3) generating a model that can incorporate various chemical and biological data to predict potential acute toxicity. We grouped the 11,992 chemicals with curated acute toxicity information from the ICCVAM Acute Toxicity Work Group (ATWG) into 2,192 clusters based on structural similarity defined by ToxPrint fingerprints. Bioactivity data for approximately 31,500 new assays were retrieved from PubChem for 4,786 ATWG chemicals. This assay data was combined with the bioactivity data previously gathered from ToxCast and a minimal

suite of assays from PubChem and/or ToxCast were identified for each cluster, based upon activity enriched for chemicals within a cluster. Additionally, to find the minimum AC50 for a chemical, we compared the cluster-specific assay AC50 against different methods for calculating the cytotoxicity value: cytotoxicity point and lower bound of cytotoxicity calculated by ToxCast, lowest burst assay AC50, and median of the lowest 25th percentile in burst assays. Of the 1,637 acutely toxic chemicals (rat oral LD50 <= 2,000 mg/kg as defined by the ATWG) with activity in ToxCast below the lower bound of cytotoxicity, 1,139 were linked to one or more cluster-specific minimal ToxCast assay. Depending on the cytotoxicity method, when the minimum AC50 value for a chemical came from cytotoxicity rather than the cluster-specific assay, the default cytotoxicity point value was used for the majority of chemicals (ranging between 70-97.4%). The minimal AC50 values calculated using the different cytotoxicity methods (excluding the lower bound of cytotoxicity) were significantly associated with the binary ATWG toxicity classification (p-value = 2.2 x 10-16). This study also demonstrated that when a cytotoxicity value was the minimum AC50 for a chemical, the default cytotoxicity point was used most. In all, 1,214 out of 1,637 chemicals with ToxCast data have an assay that is predictive for acute toxicity. Inclusion of the larger set of assays from PubChem will expand the chemical coverage and potentially identify novel targets that correspond to the known mechanism of toxicity, which we've previously shown improves the predictivity. Taken together, the results suggest that combining bioactivity and structural information may be as reproducible as traditional in vivo studies. Because the current workflow focuses on tiered testing guided by the in silico predictions, high-throughput assays are not required, which greatly expands the number of assays available for testing. Integrated models that combine the data from in silico and targeted bioactivity measurements can further improve the predictivity.
Score 5*: *2 for developing in vitro/in silico methods for acute tox + 2 for in-house validation + 1 for potential impact on avoiding animal acute tox tests*

4311
3D Nephroscreen: High-Throughput Drug-Induced Nephrotoxicity Screening on a Microfluidic Proximal Tubule Model
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Renal toxicity remains a major issue in clinical trials and stresses the need for more predictive models fit for implementation in early drug development. Here, we describe the use of a high-throughput, microfluidic platform for the detection of drug-induced nephrotoxicity. A microfluidic platform (Mimetas' OrganoPlate®) was combined with renal proximal tubule epithelial cell lines (PTEC) and exposed to fluid shear stress. A 12-compound nephrotoxicity screen across multiple laboratories was performed in collaboration with sponsors and the NC3Rs. ciPTECOAT1 or RPTECs (Sigma) seeded against an ECM gel under perfusion flow were used to establish a proximal tubule-on-a-chip. Tubules with polarized epithelium containing functional transporter expression were obtained. Drug-induced toxicity was assessed by exposing the tubules to 4 benchmark compounds with known clinical effect and 8 blinded compounds supplied by the sponsors for 24 and 48 hours. Epithelial barrier tightness and drug-transporter

interactions were evaluated. Parallel to this, cellular damage and stress were assessed using various read-outs. Finally, gene expression analysis was performed to assess AKI markers. The Nephroscreen revealed that a combination of cell viability, LDH and miRNA release were the most predictive readouts in determining nephrotoxicity. Most of the blinded compounds resulted in toxicity detected by at least one of the functional read-outs. Nephroscreen provides a reliable standardized and automatable system for efficacious identifying nephrotoxicants and revealing their mode of action.

1.Wilmer, M. J. et al. Kidney-on-a-Chip Technology for Drug-Induced Nephrotoxicity Screening. Trends in Biotechnology 34, 156-170 (2016).
2.Trietsch, S. J., Israëls, G. D., Joore, J., Hankemeier, T. & Vulto, P. Microfluidic titer plate for stratified 3D cell culture. Lab Chip 13, 3548-3554 (2013).
3.Vormann, M. K. et al. Implementation of a Human Renal Proximal Tubule on a Chip for Nephrotoxicity and Drug Interaction Studies. J. Pharm. Sci. 1-14 (2021).

Score 5*: *2 for development of nephrotoxicity assay on kidney MPS + 3 for external validation*

ICT 2022

S-24-01

Innovative toxicology approaches to predict safety of inhaled candidate drugs

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The development of inhaled drug candidates for respiratory diseases is frequently impacted by clinical dose level limitations due to lung pathology or functional respiratory effects in non-clinical safety studies. The potential in vivo safety findings include lung epithelial irritation, inflammation, immunogenicity and hypersensitivity as well as responses such as bronchoconstriction and cough. To enable the discovery of novel candidate drugs with the right safety profile, there is a need for lung toxicity assays that can be applied during drug discovery for early hazard identification and mitigation. The assays developed need to be predictive for a diverse set of modalities and tailored to its specific safety needs and unknowns. As part of our aim to predict and mitigate lung safety concerns early, prior to candidate selection, we have implemented a set of in vitro 2D and 3D lung models. Previously we have shown that in vivo toxicity can be predicted in vitro by studying cell barrier integrity by transepithelial electrical resistance (TEER) in a 3D human airway model. To increase through-put and sensitivity, we have recently developed a novel high content screening assay to allow for quantification of the tight-junction marker Occludin in an alveolar cell line. This assay has excellent predictivity for in vivo lung toxicity across a set of 20 validation compounds and is utilized as a model system for lung epithelial barrier integrity for inhaled molecules. To predict potential toxic effects across a broad range of inhaled modalities, we have explored systems with increasing physiological relevance, including in vitro lung models encompassing multiple cell types (eg. immune component, alveolar epithelial and endothelial cells) and microphysiological systems and will discuss their predictivity. Lack of mechanistic understanding in the event of observed in vivo lung toxicity, is a

key issue in the development of inhaled drugs. We have developed an approach to overcome that, by combining spatial transcriptomics, mass spec imaging and traditional histopathology in lung, for which we will present a proof of principle study.
Score 6: ***3 for new HCS assay of lung toxicity + 2 for in-house validation + 1 for adding further analytical tools to improve mechanistic understanding***

SOC-VI-09
SkinEthic™ HCE Time-to-Toxicity: on the way of being the first OECD adopted new approach methodology allowing the identification on its own of substances and mixtures for eye hazard identification
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Before a new product is approved by the authorities and placed on the market, numerous tests must be carried out. This includes testing whether the chemical induce ocular effects. Since March 2013, European regulations have banned animal testing for cosmetics purposes including for local tolerance irrespective of whether there are alternatives. For more than two decades, scientists have been trying to replace in vivo rabbit eye irritation test with non-animal methods. So far, several in vitro methods have been implemented into regulations, however none of them is able to replace the test completely due to the complexity of the endpoint and the classification schemes applied by the regulation under the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (UN GHS). Taking into account our expertise on the SkinEthic™ HCE model (test system of OECD Test Guidelines 492) and our kwowldege on protocols, the SkinEthic™ HCE Time-to-Toxicity test method was established. The new approach methodolgy (NAM) evaluates the hazard potential of a chemical based on its ability to induce cytotoxicity. The method consists of 2 protocols, one for liquids (TTL) and one for solids (TTS). Based on the viability observed for the different exposure periods (from 5 to 120-min) a classification is assigned. The method was developed with 74 training chemicals (32 liquids, 42 solids) and challenged with 52 test chemicals selected on the basis of the main in vivo drivers of classification (i.e., corneal, conjunctival and persistence effects). Application to 74 training chemicals, accuracy value was above 72%, with 75% Cat.1, 68% Cat.2 and 74.9% No Cat. correctly identified [1,2]. The relevance and reliability of both TTL and TTS protocols have been also assessed on 40 coded chemicals in a multi laboratory trial in three laboratories [3]. The within laboratory reproducibility from the 3 laboratories was 90% for liquids and 100% for solids while the between laboratory reproducibility was 80% and 100%, respectively. When considering all 151 tested chemicals, the test method has a balanced accuracy of 74% with correct predictions of 79% for Cat 1 (N = 50), 69% for Cat 2 (44) and 75% for No Cat (57), when compared to reference in vivo rabbit eye test data. Furthermore, none of the UN GHS Cat. 1 were identified as false negative leading to a conservative approach. Overall, these studies provide evidence that the test method is capable of distinguishing between the three UN GHS categories. The SkinEthic™ HCE Time To Toxicity was recommended as a full replacement to the in vivo Draize acute eye irritation test for classification of substances and mixtures by independent International experts and therefore the new Test Guideline 492B is considered for an OECD adoption. In conclusion, the approval of this NAM opens the

possibility for progress to enable to get rid of animal testing to predict chemical ocular identification.

