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Relationship Between Adverse Outcome Pathways and Chemistry-Based *in Silico* Models to Predict Toxicity

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Abstract

The current landscape of Adverse Outcome Pathways (AOPs) provides a means of organising information relating to the adverse effects elicited following exposure to chemicals. As such, AOPs are an excellent driver for the development and application of *in silico* models for predictive toxicology allowing for the direct relationship between chemistry and adverse effects to be established. Information may be extracted from AOPs to support the creation of (quantitative) structure-activity relationships ((Q)SARs) as well as to increase confidence in grouping and read-across. Any part of an AOP can be linked to these various types of *in silico* models. There is, however, an emphasis on using information from known Molecular Initiating Events (MIEs) to create models including 2D and 3D structural alerts, SARs and QSARs. MIEs can be classified according to the nature of the interaction e.g. covalent reactivity, oxidative stress, phototoxicity, chronic receptor mediated, acute enzyme inhibition, unspecific, physical and other effects. Different types of MIEs require different approaches to their *in silico* modelling. Modelling Key Events and Key Event Relationships is useful if they represent the rate limiting step or key determinant of toxicity. Modelling of metabolism and chemical interactions will become part of AOP networks, which are also driving species-specific extrapolation and respective adaptation of models. With more information and data being captured, *in silico* approaches will increasingly support the application of knowledge from AOPs to build weight of evidence and support risk assessment, e.g. in the context of Integrated Assessment and Testing Approaches (IATAs).

Introduction

As a gradual process, the toxicological testing of chemicals is undergoing a paradigm shift. For decades there has been a reliance on *in vivo* and latterly, to a more limited extent, *in vitro* testing of a chemical with the effects being recorded and used to allow for some type of risk assessment. The shift is towards an assessment of the (perturbation of) pathways of toxicity allowing for a mechanistic basis to understanding the effects of chemicals, which is often overlooked in the traditional testing paradigm. This has a number of advantages, not least in the replacement of *in vivo* testing with associated reduction in animal use and, in most cases, costs and the time involved. Advances in what is termed 21st Century Toxicology are developing tools and techniques that provide information on pathways (and hence mechanisms) that are derived from cells and cell lines more relevant to humans and other target species, rather than relying on the extrapolation from surrogate test species.[1]

The development of the toxicological pathway concept, not least stimulated by the 2007 National Academies of Sciences, Engineering, and Medicine report,[2] has seen a growth in the number of assays and hence data available, e.g. those directly associated with pathways such as from high throughput / content assays such as Tox21 / ToxCast[3] and omics[4-6] or those that may be applied indirectly from existing *in vivo* and other data.[7-9] These assays have provided an explosion in the number of pathway related data available which could form the basis of either strategies to predict toxicity, or be used to develop computational models. However, there has been no overarching means of interpreting, rationalising or utilising these data. As such, the Adverse Outcome Pathway (AOP) concept was developed in part as a response to the call for toxicology to become pathway orientated, but also to assist in the utilisation of data from such assays.[10]

It is appreciated that AOPs are a conceptual framework to support the prediction of toxicity, a full description of AOPs is not given here but is available from various authors.[10-19] In practical terms AOPs will assist in the organisation of data, assays, mechanistic knowledge and models to predict toxicity within the paradigm of 21st Century Toxicology. The ability to use the pathway-derived data to extract further information and knowledge is one of their advantages, especially when they can be formalised into computational models.[20-21] When associated with chemical structure, these models, also called *in silico* approaches, can provide a direct linkage between chemistry and adverse effect leveraging the content of the AOP to support the meaning and interpretation of the model.[22-23]

In silico models for toxicity prediction vary from structural alerts derived from structure-activity relationships (SARs) through to quantitative structure-activity relationships (QSARs) which are suitable for the prediction of potency.[24] This paper explores the linkage of these models, as well as grouping and read-across, to AOPs. At the outset it is well acknowledged that AOPs can support computational modelling.[21] In addition, the use of AOPs to support computational modelling deriving structural alerts[25-28] for toxicity prediction or as part of a grouping strategy leading to read-across, and for QSAR development, is well established.[29] Other computational approaches, beyond the *ab initio* risk assessment consideration, that utilise and extend the AOP framework are the development of Integrated Assessment and Testing Approaches (IATAs).[13, 18, 30-38]

The Links Between *in Silico* Models and the Different Steps of an AOP

Figure 1 illustrates that models, or *in silico* approaches, may potentially be utilised at all stages of the AOP to provide knowledge and information (it should be noted that the

structure of the generic AOP shown in Figure 1 is illustrative only and many AOPs do not include exposure or proceed to the ecosystem level). There are many purposes to the use of models within the AOP framework; these include:

- providing data and information,
- capturing the knowledge of the AOP, and
- extrapolation and prediction.

