PROPOSAL FOR A TEMPLATE, AND GUIDANCE ON DEVELOPING AND ASSESSING THE COMPLETENESS OF ADVERSE OUTCOME PATHWAYS

2012

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Appendix I

1. Background

1. The historical paradigm for protecting humans and the environment from adverse effects of chemicals has centred primarily on whole animal toxicity testing with single chemicals of concern. However, due to the costs and time involved, it is not practical or feasible to test exhaustively all chemicals that could adversely affect humans and ecosystems. These realities have long indicated the need for scientifically sound models and tools for predicting adverse effects of chemicals based on relatively little data. However, to date, efforts to incorporate mechanistic understanding of biological systems as a basis for effects extrapolation have been limited. Despite this, with recent and projected advances in toxicogenomics, bioinformatics, systems biology and computational toxicology, scientists seem poised to make critical breakthroughs that will revolutionise predictive toxicology and elicit a paradigm shift in regulatory toxicity testing and risk assessment. The so-called Adverse Outcome Pathway (AOP) methodology is one approach to provide a framework for this information to be collected and rationalised. The purpose of this is to provide a mode (and/or mechanism) of action basis for understanding adverse effects.

2. The current document represents an initial effort to bring together information related to Adverse Outcome Pathways (AOPs) to enable greater understanding and uptake. The primary purpose of this guidance document is not to reproduce or replace the ever-expanding volume of journal articles, reports, documents, and textbooks on AOPs but to provide an introduction to the development and evaluation of AOPs. In this context, an AOP is a conceptual construct that portrays existing knowledge concerning the pathway linkage between a direct molecular initiating event and an apical adverse outcome at a biological level of organisation that is relevant to a regulatory decision (e.g. risk assessment or classification and labelling).

2. Introduction

3. Recognising the limitations of current *in vivo* testing approaches and the rapid development of new biochemical and cellular assay systems and computational predictive methods, regulators and other stakeholders have been exploring ways to integrate existing knowledge from *in vivo* tests with the results of molecular screening and omics assays, computational predictive methods and other sources of information. The purpose of this integration is to identify better methods of making regulatory decisions.

4. Regulatory toxicology involves hazard identification, dose response assessment, exposure assessment, and risk characterisation. Over the past two decades, a variety of groups have advocated systems and pathway-based approaches to define the processes by which toxicants elicit outcomes of regulatory interest. Early applications of the pathway approach were often referred to as exposure-dose-response models or biologically based dose-response models [1, 2]. In 2001, a framework for using mode-of-action (MOA) information to determine human relevance of animal data was published by the International Programme on Chemical Safety (IPCS) [3]. The MOA describes the key events and processes, starting with interaction of an agent with the cell through functional and anatomical changes, resulting in cancer or other health endpoints. In 2007, the United States National Academy of Science (NAS) published the *Report on Toxicity Testing in the 21st Century: A Vision and a Strategy* in which the concept of a 'toxicity pathway' was very prominent [4]. At the centre of the vision for transforming toxicity testing described in this report is a reorientation of such testing to evaluate the responses of toxicity pathways (i.e. normal

cellular signalling pathways) that can be perturbed by chemical exposures in well-designed *in vitro* assays using human cells. Since the McKim Conferences on Predictive Toxicology in 2006, 2007, and 2008, the alternative term "Adverse Outcome Pathway" (AOP) has evolved [5]. An AOP describes pathways initiated via non-specific interactions (e.g. narcosis), as well as more specific ligand-receptor interactions leading to adverse outcomes [6]. Although developed for use in ecotoxicology, the AOP concept is also applicable to human health effects [7]. The pathway approach is based on the concept that toxicity results from the chemical first reaching and then interacting with an initial key target in the organism. According to this theory, an AOP is the sequential progression of events from the molecular initiating event (MIE) to the *in vivo* outcome of interest (Fig. 1). Generally, it refers to a broader set of pathways that would; 1) proceed from the MIE, in which a chemical interacts with a biological target (e.g. DNA binding, protein oxidation, or receptor/ligand interaction etc.), 2) continue on through a sequential series of biological activities (e.g. gene activation, or altered cellular chemistry or tissue development etc.), and 3) ultimately culminating in an adverse outcome of relevance to human or ecological risk assessors (e.g. mortality, disrupted reproduction, cancer, or extinction etc.) [8].

