

Lush Science Prize 2012

Research Paper

Executive Summary

Complexity of the area under review

The Lush Science Prize is designed to reward 'outstanding contributions' to 21st Century Toxicology Research. Brief literature searches revealed that 21st century toxicology is a concept or vision which requires the participation of many different scientific disciplines working to solve a variety of complex problems. Scientific journals revealed that there are thousands of scientists, in hundreds of institutions, reporting on potentially promising research results which touch on this area each year.

The need for a focus or filter

The complexity of the subject matter means that, in order for this research paper to have a rational set of criteria to identify potential winners, it needs to focus in on a specific area of research. Because of Lush Prize's wider goal, to encourage a breakthrough event around a 'proof of concept toxicity pathway study', the focus chosen for this report was on researchers 'identifying key pathways whose perturbations result in toxicity'.

Applying the filter to conference abstracts

In the period under review (2011-12), two major international conferences on alternatives to animal testing have taken place: the 8th World Congress on Alternatives and Animal Use in the Life Sciences (WC8 at Montreal); and the European Society for Alternatives to Animal Testing (EUSAAT 14 at Linz). For the purposes of this report a review of the 381 abstracts submitted to these conferences was chosen as the only practical way of getting to a short list of a manageable size in a highly complex area. 51 'apparently pathway based' studies were identified of which 19 were reporting new pathway insights.

Three research teams for the Prize short list

This report recommends that the top three scoring teams from this list should be added to the open public nominations for the Science Prize.. They were:

- B Zimmer (Doerenkamp-Zbinden Chair of In Vitro Toxicology and Biomedicine), University of Konstanz, Germany and the international team involved in Human Neural Crest Cell toxicity pathway research.
- M Lindstedt and the team at Lund University, Sweden working on biomarker analysis for skin sensitivities.
- M Whelan and the team at the Systems Toxicology Unit at the EU's Joint Research Centre Institute for Health and Consumer Protection at Ispra in Italy for work on toxicity pathways in hepatotoxicology and developmental toxicology.

It also recommends that the toxicologists on the judging panel should be invited to each nominate one further team for the 2012 Science Prize if they choose.

1. Background

In June 2012 the Lush Prize commissioned five research papers, each focusing on one element of the annual prize: Science, Lobbying, Training, Public Awareness and Young Researcher.

The purpose of each research paper was to provide the Prize Team with:

- an overview of current trends and ideas in each area
- information on who is active in each area
- information on potential prize-winners to feed into the nominations process
- general recommendations for the design and communication of the prize

This paper looks at the Science Prize for 'outstanding contributions' to 21st Century Toxicology Research. The Lush Prize website in August 2012 defined this area as follows: "21st Century Toxicology is a new approach to alternatives testing research which is exciting regulators, campaigners and companies around the world. It has become possible because of advances in biology, genetics, computer science and robotics. There are two important elements.

One is that it focuses on 'toxicity pathways'. The other is that it seeks to build up a map of all known substances and their effects on the human organism.

The vision is that, when new substances become available their molecular similarity to other substances on the map, and the pathways they take, will enable computer simulations to give greater predictive accuracy of toxicity than any animal model. They will also be able to perform such tests very quickly. Once this map is built and working, it is understood that all animal testing is likely to 'fall away'.

We are seeking nominations from specialist institutions working in the sector, though an outstanding project producing a more general workable alternative toxicity testing method where none existed before may also be considered. In addition organisations are invited to put themselves forward."

The Prize website also explains how the awards should be for projects "running in the year preceding the prize award, or in the year of the award itself. For the 2012 Prize, this means projects running in 2011 or 2012. We will accept nominations from anywhere in the world, for projects which have taken place anywhere in the world."

It should also be noted that the Lush Prize also has a 'breakthrough element' - where in any one year the full £250,000 will go to an organisation providing a 'proof of concept toxicology pathway study'.

2. Methodology

The research began with a Brief Literature Review, looking at key texts in the area of 21st Century Toxicology. The second stage involved compiling a working list of Key Institutions driving the idea forward and identifying resources and projects they may have. The next stage was to look at broader Scientific Journals in the area to see how this resource could inform the Prize decision making. The fourth stage was to look at Major International

Conferences - namely the 2011 World Council for Alternatives (WC8) and the 2012 EUSAAT Congress to see whether they could help identify particular projects.

The fifth stage involved Exploring Strategic Decisions in order to find a mechanism or core idea which would help condense the list of possible winners into a manageable size. The next stage involved applying that core – toxicity pathway – idea as a filter to the very many possible science teams working in the area.

Finally a series of Conclusions and Recommendations were made in order to provide the judging process with a short list of possible nominations from the Lush Prize team. An afterword on ethical issues requiring further guidance finishes the report.

3. Brief Literature Review

3.1 Toxicity Testing in the 21st Century: A vision and strategy, 2007, National Academy of Sciences/National Research Council (NAS/NRC), USA

The founding document of the 21st Century Toxicology idea was this seminal 2007 report. It is more than 200 pages long and freely available on the internet. In it, the Committee on Toxicity Testing and Assessment of Environmental Agents envisioned "a new toxicity testing system that evaluates biologically significant perturbations in key toxicity pathways by using new methods in computational biology and a comprehensive array of *in vitro* tests based on human biology."

