# Lush Science Prize 2022
# Background Paper

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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 tt21c research. The most promising approaches which might achieve this are considered to be:

- Research aimed at elucidating adverse outcome pathways (AOPs) that describe the mechanistic steps by which a toxicant induces an adverse effect in a human;
- Developing new *in vitro* tools, known as organs-on-a-chip (OoCs, aka microphysiological systems (MPS)) that can fully replace animals in laboratory research and testing, and;
- The use of computational, 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 judging panel by identifying key projects that are making major contributions to the field of animal-free toxicology research. From these projects, the panel may choose to select the winners for the 2022 Lush Science Prize. The Lush Prize panel is particularly interested in supporting work that elucidates AOPs for the complex areas of systemic toxicology and developmental toxicology.

This 2022 Science Background paper identifies 17 pieces of work carried out by researchers whom we believe constitute potential candidates for the Judges' shortlist. These projects received scores of 6 or 7 against the new scoring criteria for their potential to make major contributions towards providing practical non-animal tests which could be accepted by regulators. The full abstracts for these pieces of work are given in Section 5.4. We consider all to be worthy for consideration by the judges as potential prize winners.

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-ToxRisk (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 that have been held in the preceding 2

years. For this year these were the 2021 and 2022 Society of Toxicology (SoT) annual conferences, the 2021 EUROTOX conference, and the 11th World Congress on Alternative and Animal Use in Life Sciences in 2021. There was a total of around 7,500 abstracts from oral and poster presentations from these four conferences, but only 933 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 over 2,250 potentially relevant projects which we assessed as described in Section 2. Relevant abstracts were filtered and scored using the modification of the system described in the 2020 Science Background paper. In the revised 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 available for abstracts which stand out in some other way.

Overall, from 129 abstracts which scored 1 or more, 72% scored 4 or more points, with 13% (17 abstracts) scoring 6 or 7 points. The full abstracts of those projects scoring 6 or 7 are provided in Section 5.4. Details of the other abstracts which received a score are shown in the table in Section 5.5.

## 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 in the 24 months preceding the award (i.e. June 2020 – May 2022).

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

Secondly, we identified relevant conferences held in the preceding 24 months 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 four relevant conferences for 2020 – 2022, for which abstracts were available, were the 60th meeting of the Society of Toxicology, held online, March 2021[1], and their 61st meeting held in San Diego, March 2022[2]; the EUROTOX 2021 Congress, held virtually in September - October 2021[3], and the WC11 Maastricht conference, also held virtually in August-September 2021[4].

From all the abstracts which comprised the WC11 conference presentation and poster proceedings, we reviewed only those that were presented within conference themes relevant to scientific research. The selected themes were: 'Innovative Technologies' and 'Safety'. We excluded abstracts presented in the 'Ethics, Welfare, and Regulation' and 'Disease' themes.

For EUROTOX 2021 we identified sessions potentially relevant to the Lush Prize themes and reviewed all abstracts within those sessions.

For SoT 2021 meeting there were 2,188 abstracts in total, of which 95 were indexed under relevant Keyword Index headings. The relevant Keywords from the SoT 2021

[1] https://www.toxicology.org/pubs/docs/Tox/2021Tox.pdf

[2] https://www.toxicology.org/pubs/docs/Tox/2022Tox.pdf

[3] https://www.eurotox2021.com/wp-content/uploads/EuroTox-2021-Final-abstract-book-by-Elsevier.pdf

[44] https://proceedings.altex.org/data/2021-01/altex_WC11.pdf

abstract book were: Adverse Outcome Pathway; Adverse Outcome Pathway -Network; Computational Toxicology, In Silico; In Silico, Tox Database, Read-Across, QSAR; Organ-chip; and Predictive Toxicology.

Of the 3,820 abstracts presented at the SoT 2022 meeting, only the 111 indexed under relevant headings in the Keyword Index were considered. The relevant Keywords from the SoT 2022 abstract book were: Adverse Outcome Pathways; AOP; Computational Toxicology, In Silico Simulation; Organ-on-chip; and Predictive Toxicology.

Thirdly, we conducted a review of the recent literature. Firstly, we searched PubMed for research published from 01/06/2020 to 07/05/2022, 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. As a secondary source, we specifically reviewed all articles published in the ALTEX journal, for any relevant articles not identified by the PubMed searches.

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 the newly devised system which awarded points as described below.

As for previous years, because the conferences yielded a limited number of relevant abstracts and the conference abstract books presented titles and abstracts simultaneously, there was no merit to reviewing abstracts in the three stages. Thus, abstracts were either accepted or rejected for scoring and then scored in a single sweep.

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* |

For the 2020 Lush Science Prize there was a new focus on the practical application of reported work for replacing animal use in testing, and this focus persists for 2022. We have continued to use the adapted 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.

# 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, for the 2020 Lush Prize.

## 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). While the routine work of these programmes continue, there are no major updates to report.

### 3.1.2. NCATS

NCATS' Tissue Chips programme continues with its focus on disease modelling & efficacy testing, funding research to the tune of several $millions per year. Ten projects to develop systems to conduct clinical trials on a chip for rare disease and paediatric disorders were funded in 2020, with a total of $7 million.

### 3.1.3. FDA

The FDA continues to implement its Predictive Toxicology Roadmap, first published in 2017. These activities include in-house research, as well as collaborations with commercial entities. In 2020, the FDA convened a workshop of pharmaceutical industry partners to discuss the use of 'complex *in vitro* models' (CIVMs) in regulatory approval of medicines, the results of which were published this year[5]. One of the key conclusions from the workshop was the value in creating animal-derived CIVMs to increase confidence in human-based systems. The thinking behind this approach is that, if species-specific sensitivities are evident in CIVMs and show strong concordance with *in vivo* data for that species, then this could justify the reduction, or even replacement, of animal use with human CIVMs.

### 3.1.4. EPA

The EPA announced draft guidance to waive toxicity tests on animal skin for determining whether pesticides lead to adverse effects6. In December 2021 it published its updated New Approach Methods workplan7. In this document, the EPA describes its updated roadmap and identifies tangible steps to pursuing and achieving a reduction in the use of vertebrate animals for toxicity testing and related research, while ensuring that the Agency's regulatory, compliance, and enforcement activities remain fully protective of human health and the environment. In doing so, EPA will have to ensure its regulatory framework is robust and flexible enough to accommodate the

[5] Baran *et al* (2022). Perspectives on the evaluation and adoption of complex *in vitro* models in drug development: Workshop with the FDA and the pharmaceutical industry (IQ MPS Affiliate). *ALTEX* **39**, 297-314. doi.org/10.14573/altex.2112203

[6] https://www.epa.gov/newsreleases/epa-announces-guidance-waive-toxicity-tests-animal-skin

[7] https://www.epa.gov/system/files/documents/2021-11/nams-work-plan_11_15_21_508-tagged.pdf

development and the use of NAMs, and additionally continue to engage and communicate with stakeholders to incorporate their knowledge and address concerns as EPA moves away from vertebrate animal testing. In this work plan, EPA discusses the near- and long-term strategies it will deploy through 2024 to accomplish its objectives.

## 3.2. EU

### 3.2.1. EURL-ECVAM

The EURL-ECVAM Status Report 2021 describes the range of work undertaken during that year[8].

### 3.2.2. European Council

The European Pharmacopoeia is finally to phase out the requirement for the rabbit pyrogen test within 5 years[9], meaning that rabbits will no longer be subjected to fever symptoms in order to batch test the safety of medicines. This decision comes 25 years after an *in vitro* alternative was developed.

### 3.2.3. ECHA & REACH

ECHA has been controversially insisting on animal tests for chemicals whose sole use is in cosmetics, citing REACH regulations as justification. This policy has attracted a significant amount of attention and has been the subject of criticism from academics and activists alike[10,11]. Despite these comments, ECHA maintains that it upholds its mandate to require animal testing only as a last resort, where no *in vitro* or *in silico* alternative exists[12].

### 3.2.4. EU-ToxRisk

The EU-ToxRisk programme drew to a close during this Lush Prize cycle, and held its final open symposium in November 2021. This €60 million programme has produced a large number of articles and reports[13] (including one of the highest scoring papers from our analysis), and set out a paradigm for how to implement next-generation risk assessment on a large scale – see Section 4, footnote 19.

### 3.2.5. RISK-HUNT3R

This EU Horizon 2020 project[14] is the follow up to the EU-ToxRisk programme. It has been funded for 5 years with €23 million, with the aim of building on the outcomes of the EU-ToxRisk programme. The project will develop, validate, and implement integrated

[8] https://publications.jrc.ec.europa.eu/repository/bitstream/JRC127780/JRC127780_01.pdf

[9] https://www.edqm.eu/en/-/european-pharmacopoeia-to-put-an-end-to-the-rabbit-pyrogen-test

[10] https://doi.org/10.14573/altex.2104221

[11] https://doi.org/10.1007/s00204-021-03034-y

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

[13] https://www.eu-toxrisk.eu/page/en/project-results.php

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

approaches to next-generation risk assessment, based on *in vitro* and *in silico* new approach methods. It is part of a collaborative research cluster that also includes the ONTOX[15] and PrecisionTox[16] projects.

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

The OECD Test Guidelines Programme issued a new guideline in June 2021 – 'Defined Approaches for Skin Sensitisation' (DAs)[17]. Validation against an extensively curated set of human and mouse reference data indicates that this approach predicts human skin sensitization hazard better than the accepted animal test. A supporting document providing details on the data curation and performance characteristics of the DAs on skin sensitisation is due be published. This is a new type of OECD Guideline that uses several types of combined information to provide chemical safety information and will enable a non-animal approach to identifying potential skin sensitizers to be used worldwide. The project to develop this new Guideline started in 2017 and was led by the United States, the European Commission Joint Research Centre, and Health Canada, supported by a group of nominated experts.