References

[1] Alépée N., Leblanc V., Grandidier MH., Teluob S., Tagliati V., Adriaens E., Michaut V. (2020) Development of the SkinEthic HCE Time-to-Toxicity test method for identifying liquid chemicals not requiring classification and labelling and liquids inducing serious eye damage and eye irritation. Toxicol. in Vitro 69, 104960.

[2] Alépée N., Leblanc V., Grandidier MH., Teluob S., Viricel A., Adriaens E., Michaut V. (2021). SkinEthic HCE Time-to-Toxicity on Solids: A test method for distinguishing chemicals inducing serious eye damage, eye irritation and not requiring classification and labelling. Toxicol. in Vitro 75, 105203.

[3] Alépée N., Grandidier MH., Teluob S., Amaral F., Caviola E., De Servi B., Martin S., Meloni M., Nardelli L., Pasdelou C., Tagliati V., Viricel A., Adriaens E., Michaut V. (2022). Validation of the SkinEthic HCE Time-to-Toxicity test method for eye hazard classification of chemicals according to UN GHS. Toxicol. in Vitro, 80,105319. https://doi.org/10.1016/j.tiv.2022.105319.

Score 6: ***2 for development of existing assay + 3 for external validation + 1 for being a single in vitro test that replaces an approved animal test***

P01-01

Allergic respiratory diseases linked with AOP caused by chemicals in the workplace

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Purpose: Occupational allergic respiratory disease (especially occupational asthma) is an immune disease caused by antigens in the workplace. For the initial detection of this disease, we tried to identify the disease mechanism and markers using Adverse outcome pathway (AOP), which is being developed as part of an animal replacement experiment.

Methods: Using a 3D-cell model constructed with human respiratory cells (MucilairTM, SmallairTM, SmarllairTM-asthma), the cytotoxicity, oxidative damage-related and inflammation-related indicators of Methylene Diphenyl diisocyanate (MDI), Toluene diisocyanate(TDI), and Trimellitic anhydride (TMA) were examined, and morphological changes of the constituent tissues were confirmed.

Results: Cell damage caused by the test substance was confirmed in each respiratory system model d 24 hours or 48 hours after administration of the test substance using the Mucilair, Smallair and Smallair-asthma models. In addition, an increase in ROS/RNS by the test substance by model and timewas confirmed, but clear changes in cytokines and chemokines were not confirmed. Morphological changes and mucus secretion changes of each model were confirmed through histopathological examination.

Conclusion: AOP and KE related to occupational asthma were experimentally confirmed through a 3D-cell model composed of human respiratory system cells. Indices of respiratory system cell stimulation and damage and oxidative damage by each substance were changed. However, the next phase of cytokine and chemokine changes is not clear, so it is considered that additional experimental evidence is needed.

References

[1] Balogh SK, Sivars U, Hornberg E, 2018, 'A 3D Human Airway Model Enables

Prediction of Respiratory Toxicity of Inhaled Drugs In Vitro', Toxicol Sci,162(1):301–308.
[2] Blume C, Davies DE, 2013, 'In vitro and ex vivo models of human asthma', Eur J Pharm Biopharm, 84(2):394–400.
[3] Clippinger AJ, Allen D, Behrsing H, 2018, 'Pathway-based predictive approaches for non-animal assessment of acute inhalation toxicity', Toxicol Vitro, 52:131–145.
[4] Huang S, Wiszniewski L, Constant S, 2013, 'Potential of in vitro reconstituted 3D human airway epithelia (MucilAir™) to assess respiratory sensitizers', Toxicol In Vitro, 27:1151–1156.
[5] Kimber I, Poole A, Basketter DA, 2018, 'Skin and respiratory chemical allergy: confluence and divergence in a hybrid adverse outcome pathway', Toxicol Res, 26;7 (4):586–605.
[6] Sullivan KM, Enoch SJ, Ezendam J, 2017, 'An Adverse Outcome Pathway for Sensitization of the Respiratory Tract by Low-Molecular-Weight Chemicals: Building Evidence to Support the Utility of In Vitro and In Silico Methods in a Regulatory Context', Applied In Vitro Toxicology, 3(3):213–226.
[7] Welch J,Wallace J, Lansley AB, Roper C, 2021, 'Evaluation of the toxicity of sodium dodecyl sulphate (SDS) in the MucilAir™ human airway model in vitro', Regul Toxicol Pharmacol, 125:105022.
Score 5*: *2 for confirming elements of respiratory AOP + 1 for proof of principle + 1 for using 3D cell model*

P01-02

AOP-based in vitro assay development for assessment of inhalational toxicants — oxidative stress leading to decreased lung function

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The respiratory system is one of the major exposure routes to exogenous, often toxic, compounds. It is, therefore, necessary to develop reliable tools for assessing the effects of inhaled substances on human health. Currently, acute inhalational toxicity assessments for regulatory purposes are conducted in animals in accordance with OECD methods TG403, TG436, and TG433. However, 21st century toxicology approaches leaning on the 3Rs principle as well as changes in legislature, cost, and limited throughput have galvanized the development of alternative in vitro methods. Adverse outcome pathways (AOP) organize available information related to the occurrence of an a dverse outcome (AO) by providing causal relationships between a molecular initiating event (MIE) and subsequent key events (KE), thereby facilitating mechanistic understanding of a particular pathogenesis and linking exposures to adverse effects. AOPs can be utilized to derive meaningful alternative in vitro methods to complement the evaluation of inhalational toxicants. Here, we describe a conceptual framework for the development and validation of biological assays to enable MIE assessment and measurement of KE within the scope of AOP411 (oxidative stress leading to decreased lung function). AOP411 describes the impact of oxidative stress (an MIE) on ciliary beat frequency (KE1) and mucociliary clearance (KE2), which eventually lead to decreased lung function (an AO). For our model, we used well-established 3D human bronchial epithelial cell cultures (MucilAirTM, Epithelix). We quantitatively characterized the MIE by measuring the increase in reactive oxygen species (ROS) levels and decrease in antioxidant (glutathione) levels following acute and chronic exposure to different concentrations of known oxidative stress inducers. To

recapitulate the causal relationship and the temporal sequence of the KEs, we performed high-speed video-microscopy analysis with the Sisson- Ammons Video Analysis (SAVA) system to further measure ciliary beat frequency and mucociliary clearance after exposure-induced oxidative stress. Further validation of this AOP-based approach is expected to increase its acceptance among regulatory entities with a view towards decision-making in the context of risk assessment.
Score 5*: *2 for confirming elements of respiratory AOP + 1 for proof of principle + 1 for using 3D cell model*

P04-14
Identify chemicals potentially able to interfere with the endocrine system using a suite of complementary in silico models employing semi-automation
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There is a need for new approach methodologies (NAMs) to identify potential endocrine disrupting chemicals involved in key events of endocrine pathways including binding to receptor proteins. In 2018 a guidance describing how to perform hazard identification for endocrine-disrupting properties by following the scientific criteria which are outlined in EU 2017/2100 and EU 2018/605 for biocidal products and plant protection products, respectively,was published by ECHA and EFSA. In this guidance computational approaches are proposed as line of evidence for endocrine activity assessment. An in silico screening workflow which follows this guidance is described in this study. It employs 113 freely available and commercial models covering 27 receptors: 74 (Q)SARs, 17 rule-based profilers, 16 models of receptor interactions and 6 ToxCast pathway models. It addresses EATS (Estrogen, Androgen, Thyroid, Steroidogenesis) and “other” modalities as well as the Mode of Action agonist, antagonist or binding. The issues with this process is that each model has its own terminology for results, definition of whether the prediction is within the applicability domain as well as different specificity and model reliability. In order to reach a consensus outcome, it was first necessary to devise an ontology from the output of the different models into a normalised input. The model specificity, model reliability and type of model requires scoring to provide a weighting to the predictions. For example, the results of the profilers are primarily used to confirm the results of the QSAR as these approaches are known to give high rate of False positives. The molecular modelling and the in vitroToxCast results are rated according only to the reliability of the models as the applicability domain is not an issue. All the models are applied and the results are combined with an algorithm that considers existing experimental data, the reliability of the model and the uncertainty of each prediction. The predictions are then combined by receptor and an overall conclusion for each receptor is given. When models with the same level of uncertainty disagree an expert assessment of the nearest neighbours is carried out to get to a final conclusion. Positive predictions are used to give indication on the mechanism of action for the endocrine disruption. As this workflow is very labour intensive, a KNIME workflow was coded to automate the data normalisation, weighting process and report generation. Two examples are demonstrated: butylparaben, correctly predicted as active towards the estrogen receptor, and triclosan, correctly predicted to be active towards the androgen receptor.
Five active and five inactive compounds, CAS 100-21-0, 84-61-7, 98-54-4, 106-44-5, 938-16-9, 438-22-2, 108-43-0, 10161-33-8, 83792- 61-4 and 17-hydroxyestra-4,9,11-trien-3-one, are demonstrated and correctly predicted.