Its vision had five key components

1. Chemical characterisation ('a variety of computational methods')
2. Toxicity testing
3. Dose-response and extrapolation modelling
4. Population-based and human exposure data
5. Risk context

Progress, it said, would be likely to occur in four phases:

- "Phase I would focus on elucidating toxicity pathways; developing a data-storage, -access, and -management system; developing standard protocols for research methods and reporting; and planning a strategy for human surveillance and biomonitoring to support the toxicity-pathway testing approach.
- Phase II would involve development and validation of toxicity-pathway assays and identification of markers of exposure, effect, and susceptibility for use in surveillance and biomonitoring of human populations.
- Phase III would evaluate assays by running them in parallel with traditional toxicity tests, on chemicals with large datasets, and on chemicals that would not otherwise be tested as a screening process.
- Finally, the validated assays would be assembled into panels in Phase IV for use in place of identified traditional toxicity tests."

We are still at Phase I, and therefore any outstanding activities in this phase could be potential prize winners.

It also produced two lists of "Key Questions to Address in Implementation" which could be useful to further define areas of work worthy of a Prize.

1 Knowledge Development Questions

Toxicity-Pathway Identification—What are the key pathways whose perturbations result in toxicity?

Multiple Pathways—What alteration in response can be expected from simultaneous perturbations of multiple toxicity pathways?

Adversity—What adverse effects are linked to specific toxicity-pathway perturbations? What patterns and magnitudes of perturbations are predictive of adverse health outcomes?

Life Stages—How can the perturbations of toxicity pathways associated with developmental timing or ageing be best captured to enable the advancement of high-throughput assays?

Effects of Exposure Duration—How are biologic responses affected by exposures of different duration?

Low-Dose Response—What is the effect on a toxicity pathway of adding small amounts of toxicants in light of pre-existing endogenous and exogenous human exposures?

Human Variability—How do people differ in their expression of toxicity-pathway constituents and in their predisposition to disease and impairment?

2 Method Development Questions

Methods to Predict Metabolism—How can adequate testing for metabolites in the high-throughput assays be ensured?

Chemical-Characterization Tools—What computational tools can best predict chemical properties, metabolites, xenobiotic cellular and molecular interactions, and biologic activity?

Assays to Uncover Cell Circuitry—What methods will best facilitate the discovery of the circuitry associated with toxicity pathways?

Assays for Large-Scale Application—Which assays best capture the elucidated pathways and best reflect in vivo conditions? What designs will ensure adequate testing of volatile compounds?

Suite of Assays—What mix of pathway-based high- and medium-throughput assays and targeted tests will provide adequate coverage? What targeted tests should be developed to complement the toxicity-pathway assays? What are the appropriate positive and negative controls that should be used to validate the assay suite?

Human-Surveillance Strategy—What surveillance is needed to interpret the results of pathway tests in light of variable human susceptibility and background exposures?

Mathematical Models for Data Interpretation and Extrapolation—What procedures should be used to evaluate whether humans are at risk from environmental exposures?

Test-Strategy Uncertainty—How can the overall uncertainty in the testing strategy be best evaluated?

The report also points to institutional development as an important factor. "A critical factor for success is the conduct of the transformative research to establish the scientific basis of new toxicity-testing tools and to understand the implications of test results and the application in risk assessments used in decision-making. The committee concludes that an appropriate institutional structure that fosters multidisciplinary intramural and extramural research is needed to achieve the vision."

It is apparent at this early stage that, although the vision itself is simple enough to

communicate, its implementation involves considerable complexity involving a wide range of scientific disciplines.

3.2 Current Standing and Future Prospects for the Technologies Proposed to Transform Toxicity Testing in the 21st Century 2010

Erwin van Vliet. Johns Hopkins University, Bloomberg School of Public Health, Center for Alternatives to Animal testing (CAAT) From ALTEX Vol 28, No.1

This article, three years later, provides an excellent overview of problems and opportunities on the way to the 21st century toxicology vision. It also provides a useful overview for lay readers and is freely available in full on the internet. The author breaks the potential new toxicity testing technologies down into the following types.

1. Promising *in vitro* models

- Primary monolayer and three-dimension cell cultures
- Primary cell models reproducing the liver
- Primary cell models reproducing the central nervous system
- Primary cell models reproducing the kidney
- Primary cell models reproducing the skin
- Human stem cell models
 - Human embryonic and adult stem cells
 - Induced pluripotent stem cells
 - Stem cell applications for hepatotoxicity testing
 - Stem cell applications for cardiotoxicity testing
 - Stem cell applications for developmental toxicity testing

2. Non mammalian model organisms

- Nematode
- Zebra fish
- Fruit fly

3. High Throughput Toxicity Testing

- The EPA ToxCast program

4. Imaging Technologies

- High content imaging technologies

5. Omics technologies

- Genomics
- Toxicogenomics
- Proteomics
- Metabolomics

6. The Systems Biology Approach

7. Testing Strategies

He also lists between five and ten "developmental requirements" for each technology type. They are too detailed to be included here, but offer a potentially useful third-party filter to the question "does this project contribute to 21st century toxicology development."