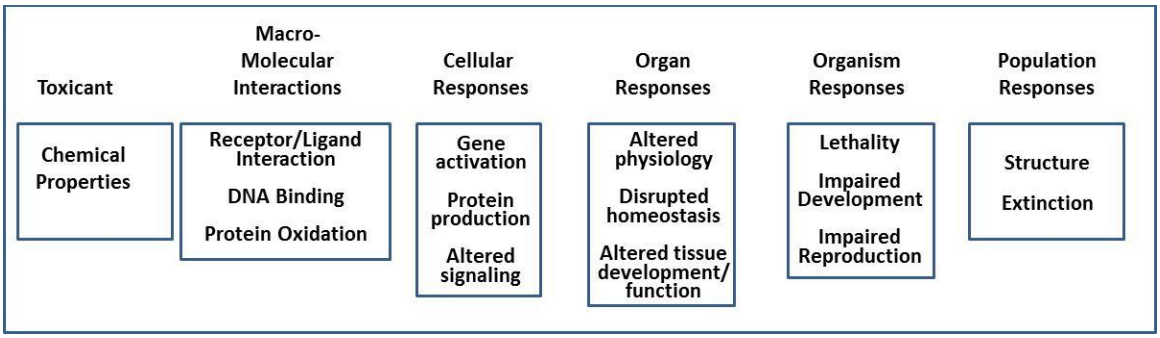

Figure1. A schematic representation of the Adverse Outcome Pathway (AOP) illustrated with reference to a number of pathways.

5. An AOP is a representation of existing knowledge concerning the linkage(s) between a MIE and an adverse outcome at the individual or population level [9]. While AOPs may be depicted as linear processes, the amount of detail and linear character of the pathway between a molecular initiating event and adverse outcome can vary significantly. This is especially true for human health endpoints, where effects are the result of multiple organ interactions (e.g. skin sensitisation), multiple events (e.g. repeat dose toxicity), accumulation over time (e.g. neural toxicity), or are related to a specific life stage of an organism (e.g. developmental toxicity). Nonetheless, although a number of biochemical steps are required for a toxic response to be realised, the molecular initiating event is a prerequisite for all subsequent steps [10].

6. Additionally, an AOP is based on the fact that chemical interactions are at the molecular level and not at the whole organism level. Thus, adverse effects observed *in vivo* are the result of biological cascade initiated by the chemical structure of the toxicant. Hence, an AOP is designed to avoid mixing information from multiple mechanisms (i.e. different molecular initiating events which can cause the same *in vivo* outcome through different AOPs). However, it should be noted that each component of this pathway may itself be influenced by other pathways ongoing within the biological system being modelled.

3. The use of AOPs

7. A well-identified AOP, with an accurately described sequence of events through the different levels of biological organisation in organisms, provides valuable pieces of mechanistic information which can be used for many purposes [11].

3.1. Use of AOPs for developing chemical categories

8. AOPs have been linked to developing a chemical category and to derive traditional structure-activity relationships (SARs) [7]. In order to use AOPs to develop chemical categories there are three information libraries which must be collated, programmed, and integrated; 1) a library of effects used in hazard assessment, 2) a library of molecular initiating events, and 3) a library of AOPs [11].

3.2. Use of AOPs for the Test Guideline Programme

9. By identifying and describing the key events, the AOPs could inform the work of the Test Guideline Programme. Indeed, when the key events are identified and scientifically proven, one could propose developing *in vitro* and *ex vivo* assays that detect direct chemical effects or responses at the cellular or higher levels of biological organisation [8]. Reversely, by linking proposals for the development of *in vitro* test methods to key events in an AOP, the relationship to hazard endpoints relevant for regulatory purposes can be established.

3.3. Use of AOPs for the development of Integrated Approaches to Testing and Assessment

10. An AOP, for any given hazard endpoint, can be the basis for developing an integrated approach to testing and assessment (IATA) or an integrated testing strategy (ITS) for that hazard endpoint. The application of IATA and ITS may lead to the refinement, reduction and/or replacement of conventional *in vivo* testing.