[15] https://ontox-project.eu

[16] https://precisiontox.org/

[17] https://www.oecd.org/chemicalsafety/testing/oecdguidelinesforthetestingofchemicals.htm

## 4. Literature Highlights

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

A paper by Zhong *et al* published outside of the timescale of this Lush Prize cycle, but important nonetheless, shows developmental neurotoxicity of an antidepressant commonly prescribed to pregnant women[18]. Selective serotonin reuptake inhibitors are often used to treat depression in pregnant women, but Zhong *et al* showed that a widely used inhibitor, paroxetine, decreased the expression of synaptic markers, neurite outgrowth, and the oligodendrocyte cell population, compared with controls, in an organotypic model of brain development.

Among the many papers published by the EU-ToxRisk programme (which include one of our highest scoring papers) the overview, by Moné *et al*, of how non-animal approaches can impact next-generation risk assessment, is particularly noteworthy[19]. They describe how the EU-ToxRisk programme used case studies and close collaboration with regulators and scientific stakeholders to create a framework of how to implement next-generation risk assessment. This framework could form the basis of future projects to advance non-animal safety testing.

A second t4 Workshop on MPS systems and the impact they are having on patient benefits and animal welfare in drug discovery[20] convened in 2019. This report by Marx *et al* offers a relatively pessimistic view of the rate of uptake and impact of MPS technologies on drug discovery and toxicological risk assessment so far but offers solutions and a roadmap towards regulatory acceptance of MPS models.

Two papers discussing the ethical issues around foetal bovine blood collection to produce foetal bovine serum (FBS) were published in ALTEX in 2021. Versteengen *et al*[21] put the industry view that foetal blood collection is humane and is a practical use of an otherwise wasted by-product of animal slaughter for food. Weber *et al* refute these arguments in their reply[22], and also highlight the scientific issues with using FBS for human cell-based research and testing. These issues will be further discussed in special edition of the ATLA Journal on FBS, due to be published in the autumn of 2022.

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[18] Zhong *et al* (2020). Antidepressant paroxetine exerts developmental neurotoxicity in an iPSC-derived 3D human brain model. *Front. Cell. Neurosci.* **14**, article 25. doi.org/10.3389/fncel.2020.00025

[19] Moné *et al* (2020). Setting the stage for next-generation risk assessment with non-animal approaches: the EU-ToxRisk experience. *Arch. Toxicol.* **94**, 3581-3592. doi.org/10.1007/s00204-020-02866-4

[20] Marx *et al* (2020). Biology-inspired microphysiological systems to advance patient benefit and animal welfare in drug development. *ALTEX* **37**, 365-394. doi.org/10.14573/altex.2001241

[21] Versteegen *et al* (2021). Animal welfare and ethics in the collection of fetal blood for the production of fetal bovine serum. *ALTEX* **38**, 319-323. doi.org/10.14573/altex.2101271

[22] Weber *et al* (2021). Reply to comment 'Animal welfare and ethics in the collection of fetal blood for the production of fetal bovine serum. *ALTEX* **38**, 324-326. doi.org/10.14573/altex.2103191

Marty *et al*[23] proposed a method to track and measure the impact of new approach methods (NAMs) in reducing animal use. This collaboration between the Dow Chemical Company and PETA demonstrates the concrete value, both financial and ethical, in implementing NAMs wherever possible.

Dirven *et al* conducted a systematic review of the performance of preclinical models in predicting drug-induced liver injury (DILI) in humans[24]. They compared two anti-diabetic drugs from the same class, one of which has been withdrawn from the US market because of DILI and one of which has not. Neither drug had biomarkers indicating a hazard in *in vivo* studies, but there were significant differences in their activity in *in vitro* toxicity assays (ToxCast database, 129 vs 60 activity hits) and in adverse effect reporting. The authors conclude that animal and human trials failed to identify the potential of the withdrawn drug to cause DILI, while *in vitro* data showed marked differences in the off-target activity profile of the two drugs.

Kolle and collaborators[25] suggest four preconditions for the implementation of 'defined approaches' (combinations of non-animal methods that together can replace *in vivo* Test Guidelines). These preconditions include the observations that reference data should not replicate the limitations of the murine local lymph node assay, and that methods and models should be validated before they are used in OECD-adopted DAs.

In 2021, Stem Cell Reports published a special issue[26] on OoC, with an emphasis on the strength of combining stem cell-derived, differentiated cells with 3D cell culture and OoC systems. Among the papers in this issue was a report of the European Commission and European Standardisation Organisations' efforts to identify the needs and priorities for organ-on-chip standards development[27]. Separately, Massimo Mastrangeli and Janny van den Eijnden-van Raaij[28] highlighted the progress made in the development of OoC systems and their application in drug development and disease modelling. They highlight the OoC roadmap developed by the EU Horizon 2020 programme's ORCHID project.

A letter by Vulto and Joore, to Nature Reviews Drug Discovery[29] highlighted the level of adoption of OoC technology by the pharmaceutical industry and provided examples of

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[23] Marty et al (2022). Animal metrics: Tracking contributions of new approach methods to reduce animal use. ALTEX 39, 95-112. doi.org/10.14573/altex.2107211

[24] Dirven *et al* (2021). Performance of preclinical models in predicting drug-induced liver injury in humans: a systematic review. *Scientific Reports* **11**, 6403. doi.org/10.1038/s41598-021-85708-2

[25] Kolle *et al* (2020). Replacing the refinement for skin sensitization testing: Considerations to the implementation of adverse outcome pathway (AOP)-based defines approaches (DA) in OECD guidelines. *Regul. Toxicol. Pharmacol.* **115**, article 104713. doi.org/10.1016/j.yrtph.2020.104713

[26] Mummery & Loskill (2021). Welcome to the special issue on organs-on-chip from the guest editors. *Stem Cell Reports* **16**, 2029-2032. doi.org/10.1016/j.stemcr.2021.08.013

[27] Piergiovanni *et al* (2021). Putting science into standards workshop on standards for organ-on-chip. *Stem Cell Reports* **16**, 2076-2077. doi.org/10.1016/j.stemcr.2021.07.010

[28] Mastrangeli & van den Eijnden-van Raaij (2021). Organs-on-chip: The way forward. *Stem Cell Reports* **16**, 2037-2043. doi.org/10.1016/j.stemcr.2021.06.015

[29] Vulto & Joore (2021). Adoption of organ-on-chip platforms by the pharmaceutical industry. *Nat. Rev. Drug Discovery* **20**, 961-962. doi.org/10.1038/s41573-021-00323-0

where OoC technologies have contributed to the reduction in development costs of pharmaceutical compounds.

Schneider et al[30] discuss the difficulties associated with validating complex systems such as OoCs and propose that initiatives such as "open technology platforms, and collaboration between OoC developers and risk assessors may prove an expedient strategy to build confidence in OoCs for application in safety and efficacy assessment".

Finally, Busek *et al*[31] surveyed academic users of OoC platforms and gathered responses from 187 users in 35 countries. They concluded that "current commercial OoC platforms provide a substantial level of robustness and usability—which is also indicated by an increasing adaptation of the pharmaceutical industry—but a lack of complexity can challenge their use as a predictive platform. Self-made systems, on the other hand, are less robust and standardized but provide the opportunity to develop customized and more complex models, which are often needed for human disease modelling".

[30] Schneider *et al* (2021). Applicability of organ-on-chip systems in toxicology and pharmacology. *Crit. Rev. Toxicol.* **51**, 540-554. doi.org/10.1080/10408444.2021.1953439

[31] Busek *et al* (2022). Academic user view: Organ-on-a-chip technology. *Biosensors* **12**, 126. doi.org/10.3390/bios12020126

# 5. Outcome: Candidate Abstracts Identified for the Judges

## 5.1 Conference Abstract Selection

As described in the Methodology, we reviewed abstracts from the Society of Toxicology 60th and 61st Annual Meetings in 2021 and 2022 respectively, the EUROTOX 2021 meeting, and the 11th World Congress on Alternatives and Animal Use in the Life Sciences, 2021 (WC11).

From the around 1,100 abstracts which comprised the WC11 conference presentation and poster proceedings, we reviewed only the 456 that were presented within relevant conference themes: 'Innovative Technologies' and 'Safety'. Of these 456 abstracts reviewed, 31 were selected for scoring.

For EUROTOX 2021 the total number of abstracts for review within relevant oral presentation and poster sessions was 271. Of these, only 6 were scored.

From the total of 2,188 abstracts presented at the Society for Toxicology's 2021 meeting, 95 were identified as potentially relevant based on the Abstract book's relevant Keywords. Of the 95, 13 were scored.

Of the 3,820 abstracts presented at the Society for Toxicology's 2022 meeting, only the 111 abstracts indexed under relevant Keywords were considered. Of these, 18 abstracts were identified for scoring.

Overall, from a total of 933 potentially interesting abstracts identified at the four relevant conferences in the last two years, a total of 68 (7%) were selected as being suitable for scoring.

## 5.2 Published Paper Abstract Selection

From the PubMed search we identified a total of 2,254 articles published in the last two years and of potential interest to Lush. This represents a significant increase in publication rate compared with the 772 papers identified in the 18 months (May 2018 – Nov 2019) preceding the previous, 2020, Lush report. Of these 2,254 articles, 932 (41%) relevant titles were from the "Adverse Outcome Pathways" and "AOP" searches; a further 309 (14%) relevant projects from the "Organ on a chip" search, 93 (4%) from the "Microphysiological system(s)" search; and finally, an additional 920 titles (41%) 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 2254 titles by around 80%. Of the remainder, after review of abstracts in stage 3, 61 abstracts were scored (13 from the AOP searches, 17 from the OoC searches, 12 from the Microphysiological system(s) search, and 19 from the Computox searches). This represents only a modest increase in number of scoring published abstracts over those considered in the 2020 Lush report.

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 129 abstracts describing work which scored at least one point according to our given criteria. This is an increase of 30% in scoring abstracts from the 2020 Lush report.

The distribution of scores was as follows: none of the abstracts scored only 1 point for standing out in some way; only 2 scored 2 for bringing new knowledge or tools to a previously identified AOP, OoC model, or computox tool; 34 scored 3; 47 scored 4; 29 scored 5; and 14 abstracts scored 6 points. Unlike in the previous, 2020, report, 3 abstracts scored the realistic maximum likely score of 7 points,

The distribution of scores allowed us to select those scoring the highest (6 or 7 points, 13% of all abstracts scored) for recommendation as potential Science Prize nominees. All 17 of these high-scoring abstracts are shown in full in Section 6.4. For abstracts of published papers, the DOI (digital object identifier) for that paper is provided. For conference abstracts, we give the abstract or poster number for identification – the conference abstract books can be obtained from the links provided in Section 2. The abstracts scoring up to and including 7 points are fully listed in the Table in Section 5.5.

