Application of the Adverse Outcome Pathway (AOP) approach for cardiotoxicity endpoints

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

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EURL ECVAM European Union Reference Laboratory for Alternatives to Animal Testing

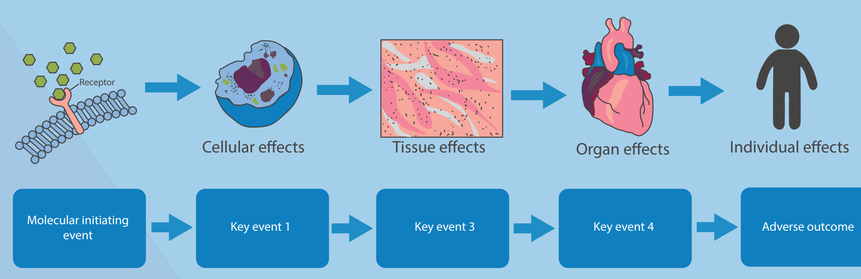
What is the AOP Framework?

An AOP (Adverse Outcome Pathway) is a way of linking a chemical interaction at the molecular level with an adverse outcome at the organ/organism level, through understanding the key events that occur at different levels of biological organisation (see Figure 1).

Establishing AOPs and identifying the data gaps within the pathways can lead to development and application of *in vitro* and *in silico* techniques that assess the propensity for a substance to elicit the key events identified in the AOP, to help predict whether an adverse outcome is likely. If non-traditional (non-animal) methods are routinely used to assess the occurrence of key events, confidence in these methods may be increased. These factors may ultimately result in a reduced reliance on the need for animal toxicity tests (see also Burden *et al*, 2015).

To enable the wider application of this mechanistic approach to product development and routine safety assessment, an AOP knowledge base has been established by the OECD to standardise the integration and organisation of the large amount of data available on different and interconnected AOPs, using an online AOP Wiki resource.

Figure 1: The Adverse Outcome Pathway



MIE: The initial point of chemical interaction on a molecular level within the organism, resulting in a perturbation that starts the AOP.	KE(s): A change in biological state that is both measurable and essential to the progression of a defined biological pertubation, leading to a specific adverse outcome. There needs to be robust scientific evidence linking the KEs, with direct relationships defining upstream and downstream KEs.	AO: Specialised key event that is generally accepted as being of regulatory significance.

Why develop AOPs for cardiotoxicity?

A number of reasons exist to develop AOPs in this area.

1. 3Rs Potential: high number of *in vivo* tests are carried out to assess potential for cardiotoxicities. Use of *in vitro* or *in silico* tests to assess effects at earlier key events could reduce the requirement for tests in animals, as well as improving predictions and/or identifying cardiovascular liabilities earlier in development.
2. 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.
3. Novelty: this area is not already represented in the OECD AOP work plan.
4. Feasibility/data availability: a lot of mechanistic *in vitro* data already exist that could be utilised without further experimental work.
5. 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.
6. Clinical need: Better protection of human health through better understanding of mechanisms and fewer adverse effects.

Methods and provisional results

The NC3Rs has established a network of collaborators from academia, industry and clinicians with expertise in the area of cardiovascular research. By mapping existing knowledge from literature, the working groups aim to define key events along the pathways of interest and to identify gaps for further investigation.

The expert working group agreed to develop AOPs for two cardiotoxicities of concern to the industry, as outlined below:

Cardiotoxicity AOP (1) – L-type Ca^{2+} block leading to heart failure:

This AOP (see Figure 3b) describes molecular processes following binding and block of the L-type Ca^{2+} channel (the molecular initiating event) via key events involving internal Ca^{2+} disruption, decreased Ca^{2+} binding to Troponin C, decreased force of contraction at a cellular/fibre level then at the tissue/organ level (reduced left ventricular ejection fraction) and finally the adverse outcome of heart failure. Whilst this is a simplified pathway description, it illustrates AOP principles describing cellular mechanisms that could be assessed *in vitro* prior to animal testing.

