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 4 | December 2016

NC3Rs survey: current knowledge and awareness of pathways-based approaches

We recently conducted a community-wide survey to collect information on current awareness and experience with AOPs

We would like to thank everyone who took the time to complete the survey. We received 83 responses from a range of sectors across human and environmental toxicology including academia, industry, consultancy and regulatory agencies. We have conducted a preliminary analysis of the results.

Overall, the majority of respondents reported that they or their organisation currently utilise pathways/mechanistic based approaches, with most stating that they/their organisation used such approaches for safety assessment, followed by basic research. Respondents indicated that the main potential benefits of these approaches include improved mechanistic understanding and predictivity, in addition to reduced reliance on animal toxicity testing.

Over 70% of respondents indicated they were fully aware and comfortable using terminology associated with the AOP concept. Most respondents (86%) reported it was possible or likely they or their organisation would contribute to the OECD's AOP framework – mainly by filling in data gaps within existing AOPs, or by developing new AOPs.

The top three aspects that respondents indicated would encourage them to invest time in applying the AOP concept in their own work were:

- Increased confidence in the potential benefits;
- Regulatory buy-in; and
- Increased funding.

Notably, there were differences between the top answers to this question depending on which sectors respondents represented.

We plan to share a more detailed analysis in the near future, please see forthcoming editions of AOP News.

We will use the findings from this survey to help shape our future activities to continue to support the role of mechanistic/pathways-based approaches in enabling safety assessment using non-animal data.

In this issue:

Links & Resources

- NC3Rs AOP resource page
- OECD's AOP framework
- AOP-Wiki

The cardiotoxicity AOP workstream

Cardiotoxicity was identified as an area of potential interest for AOP development at the 2014 NC3Rs workshop 'Applying pathways-based approaches across the biosciences'.

A number of reasons make this an attractive topic:

- ***3Rs Potential***: high numbers of *in vivo* tests are currently carried out to assess the cardiotoxicity potential of new pharmaceuticals. The use of *in vitro* or *in silico* tests to assess or identify effects at earlier key events (KEs) could reduce the requirement for tests in animals, as well as improving predictions and/or identifying cardiovascular liabilities earlier in development.
- ***Industry/business need***: cardiotoxicity is a major cause of drug attrition due to safety concerns and there is a need to develop more predictive, human-relevant testing strategies.
- ***Novelty***: this area was not already represented in the OECD AOP work plan.
- ***Feasibility/data availability***: a lot of mechanistic *in vitro* data already exist that could be utilised without further experimental work.
- ***Scientific community engagement***: this is an area with an established network of committed scientists. Engaging the pharmaceutical industry would extend the use of AOPs across sectors.
- ***Clinical need***: better protection of human health through better understanding of mechanisms and fewer adverse effects.

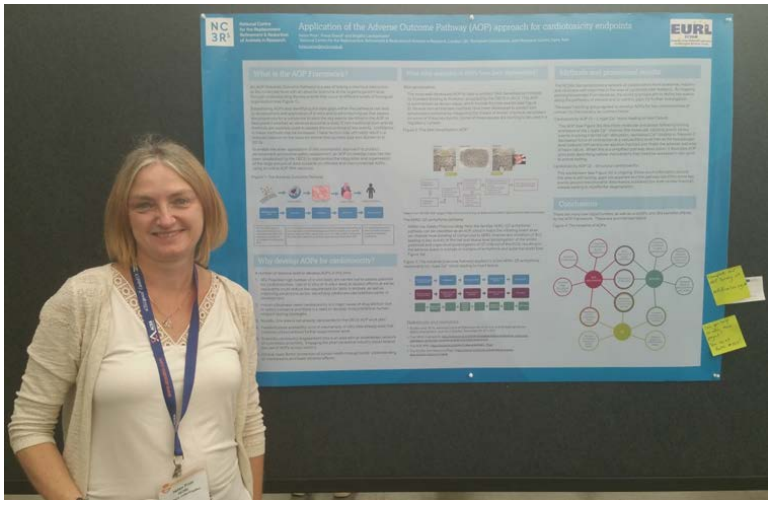
National Centre for the Replacement Refinement & Reduction of Animals in Research

