National Centre for the Replacement Refinement & Reduction of Animals in Research

Adverse Outcome Pathway News

For scientists, risk assessors and regulators with an interest in pathways-based approaches

Issue 2: February 2016

Upcoming NC3Rs workshop: NOW OPEN FOR REGISTRATION!

In 2014, we launched a programme to support the wide-scale development and application of pathways-based approaches. The aim of this programme is to improve the science of human and environmental safety assessment and ultimately reduce the reliance on animal toxicity testing. As part of this initiative, a two day workshop, *'Applying pathways-based approaches across the biosciences',* was held in May 2014. Since then there has been a substantial increase in the field's investment into developing pathways-based approaches, including a rapid rise in the number of adverse outcome pathways (AOPs) within the AOP-Wiki.

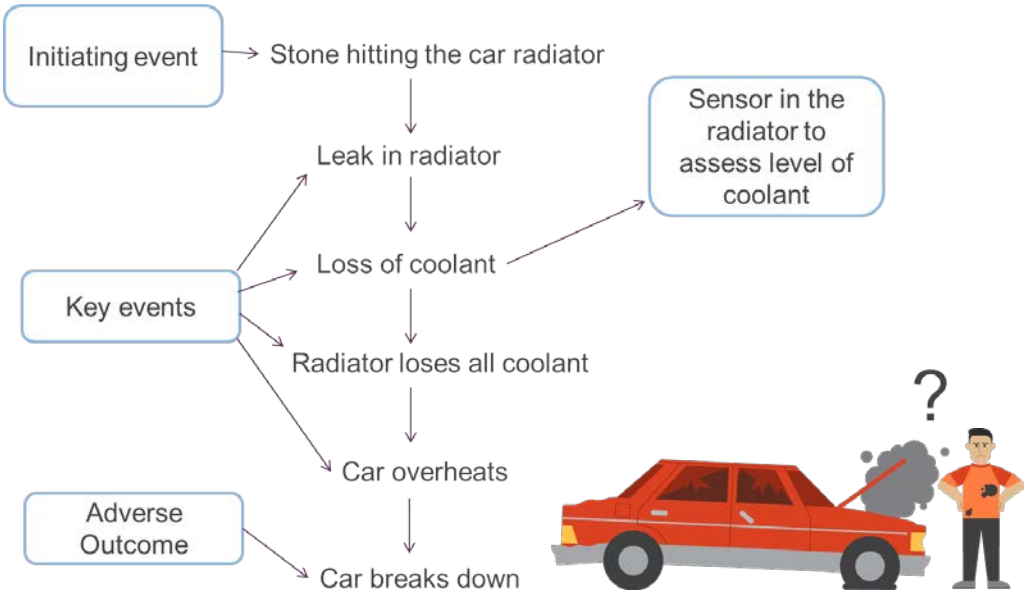
The knowledge gained so far now has potential to be translated into new approaches for safety assessment. To maximise this we are holding a follow-up workshop, '*Pathways-based approaches across the biosciences: Towards application in practice'* in central London on **28 April 2016**.

The agenda includes:

1) Case studies: practical applications of pathways-based approaches, including industry perspectives;
2) Adverse outcome pathways: perspectives for future development and application;
3) Next steps to enable wider application of pathways-based approaches;
4) An interactive expert panel discussion entitled '*The big conundrum – what constitutes validation*?'.

The workshop will be chaired by Professor Ian Kimber OBE (Professor of Toxicology, University of Manchester), with a keynote presentation from Dr Kevin Crofton (Deputy Director of the National Center for Computational Toxicology, US Environmental Protection Agency). Attendance is free, but registration is essential. To register please click here.

An analogy: how would an AOP look if we apply the concept to 'adverse events' that happen in our daily lives?



In this issue:

Links & resources:

- NC3Rs AOP resource page
- OECD's AOP framework
- AOP-Wiki
- Register for NC3Rs workshop

An industry perspective: pharmaceuticals

Maria Beaumont and James Louttit, GlaxoSmithKline

In many ways there are clear similarities between the AOP goals of the chemical industry and the pharmaceutical sector. Our 'pharma' vision is to define a multidisciplinary and mechanistically-based approach to modernise nonclinical safety assessment, reduce attrition in early discovery, improve translation into the clinic and positively impact the 3Rs. This will require new ways of working, and the use of an AOP framework to leverage existing data and/or harness the power of various data-generating platforms will underpin this approach.