Computational, or *in silico*, models for toxicity prediction are many and varied. The focus of this paper is the relationship of chemistry-based models for toxicity prediction with AOPs. Briefly, as discussed in more detail below, *in silico* models are especially useful to capture and predict the Molecular Initiating Event (MIE) of an AOP, such as binding to biological molecules or receptors, and thus predict which chemicals can potentially be associated with adverse effects triggered by the MIE. (Q)SARs can also model Key Events or Key Event Relationships further downstream in an AOP, which is relevant for toxicity prediction in particular if these events are rate-determining steps in an AOP. In this case biokinetic modelling will support the prediction of which steps in an AOP actually take place in specific exposure situations, and can contribute to the prediction of which target organs a chemical will be distributed to and the actual target organ concentrations. Modelling exposure related to the source and models determining e.g. oral or skin absorption or bioavailability as a whole, will support quantification of the effects occurring as a result of the AOP. Overall, a higher level of modelling, such as multiscale modelling, will be required to capture all elements of the AOP to link exposure to risk. Extending the consideration of adverse outcomes to the individual, population or ecological models can extrapolate and predict adverse effects at the population or ecosystem level.

FIGURE 1 HERE

Figure 2 summarises the main differences where models may drive the development of AOPs and *vice versa*. It illustrates that on one hand AOPs can support the building of predictive models, to base them on mechanistic information and give them relevance for toxicity assessment, and on the other hand, computational models can help to compile AOP pathways. When both robust mechanistic information and computational models for e.g. bioactivities are available, this will allow for the better prediction of relevant adverse effects or use in an IATA. As such, the ultimate aim of building models and organising mechanistic information within the AOP framework is to provide relevant prediction of adverse effects to allow for adequate chemical safety assessment.

FIGURE 2 HERE

It is important to also consider that there will be a cyclical process whereby models will be developed on the basis of a (putative) AOP which will then be refined as more information is passed back to the model – likewise this will assist in the refinement of the AOP. More broadly, the relationship between AOPs and *in silico* chemistry based models can be considered as being:

i) models using information derived from the AOP,

ii) models that support the use or application of information from the AOP e.g. an AOP being used to provide evidence for the *ab initio* risk assessment of a substance and build a mode-of-action hypothesis,[39]

iii) AOPs supporting the mechanistic interpretation of a model or providing evidence to build a weight of evidence to support risk assessment (cf. read-across case studies).[40-43]

This paper attempts to provide an overview of the types of models that may be applied at different stages of the AOP, taken in the broadest context from sources to ecosystem, with a particular focus on the mechanistic and toxicological aspects. This paper covers the mechanistic aspects of the AOP, it does not consider exposure modelling, *in silico* models for the apical endpoint (e.g. traditional (Q)SARs) or ecosystem level models.

Predictive Models Based on AOP-Related Mechanistic Information

Models of the Molecular Initiating Event

Information from the Molecular Initiating Event (MIE) is one of the key drivers for *in silico* models derived from an AOP.[25-28] Before computational modelling approaches are considered, it should be recognised that there are different types of MIEs and each may require a different modelling approach. These have been summarised in general terms in Table 1, along with examples and the types of models that can be derived from the different types of MIE. [22, 23, 44-74] Table 1 provides by no means an exhaustive list and practitioners will no doubt wish to expand this according to their needs and experience. The key to understand why this is important is in the concept of, if the MIE is known, then a model can be developed for it.

TABLE 1 HERE

On the basis of knowledge of different types of MIEs, it is thus possible to classify AOPs with regard to the type of MIE. In order to illustrate the types of MIEs, Table 2 lists the citable AOPs (at the time of preparation of the manuscript) listed on the AOP Wiki

(https://aopwiki.org/)[75] which is one component of the OECD-sponsored AOP Knowledge Base (AOP-KB, http://aopkb.org/).[76] This wiki represents the central repository for all AOPs developed as part of the OECD AOP development effort by the Extended Advisory Group on Molecular Screening and Toxicogenomics. The AOP Wiki is not, of course, a complete list of AOPs with many others becoming available. However, what is clear from Table 2 is that the majority of citable AOPs on the AOP Wiki are for what is termed "chronic receptor mediated" toxicity, with a smaller number classified as being "covalent reactivity". This is probably due to the AOPs being defined, at least at the start of the process of their compilation, around common, well-known and familiar modes of action. Table 2 was also supplemented by literature examples of AOPs where none were available as being citable from the AOP Wiki. It must be appreciated that these literature AOPs are likely to be less developed (in the formal sense of the AOP Wiki) at the time of writing.[12, 29, 62, 67, 77-78]