3.4. Use of AOPs for further development of the OECD QSAR Toolbox

11. In the context of the development of the OECD QSAR Toolbox, the guidance how to implement the AOP into the Toolbox needs to be developed. Once there is agreement on how to implement AOPs into the workflow of the Toolbox to build categories, work can start on actual implementation. In parallel, the OECD QSAR Toolbox Management Group is planning to consider the identification of existing QSARs that predict Molecular Initiating Events and that can be implemented as profilers in the Toolbox [11].

4. Aims of this Document

12. The aim of this document is to propose a scheme to report an AOP, together with guidance on developing the AOP. It should give an insight into which pieces of information are necessary to identify an

AOP and how to present them. It will also provide assistance on how to undertake the assessment of an AOP in terms of its completeness and relevance.

5. Development of an Adverse Outcome Pathway (AOP)

5.1. Identification of the Main Blocks of Information of an AOP

13. To identify the information associated with an AOP, the concept of a "template" is presented here. This is a generic form of the AOP that allows the information to be captured. In general, the AOP template consists of three main information blocks: the molecular initiating event (MIE), intermediate events and the final, or apical, adverse outcome. For any AOP, each of the three main information blocks should be clearly identified. While the development of the AOP can be started from any of these blocks, depending on what knowledge is available at the beginning of the exercise, typically AOP development begins with either the MIE or the apical endpoint of interest. The latter reflects the fact that an AOP is anchored at its two ends by the chemical/biological interaction and outcome of interest (Fig. 2).

14. Usually, the apical endpoint is associated with an *in vivo* OECD Test Guideline. However, in cases such as cell proliferation or bioenergetics, the apical endpoint may be at a lower level of biological organisation. In any case, a given apical endpoint will be associated with a finite set of possible MIEs. Similarly, a given MIE will be associated with a finite set of possible apical endpoints. But each AOP will have only one MIE and one apical endpoint.

15. The identification of the adverse outcome relevant to the assessment is one of the steps in the developing of the AOP. It is essential to define this apical effect clearly, as it determines the most relevant mechanistic information and thereby intermediate effects related to this endpoint, which are leading to the next step, where the key events should be identified. The third building block of the AOP is the molecular initiating event. This step should explain how the chemical interacts with biological (macro)molecules and allows the applicability domain for the stated adverse effect to be defined.

16. To develop the AOP, different types of data can be utilised, These include: structural alerts that are reflective of the types of chemicals that can initiate a pathway, *in chemico* methods that measure the relative reactivity or chemical-biological interactions, *in vitro* assays that confirm the subsequent cellular responses (e.g. gene expression) and, ultimately, *in vivo* tests that measure endpoints that are directly relevant to the adverse outcome that drives regulatory decision making [12]. This information can be used to identify key steps in the AOP and provide scientific evidence supporting the AOP.

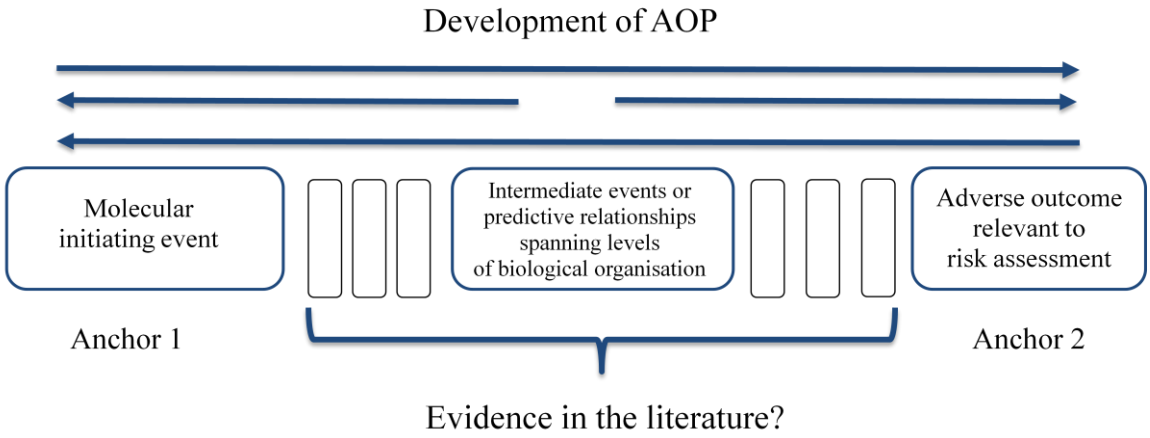

Figure 2. A Schematic Diagram for the development of an AOP Starting at any of the Three Main Blocks of Information.