## 5.4 High Scoring Abstracts

This year 17 projects received the highest scores of either 6 or 7 for reporting new or significantly improved AOPs, *in silico* assays, validation techniques, computational modelling approaches, or organ-on-a-chip models, and demonstrating their practical potential with some level of validation. The 17 abstracts are given below.

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

**New approach methods (NAMs) supporting read-across: Two neurotoxicity AOP-based IATA case studies.**

Source: PubMed.

W Van der Stel[1], G Carta[2], J Eakins[3], J Delp[4], I Suciu[4,5], A Forsby[6], A Cediel-Ulloa[7], K Attoff [6], F Troger[8], H Kamp[9], I Gardner[10], B Zdrazil[8], MJ Moné[1], GF Ecker[8], M Pastor[11], JC Gómez-Tamayo[11], A White[12], EHJ Danen[1], M Leist[4], P Walker[3], P Jennings[2], S Hougaard Bennekou[13], B Van de Water[1].

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[3] Cyprotex Discovery Ltd., Alderley Park, Macclesfield, Cheshire, United Kingdom.

[4] Chair for In vitro Toxicology and Biomedicine, Department of Inaugurated by the Doerenkamp-Zbinden Foundation, University of Konstanz, Konstanz, Germany.

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ALTEX. 2021;38(4):615-635. doi: 10.14573/altex.2103051. Epub 2021 Jun 10.

**Score 7: 3** for comprehensive deployment of read-across approaches + **3** for level of practical usefulness (external validation), + **1** for underpinning use of computational toxicology to develop new AOPs and read-across approaches.

*Abstract*

Read-across approaches are considered key in moving away from in vivo animal testing towards addressing data-gaps using new approach methods (NAMs). Ample successful examples are still required to substantiate this strategy. Here we present and discuss the learnings from two OECD IATA endorsed read-across case studies. They involve two classes of pesticides – rotenoids and strobilurins – each having a defined mode-of-action that is assessed for its neurological hazard by means of an AOP-based testing strategy coupled to toxicokinetic simulations of human tissue concentrations. The endpoint in question is potential mitochondrial respiratory chain mediated neurotoxicity, specifically through inhibition of complex I or III. An AOP linking inhibition of mitochondrial respiratory chain complex I to the degeneration of dopaminergic neurons formed the basis for both cases but was deployed in two different regulatory contexts. The two cases also exemplify several different read-across concepts: analogue versus category approach, consolidated versus putative AOP, positive versus negative prediction (i.e., neurotoxicity versus low potential for neurotoxicity), and structural versus biological similarity. We applied a range of NAMs to explore the toxicodynamic properties of the compounds, e.g., in silico docking as well as in vitro assays and readouts – including transcriptomics – in various cell systems, all anchored to the relevant AOPs. Interestingly, although some of the data addressing certain elements of the read-across were associated with high uncertainty, their impact on the overall read-across conclusion remained limited. Coupled to the elaborate regulatory review that the two cases underwent, we propose some generic learnings of AOP-based testing strategies supporting read-across.

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**The EU-ToxRisk method documentation, data processing and chemical testing pipeline for the regulatory use of new approach methods.**

Source: PubMed.

A Krebs[1,2], BMA van Vugt-Lussenburg[3], T Waldmann[1,4], W Albrecht[5], J Boei[6], B Ter Braak[7], M Brajnik[8], T Braunbeck[9,] T Brecklinghaus[5], F Busquet[10], A Dinnyes[11], J Dokler[8], X Dolde[1], TE Exner [8], C Fisher[12], D Fluri[13], A Forsby[14,15], JG Hengstler[5], AK Holzer[1], Z Janstova[11], P Jennings[16], J Kisitu[1,2], J Kobolak[11], M Kumar[17], A Limonciel[16], J Lundqvist[14,15], B Mihalik[11], W Moritz[13], G Pallocca[10], APC Ulloa[14], M Pastor[18], C Rovida[10], U Sarkans[19], JP Schimming[20], BZ Schmidt[21], R Stöber[5], T Strassfeld[13], B van de Water[20], A Wilmes[16], B van der Burg[3], CM Verfaillie[17], R von Hellfeld[9], H Vrieling[6], NG Vrijenhoek[20], M Leist[22,23].

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Arch Toxicol. 2020 Jul;94(7):2435-2461. doi: 10.1007/s00204-020-02802-6. Epub 2020 Jul 6.

**Score 7: 3** for new, unified strategy for comprehensive testing and validation of procedures **+ 3** for level of practical usefulness (external validation), **+ 1** importance of work in avoiding animal testing in future

*Abstract*

Hazard assessment, based on new approach methods (NAM), requires the use of batteries of assays, where individual tests may be contributed by different laboratories. A unified strategy for such collaborative testing is presented. It details all procedures required to allow test information to be usable for integrated hazard assessment, strategic project decisions and/or for regulatory purposes. The EU-ToxRisk project developed a strategy to provide regulatorily valid data, and exemplified this using a panel of > 20 assays (with > 50 individual endpoints), each exposed to 19 well-known test compounds (e.g. rotenone, colchicine, mercury, paracetamol, rifampicine, paraquat, taxol). Examples of strategy implementation are provided for all aspects required to ensure data validity: (i) documentation of test methods in a publicly accessible

database; (ii) deposition of standard operating procedures (SOP) at the European Union DB-ALM repository; (iii) test readiness scoring according to defined criteria; (iv) disclosure of the pipeline for data processing; (v) link of uncertainty measures and metadata to the data; (vi) definition of test chemicals, their handling and their behavior in test media; (vii) specification of the test purpose and overall evaluation plans. Moreover, data generation was exemplified by providing results from 25 reporter assays. A complete evaluation of the entire test battery will be described elsewhere. A major learning from the retrospective analysis of this large testing project was the need for thorough definitions of the above strategy aspects, ideally in form of a study pre-registration, to allow adequate interpretation of the data and to ensure overall scientific/toxicological validity.

DOI: 10.1007/s00204-020-02802-6 PMCID: PMC7367925 PMID: 32632539

### 9 An adverse outcome pathway (AOP)-informed integrated approach to testing and assessment (IATA) as a tool to conduct a developmental neurotoxicity (DNT) hazard characterization

Source: WC11.

A Bal-Price[1], K Crofton[2], Ma Paparella[3], M L Martino[4], P Martina Panzarea[4], F Barrucci[4], A F. Hernandez-Jerez[5], S Hougaard Bennekou[6], I Tzoulaki[7], M Wilks[8], I Mangas[4] and A Terron[4]

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**Score 7: 2** for demonstration of use of IATA combining in silico & in vitro approaches **+ 4** for level of practical usefulness ((almost) approved), + **1** for practical impact

*Abstract*

New approaches in toxicology including the AOP and IATA concepts, in vitro assays based on human cells and in silico modelling used in an integrated manner, may pave the way to a more efficient and predictive assessment of DNT, solving various regulatory challenges (Bal-Price et al., 2018). Towards this goal, the EFSA PPR Panel has developed an AOP-informed IATA as a tool to conduct DNT hazard characterization for IATA. Deltamethrin and flufenacet were selected for IATA case studies. A systematic literature review was conducted for human epidemiological studies, animal data (including in-vivo regulatory studies), in-vitro and zebrafish data. The AOP framework was applied to integrate information from these lines of evidence, and the DNT in vitro data from battery of assays (DNT-IVB) anchored to key neurodevelopmental processes. Uncertainty analyses were performed for each type of evidence to support conclusions on the hazard identification/characterization, and to express the uncertainty in a probabilistic way. This stepwise approach resulted in the development of an evidence-based AOP network for deltamethrin with a probabilistic quantitative estimation of the weight-of-evidence (WoE) using a Bayesian network approach. This AOP network consisted of two MIEs leading to altered behavioral function (the adverse outcome). The case studies showed the applicability of the DNT-IVB for hazard characterization and illustrated the usefulness of a developed AOP network and probabilistic quantification of the WoE for

regulatory decision making. Mechanistic understanding facilitated a human-relevant adverse outcomes interpretation, supporting the contextualization of these studies in the risk assessment process. Based on this information and experimentally generated new in vitro data, an OECD Guidance Document on in vitro DNT test methods within the context of an IATA is under development (Sachana et al., 2019) in collaboration with EFSA and DNT experts from the OECD member countries and should be finalized before the end 2021.

References

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Sachana, M., Bal-Price, A., Crofton, K. M. et al. (2019). International regulatory and scientific effort for improved developmental neurotoxicity testing. Toxicol Sci 167, 45-57.

**Skin sensitization in silico protocol.**

Source: PubMed.

C Johnson[1], E Ahlberg[2], LT Anger[3], L Beilke[4], R Benigni[5], J Bercu[6], S Bobst[7], D Bower[8], A Brigo[9], S Campbell[10], MTD Cronin[11], I Crooks[12], KP Cross KP[8], T Doktorova[13], T Exner[13], D Faulkner[14], IM Fearon[15], M Fehr[16], SC Gad[17], V Gervais[18], A Giddings[19], S Glowienke[20], B Hardy[13], C Hasselgren [3], J Hillegass[21], R Jolly[22], E Krupp[23], L Lomnitski[24], J Magby[25], J Mestres[26], L Milchak[27], S Miller [8], W Muster[9], L Neilson[28], R Parakhia[29], A Parenty[20], P Parris[30], A Paulino[31], AT Paulino[31], DW Roberts[10], H Schlecker[32], R Stidl[33], D Suarez-Rodrigez[34], DT Szabo[35], RR Tice[36], D Urbisch[37], A Vuorinen[16], B Wall[25], T Weiler[18], AT White[19], J Whritenour[38], J Wichard[32], D Woolley[39], C Zwickl [40], GJ Myatt[8].