EURL ECVAM European Union Reference Laboratory for Alternatives to Animal Testing

In collaboration with EURL-ECVAM, a 'Cardiotoxicity Brainstorming meeting' was held in July 2015, bringing together experts in the field of cardiovascular research from industry, academia and clinical sectors, at which two distinct topics were chosen for further exploration. These topics - negative cardiac contractility and structural cardiotoxicity - were then examined in detail at a further workshop in November 2015 by small subgroups of experts.

To progress this project and introduce the AOP concept and utility of these potential pathways to a new audience of relevant scientists, Helen Prior (NC3Rs programme manager, with a background in cardiovascular safety pharmacology), submitted an abstract to the Safety Pharmacology Society Annual meeting in Vancouver (September 2016) for a poster presentation. This abstract, entitled 'Application of the Adverse Outcome Pathway (AOP) Approach for Cardiotoxicity Endpoints', was also selected for a short oral communication, which gave this topic additional exposure to an audience of approximately 50 people.

In June 2016, one of the cardiovascular topics was accepted as a project onto the OECD work plan under the title 'L-type Ca^{2+} channel block leading to heart failure'. Work is currently ongoing to populate this pathway.



Any scientists with cardiovascular/cardiotoxicity expertise interested in assisting us with this task are encouraged to contact us at aops@nc3rs.org.uk.

An industry perspective: industrial chemicals

Dr Chantal Smulders, Shell

Risk assessment of industrial chemicals needs human-relevant data. AOP-based testing strategies in combination with exposure and dose-response considerations can improve estimates of human risk.

Global chemical safety regulations have developed at an increasing pace in the past decade, with examples being the European Union's Regulation, Evaluation, and Authorisation of Chemicals (REACH) and the new Lautenberg Act amending the Toxic Substances Control Act (TSCA) in the United States. The majority of the hazard information requirements in these regulations still rely on animal testing data, despite the availability of new test systems not using animals.

Do the current animal tests provide the most accurate information for human risk assessment? It is not uncommon in chemical safety testing that significant resources, including animals, time, and cost is spent on long-term animal studies, followed by even more resources, to demonstrate the outcomes of the animal tests are not relevant for human risk assessment. This is one sign that indicates that a new way of testing is required – a way that provides a more accurate estimate of actual risk to humans.

Testing strategies based on AOPs, or more specifically, the molecular initiating events (MIEs) that could lead to adverse outcomes in humans could result in scientifically more accurate estimates of human risk. For skin sensitisation, an AOP-driven testing strategy has been successfully developed and regulatory acceptance and implementation is progressing. For longer-term toxicity endpoints the challenge remains, not least because a network of different AOPs and MIEs are involved. The CRACK IT PREDART project was an ambitious one which aimed to develop an AOP-based testing strategy in non-vertebrate species (slime mold and nematode) and zebrafish embryos for developmental and reproductive toxicity (DART). The basic assumption is that the most crucial AOPs for these endpoints are conserved in evolution. The challenge is to overlay the pathways from these species with rodent and human pathways to verify which phenotypic effects are relevant for adverse effects in rodents and humans. The assays in their current state have proven to be useful tools for early identification of potential human developmental or reproductive toxicants. However, more work needs to be done in the identification and overlay of AOPs and MIEs in key species. Do we need to know all the AOPs that could lead to developmental or reproductive toxicity for a reliable testing strategy, and do all these AOPs need to be fully characterised? Elucidation of all possible AOPs for these endpoints is a never-ending story; however, if we could identify the most important MIEs covering the majority of potential adverse outcomes we already have a very usable basis for a testing strategy. The more the new testing strategy is used, the more knowledge we will gain, and this can drive acceptability of the new testing strategy for human risk assessment.

