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

Workshop report:

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

Workshop: 28 April 2016
Report published: 29 July 2016

Natalie Burden
NC3Rs

Fiona Sewell
NC3Rs

Ian Kimber OBE
University of Manchester

Pioneering Better Science

Background

In recent years, there has been a growing interest in the application of mechanistic or pathways-based approaches for human and environmental safety assessment of chemicals and pharmaceuticals. This includes the development of the Adverse Outcome Pathway (AOP) concept. This concept links a molecular initiating event (MIE), caused by a chemical or drug interaction at a molecular or cellular level, with an undesired ('adverse') effect in an organism or population, through a scientifically proven chain of causally related 'key' events (KEs). *In vitro* and *in silico* methods can in some instances be used in place of animal toxicity tests to determine whether a chemical or drug induces the KEs within the biochemical pathway of interest, and thereby predict the likelihood of a subsequent adverse outcome. Identification of hazardous compounds earlier in drug or product development will reduce the number of compounds that go on to further compulsory tests in animals. This has potential to reduce the levels of attrition resulting from undesired effects discovered late in the development process, particularly for pharmaceutical candidates.

In 2014, the NC3Rs launched a programme to support scientists in the development and application of pathways-based approaches to improve the identification and characterisation of hazards with reduced reliance on animals. With the support of an expert Steering Group comprised of scientists from academia, industry and regulatory agencies, we held our first workshop in this area, 'Applying pathways-based approaches across the biosciences', in May 2014. This resulted in a peer reviewed publication (Burden *et al.*, 2015), and the launch of a project to develop an AOP in the field of cardiotoxicity in collaboration with EURL-ECVAM and an expert working group. We subsequently launched a resource webpage and a regular 'AOP News' bulletin to support scientists and regulators interested in further developing and applying the AOP concept.

Since the 2014 workshop there has been substantial progress in the science related to the area of pathways-based approaches and AOPs, and the continued establishment of frameworks designed to advance the application of pathways-based approaches. Our focus has now broadened to examine the application of pathways-based approaches in practice and in April 2016 we held a second workshop which focused on the current status and potential future applications of these approaches. The key objectives of the 2016 workshop were to:

- Increase awareness among the scientific community of developments in the field;
- Expand and consolidate 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.

To sign up to alerts for the latest AOP News, please email aops@nc3rs.org.uk

Background

A copy of the workshop programme can be found in Annex 1. The presentations covered:

i. case studies that demonstrated the current application of pathways-based approaches;

ii. lessons learned from previous initiatives in this area, and current initiatives underway to support their application;

iii. 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 that addressed the question: 'The big conundrum – what constitutes validation?'. The workshop was attended by almost 100 scientists drawn from the pharmaceutical, industrial chemical and agrochemical industries (45%), academia (29%), regulatory agencies (7%), and other relevant organisations (19%) including: consultancy companies and contract research organisations, the European Commission, and the Organisation for Economic Cooperation and Development (OECD). To gain an understanding of the audience's intended use of pathways-based approaches, an interactive voting system was used at the start of the workshop. In response to the question 'what are you looking to develop AOPs for?' 29% of the audience responded 'to increase mechanistic understanding'; 20% 'for regulatory purposes'; 10% 'for screening or prioritisation of products or compounds'; and 2% 'other'. The majority (39%) responded that they were interested in all three types of application.

This report summarises the main themes presented and discussed at the workshop, and provides a basis to inform future activities in this area to enable the wider application of pathways-based approaches within routine practice.

Application of pathways-based approaches: current status

Case studies were presented during the workshop to demonstrate current practical applications of pathways-based approaches. Generally, these are not yet applicable in a regulatory context. There is however scope for their application in screening programmes to identify harmful substances early in the development process. This would allow movement away from a 'black-box' approach so that more informed decisions can be made regarding which candidates to take forward into regulatory studies involving animals. This would serve to reduce the number of animals used, save time and resource, and accelerate the development process.