5.1.1. Identification of the Apical Adverse Effect

17. An adverse effect can be defined based on different levels of biological organisation: cellular/tissue, organ, organ system, individual, population or ecosystem. The adverse outcomes can be also divided into; 1) long term health endpoints where effects are the results of multiple events (e.g. repeat dose toxicity) or accumulation over time (e.g. neural toxicity) or are related specifically to a particular life stage of the organism (e.g. developmental toxicity), 2) local effects, where MIEs are likely to be closely aligned with the *in vivo* outcome (e.g. skin sensitisation, skin and eye irritation). It is essential to clearly and precisely define the apical adverse effect as one of the anchors of the AOP. This helps to define the mechanistic sequence of events leading to this outcome.

5.1.2. Recognition of Intermediate Steps and Key Events Leading to the Apical Outcome

18. The response matrix is the collection of intermediate events which lie between the apical outcome of interest and the MIE. This matrix can be quite large but experience has shown that significant portions can be scaled. This matrix includes *in vitro* and 'omics' endpoints (i.e. modern toxicology), as well as traditional *in vivo* endpoints (e.g. histopathology) that arise from standard test guidelines. As the response matrix expands, it gives rise to the sense of complexity in toxicity.

19. Before the identification of intermediate events leading to adverse outcome, an understanding of the normal physiological pathways of the AOP is essential (e.g. reproductive processes, liver functions). This will help in the recognition of the complex networks of processes on the different level of biological organisation, which can be disrupted. During the identification of key steps, a review of the existing literature is required to find out as much information as possible about the plausible mechanism and the intermediate steps leading to the apical outcome. This aspect is crucial for the development of the AOP. It requires manual evaluation of the scientific literature to determine relevant intermediate events and their

usefulness as key events in developing the AOP. Usually, multiple intermediate events are identified. Therefore, the assembled knowledge has to be filtered and selected to match the single AOP.

20. Key events are steps along the pathway that represent pivotal events at the different levels of biological organisation. To be a key event, the intermediate step must be able to be evaluated experimentally. That is to say, the event must be able to be used in a hypothesis which can then be tested. There are no rules as to which types of data have to, or can be used to support a key event. However, such data should be reliable and relevant to the specific adverse outcome.

21. There is no specification as to how many key events have to be defined. The number clearly depends on where in the biological organisation the apical outcome is located (e.g., cell, organ or population level). It is intuitive that key events at different levels of biological organisation are of greater value than multiple events at the same level of organisation.

5.1.3. Definition of the Molecular Initiating Event (at the Site of Action)

22. Chemical-induced perturbations of biological systems are at the molecular level. Most chemicals can interact with more than one molecular target. Chemicals have different affinities for different targets. The most potent affinity typically drives the apical toxicity. The molecular initiating event represents the primary anchor or "the foundation" of the AOP, therefore it is very important to identify clearly the beginning of the cascade leading to the specified adverse outcome. Many molecular initiating events are defined in the form of "receptor binding", others are based on the principles of organic chemistry (electrophile-nucleophile reactivity). The understanding of the molecular initiating event allows for the definition of the properties of chemicals inducing the perturbation, such as bioavailability, structural requirements (especially for receptor binding) and metabolic transformation. The understanding of the chemistry of potential inducers helps to define the applicability domain for the AOP.

23. In the ideal scenario, when the initiating event is well-defined, not only should the potential inducer of that event be recognised but also the site of action, which implies the type of biological (macro)molecule that interacts with the target chemical. For some apical outcomes, especially based on receptor binding mechanisms, the identification of the site of action is very important, as the "shape" and other properties of the receptor define structurally the type of molecules which can bind to it. However, there are a number of endpoints for which the identification of site of action is quite difficult, or even impossible, or they cannot be defined precisely.