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Regul Toxicol Pharmacol. 2020 Oct;116:104688. doi: 10.1016/j.yrtph.2020.104688. Epub 2020 Jul 1.

**Score 6: 3** for comprehensive in silico testing protocol **+ 3** for level of practical usefulness (external validation).

*Abstract*

The assessment of skin sensitization has evolved over the past few years to include in vitro assessments of key events along the adverse outcome pathway and opportunistically capitalize on the strengths of in silico methods to support a weight of evidence assessment without conducting a test in animals. While in silico methods vary greatly in their purpose and format; there is a need to standardize the underlying principles on which such models are developed and to make transparent the implications for the uncertainty in the overall assessment. In this contribution, the relationship between skin sensitization relevant effects, mechanisms, and endpoints are built into a hazard assessment framework. Based on the relevance of the mechanisms and effects as well as the strengths and limitations of the experimental systems

used to identify them, rules and principles are defined for deriving skin sensitization in silico assessments. Further, the assignments of reliability and confidence scores that reflect the overall strength of the assessment are discussed. This skin sensitization protocol supports the implementation and acceptance of in silico approaches for the prediction of skin sensitization.

DOI: 10.1016/j.yrtph.2020.104688 PMCID: PMC7518315 PMID: 32621976

**Applying knowledge-driven mechanistic inference to toxicogenomics.**

Source: PubMed.

IJ Tripodi[1], TJ Callahan[2], JT Westfall[3], NS Meitzer[4], RD Dowell[5], LE Hunter[6].

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Toxicol In Vitro. 2020 Aug;66:104877. doi: 10.1016/j.tiv.2020.104877. Epub 2020 May 6.

**Score 6: 3** for a new computox tool **+ 2** for level of practical usefulness (in-house demonstration), **+ 1** for potential utility

*Abstract*

When considering toxic chemicals in the environment, a mechanistic, causal explanation of toxicity may be preferred over a statistical or machine learning-based prediction by itself. Elucidating a mechanism of toxicity is, however, a costly and time-consuming process that requires the participation of specialists from a variety of fields, often relying on animal models. We present an innovative mechanistic inference framework (MechSpy), which can be used as a hypothesis generation aid to narrow the scope of mechanistic toxicology analysis. MechSpy generates hypotheses of the most likely mechanisms of toxicity, by combining a semantically-interconnected knowledge representation of human biology, toxicology and biochemistry with gene expression time series on human tissue. Using vector representations of biological entities, MechSpy seeks enrichment in a manually curated list of high-level mechanisms of toxicity, represented as biochemically- and causally-linked ontology concepts. Besides predicting the canonical mechanism of toxicity for many well-studied compounds, we experimentally validated some of our predictions for other chemicals without an established mechanism of toxicity. This mechanistic inference framework is an advantageous tool for predictive toxicology, and the first of its kind to produce a mechanistic explanation for each prediction. MechSpy can be modified to include additional mechanisms of toxicity, and is generalizable to other types of mechanisms of human biology.

DOI: 10.1016/j.tiv.2020.104877 PMCID: PMC7306473 PMID: 32387679

**Development of a novel dual reproductive organ on a chip: recapitulating bidirectional endocrine crosstalk between the uterine endometrium and the ovary.**

Source: PubMed.

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Biofabrication. 2020 Oct 16;13(1). doi: 10.1088/1758-5090/abbd29.

**Score 6: 3** for developing 2 organ reproductive system chip with endocrine crosstalk + **2** for level of practical usefulness (in-house validation), + **1** for developing a reporter gene toxicity assay within the system

*Abstract*

Conventional 2D or even 3D in vitro culture models for human reproductive organs cannot properly recapitulate the bidirectional endocrine crosstalk between the uterine endometrium and the ovary. This crosstalk is essential for maintaining the various physiological features and functions of each tissue. Moreover, most in vitro models for the female reproductive tract also fail to mimic its multicellular structure. We therefore developed a novel 'dual reproductive organ on a chip' that reflects the bidirectional endocrine cross-talk and the complex multicellular structures by integrating various cellular components of both the human uterine endometrium and the ovary with several biodegradable natural polymers. Indeed, the bidirectional endocrine crosstalk between these two tissues is achieved through media sharing between channels, and it can markedly improve the viability of loaded cells within each chamber of the chip platform. In addition, we also identified a reliable reproductive toxicity marker, SERPINB2, which is significantly increased in response to various toxic exposures in both endometrial and ovarian follicular cells. Based on these findings, we next established a SERPINB2 luciferase reporter system that was specifically designed for detecting and quantifying the toxicity of certain substances. By introducing this SERPINB2 luciferase reporter system into the loaded cells within the chip platform, we ultimately developed an effective 'dual reproductive organ-on-chip' that was successfully used to predict the reproductive toxicity of various hazardous materials.

DOI: 10.1088/1758-5090/abbd29 PMID: 32998123

**2551 Construction of a AOP Network Related to Metabolism Disorders Induced by an Endocrine-Disrupting Chemical Mixture Using Artificial Intelligence and Systems Toxicology**

Source: SoT 2021.

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**Score 6: 3** for a new AOP tool **+ 2** for level of practical usefulness (in-house demonstration), + **1** for use of computational toxicology to develop new AOPs

*Abstract*

Metabolic disorders are among the main adverse health outcomes that have been associated with endocrine disrupting chemicals (EDCs). It is essential to have a better understanding of the mode of action of suspected EDCs, and the biological pathways that they may be perturbed to identify their real impact on the human population. The concept of Adverse Outcome Pathways (AOP) provides a practical organizing framework of perturbations at different levels of the biological organization by linking molecular initiating events (MIE) to an adverse outcome (AO) across several intermediate key events (KE). We investigated the applicability of an integrated systems toxicology approach to develop a AOP network related to metabolism disorders. First, a new tool called AOP-helpFinder was used to identify metabolic effects associated with selected EDCs such as biphenols, PFAS, PCBs, brominated compounds. AOP-helpFinder combines text mining and graph theory to automatically screen abstracts from the PubMed database. The tool searched for co-occurring terms among two lists: one that contains the studied EDCs, and one with the biological events (from the AOP-wiki database and from in house experts). This step allowed to decipher links between each EDC and biological events, and was followed by a manual curation to select the most relevant publications, whose reliability was assessed by a dedicated tool. Then, an AOP network was proposed, which was enriched by integration of databases information (e.g. CompTox).The AOP-helpFinder tool allowed to establish linkage between the EDCs and 80 events from 15414 articles. The most relevant events were related to lipids accumulation/obesity, oxidative stress, diabetes, and liver steatosis. Among them, 20 events were related to nuclear receptors and transcriptional factors. After an individual analysis of each EDC, the findings were merged to mimic an EDC mixture. An AOP network that reflects biological key events that could be triggered by several EDCs was then proposed. These findings highlight the increasingly relevant use of computational tools in predictive toxicology.

**2530 Drugshot, an Appyter for Querying Biomedical Search Terms to Receive Prioritized Lists of Small Molecules**

Source: SoT 2021.

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Icahn School of Medicine at Mount Sinai, New York, NY. Sponsor: A. Ma'ayan, Society of Environmental Toxicology and Chemistry

**Score 6: 3** for a new AOP tool **+ 2** for level of practical usefulness (in-house demonstration), **+ 1** for wide utility of computox tool

*Abstract*

Current methods for screening compound toxicity are mostly confined to costly in vitro and in vivo experiments. While there are currently existing computational methods that can assess toxicity of novel compounds, most are tailored to specific use cases and do not take advantage of the aggregated knowledge available from biomedical literature to make novel predictions. Drugshot is a web-based software application that enables users to enter arbitrary search terms into a simple input form. Once submitted, Drugshot deploys a downloadable Jupyter notebook in the cloud with the results which contain ranked lists of associated and predicted compounds relevant to the search terms. The associated compound list ranks each compound according to total co-mentions of the drug and the search terms from shared PubMed IDs. Additionally, lists of compounds predicted to be associated with the search terms are generated based on co-occurrence in the literature, co-expression from L1000 drug-induced gene expression profiles, and based on chemical structural similarity. Through its search functionality and abstraction of drug lists from different sources, Drugshot can facilitate hypothesis generation by suggesting associated and predicted drug lists related to any biomedical term of interest. We validated the utility of Drugshot with a case study of endocrine disruptors to prioritize top compounds from the literature, and predict novel compounds that may disrupt endocrine function.

Drugshot is freely and openly available at: https://appyters.maayanlab.cloud/#/DrugShot.

**2538 Gaining Confidence in Computational Models for Risk Assessment: Combining Approaches and Understanding Uncertainty**

Source: SoT 2021.

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**Score 6: 3** for combining computox toolstool + **2** for level of practical usefulness (in-house demonstration), + **1** for use of computational toxicology to identify new MIEs

*Abstract*

Molecular Initiating Events (MIEs) are good targets for in silico modelling, as they are well defined chemical-biological interactions. Computational approaches based on the chemistry of chemical binders have been developed to make predictions at pharmacologically important human MIEs. These approaches have been combined to provide a high performing model and increase confidence in its predictions, and Bayesian learning has been implemented to provide activity predictions with an understanding of uncertainty.

Three computational techniques have been used to predict MIEs. These include structural alerts developed automatically using maximal common substructure searches, random forest models constructed using 200 physicochemical descriptors as the input, and neural networks in Python 3 using TensorFlow. All three computational approaches consistently provide models with over 90% accuracy against test data. Combining these models in a decision-making context allows us to use the advantages of each method to provide the best possible prediction. This procedure shows an increase in model performance when compared to any individual model. Applicability domains and confidence scores for test chemicals can be used to better understand how new chemicals compare to the data set used in model construction. To extend these models to qualitative activity prediction, Bayesian learning neural networks have been constructed. By using probability distributions throughout, the network can produce an output prediction with a mean and standard deviation. This uncertainty accounts for both how close the new example is to the existing data and how much variation exists within the training set. These networks produce quantitative activity estimates with errors within one log unit, on external validation data, and help distinguish between molecules similar to and different from the training data. Next-generation risk assessment requires information on molecular potency and uncertainties, and decision-makers must have confidence in the tools being used. These models provide additional understanding and confidence, vital for their use in risk assessment.