TABLE 2 HERE

It is striking from Table 1 that the majority of models derived from AOPs utilise 2-D approaches to capture molecular fragments responsible for toxicity. There is a rich history of such "structural alerts" and their conversion into usable computational models.[20] There are many good reasons for this, e.g. they are easy to define and comprehend – hence aiding in their transparency. Technologies such as SMARTS strings have made chemistry fragments easy to code and be handled computationally.[79] They have also been developed into a number of commercially and freely available expert systems. Most notable amongst the freely available systems is the OECD QSAR Toolbox which for some endpoints,[80] e.g. skin sensitisation, provides a direct linkage to the AOP and the possibility to build a read-across argument on the basis of data from the AOP.[81, 82] They are particularly suited when a

structural fragment drives a particular chemical and /or biological interaction e.g. covalent binding to DNA or proteins.[44-45] The disadvantage is that 2-D structural alerts implicitly are not able to capture 3-D properties and configurations that are required for receptor binding. 2-D alerts, e.g. those coded as SMARTS strings, can be extended and made more sophisticated when considered as "chemotypes", i.e. alerts extended with further information on molecular structure such as atomic charges; these chemotypes may be coded in the so-called Chemical Structure Reactivity Markup Language (CSRML).[3, 83]

Receptor mediated MIEs leading to toxicity are more difficult to capture in terms of models. However, there is a growing appreciation for the needs for such models to assist in the prediction of chronic toxicity. 3-D or conformationally dependent properties can, to a limited extent, be captured with extended SMARTs strings which represent particular scaffolds.[22, 23] However, this is a relatively crude approach that may be suited for grouping and read-across but less for toxicity prediction when understanding stereoisomerism may be important. Chemotypes are more suitable for the capture and modelling of 3-D effects as they can supplement 2-D structural alerts with other properties.[83] Most suited, although requiring the greatest level of expertise for application, are techniques derived from drug design e.g. molecular modelling of the receptor-ligand interaction and / or development of toxicophores.[57-58] There are also many reported studies (beyond the scope of this paper) on modelling effects associated with endocrine disruption – most notably binding to the oestrogen receptor. As an example, the recent CERAPP project, where a large variety of mostly-QSAR type models were developed, demonstrates the relevance of this approach.[84] Extending the endocrine disruption paradigm, the work of Wu et al[59] mapped and compiled known mechanisms of developmental toxicity, supporting them with data and structural alerts. This is a scheme which allows for the interpretation and prediction of developmental

toxicity rapidly, but at this time is, at best, only partially supported by AOPs. However, this approach maps out where development of AOPs is required.

There are a variety of modelling approaches relating to toxicities associated with unspecific MIEs, i.e. where there is no single definable interaction analogous to receptor binding or covalent interactions. For instance, models may be based on 2-D properties associated with giving the molecule the capability to act in the required manner, e.g. the amine functionality promoting membrane accumulation leading to phospholipidosis.[68,69] Alternatively, models may be related to the properties that govern the unspecific effect e.g. hydrophobicity being the determining feature for non-polar narcosis.[66]

Overall, there is a need to identify the MIE and appropriate modelling techniques for it – this is crucial information that can be derived from an AOP. (Q)SARs should be benchmarked against the MIE to ensure that the modelling approach is valid for the endpoint of interest. Inappropriate modelling approaches run the risk of spurious and overfit models.

Models of Key Events and Key Event Relationships

As indicated in Figure 1, the modelling of key events is further along the AOP towards apical endpoints and may be considered more relevant and quantitative than the MIE. Models may be developed for data derived from assays relating to individual Key Events, these may, for instance, be in the form of QSARs. However, there are two distinct issues in the modelling of data for Key Events, namely the difficulty in obtaining such data (although initiatives such as ToxCast and other High Throughput Screening projects may assist here)[3] and their relevance, especially when considered in isolation from the MIE, in comparison to, for instance, the MIE itself. However, when relevant data are available that are founded in

the preceding Key Events within the AOP, the modelling of Key Events and Key Event Relationships may provide a more quantitative prediction of (adverse outcome) potency providing they are the rate limiting step. In addition, should a Key Event or Key Event Relationship not occur routinely following interaction with the MIE, and require further stimulation, e.g. another MIE or reaching a critical concentration (a Point of Departure for the Key Event), then models may provide more useful information than one based on the MIE alone. However, should the Key Event provide no more meaningful information than the MIE, then it may be more appropriate to model the MIE as it is more fundamental and likely to be more rapid and less expensive to obtain data for.

Any models for Key Events will be similar to those for MIEs, i.e. 2-D structural alerts for specific molecular fragments, toxicophores for receptor interactions. However, there are relatively few assay data available relevant to Key Events and even fewer, if any, models. One good source of information has been from the Ames test and related assays for genotoxic carcinogenicity. The Ames test in this instance is a good surrogate for the MIE involving interaction with DNA but less likely to be appropriate for the later Key Events in this AOP. Another source of data would be for skin sensitisation where there is a well developed AOP[13] and where the AOP has been converted into an IATA.[33] Despite the proliferation of assays for the different stages of the AOP, with the exception of models for protein reactivity,[49, 85] no viable models for e.g. HClat, etc have been published. Thus *in silico* models would have the potential to eventually replace assays within the IATA, but have not yet been fully recognised, such models would assist in hazard identification and ultimately risk assessment. The reliability and relevance of *in silico* models to predict an endpoint within an IATA will be increased if the models are mechanistically based and cover different

steps of an acknowledged AOP in the context of a consensus approach, i.e. concordant results with independent prediction models.