3.3 AXLR8: Alternative Testing Strategies: Progress Report 2011 & AXLR8-2 Workshop Report on a 'Roadmap to Innovative Toxicity Testing' Berlin: AXLR8 Consortium, 2011

AXLR8 is a leading player in European moves towards 21st century toxicology (see also 4.3 below). Its annual report provides a regular update on developments in Europe. The 2011 report provides many useful insights into the 21st Century Toxicology concept including the following perspective offered by the Hamner Institute:

"The NRC report discusses approaches for deliberate implementation over the coming 10-20 year period with emphasis on filling out a list of toxicity pathways as quickly as possible. The Hamner Institutes of Health Sciences Programme in Chemical Safety Sciences has suggested an alternative approach to accelerate implementation. The Hamner proposes to work on a series of 10 or so prototypes to evaluate the steps required to use toxicity pathway assay results for human health risk assessment.

These steps include:

- (1) Mapping and modelling the cellular circuitry controlling each of the toxicity pathways
- (2) Developing appropriate, well-designed pathway assays
- (3) Examining relationships between pathway perturbations and adverse responses
- (4) Providing modelling tools to interpret the relationship of test-tube concentrations with those expected in people exposed to environmental levels of test compounds, and
- (5) Integrating results from these various studies to provide human health risk assessments."

In a report on Unilever's work on skin sensitisation in the 2011 AXLR8 Report, Gavin Maxwell explained how the immunobiology of skin sensitisation has been investigated for many years and, as a consequence, the toxicity pathways involved in this specific area are relatively well understood.

"Several non-animal test methods have been developed to characterise these key toxicity pathways. Reassuringly, not one but several key pathways were found to drive the sensitiser-specific T cell response, and these pathways could be broadly categorised into the following groups:

- skin bioavailability;
- protein reactivity;
- skin inflammation;
- DC maturation (including DC activation in skin and DC migration to dLN);
- T cell proliferation."

3.4 Thomas Hartung on 21st Century Toxicology

Thomas Hartung is a key thinker and writer on 21st Century Toxicology. He is Doerenkamp-Zbinden Chair for Evidence-based Toxicology and Director of the Center for Alternatives to Animal Testing, Department of Environmental Health Sciences Johns Hopkins Bloomberg School of Public Health (see also 4.4 below).

In 'Toxicology for the Twenty-first Century' (*Nature*, July 2009) he explains how: "The opportunity to create a new regulatory toxicology lies in a programme, similar to the Human Genome Project, that analyses the interaction of small molecules with cells. Such a programme will provide the molecular biological tools to switch cellular pathways of toxicity, knowledge that is needed for a new way of approaching toxicology."(p212)

In "A toxicology for the 21st Century - mapping the road ahead. 2009 Tox. Sci. 109(1)" he agrees with the NAS/NRC report above that: "The sheer dimensions of the tasks ahead will require a trans-disciplinary, trans-national, trans-stakeholder, and trans-industrial sectors approach. Information hubs such as AltWeb (<http://altweb.jhsph.edu/>), AltTox (<http://www.alttox.org/>), EPAA (<http://ec.europa.eu/enterprise/epaa/>), EBTox (<http://www.ebtox.org/>), ECVAM (<http://ecvam.jrc.it/>), and the Center for Alternatives to Animal Testing (<http://caat.jhsph.edu/>) have a key role here."

4. Key Institutions

There are thousands of institutions whose work touches on - or includes work on elements within - 21st century toxicology. These will include:

- Research centres performing research on or within any one of the 22 technologies identified by Erwin van Vliet in 3.2 above;
- national centres for the promotion of 3RS (reduction, refinement and replacement of animal testing);
- organisations involved in the validation of alternative tests such as the OECD, ECVAM, and ICCVAM;
- organisations focused on replacing animal testing in pharmaceutical research such as the UK NGOs FRAME and the Dr Hadwen Trust.
- commercial companies involved in toxicity testing, many of whom are involved in collaborative programs with others on this list.

The breadth and complexity of this field, interpreted at its widest, is a recurring issue in this Report which is touched upon in more detail in Strategic Issues X.X below.

There are however six inter-related institutions or programs which currently stand out as driving forward the 21st century toxicology vision as an abstract idea, and these are outlined in more detail below. To some extent this should be viewed as a working list and we welcome suggestions as to other organisations which may be added to refinements of this research in this and future years.

