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 1: October 2015

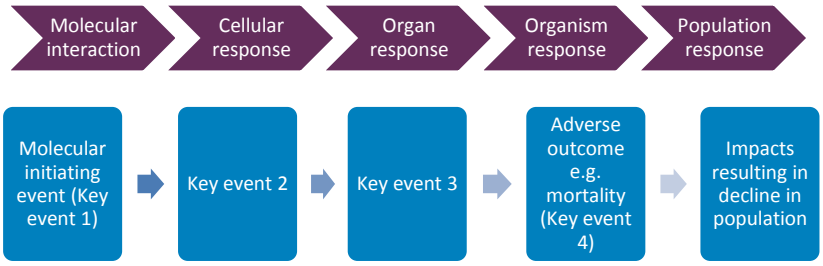
An introduction to the AOP Concept

In recent years, there has been increasing interest from the academic and industry sectors in using cell-based and computational approaches to investigate how pharmaceuticals and environmental chemicals interact with biological systems, to help predict whether they are harmful to humans and/or the environment. The vision for the future is that such approaches could be used to determine whether a chemical or drug of interest causes the critical (or 'key') events within the biochemical pathways known to result in adverse outcomes in organisms or populations. Advances in this area could enable the prediction of an adverse outcome within an organism in the absence of animal studies.

Such a scenario will be dependent on:

a) the key events of the pathways being categorically linked to each other and the adverse outcome in question;
b) the appropriate tools being available to assess the propensity for chemicals to induce the key events.

The biochemical pathways that will need to be developed and used can be described as 'Adverse Outcome Pathways' (AOPs), and can be visualised as:



To enable the wider application of such a mechanistic approach to product development and routine safety assessment, a framework has been established by the OECD to standardise the integration and organisation of the large amount of data available on different and interconnected AOPs. The framework offers a means to i) share information on a large scale; ii) better substantiate the links between pathway components and between pathways to establish networks; and iii) bring the necessary scientific disciplines together. The ultimate aim of the framework is to build sufficient confidence in the AOPs, so that adverse outcomes can be predicted through generation of the relevant mechanistic information, and practically applied in the safety assessment.

There is potential for the knowledge gained through the development of AOPs to be utilised in both early screening (i.e. for compound prioritisation) and regulatory risk assessment. The intended use for a specific AOP will determine the level of detail it captures, for example for regulatory purposes there will need to be a higher level of confidence in the linkages within the pathway and fewer data gaps than if it were used for screening purposes.

In this issue:

Links & resources:

- NC3Rs AOP resource page
- OECD's AOP framework
- AOP Wiki
- Human Toxicology project consortium presentations:

  AOPS 101

  AOPS 201

An industry perspective: chemicals

Carl Westmoreland, Unilever

The 3Rs opportunities with Adverse Outcome Pathways

Safety assessors, such as those in industry and in regulatory agencies worldwide, have traditionally determined acceptable (i.e. 'safe') levels of exposure to new chemicals by combining toxicology data from experimental animals with predicted levels of exposure in humans and environmental species. The role of animal testing in safety assessment is increasingly questioned and there are currently several activities on-going globally looking at opportunities to reduce, refine and replace animal use in this area.
Knowledge of the pathways which eventually lead to toxicity could enable safety assessments in the future to be conducted based on mechanistic understanding and 'Twenty First Century' science, ultimately replacing the need for toxicology tests in animals. The AOP framework enables experimental in vitro data or in silico predictions associated with key events in a toxicity pathway to be generated on a new chemical. This information can be integrated in a transparent and mechanistic way, with biological knowledge of the pathways to inform decisions on the safety of the chemical.

The aim of the AOP approach is to provide a toolbox of predictive, robust in vitro assays, in combination with *in silico* predictions. The toolbox can be used to assess the critical mechanistic steps involved in the induction of an adverse outcome rather than the toxicity effects themselves. This will reduce the reliance on whole animal testing for hazard assessment.

For AOPs to be of use in the future for conducting safety assessments without using animals, it is clear that (eco)toxicological risk assessment processes and associated regulations will need to evolve to embrace this new science. Many technical challenges still remain to be solved before this vision is realised. Central to these challenges is the need for AOPs to produce a quantitative output (i.e. dose-response information to provide a quantifiable link between the key events and the adverse outcome). Only if risk assessors can have confidence in the dose response predictions of AOPs, can this information be used for determining acceptable levels of exposure by combining it with predicted levels of exposure to those chemicals in humans and environmental species. An important aspect of this integration is the fundamental role of mathematical modelling in simulating underlying chemical and biological processes, to provide context to the experimental data and to explicitly capture uncertainties when determining acceptable levels of exposure to new chemicals.