5.2. Data Summation

24. After the compilation of all information for the adverse outcome, it is necessary to report them systematically. Part II of this document presents the template on how to report the development of the AOP.

25. At the beginning, the collected data should be used to present the whole adverse pathway step–by-step starting from the characterisation of the route of exposure and chemical properties, to the identification of the molecular initiating event and site of action, if possible. After that, the responses at the macromolecular, cellular/tissue, organ, organism, and population\ecosystem levels should be identified; the final stage depends on the level of biological organisation of the adverse outcome. This report on the knowledge relating to the AOP is often based on one of a few well-studied model toxicants. Following this, a concise summary of the qualitative understanding of the AOP has to be undertaken. For this purpose, the key events, documentation of the experimental support for each event, and a subjective evaluation of the strength of the scientific evidence for that event need to be listed, as summarised in Table 1.

Table 1: Summary information on the key events of the AOP.

Key Events	Experimental Support	Strength of Evidence
Molecular Initiating Event		
Key Event 1		
Key Event (n-1)		
Key Event n		
Adverse Outcome		

26. The reporting of the experimental support for each key event and its evaluation is very important in the AOP documentation. This is because the reporting and evaluation is the first step in the assessment of the current usefulness of the AOP. Therefore, there is benefit in attempting to standardise the process of evaluating the strength of evidence providing some criteria and categories. The evidence can be assessed, for example, as being very strong, strong, moderate, weak or extensive, adequate, considerable etc. The criteria on how to assign the classification should also be proposed to assist in the evaluation of the evidence in a uniform manner. For example, the following criteria could be considered: the number and type of assay (validation, international acceptance, reproducibility and repeatability); the strength of the association of the assays with key events, the number of chemicals tested for each endpoint etc. Currently, no set of criteria for evaluating the strength of evidence exist, and it is strongly recommended that theybe developed to assist in the evaluation of an AOP.

27. An additional form of data summation is the flow diagram of the intermediate events associated with the AOP. This graphical version of the AOP shows visually the sequence of events at the different levels of biological organisation. Figure 3 presents the example flow diagram of the pathways associated with skin sensitisation [15].

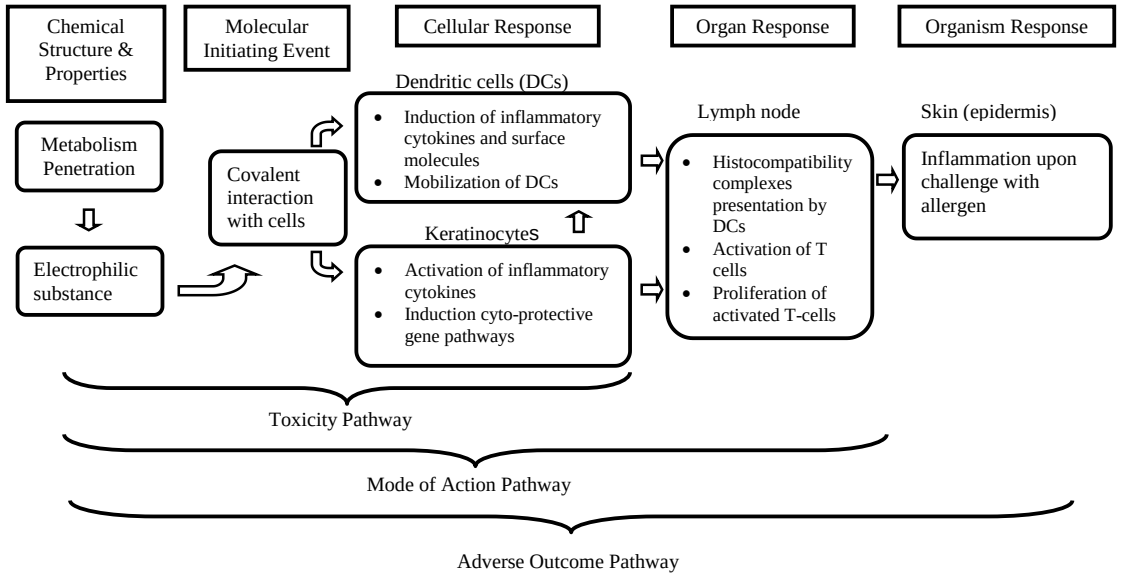

Figure 3. Flow diagram of the pathways associated with skin sensitisation (adapted from [15]).