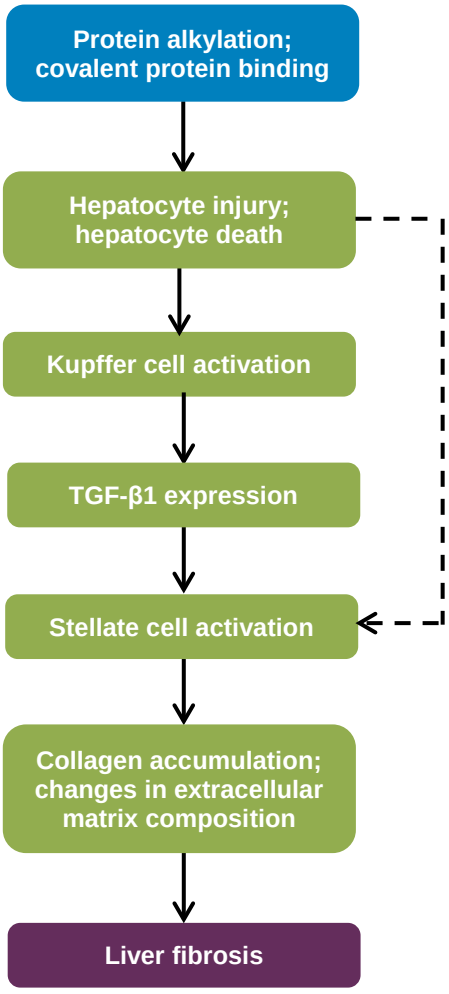
A crucial challenge for the chemical industry however, is that most of the manufactured chemicals are commodities intended for socio-economic benefit, and not intended to have a biological effect. Examples include solvents, feed stocks for plastics, and automotive fuels. Most of these industrial chemicals have no intended biological mode of action, and safety studies in animals often show non-specific effects at high doses. It is therefore of pivotal importance that exposure and dose-response are taken into account in the development of AOP-based assays. Only by doing this thoroughly, will we be able to discriminate adaptive responses from specific adverse effects, and specific adverse effects from generic toxicity caused by system overload.

Highlights

- The majority of the hazard information requirements in chemical safety regulations still rely on animal testing data, despite the availability of new test systems not using animals.
- AOP-based testing strategies could result in more robust estimates of human risk although it is of pivotal importance that exposure and dose-response are taken into account in the development of AOP-based assays - especially for industrial chemicals which generally have no intentional biological effect, and for which safety studies in animals often show non-specific effects at high doses.
- Elucidation of all possible AOPs for complex endpoints such as DART is a never-ending story; however, if we could identify the most important MIEs covering the majority of potential adverse outcomes we already have a very usable basis for a testing strategy.

AOP spotlight: Protein alkylation leading to liver fibrosis

We ask Dr Brigitte Landesmann, Scientific Officer at the Chemicals Safety and Alternative Methods Unit, European Commision's Joint Research Centre (JRC), author of AOP 38, and end-user Dr James Sidaway, founder of Phenotox Ltd., to give their perspectives on its development, potential impact on the 3Rs, and application in practice.



This AOP links the MIE, protein alkylation, through to the adverse outcome (AO) of liver fibrosis.

The liver is potentially the most vulnerable organ to toxicants, due to its key role in metabolism. Hepatotoxicity is therefore a key consideration for assessment of the risk to human health. Liver fibrosis in particular is a common type of hepatotoxicity and is often associated with chronic or repeated exposure, and is one of the endpoints considered for regulatory purposes.

Due to the complex processes involved, an adequate cell model is currently not available and an *in vitro* evaluation of fibrogenic potential is therefore not feasible. A sufficiently detailed description of the AOP to liver fibrosis might support chemical risk assessment by indicating early markers for downstream events and facilitate a testing strategy without the need for a sophisticated cell model. Identified uncertainties and knowledge gaps resulting from development of this AOP can direct future research by priority setting and targeted testing. The KE descriptions can be used for hazard identification and read-across to assess the toxic potential of an untested substance. (*Text adapted from the AOP Wiki entry*).