Score 5*: ***3 for devising semi-automated combination of existing tools for identifying potential endocrine disruptors + 2 for in-house validation***

P17-18

Performance of a new defined approach for surfactants for eye hazard assessment based on in vitro test methods

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Cosmetics Europe (CE) has developed two defined approaches (DAs) for non-surfactant liquid chemicals for eye hazard identification, i.e. addressing serious eye damage (UN GHS Cat. 1), eye irritation (UN GHS Cat. 2) and (the absence thereof; UN GHS No Cat.) (Al.p.e et al., 2021). However, neither a single in vitro test method nor a testing battery has been developed to assess eye hazard potential of surfactants across the whole range of UN GHS categories. CE has recently developed a defined approach (DA) to predict this endpoint for liquid, semi-solid and solid chemicals having surfactant properties. The DA for surfactants is based on the combination of Reconstructed human Cornea-like Epithelium test methods (RhCE, OECD TG 492) and a modification of the Short Time Exposure test method (STE, OECD TG 491). The reference set used to develop the DA contained cationic, anionic, non-ionic and zwitterionic surfactants. Furthermore, the most important drivers of in vivo Cat. 1 and Cat. 2 classification were represented. Regarding the No Cat., the 2 main subgroups (CO = 0 and CO>0)were also included. In a first tier of the DA, an RhCE test method (OECD TG 492: EpiOcular™ EIT or SkinEthic™ HCE EIT) is used to distinguish No Cat. from classified substances. In case the surfactant results in a positive call based on an RhCE method, the STE method is used to further sub-categorize. Surfactants that result in a viability < 20% when tested at 5% w/v and 0.5% w/v with the STE are classified Cat. 1, in all other cases the surfactant is classified Cat. 2. The performance of the DA was assessed against the proposed minimum performance of 75% for Cat. 1, 50% for Cat. 2 and 70% for No Cat. agreed by the OECD experts. The balanced accuracy of the DA was 79.3% (N = 40), 83.3% of Cat. 1 (N = 18), 77.8% of Cat. 2 (N = 9) and 76.9% of No Cat. (N = 13) were correctly identified. These values were greater than the proposed minimum values. In conclusion, combination of an RhCE method with the STE test method demonstrates their potential to successfully distinguish between the 3 UN GHS categories for eye hazard identification.

Score 5*: ***2 for development of Defined Approach for eye hazard potential of surfactants + 3 for external validation.***

ESTIV 2022

O-3B-2

Integration of human-stem-cell-based embryoid bodies into a microfluidic multi-tissue platform for systemic embryotoxicity testing

ABSTRACT #303

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Assessing compound embryotoxicity constitutes a central part of every drug development process. However, current in vitro assays do not include complex embryo maternal interactions during pregnancy and are mostly based on the use of murine-derived cell models, which are of limited predictive power due to considerable inter-species differences. Here, we present a multi-organ platform, which combines a microphysiological model of the placental barrier with 3D embryoid bodies (EBs), derived from human induced pluripotent stem cells (hiPSC). The platform consists of two independent fluidic networks, representing maternal and embryonic blood circulation. Both fluidic networks are separated by a semipermeable membrane, which serves as a scaffold to form and culture a human placental- trophoblast barrier in the maternal culture compartment. The hiPSC-derived EBs are cultured in immediate vicinity to the placental barrier in a hanging drop on the embryonic side, which enables direct interaction and molecule exchange between the tissue models through the liquid phase. In a first step, we successfully established the formation and cultivation of hiPSC-derived EBs in our microfluidic device and compared their growth behavior and morphology to those achieved with standard well plates. To evaluate toxicity effects on embryonic development, we established an optical clearing method to visualize the spatial distribution and differentiation of hiPSCs into derivatives of the three germ layers. We further developed a qPCR- based panel of genes, expressed during early embryonic development, to evaluate altered gene expression patterns in differentiating EBs. In a next step, we integrated the placental barrier into the system and confirmed hEB growth and differentiation under co-culture conditions. These results show the potential of the platform to mimic physiologically relevant conditions on chip and lay a promising foundation to study the effects of compounds at the embryo-maternal interface in an entirely human-based system.

References

[1] J.A. Boos, P.M. Misun, A. Michlmayr, A. Hierlemann, and Olivier Frey "Microfluidic Multi- tissue Platform for Advanced Embryotoxicity Testing In Vitro," Adv. Sci., 6, 13, 2019.

Score 5*: *3 for novel MPS of human placenta and embryoid bodies for embryotoxicity testing + 1 for proof of principle + 1 for developing a gene panel to assess alterations in gene expression*

P-4a-13

Physiological map to study kidney toxicity in the ONTOX project

ABSTRACT #428

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Background and Objectives Continuous improvements of computational approaches also increase the predictive performances of toxicological in silico models [1]. However, being mainly based on animal test data, these computational models lack a good correlation with human toxicity, and, being often based uniquely on chemical structures, they are unable to explain toxicological processes. To overcome these limitations, we have developed a new semi-automated strategy to build a Physiological Map (PM), a framework to study human toxicological mechanisms. Materials and Methods Our method is useful to build a PM or to validate an existing PM. To retrieve information, a manual literature review was accompanied by computational interrogation of ontologies (e.g. Gene Ontology), thus creating a network of genes, proteins, molecules and phenotypes [2]. The network was converted manually into a PM using the CellDesigner software and visualized on the web using the MINERVA platform. The entire procedure was supported and revised by field experts. Results We present here the human kidney PM, developed in the framework of ONTOX, a European project aimed at improving risk assessment avoiding the use of animal tests [3]. With the purpose to better understand tubular necrosis and nephrolithiasis, the PM represents the normal physiology in proximal tubule, the loop of Henle, distal tubule, and collecting duct cells, displaying the vitamin D metabolism and the urine production processes: filtration, reabsorption and secretion. Discussion and Conclusions Our method assists the user to build a PM even starting from limited data. The PM is initially a static representation of physiological processes, also useful to study and develop new adverse outcome pathways. Subsequently, we could add kinetic parameters, transforming the PM into a dynamic model able to represent cellular perturbations. This approach presents an opportunity to investigate human toxicities, improving the toxicological predictions from a qualitative and quantitative perspective.

References

[1] Manganelli S, Gamba A, Colombo E, Benfenati E (2022) 'Using VEGAHUB Within a Weight-of-Evidence Strategy'. In: Benfenati E. (eds) In Silico Methods for Predicting Drug Toxicity. Methods in Molecular Biology, vol 2425. Humana, New York, NY. https://doi.org/10.1007/978-1-0716-1960-5_18 [2] Gamba A, Salmona M, Bazzoni G (2020) 'Quantitative analysis of proteins which are members of the same protein complex but cause locus heterogeneity in disease', Sci Rep 10, 10423. https://doi.org/10.1038/s41598-020- 66836-7 [3] Vinken M., et al. (2021) 'Safer chemicals using less animals: kick-off of the European ONTOX project', Toxicology 458, 152846. https://doi.org/10.1016/j.tox.2021.152846

Score 5*: *3 for creation of a physiological map of kidney toxicity + 1 for proof of principle + 1 for using computational methods to identify new AOPs*

P-4a-18

Designing physiological maps as a tool to study liver toxicology
ABSTRACT #388

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Background and Objectives: Physiological Maps (PMs) are conceptual constructs that incorporate information as mechanistic representations of biological processes1. PMs can be used qualitatively and quantitatively as a mechanistic basis for improving Adverse Outcome Pathways (AOP) and supporting model rationale for several purposes. Within the ONTOX project, we have created two PMs to study the following chemical-induced liver diseases: steatosis and cholestasis. The purpose of the PMs is to improve current AOP networks and develop ontologies to support liver toxicity prediction. Material and Methods: We adapted the workflow from the Disease Maps project2 to construct the maps. First, relevant physiological literature was curated with the support of domain experts. Then, we listed fundamental mechanisms to be mapped and screened online databases for previously described pathways. Finally, we integrated pathways and literature data using the CellDesigner software and displayed them using the MINERVA platform3. Results: The maps include all the processes known in the current state of the art to trigger the corresponding AOP network on cholestasis and steatosis. However, they are not restricted to the currently available AOPs but also include other physiological processes vital to liver physiological functioning and homeostasis. Moreover, these maps expand beyond the liver, encompassing routes in different compartments critical for understanding liver mechanistic processes. Discussion and Conclusion: We designed these maps using expert-curated literature and previously available community- developed pathways, focusing on physiological functions and the human genome, transcriptomics, and proteome. They must be constantly updated to serve the community as a dynamic tool. Besides, PMs will become a more robust and multi-layered tool with the incorporation of quantitative kinetic and chemical information in future versions, which will be developed further as chemical-induced disease ontologies1. Such tools will offer a more comprehensive understanding of the liver-specific pathways regulated by chemical compounds, allowing for future toxicity prediction.