4.1 Tox21 Program

"The Toxicology in the 21st Century (Tox21) program is an on-going collaborative effort among four U.S. Government agencies: the National Toxicology Program (NTP) of the National Institute of Environmental Health Sciences, the Environmental Protection Agency (EPA), and Food and Drug Administration (FDA), and the National Institutes of Health Chemical Genomics Center (NCGC). Tox21 is developing and deploying a wide range of high-throughput *in vitro* biological testing and computational technologies to identify the activities and mechanisms of action of thousands of chemicals, with the goal of providing a science- and data-driven basis for *in vivo* chemical testing prioritization and risk assessment. After a pilot phase that began in 2005 and Tox21 Phase I that began in 2008, the Tox21 program entered Phase II in March 2011 with the completion of a testing library of 11,000 environmental and pharmaceutical chemicals, a dedicated robotics system capable of testing the entire Tox21 library in triplicate 15-concentration quantitative high-throughput screening (qHTS) format across a different *in vitro* assay every week, informatics databases and algorithms to analyse, visualise, and model the data, and targeted testing paradigms to examine the predictive and *in vivo* relevance of the models created. Tox21 also has a robust technology development component focused on the enumeration of all potential toxicity pathways, incorporation of metabolism and cell-cell interactions into *in vitro* assays, and the incorporation of exposure information into the models developed."¹

4.2 The US Environmental Protection Agency's Toxcast program

Toxcast is a project managed by one of the partners within the wider Tox21 program.

"ToxCast addresses many of the issues raised in the NRC report. It is a multi-year, multi-million dollar effort to comprehensively apply batteries of *in vitro* tests against chemicals with known toxicological phenotypes derived from traditional guideline studies for cancer, reproductive impairment and developmental disorders...With a commitment to transparency and public release of all data (see epa.gov/actor for access), it is the most strategic and coordinated public sector effort to transform toxicology."²

Further data from its e1k and Tox21 libraries were released to the public in March 2012.

The EPA is also working on DSSTox - a public forum for publishing chemical structure and toxicity data.

www.epa.gov/ncct/toxcast/

4.3 CAAT: Johns Hopkins University Center for Alternatives to Animal Testing

CAAT is a non-profit organisation based in Baltimore promoting humane science by supporting the creation, development, validation, and use of alternatives to animals in research, product safety testing and education. It has a European base at the University of Konstanz in Germany. It is perhaps the key NGO in the alternatives to animal testing movement.

It organises a number of key information resources which have been invaluable for this

¹Tox21 Special Session at the 8th World Congress (P) C. P. Austin 2011

²Alternative Testing Strategies: Progress Report 2011 & AXLR8-2 Workshop Report on a 'Roadmap to Innovative Toxicity Testing' Berlin: AXLR8 Consortium, 2011 p233

research including altweb: The Global Clearinghouse for Information on Alternatives to Animal Testing

On September 2011 it was announced that CAAT had received a \$6m grant from the National Institutes for Health for a consortium to develop a new technological methodology for mapping the molecular pathways of toxicity within cells.³

4.4 AXLR8

AXLR8 is a European Union funded project focused on "accelerating a transition in Europe toward a 'toxicity pathway'-based paradigm for chemical safety assessment through internationally co-ordinated research and technology development with the common goals of improved health and environmental protection."

It was established on the initiative of United Kingdom branch of Humane Society International (HSI-UK), the Freie Universität Berlin (FUB), and the Centre for Advanced R&D on Alternative Methods at the Flemish Institute for Technological Research (CARDAM-VITO).

A core publication is its annual progress report covered in 3.3 above.

4.5 HSI and the Human Toxicology Project Consortium

The Humane Society of the United States is the US's largest animal protection NGO working across a wide range of animal issues. It has long championed the cause of 21st Century Toxicology and was the founder of the Human Toxicology Project Consortium. The Consortium argues that the National Academy of Sciences' report's vision needs a project which is "well-funded, coordinated, and international—on the scale of the Human Genome Project of the 1990s. The HSUS and the Human Toxicology Project Consortium are calling upon the United States government and other governments, corporations, and stakeholders to commit to the Human Toxicology Project and back the project with \$100 million per year in public and corporate funding for the next decade."

HSUS and Proctor and Gamble have also set up the excellent www.alttox.org - a forum and information hub on non-animal methods for toxicity testing.

4.6 OECD Advisory Group on Molecular Screening and Toxicogenomics

This group is fast becoming an international focal point for work on elucidating and cataloguing 'adverse outcome pathways' and getting so-far-inactive member countries more engaged in this arena. "Regrettably the OECD's public website is horribly out-of-date and doesn't do justice to the dynamic work that's going on behind-the-scenes, but hopefully this will improve over time."⁴

³ invitrojobs.com news archive: CAAT has received grant from NIH for mapping of toxicity

⁴ Email from Troy Seidle at HSI

5. Scientific Journals

Reading the last two years of news feeds and forum posts on the websites of the Key Institutions above failed to reveal announcements of 'outstanding research' deserving of a prize in the field of 21st century toxicology. Quite rightly, peer-reviewed journals are the proper way for this kind of 'announcement' to be made.