Dr Landesmann describes:

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

Dr Sidaway describes:

- Potential applications within pharmaceutical risk assessment;
- How the AOP can be used now;
- Potential impacts for the 3Rs;
- Related AOPs that would have particular relevance for the pharmaceutical industry.

Interview with Dr Brigitte Landesmann, author of AOP 38

What were the initial reasons and drivers for developing this AOP?

This AOP development activity started in 2011 in the context of the SEURAT-1 (Safety Evaluation Ultimately Replacing Animal Testing) research project, which aimed to develop alternative models for safety assessment based on mechanistic knowledge.
For that purpose AOPs related to chronic liver injury were developed to support the design of studies for predicting selected types of repeated-dose toxicity. The liver was one of the project's target organs and liver fibrosis typically results from chronic injury.
Along with the ongoing evolution of the AOP concept, the initial pathway description has been revised, extended, and modified and it was among the first AOPs that were developed according to OECD guidance.

What approach was taken to develop this AOP?

As described above, the adverse outcome (AO) was chosen according to the needs of the SEURAT-1 research project; protein alkylation was the common MIE for the two SEURAT-1 reference chemicals for liver fibrosis. Thus the AOP's two anchor points have been defined and an intensive literature search identified studies relevant to the understanding of the pathway to liver fibrosis and the identification of further KEs in between at different levels of biological organisation. Special emphasis was put on the inclusion of human data. The literature research was carried out mainly by myself, but the draft AOP was repeatedly discussed with colleagues within the unit, as well as with SEURAT-1 collaborators. In October 2014 a workshop was organised to discuss this AOP and its further development with external experts in this field. Finally, this AOP has now undergone the complete OECD review process, has been endorsed and published in a new OECD Series on Adverse Outcome Pathways. Extensive discussions in the course of this review process have provided important feedback that was also incorporated in the AOP description.

What is the intended application of your AOP?

This AOP was initially developed to support the design of studies for predicting liver toxicity. Since liver fibrosis results from a complex interplay between various hepatic cell types, receptors and signalling pathways, elaborate multi-cell models are required for investigation *in vitro*. Though the available selection of liver models is continuously increasing and novel cell culturing strategies are being devised, there is still no valid cell model available for mimicking fibrogenesis.

The identification of early (upstream) markers for the final outcome could support chemical risk assessment by facilitating a testing strategy without the need for an elaborate cell model.
An improved mechanistic understanding helps in identifying uncertainties and knowledge gaps and can direct future research.

In its current state of development, can the AOP be used for its intended application?

To some extent. The pathway description was used as a blueprint for experimental design, which has been utilised in other projects beyond SEURAT-1. Some uncertainties were revealed that are worth further investigation.

What next steps are needed before the AOP can be used for its intended application?

Quantitative data are necessary for applicability in risk assessment; the investigation of quantitative aspects regarding how much change and for how long in an upstream KE is needed to cause a corresponding change in the next downstream KE remains an ambitious goal. The AOP description can support devising a suitable cell model to further explore the sequence of events, especially in quantitative terms and to investigate the significance of early markers for the prediction of the AO.

How can the AOP or information within the AOP be utilised in the meantime?