There are many new technologies available which provide opportunities to develop and apply innovative approaches to hazard and risk assessment, and which will inform the development of AOPs. Alternative non-protected species[1] (such as zebrafish embryos and the invertebrates *C. elegans* and *Drosophila*) are also beginning to offer useful means of screening chemicals quickly and cheaply. Such species are now being used within the chemicals industry for the early identification of developmental and reproductive toxicity, through the investigation of conserved AOPs. Well-developed AOPs support the evaluation and validation of alternative strategies for safety assessment. For example, the AOP 'disruption to thyroid hormone signalling in fish leading to impaired swim bladder inflation'[2] has informed the development of non-animal assays which measure the propensity for substances to elicit KEs in the pathways of interest.

Mechanistic data from non-animal methods are also now being used to identify biomarkers that are linked to adverse effects. Robust biomarkers – particularly those causally linked to adverse outcomes – could play a key role in predictive toxicology, and in the assessment of toxic potency. Information on the ability of substances to induce such biomarkers could also be used to support read-across arguments, and help to avoid the need for *in vivo* studies.

Finally, there are specific areas of toxicity testing, such as the identification and assessment of endocrine disrupting chemicals, where there remains a strong need and regulatory drive to develop and apply AOP-structured information.

1. In this context, non-protected means not classified as a protected animal under the UK's Animals (Scientific Procedures) Act 1986: www.legislation.gov.uk/ukpga/1986/14/contents.
2. Covered in the AOP wiki index numbers 155-159 (https://aopwiki.org).

Much progress has already been made to (a) identify and characterise relevant AOPs and (b) develop integrated approaches that allow application of the knowledge held within AOPs. This information is currently being used to inform new activities in this area ultimately to support the wider application of AOPs. Updates were presented on the following activities and initiatives:

- The OECD's AOP development programme is an integral component for many of the OECD's activities related to toxicity testing and safety assessment, and has evolved considerably since its inception. The OECD-sponsored AOP KnowledgeBase is a central repository for AOPs developed through the OECD's AOP development effort. It is designed to enable the use of mechanistic information for regulatory purposes in a systematic manner. The KnowledgeBase is already being used by a wide range of AOP developers. Recently, the OECD recognised the need for a framework to support the development of Integrated Approaches to Testing and Assessment (IATA) strategies for defined hazard endpoints, so that the mechanistic information that is stored within AOPs (such as those in the KnowledgeBase) can be utilised within defined regulatory contexts.

- SEURAT-1 (Safety Evaluation Ultimately Replacing Animal Testing) was a large-scale initiative funded jointly by the European Commission and Cosmetics Europe that aimed to fill gaps in scientific knowledge and accelerate the development of non-animal approaches for the assessment of the potential for systemic toxicity following repeat dose exposure. This five year initiative, which ended in December 2015, set out to systematically organise existing information and set up testing strategies for toxicity prediction using a case-study approach. This was a first step in determining whether existing information and new testing strategies based on improved mechanistic understanding could provide sufficient evidence to support integrated safety assessment relying only on alternative (non-animal) methods. Experience gained during the course of this project will be used to support the new EU-ToxRisk project.

- The EU-ToxRisk project is a six year, large scale Horizon 2020 supported initiative launched in January 2016 with the ultimate goal of delivering reliable, animal-free hazard and risk assessment of chemicals. The project is utilising an AOP-driven approach and focuses on the areas of central nervous system, lung, liver, kidney and developmental and reproductive toxicity. This work will continue to make use of the case study strategy employed in SEURAT-1 and has a specific goal of ensuring that the new approaches developed are accepted and implemented in regulatory contexts.

The next steps towards wider application in practice

Although much investment is being made by organisations such as the OECD to support the systematic development of high quality AOPs, their application in regulatory risk assessment and for risk management decisions will be limited unless there is increased confidence and agreement on whether substances of interest genuinely trigger these AOPs in their entirety. There is also a need to be able to extrapolate quantitative information on the relationships between tipping points for KEs in AOPs to *in vivo* exposures, so that the predictions made can be considered in the context of actual levels of human or environmental exposure. Some case studies which use quantitative, AOP-based approaches to develop prototype non-animal risk assessment have been developed (e.g. MacKay *et al*. 2013, Adeleye *et al*. 2015). The continuing challenge will be to align the knowledge of toxic mechanisms/AOPs and dosimetry with exposure considerations.