The pharmaceutical industry has a wealth of precompetitive data that, if made more accessible, could be used in the development of AOPs to help answer one of the key questions: '*given an adverse event, what primary molecular site of interaction/pharmacology and/or drug molecules might initiate that event*?'. Consequently, the development of such AOPs will also enable us to answer the question '*given a drug molecule and/or a primary molecular site of interaction/pharmacology what potential adverse events could arise?'*. Thus, knowledge of AOPs could support the pharmaceutical industry in making go/no go decisions.

Last year, GSK started to embrace AOPs through a series of case studies that helped to 'socialise' the underlying concepts with colleagues working in safety assessment and drug metabolism. We used the generic AOP concept linking a molecular initiating event (MIE) with an adverse outcome and tasked breakout teams to assemble AOPs using previously published data on cardiac, renal and hepatotoxicity themes. Where possible, literature data was supplemented with existing in-house data (e.g. in early target review documents, nonclinical reports, investigator brochures, attrition data sets and screens) to show how AOPs could be developed in practice. Colleagues gained hands-on experience constructing mock AOPs that were relevant to pharma.

By taking data for a compound's activity at individual cardiac ion channels (MIE) for example, and using a mathematical simulation to predict how these activities will combine and cause key events (e.g. modification of cardiac action potential and repolarisation), we can identify compounds that are more or less likely to generate specific types of cardiac arrhythmias (adverse outcome). This approach forms part of the CiPA (comprehensive *in vitro* proarrhythmia assay) initiative, a recent FDA/Industry/Academic consortium which is developing a novel regulatory paradigm for assessment of pro-arrhythmic activity in medicines in development.

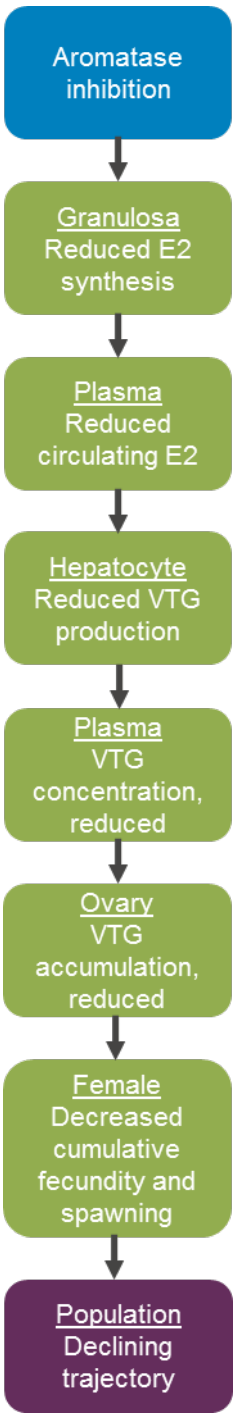
However, AOPs are not yet quantitative and we may be years away from a genuine systems toxicology vision. In the more immediate future, we see harmonisation of ontologies aligned to the rest of biology to drive universal standards for AOPs as a key next step. This will complement ongoing developments in target sciences and the Innovative Medicines Initiative eTOX project, and can build on current opportunities to employ pathways-based approaches for early screening purposes to ensure better candidate selection and therefore a reduction in the number of animals used for regulatory testing.