Chronic hepatic injury regardless of the causing stimuli (i.e. toxic, metabolic, inflammatory, parasitic, or vascular), may lead to liver fibrosis by the same mechanisms and thus this pathway description is also relevant for disease progression. An integration of clinical data would further characterise the pathway and be beneficial both for clinicians and toxicologists by informing relevant *in vitro* testing and facilitating the extrapolation of *in vitro* information to disease and regeneration mechanisms *in vivo.*
The complex mechanism of fibrogenesis does not only affect a single organ, but causes a systemic response which equally damages other organs and tissues. The described findings in liver fibrosis parallel those in studies of fibrogenesis in other organs; therefore, findings in the liver could be extended to studies of fibrosis in the lungs, the kidneys, the heart and other organs.
Distributed mechanistic information from the literature regarding fibrogenesis is assembled and displayed in

a coherent way and made available for use in other projects and risk evaluations. Some knowledge gaps, like the specification of targets for protein alkylation, the role of EMT (epithelial–mesenchymal transition) as an early marker and the impact of type, amount and rate of hepatocyte injury are highlighted for further investigation. Nanomaterials (NMs) are also known for causing liver inflammation and fibrosis. We could demonstrate that chemical- and NM-induced toxicological processes share downstream events. The differences are primarily related to differences in toxicokinetics and the nature of the initial KE. The mechanistic knowledge captured in this AOP can be utilised to fill knowledge gaps related to NM toxicity.

What challenges did you encounter whilst developing this AOP?

It was difficult to find suitable mechanistic data for developing this AOP, because classical *in vivo* studies are mainly focused on the apical endpoint and rarely describe mechanistic sequences in detail. Absence of a KE description in published experimental studies on fibrogenesis does not necessarily mean that this KE did not occur, but rather that it has not been investigated or described. Due to the pathogenic complexity of liver fibrosis and the lack of a suitable cell model, data from studies investigating liver fibrosis are rather scarce. Single cell cultures of various liver cell types allow studying the individual cell responses to injury and provide the opportunity to understand the roles that different liver cell types play in fibrogenic processes. Several co-culture models allow the investigation of interactions between some individual actors *in vitro*. But in general, these studies were not designed to investigate the linkages between various actors.

Chronic inflammation and oxidative stress are essential contributors to fibrogenesis; they are ongoing processes throughout the pathway and mutually interconnected with most of the KEs; therefore, it's difficult to pinpoint them to a specific position within the pathway (which does not allow branches or loops). It has been extensively and repeatedly discussed with the AOP developer community how to best represent these events within the AOP. For this AOP, processes related to inflammation and oxidative stress are described within the respective KEs, thus moving them away from visibility at first sight, which does not adequately reflect their important role in fibrogenesis. These processes are important contributors to a variety of adverse reactions and currently various ways of describing them can be found in the Wiki. Discussions are still ongoing for finding a generally accepted way to integrate these multifaceted and multiple interrelated collective terms into AOP depiction, which is indispensable for interlinking and AOP network-building.

How do you think use of this AOP in practice within the wider landscape could impact on the 3Rs?

The structured mechanistic knowledge included in this AOP supports the development of integrated testing strategies, which rely on using *in vitro* methods, that in combination with in silico approaches, facilitate *in vivo* predictions of toxicity. Described KEs can be used for hazard identification, read-across, and targeted testing.

Any further comments?

Fortunately the AOP developer community is growing and more and more AOP descriptions are being published. Regrettably not all this AOP knowledge is also contained within the AOP Wiki. The vision of building a comprehensive AOP network that can depict the complexity of potential interactions and that corresponds to systems biology can only be realised through manifold contributions and collaboration from all involved parties. Therefore, I appeal to all AOP authors, who have not developed their AOP in the Wiki, to take this little extra effort and put their data into the Wiki.

What advice would you give to researchers about to embark on developing an AOP?

- AOP development can be quite a tedious ask, but it is also illustrative, satisfactory, and enjoyable as the pathway takes shape and the various 'puzzle stones' fit together. My strongest advice is not to work in isolation, but to **collaborate with colleagues**, preferably from various **different disciplines**. Discussion beyond the borders of one's own expertise are enlightening and fruitful and each background provides a different aspect, thus contributing to diversity and completeness of the whole.
- The Wiki provides an ideal platform for discussion and exchange. Before starting one's own development project it is advisable to **look into the Wiki** to see whether there are already suitable elements (KEs or KERs) that can either be integrated into one's own pathway or interlinked with it. To **facilitate sharing**, the modules should be described as generically as possible (still being as specific as necessary).