**4018 Comprehensive Analysis of the Literature to Construct AOP Network Related to Metabolism Induced by Endocrine Disruptors Using the AOP-helpFinder Tool**

Source: SoT 2022.

K. Bernal[1], F. Jornod[2], E. Zgheib[1], E. Blanc[1], M. Kim[3], and K. Audouze[1].

[1] Université de Paris, Paris, France

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**Score 6: 3** for the development of a new AOP tool **+ 2** for level of practical usefulness (in-house validation), **+ 1** for increasing the utility of the AOP-Wiki

*Abstract*

Adverse outcome pathways (AOP) are a conceptual framework that support the use of alternative toxicological approaches in chemical risk assessment. AOPs provide structured organizations of known biological perturbations, starting from an initial molecular event (MIE) to an adverse outcome (AO) across several intermediate key events (KE). To help the

development of AOPs and AONs (AOP networks), which are a combination of several AOPs sharing at least one biological event, we have developed a tool named AOP-helpFinder (http://aop-helpfinder.u-paris-sciences.fr/ ). AOP-helpFinder is based on artificial intelligence, more precisely using natural language processing (NPL) and graph theory to systematically and rapidly explore all available abstracts stored in the PubMed database. It will identify and extract known associations between stressors of interest and KEs, therefore supporting the development of AOPs. Recently a webserver has been created to facilitate the use of this advanced bioinformatics tool. The webserver operates with an updated version of the tool, that allows to refine (using machine learning, i.e. lemmatization for text normalization) and reduce (capability to search only in the results/conclusion part of an abstract) the searches. Here we investigated the applicability of using the AOP-helpFinder tool with an integrated systems toxicology approach to develop an AON related to metabolic disorders, that are among the main adverse health outcomes associated with endocrine-disrupting chemicals (EDCs). Co-occurring terms (i.e. EDCs and biological events (MIE, KE, AO) from the AOP-wiki database and from in house experts) were identified by the AOP-helpFinder tool. This step allowed to decipher known links between EDCs and biological events and was followed by a manual curation to select the most relevant publications. Then, an AON was proposed, which was enriched by integration of complementary data extracted from other databases (e.g. CompTox). Computational approaches that allow Identification, extraction and integration of existing knowledge, appear to be essential to have a better understanding of the mode of action of suspected EDCs, and the biological pathways that may be perturbed in order to identify their real impact on the human population.

**4662 Mechanism-Driven Modeling of Drug-Induced Liver Injury Using Structural Alerts and an Oxidative Stress Screening Assay**

Source: SoT 2022.

X. Jia[1], X. Wen[2], D. P. Russo[1], L. M. Aleksunes[2], and H. Zhu[1].

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[2] Rutgers Ernest Mario School of Pharmacy, Piscataway, NJ.

**Score 6: 3** for a new AOP tool **+ 2** for level of practical usefulness (in-house demonstration), **+ 1** for wide utility of in silico tool

*Abstract*

Evaluation of preclinical and clinical DILI is expensive, time-consuming, and often requires many subjects to detect hepatotoxicity signals. In vitro testing assays, which were used to identify relatively simple toxicity mechanisms, are not sufficient to predict complex chemical toxicities such as DILI. As an advanced framework of risk assessment, Adverse Outcome Pathways (AOPs) describe mechanisms that involve complex chemical toxicities. We aimed to apply a computational framework to developing an AOP that is predictive of DILI. For this effort, we employed complementary computational modeling and in vitro assays focusing on cytotoxicity and oxidative stress via activation of the antioxidant response element (ARE). We collected and curated a dataset of drug compounds (n=704) with known DILI labels as modeling set, which were used to search PubChem for relevant assay data. The ARE assay, which identifies chemicals that have the potential to induce oxidative stress, shows high correlations to DILI (PPV=0.82). Next, we developed quantitative structure-activity relationship (QSAR) models based on ARE data to predict the oxidative stress response for compounds lacking ARE test results. Furthermore, potential toxicity alerts were identified from chemical fragments that correlated to DILI. The mechanistic DILI model consists of the identified structural alerts as molecular initiating event and in vitro ARE activation as a key event. To experimentally validate the ARE QSAR model and the resulted mechanistic DILI model, 11 compounds in the modeling set and 12 new external compounds were selected and experimentally tested using our in-house ARE screening assay. The mechanistic model showed good DILI predictivity (accuracy = 0.78) for modeling set and new compounds. Potential false positive DILI predictions caused by only using ARE results can be corrected by incorporating the alerts and vice versa. This

mechanistic model illustrates a potential toxicity pathway from initial chemical features to associated cellular responses, and this strategy can be applied to develop predictive models for other complex toxicity endpoints.

### 4673 Adverse Outcome Pathway Guided Acute Oral Toxicity Testing

Source: SoT 2022.

M. D. Nelms[1], S. W. Edwards[1], V. Hench[1], J. Ponder[2], and K. Sullivan[2].

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**Score 6: 3 for** chemoinformatics analysis of ht assay data **+ 2** for level of practical usefulness (in-house demonstration), **+ 1** for importance of in vitro/in silico tool for acute toxicity

*Abstract*

Adverse outcome pathways can facilitate tiered testing strategies for acute oral toxicity testing by providing a mechanistic framework to identify the appropriate non-animal methods and connect them to apical adverse outcomes. This study used chemical structure and bioactivity measurements to improve confidence in pre-existing in silico approaches and identify in vitro assays for tiered testing strategies to define acutely toxic chemicals. We performed a comprehensive analysis of 11,992 chemicals with curated acute toxicity information from the ICCVAM Acute Toxicity Work Group (ATWG). Chemicals were segregated into 2,192 clusters based on shared structural characteristics defined by ToxPrint fingerprints. ToxCast assays, where activity was enriched for chemicals within a cluster, were used to identify the minimum number of assays needed to detect all acutely toxic chemicals. Of the 1,627 acutely toxic chemicals (rat oral LD50 <= 2,000 mg/kg as defined by the ATWG) with activity in ToxCast below the range of cytotoxicity, 1,139 were linked to one or more structure-guided ToxCast assays. While 300 assays were required to detect all acutely toxic chemicals with false discovery rate of 3%, selecting assays based on a chemical's structural cluster means that no single chemical requires more than four assays and 98% require two assays or less. Cluster-specific ToxCast activity was significantly associated with both the binary ATWG toxicity classification (p-value = $2.2 \times 10^{-16}$) and the five United Nations Globally Harmonized System (GHS) categories for acute oral toxicity (estimated p-value = $5 \times 10^{-4}$). The structural clusters alone were significantly associated with GHS categories (estimated p-value = $5 \times 10^{-5}$) as well. Further evaluation of the assays associated with chemical clusters enriched for acutely toxic chemicals confirmed that assay performance is improved when the assay is directly linked to the mechanism of toxicity though several assays with indirect links to the toxicity mechanism showed good performance. This study also confirmed previous observations that cytotoxicity can predict acute toxicity. Our results suggest that a combination of in silico approaches, such as the Collaborative Acute Toxicity Modeling Suite (CATMoS), and bioactivity information guided by chemical structure and toxicological mechanism represents an efficient tiered testing strategy sufficient to reduce or eliminate animal testing for acute oral toxicity.

### 4674 QSAR Modeling of Lowest and Highest In Vitro Potency Levels for Inhibition of Human Hedgehog (Hh) Signaling Pathway and Screening of 11,096 REACH-Registered Substances

Source: SoT 2022.

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Danmarks Tekniske Universitet, Kongens Lyngby, Denmark.

**Score 6: 3** for chemoinformatics analysis of ht assay data **+ 2** for level of practical usefulness (in-house demonstration), **+ 1** for importance of in vitro/in silico tool for developmental toxicity

*Abstract*

Hedgehog (HH) signalling is an evolutionary conserved signal transduction network essential for normal development across vertebrates. The HH signalling pathway is made up of several ligands, receptors and co-factors that instruct cells and tissues to differentiate and organise themselves in a spatiotemporal manner. Consequently, dysregulated HH signalling can lead to a plethora of disorders such as brain malformations, facial and limb deformities, ciliopathies (collection of syndromes and disorders), and cancers. In this study, we performed a comprehensive in-house systematic procedure to extract the most robust positive and negative experimental results for 9,667 substances from the US Toxicology in the 21st Century (Tox21) high-throughput in vitro assay data. Quantitative structure-activity relationship (QSAR) models with binary outputs for their inhibitory effect on the HH signalling pathway were developed at two potency thresholds. Rigorous cross-validation, as well as external validations with 20% left-out test sets independent of model development and selection, demonstrated robust and highly predictive models with high balanced accuracies. The models were applied to screen 11,096 registered substances under the European Union (EU) chemicals regulation, REACH (Registration, Evaluation, Authorisation and Restriction of Chemical substances). These predictions can be used, for example, for priority setting to support read-across and in weight-of-evidence (WoE) assessments of chemicals.

**4675 Estimation of No-Observed-Adverse-Effect Level (NOAEL) by Read-Across for Large Inventories of Structurally Related Chemicals and Metabolites**

Source: SoT 2022.