There is also the possibility to model Key Event Relationships, such models may assist in the quantification and understanding of the relationship. The benefit here is that, should the Key Event Relationship be the rate limiting step, then a model for that specific relationship, which may resemble a QSAR or quantitative activity-activity relationship (QAAR), could hold the key to making a quantitative prediction of potency. Currently there are few, or no examples of (Q)SAR or QAAR models for Key Event Relationships, although some *in silico* models for Key Event Relationships are becoming available, especially in the form of quantitative AOPs – as described in the next section.

Ongoing efforts to formalise relationships in AOPs through ontologies[86] and to capture response-response relationships and corresponding data e.g. in the Effectopedia module of the AOP-KB (http://effectopedia.org/)[87] will further support future model development in this area.

Models Related to the Whole AOP

It is conceivable that the ultimate aim of modelling the AOP may result in the development of an algorithm, or sequence of models[21], to include all steps of an AOP. However, it is recognised that this is a long-term aspiration and will not, necessarily, be required if there is the possibility to predict the adverse outcome with a defined level of confidence. Currently there are no chemistry-based approaches i.e. (Q)SARs that attempt to describe the AOP as whole. In the longer term, there may be value in this as it may assist in the implementation of models from the AOP in the form of IATA.[88, 89] However, there will be limited value going

beyond chemistry-based models for the MIE and possibly the rate-limiting Key Event Relationship. There is increasing interest in quantitative AOPs (qAOPs) which attempt to formalise the Key Event Relationships from the MIE to the Adverse Outcome, e.g. Conolly et al[89] developed a qAOP that linked the inhibition of cytochrome P450 19A aromatase (the MIE) to population-level decreases in the fathead minnow. The qAOP itself consisted of three linked computational models for relevant parts of the AOP.

A further approach that has yet to be fully applied to AOPs is that of multi-level, or multi-scale modelling.[90] These approaches attempt to derive inter-connected models for each component of the AOP in a manner that is representative of the overall AOP. As such it is a data hungry approach. These models may be fed with data from public data sources such as ChEMBL[22,23, 91-93] and ToxCast/Tox21.[94] The use of ToxCast data in this context remains an opportunity and a challenge, with more data and a better appreciation of how to use the data required.[95]

Specific Considerations for *in Silico* Modelling with Regard to AOPs

Transformations and Interaction of Chemicals

Until now, metabolic activation has seldom been considered as part of an AOP, however it is necessary for chemistry-based *in silico* modelling (and the use of information from an *in vitro* assay) if a realistic toxicological profile is required and only the parent structure is available. For instance, it is important to determine if the parent molecule will be metabolised into a more toxic form or detoxified. A large number of *in silico* approaches are available to predict potential metabolites,[96] some freely available and others commercial. The performance of such predictive methods is not always ideal with over-prediction of

metabolites being one problem, and it still being very difficult to identify stable metabolites and kinetics/rates of transformation being another.[97] There is, however, the possibility that knowledge of metabolic events in AOPs may provide better knowledge to support improved toxicity prediction.

Another possibility to use knowledge from AOPs to support the capture of information and development of *in silico* models is with effects such as chemical interactions. In this case, should a key stage of an AOP require the inhibition of an enzyme (either from the target or another molecule), this knowledge could be incorporated into a model. As yet, there are no approaches that have used the information from an AOP in this way – this is something that would be of immense benefit for understanding and modelling drug-drug interactions as well as predicting effects from combined exposure to chemicals, taking into consideration possible synergistic effects – even over time - which are not accounted for when evaluating chemicals individually.

As AOPs naturally evolve from linear constructs to networks of inter-related effects[21], metabolism and interactions will become integral to the development of AOP networks within an ontology framework, a formalised way to organise AOP knowledge and capture AOP relationships.[86] This provides the possibility, for example, to include models for metabolism, particularly the ability to bind to a CYP or enzyme and the rate of binding / inhibition. In addition models will be required to predict relevant metabolites that may go elsewhere in the AOP network, or to completely independent AOPs. The other possibility is that knowledge of metabolism, metabolite formation and rates of formation which is contained within the AOP network will provide information for the models themselves, thus the AOP will drive the model.