References

1. Vinken, M. et al. Safer chemicals using less animals: kick-off of the European ONTOX project. Toxicology 458, 1–7 (2021).
2. Mazein, A. et al. Systems medicine disease maps: community-driven comprehensive representation of disease mechanisms. npj Syst. Biol. Appl. 4, (2018).
3. Hoksza, D., Gawron, P., Ostaszewski, M., Hasenauer, J. & Schneider, R. Closing the gap between formats for storing layout information in systems biology. Brief. Bioinform. 21, 1249–1260 (2019).

Score 5*: ***3 for creation of a physiological map of liver toxicity + 1 for proof of principle + 1 for using computational methods to identify new AOPs***

EUSAAT 2022

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Building virtual cohorts via the integration of public data

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"In silico clinical trials" refers to the development of patient-specific models to form virtual cohorts for testing the safety and/or efficacy of new drugs and of new medical devices. This type of approach can be used in all stages of drug discovery, from screening to pre-clinical trials and greatly reduce drug development time and animal testing. In silico preclinical trials could potentially address the translational issues in several ways by evaluation of a proof of concept in the early phases of the drug development process [1]. Current in silico methods, namely those based on Artificial Intelligence and Machine Learning, depend on possessing great volumes of data, a problem not always easy to overcome. Transcriptomics have the potential of characterizing individuals' biological and disease state [2]. Resorting to the integration of publicly available data is a viable way getting new insights into disease mechanisms, and to simulate patient populations [3]. Nevertheless, public datasets are subjected to different processing and normalization procedures [4] which often prevents data integration and its usage in Big Data experiments. Although sequencing data sets (e.g., RNA-Seq) are becoming more abundant, the larger proportion of public transcriptomic data streams from microarray (MA) analysis [5]. With the aim building of cohorts of virtual individuals, in this work we present a method for MA data processing and normalization that allows the analysis across previously processed datasets. We accomplish this goal by, first, predicting and reverting mathematical transformations that may have been applied to MA data; and second, applying a normalization technique that considers the expression of House Keeping genes across the whole set of samples in our global database. Our method is shown to decrease batch effects among samples, thus allowing the extraction of biological conclusions from integrated datasets. As means of assessing this approach in a biological context, we've tested a prostate cancer dataset containing data from 63 different GEO experiments with differential gene expression of 817 samples, and the method was capable of reproducing discoveries reported in other prostate cancer-centered publications. This method corresponds to one of the key initial steps to building virtual patients and conduct in silico clinical trials. Furthermore, we think that it facilitates the reuse of existing datasets, allowing researchers to perform analysis across larger cohorts, and reducing the needs for extensive animal experimentation required for hypothesis building and validation.

References

[1] Pappalardo, F., Fichera, E., Paparone, N. et al. (2016). Bioinformatics 32, 2672-2680. doi:10.1093/bioinformatics/btw293
[2] Byron, S. A., van Keuren-Jensen, K. R., Engelthaler, D. M. et al. (2016). Nat Rev Genet 17, 257-271. doi:10.1038/nrg.2016.10
[3] Kato, S., Kim, K. H., Lim, J. L. et al. (2020). Nat Commun 11, 1-9. doi:10.1038/s41467-020-18613-3
[4] Cheng, L., Lo, L. Y., Tang, N. L. S. et al. (2016). Sci Rep 6, 1-11. doi:10.1038/srep18898
[5] Thompson, J. A., Tan, J. and Greene, C. S. (2016). PeerJ 4, e1621. doi:10.7717/peerj.1621

Score 5*: *3 new computox approach to preclinical trials + 1 for proof of principle + 1 for potential range of applicability*

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Human test methods for developmental neurotoxicity (DNT) evaluation: Set-up, scientific validation and statistical analyses

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Testing for developmental neurotoxicity (DNT) is currently performed in rats according to OECD/US-EPA guidelines. These methods are resources demanding, have unknown sensitivity and uncertainties in their interpretation. To overcome these issues and allow a large number of chemicals to be tested for DNT, a DNT in vitro testing battery (DNT IVB) has been assembled under the guidance of the European Food Safety Authority (EFSA) in collaboration with the Danish- and US-Environmental Protection Agency and under the umbrella of the Organisation for Economic Cooperation (OECD). As an integral part of the DNT IVB we set up the Neurosphere Assay consisting of primary human neural progenitor cells (hNPC). This test system pictures the neurodevelopmental processes NPC proliferation and migration, neuronal and glia differentiation, neurite outgrowth as well as neuronal and oligodendroglial migration. The individual test methods representing these endpoints were scientifically validated by demonstrating specific cell morphologies, marker expressions, presence of neurodevelopmental processes, physiological signaling responses and toxicological adverse effects. 120 compounds representing different chemical classes were tested in the respective assays generating concentration-response data. Based on this data set, different biostatistical methods for data evaluation were compared with regards to compound classification and an R-based data evaluation pipeline was set up accordingly. In addition to the Neurosphere Assays, a test method for assessing human neuronal network formation and function – the human NNF assay – was established using human induced pluripotent stem cell-derived neurons and primary human astrocytes grown on microelectrode arrays. This assay was challenged with more than 30 pesticides and data was evaluated using the established biostatistical pipeline. We present assays and testing results for a large variety of neurodevelopmental endpoints that contribute to an OECD-supported DNT IVB. More data are currently being generated to increase knowledge on the IVB's applicability domains for reducing uncertainty in its performance.

Score 6: ***3 for developing a multi-endpoint DNT test battery + 2 for in-house validation + 1 providing concrete support for OECD DNT testing programme***

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cellasys #8: A microphysiometric test to identify serum-free cell culture media

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Most Life Science labs culture cells using fetal bovine serum (FBS), which is collected from fetuses when pregnant cows are slaughtered [1]. It is estimated that the worldwide annual production of FBS is about 600,000 to 800,000 liters, collected from around 1 to 2 million fetuses. While this vast number of fetuses per year entails not only severe

ethical issues, the usage of FBS is also associated with substantial quality and reproducibility concerns (e.g., unknown components that also vary from batch to batch) [2]. For these reasons, scientists are trying to transit from serum-based cell culture media (CCM) to chemically defined CCM without FBS. To do so, weaning experiments are usually carried out to evaluate new serum-free CCM formulations. Here, cells are gradually switched from a serum-based CCM to a serum-free alternative. However, such experiments take weeks to months [3], resulting in labor-intense and, hence, time-consuming tasks. To address this issue and foster the acceleration of serum-free CCM, the talk presents a methodology to identify serum-free CCMs as well as a case study with L929 cells. Building upon our established microphysiometry technology [4], we implemented a standardized and automated testing schema, referred to as cellasys #8, designed to quickly identify the impact of serum-free CCMs on cellular metabolism and morphology. In detail, miniaturized pH, oxygen and impedance microsensors allow real-time measurements to identify changes in cell adherence and vitality. In combination with an automated and integrated medium change from serum-based to serum-free CCM, the test setup allows the evaluation of two crucial features: Firstly, the culture with a novel CCM is evaluated to identify its suitability and, secondly, the recovery phase is evaluated when cells are cultured again with serum-based CCM afterward. To demonstrate the applicability of the cellasys #8 test, we evaluated the suitability of three serum-free CCM formulations for the culture of L929 cells: (i) DMEM, (ii) DMEM/F12, and (iii) DME/F12 + ITS. DME/F12 + ITS was selected, as it is an established serum-free CCM for L929 cells. While the cellasys #8 test indicated changes in metabolism and morphology with DMEM and DME/F12, no changes were recorded during the treatment as well the recovery phase when cells were cultured with DME/F12 + ITS. This case study demonstrates that the cellasys #8 test is able to successfully identify serum-free CCM formulations at increased speed and reduced costs.