J. Rathman[1,2], C. Yang[1], J. V. Ribeiro[1], A. Mostrag[1], T. Magdziarz[3], and B. Hobocienski[1].

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**Score 6: 3** for QSAR comparisons driven by biological effect profiles **+ 2** for level of practical usefulness (in-house demonstration), **+ 1** for utility

*Abstract*

Although chemical safety assessment using quantitative structure-activity relationships (QSAR) and rule-based systems is becoming more acceptable, these approaches are not well-suited to endpoints for which the underpinning data are weakly grounded in specific chemical-biological interactions. One example where a new paradigm is needed is for estimating the no-observed-adverse-effect level (NOAEL), required in the assessment of systemic toxicity. When an experimentally determined NOAEL for a query compound of interest is unavailable, it can be estimated by a read across approach based on analog compounds having experimental data. This study presents a novel method whereby a combination of statistically robust chemoinformatics analysis, highly curated NOAEL databases from the COSMOS and Antimicrobial Threshold of Toxicological Concern (TTC) projects, and mechanistic grouping of >1300 compounds are applied to estimate confidence bounds for the NOAEL of a target compound. The central hypothesis is that similar compounds (structures, properties, biological effects) likely have similar toxicity profiles and, hence, similar NOAEL values. Analog quality (AQ) quantifies the suitability of an analog candidate for read across to the target by considering similarity from structure, physicochemical and biological perspectives. Biological similarity is based on experimental assay data; we present an approach in which assay vectors-based on defined aggregations of Tox21/ToxCAST assay data are used as biological fingerprints that capture target-analog similarity relevant to specific effects of interest, for example hormone receptors (ER/AR/PR). Once one or more analogs have been qualified for read across, a decision theory approach is used to estimate confidence bounds for the NOAEL of the target. We demonstrate how the confidence interval is dramatically narrowed when analogs are constrained to biologically related profiles. While for regulatory purposes read across often focuses on a single target, in other scenarios it is desirable to estimate NOAEL values for a

large number of structures, for example when screening virtual compound libraries. To this end we present an implementation of our NOAEL estimation technique that allows batch processing of large numbers of structures. This workflow was validated by use cases for large sets of bisphenols and pesticide metabolites. The process can be reduced to algorithmic workflows in a computational system to enable the efficient assessment of a large number of substances.

### 596 From case studies to a regulatory guidance: The EU-ToxRisk NAM-assisted RAx advisory document.

Source: WC11.

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[3] Fraunhofer Institute (ITEM), Hanover, Germany;

[4] TNO, Zeist, The Netherlands;

[5] Danish Technical University, Copenhagen, Denmark.

**Score 6: 2** for demonstration of use of read-across using in silico & in vitro approaches **+ 3** for level of practical usefulness (external validation), **+ 1** for practical utility

*Abstract*

Grouping/category approaches for read-across have evolved over the last decades as important risk assessment tools in order to attempt filling data gaps in particular for complex endpoints such as repeated dose or developmental and reproductive toxicity without performing additional animal studies (ECHA 2014). To date many read-across cases fail to demonstrate toxicokinetic and toxicodynamic similarities. New concepts involving an integrated application of in vitro and in silico tools are needed to better characterize common properties of structural similar chemicals, collectively called New Approach Methodologies (NAMs). EU-ToxRisk has developed a NAM-based read-across strategy (Escher et al., 2019) and systematically evaluated this strategy in several experimental case studies addressing repeat dose toxicity and developmental and reproductive toxicity. Case studies involved both in silico and in vitro animal-free approaches targeting both toxicokinetics and toxicodymanics aspects. The case studies have been systematically reported using read-across reporting templates from ECHA and OECD, and subsequently been evaluated by an international regulatory authority panel under guidance of the OECD. These case studies have resulted in important learnings which led to an EU-ToxRisk read-across regulatory advisory document. These learnings will be presented in this presentation. Altogether our work strongly encourages the toxicological community to integrate a mechanism-based risk assessment and its regulatory acceptance for read-across assessment.

Reference

Escher, S. E., Kamp, H., Bennekou, S. H. et al. (2019). Towards grouping concepts based on new approach methodologies in chemical hazard assessment: The read-across approach of the EU-ToxRisk project. Arch Toxicol 93, 3643-3667. doi:10.1007/s00204-019-02591-7

### 444 Application of newly validated route-specific in vitro genotoxicity assays to support the safety assessment of cosmetic ingredients

Source: WC11.

R Fautz[1], K Reisinger[2], S Hoffmann[3], N J. Hewitt[4], G Ouédraogo[5], J Kenny[6], B Wall[7] and S Pfuhler[8]

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[7] Colgate-Palmolive, Piscataway, NJ, United States;

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**Score 6: 2** for case study of newly validated genotox tests **+ 4** for level of practical usefulness (approved)

*Abstract*

New in vitro skin models have been established as follow up assays to improve the prediction of potential genotoxicity of cosmetic ingredients in the absence of in vivo data. The reconstructed skin micronucleus test (RSMN) and RS Comet assays are now validated and have been accepted into the OECD test guideline development program. Here, we demonstrate their application to safety assessment by conducting a case study based on the oxidative hair dye, 4-nitro-1,2-phenylenediamine (B24). The strategy is based on an endpoint-triggered follow up of positive results from the Ames and in vitro micronucleus (MNvit) 2-test battery. For topically applied chemicals, the RSMN assay is recommended for MNvit positive chemicals, whereas, Ames positives should be tested in the RS Comet assay. B24 was positive in the Ames assay but negative in the MNvit assay; therefore, it was tested coded in the RS Comet assay. In experiment 1, B24 was non-cytotoxic and did not induce DNA breaks in keratinocytes or fibroblasts up to the lowest precipitating dose (50 mg/cm2). In experiment 2, in the presence of the repair inhibitor, aphidicolin, there was no statistical increases in %tail DNA that exceeded the historical controls up to 50 mg/cm2. B24 was concluded to be negative, which is in accordance with negative results in the HPRT mammalian cell gene mutation assay. The conclusion from this case study is that while B24 causes mutagenicity in bacteria, it is not genotoxic in mammalian cells or in a higher tier assay using human skin equivalents. The RS Comet assay allows an assessment under the relevant exposed conditions, i.e., topically, and in a test system containing relevant metabolizing enzymes. Further case studies are under way to evaluate other scenarios from the testing strategy.

### 256 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

Source: WC11.

TP Tao[1], J Kühnl[2], K Brandmair[2], S Gerlach[2], A Winter[1], U Marx[1], I Maschmeyer[1], J Przibilla[3], F Kern[3], A Schepky[2], Ca Jacques-Jamin[4], C Genies Pierre[4] and N Hewitt[5]

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[2] Beiersdorf AG, Hamburg, Germany

[3] Pharmacelsus GmbH, Saarbrücken, Germany

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[5] Cosmetics Europe, Brussels, Belgium

**Score 6: 3** for demonstration of applicability of skin/liver chip in cosmetics testing **+ 2** for level of practical usefulness (in-house validation), **+ 1** for potential importance in OoC application

*Abstract*

The Cosmetics Europe's Long Range Science Strategy (LRSS) aims to establish the link between dermal and systemic exposure route. In this project, we have used TissUse's microfluidic platform, the HUMIMIC Chip2 model, incorporating skin (Epi-Derm™) and liver organoids (consisting of HepaRG™ and stellate cells), to investigate the influence of exposure scenarios on the bioavailability and metabolic fate of chemicals. The aromatic amine hair dye,

4-amino-2-hydroxytoluene (AHT), was selected as a case study chemical to determine whether the Chip2 model could be used to mimic the first-pass effect in the skin that was observed in in vivo studies in rats. Both organoids were well maintained over 6 days, as indicated by TEER, metabolic analysis and viability markers. The kinetics of AHT and several of its metabolites, including N-acetyl-AHT and AHT-sulfate, differed between topical and systemic application. Importantly, topical application resulted in a higher peak concentration of N-acetyl-AHT and increase of its area under the curve (AUC) by 275%, demonstrating that a first-pass effect of N-acetylation in the skin had occurred. There was a concomitant decrease in the peak concentration and AUC of AHT-sulfate after topical compared to systemic application. These results were in accordance with in vivo observations, where the ratios of these two metabolites were altered by the application route. In conclusion, these data demonstrate that the Chip2 maintains the functions of skin and liver organoids for several days. Importantly, the Chip2 model recapitulated the route-specific alteration in the metabolite profile of AHT observed in vivo. This type of information is important for the risk assessment of topically-applied compounds which may also undergo first-pass metabolism in the skin and whose systemic effects are altered accordingly.

## 5.5 All Scored Abstracts

The Table lists details (Title, Authors, source, and score) of all the 129 abstracts scoring 1 or more. The Table is ordered by score (from 7 – 1) and by source of abstract – PubMed, SoT21, SoT22, WC11 and EUROTOX 21. For abstracts of published papers, the abstract title in the Table is a hyperlink to the DOI (digital object identifier) for that paper. For conference abstracts, we give the abstract or poster number for identification.

| Title | Authors | Source | Score |
|---|---|---|---|
| New approach methods (NAMs) supporting read-across: Two neurotoxicity AOP-based IATA case studies. | Van der Stel W et al. | PubMed | 7 |
| The EU-ToxRisk method documentation, data processing and chemical testing pipeline for the regulatory use of new approach methods. | Krebs A et al. | PubMed | 7 |
| 9 An adverse outcome pathway (AOP)-informed integrated approach to testing and assessment (IATA) as a tool to conduct a developmental neurotoxicity (DNT) hazard characterization | Bal-Price A et al. | WC11 | 7 |
| Skin sensitization in silico protocol. | Johnson C et al. | PubMed | 6 |
| Applying knowledge-driven mechanistic inference to toxicogenomics. | Tripodi IJ et al. | PubMed | 6 |
| Development of a novel dual reproductive organ on a chip: recapitulating bidirectional endocrine crosstalk between the uterine endometrium and the ovary. | Park SR et al. | PubMed | 6 |