It is important to keep in mind, that AOPs are a **pragmatic simplification** and do not intend to provide a complete and detailed description of every aspect of the biology involved in the sequence of events that leads to an AO. However, the **choice of the relevant level** of detail in AOP description is crucial, because too many details might distract from understanding the main pathway while being too concise holds the risk of overlooking relevant processes.

Interview with Dr James Sidaway, pharmaceutical toxicologist

What do you think are the potential applications of this AOP in your field of work?
Covalent protein alkylation by reactive chemicals is a key mechanism of chemical toxicity. For example reactive metabolite mediated toxicities are a major concern for the pharmaceutical industry. Several screening and integrated approaches are in use for hazard identification and to a lesser extent risk assessment within the pharmaceutical industry but there is little consensus on the optimal strategy. The 'Protein Alkylation leading to Liver Fibrosis' AOP is a comprehensive summary of the evidence for and uncertainties of the KEs that lead to the adverse outcome. This is one adverse outcome in a spectrum of reactive metabolite related adverse events, such as liver necrosis and toxicities in other organs, including skin and lung which are relevant to pharmaceutical risk assessment. In this context this AOP is a good first step in achieving a comprehensive reactive metabolite toxicity AOP framework that might be used by the pharmaceutical industry to improve screening and integrated approaches.

If the AOP is not yet sufficiently developed for all the applications you have mentioned, how can the AOP or information within the AOP be utilised in the meantime?
The AOP is a very useful and comprehensive information repository. It could be used to identify other relevant AOPs in the area of protein alkylation/reactive metabolite toxicities where there is overlap with KEs.

What are the next steps needed before the AOP can be used for the applications you have described? What do you perceive are the challenges that need to be overcome for the intended application to be realised?
A comprehensive set of AOPs that are relevant for all reactive metabolite toxicities could serve to identify future research opportunities for greater development of relevant non-animal models for screening. It could also be used as a framework for achieving consensus on the optimal testing strategy. One challenge is how to integrate AOPs that are clearly related (e.g. skin sensitisation). Greater engagement with industry and academic experts in this area could also facilitate new AOPs to be developed that are relevant to all chemical industry sectors. Another challenge is how to incorporate the bioactivation pathways that lead to reactive metabolite formation which are often assessed in early drug metabolism and toxicity studies.

How do you think utilisation of this AOP in your industry could impact on the 3Rs?
A single AOP will have limited 3Rs impact on human risk assessment in the pharmaceutical industry. The 3Rs impact may ultimately be evident when there is a comprehensive set of AOPs that has led to development and utilisation of acceptable non-animal methods.

Are there any related AOPs that would have particular relevance for your field that you would like to see developed?
AOPs which include mitochondrial mechanisms of drug-induced liver toxicity and the molecular initiating events from the bioactivation pathways would be relevant.

Further reading

Wiki entry for AOP 38: Protein alkylation leading to liver fibrosis
https://aopwiki.org/wiki/index.php/Aop:38

Horvat T *et al.* (2016). Adverse outcome pathway development from protein alkylation to liver fibrosis. *Arch. Toxicol.* doi:10.1007/s00204-016-1814-*8*

Willett C *et al*. (2014). Pathway-based toxicity: history, current approaches and liver fibrosis and steatosis as prototypes.
ALTEX. 31 (4): 407-421. doi:10.14573/altex.1401283

Willett C *et al*. (2014). Building shared experience to advance practical application of pathway-based toxicology: liver toxicity mode-of-action.
ALTEX. 31 (4): 500-519. doi: 10.14573/altex.1401281

Latest news – OECD publications special

New Series on AOPs published in the OECD i-Library

Includes documents for each of the newly 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

Plus a users' handbook supplement to the guidance document for developing and assessing AOPs.