Interspecies Relationships of Toxicity and Sensitivity

AOPs provide a rational means to extrapolate toxicity across species. The relative similarity of acute aquatic potency within groups of compounds acting by a similar mechanism of action, especially non-polar narcosis has been known for many years[98] and can be applied to successful QSAR development.[29] Similarly, the need to take care for other mechanisms of action, particular those associated with species-specific metabolism[99] or reactivity[100] is important and can be related to the individual AOPs. Whilst generalist extrapolation approaches may work for lethal potency, more sophisticated modelling will be required for receptor mediated responses. Knowledge of the essential receptors in an AOP can help drive the extrapolation of AOPs from one species to another. Thus whilst AOPs are chemical-agnostic, they are also species-specific and therefore can drive the adaption/applicability of predictive models. The web-based SeqAPASS tool is one such approach, utilising information on receptor homology to allow for extrapolation of effects, being wholly supported by the AOP.[101-102]

Models for Exposure and Bioavailability – Adding Value to AOPs

Whilst not strictly part of the AOP, computational models for exposure and bioavailability are useful for their implementation.[103] These can impact at various points whilst using information from an AOP. Models for exposure and bioavailability also vary in their complexity. Another area of progress is the development of Aggregate Exposure Pathways which will provide information to derive models from.[104]

There are a number of SAR approaches that can be used to screen out compounds that are not likely to reach the site of the MIE. Very commonly used (especially as a drug design tool) is the Lipinski Rule of 5[105] and various adaptions.[106] The premise here is that a small number of easily calculated properties can identify compounds with low solubility which is taken as being indicative of poor oral absorption – thus if this is required for systemic exposure then risk assessment may be able to discount oral exposure as a likely route to stimulate the AOP. In a similar manner, Ates et al developed a small number of physico-chemical properties that are seen to be determinants of poor absorption through the skin.[107]

There are also QSARs for effects such as bioavailability which may be used as part of the risk assessment process. In addition QSARs can provide an estimate of exposure following absorption through the skin,[107-108] blood-brain barrier,[109] cornea[110] and various other membranes.[96] Whilst such QSARs may be useful, to assess organ level concentrations following different types of exposure and doses requires the use of physiologically based kinetic (PBK) models.[111-112]

AOP-Derived Models Driving Grouping and Domain Definition

In addition to supporting models such as (Q)SARS, the information from MIEs is a key means of grouping compounds providing a mechanistic basis and transparency.[113-114] *In silico* 2-D profilers of structural alerts, built on knowledge of MIEs have provided a means to group compounds for a number of adverse outcomes including skin sensitisation;[79] respiratory sensitisation;[46] phospholipidosis;[68-69] mutagenicity;[44, 115] hepatotoxicity;[116] reproductive toxicity[59] and testicular toxicity.[117] Successful grouping allows for read-across to fill data gaps for these types of endpoints.

The MIE can also be used to define the chemical domain of an AOP through intelligent testing using assays derived around the MIE. For instance, the definition of mechanistically derived domains of reactivity, as measured by *in chemico* testing, has been shown to assist in the identification of chemical domains associated with protein reactivity, which in turn may be related to effects such as skin sensitisation or elevated acute toxic potency.[118-119]

The value of AOPs to support read-across was demonstrated in a series of case studies.[40-43] The case studies demonstrated how the read-across hypothesis and justification were strengthened with knowledge of data from New Approach Methodologies (NAMs) that were related to the AOP. [120]

Conclusions and Recommendations

AOPs provide a framework to organise information within the context of 21st Century Toxicology. As such, they are a good driver for the creation, development and application of in *silico* models to predict toxicity. Indeed, AOPs provide the direct mechanistic relevance and transparency that is a pre-requisite for *in silico* models of toxicity. The ability to be able to reference back to an AOP has been shown to be crucial for the justification of grouping and read-across and to allow for the development of IATA. As further information and data are captured, *in silico* models provide the means to create knowledge and apply it in a rational manner to predict toxicity.

Currently most *in silico* models from AOPs are derived from the MIE. Qualitative models can be derived from knowledge of the MIE, in theory with limited knowledge and without many data to support the hypothesis e.g. an alert for skin sensitisation may be developed on the

basis of only a single data point if there is a clear mechanistic (i.e. organic chemistry) understanding. Quantitative models for the MIE, as well as for Key Events and Key Event Relationships require a more complete data set with information from a greater number of compounds covering a range of activity and properties. MIEs, in terms of modelling, can be classified into a number of types (see Tables 1 and 2). It is useful to classify MIEs in this manner. Capturing the type of MIE will allow for possibilities for modelling from an AOP to be identified; these could be stored in the AOP Wiki in the first instance and then progressed to Effectopedia as well as tools such as the OECD QSAR Toolbox. In order to maximise the use of information from AOPs there should be a shift to storing information on the MIE in a manner that could be translated into an *in silico* model e.g. classifying MIEs in tools such as Effectopedia and providing a direct linkage from there to a chemistry based model. Currently most *in silico* models derived from the MIE are based on 2-D descriptions of chemistry (with some notable exceptions). Most models are for MIEs associated with receptor binding, with covalent interactions also well represented.