References

[1] Van der Valk, J. (2022). Science 375, 143-144. doi:10.1038/537433a
[2] Van Der Valk, J. et al. (2018). ALTEX 35, 1-20. doi:10.14573/altex.1705101
[3] Gstraunthaler, G. and Lindl, T. (2021). Spektrum Verlag, Heidelberg. doi:10.1007/978-3-662-62606-1
[4] Wiest, J. (2022). Organs Chip 4, 100016. doi:10.1016/j.ooc.2022.100016

Score 5*: *3 for new, universal, assay to evaluate serum-free culture medium formulations + 1 for proof of principle + 1 for potential impact on animal (foetal calves) welfare*

174

Development of physiologically relevant in vitro inhalation model to predict acute respiratory toxicity of mists and volatile liquids

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Acute respiratory toxicity (ART) testing is required to assess the health effects of inhaled substances. OECD accepted methods utilize GHS categorization that is based on animal death. There is no validated in vitro ART assay, even though animal tests have been discredited as predictors of human responses and on ethical grounds. The goals of this work were to develop physiologically relevant ART tests using the

EpiAirway™ tissue model, demonstrate interlaboratory transferability, and correlate the results to an established categorization system relevant to human respiratory irritation. Test articles (TA, n = 53) were applied to EpiAirway tissues (0.6 cm2) at MatTek (USA) and IVLSL (Slovakia) with ART protocols developed for exposure to mists/sprays (Direct Application Protocol, DAP) and vapors/volatile liquids (Vapor Cap Protocol, VCP). In both protocols, tissues were exposed for 4 hours to 4 fixed doses of the TA (0.5, 2, 10, 20 mg/tissue, diluted in corn oil or water). In the DAP, TAs were applied to the apical tissue surface and in the VCP – to an absorbent material in a specially designed cap that forms a tight seal above the tissue allowing exposure to TA vapor. The effects on tissue viability (MTT assay) and barrier properties (Transepithelial Electrical Resistance, TEER) were determined. The effective doses which reduced tissue viability by 25% (ED-25) or by 75% (ED-75) were mathematically interpolated for the DAP and VCP methods, respectively. The ED-25 and ED-75 were correlated to the acute irritation Health Effects (HE) Codes (HE14/15/16) listed by OSHA, which are relevant to human exposure. Using the MTT assay, the DAP discriminated between HE14/15/16&NH with a Sensitivity/Specificity/Accuracy (S/S/A) of 77.6/87.6/82.6% (MatTek) and 75.5/86.1/80.8% (IVLSL); correlation to GHS Cat.1&2/3&4/5&NC gave results of 63.5/76.1/69.8% (MatTek) and 63.8/76.1/70.0% (IVLSL) S/S/A. The VCP discriminated between HE codes with S/S/A of 80.9/90.5/85.7% (MatTek) and 77.6/90.0/83.8% (IVLSL); correlation to GHS was 70.1/82.9/76.5 S/S/A (MatTek) and 71.1/82.2/76.7% (IVLSL). Both protocols demonstrated high predictivity of human HE Codes, which are more relevant to human respiratory toxicity than the GHS categories. Good inter-laboratory reproducibility was observed for the VCP. The VCP and DAP provide physiologically relevant, organ-specific in vitro tests that can improve the predictivity of human responses and significantly reduce the number of animals being used.

Score 5*: *2 for the development of EpiAirway as an in vitro ART assay + 3 for external validation*

WC12

Oral abstracts

85

Development of a microphysiological skin-liver-thyroid Chip3 and its application to evaluate the effects on thyroid hormones of topically applied cosmetic ingredients under consumer-relevant conditions

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All cosmetic ingredients registered in Europe must be evaluated for their safety using non-animal methods. Microphysiological systems (MPS) offer a more complex higher

tier model to evaluate chemicals. We investigated whether thyroid follicles could be incorporated to evaluate the potential of topically applied chemicals to cause endocrine disruption. This combination of models in the HUMIMIC Chip3 is new; therefore, we describe here how it was optimized using two chemicals known to inhibit thyroid production, daidzein and genistein. The MPS was comprised of Phenion® Full Thickness skin, liver spheroids and thyroid follicles cocultured in the TissUse HUMIMIC Chip3. Endocrine disruption effects were determined according to changes in thyroid hormones, thyroxine (T4) and 3,3',5-triiodothyronine (T3). The skin-liver-thyroid Chip3 model was used to determine a consumer-relevant exposure to daidzein present in a body lotion based on thyroid effects. A "safe dose" of 0.235 µg/cm2, i.e., 0.047% applied in 0.5 mg/cm2 of body lotion was the highest concentration of daidzein which does not result in changes in T3 and T4 levels. This concentration correlated well with the value considered safe by regulators. In conclusion, the Chip3 model enabled the incorporation of the relevant exposure route, metabolism in the skin and liver, and the bioactivity endpoint into a single model. These conditions are closer to those in vivo than 2D cell/tissue assays lacking metabolic function. Importantly, it also allowed the assessment of repeated doses of chemical and a direct comparison of systemic and tissue concentrations with toxicodynamic effects over time, which is more realistic and relevant for safety assessment.

Score 5*: ***3 for MPS for endocrine disruption by topically applied chemicals + 1 for proof of principle + 1 for potential impact***

613

OECD validation of the ToxTracker assay for genotoxic mode of action assessment

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ToxTracker is a mammalian stem cell-based reporter assay that detects activation of specific cellular signaling pathways upon chemical exposure. ToxTracker contains six different GFP-tagged reporter cell lines that together allow the accurate identification of genotoxic substances and discrimination between induction of DNA damage, oxidative stress and/or protein damage in a single test. More recently, the assay was extended to allow the discrimination between clastogenic and aneugenic compounds. The ToxTracker assay was evaluated in a large international inter-laboratory validation study, approved by the OECD. The goal of this prospective validation study was to explore the applicability of ToxTracker for regulatory applications, establish the transferability and reproducibility of the assay and to explore how it can be applied to improve the in vitro genotoxicity testing strategies. The validation has been conducted strictly following OECD guidance document 34. ToxTracker was transferred to seven laboratories. The validation labs were trained to perform the assay and tested a training set of compounds to show their proficiency to run ToxTracker. Next, the labs evaluated a selection of 64 coded, well-established genotoxic and non-genotoxic chemicals with each compound being tested in three labs independently. The accuracy to predict genotoxicity was 89%. Also, the intra- and inter-laboratory reproducibility were determined. The mechanistic information that was provided by ToxTracker was used to gain insight into the MoA of genotoxic compounds and to explain positive results from the standard in vitro genotoxicity assays.

Score 5*: ***2 for further development of ToxTracker assay + 3 for external validation***

650

Development of a 3D genotoxicity model for assessment of cosmetic formulations

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A variety of in vitro assays exist to examine the genotoxic potential of compounds, however the application to real-life exposure is questioned. We show that 3D tissue models can be effectively combined with an in vitro animal-free genotoxicity screen, overcoming insoluble formulations and with dosing that mimics real-life application. This system allows investigation into whether potential genotoxic compounds can pass through a reconstructed skin barrier, and if so, remain genotoxic following exposure to skin metabolic enzymes. Furthermore, use of the animal-free Blue-Screen test allows for identification of all 3 classes of genotoxins; mutagens, clastogens and aneugens. In summary, we created a co-culture system consisting of TK6 cells and EpiDerm tissue models which was validated using a panel of known genotoxic and non-genotoxic agents. Five concentrations of each chemical were dosed onto the apical side of the tissues for 48 h. TK6 cells were collected 48 h post-dosing and quantified for genotoxicity and cytotoxicity measurements. Results show that the co-culture system was at least 70% concordant with the results gained from a BlueScreen test and in depth analysis of metabolic profiles between human liver S9 and the EpiDerm tissue models explained the remaining differences. This demonstrates the greater physiological relevance of incorporating skin metabolism and a functional barrier to aid to model systemic genotoxicity following skin absorption. The test has been used to assess genotoxicity of final formulations that don't require dilution (e.g. hair dyes) mimicking real-life application to determine how the skin barrier can modulate genotoxic potential.