Highlights:

- *The AOP framework and approach to safety assessment has great potential for the 3Rs.*
- *Knowledge of the pathways which lead to toxicity could enable safety assessments to be based on mechanistic understanding.*
- *Improved mechanistic understanding will enable the development of new predictive assays.*
- *An AOP toolbox of predictive* in vitro *and* in silico *methods will reduce the reliance on whole animal testing for hazard assessment.*
- *AOPs will need to be quantitative if they are to be of use in the future for conducting safety assessments without using animals.*

What you need to know: OECD's development programme and the AOP Wiki

In 2012, the OECD (Organisation for Economic Co-operation and Development) launched a programme of work which now serves as the central framework for AOP development. As part of this programme the OECD is participating in a collaborative effort to develop a web-based platform, known as the AOP Knowledgebase (AOP-KB), where "authors" of an AOP can enter information pertinent to the pathway of interest. The first AOP-KB module is the "AOP Wiki" which has been designed as an interactive and virtual repository for AOP development. All stakeholders from academia, governmental agencies and industry are invited to use the Wiki either as a source of information, or as active contributors posting comments and content. This expert contribution from third-parties is strongly encouraged to maximise the benefits of "crowd sourcing". AOPs within the AOP Wiki are living documents and are intended to be revised as and when new information becomes available. Ultimately, some of the AOPs in the Wiki will be subject to expert reviews and subsequently published by the OECD. Further modules within the AOP-KB, designed to facilitate the collaborative development and utilisation of AOPs, will be made available for use in the future.

Feedback on the OECD's AOP Training

Anna Bottomley, NC3Rs Programme Manager, attended the OECD Headquarters in Paris last month for a training session on the the development of AOPs

The OECD hands-on training approach was focussed on turning real-life data into working AOPs. One example involved mapping how aromatase inhibition leads to decreased ovulation and spawning in fish. Participants were provided with a dataset, including *in vitro, in vivo* and *ex vivo* data available from the literature, which was used to guide them through each step of the development process. The difficulties faced in practice were analysed in plenary discussions and expert panelists gave their opinions on best practice. A practical session on entering information into the AOPwiki gave participants the knowledge needed to begin to develop AOPs in their specific area of interest.

"The practical information gained will be invaluable when we initiate our project to develop an AOP in the area of cardiovascular toxicity"

Latest news

NC3Rs publication

Our latest publication, with key opinion leaders in toxicology explores how the development and application of AOPs could benefit chemical safety assessment whilst reducing reliance on animals.

Reference:

Burden N, Sewell F, Andersen ME, Boobis A, Chipman JK, Cronin MT, Hutchinson TH, Kimber I & Whelan M.
"Adverse Outcome Pathways can drive non-animal approaches for safety assessment" non-animal approaches for safety assessment." *Journal of Applied Toxicology*. 5 May 2015. doi/10.1002/jat.3165/full

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NC3Rs project to develop an AOP for cardiotoxicity

We are working with a group of experts to convert pathways relevant to cardiotoxicity into AOPs.

Painting the future animal-free safety assessment of chemical ingredients: Achievements of SEURAT-1

4 Dec 2015 BRUSSELS, BELGIUM

This concluding symposium is an opportunity to learn about the recent achievements of the SEURAT-1 (Safety Evaluation Ultimately Replacing Animal Testing) cluster, and their impact in the field of alternative testing strategies. High-level presentations will showcase the SEURAT-1 success stories in an accessible manner and an open Exhibition will then allow for deeper discussions and networking. Registration is open until 6 November: http://www.seurat-1.eu/pages/library/events/seurat-1-symposium.php

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HeMiBio International Symposium

Biology meets technology for liver toxicity testing

2-3 December 2015 LEUVEN, BELGIUM

Includes a session on implementation of the AOP concept in HeMiBio, with a focus on liver toxicity. Registration opens July 2015.
www.hemibio.eu/events/events-details

In the next issue:

- AOP spotlight: We explore the scientific and 3Rs benefit offered by the application of a well-developed AOP
- A pharmaceutical perspective on AOPs
- Latest news

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