Key Events and Key Event Relationships may also prove useful for modelling, however, with notable exceptions such as receptor binding data and endpoint data such as those for the Ames assay, there is a lack of data. The lack of data is particularly acute for "intermediate" Key Events and Key Event Relationships i.e. those between the MIE and the adverse outcome. With increasing data becoming available in the framework of 21st Century Toxicology and high-throughput measurements, the data gaps may be filled in the future. However, they will only be useful if they represent the rate limiting step or key determinant of toxicity. These should form the basis of QSARs in particular as there is a desire to make the predictions more quantitative. The accurate prediction from chemical structure of

metabolites and their rates of formation have historically been very difficult; this is an area where improvements are required, especially as networked AOPs become a reality.

Modelling of the whole AOPs, or qAOPs, will also require further data. At the moment a multitude of full chemistry-based computational models for a complete AOP seems unlikely. However, consideration of the AOP framework, especially as the new and / or updated tools become available, will both support model development and allow for the identification of gaps where either models or AOPs are required.

In conclusion, ideally *in silico* models will ultimately allow for the complete replacement of toxicological testing whereby only a chemical structure is required to make a hazard and / or risk assessment – however we are a long way from achieving this. As the frameworks provided by AOPs (or whatever framework is utilised) to organise information and data become more sophisticated, the needs of modelling and the intimate and intricate relationship between the AOP and the models should not be forgotten but should be at the heart of AOP development and data capture.

Author Disclosure Statement

The authors disclose no competing financial interests exist.

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Table 1. Types of MIEs with associated adverse outcomes and types and examples of appropriate modelling approaches

Type of MIE	Description	Adverse Outcome	Appropriate Type(s) of Modelling Approach(es)	Examples of Models
Covalent Reactivity	Covalent binding of the compound with a biological macromolecule e.g. DNA, cellular membrane proteins, immunoproteins	Mutagenicity, skin sensitisation, respiratory sensitisation, liver fibrosis, acute toxicity evaluated above baseline	2-D structural alerts for specific molecular fragments, quantum chemical calculations of reactivity	DNA binding;[44] protein binding;[45] respiratory sensitisation; [46] DFT calculations[47-49]
Free Radical / Oxidative Stress	Cellular or tissue damage caused by free radicals promoted by e.g. redox cycling	Mutagenicity, tissue damage, aging, mitochondrial toxicity	2-D structural alerts for specific molecular fragments, calculation of redox potential	2-D alerts for excess aquatic toxicity;[50-52] mitochondrial toxicity[53]

Phototoxicity	Damage caused by a reactive molecule or radical following excitation by UV light	Mutagenicity, tissue damage, aging	2-D structural alerts for specific molecular fragments, quantum chemical calculations of chemical stability	2-D alerts and QSARs for excess aquatic (photo)toxicity[54-56]
Chronic Receptor Mediated	Stimulation or disruption of a normal physiological process, e.g. hormonal control, through (antagonistic or agonistic) binding (usually reversible) to a receptor	Many and varied adverse outcomes e.g. disruption of endocrine function leading to reproductive impairment, cancer etc	2-D alerts for scaffolds associated with binding. 3-D toxicophores, receptor-ligand docking, molecular dynamics and other associated molecular modelling techniques	2-D alerts for nuclear receptor binding leading to steatosis;[22, 23,] 3-D toxicophores for PPARγ binding leading to steatosis;[57-58] 2-D alerts and QSARs leading to reproductive toxicity and other endocrine effects[59-61]

Acute Enzyme Inhibition	Irreversible inhibition of a physiological enzyme e.g. acetylcholinesterase.	Rapid acute toxicity elevated above baseline	2-D structural alerts for specific molecular fragments. 3-D toxicophores	2-D and 3-D QSARs for excess aquatic toxicity as a result of enzyme inhibition[62-64]
Unspecific	Unspecific events that occur at any relevant site e.g. reversible membrane disruption.	Narcosis referring to basal cytotoxicity, resulting in anaesthesia and lethality, phospholipidosis leading to liver failure	Relevant physico-chemical properties e.g. log P in aquatic environment, vapour pressure in air. 2-D structural alerts for specific molecular fragments	QSARs for non-polar narcosis to aquatic species;[65-67] 2-D alerts for phosopholipidosis[68-69]
Physical	Disruption of membranes due to the (physical / chemical) characteristics of a molecule	Skin, eye, nasal, respiratory (other membrane) irritation and corrosion	2-D structural alerts for specific molecular fragments. Measures of acidity / basicity;	2-D alerts for skin / eye irritation / corrosion;[70-71] pH for irritation /

			surfactant activity e.g. critical micelle concentration	corrosion;[72] QSAR for irritation[73]
Other[a]	Any other effect that may be related to promoting an adverse outcome e.g. weather, temperature or anthropogenic factors	Any however usually associated with population decline	Any relevant effect or property	Climate as a stressor[74]

[a]It is acknowledged that AOPs in this category do not have a chemical interaction. This category of MIE is included in this table to enable any framework to categorise AOPs to be fully inclusive of all possible interactions. It should be noted that non-chemical interactions may need to be defined separately.