Using the AXLR8 2011 Report as a starting point, a list of 31 most referred journals was made as follows.

| | | |
|--|--|--|
| Alternatives to Laboratory Animals ALTEX Arch Toxicol Chem Res Toxicol Crit Rev Toxicol Curr Protoc Cell Biol Environ Health Perspect Food Chem Toxicol Int J Pharm J Appl Toxicol. | J Clin Invest J Dermatol J Immunol J Invest Dermatol Skin Pharmacol Physiol Photochem Photobiol J. Mol. Cell. Biol. J Vis Exp Molec Cell Nat Rev Cancer | Regulat Toxicol Pharmacol Reprod Toxicol Science Stem Cell Res. Stem Cell Rev Rep. Toxicol Toxicol Appl Pharmacol. Toxicol In Vitro Toxicological Sciences (see also above) Xenobiotica |
|--|--|--|

ALTEX (Alternatives to Animal Experimentation) is a quarterly journal for new paths in biomedical science. It is "The Official organ of CAAT (Center for Alternatives to Animal Testing, Johns Hopkins University, Baltimore), EUSAAT (European Society for Alternatives to Animal Testing, Vienna), t4 (Transatlantic Think Tank of Toxicology, Baltimore, Konstanz, Utrecht). It is a quarterly journal with around four main articles in each issue. Reviewing this publication for the 2011/12 period was not difficult but it did not reveal any particularly outstanding contributions.

Toxicology In Vitro was the next journal explored. With 8 issues per year containing an average of 30 articles each, it would have required reading around 420 abstracts for the period under review to search this journal alone. Furthermore an exploration of the journal aggregating website sciencedirect.com revealed that although there were only 21 journals with 'toxicology' in the title, there were 1078 in the wider field of 'biochemistry, genetics and molecular biology'.

The resources available for this research were insufficient for exploring these journals properly. Although in future years some targeted approach around scientific journals may be possible, in 2012 another approach was necessary. This involved looking at the output from two international conferences.

6. Major International Conferences

6.1 8th World Congress on Alternatives and Animal Use in the Life Sciences (WC8)

The World Congresses on Alternatives and Animal Use in the Life Sciences are the longest running series of international scientific conferences dedicated to the replacement, reduction, and refinement (3Rs) of animal use in research and safety testing. Since 1993, these conferences have been held every 2-3 years, and have attracted a large international group of stakeholders and leading experts interested in scientific approaches to the development and use of methods that replace, reduce, and/or refine animal-based laboratory methods.

The 8th world conference was held in Montreal in August 2011. 275 abstracts, including both oral and poster presentations, were submitted to the conference and are published in full as a free pdf on the ALTEX website. Reading and evaluating these abstracts with a view to identifying potentially outstanding work was a central part of research for this paper and a breakdown and analysis of them occurs in part 7 below.

The 9th world congress is to take place in Prague 2014.

6.2 Abstracts of EUSAAT 2012 (14 Annual Congress of EUSAAT) and Linz 2012 (17th European Congress on Alternatives to Animal Testing).

The European Society for Alternatives to Animal Testing is involved in the dissemination and validation of alternative methods to animal testing and the promotion of research in the field of the 3Rs. It holds its congress every two years and its 14th congress was due to be held on September 5th to 8th 2012 in Linz - just after the deadline for this research paper.

Fortunately, submitted abstracts were published online on the EUSAAT website in August allowing them to be read and evaluated as part of this research. 111 abstracts were submitted and a breakdown and analysis of these also occurs in part 7 below.

7. Exploring Strategic Decisions

The goal of the Lush Science Prize is, as we have seen in 1 above, to reward an outstanding contribution to 21st century toxicology research. As we have seen in 3 above, 21st century toxicology is a concept or vision which requires the participation of many different scientific disciplines working to solve a variety of complex problems. A brief look at the scientific journals (and the conference abstracts) reveals that there are thousands of such scientists reporting on potentially promising research results each year.

How is the Lush Prize to identify whether a promising research outcome in say proteomics is any more 'outstanding' than a similarly promising outcome in stem cell developmental toxicology testing?

One solution is to use the expertise of the toxicologists on the judging panel and this is one suggestion made in the conclusions at 10 below.

A second option is for the prize to select a single area or question - perhaps from the NAS/NRC report such as 'how can adequate testing for metabolites in the high throughput assays be assured?'

As mentioned in 1 above, a central goal of the Prize is to focus attention on 'proof of concept toxicity pathway study' offering a high value breakthrough award in this area. To some degree the smaller annual awards are a holding exercise until such a breakthrough occurs. If the Prize were to make the annual science awards focused on an outstanding contribution to 'identifying key pathways whose perturbations result in toxicity' (see NAS/NRC Knowledge Development Questions at 3.1 above) then this means:

- (a) That it can keep the attention of all toxicologists - not just those in one discipline, and
- (b) That it can help communicate the breakthrough element of the prize by having a consistent 'story' focussing on pathways.

Given that some kind of screening process is necessary for this Research Report to get to an answer to the question 'what have been the outstanding 21st century toxicology contributions of 2011 and 2012', work around toxicity pathways was therefore chosen as the key question to identify a short list of potential science award winners from those submitting abstracts to the two major conferences in 6 above.

8. Short listing pathway-based research from the two major conferences

Although researching abstracts submitted to two conferences is no guarantee of having identified all potentially significant research in the field of 21st century toxicology, for the purposes of this report it was chosen as the only practical way of getting to a short list of a manageable size in a highly complex area.