Highlights:

- *Whilst not formalised as AOPs the pharmaceutical industry have actually been using pathways-based approaches for some time.*
- *GSK can already envisage the use of AOPs by discovery-facing and/or investigative toxicologists who routinely consider primary mode of drug action on their targets.*
- *Safety pharmacology has been using the framework of MIE, key events and adverse outcomes for a number of years to aid in risk assessment of compounds for potential pro-arrhythmic actions of drugs under evaluation.*
- *Quantitative AOPs, using mathematical models to interrogate the underlying biological pathways, are the ultimate aspiration.*
- *Better selection of lead compounds will reduce the number of animal studies carried out for compounds that are destined to fail in the clinic.*
- *By using a multidisciplinary approach GSK can imagine a bright future where applying an AOP framework could get easier, with increased regulatory acceptance of new AOP-based approaches and reduced reliance on animals.*

AOP Spotlight : Aromatase inhibition leading to reproductive dysfunction in fish

We ask **Dr Dan Villeneuve** (US EPA), author of AOP 25, and end-user **Dr James Wheeler**, an ecotoxicologist at Dow AgroSciences, to give their perspectives on its development, potential impact on the 3Rs, and application in practice.



This AOP links the molecular initiating event, inhibition of cytochrome P450 aromatase (CYP19), through to an adverse effect on fish reproduction, which could potentially result in population decline. The AOP has been developed mainly using data from the fathead minnow (*Pimephales promelas*), a common model fish species used in ecotoxicology research and regulatory applications. However, knowledge of basic comparative reproductive endocrinology, including cross-species conservation of CYP19 structure and function, suggests that this AOP could be applicable to other oviparous vertebrates[1].

A number of environmental contaminants, including some pesticides and drugs, have been shown to inhibit the activity of CYP19. Exposure of reproductively-active female fish to aromatase inhibitors decreases the activity of ovarian CYP19, resulting in a cascade of downstream key events, such as a depression in plasma E2 concentrations, subsequent decreases in plasma vitellogenin, lowered deposition of vitellogenin into developing oocytes, and depressed egg production that can be translated, via modelling, into population declines.

This pathway will be of interest to both academic and industry scientists, as the information it provides could support the identification of chemicals with potential to adversely affect fish populations. This AOP can be found in the AOP-Wiki and has recently undergone expert external review.

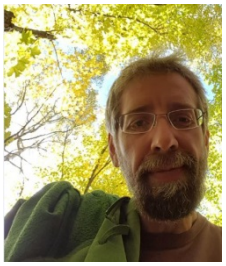
Dr Villeneuve describes:

- Why this AOP was developed;
- The approach taken to develop the AOP;
- The intended application of the AOP;
- Challenges along the way;
- How this AOP could support the 3Rs;
- His advice for researchers planning to develop AOPs.

Dr Wheeler gives his views on:

- Potential applications within agrochemical environmental safety assessment;
- How the information in the AOP can be used now, and the potential impacts on the 3Rs;
- Related AOPs that could be useful in this area of testing.

[1]Becker RA *et al.* (2015) Increasing Scientific Confidence in Adverse Outcome Pathways: Application of Tailored Bradford-Hill Considerations for Evaluating Weight of Evidence. *Regulatory Toxicology and Pharmacology* 72:514-537.



Interview with Dr Dan Villeneuve, research toxicologist

Why was this AOP initially developed?

The AOP linking aromatase inhibition to reproductive dysfunction in fish emerged from an integrated research program launched in 2005 (see Ankley *et al.* 2009) which was aimed at elucidating the diversity of mechanisms through which chemicals could directly act on components of the hypothalamic-pituitary-gonadal axis to perturb critical reproductive functions. The intent was to use that mechanistic understanding to develop alternative tests that could detect endocrine active chemicals in a more rapid, cost effective and specific manner than could be achieved through the fish short term reproduction assay (FSTRA) that was developed for the US Endocrine Disruptor Screening Program (EDSP). Due to its function as a rate-limiting enzyme involved in the synthesis of 17β-estradiol, a key reproductive hormone, aromatase inhibition was one of the first molecular initiating events (MIE) examined as part of this research effort.

How was this AOP developed?

The AOP was largely based on novel experimental data generated in our laboratory over the span of nearly 10 years. The study designs included measurement of endpoints at multiple levels of biological organization and a number of time-course experiments were employed to elucidate temporal concordance among the responses as well as to understand compensatory and feedback behaviors that occur within the axis and dictate complex dose-response time-course behaviors. The experimental data was supplemented with literature searches. More recently, through OECD-led review of the AOP, we have received additional feedback from experts in the field. We are in the process of incorporating additional supporting information based on those expert reviews.