Score 5*: *3 for creating an assay with clear practical application + 2 for in-house validation*

700

Development and execution of an occupational next generation risk assessment (NGRA) on an exclusive use cosmetic ingredient under EU REACH: A case study on C12-15 alkyl benzoate

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Next-Generation Risk Assessment (NGRA) is as an exposure-led, hypothesis-driven approach that integrates new approach methodologies (NAMs) to assure safety without animal testing. There are some examples in the literature highlighting NGRA for consumer safety assessment of cosmetic ingredients (Dent et al., 2018; Baltazar et al., 2020), but none currently outlining how this might be done to assure occupational safety. Therefore, in this case study, NGRA was applied in an occupational safety

assessment for an ingredient exclusively used in cosmetics (INCI: C12-15 Alkyl Benzoate), in order to avoid animal testing requested under the EU REACH regulation, following a compliance check from the European Chemicals Agency. Modelling was used to estimate worker external dermal and inhalation exposure to the substance from handling, during formulation into finished cosmetic products. These external exposure estimates were combined with in vitro ADME data and converted to internal concentrations (plasma Cmax) using physiologically based kinetic (PBPK) modelling. Systemic toxicity was assessed using a suite of in vitro NAMs to identify points of departure (PoDs) for a variety of biological effects and bioactivity. These assays indicated C12-15 Alkyl Benzoate exhibits little bioactivity, and enabled bioactivity:exposure ratios (BERs) to be calculated which prove it is safe for workers and that risks are adequately controlled under normal occupational use conditions. This case study highlights how an NGRA approach can be used to reach an occupational safety decision and formulate a scientific basis to avoid animal testing under EU REACH and similar schemes.
Score 5*: ***3 for new NGRA approach to show occupational safety for cosmetic ingredient that complies with REACH without animal use + 1 for proof of principle + 1 methods integration***

Poster abstracts

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Insights from profiling transcription factor transactivation with CYP450 metabolism integration
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Profiling chemical effects on transcription factor activity can help characterize the mechanisms by which chemicals perturb biological systems. Such profiles can contribute to a predictive approach to characterizing chemical effects that avoids animal testing. The Attagene cis-FACTORIAL™ assay uses a reporter system to quantify the activity of 46 transcription factors to provide a quantitative assessment of chemical effects. A new version of this assay, CYP-FACTORIAL™, adds nine key cytochrome P450 (CYP450) enzymes to evaluate effects on transcription factor activity with and without CYP-mediated Phase 1 metabolism. This supports evaluation of whether CYP-mediated oxidation results in an altered bioactivity profile. This study examined activity of 24 chemicals across four test concentrations in the cis-FACTORIAL™ and CYP-FACTORIAL™ assays. Results suggest that alterations in CYP450 metabolism have the greatest effects on transcription factors activating the estrogen receptor (ER), aryl hydrocarbon receptor (AhR), and oxidative stress response (NRF2) pathways. Comparisons of profiles of test vs. reference chemicals identified a highly conserved polycyclic aromatic hydrocarbon toxicity signature involving activation of AhR, NRF2, and ER. Interestingly, a profile in which ER and AhR are activated but NRF2 is not activated correlated to non-toxic compounds, suggesting the possibility of using differences between signatures to predict toxic outcomes. Integrating the profiling approach with metabolism in a multiplexed in vitro assay system allows this assay platform to provide insight into chemically induced bioactivity and thus facilitates the

development of mechanistically based human-relevant predictive testing approaches.
Score 5*: *2 for development of existing assay + 2 for in-house validation + 1 universality of approach across different types of toxicity*

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Tissue-specific network analysis to predict the hepatotoxicity of chemicals

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Development of new approach methodologies to characterize a potential hazard of cosmetic ingredients is a pivotal topic since the animal testing ban. Qualitative and quantitative methods to identify biological similarities, mechanism of action (MoA) and point of departure (PoD) are needed. Omics data have emerged as valuable source of knowledge to address these topics. In recent years, toxicogenomic datasets emerged to document systemic gene expression and their downstream effects on toxicological endpoints. Among the many options, network analysis appears to be very promising computational methods and have been proven useful for integration and exploration depicting complex interactions. In this work, we used network analysis to characterize and evaluate the hepatotoxicity level of chemicals. We constructed a multi-layer, liver-specific molecular network by integrating multi-omics datasets including gene expression of human HepG2 cell line, protein-protein interactions, drug targets, pathways and adverse events. An analytical framework consisting of network signal diffusion and module detection was developed to help predict mechanisms of drug-induced liver toxicity. This approach was benchmarked on gene signature of hepatotoxic and non-hepatotoxic compounds obtained from transcriptomic dataset. Liver specific networks analysis allowed to go beyond conventional transcriptomics exploration by 1) refining enrichment and highlighting relevant MoAs, 2) connecting genes and pathways to proteins, drugs and adverse effects to enrich biological interpretation and 3) detecting modules allowing quantification of pathways activation, paving the way for PoDs. This framework has been packaged in an R tool that builds networks to identify potentially hepatotoxic chemicals and their MoA.
Score 6: *3 for new liver "molecular network" + 2 for in-house validation + 1 for integrating omics data with computational interpretation tool*

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Developmental neurotoxicity in vitro assays applied for molecular initiation and key event identification to create an AOP network related to cognitive function defects

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Cognitive functions such as learning, memory, decision-making, problem-solving, and attention are key factors contributing to our personalities. They are established during brain development via a series of complex and interdependent processes that are

particularly vulnerable to chemical insults. Despite its significance, only a fraction of our chemical exposome has been assessed for its developmental neurotoxicity (DNT) potential. For data gap closure by fast and cost-effective compound evaluation, a DNT in vitro battery (IVB) has been set up. To build confidence in IVB tests, the aim of this study was to map key neurodevelopmental events measured in neurospheres in combination with transcriptome analyses to putative adverse outcome pathways (AOPs). Human 3D neurospheres were utilized to measure the effects of different compound classes on key neurodevelopmental events, i.e., neural progenitor cell (NPC) proliferation, migration, and lineage differentiation as well as thyroid hormone (TH)-dependent oligodendrocyte maturation by high content image analyses. These endophenotypic outcomes were combined with respective transcriptome analyses. We identified three independent modes-of-action (MoAs) interfering with oligodendrocyte development (TH disruption via receptor binding, oxidative stress, and disturbance of cholesterol homeostasis) in differentiating NPC. Furthermore, we confirmed a migration endophenotype caused by compound-protein interaction. All identified KEs were integrated into already existing DNTAOPs resulting in an AOP network related to cognitive function defects. This study demonstrates the power of combining endophenotypic with transcriptomic analyses to elucidate compounds' MoA and create AOPs and AOP networks for gaining confidence in DNT hazard assessment by using the DNT IVB in a regulatory context.
Score 5*: *3 for creating new AOP network for cognitive function + 2 for in-house validation*

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Acutox: An animal product-free assay for predicting acute oral toxicity
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Acute oral toxicity is currently assessed using the rodent LD50 test following OECD test guidelines 420, 423 and 425. These tests are widely criticized on scientific and ethical grounds, as they lack reproducibility and relevance to human exposure. We have shown that an in vitro cytotoxicity assay can be used to predict acute oral toxicity. The aim of this work was to develop an enhanced, animal product-free, metabolically relevant in vitro assay capable of predicting EPA and GHS acute oral toxicity category classifications. Adult human dermal fibroblasts were grown in animal-product free media containing human serum and dosed with a cohort of 70 chemically diverse test articles with known, well curated rodent LD50 categories spanning all EPA and GHS classifications. Viability was assessed using both the Neutral Red Uptake and MTT assay, and IC50 calculated for all 70 test articles. These data were used to create a prediction model capable of predicting EPA classification. This scientifically and ethically robust screening or replacement alternative to the rodent LD50 assay can be run alongside in silico approaches to improve the prediction of acute oral toxicity classification and labelling and is considered to be suitable for inclusion in a weight of evidence approach for acute oral toxicity classification. The Acutox assay contributes to a registrants 3Rs initiative by replacing aspects of in vivo acute oral toxicity testing and offers a novel animal product-free and metabolically relevant, human in vitro approach to acute toxicity.