5.3. AOP assessment

28. In the OECD approach, it is considered critical to be able to gauge the reliability and robustness of an AOP. This should be done by evaluating the experimental support of the AOP. In such assessment, the qualitative and quantitative understanding of the AOP has to be analysed. This means that key steps should be clearly identified and scientifically proven, both qualitatively and (if possible) quantitatively. An important aspect of quantifying an AOP is the threshold and scale of the linkage between key events in the pathway. Moreover, the assessment of the quantitative understanding of an AOP should determine the response-to-response relationships required to scale *in vitro* effect(s) to *in vivo* outcomes. Usually the assessment of the experimental evidence and empirical data clearly support the qualitative understanding of the AOP in the identification and characterisation of the potential inducer of an adverse effect. However, the same assessments very often reveal hurdles with the prediction of the relative potency of the inducer, because of the lack of necessary data. Therefore the assessment of the quantitative understanding of an AOP is more problematic than the qualitative.

29. The first stage of the assessment of AOP is performed during the data summation, where every key step is documented together with the scientific evidence and its evaluation. An additional aspect of evaluating the AOP is the implementation of the Bradford Hill criteria [13] to assess the Weight-of-Evidence supporting the AOP. In this assessment, the author of the AOP has to make a decision with regard to the following criteria:

- Concordance of dose-response relationships
- Temporal concordance among the key events and adverse outcome
- Strength, consistency, and specificity of association of adverse outcome and initiating event

- Biological plausibility, coherence, and consistency of the experimental evidence
- Alternative mechanisms that logically present themselves and the extent to which they may distract from the postulated AOP. It should be noted that alternative mechanisms of action, if supported, require a separate AOP
- Uncertainties, inconsistencies and data gaps.

5.4. Confidence in AOP

30. The final step in the reporting of the AOP is a statement regarding the confidence associated with this AOP. Confidence in an AOP is increased by a more comprehensive understanding of the nature of the interaction between the chemical and the biological system, coupled with mechanistic understanding of the biological response. The confidence is ascertained by addressing the following questions:

1) How well characterised is the AOP?
2) How well are the initiating and other key events causally linked to the outcome?
3) What are the limitations in the evidence in support of the AOP?
4) Is the AOP specific to certain tissues, life stages / age classes?
5) Are the initiating and key events expected to be conserved across taxa?

31. In summary, an AOP should be based on a single, defined molecular initiating event and linked to a stated *in vivo* hazard outcome(s). During the development process of the AOP, few or more toxic pathways could be determined that can be linked to the same or different molecular initiating event(s), but in the end, a single pathway linked to the specific initiating reaction should be identified.

32. An AOP may be considered either plausible or probable, depending upon the extent (i.e. depth and breadth) of the available scientific evidence supporting the AOP and the extent to which the key events have been experimentally tested and found to be consistent with data for other key events. Accordingly, an AOP may be considered a dynamic entity, as it can be continually updated and refined as new information can be incorporated into the general understanding of the pathways. An evaluation of the scientific evidence supporting a proposed AOP can be conducted by answering a predetermined set of questions.

5.5. Minimal Information Requirements for an AOP

33. It is important to define the minimal requirements for information associated with the developed AOP. The acceptance of the AOP requires an understanding of key processes or critical events measured along the pathway. The crucial steps in establishing an AOP are the MIE and a well-understood apical outcome, as these are the anchors of an AOP. Therefore, it is critical to identify the chemical-biological interaction and the outcome elicited by this MIE. The identification and characterisation of key events depend on the level of knowledge about this hazard effect. There are examples of relatively well-recognised endpoints, such as skin sensitisations, for which the AOPs are accurately developed. However, it has to be kept in mind that for many endpoints there is a lack of relevant information allowing for the definition of the

sequence of events leading to the apical outcome. In this case, it is important to have mechanistic understanding between the initiating event and adverse effect. Moreover, it is necessary to understand the basis of normal physiology (e.g. nervous system function, reproductive processes, differentiation of tissues) of the AOP. The AOPs identified must not contradict any steps of normal biological processes, since they need to be biologically plausible. Even if some steps are not known with certainty, the overall process must agree with what is known about the particular biology being considered [14]. It is important to understand the linkages and scaling factors as the pathway moves up the level of biological organisation, especially for events which depend on potency in the *in vivo* outcome [7]. As the process of AOP development proceeds and more are recorded, there will be a better understanding of what may, ultimately, constitute the minimum requirements for an AOP. The absolute minimum will be the MIE, the adverse outcome, and at least one meaningful key event. However, recommendations of such minimum requirements must be treated with caution at this time.

