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 3 | July 2016

Recent NC3Rs workshop: Report published

In April 2016, we held our second workshop on pathways-based approaches, focusing on their application in practice.

The first workshop was held in May 2014. Since then there has been substantial progression of the science in this area, and the continued establishment of frameworks designed to advance the application of pathways-based approaches. Our focus has now shifted to examine the application of pathways-based approaches in practice. The key objectives of the 2016 workshop were to:

- Increase awareness among the scientific community of developments in the field;
- Expand the multidisciplinary community needed to accelerate the development and application of pathways-based approaches;
- Encourage the transition towards application of the knowledge within established AOPs for product development and/or regulatory safety assessment, to ensure that the 3Rs benefits of utilising mechanistic approaches are maximised.

The areas covered during the workshop included:

- Case studies that demonstrated the current application of pathways-based approaches;
- Lessons learned from previous initiatives in this area, and current initiatives underway to support their application;
- The next steps that will be needed to enable wider application of pathways-based approaches.

A keynote presentation was given by Dr Kevin Crofton from the National Center for Computational Toxicology, US Environmental Protection Agency, and the day concluded with a roundtable panel discussion: '*The big conundrum – what constitutes validation*?'. The workshop was attended by almost 100 scientists drawn from the pharmaceutical, industrial chemical and agrochemical industries, academia, regulatory agencies, and other relevant organisations including: consultancy companies and contract research organisations, the European Commission, and the Organisation for Economic Cooperation and Development (OECD).

Further information can be found in the workshop report. Presentations from the day can be accessed here.

In this issue:

Links & Resources

- NC3Rs AOP resource page
- OECD's AOP framework
- AOP-Wiki
- OECD's AOP framework
- Complete the NC3Rs AOP survey

An industry perspective: agrochemicals

Dr Tina Mehta, Dow AgroSciences

The ultimate vision for safety assessment of agrochemicals is movement away from hazard characterisation towards more human and/or environmentally-relevant assessments, through the development and use of more appropriate test systems.

Currently, the development of agrochemical products requires hazard characterisation for mammalian and environmental toxicity which involves an intensive and ever-increasing programme of animal testing globally. Product evaluation also comprises toxicological relevance assessments for dietary and groundwater metabolites and technical substance impurities, and equivalence assessment for alternative sources of manufacture and mixtures assessments, often with different data requirements globally and limited risk assessment approaches. The regulatory requirements need to be re-addressed and a more scientific approach is necessary, to provide better human health and environmental protection and simultaneously impact the 3Rs. Exposure-based safety assessments, incorporating the principles of Risk 21 and utilising an appropriate 'toolbox-based approach' will provide the solution for this. Technological advances (for example, -omics, 3D cells, stem cells, organ-on-a-chip) are now providing significant alternative test models or systems to support better prediction of potential adverse risks. This allows 'fit-for-purpose', integrated, intelligent testing strategies, with informed weight-of-evidence that will increase the speed, efficiency and accuracy of regulatory decision-making for human health, ecotoxicology and environmental protection. Global acceptance of alternative approaches is an important consideration and developing a consensus on relevance, application and regulatory decision-making is crucial. Having an open mind to new and better approaches, developing trust in all stakeholder scientists, and removing bias from science will help the common goal be achieved.

A great example of the utility of alternatives and impact on the 3Rs is acute toxicity testing for agrochemical products. Traditionally, a "6-pack" of studies is required for classification and labelling (C&L) purposes, utilising up to 69 animals per product. This includes acute oral, dermal and inhalation toxicity, skin and eye irritation and skin sensitisation studies. Recent developments have shown that *in vivo* testing is no longer required to address these toxicity endpoints to provide C&L information – a non-animal approach can be used to adequately protect the end-user. This relies on a combination of recognised calculation methods, *in vitro* tests for both skin and eye irritation, and the AOP for skin sensitisation (combining two or three *in vitro* assays). This is the first time that an AOP will be used to inform hazard assessment practice for regulatory purposes, and represents an important milestone. Recently an OECD Acute Toxicity Waiver Guidance Document has been developed and approved[1] which further supports the utility of alternative approaches or waiver opportunities to address these endpoints. There is a valuable opportunity here for global harmonisation and a huge reduction in the use of animals, if all regulators follow and implement this guidance.

[1]OECD (2016), Guidance document on considerations for waiving or bridging of mammalian acute toxicity tests. ENV Publications, Series on Testing and Assessment, No. 237.