Score 6: ***3 for new animal product-free acute tox assay + 2 for in-house validation + 1 for including phase 1 metabolism***

MPS 2022

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A PBPK-compliant human intestine-liver-brain-kidney chip for QIVIVE in drug development

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Microphysiological systems (MPS) have proven to be a powerful tool for recreating human tissue- and organ-like functions. However, establishing complex human *in vitro* ADME models involving co-culture of key organs to mimic physiologically based pharmacokinetic (PBPK) distribution behavior still present a challenge. In our recent study, we developed a PBPK compliant ADME 4- Organ-Chip (Chip4) with a downscale factor of 1:100,000 of the human body. The integration of an intestinal barrier model for absorption and first-pass metabolism, liver microtissues for main metabolism, a kidney model with proximal tubular-like cells and podocytes for excretion, and neuronal spheroids as a potential target organ were optimized in the chip and co-cultured for 14 days. The setup was repeatedly exposed to Haloperidol, an antipsychotic medication and to Carbamazepine, a tricyclic compound with anticonvulsant properties through different routes. Results on direct as well as metabolite induced effects on organ-specific levels will be presented. Subsequently this data formed the basis for the development of an *in silico* PBPK model for compound prediction.

Score 5*: ***3 for complex 4 organ chip + 1 for proof of principle + 1 for PBPK compliance***

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InterOrgan multi-tissue chip system for linking matured tissue niches by vascular flow

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Human *in vitro* tissue platforms for studies of integrated human physiology in health and disease are becoming increasingly predictive of clinical data. At present, establishing physiological communication between multiple tissues while preserving their individual phenotypes remains a major challenge that must be overcome to model whole-body physiology and systemic diseases. To this end, we established a scientific premise for developing a bioengineered multi-organ platform for modeling human physiology, that

integrates four human organ systems: heart, liver, bone, and skin, all equipped with endothelial barriers and connected by vascular perfusion with circulating immune cells (Ronaldson-Bouchard et al., in press). A major innovative component of the platform is in the biomimetic approach to functional integration, by (i) maintaining a local regulatory niche for each tissue, (ii) connecting tissue compartments by a vascular perfusate containing immune cells, and (iii) establishing a semi-permeable endothelial barrier between the circulatory and tissue compartments. The platform is modular, configurable, PDMS-free and enables real-time imaging and monitoring of cell and tissue functions. Tissues linked by vascular perfusion maintained their molecular, structural, and functional phenotypes over four weeks of culture and showed the expected tissue specific responses during drug screening applications. Multiplexed analysis of complex proteomic data with application of the tools and methodologies from systems biology, physiology and bioengineering highlights the need for maintaining the tissue specific niche during multi-tissue culture to preserve individual tissue maturation over extended timelines. We demonstrated that tissues linked by vascular perfusion preserve individual tissue fidelity, reveal a more clinically relevant PK/PD profile, recapitulated multi-organ toxicity of doxorubicin observed in pediatric and adult clinical study, and enabled identification of clinically relevant early miRNA biomarkers of cardiotoxicity. Overall, the InterOrgan platform can facilitate clinical translation by enabling physiologic communication of phenotypically stable engineered human tissues.

Reference

Ronaldson-Bouchard et al. (in press). Nat Biomed Eng.

Score 5*: *3 for new multiorgan chip + 1 for proof of principle + 1 for including vasculature and immune competency*

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Perfused Organ Panel™ microphysiological system with synthetic hemoglobin, blood substitute, builds confidence in mitochondrial and xenobiotic metabolism of 3D liver models

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Oxygen is the key to energy production and Phase I xenobiotic metabolism in mammals. It is the final electron acceptor in the mitochondrial electron transport chain providing the bulk of cell energy through oxidative phosphorylation. Many reactive metabolites are formed by cytochrome CYP450 oxidation of drugs and chemicals. CYP450 monooxygenases are membrane-bound heme proteins that use molecular oxygen to catalyze over 95% of intracellular oxidation reactions and the oxidation of exogenous chemicals. Oxygen is essential for catalytic activity of oxygenases, redox reactions, energy production, and the function of ATP-dependent transporters, all of which contribute to human-relevant cell and chemical metabolism *in vitro*. In humans, over 98% of oxygen is delivered to tissues by dissociation from hemoglobin contained in erythrocytes. Only 2% of oxygen is delivered dissolved in blood plasma. Without hemoglobin, oxygen availability for tissues *in vivo* and *in vitro* is low due to its low solubility in blood plasma and culture media. This may contribute to cellular de-differentiation, loss of xenobiotic competence, and the emergence of glycolytic phenotypes that can evade reactive metabolites and free radicals misrepresenting potential *in vivo* toxicity. To address these challenges, Lena Biosciences developed Perfused Organ Panel™ microphysiological system with a synthetic hemoglobin, Blood

Substitute. The platform restored oxidative phosphorylation, the dominant mode of energy production in normal cells *in vivo*, providing 4x higher oxygen consumption rate in HepG2 cells without any evidence of oxidative stress compared to 2D. Significantly higher respiratory metabolism for identification of mitochondrial liabilities and significantly higher CYP450 activity (log-fold higher in primary cells) was shown in diverse liver models, including primary human hepatocytes, primary mouse hepatocytes, differentiated HepaRG cells, iPSC-derived hepatocytes, and HepG2 cells cultured in the platform. Perfused Organ Panel™ MPS builds mitochondrial and xenobiotic metabolism confidence in 3D models for human-relevant toxicity testing *in vitro*.

Score 5*: *3 for MPS with synthetic haemoglobin + 1 for proof of principle + 1 for demonstrating importance of oxygen carrier*

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A multiscale computational framework for modeling microphysiological systems

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Over the past few decades, the use of mathematical models to support various stages of drug development and to predict cellular behavior has seen significant increase. Of particular interest is the effective use of pharmacokinetic/pharmacodynamic-toxicity (PK/PD-Tox) models for dose optimization, toxicity threshold identification, translation of *in vitro* compound potency to the *in vivo* setting, reduction in the number of *in vivo* studies and improvement of results translation from preclinical species into the clinical setting. The majority of these models use simplified lumped compartments to simulate *in vitro* drug transport, absorption, distribution, metabolism and excretion. This approach uses ordinary differential equations with non-physiological parameters to represent complex biological processes and tissues, which may fail to adequately capture relevant gradients or zonation in the *in vitro* environment. Alternatively, high-fidelity first principles-based models that use spatially resolved geometries and multiphysics approaches to describe species transport and elimination are able to capture these gradients, but are computationally expensive. In microfluidic organ-on-chip systems, use of high-fidelity models may be more appropriate design for analyzing flow patterns, pressure drops, wall shear stress profiles, membrane mechanical loads, etc.; however, reduced-order models are more suitable for modeling long-term drug transport, and PK/PD-Tox effects. Here we present and demonstrate a multiscale modeling approach combining lumped and spatially resolved first principles-based mathematical models for capturing the intricate biophysical details captured by microphysiological systems. These models have been used to i) maximize chip performance (i.e., optimize flow rate to achieve physiologically relevant shear stress and adequate oxygen/nutrient distribution across the system), ii) identify/describe underlying mechanisms to elucidate the PK-PD relationship (i.e., production of toxic byproducts that induce cell death) and iii) support *in vitro-in vivo* translation (i.e., scaling metabolism from chip to human). Examples of validated results highlighting different aspects of the framework will be presented.

Score 5*: *3 for new computational modelling approach for PK/PD in MPS + 1 for proof of principle + 1 for enhancing performance and translation of MPS*

MPS2023

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A novel microfluid liver-on-chip model: Application in regulated genotoxicity testing

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Genotoxicity assessment of test compounds are based on a combination of tests to assess different genotoxic endpoints associated with human diseases: mutagenicity, clastogenicity and aneuploidy. To this day, there is no single genotoxicity test capable of detecting all mechanisms, and all assays involve rodent metabolic activation system or in vivo rodents testing, which could lead discrepancy from human accuracy predictivity response. The objective of this work was to develop a single in vitro assay able to accurately address the different in vitro and in vivo genotoxicity endpoints using a human cells metabolically competent in vitro system to increase human-relevant predictivity outcome and replace animals testing. Different models were evaluated, and the most promising evaluated model consists of a fluidic flow microphysiological liver-on-chip system using the PhysioMimix™ barrier plate supplied by CN-Bio co-cultured with human lymphoblastoid TK6 cells in Transwell® insert. The preliminary results obtained with direct genotoxicants, methanesulfonate and ethyl methanesulfonate, and indirect genotoxicants, benzo[a]pyrene and cyclophosphamide, demonstrated results in line with expected in vivo responses for the end points evaluated, namely the comet assay and the micronucleus test. Measured levels of urea and albumin, in addition to the CYP enzymes activity, also demonstrated appropriate liver properties with metabolic competency. In conclusion, the human cells model showed appropriate metabolic properties competency without requiring additional rodent metabolic activator, and the capability of appropriately addressing genotoxic adverse outcomes within a single system, i.e., induction of chromosomal damage or damage to the mitotic apparatus (micronucleus test) and DNA strand breakage (comet assay). The next steps in the development of the model will be to integrate the evaluation of mutagenicity by duplex sequencing analysis and to increase the number of compounds evaluated in the model to have a better view of its accuracy.