Mathematical models have potential to play a significant role in predicting the propensity for substances to cause toxic effects, and there are many benefits to their use such as reduced cost and avoidance of the use of animals. As these models can be highly complex it is challenging to integrate the data they generate into risk assessments that have been historically driven by animal data. Improved understanding of the underlying biology offered by AOP development will result in decreased uncertainty and increased confidence within mathematical models, allowing the data they generate to become more accessible to risk assessors and thereby increasing the utility of these approaches.

As the aspiration to incorporate the information within AOPs into regulatory risk assessment grows, so does the need for agreement on how best to integrate information from different sources to enable decision making. The aim of the OECD's IATA framework is to provide guidance on the scientific and practical considerations that will be needed to allow the application and integration of information from multiple different approaches for safety assessment purposes.

The resulting recommendations need to be flexible enough to allow the evolving methodology to be embraced but prescriptive enough to enable their implementation and ultimate acceptance. The integration of several methods will require a certain level of standardisation, with agreed criteria so that there is confidence in the data generated from individual methods and assays, including those which measure effects on key events. As it is not practical or sustainable for all new methods to undergo formal validation processes, the development of standards for non-animal approaches will enable the wider use of new technologies as they emerge from the science base, to accelerate the application of mechanistic approaches. It is critical that validation processes evolve so that they are able to keep pace with the development of new scientific opportunities, and not be the limiting factor in their application.

Key messages from the workshop

The overarching theme emerging from this workshop is that AOPs do not need to be 'perfect' - i.e., fully developed, quantitative and capturing all known biology - to be useful. It will never be possible to characterise fully and 'finish' an AOP, because mechanistic knowledge will continue to evolve over time. The level of confidence and/or certainty needed within an AOP is very much dependent on its intended application. It is clear from the case studies presented that different levels of characterisation may be required before an AOP (or AOPs) can be used to inform the development of assays that provide useful information on the potential for a substance to exert toxic effects. The key question that should be asked is whether the AOP is fit for the purpose for which it has been developed – i.e., is the level of information within it sufficient and accurate enough to enable the scientific question at hand to be answered? AOPs that are more primitive, i.e. not well-characterised or quantitative, will still have value in informing on data gaps and supporting regulatory decision making, in the context of IATA and weight of evidence approaches.

Concluding remarks

There continues to be an appetite for a paradigm shift towards a more mechanistically-driven approach to toxicity testing for safety assessment. Progress is being made in the use of new technologies that provide information on the effect of substances on MIEs and KEs, particularly for use as early screens. It is important that the expectations of an AOP (in terms of level of information and confidence in the information it contains) reflect the purpose for which the information is being applied. To gain greater application of pathway-based approaches in a regulatory context, it is essential that the relevant communities continue to engage with the AOP concept and support the ongoing initiatives to enable the greater use of mechanistic knowledge for decision making. There is also a need to ensure that the level of scientific knowledge and expertise is appropriate to ensure the development of high quality robust and reliable AOPs. To ensure this transition into widespread use within a regulatory context, there were several issues raised that remain to be addressed. These include:

- How best to evaluate the quality and relevance of proposed AOPs.
- How to align AOPs with exposure considerations, to ensure that the AOPs are relevant to risk assessment.
- How best to integrate data generated from multiple sources.
- Establishing how AOPs can be used to decrease uncertainty within newer alternative methods used to assess toxicity.

Taking these steps will be essential to ensure that (a) the information contained within AOPs is transformed from explanations of toxicity into useful predictive tools, and (b) the opportunities that exist to conduct animal-free safety assessments are maximised, to ensure the largest possible impact on the 3Rs.

Acknowledgements

The NC3Rs is grateful to the speakers, panellists and participants of the workshop.

References

Adeleye Y, Andersen M, Clewell R, Davies M, Dent M, Edwards S, Fowler P, Malcomber S, Nicol B, Scott A, Scott S, Sun B, Westmoreland C, White A, Zhang Q, Carmichael PL (2015). Implementing Toxicity Testing in the 21st Century (TT21C): Making safety decisions using toxicity pathways, and progress in a prototype risk assessment. *Toxicology* 332:102-11

Burden N, Sewell F, Andersen ME, Boobis A, Chipman JK, Cronin MT, Hutchinson TH, Kimber I and Whelan M (2015). Adverse Outcome Pathways can drive non-animal approaches for safety assessment. *Journal of Applied Toxicology* 35(9):971-5. Open Access.