How do you see the AOP being used?

The AOP was originally intended to support the development and use of alternative methods for screening chemicals for endocrine activity. As quantitative understanding of this AOP has evolved, we are moving toward potential applications to risk-based assessments. As part of a broader collaborative effort, we have developed a series of computational models that align with various key events along this AOP. We are now in the process of testing those model predictions to evaluate whether this AOP can be applied in a more quantitative manner.

Is the AOP ready to be used for this purpose?

Yes, in its current state of development, we feel the AOP provides a strong basis for using either *in silico* prediction or *in vitro* evidence of aromatase inhibition to identify a chemical as having potential to cause reproductive impairment in fish. Thus, there is immediate application for both hazard identification and hypothesis-driven testing.

What challenges did you encounter along the way?

This AOP was developed whilst the core principles of AOP development and OECD guidance on describing AOPs were being actively developed and revised. Consequently, there were changes and different versions along the way as the guidance and principles took shape. Additionally, conducting the weight of evidence evaluation can be quite challenging, since many studies in the extant literature were not designed with AOP development in mind. It is ideal to have studies that measured multiple key events in the same study and also tested multiple doses and collected data at multiple time points. Unfortunately, such studies are rare. We were fortunate, in this case, to have many of those types of data available, which made the weight-of-evidence evaluation a bit more manageable.

In many ways, this has been one of the most straight-forward AOP development projects we have been involved in. The basic biology (basis for biological plausibility) is quite well understood and supporting evidence, from our work and that of others, was available but not overwhelming. At present, we are most interested in challenging this AOP with new chemicals that have been identified as aromatase inhibitors to see how well these general patterns of response hold up. So far so good!

How could using the information from this AOP in practice support the 3Rs?

We think that use of this AOP supports the idea that *in vitro* methods for screening for aromatase inhibitors, such as the use of the H295R cell-bioassay or the two aromatase assays currently employed by ToxCast (Tox21_Aromatase_Inhibition; NVS_ADME_hCYP19A1), are viable alternatives to the 21 day FSTRA for identifying aromatase inhibitors. Consequently, we feel that application of this AOP could lead to a reduction in the number of animals employed for endocrine disruptor screening.

What advice would you give other researchers considering developing an AOP?

Four general pieces of advice I would give to researchers about to embark on developing an AOP are the following:

Get to know the AOP-KB: Before beginning an AOP development project, browse/search the adverse outcome pathway knowledge-base to see if similar AOPs, or AOPs that include similar key events have already been described. If someone has already started a similar AOP it may be more efficient to simply contribute to an existing effort. AOPs are designed to be modular. If key events or key event relationships relevant to your AOP have already been described, there is no need to reinvent them. Instead, it will be more efficient to expand and/or enhance them.

Keep it simple: You don't need to cram everything you know into a single AOP. Not every detail of the biology or every modulating factor that can affect the biology needs to be represented as a key event in the AOP. Simpler is often better. Try to focus on critical check-points that can be readily evaluated experimentally, rather than detailed mechanisms that can often only be verified through very elaborate and focused experimentation.

Make it fun: Developing an AOP can be a lot of work. It is much easier, and often more fun, if it is an area you are familiar with and have interest in. If you need to develop an AOP outside your primary area of expertise/interest, try to engage appropriate subject matter experts to help. Ideally, AOP development should be a collaborative exercise.

Design experiments with AOP development in mind: If you are conducting experiments intended to support AOP development, it is very helpful to be familiar with the types of weight of evidence considered when evaluating AOPs. The optimal ways to design experiments to establish dose-response concordance, temporal concordance, essentiality etc. among key events often require different experimental designs than are often applied in other types of toxicity testing and research.

Responses to these interview questions represent the personal views of Dan Villeneuve. They neither constitute, nor necessarily reflect, official US EPA policy.

Interview with Dr James Wheeler, regulatory ecotoxicologist

How could information from this AOP be currently used in your field?