6. Examples of the AOP documentation

34. During the last few years, there has been a growing interest in the adverse outcome pathways as a transparent linkage between the exposure and the adverse effect. As a result, a small number of AOPs have been proposed up to now, such as skin sensitisation initiated by covalent binding to proteins [15]; voltage gated sodium channels mediated neurotoxicity [9]; estrogen receptor-mediated reproductive impairment [9]; acute aquatic toxicity initiated by weak acid respiratory uncoupling [9]; haemolytic anaemia induced by anilines following repeated dose exposure and nephrotoxicity induced by 4-aminophenols [9]; cardiotoxicity in fish induced by 2,3,7,8-tetrachlorodibenzeno-p-dioxin [16]. Most of the adverse outcome pathways can be found in the report from a recent OECD workshop [9]. Analysing all these documents, significant differences can be identified in the documentation of the AOPs. Different levels of information are available among these reports; for some of them, no clear assessment of the AOP is made. This confirms the importance of the standardisation procedure during the development and documentation of an AOP.

7. Conclusion

35. To implement a predictive strategy for risk assessment, results from *in vitro* toxicity assays focused on cellular responses to molecular initiating events will need to be extrapolated to effects on organisms and ultimately to populations. This can be achieved by developing the AOP which links an MIE with adverse effects. As they are intended to be used by the regulatory agencies, it is important to standardise the way in which AOPs will be developed and documented.

36. The AOP should provide a transparent, chemical and biological mechanistically-based framework for developing or refining chemical categories, as well as proposing and prioritising targeted *in vitro* and *in vivo* testing. By understanding the likelihood of effects at the chemical level and/or lower levels of biological organisation from structure-activity relationships (SARs), and *in chemico* and *in vitro* assays, one could efficiently determine if additional tests at higher levels of biological organisation (e.g. *in vivo* assays) are required [12]. However, whilst the potential has been shown (e.g. the work of Meek et al.,

[18]), the depth and breadth of available data do not currently allow for a large proportion of decisions associated with quantitative risk assessment to be made.

37. As indicated by Bauch et al., not all key events in an AOP may have to be satisfied in order to make an assessment [17]. Justification of an AOP will involve consideration of the information concerning the molecular initiating event, other key events, and the apical outcome which is the basis of the assessment, as well as the Weight-of-Evidence for each event. What is considered sufficient justification of an AOP will be use-dependent, with a greater justification required for applications with greater potential impact [12]. For developing an IATA or an ITS, a consistency across several levels of biological organisation, including anchoring to the apical effects, is likely to be required. However, for category formation, the understanding of a single key event is sufficient to group potential chemicals inducing this effect.

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Part II

The AOP Template

To standardise the documentation of an AOP, a scheme on how to conduct this process is proposed. The author of the AOP should fill every field in the AOP format. If the field is not pertinent to the proposed pathway, for example, the adverse outcome is localised in the organ or tissue level, so the identification of responses on the higher level–individual or population/ecosystem is not appropriate then it should be stated as not applicable. In addition, instances where information is missing or lacking should be stated clearly.

1. The Adverse Outcome Pathway identifier

Name the AOP by defining a clear and concise adverse outcome together with molecular initiating event.

2. Date of publication of AOP

Report the date (day/month/year) of AOP publication.

3. Date of updating the AOP

Indicate the date (day/month/year) of any update of the AOP. The AOP can be updated for a number of reasons, such as additions of new information and corrections of information.

4. The introduction

Give short background on the current knowledge about the endpoint of interest.

5. Summary of the AOP

Report briefly the knowledge about the AOP following steps:

5.1. Characterisation of the exposure
Define the route of exposure.

5.2. Characterisation of chemical properties
Identification of properties required to initiate the adverse effect (bioavailability, reactivity, metabolism).