| Title | Authors | Source | Score |
|---|---|---|---|
| 2530 Drugshot, an Appyter for Querying Biomedical Search Terms to Receive Prioritized Lists of Small Molecules | Kropiwnicki E et al. | SoT 2021 | 6 |
| 2551 Construction of a AOP Network Related to Metabolism Disorders Induced by an Endocrine-Disrupting Chemical Mixture Using Artificial Intelligence and Systems Toxicology | Zgheib E et al. | SoT 2021 | 6 |
| 2538 Gaining Confidence in Computational Models for Risk Assessment: Combining Approaches and Understanding Uncertainty | Allen T E et al. | SoT 2021 | 6 |
| 4018 Comprehensive Analysis of the Literature to Construct AOP Network Related to Metabolism Induced by Endocrine Disruptors Using the AOP-helpFinder Tool | Bernal K et al. | SoT 2022 | 6 |
| 4662 Mechanism-Driven Modeling of Drug-Induced Liver Injury Using Structural Alerts and an Oxidative Stress Screening Assay | Jia X et al. | SoT 2022 | 6 |
| 4673 Adverse Outcome Pathway Guided Acute Oral Toxicity Testing | Nelms M D et al. | SoT 2022 | 6 |
| 4674 QSAR Modeling of Lowest and Highest In Vitro Potency Levels for Inhibition of Human Hedgehog (Hh) Signaling Pathway and Screening of 11,096 REACH-Registered Substances | Moeller C A et al. | SoT 2022 | 6 |
| 4675 Estimation of No-Observed-Adverse-Effect Level (NOAEL) by Read-Across for Large Inventories of Structurally Related Chemicals and Metabolites | Rathman J et al. | SoT 2022 | 6 |
| 596 From case studies to a regulatory guidance: The EU-ToxRisk NAM-assisted RAx advisory document | van de Water B et al. | WC11 | 6 |
| 444 Application of newly validated route-specific in vitro genotoxicity assays to support the safety assessment of cosmetic ingredients | Fautz R et al. | WC11 | 6 |
| 256 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 T-P et al. | WC11 | 6 |
| Do Similar Structures Have Similar No Observed Adverse Effect Level (NOAEL) Values? Exploring Chemoinformatics Approaches for Estimating NOAEL Bounds and Uncertainties. | Yang C et al. | PubMed | 5 |

| Title | Authors | Source | Score |
|---|---|---|---|
| Systematic Identification of Molecular Targets and Pathways Related to Human Organ Level Toxicity. | Xu T et al. | PubMed | 5 |
| Unbiased approach for the identification of molecular mechanisms sensitive to chemical exposures. | Suvorov A et al. | PubMed | 5 |
| Development and validation of the TGx-HDACi transcriptomic biomarker to detect histone deacetylase inhibitors in human TK6 cells. | Cho E et al. | PubMed | 5 |
| High throughput data-based, toxicity pathway-oriented development of a quantitative adverse outcome pathway network linking AHR activation to lung damages. | Jin Y et al | PubMed | 5 |
| A cross-industry collaboration to assess if acute oral toxicity (Q)SAR models are fit-for-purpose for GHS classification and labelling. | Bercu J et al. | PubMed | 5 |
| Predicting drug-induced hepatotoxicity based on biological feature maps and diverse classification strategies. | Su R et al. | PubMed | 5 |
| Integrative systems toxicology to predict human biological systems affected by exposure to environmental chemicals. | Taboureau O et al. | PubMed | 5 |
| Construction of a high fidelity epidermis-on-a-chip for scalable in vitro irritation evaluation. | Zhang J et al. | PubMed | 5 |
| Cell and tissue system capable of automated culture, stimulation, and monitor with the aim of feedback control of organs-on-a-chip. | Konishi S et al. | PubMed | 5 |
| 32961 Improving and Updating the Population Life-Course Exposure to Health Effects Model (PLETHEM): An Online Tool and R Package for PBPK Modeling | Fitzpatrick JM et al. | SoT 2021 | 5 |
| 2520 Reproducibility and Reliability of High-Throughput Transcriptomics for Chemical Safety Screening | Everett LJ et al. | SoT 2021 | 5 |
| 2788 On the Way to Proactive Compound De-risking Using Artificial Intelligence | Rouquie D. et al | SoT 2021 | 5 |
| 3033 A Human Embryonic Stem Cell-Based High-Throughput Platform with AI Technology to Screen for Developmental Toxicants | Chen Y et al. | SoT 2021 | 5 |

| Title | Authors | Source | Score |
|---|---|---|---|
| 2134 Evaluation of the Utility of the Beta Human Liver Emulation System (BHLES) for Toxicity Testing in a Regulatory Setting Using Model Compounds | Eckstrum K et al. | SoT 2021 | 5 |
| 2970 A Human Pluripotent Stem Cell-Based Assay, devTOX quickPredict, Accurately and Reproducibly Predicts the Developmental Toxicity Potential across a Diverse Set of Chemicals | Palmer J A et al | SoT 2021 | 5 |
| 4002 Knowledge-Guided Deep Learning Models of Drug Toxicity Improve Interpretation | Hao Y et al. | SoT 2022 | 5 |
| 4648 Adverse Outcome Pathways to Guide the Development of IATA | Saarimäki L A et al. | SoT 2022 | 5 |
| 4652 Identifying Perturbations That Modulate ESR1 Expression with Appyters | Xie Z et al. | SoT 2022 | 5 |
| 4007 Viewing and Interrogating AOP-Wiki Knowledge as a Network within Wiki Kaptis | Kane S et al. | SoT 2022 | 5 |
| 4668 Role of a Chemoinformatics Platform in Industry Research Programs and Risk Assessments for Cosmetics Ingredients | Ouedrago G et al | SoT 2022 | 5 |
| 868 A high-throughput, microfluidic platform for drug screening on vascularized 3D tissues | Joore J | WC11 | 5 |
| 67 Blood vessels in organs-on-chips | van de Meer A | WC11 | 5 |
| 851 In silico approaches to link adverse outcomes to molecular initiating events through AOPs | Allen T et al. | WC11 | 5 |
| 262 An industry perspective on strategies for integrating new approach methodologies for next generation risk assessment: Coumarin as a case study | Baltazar M T et al. | WC11 | 5 |
| 730 Automating multi-organ-chip assays and analysis for improved standardization and reproducibility | Hübner J et al. | WC11 | 5 |
| 923 Towards an ADME competent 4-organ-chip | Atac B et al. | WC11 | 5 |
| 1006 Development of an in silico platform to assess developmental and reproductive toxicity (DART) | van der Voet M et al. | WC11 | 5 |
| P11-02 ReproTracker: A Human Stem Cell-Based Biomarker Assay for In vitro Assessment of Developmental Toxicity | Jamalpoor A et al. | EUROTOX 2021 | 5 |

| Title | Authors | Source | Score |
|---|---|---|---|
| Exploration of the DARTable Genome- a Resource Enabling Data-Driven NAMs for Developmental and Reproductive Toxicity Prediction | Janowska-Sejda EI et al. | PubMed | 4 |
| AOP Report: Uncoupling of Oxidative Phosphorylation Leading to Growth Inhibition via Decreased Cell Proliferation | Song Y & Villeneuve DL | PubMed | 4 |
| Predictive Model for Drug-Induced Liver Injury Using Deep Neural Networks Based on Substructure Space. | Kang MG & Kang NS. | PubMed | 4 |
| Development of blood brain barrier permeation prediction models for organic and inorganic biocidal active substances. | Shin HK et al. | PubMed | 4 |
| High-Throughput Transcriptomics Platform for Screening Environmental Chemicals. | Harrill JA et al. | PubMed | 4 |
| Risk-Based Chemical Ranking and Generating a Prioritized Human Exposome Database. | Zhao F et al. | PubMed | 4 |
| Comparing in vitro human liver models to in vivo human liver using RNA-Seq. | Gupta R et al. | PubMed | 4 |
| Derivation, characterisation and analysis of an adverse outcome pathway network for human hepatotoxicity. | Arnesdotter E et al | PubMed | 4 |
| An adverse outcome pathway on the disruption of retinoic acid metabolism leading to developmental craniofacial defects. | Menegola E et al. | PubMed | 4 |
| A toxicity pathway-oriented approach to develop adverse outcome pathway: AHR activation as a case study. | Jin Y et al. | PubMed | 4 |
| Analysis of reproducibility and robustness of a human microfluidic four-cell liver acinus microphysiology system (LAMPS). | Sakolish C et al. | PubMed | 4 |
| Transforming early pharmaceutical assessment of genotoxicity: applying statistical learning to a high throughput, multi end point in vitro micronucleus assay. | Wilson A et al. | PubMed | 4 |
| Application of the DILIsym® Quantitative Systems Toxicology drug-induced liver injury model to evaluate the carcinogenic hazard potential of acetaminophen. | Eichenbaum G et al. | PubMed | 4 |

| Title | Authors | Source | Score |
|---|---|---|---|
| In vitro and in silico genetic toxicity screening of flavor compounds and other ingredients in tobacco products with emphasis on ENDS. | Hung P et al. | PubMed | 4 |
| Revealing cytotoxic substructures in molecules using deep learning. | Webel H et al. | PubMed | 4 |
| Demonstration of the first-pass metabolism in the skin of the hair dye, 4-amino-2-hydroxytoluene, using the Chip2 skin-liver microphysiological model. | Tao TP et al. | PubMed | 4 |
| Human IPSC-Derived Model to Study Myelin Disruption. | Chesnut M et al. | PubMed | 4 |
| Characterizing the reproducibility in using a liver microphysiological system for assaying drug toxicity, metabolism, and accumulation. | Rubiano A et al. | PubMed | 4 |
| Characterization of application scenario-dependent pharmacokinetics and pharmacodynamic properties of permethrin and hyperforin in a dynamic skin and liver multi-organ-chip model. | Kühnl J et al. | PubMed | 4 |
| Toxicity of topically applied drugs beyond skin irritation: Static skin model vs. Two organs-on-a-chip. | Tavares RSN et al. | PubMed | 4 |
| Impact of aerosols on liver xenobiotic metabolism: A comparison of two methods of exposure. | Bovard D et al | PubMed | 4 |
| Human neural tube morphogenesis in vitro by geometric constraints. | Karzbrun E et al. | PubMed | 4 |
| A new microfluidic method enabling the generation of multi-layered tissues-on-chips using skin cells as a proof of concept. | Valencia L et al. | PubMed | 4 |
| A 3D microfluidic liver model for high throughput compound toxicity screening in the OrganoPlate®. | Bircsak KM et al. | PubMed | 4 |
| Enhanced predictive capacity using dual-parameter chip model that simulates physiological skin irritation. | Jeon B et al. | PubMed | 4 |
| Repeated dose multi-drug testing using a microfluidic chip-based coculture of human liver and kidney proximal tubules equivalents. | Lin N et al. | PubMed | 4 |