This AOP is useful to inform on priority setting for the screening of substances for endocrine activity, and could be used for programmes such as the US EPA's Endocrine Disruptor Screening Program. The AOP links outcomes from fish screening assays typically employed to establish whether a chemical has the potential to interact with the endocrine system of fish (in this case the steroidogenic pathway), with an adverse outcome. It has utility to screen unknown substances for potential aromatase activity as part of endocrine activity screening. It will also assist in the interpretation of endocrine screening studies and the design of additional tests to further investigate any observed steroidogenic activity. Within a regulatory context, the applicability domain of this AOP is currently limited; the AOP lacks quantitative aspects and only covers a narrow range of a fish lifecycle. For example, the data underpinning the adverse effect (reproductive dysfunction) relies largely on experimental data from fish endocrine screening assays (e.g. OECD TG 229 or variants thereof), thus limiting the applicability domain to adult, sexually mature female fish. Different life stages may be more sensitive or exhibit different adverse effects and may therefore be of greater relevance to the overall environmental risk assessment. Indeed, exposure to some aromatase inhibitors at early life stages over the period of sexual differentiation can affect the sex ratio of fish. Consequently, this AOP may only be currently applicable for prioritisation of chemicals for further screening.

What would be needed to increase the utility of the AOP in your field?

To broaden the use of this AOP into a risk assessment context, the following would be needed:

- A network of AOPs to cover effects in other life stages and in male fish, as risk assessments are intended to protect all life stages.
- Development of AOPs that examine other non-aromatase modes of action. These could potentially contain the same key events, therefore it would be useful to include information to enable discrimination of aromatase active substances from those which affect the same key events through a non-endocrine mechanism (e.g. liver toxicity).
- Definition of the minimum data that is needed to make a decision based on this AOP. This will be dependent on the intended application, i.e. whether it is to be used for prioritisation and screening or direct regulatory use.
- An increase in quantitative understanding of *in vitro* to *in vivo* extrapolation to enable the extension to computational tools to model the key events. Although the development of more quantitative tools is happening (e.g. see Watanabe *et al.* 2016), there is still a gap for the acceptance of validated approaches. Furthermore, relatively sophisticated population models capable of integrating predictions of fecundity and other adverse effects (e.g. sex ratio skew) would be required for the translation to population relevant changes – the 'currency' of environmental risk assessment.

One of the key challenge areas will be establishing the confidence that regulatory actions derived from AOP application are sound and maintain high levels of environmental protection.

How could using the information from this AOP in practice support the 3Rs?

If used effectively the AOP could be used to prioritise *in vivo* testing for the screening of chemicals for potential endocrine activity, meaning that fewer *in vivo* tests and thus animals will be required. It can also be used as a framework to inform weight-of-evidence for suspected endocrine active substances. It may also be used to design 'intelligent testing strategies' to address specific questions rather than defaulting automatically to large, animal intensive higher tier tests.

Out and about!

NC3Rs Programme Manager Dr Fiona Sewell attended the final SEURAT-1 symposium

December last year marked the end of SEURAT-1 (Safety Evaluation Ultimately Replacing Animal Testing), a five-year research initiative introduced by the European Commission to work towards animal-free safety assessment.

SEURAT-1 consisted of six complementary research projects involving 70 partners from academia, industry and small and medium-sized enterprises (SMEs), co-funded by the European Union and Cosmetics Europe, each contributing €25 million. The projects aimed to replace *in vivo* repeated dose systemic toxicity tests that are currently used for the assessment of chemicals for human safety, through improved understanding of molecular mechanisms and the delivery of a set of alternative non-animal tools and technologies. It was facilitated by the coordination and support action, COACH, to ensure a fully integrated approach.

The projects have focused on liver toxicity and have developed a number of transferable technologies that are able to provide toxicological information for new and existing chemicals to inform risk assessment. However, there is still a way to go before these methods will be able to replace repeat-dose testing in animals. Future efforts need to focus on increasing confidence in the predictivity of these new methods, with the aim of regulatory acceptance.

Originally named SEURAT-1 to indicate that this was only the first step in reaching the final goal of replacing animal testing, there are no plans for a SEURAT-2. However, aspects of SEURAT-1's work will be continued in the recently launched EU-ToxRisk project.