New OECD Guidance Documents published

- Guidance Document on reporting of defined approaches within integrated approaches to testing and assessment (IATA)
- Guidance document on reporting of defined approaches and individual information sources to be used within IATA for skin sensitisation

Events

Applying exposure science to increase the utility of non-animal data in efficacy and safety testing

15 – 16 February 2017, LONDON, UK

The NC3Rs is co-hosting a two-day workshop with Unilever to increase awareness and build confidence in the application of exposure-driven approaches to support decision-making using data from non-animal approaches.

The meeting will bring together academic and industry researchers across multiple disciplines to share their knowledge and experiences in applying exposure science to increase the utility of *in vitro* and *in silico* data for decision-making, efficacy and safety assessment. The workshop will be attended by expert scientists from academia, government and regulatory agencies and the (agro)chemicals, consumer products, and pharmaceutical industries

Attendance is free, but registration is essential. The closing date for registration is 9 January 2017.

British Toxicology Society (BTS) Annual Congress 2017

3 – 5 April 2017, LIVERPOOL, UK

At next year's BTS meeting, there will be a symposium jointly organised by the NC3Rs focused on complex *in vitro* models (and other non-animal approaches) to drive exposure-based risk assessment. There will also be a session on AOP-based approaches to safety assessment.

For further details, visit the BTS website.

Ask an expert

Drs João Barroso, and Silvia Casati, both at the Chemicals Safety and Alternative Methods Unit/EU Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) at the European Commission's JRC, answer the question:

'What's next for the application of the skin sensitisation AOP in safety assessment?'

In 2013, prompted by the increasing knowledge on the key mechanisms of the skin sensitisation pathway, the European Commission JRC's EURL ECVAM released a strategy paper that outlined what needed to be accomplished to fully establish alternative approaches to the hazard assessment and classification of substances for skin sensitisation. To this end, on behalf of the EU, EURL ECVAM has played a leading role at the OECD in the adoption of the first three Test Guidelines (OECD TGs 442C, 442D and 442E) on validated non-animal test methods addressing specific chemical and biological mechanisms covered by KEs of the skin sensitisation AOP. Two additional *in vitro* methods (LuSens and U-SENS) have been peer reviewed in 2016 by the EURL ECVAM Scientific Advisory Committee and others are currently under evaluation. These efforts paved the way for the revision of the REACH legal text and the ECHA Guidance on Information Requirements and Chemical Safety Assessment, making the use of alternative methods for skin sensitisation testing the primary choice.

Owing to the fact that currently adopted non-animal methods must be actually combined in some way to arrive at a conclusion regarding skin sensitisation potential and potency of a chemical, EURL ECVAM led the drafting of OECD Guidance Documents 255 and 256 on the reporting of Defined Approaches (DAs) based on the integration of various relevant mechanistic data, including those derived from the validated *in chemico* and *in vitro* methods, using fixed Data Interpretation Procedures. These approaches show how the AOP can be used to inform regulatory decisions on the potential hazard and/or risk of chemicals and some have comparable or better performance than the Local Lymph Node Assay (LLNA) in predicting skin sensitisation responses in humans. Importantly, their consistent reporting was a first step towards a globally harmonised approach for skin sensitisation assessment using non-animal data. The next step is to capture these DAs within a suitable and recognised regulatory instrument such as an OECD TG so that they are ultimately given the same status as existing *in vivo* TGs, for example in relation to the Mutual Acceptance of Data by OECD member countries. The EU (through the JRC's EURL ECVAM), the USA and Canada have recently submitted a joint project proposal to the OECD to move in this direction. The goals of the project are to develop an assessment framework for non-animal DAs that are intended to serve as alternatives to the current animal tests, to evaluate the existing DAs on the basis of that framework and, ultimately, to draft a Performance Based Test Guideline including those DAs able to meet the assessment criteria.

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

In the next issue:

- An overview of the upcoming SETAC Pellston workshop: Advancing the Adverse Outcome Pathway Concept – An International Horizon Scanning Approach
- 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.