5.3. Identification of the molecular initiating event
Name and describe the MIE.

5.4. Identification of the site of action
Name the site of the chemical (re)action which initiates the adverse pathway.

5.5. Identification of the responses at the macromolecular level
Describe how the biochemical pathway is affected by the chemical on the molecular target.

5.6. Identification of the responses on the cellular/tissue level
Describe the cellular/tissue outcomes, based on available information.

5.7. Identification of the responses on the organ level
Describe the organ level responses, based on available information.

5.8. Identification of the responses on the organism level
Describe the key organism response, based on available information.

5.9. Identification of the overall effect on the population or ecosystem
Describe how the population or ecosystem is affected by the toxic pathway.

6. Summary of the Key Events of the AOP

Summarise the qualitative understanding of the AOP by listing them in a table that summarises the key events, documentation of the experimental support for each event, and a subjective evaluation of the strength of the scientific evidence for that event (e.g. strong, well established, adequate) (See Table 1).

Include also the flow diagram of the intermediate events associated with AOP.

7. Scientific evidence supporting the AOP

Include any available information supporting the steps/key events in the AOP. This can include any type of data: in vivo, in vitro, in silico, in chemico, toxicogenomics. Each key event should be considered separately in a single sub-section.

8. Assessment of the AOP

8.1. Assessment of the Weight-of-Evidence supporting the AOP

Answer the Hill criteria:

8.1.1. Concordance of dose-response relationships

Report any reference/study giving evidence of dose-response relationship.

8.1.2. Temporal concordance among the key events and adverse outcome

State the agreement between the sequences of biochemical and physiological events leading to the adverse outcome together with the evidence in the literature.

8.1.3. Strength, consistency, and specificity of association of adverse outcome and initiating event

Give the scientific evidence on the linkage between initiating event and adverse outcome.

8.1.4. Biological plausibility, coherence, and consistency of the experimental evidence

Explain the logic, coherence and consistency along with the experimental data supporting the AOP. Describe how the experimental evidence is logical and consistent with the mechanistic plausibility proposed by the theory explaining the initiation of the adverse outcome. If possible, describe the coherence of experimental results for multiple chemicals across different species.

8.1.5. Alternative mechanism(s) that logically present themselves and the extent to which they may distract from the postulated AOP. It should be noted that alternative mechanism(s) of action, if supported, require a separate AOP.

Report other possible mechanisms that can lead to the adverse outcome and state if they can be covered by this AOP.

8.1.6. Uncertainties, inconsistencies and data gaps

Include any uncertainties about the experimental details, such as uncertainties regarding the differences in sensitivity of different biological targets (e.g. cysteine versus lysine, Type I pyrethroid versus Type II), the measurements of biological activity in different assays. Describe inconsistencies within the reported data, such as differences between in vivo responses for very similar chemicals, and report any data gap that cause the weakness of the AOP.

8.2. Assessment of the quantitative understanding of the AOP

Include an evaluation of the experimental data and models to quantify the molecular initiating event and other key events. If possible, describe transparent determination of thresholds and response-to-response relationship to scale in vitro and in chemico effects to in vivo outcomes.

9. Confidence in the AOP

Discuss the summary of the scientific evidence supporting the AOP by answering the following questions:

9.1. How well characterised is the AOP?

Describe how well the adverse outcome is understood qualitatively and quantitatively.

9.2. How well are the initiating and other key events causally linked to the outcome?

Give short statement on the relationship between each key event and adverse outcome.

9.3. What are the limitations in the evidence in support of the AOP?

Indicate any lack or disagreement in the scientific evidence supporting the AOP.

9.4. Is the AOP specific to certain tissues, life stages / age classes?

Indicate if there are critical life stages, where exposure must occur, to results in the adverse effect. Or specify if there are key events along the pathway which are dependent on the life stage, although the AOP is known to be initiated regardless of life stage. Indicate also if the AOP is associated also with age- or sex-dependence.

9.5. Are the initiating and key events expected to be conserved across taxa?

State if the key events for this AOP appear to be conserved across any group of animals (e.g. mammals).

10. Reference(s)

List the bibliographic references to original papers, books or other documents used to support the AOP.