Cosmetics Europe also plan to continue to advance the development of Alternatives to Animal Testing (AAT) through the Long Range Science Strategy (LRSS), a research consortium which aims to fund, direct and promote the successful development of AAT-based test methods and approaches for safety assessment and to facilitate regulatory acceptance.

Latest news

Interesting new findings from Tox21 data published

The Tox21 programme is a collaboration between the National Institute of Health (NIH), the US Environmental Protection Agency (EPA) and the US Food and Drug Administration (FDA). It aims to improve toxicity testing methods to enable faster and more efficient evaluation of chemical effects on human health. Researchers are testing a collection of 10,000 environmental chemicals and approved drugs for their potential to disrupt biological pathways that may result in toxicity. It utilises high-speed, automated screening using cells and isolated molecular targets instead of laboratory animals.

Recently published in *Nature Communications*, the first comprehensive analysis of the Tox21 effort examines results from a panel of 30 human cell-based assays focused on nuclear receptor signalling and stress response pathways. The results, along with knowledge of chemical structure, were used to identify chemical structure-activity signatures and inform the development of models that predict *in vivo* toxicity endpoints. The models based on data from *in vitro* assays were distinctly better at predicting human toxicity endpoints than animal toxicity. These findings have potential to help prioritise the chemicals that need to go through to compulsory animal tests and the data generated could be used to inform other pathways-based approaches.

So far the results are encouraging but more work is needed to enable the paradigm shift towards non-animal approaches for safety assessment of chemicals for human health. This includes building awareness with regulatory agencies as well as expanding the focus of the studies to include assays to cover additional pathways and targets that could be relevant for toxicity.

EU-ToxRisk project launched

In January, the 39 partners within the EU-ToxRisk consortium came together to officially launch the project. This large-scale, €30 million Horizon 2020-supported collaborative project brings together academia with small and medium-sized enterprises, large industry, contract research organisations and regulatory bodies. It aims to develop a mechanism-based, human relevant, non-animal approach to risk assessment that can replace current regulatory repeat-dose toxicity and reproductive and developmental toxicity tests. The NC3Rs is one of the project partners and will play a role in facilitating regulatory engagement. Look out for more information on EU-ToxRisk in the next edition of AOP News.

Events and training

Toxicogenomics and Systems Toxicology SafeSciMET Course

29 February - 4 March 2016 LEIDEN, THE NETHERLANDS

This course deals with established and novel methods in toxicogenomics and systems toxicology research and their application in drug safety by introducing and comparing the principles of the various genomics and bioinformatics tools used. Students will gain understanding of the design of such studies, their statistical evaluation and the biological interpretation of the data, including cross-platform consistency and extrapolations from *in vitro* to *in vivo* and from animal to man. Emphasis is given to development, validation and use of relevant biomarkers in toxicology and early phase clinical development. Regulatory aspects relevant for toxicogenomics data and drug registration within the context of risk assessment are also covered: www.safescimet.eu/courses/

Society of Toxicology Annual Meeting 2016

13-17 March 2016 NEW ORLEANS, USA

Continuing education course: 13 March
Adverse Outcome Pathway (AOP) Development and Evaluation

This training course will inform participants on the core principles of AOP development and assessment and the OECD efforts to support this effort. Guidance will be given on how to assemble and evaluate the evidence supporting the AOPs using established best practices. There will also be a live demonstration, where an AOP is developed from a training case study with their assistance and entered into the AOP-Wiki. The value of AOP development will be demonstrated via examples from the European Food Safety Agency and by considering integrated approaches to testing and assessment using the skin sensitisation AOP. www.toxicology.org/events/am/AM2016/ce.asp

Continuing education course: 15 March
Creating an Adverse Outcome Pathway in the AOP-Wiki course

This is a satellite session to the main meeting. Participants will gain experience entering an adverse outcome pathway into the AOP-Wiki. Dr Steven Edwards of the US EPA will provide a brief introduction into the AOP-Wiki, and course instructors will be available to provide guidance and answer questions as participants work through and present a case example. Please indicate your interest in attending by contacting Kristie Sullivan at ksullivan@pcrm.org.