| Title | Authors | Source | Score |
|---|---|---|---|
| 2367 Assessing Machine-Learning Methods in the Identification and Quantification of Environmental Chemical-Key Event Pairs Associated with Adverse Health Outcomes | Mortensen HM | SoT 2021 | 4 |
| 2129 Development of a 3D In Vitro Screening Model to Monitor Kidney Proximal Tubule Toxicity | Dorau M et al | SoT 2021 | 4 |
| 2798 Estimating EC3 (Effective Concentration for a Stimulation Index of Three) Confidence Bounds and Uncertainties for Skin Sensitization Based on Structure Similarity and Assay Profiles | Rathman J et al. | SoT 2021 | 4 |
| 3321 Bayesian Benchmark Dose Estimation with BBMD: Inference from Genomic Data | Ji C et al | SoT 2022 | 4 |
| 3991 AI/ML Models to Predict DILI Severity Using Chemical Properties and Predicted Off-Target Interactions | Rao M S et al. | SoT 2022 | 4 |
| 3992 High-Throughput High Content Imaging of Environmental Toxicants Reveals Novel Morphometric Phenotypes | Cemalovic N et al. | SoT 2022 | 4 |
| 4020 Predicting Molecular Initiating Events from High-Throughput Transcriptomic Screening Using Machine Learning | Bundy J L et al. | SoT 2022 | 4 |
| 4025 Refining Reference Chemicals and Signatures of Activity Using High-Throughput Transcriptomics for Advancing Predictive Toxicology | Taylor LW et al. | SoT 2022 | 4 |
| 4444 Assessing Drug-Induced Liver Injury Using a Sensitive and Selective Human Liver Microphysiological System and Clinical Biomarkers | Novac O et al. | SoT 2022 | 4 |
| 644 STopTox: An in-silico alternative to animal testing for acute systemic and topical toxicity | Muratov E et al. | WC11 | 4 |
| 292 Modeling blood-brain barrier permeation in the autologous stem cell-derived Chip4 | Koenig L et al. | WC11 | 4 |
| 275 Evaluation of a new approach methodology toolbox for the next generation risk assessment of systemic toxicity | Cable S et al. | WC11 | 4 |
| 604 High throughput transcriptomics to derive mode-of-action and potency information to support read-across approaches | van de Water B | WC11 | 4 |

| Title | Authors | Source | Score |
|---|---|---|---|
| 948 A standardized platform for miniaturized cortical organ | van der Kroeg M et al. | WC11 | 4 |
| 728 A 3D-printed microplate insert for high-throughput and ultra-long term high resolution imaging of live human brain organoids: A new platform to replace animal models in brain cancer research | Oksdath Mansilla M et al. | WC11 | 4 |
| 684 Exploring 3D bioprinting technology for the development of complex reconstructed skin model with hair follicle structure and automation of the fabrication of hair follicle spheroids | Motter Catarino C et al. | WC11 | 4 |
| 555 Defining the reproducibility and applicability domain of devTOX quickPredict, a human pluripotent stem cell-based developmental toxicity assay | Palmer J A et al. | WC11 | 4 |
| 660 Better than Matrigel? Alternative cell culture coatings for induced pluripotent stem cell culture and renal podocyte differentiation | Murphy C et al. | WC11 | 4 |
| 775 Subacute 28-day respiratory toxicity assay using an in vitro human airway model | Markus J et al. | WC11 | 4 |
| 1004 OPERA, an open-source and open-data suite of QSAR models | Mansouri K et al. | WC11 | 4 |
| S24-02 In silico approaches to link adverse outcomes to molecular initiating events through AOPs | Oliveira AA et al. | EUROTOX 2021 | 4 |
| A semi-automated workflow for adverse outcome pathway hypothesis generation: The use case of non-genotoxic induced hepatocellular carcinoma. | Doktorova TY et al. | PubMed | 3 |
| Improving QSAR Modeling for Predictive Toxicology using Publicly Aggregated Semantic Graph Data and Graph Neural Networks. | Romano JD et al. | PubMed | 3 |
| The unreliability of the reliability criteria in the estimation of QSAR for skin sensitivity: A pun or a reliable law? | Toropov AA & Toropova AP | PubMed | 3 |
| In Silico Assessment of Acute Oral Toxicity for Mixtures. | Chushak Y et al. | PubMed | 3 |
| Discriminant models on mitochondrial toxicity improved by consensus modeling and resolving imbalance in training. | Tang W et al. | PubMed | 3 |

| Title | Authors | Source | Score |
|---|---|---|---|
| Microfluidic organ-on-chip system for multi-analyte monitoring of metabolites in 3D cell cultures. | Dornhof J et al. | PubMed | 3 |
| Condensed ECM-based nanofilms on highly permeable PET membranes for robust cell-to-cell communications with improved optical clarity. | Choi B et al. | PubMed | 3 |
| Rapid Prototyping of Multilayer Microphysiological Systems. | Hosic S et al. | PubMed | 3 |
| Integration of Electrospun Membranes into Low-Absorption Thermoplastic Organ-on-Chip. | Chuchuy J et al. | PubMed | 3 |
| Multiphoton-Guided Creation of Complex Organ-Specific Microvasculature. | Rayner SG et al. | PubMed | 3 |
| Microfluidic skin chip with vasculature for recapitulating the immune response of the skin tissue. | Kwak BS et al. | PubMed | 3 |
| Evaluation of the utility of the Beta Human Liver Emulation System (BHLES) for CFSAN's regulatory toxicology program | Eckstrum K et al. | PubMed | 3 |
| Simulating drug concentrations in PDMS microfluidic organ chips. | Grant J et al. | PubMed | 3 |
| Human Blood Vessel Organoids Penetrate Human Cerebral Organoids and Form a Vessel-Like System. | Ahn Y et al. | PubMed | 3 |
| High-throughput organ-on-chip platform with integrated programmable fluid flow and real-time sensing for complex tissue models in drug development workflows. | Azizgolshani H et al. | PubMed | 3 |
| Second-generation lung-on-a-chip with an array of stretchable alveoli made with a biological membrane. | Zamprogno P et al. | PubMed | 3 |
| IFlowPlate-A Customized 384-Well Plate for the Culture of Perfusable Vascularized Colon Organoids. | Rajasekar S et al. | PubMed | 3 |
| Engineering neurovascular organoids with 3D printed microfluidic chips | Salmon I et al. | PubMed | 3 |
| 3D Microvascularized Tissue Models by Laser-Based Cavitation Molding of Collagen | Enrico A et al. | PubMed | 3 |

| Title | Authors | Source | Score |
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| 2962 Mass Balance Model for Simulation of In Vitro Dynamic Chemical Distribution with Repeat Dosing | Bloch S et al. | SoT 2021 | 3 |
| 4031 Assessment of the Dermal Sensitization Potency of Extractables and Leachables Using Existing Data and In Silico Methods | Chilton ML et al. | SoT 2022 | 3 |
| 4663 Identification of Chemicals Associated with Retinoid Signaling Pathway Disturbance and Skeletal Dysmorphogenesis via New Approach Methods' Model and Adverse Outcome Pathway Development | Pierro JD et al. | SoT 2022 | 3 |
| 46 IATA as an opportunity for next generation risk assessment | Ouedraogo G | WC11 | 3 |
| 324 Optimization of an in vitro placental transfer assay for screening purposes | Gomes C et al. | WC11 | 3 |
| 283 Semantic modelling of adverse outcome pathways and the implementation in reproducible workflows | Martens M et al. | WC11 | 3 |
| 931 A 3D autologous iPSC-derived hair bulb model | El Baraka O et al. | WC11 | 3 |
| 291 A digital tool based on transcriptomic data for the integration of biological fingerprint analogies in the read-across approach | Riu A et al. | WC11 | 3 |
| 497 Fully human skin-on-a-chip with a modular architecture and integrated sensors for drug screening and disease modelling | Zoio C et al. | WC11 | 3 |
| 511 Chemically selective and label-free characterization of pancreas organoids inside hydrogel matrices | Jung N et al. | WC11 | 3 |
| 1111 Comparative toxicological analysis of an iPSC derived airway epithelium model with a primary bronchial airway epithelium model at an air liquid interface using TempoSeq | Djidrovski I & Armstrong L | WC11 | 3 |
| S02-03 Human 3D brain model to study developmental neurotoxicity | Pamies D et al. | EUROTOX 2021 | 3 |
| S03-02 Use of chemical informatics, quantum chemistry modelling and artificial intelligence algorithms to predict molecular initiating events | Allen TE et al. | EUROTOX 2021 | 3 |

| Title | Authors | Source | Score |
|---|---|---|---|
| S06-02 Creation of functional pluripotent stem cell-derived hepatocyte-like cell and more complex liver models | Verfaillie C et al. | EUROTOX 2021 | 3 |
| P06-26 Predicting drug-induced liver injury in vitro by combining human 3D liver models and organ-on-chip technology | Cox B et al. | EUROTOX 2021 | 3 |
| A novel organ-chip system emulates three-dimensional architecture of the human epithelia and the mechanical forces acting on it. | Varone A et al. | PubMed | 2 |
| 853 An integrated approach to testing and assessment for evaluating inhalation risk | Wolf D | WC11 | 2 |

# 6. Conclusions

This review of nearly 3,200 research projects described either in the 2021 and 2022 SoT conferences, the 2021 EUROTOX conference proceedings, and the WC11, or in the published literature, yielded 17 abstracts describing projects by investigators whom we believe should be nominees for the 2022 Lush Science Prize. These abstracts are presented in Section 5.

This is the first time that a full two-year cycle of research has been reviewed for consideration for the Lush Science Prize, and we expected a significant increase in the number of relevant papers. This has proved to be the case, despite the effects of COVID-19 lockdowns around the world. While we have found more relevant papers for the current prize cycle than last time (nearly 3,200 in 2022 (2 years) compared with around 1,700 in 2020 (18 months)), this is still many fewer than in the one year cycle of 2018 (4,100). Nevertheless, the number of scoring abstracts continues to rise (129 in 2022 vs 99 in 2020). The modified scoring system, which aims to take into account the practical readiness of reported work, continues to create a wider spread of scores, so that now only 13% of abstracts were considered high scoring (17 abstracts). The interdisciplinary and collaborative nature of the research is frequently well-illustrated by the multi-authorship of much of the work.

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 2022 Science Prize.