The second issue is that the author of this report is a journalist and consultant and not a toxicologist. It is entirely possible that poor technical knowledge means that pathway-based studies have been missed in the technical language of the abstracts. As a back up system, the author has word-searched for 'pathway' across the two main conference documents.

The two tables below show the number of 'apparently pathway-based studies' appearing in the various subsections of each of the conference reports. The expression 'apparently pathway based' is used because of the technical skills gap mentioned above.

Appendix I of this report contains all 35 abstracts from WC8 selected by this method, and Appendix II all 16 abstracts from EUSAAT 14.

| World Congress on Alternatives 2011 | Number of abstracts | Apparently pathway based |
|---|---------------------|--------------------------|
| I-1 Potency and safety testing of human vaccines | 25 | |
| I-2 Addressing systems toxicology | 35 | 5 |
| I-3 Biological and biotechnology-based therapeutics | 12 | |
| I-4 Regulatory testing paradigms and validation of alternative test methods for detecting oestrogen active substances; impact on the Three Rs | 10 | |
| I-5 Nanotoxicology and the Three Rs | 5 | |
| I-6 Advances in alternative methods for ecotoxicology | 16 | 2 |
| I-7 Potency and safety testing of veterinary vaccines | 6 | |
| I-8 Safety testing for chemically-induced eye injuries: Recent Three Rs advances | 31 | |
| I-9 Advances in Three Rs alternatives for reproductive and developmental toxicity | 15 | 5 |
| I-10 Safety testing for carcinogenicity and genetic toxicity: Recent Three Rs advances | 30 | 5 |
| I-11 Safety testing for skin sensitisation hazards: Recent Three Rs advances | 65 | 8 |
| I-12 Epigenetics and its increasing relevance in toxicology and risk assessment | 4 | 1 |
| I-13 Toxicity testing in the 21 st century | 9 | 6 |
| I-15 Shellfish toxin testing: How are the Three Rs being progressed in this field? | 3 | 1 |
| I-16 Alternatives for potency testing of rabies vaccines | 3 | |
| I-18 Report on the ICCVAM International Workshop on Vaccines | 2 | |
| I-19 Toxicity testing strategies – progress in skin sensitisation testing: | 5 | 2 |
| TOTALS | 276 | 35 |

| EUSAAT 14 | Number of abstracts | Apparently pathway based |
|---|---------------------|--------------------------|
| LECTURES | 55 | 9 |
| FEATURED SESSIONS (POSTER PRESENTATIONS) | | |
| I: Progress in 3Rs Research: EU FP6 & FP7 Projects | 0 | |
| II: Chemicals — REACH and Animal Welfare | 6 | 1 |
| III: 7th Amendment of EU Cosmetics Directive | 3 | |
| IV: Directive 2010/63/EU and Other Legal and Ethical Topics | 1 | |
| V: 3Rs Progress in Other Sectors | 5 | |
| VI: Inhalation and Nanotoxicology | 7 | 1 |
| VII: 3R Goes 3D — Implementation of 3D Methods in Toxicity Testing | 6 | |
| VIII: Free Communications | 7 | |
| IX: 21st Century Non-animal Tools For Basic and Biomedical Research | 10 | 3 |
| X: Good Cell Culture Practice | 3 | |
| EUSAAT–ETS JOINT SYMPOSIUM | 8 | 2 |
| TOTALS | 111 | 16 |

8.1 Scoring these pathway-based studies

The 51 titles from this working list were still too many for a judging short list. In order to try to rationally shorten the list a simple scoring system was used.

| | |
|--|---------|
| Does it appear to be reporting a new pathway discovery? | Score 3 |
| If it is working with apparently previously understood pathway research, does it bring new knowledge or tools? | Score 2 |
| Does it stand out in any other way? | Score 1 |

Individual scores for each study appear in the Appendices.

WC8 - 14 Studies Scoring 3 or more

| | | |
|--|-----|-----------------------|
| Signature biomarker analysis for prediction of skin sensitizers using a cell-based in vitro alternative to animal experimentation I-11-137 | 3+1 | Sweden |
| BLTK1 murine Leydig tumor cells: a novel model for evaluating the steroidogenic effects of reproductive and developmental toxicants I-9-300 | 3 | Michigan |
| Effects of developmental toxicants on microRNA expression during neural differentiation of murine embryonic stem cells I-9-261 | 3 | Berlin |
| The developmental neurotoxicity of lead in rat primary aggregating brain cell cultures using transcriptomics and metabolomics approaches I-9-330 | 3 | JHU |
| Embryonic stem vs. embryonic carcinoma cells: an miRNA perspective on developmental toxicology I-9-627 | 3 | JRC Italy |
| Characterization of molecular events underlying induced morphologically transformed (MT) phenotypes in the Syrian Hamster Embryo (SHE-MT) assay I-10-322 | 3 | London Poss AT |
| Contact sensitizers modulate the arachidonic acid metabolism of PMA-differentiated U-937 monocytic cells activated by LPS I-11-230 | 3 | L'Oreal |
| A new perspective to evaluate sensitizing agents using microarray analyses I-11-276 | 3 | Brazil |
| Functionality and specificity of gene markers for skin sensitization in dendritic cells I-11-542 | 3 | Belgium |
| A test strategy to detect developmental toxicants that affect neural development using human embryonic stem cells I-12-589 | 3 | Konstanz |
| Taking a mode-of-action approach to designing a hepatotoxicity screening strategy using the HepaRG cell model and high content imaging I-13-685 | 3 | Italy (some JRC team) |
| Data integration and analysis approaches for toxicogenomics applications in the 3Rs: McDSA, ConXbase and ToxProfiler I-2-450 | 2+1 | Computational Neth. |
| Assessment of mitochondrial toxicity of environmental chemicals using a quantitative high-throughput screening approach I-6-478 | 2+1 | SARS TOX21 |
| Peptide reactivity assay using spectrophotometric analysis for screening of skin sensitizers I-11-259 | 2+1 | HTP Korea |