British Toxicology Society Annual Congress 2016

3-6 April 2016 MANCHESTER, UK

Relevant sessions include a symposium on 'New Frontiers in Predictive Approaches to Safety Assessment' and a presentation in the '*In Vitro* Methods for Genotoxicity and Carcinogenicity' symposium on 'Adverse Outcome Pathways in genotoxicity and carcinogenicity' by Andrew Scott, Unilever: www.thebts.org/Meetings/BTSAnnualCongress2016.aspx

Pathways-based approaches across the biosciences: Towards application in practice

28 April 2016 CENTRAL LONDON, UK

For more information, please see page 1 of this edition of AOP News. Agenda and registration: www.nc3rs.org.uk/events/pathways-based-approaches-across-biosciences-towards-application-practice

2016 World Congress on *In Vitro* Biology

11-19 June 2016 SAN DIEGO, USA

At this international event participants will have the opportunity to learn about both basic and state-of-the-art cell culture and biotechnology research. It offers the opportunity to connect with scientific colleagues, share your scientific and personal accomplishments, and attend sessions that discuss cutting edge research in the disciplines of *in vitro* science and biotechnology. For more information please go to https://sivb.org/meetings/

Funding opportunity

LRI Innovative Science Award 2016 Competition

The European Chemical Industry Council (Cefic), in conjunction with the Society of Environmental Toxicology and Chemistry (SETAC), the Association of European Toxicologists and European Societies of Toxicology (EUROTOX), the International Society of Exposure Sciences (ISES) and Chemical Week, is offering a €100,000 award to support promising new research in the field of novel approaches to the characterisation of molecular initiating events (MIEs), or other key events (KEs), in pathways of human and environmental toxicity. The deadline for applications is 18 March 2016. To find out more and apply go to: http://cefic-lri.org/funding-opportunities/apply-for-the-lri-award/

Publication highlights

Perkins EJ, Antczak P, Burgoon L *et al.* (2015). **Adverse outcome pathways for regulatory applications: Examination of four case studies with different degrees of completeness and scientific confidence**. *Toxicol Sci* 148(1):14-25. doi: 10.1093/toxsci/kfv181

Huang, R, Xia M, Sakamuru S *et al.* (2016). **Modelling the Tox21 10 K chemical profiles for *in vivo* toxicity prediction and mechanism characterization**. *Nat Commun* 7:10425. doi:10.1038/ncomms10425

Ask an expert!

Do you have any burning queries about AOP development? Or is there something you always wanted to know about pathways-based approaches? You can put your questions to our panel of experts from industry, government agencies and academia. The panel is overseen by Professor Ian Kimber, University of Manchester. Our panel's answers will appear in the next edition of AOP News.

Send your questions to:
aops@nc3rs.org.uk

Complete our survey

Click here to participate in an NC3Rs community-wide survey to collect information on current knowledge and experience in the area of pathways-based approaches

In the next issue:

- An agrochemical perspective on AOPs
- An introduction to EU-ToxRisk
- Your AOP questions answered
- Latest news

Contact us: If you would like to subscribe to AOP News or you have any queries or suggestions for future content, please contact us at: **aops@nc3rs.org.uk**

Ever considered joining the NAT SIG?

The Non-Animal Technologies Special Interest Group (NAT SIG) is delivered by the NC3Rs in partnership with Innovate UK and the Knowledge Transfer Network (KTN). It has been established to identify and connect key skill sets and capabilities in non-animal technologies (NATs) and deliver the recommendations from the Non-Animal Technologies roadmap, a strategic cross-funder vision for the UK as a market leader in NATs.

What does the NAT SIG do?

The NAT SIG connects research and business sectors in building a community of technology developers and end-users to create and apply new models for improved safety and efficacy testing across a range of industries. The platform showcases the latest news, events and funding opportunities relevant to the area.

Benefits of membership

Become part of this rapidly growing online community by joining this group. Membership is free and benefits include:

- Opportunities to help shape the UK's non-animal technologies sector.
- Automatic notification of relevant funding competitions, events and news.
- Opportunity to contribute to discussions.
- Potential to identify members with complementary expertise with whom to collaborate.

To find out more, please visit https://connect.innovateuk.org/web/non-animal-technologies