Table 2. Examples of currently available AOPs classified according to the type of MIE. Where available "citable" AOPs from the AOP Wiki (https://aopwiki.org/) have been used in preference to those available in the literature.

AOP Title	MIE of AOP	Adverse Outcome	OECD Project Number or Reference; AOP Wiki ID
Covalent Reactivity			
Covalent Protein binding leading to Skin Sensitisation	Covalent binding to immunoprotein	Skin sensitisation	OECD 1.1 ID: 40
Alkylation of DNA in male pre-meiotic germ cells leading to heritable mutations	DNA alkylation	Heritable mutations	OECD 1.11 ID: 15
Protein Alkylation leading to liver fibrosis	Protein alkylation	Liver fibrosis	OECD 1.14 ID: 38

AFB1: Mutagenic mode-of-action leading to hepatocellular carcinoma	Formation of pro-mutagenic DNA Adducts	Hepatocellular carcinoma	OECD 1.8 ID: 46
Free Radical / Oxidative Stress			
No AOP currently citable on the AOP Wiki (although some are in draft form) or available in the literature			
Phototoxicity			
No AOP currently citable on the AOP Wiki or available in the literature			
Chronic Receptor Mediated			

Inhibition of thyroperoxidase and subsequent adverse neurodevelopmental outcomes in mammals	Inhibition of thyroperoxidase	Neurodevelopmental outcomes	OECD 1.10 ID: 42
Disruption of VEGFR signalling leading to developmental defects	Inhibition of VegfR2	Developmental outcomes	OECD 1.6 ID: 43
Sustained AhR activation leading to rodent liver tumours	Binding to AhR	Liver tumours	OECD 1.7 ID: 41
Androgen receptor agonism leading to reproductive dysfunction	Agonism of androgen receptor	Reproductive dysfunction	OECD 1.12 ID: 23
Aromatase inhibition leading to reproductive dysfunction	Aromatase Inhibition	Reproductive dysfunction	OECD 1.12 ID: 25
Oestrogen receptor antagonism leading to reproductive dysfunction	Binding to the oestrogen receptor	Reproductive dysfunction	OECD 1.12 ID: 30

Chronic binding of antagonist to N-methyl-D-aspartate receptors (NMDARs) during brain development leads to neurodegeneration with impairment in learning and memory in aging	Binding to NMDAR	Neuroinflammation leading to neurodegeneration	OECD 1.13 ID: 12
Chronic binding of antagonist to N-methyl-D-aspartate receptors (NMDARs) during brain development induces impairment of learning and memory abilities	Binding to NMDAR	Impairment of learning and memory abilities	OECD 1.22 ID: 13
Aromatase (Cyp19a1) reduction leading to impaired fertility in adult female	Binding to aromatase	Impaired fertility	OECD 1.21 ID: 7
PPARα activation in utero leading to impaired fertility in males	Binding to PPAR	Impaired fertility	OECD 1.21 ID: 18
Binding of agonists to ionotropic glutamate receptors in adult brain causes excitotoxicity that	Binding to Glutamate Receptor	Learning and memory impairment	OECD 1.23 ID: 48

mediates neuronal cell death, contributing to learning and memory impairment			
Inhibition of the mitochondrial complex I of nigra-striatal neurons leads to parkinsonian motor deficits	Binding of inhibitor to NADH-ubiquinone oxidoreductase (complex I),	Motor function, impaired	OECD 1.33 ID: 3
Acute Enzyme Inhibition			
Inhibition of acetylcholinesterase leading to lethality	Irreversible binding to acetylcholinesterase	Lethality	[12, 62]
Unspecific			

Reversible membrane disruption leading to anaesthesia e.g. non-polar narcosis, basal cytotoxicity	Unspecific membrane disruption	Anaesthesia, lethality	29, 67, 77-78
Physical			
Intracellular acidification induced olfactory epithelial injury leading to site of contact nasal tumours	Decrease in intracellular pH	contact nasal tumours	OECD 2.7
Other			
No AOP currently citable on the AOP Wiki (although some are in draft form) or available in the literature			