EUSAAT 5 Studies scoring 3 or more

| | | |
|---|-----|---------------------|
| Evaluation of Developmental Toxicants and Signalling Pathways in a Functional Test Based on the Migration of Human Neural Crest Cells | 3+1 | Konstanz |
| In Vitro Assessment of Developmental Neurotoxicity by Using 3D cultures: Species Can Make a Difference | 3 | Dusseldorf |
| Use of a Human Embryonic Stem Cell-based Test System For Neurodevelopmental Toxicity Testing and Epigenetic Profiling | 3 | Konstanz |
| A Three-dimensional hESC-derived System to Assess Chronic Nanotoxicity to the Developing Nervous System | 3 | Konstanz |
| Acetaminophen Changes the Bioavailability of Substances Across an Intestinal Barrier Model | 3 | Linz Pharmaceutical |
| Embryonic Stem Cell Approaches Towards Biomarkers of Embryotoxicity | 3 | Neth. |

It should be noted that this current focus on identifying toxicity pathways means that computational projects (which will only record existing pathways rather than discover new ones) will be unlikely to make the final list. A new focus in future years would be required to give them a chance.

9. Outstanding Research

Although the goal of this Research Paper was to find 5 potential award winners, with this scoring method two stood out. The abstracts are reproduced in full here.

Evaluation of Developmental Toxicants and Signalling Pathways in a Functional Test Based on the Migration of Human Neural Crest Cells (*P)

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Information on the potential for developmental neurotoxicity (DNT) is scarce for the majority of chemicals. To guarantee high consumer safety, more information is required. However, test capacities for further animal-based testing are limited and associated with ethical problems. Therefore, new animal-free approaches with higher throughput are required. A screening strategy using different assays, based on relevant human cell types combined in a test battery, has therefore been proposed by the EPA and other international key authorities. The feasibility of such test batteries has already been shown in the fields of genetic toxicology and skin sensitisation. Such a test battery does not currently exist for DNT. For this reason, many laboratories, including ours, are developing assays to model neurodevelopment *in vitro* for toxicity screening. Impaired neural crest (NC) function is one of the most prominent causes of the teratogenic effects of chemicals. It is estimated that one third of all congenital birth defects are associated with neural crest

cells and their derivatives. Testing of toxicant effects on the NC is highly desirable, and should form an element of a future DNT test battery. We aimed to develop a robust and widely applicable human-relevant NC function assay, allowing sensitive screening of different toxicants, and a definition of toxicity pathways, as well as evaluation of such an assay as part of a larger DNT *in vitro* test battery. We generated NC cells from human embryonic stem cells and, after establishing a migration assay for NC cells (MINC assay), we tested environmental toxicants, as well as inhibitors of physiological signal transduction pathways. Methylmercury (50nM), valproic acid (> 10 μ M) and lead acetate (1 μ M) affected the migration of NC cells more potently than the migration of other cell types, including central nervous system progenitor cells. The MINC assay correctly identified the neural crest toxicants, triadimefon and triadimenol (two pesticides). Additionally, it highlighted different sensitivities to various organic and inorganic mercury compounds. Negative control compounds, such as mannitol or acetaminophen, did not alter NC cell migration. By using classic pharmacologic inhibitors and by performing large-scale microarray gene expression profiling, we found several signalling pathways that are relevant for the migration of NC cells in the MINC assay, which could therefore be potential targets for different toxicants. In preliminary experiments, we found that chemicals specifically targeting those pathways indeed have adverse effects on NC cell migration in the MINC assay. The MINC assay faithfully models human NC cell migration, and reveals impairment of this function by developmental toxicants with good sensitivity and specificity. Additionally, the MINC assay can be used to identify important pathways of toxicity (PoT) in the area of NC cell migration. We therefore believe that our MINC assay could play an important role as part of a DNT *in vitro* test battery.

It should be noted that some members this team at University of Konstanz also scored highly in three other partnerships in the ranking tables above.