Highlights

- The paradigm shift in agrochemical risk assessment will require the development of an appropriate toolbox which includes a multitude of predictive approaches, such as read-across, cheminformatics, threshold concepts, *in silico* and *in vitro* methods, often considered or developed under the umbrella of the AOP framework.
- Such advances will be realised by improved coordination, partnerships, cross-industry learnings, data sharing and a willingness to drive scientific excellence.
- Exposure-based assessments will focus the scientist to consider relevant risks arising from different exposure situations and to design a safety characterisation programme to address relevant concerns, incorporating the AOP framework.
- Ultimately this will restrict the need to generate *in vivo* data only when addressing more specific triggers and endpoints, and these animal data will be used to provide confirmation that adverse outcomes occur.
- The current regulations need to be revised to reflect this change in paradigm to allow more flexibility in combining innovative approaches and thus reduce reliance on the more traditional ones, to give the public a greater reassurance in the safety of products.

Horizon 2020 project underway to develop non-animal mechanistic-based approaches for regulatory risk assessment

The EU-ToxRisk project was launched earlier this year, after being awarded the recent Horizon 2020 call on the topic of 'New approaches to improve predictive human safety testing'.

This large-scale, €30 million collaborative project is being co-ordinated by Bob van de Water, Professor of Toxicology at Leiden University (The Netherlands) with the support of the Executive Office, Steering Team and Scientific Advisory Board. It brings together a consortium of 39 partners consisting of academics, small and medium-sized enterprises, large industry, contract research organisations and regulatory bodies. The NC3Rs is a partner in this consortium.

The aim of the project is to develop a mechanism-based, human relevant, non-animal approach to risk assessment that can predict repeated dose toxicity in likely target organs, namely the liver, kidney, lung and central nervous system as well as reproductive/developmental toxicity. The project will utilise the AOP framework to explore ways to integrate mechanistic understanding to inform integrated approaches for strategies ranging from read-across techniques through to *ab initio* assessments. An integral component of the new strategies will be the consideration of exposure information, for example through the use of physiologically-based pharmacokinetic (PBPK) models. A case study approach will be used to drive the project. Six case studies have been defined with particular focus on read-across approaches; data generation is currently ongoing.

Partners who were previously involved in the SEURAT-1 project will ensure that relevant learnings will be shared. During the course of this project, only non-animal approaches will be utilised and the tissues/cells used will be human or human-derived.

Over 100 people attended the official launch of the project in January 2016, and the first General Assembly was held at the end of June in conjunction with a Summer School for principle investigators, postdocs and PhD students working on the project. The full EU-ToxRisk website is currently under construction, although for those interested in finding out more there will be a special session at the 52nd Eurotox Congress in Seville, Spain in September outlining the work of the consortium in more detail. Further updates on this project will be featured in future editions of AOP News.

Locations of the 38 European partners in the EU-ToxRisk project. One partner is based in the USA.

How to determine the level of confidence in an AOP: Weight of evidence assessment for causality

The **Bradford Hill criteria** are a group of minimal conditions necessary to provide adequate evidence of a causal relationship between an incidence and a possible consequence. These have been used as the basis the weight of evidence considerations for causality for determining the level of confidence in an AOP. These criteria are summarised in the table below, where they are ranked in order of importance.

1. Biological Plausibility		
Is there a mechanistic relationship between Key Event (KE)$_{up}$ and KE$_{down}$ consistent with established biological knowledge?		
High (strong) confidence Extensive understanding based on published literature with broad acceptance in the scientific community.	**Moderate confidence** Coherence with accepted biological relationships but scientific understanding not fully established.	**Low (weak) confidence** Empirical support, but the structural or functional relationship is not understood.
2. Essentiality		
Are downstream KEs and/or the adverse outcome prevented if an upstream KE is blocked?		
High (strong) confidence Direct experimental evidence (e.g., antagonism, knockout models, etc.)	**Moderate confidence** Indirect experimental evidence (e.g., increase in KE$_{up}$ leads to an increase in KE$_{down}$).	**Low (weak) confidence** Lack of evidence, or contradictory experimental evidence.
3. Empirical Support		
Does the empirical evidence support that a change in KE$_{up}$ leads to an appropriate change in KE$_{down}$? Does KE$_{up}$ occur at lower doses, earlier time points, and higher in incidence than KE$_{down}$? Are there any uncertainties and inconsistencies?		
High (strong) confidence Extensive evidence to support KE dependent changes in downstream events, as well as temporal, dose-response and incidence concordance, with no or few critical data gaps or conflicting data.	**Moderate confidence** Demonstrated dependent change in KEs but some evidence inconsistent with an expected pattern that may be explained by factors such as experimental design, or technical considerations, etc.	**Low (weak) confidence** Limited or no studies reporting dependent change in KEs i.e. endpoints never measured in the same study or not at all, and/or significant inconsistencies.