MacKay C, Davies M, Summerfield V, Maxwell G (2013). From pathways to people: applying the adverse outcome pathway (AOP) for skin sensitization to risk assessment. *ALTEX* 30(4):473-86.

Annex 1: Workshop Programme

Agenda	
08.30 – 09.00	**Registration**
09.00 – 09.10	**Welcome and introduction** Professor Ian Kimber (Chair), University of Manchester, UK
09.10 – 09.20	**3Rs benefits and potential applications of pathways-based approaches for pharmaceutical and chemical safety assessment** Dr Fiona Sewell, NC3Rs, UK
Case studies: practical applications of pathways-based approaches	
09.20 – 10.15	**Comprehensive target screening by label-free cell microarray profiling to reduce animal efficacy and toxicology studies in drug discovery** Dr James Sidaway, Phenotox, UK **Application of non-mammalian assays in the prediction of developmental and reproductive toxicity potential to mammals** Dr Chantal Smulders, Shell, Netherlands and Dr Marjolein Wildwater, HAN University of Applied Sciences, Netherlands
10.15 – 10.45	**REFRESHMENTS**
10.45 – 12.00	**An alternative testing strategy for the fish early life-stage (FELS) test** Dr Dries Knapen, Universiteit Antwerpen, Belgium **Use of mechanistic data to support read-across case study - experiences of the VPA case study** Dr Sylvia Escher, Fraunhofer Institute for Toxicology and Experimental Medicine, Germany **Adverse Outcome Pathways focusing on endocrine active chemicals** Dr James Wheeler, Dow AgroSciences, UK
12.00 – 13.00	**LUNCH and poster viewing**
Adverse outcome pathways: perspectives for future development and application	
13:00 – 14:20	**The OECD framework for AOP development and application** Dr Magdalini Sachana, OECD, France **Lessons learned from SEURAT-1** Dr Elisabet Berggren, European Commission Joint Research Centre, Italy **EU-ToxRisk – The 'flagship' program on mechanism-based toxicity testing and risk assessment** Dr Hennicke Kamp, BASF, Germany on behalf of the EU-ToxRisk consortium **Perspective on next steps for application in practice** Professor Alan Boobis, Imperial College London, UK
14.20 – 14.40	**REFRESHMENTS**

Annex 1: Workshop Programme

Next steps to enable wider application of pathways-based approaches	
14.40 – 16.00	**The importance of exposure considerations** Dr Carl Westmoreland, Unilever, UK **Dealing with uncertainty and increasing confidence when applying mathematical models in AOP-led risk assessments** Dr John Paul Gosling, University of Leeds, UK **Integrated approaches to testing and assessment** Dr Maurice Whelan, European Commission Joint Research Centre, Italy **The drivers and benefits of standards for *in vitro* assays: an SME perspective** Dr Marie-Ann Ewart, AvantiCell, UK
16.00 – 16.15	**REFRESHMENTS**
Keynote presentation	
16.15 – 17.00	**State of the Science of Adverse Outcome Pathways** Dr Kevin Crofton, National Center for Computational Toxicology, Environmental Protection Agency (EPA), USA
Roundtable discussion	
17.00 – 17.45	**The big conundrum – what constitutes validation?** Moderated by Dr Natalie Burden, NC3Rs; supported by the NAT SIG **Professor Alan Boobis**, Imperial College London, UK **Dr Kevin Crofton**, EPA, USA **Dr David Jones**, Medicines and Healthcare products Regulatory Agency, UK **Professor Ian Kimber**, University of Manchester, UK **Dr Carl Westmoreland**, Unilever, UK **Dr Maurice Whelan**, European Commission Joint Research Centre, Italy **Dr James Wheeler**, Dow AgroSciences, UK
Wrap-up and close of meeting	
17.45 – 18.00	**Professor Ian Kimber**
Networking reception	
18.00 – 19.00	Networking reception supported by the NAT SIG