Signature biomarker analysis for prediction of skin sensitizers using a cell-based *in vitro* alternative to animal experimentation(P)

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Atopic contact dermatitis is a common inflammatory skin disease that affects a significant proportion of the population, and the incidence is increasing due to repeated exposure to sensitising chemicals. The REACH regulation requires that all new and existing chemicals within the EU should be tested for hazardous effects. As the identification of potential sensitizers currently requires animal testing, this will have a huge impact on the number of animals needed for testing. Further, the 7th Amendment to the Cosmetics Directive (76/768/EEC) imposed a ban on using animals for testing cosmetic ingredients for all human health-related effects by 2013. Thus, development of reliable *in vitro* alternatives to animal experimentation for the assessment of the sensitising capacity of chemicals is urgent. We have developed a cell-based assay, based on the monocytic cell line MUTZ-3, for the purpose of testing the propensity of new chemicals to cause sensitisation. We have stimulated the cell line with >40 skin sensitizers, irritants and controls for 24 h in optimal growing conditions (\geq 90% relative viability) and analysed the activity with genome-wide transcriptional profiling. By employing advanced computational statistics, we have identified biomarker signatures which distinguish sensitizers from controls with 90%

accuracy. Thus, we have identified a potent predictive biomarker signature for skin sensitisation and demonstrated that the mRNA microarray is a powerful assay in itself. Being based on a human biological system, the assay is considered to be more relevant and more accurate for predicting sensitisation in humans than the traditional animal-based tests. Further, the identified marker profiles are believed to describe biological pathways involved in sensitisation.

For a third potential award winner, the Systems Toxicology Unit at the EU's Joint Research Centre Institute for Health and Consumer Protection at Ispra in Italy was the only other group with more than one study scoring 3 in the WC8 list.

Taking a mode-of-action approach to designing a hepatotoxicity screening strategy using the HepaRG cell model and high content imaging (P*)

M. Mennecozzi, B. Landesman and M. Whelan Institute for Health and Consumer Protection, Ispra, Italy

The liver is central to the metabolism of xenobiotics and faced with harmful effects of toxic substances. Evaluating the risk of liver toxicity is a major issue and there is still no established *in vitro* screening strategy to reliably identify potentially hepatotoxic chemicals. In the approach described here, a mode-of-action targeted analysis of the literature has been used to identify toxicity pathways and the key biological events associated with them. This knowledge has then been used to design a multi-parametric HTS experiment to classify chemicals based on their likely association with a specific mode-of-action. We used a metabolically competent cellular model, HepaRG, and high content imaging implemented on a HTS platform. The HepaRG cell line expresses the major liver functions, including P450s, phase II enzymes, transporters and nuclear receptors at levels comparable to those found in primary hepatocytes. The high content screening approach we adopted is based on automatic analysis of image-sets acquired with an epifluorescent microscope for the quantification of immuno-fluorescently stained biomarkers expressed by treated HepaRG cells. A quantitative high throughput screening format was employed using a 96-well plate format, which facilitated the testing of a set of 92 reference chemicals and drugs with known hepatotoxic activity. Multiple cellular phenotypic changes were analysed by staining with fluorescent dyes for identification and quantification of response parameters. A biostatistical model was then developed to associate the test chemicals with different mode-of-action based categories. A systematic comparison of the classification results with literature findings allowed a preliminary validation of the approach.

10. Conclusions and recommendations

The complexity of the subject matter with 21st Century Toxicology Research means that, in order for the Science Prize to have a rational set of criteria to identify potential winners, it needs to focus in on a specific area of research. Because of Lush Prize's wider goal to encourage a breakthrough event around a 'proof of concept toxicity pathway study', it is recommended that the Science Prize focus should initially be on 'identifying key pathways whose perturbations result in toxicity'. This was, appropriately perhaps, the first 'Knowledge Development Question' listed in the ground-breaking 2007 report 'Toxicity Testing in the 21st Century'.

Applying this question as a filter to 381 research papers submitted to two major international conferences on alternatives to animal testing allowed the identification of three research teams as potential prize winners.

They were:

- B Zimmer (Doerenkamp-Zbinden Chair of In Vitro Toxicology and Biomedicine), University of Konstanz, Germany and the international team involved in Human Neural Crest Cell toxicity pathway research.
- M Lindstedt, and the team at Lund University, Sweden working on biomarker analysis for skin sensitivities.
- M Whelan and the team at the Systems Toxicology Unit at the EU's Joint Research Centre Institute for Health and Consumer Protection at Ispra in Italy for work on toxicity pathways in hepatotoxicology and developmental toxicology.

Subject to fitting in with the open public nominations for the Science Prize, these three should be added to the short list for the 2012 judging panel in October.

In addition, the report also recommends as follows:

1. Given the complexity of this field of research, and the lack of a methodology which ensures a complete analysis of all the available options, the toxicologists on the judging panel should be invited to each nominate one further team for the 2012 Science Prize if they choose.
2. Subject to consultation with the toxicologists on the judging panel, the 'identification of new toxicity pathways' should become part of the communication strategy for the 2013 Science Prize.

If there is disagreement among the judges about the quality of the Science Prize nominations in this or future years, there is always the opportunity to make the award to any of the key institutions appearing in 3 above. To some degree these institutions make ongoing 'outstanding contributions' to 21st century toxicology because they are choosing to help drive the changes. There maybe crossovers however with other Prize areas in this regard.