For further reading see:

- Becker *et al.* (2015). **Increasing Scientific Confidence in Adverse Outcome Pathways: Application of Tailored Bradford-Hill Considerations for Evaluating Weight of Evidence.** *Regulatory Pharmacol* 72(3):514-37. doi: 10.1016/j.yrtph.2015.04.004.
- Meek *et al.* (2014). **Mode of action human relevance (species concordance) framework: Evolution of the Bradford Hill considerations and comparative analysis of weight of evidence.** *J Appl Toxicol* 34(6):595-606. doi: 10.1002/jat.2984.
- OECD AOP handbook

Latest news

Standards in non-animal technologies - webinar slides and voting results published

Following the Non-Animal Technologies (NATs) cohort meeting: 'Standards in non-animal technologies' in February, hosted in collaboration with Innovate UK and the British Standards Institute, the NC3Rs and Stevenage Bioscience Catalyst hosted a webinar to report the outcomes and proposed next steps. Attendees then voted to select the most pressing priority area.

The slides and results of the voting are available here.

OECD endorses AOPs and accepts new AOPs on to work plan, including one co-led by the NC3Rs

Five AOPs were endorsed* at the annual meeting of the Working Group of National Co-ordinators of the Test Guidelines programme (WNT) meeting in April and nine new AOPs were accepted onto the OECD work plan. Information on these AOPs can be found in the AOP Wiki.

Endorsed AOPs:

- Alkylation of DNA in male pre-meiotic germ cells leading to heritable mutations.
- Aromatase inhibition leading to reproductive dysfunction (in fish).
- Protein alkylation leading to liver fibrosis.
- Chronic binding of antagonist to N-methyl-D-aspartate receptors (NMDARs) during brain development induces impairment of learning and memory abilities.
- Binding of agonists to ionotropic glutamate receptors in adult brain causes excitotoxicity that mediates neuronal cell death, contributing to learning and memory impairment.

*These AOPs have been developed under the auspices of the OECD AOP Development Programme, overseen by the Extended Advisory Group on Molecular Screening and Toxicogenomics (EAGMST), which is an advisory group under the WNT. These AOPs have been reviewed internally by the EAGMST, externally by experts nominated by the WNT, and has been endorsed by the WNT. Through endorsement of this AOP, the WNT expresses confidence in the scientific review process that these AOPs have undergone and accepts the recommendation of the EAGMST that the AOPs be disseminated publicly. Endorsement does not necessarily indicate that the AOPs are now considered a tool for direct regulatory application.

Accepted AOPs:

- Adverse outcome pathways for cranio-facial skeletal defects.
- Blocking of L-type Ca++ channels leading to heart failure (reduction in left ventricular ejection fraction, LVEF).
- Hand1 gene dysregulation leading to embryotoxicity.
- Adverse outcome pathways of proximal tubule injury mediated by covalent protein binding and lysosomal overload.
- Dysregulation of IL-2 transcription leading to immunotoxicity.
- ROS induces phototoxic reactions.
- Oxidative stress leading to hypertension.
- EGFR activation leading to mucus hypersecretion.
- Lysosomal damage leading to liver inflammation.

The AOP 'Blocking of L-type Ca++ channels leading to heart failure (reduction in LVEF)' is being led by the NC3Rs in collaboration with EURL-ECVAM. More information on this AOP can be found in the next edition of AOP News.

Publication highlights

A report has recently been published by researchers at Brunel University exploring the need to consider chemical-specific properties, pharmacokinetics, and internal exposure dynamics when developing quantitative AOPs:

Margiotta-Casaluci L *et al.* (2016). **Internal exposure dynamics drive the Adverse Outcome Pathways of synthetic glucocorticoids in fish.** *Sci Rep* 6:21978. doi: 10.1038/srep21978.