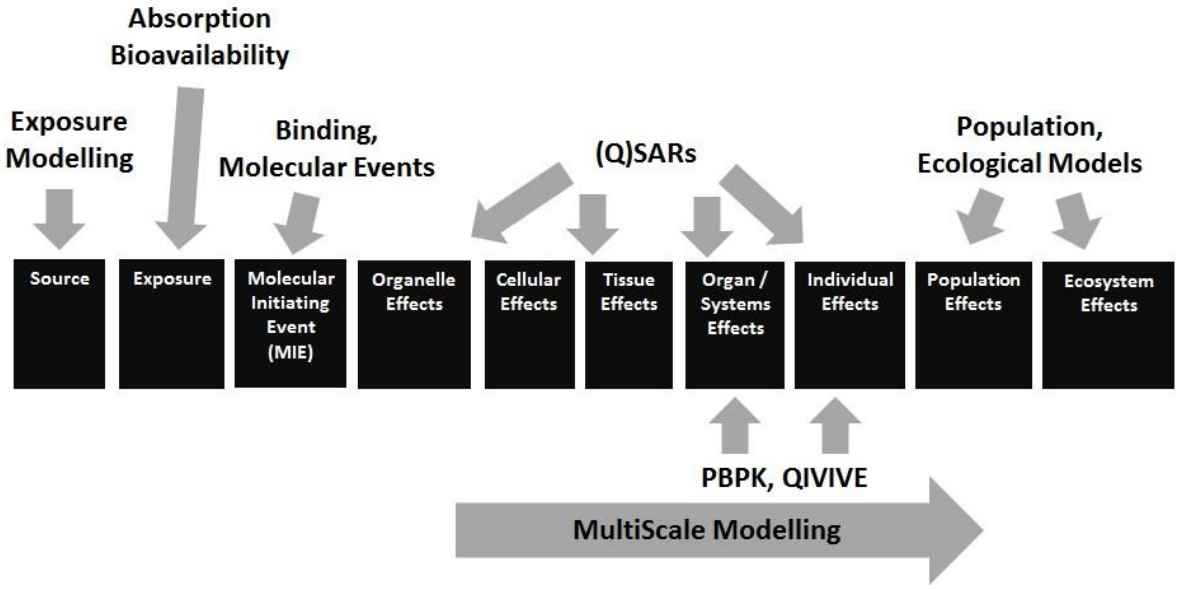

Figure 1. Schematic of a generic Adverse Outcome Pathway (from source to ecosystem) with the types *of in silico* models that may be associated with each Key Event.

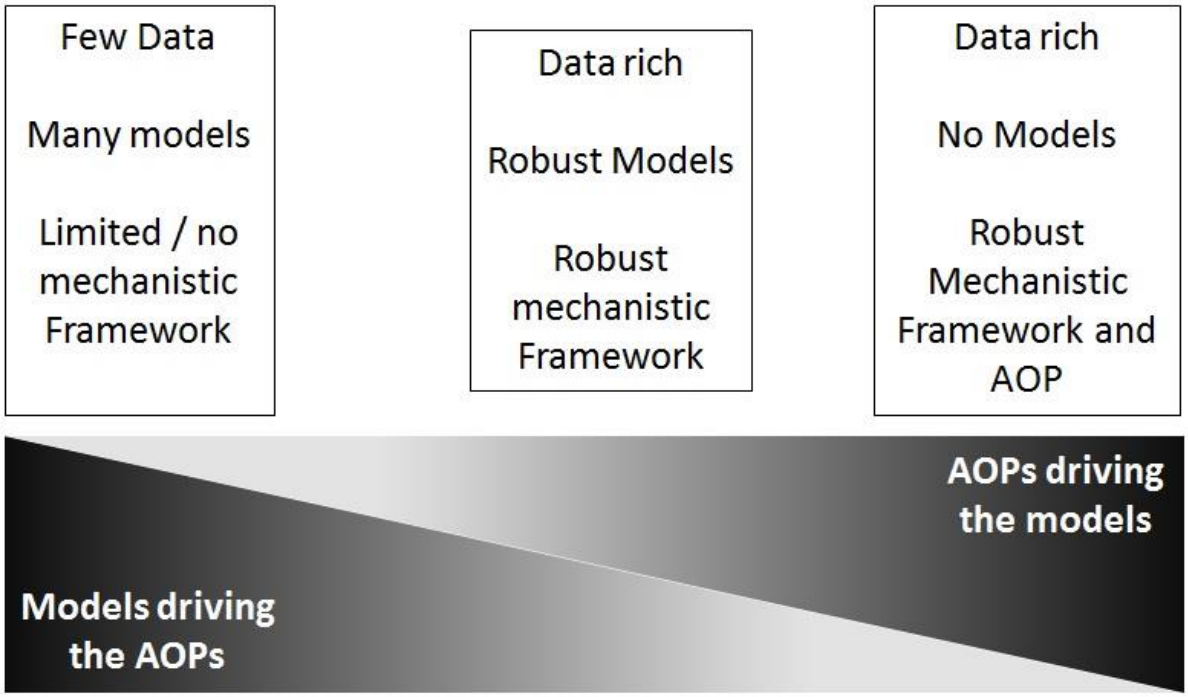

Figure 2. Main differences where models may drive the development of AOPs and vice versa.