Cardiotoxicity AOP (2) – Structural cardiotoxicity:

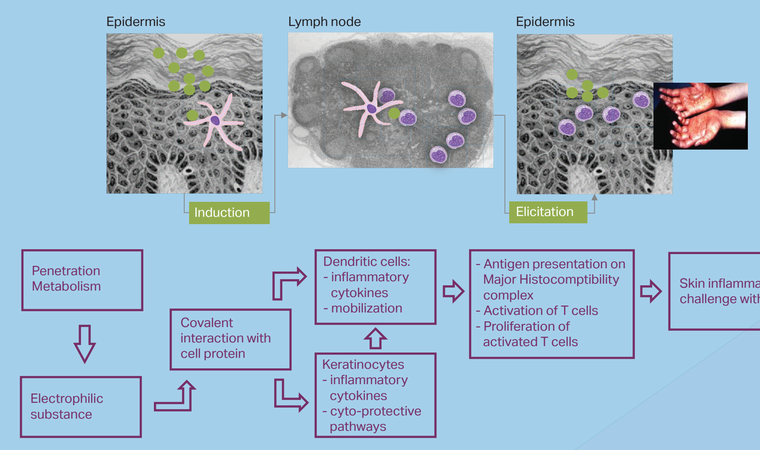
This workstream (see Figure 3c) is ongoing. Since much information around this area is still lacking, gaps are apparent and the pathway identifies some key events around mitochondrial disturbance, sustained low level cardiac troponin release leading to myofibrillar degeneration.

What other examples of AOPs have been implemented?

Skin sensitisation

The most well-developed AOP to date is entitled 'Skin Sensitisation Initiated by Covalent Binding to Proteins', accepted by the OECD in 2012. This AOP is summarised as eleven steps, which include four key events (see Figure 2). Several non-animal test methods have been developed to predict skin sensitisation potential by measuring the impact of known chemical sensitisers on some of these key events. Some of these assays are starting to be used in a regulatory context.

Figure 2: The Skin Sensitisation AOP

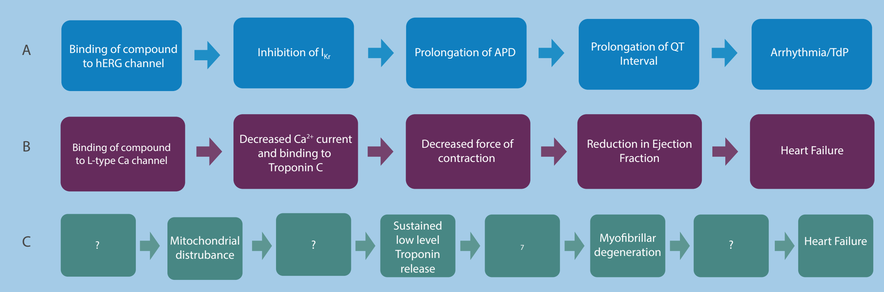


Taken from the NC3Rs AOP page: https://www.nc3rs.org.uk/pathways-based-approaches-resource-page

The hERG-QT-arrhythmia pathway

Within the Safety Pharmacology field, the familiar hERG-QT-arrhythmia pathway can be classified as an AOP, since it maps the initiating event at an ion channel level (binding of compound to hERG channel and inhibition of IKr), leading to key events at the cell and tissue level (prolongation of the action potential) and organ level (prolongation of QT-interval of the ECG), resulting in the adverse event in animals or humans of arrhythmia and potential death (see Figure 3a).