Events

52nd Congress of EUROTOX

4 - 7 September 2016, SEVILLE, SPAIN

Relevant sessions include:

- A multidisciplinary approach for novel developmental neurotoxicity risk assessment contributing to the AOP concept.
- The H2020 EU-ToxRisk project: a novel flagship program for mechanism-based safety sciences and risk assessment.
- Application of human-based system toxicology for preclinical safety assessment of pharmaceuticals.
- Integration of *in vitro* systems to predict toxicity from repeated exposure.

For more information and to register, click here.

Accelerating the uptake of mathematical models in safety & efficacy testing

14 & 15 September 2016, LONDON, UK

The NC3Rs is working with the international Health and Environmental Sciences Institute (HESI) to host a two day workshop to accelerate the acceptance of mathematically-derived data to improve the predictivity of efficacy and safety testing for drugs and chemicals.

The workshop will bring together mathematicians and life scientists from across industry and academia, plus regulators and senior decision-makers. Participants will share their knowledge and experience of integrating mathematical modelling approaches in product development, and define a future landscape for supporting the acceptance of this data in safety and efficacy decision-making.

Attendance is free, but registration is essential. The closing date for registration is 17 August 2016.

Drug Metabolism Group 2016 Summer meeting

19 September 2016, LONDON, UK

This meeting, organised by the Drug Metabolism Group, is on the theme of 'Non-Animal Technologies for Drug Metabolism' and will take place at Imperial College London. It aims to provide a forum for discussion for all those involved in drug metabolism and related toxicology, with a focus on highlighting approaches that aim to replace, reduce, or refine the use of animals. Early career researchers are encouraged to attend. Attendance is free; more information and registration can be found here.

European Society of Toxicology *In Vitro* (ESTIV) 2016 meeting

17 – 20 October 2016, JUAN-LES-PINS, FRANCE

This focus of this year's ESTIV meeting is on *in vitro* toxicology for safety assessment. Emphasis will be specifically on how new technologies can strengthen the interpretation and application of *in vitro* methods in toxicological research and risk assessment.

The ESTIV 2016 congress offers a venue for research or industry toxicologists to meet and exchange scientific and regulatory information and knowledge. Young scientists are particularly encouraged to present their work and discuss results with senior scientists.

To register for this event, visit the ESTIV website.

Ask an expert

Professor Maurice Whelan, Head of the Chemicals Safety and Alternative Methods Unit at the European Commission's Joint Research Centre, and the head of the JRC's EU Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM), answers the question: *'How will data from different assays which provide information on key events be assimilated to support decision making?'*

There are many ways that mechanistic data associated with key events of an AOP can be utilised to support chemicals safety assessment in a variety of sectors. At the OECD, the term Integrated Approaches to Testing and Assessment (IATA) has been defined recently to refer to ways of combining data from non-animal methods, using AOP knowledge as the underlying mechanistic rationale, to address different questions about a chemical's potential hazard or risk. Key event data can be integrated to inform decisions in a variety of ways including weight-of-evidence, read-across, or fixed data interpretation procedures. Having AOP knowledge aids in the selection of the right assays, deciding on the importance attributed to the data derived from them, and in the identification of possible data gaps.

It is important to realise that although the AOP framework has emerged in recent years, the use of mode-of-action data to inform decision making is of course nothing new. The considerable work done by the World Health Organisation's International Programme on Chemical Safety in particular has described and demonstrated how such data can be systematically incorporated into various risk assessment scenarios. Making curated mechanistic knowledge more readily available via the AOP Knowledge Base will hopefully broaden the uptake and impact of mode-of-action based approaches within an IATA framework.

And there are clear signs that *in vitro* key event data can be used in lieu of traditional animal data. Just recently a formal proposal has been made to change the REACH legislation, to make data from *in vitro* assays linked to three key events of the skin sensitisation pathway the default information requirement, instead of the Local Lymph Node Assay conducted on mice.

Send your questions to be answered in future editions of AOP News to: aops@nc3rs.org.uk

NC3Rs AOP survey

There is still time to participate in an NC3Rs community-wide survey to collect information on current knowledge and experience in the area of pathways-based approaches. The survey will take no longer than ten minutes to complete and can be found here. The survey will close on 31 October 2016.

In the next issue:

- An industrial chemicals industry perspective on AOPs.
- AOP Spotlight.
- Introduction to the NC3Rs/ECVAM-led AOP.
- Your AOP questions answered
- Latest news.

Contact us

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