Score 5*: *3 for combining OoC with standard genotox tests to humanise them + 1 for proof-of-principle + 1 for making the effort to use OoC in a real world tox testing scenario*

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Automation of multi-organ-chip assays

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With the approval of the FDA Modernization Act 2.0 the requirement to use animal testing for drug development has finally been eliminated – paving the way for innovative animal-free technologies like Multi-Organ-Chips. However, there are still major challenges ahead. Currently, the full potential of MPS cannot yet be fully exploited. The high complexity of MPS-based assays leads to a high amount of manual work in the execution and analysis of the assays. This problem can be solved by automating the

execution of the assays. Here we present the HUMIMIC AutoLab, a newly developed system for full automation of complex MPS based assays. The system has its own incubation system and brightfield- and fluorescence microscope. A class II safety cabinet allows sterile handling. The built-in 4°C refrigerator allows the system to operate for up to four days without user interaction. The HUMIMIC AutoLab can culture up to 24 MOC's simultaneously. Media exchange, sampling, substance application, microscopy and more are automatically performed. Several assays were performed to test the system. For example, the system demonstrated the ability to dynamically culture a MatTek EpiIntestinal™ model in combination with a HepaRG- based spheroid model in a HUMIMIC Chip2, in a 14-day experiment. In a PB/PK experiment, it was shown that the HUMIMIC AutoLab can run hourly substance applications and media changes to mimic physiological substance profiles. During the execution of the assays, it was shown that the automatic sampling and the integrated microscope can generate significantly more data points than in a manual experiment. Combining this data with deep learning techniques in the next step, more readouts and analysis could be generated or performed without additional effort. Based on the HUMIMIC AutoLab, it can be shown that in the future MPS based assays can be performed with a higher throughput with less manual effort. MPS-Assays performed with automated systems lead to a higher standardization and reproducibility, high-content data and the possibility for AI-based analysis. Together, these are important factors in advancing regulatory acceptance and industry adoption of MPS-based assays.
Score 5*: ***3 for automation and parallelisation of OoC assays + 1 for proof of principle + 1 for significant advance in making OoC useful***

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On the way to a digital twin in preclinical studies – how automation and continuous data acquisition enable AI-based in silico models

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Performing in vitro assays using microphysiological systems requires a great deal of manual effort, which often hinders their use for industrial high-throughput testing. To overcome this, we have developed a robot for standardized long-term handling of TissUse's Multi-Organ-Chips. Our system allows the automation of assays featuring up to 24 chips with different degrees of complexity. The most complex chip currently automated is the HUMIMIC Chip4, which consists of a blood circuit and an excretory circuit and can sustain a long-term (several weeks) culture of up to four autologous organoids. Data are continuously recorded automatically during the execution. Examples are temperature data, pump and flow data as well as various optical readouts using brightfield and fluorescence microscopy. These include continuous morphological readouts such as number of cells, size changes or structural changes, but also chemical readouts such as pH values and value changes, CO2 or oxygen concentration. In this work, we will show that the advantage of automation goes beyond the mere reduction of manual work time, including higher reproducibility and scalability, which is the basis for validation and standardization. The combination of holistic planning software, continuous data acquisition in databases and the integration of

machine learning and artificial intelligence tools (GANs, CNNs) enables the implementation of virtual or so-called soft-(ware) sensors, which complement the directly measurable chip health metrics. By coupling this in-process data with hybrid AI models and expert knowledge, we are establishing the roadmap to a digital twin for preclinical studies.

Score 5*: ***3 for step-change in throughput of multi-organ MPS + 1 for proof of principle + 1 for integrating AI analysis of on-line data from MPSs***

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Scalable application of RosetteArray™ technology for modeling the complex etiology of human neural tube defects and screening for risk factors

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Neural tube defects (NTDs) remain the second most common congenital malformation. Given their complex omnigenic and environmental etiology, rodent models have limited utility for investigating NTD pathophysiology and screening for prophylactic interventions. Alternatively, human pluripotent stem cell (hPSC)-derived neural rosettes model in vivo neurulation and can be used in precision medicine screens. Using foundational hPSC neural differentiation [1,2] and bioengineering [3] protocols, we developed RosetteArray technology to standardize neural rosette derivation in a micropatterned 96-well plate screening format and combine it with adaptive confocal imagining and AI-based image analysis to create a transformative platform for developmental neurotoxicity (DNT), modeling NTD etiology, and screening for novel NTD prophylactics. Here, we present the platform's capability to detect pharmaceuticals, agrochemicals, and genetic mutations with known clinical NTD risk. First, reproducible derivation of forebrain and spinal cord RosetteArrays from direct seeding of cryopreserved cells is demonstrated. Second, we present a preliminary DNT screen (24 pesticides and 6 NTD-associated substances) in which the RosetteArray performs with 91% sensitivity and 100% specificity and includes integration of simulated human metabolism. Third, we describe differential responses between forebrain and spinal RosetteArrays to a teratogen and a genetic NTD risk factor, thereby supporting inclusion of both region-specific assays in future screens for more comprehensive coverage of neural tube morphogenesis. Fourth, we present modelling of a clinically-relevant multifactorial NTD scenario where a mutant hPSC line with a NTD genetic predisposition only shows a risk phenotype in the presence of an environmental risk factor. Lastly, we discuss ongoing experiments using iPSC lines derived from patients with Spina Bifida, the most prevalent clinical NTD, to develop a precision medicine prophylactic screen. Collectively, these results support scalable implementation of the RosetteArray platform for investigating NTD etiology, conducting DNT screens to identify risk factors, and developing precision medicine approaches for discovering novel NTD risk-reducing prophylactics.

References

[1] Lippmann, E. S. et al. (2015). Stem Cell Reports 4, 632-644.
[2] Iyer, N. R. et al. (2022). Sci Adv 8.

[3] Knight, G. T. et al. (2018). eLife 7, e37549.
Score 6: *3 for new screening tool for neural tube development and DNT + 2 for in-house validation + 1 for integrating genetic predisposition to model for compound screening*

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Development of a microphysiological skin-liver-thyroid Chip3 and its application to evaluate the effects on thyroid hormones of topically applied cosmetic ingredients under consumer-relevant conditions
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All cosmetic ingredients registered in Europe must be evaluated for their safety using non-animal methods. Microphysiological systems (MPS) offer a more complex higher tier model to evaluate chemicals. Having established a skin and liver HUMIMIC Chip2 model demonstrating how dosing scenarios impact the kinetics of chemicals, we investigated whether thyroid follicles could be incorporated to evaluate the potential of topically applied chemicals to cause endocrine disruption. This combination of models in the HUMIMIC Chip3 is new; therefore, we describe here how it was optimized using two chemicals known to inhibit thyroid production, daidzein and genistein. The MPS was comprised of Phenion® Full Thickness skin, liver spheroids and thyroid follicles co-cultured in the TissUse HUMIMIC Chip3. Endocrine disruption effects were determined according to changes in thyroid hormones, thyroxine (T4) and 3,3',5-triiodothyronine (T3). A main part of the Chip3 model optimization was the replacement of freshly isolated thyroid follicles with thyrocyte-derived follicles. These were used in static incubations to demonstrate the inhibition of T4 and T3 production by genistein and daidzein over 4 days. Daidzein exhibited a lower inhibitory activity than genistein and both inhibitory activities were decreased after a 24 h preincubation with liver spheroids, indicating metabolism was via detoxification pathways. The skin-liver-thyroid Chip3 model was used to determine a consumer- relevant exposure to daidzein present in a body lotion based on thyroid effects. A "safe dose" of 0.235 µg/cm2, i.e., 0.047% applied in 0.5 mg/cm2 of body lotion was the highest concentration of daidzein which does not result in changes in T3 and T4 levels. This concentration correlated well with the value considered safe by regulators. In conclusion, the Chip3 model enabled the incorporation of the relevant exposure route (dermal), metabolism in the skin and liver, and the bioactivity endpoint (assessment of hormonal balance, i.e., thyroid effects) into a single model. These conditions are closer to those in vivo than 2D cell/tissue assays lacking metabolic function. Importantly, it also allowed the assessment of repeated doses of chemical and a direct comparison of systemic and tissue concentrations with toxicodynamic effects over time, which is more realistic and relevant for safety assessment.

Score 5*: ***3 for new model of endocrine disruption by topically applied chemicals + 1 for proof of principle + 1 for highlighting potential of MPS for tox applications in cosmetic industry***