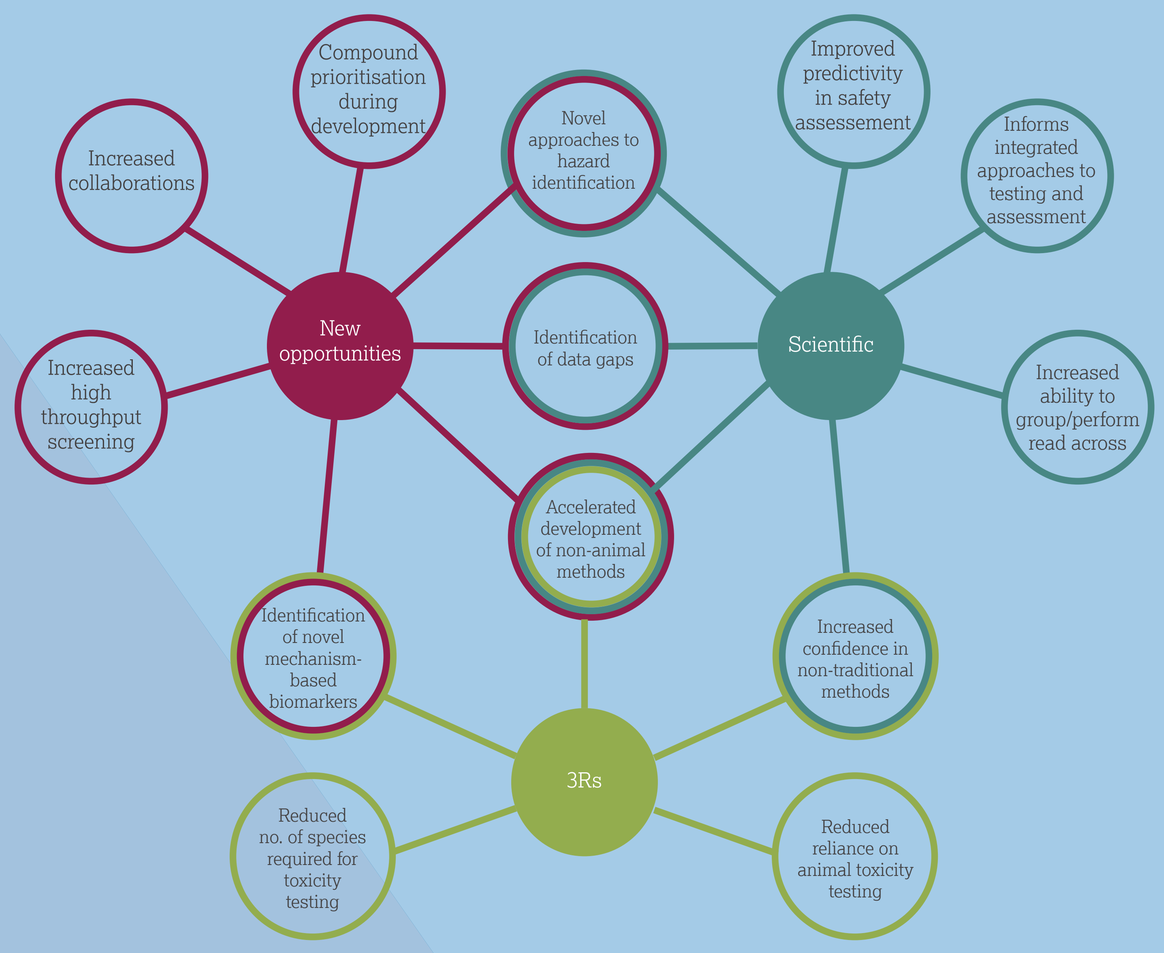
Figure 3: The Adverse Outcome Pathway applied to a) the hERG-QT-arrhythmia relationship b) L-type Ca^{2+} block leading to heart failure and c) Structural Cardiotoxicity.



Conclusions

There are many new opportunities, as well as scientific and 3Rs benefits offered by the AOP framework. These are summarised below.

Figure 4: The benefits of AOPs



References and resources

- Burden *et al*, 2015. Adverse Outcome Pathways can drive non-animal approaches for safety assessment. *Journal of Applied Toxicology* 35: 971-975.
- The OECD Framework: http://www.oecd.org/chemicalsafety/testing/adverse-outcome-pathways-molecular-screening-and-toxicogenomics.htm
- The AOP WIKI: https://aopwiki.org/wiki//index.php/Main_Page
- The NC3Rs AOP Resource Page: https://www.nc3rs.org.uk/pathways-based-approaches-resource-page