APPENDIX I

COLLECTION OF WORKING DEFINITIONS

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1. INTRODUCTION

Over the past half decade, a variety of approaches have been proposed to incorporate mechanistic information into toxicity predictions. These initiatives have resulted in an assortment of terms coming into common use. Moreover, the increased usage of 21st Century toxicity testing, with a focus on advanced biological methods, has brought forward further terms. The resulting diverse set of terms and definitions has led to confusion among scientists and organisations. As a result, one of the conclusions and recommendations from the OECD Workshop on Using Mechanistic Information in Forming Chemical Categories was the development of a standardised set of terminology [1]. It was recognised that such a glossary would assist in the understanding of the Adverse Outcome Pathway (AOP) concept as well as its recording, completion of the template and ultimate acceptance. Moreover, the use of a common ontology will also help to apply AOP concepts in developing (Q)SARs and chemical categories to advance the use of predictive techniques in assessments.

2. AIMS

The purpose of this document is to collect definitions relevant to the AOP and general toxicity pathway concepts. To do this, the literature has been searched to find multiple definitions of terms relevant to AOPs. The ultimate goal would be to provide a harmonised set of definitions. It is appreciated, however, that such definitions may not be agreed in a formal sense, but would provide an illustration of the various terms. Such harmonised definitions will be provided for terms which are agreed by OECD or WHO. In that case, only that one definition is given.

3. GLOSSARY

Recently a number of concepts on how to incorporate mechanistic knowledge into toxicity prediction have emerged. Figure 1 shows the relationships between closely related terms which are frequently used in modern toxicology. It is crucial to define these terms precisely and clearly state the differences between them. Therefore, the working definitions for toxicity pathway, mode of action, adverse outcome pathway, and source to outcome pathway have been developed to indicate explicitly the differences between them. In addition, this glossary includes other terms relevant to AOP definition and use. These have been collected from the literature and, in most cases there are multiple definitions from different sources. The single definition is provided only for those terms, which have description approved by OECD or WHO. The terms are organised in alphabetic order.
Fig. 1. Representation of the relationships between Toxicity Pathways, Mode of Action Pathways, Adverse Outcome Pathways, and Source to Outcome Pathways. The black bars represent the breadth of research common to these concepts. The gray bars represent the theoretical extent of the concepts (adapted from Croft 2010, OECD AOP Meeting Definition [1]).

Adverse Outcome Pathway (AOP)

An AOP can be defined in the context of Figure 1. As such it relates to a linear sequence of events from the exposure of an individual to a chemical substance through to an understanding of the adverse (toxic) effect at the individual level (for human health) or population level (for ecotoxicological endpoints). The key events in an AOP should be definable and make sense from a physiological and biochemical perspective. AOPs incorporate the toxicity pathway and mode of action. AOPs may be related to other mechanisms and pathways as well as detoxification routes.

AOPs span multiple levels of biological organisation. AOPs often start out being depicted as linear processes, however, the amount of detail and linearity characterising the pathway between a molecular initiating event and an adverse outcome within an AOP can vary substantially, both as a function of existing knowledge and assessment needs [1].

Representation of existing knowledge concerning the linkage between the molecular initiating event and an adverse outcome at the individual or population levels [2].

Each adverse outcome pathway is a set of chemical, biochemical, cellular, physiological, behavioural, etc. responses which characterise the biological effects cascade resulting from a particular MIE. The term “adverse outcome pathway” has been selected so not to cause confusion with the term “Toxicity Pathway”,

[1] OECD AOP Meeting Definition
[2] OECD AOP Meeting Definition
which is used by the US National Research Council in its document, Toxicity Testing in the Twenty-first Century: A Vision and a Strategy, where the focus is on “omics” and high throughput \textit{in vitro} data [3].

A conceptual framework that links a molecular-level initiating event with adverse effects relevant for risk assessment [4].

The sequence of events between cellular response and adverse outcome on an individual organism or population of organisms is an AOP [5].

\textit{Adverse Response}

Changes that occur that result in impairment of functional capacity, often due to an insult that exceeds the capacity of the adaptive response to permit a return to the homeostatic state. Outcomes might include changes in morphology, development, lifespan, or growth of the organism. Although harder to define at the molecular level, potentially adverse responses might include alternations in gene expression, protein synthesis, or cell regulation [6].

\textit{Adverse effect}

Change in the morphology, physiology, growth, development, reproduction, or life span of an organism, system, or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other influences [7].

\textit{Adverse event}

Occurrence that causes an adverse effect.

\textit{Note}: An adverse event in clinical studies is any untoward reaction in a human subject participating in a research project; such an adverse event, which may be a psychological reaction, must be reported to an institutional review board [8].
**Apical endpoint**

Traditional, directly measured whole-organism outcomes of exposure in *in vivo* tests, generally death, reproductive failure, or developmental dysfunction [4].

Observable effects of exposure to a toxic chemical in a test animal. The effects reflect relatively gross changes in animals after substantial durations of exposure [9].

An observable outcome in a whole organism, such as a clinical sign or pathologic state, that is indicative of a disease state that can result from exposure to a toxicant [10].

**Applicability Domain**

The physicochemical, structural, or biological space and information that was used to develop a (Q)SAR model, and for which that model gives predictions with a given level of reliability [11].

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1 All available definitions for Applicability Domain apply to QSARs. Therefore, there is a need to develop definitions suitable for AOPs in the future.
The applicability domain of a (Q)SAR model is the response and chemical structure space in which the model makes predictions with a given reliability [11].

The applicability domain defines the constraints of the training set compounds of a (Q)SAR model, allowing a user to choose the most suitable model, or use a given model within its own predictive capacity [12].

The domain of applicability of a (Q)SAR model is the chemical structure and response space in which the model makes predictions with a given reliability. It can be thought of as a theoretical region in multidimensional space in which the model is expected to make reliable predictions. It depends on the nature of the chemicals in the training set, and the method used to develop the model and helps the user of the model to judge whether the prediction for a new chemical is reliable or not [9].

**Bioavailability**

Fraction of an administered dose that reaches the systemic circulation or is made available at the site of physiological activity. Usually, bioavailability of a substance refers to the parent compound, but it could refer to its metabolite. It considers only one chemical form. Please note: bioavailability and absorption are not the same. The difference between e.g. oral absorption (i.e. presence in gut wall and portal circulation) and bioavailability (i.e. presence in systemic blood and in tissues) can arise from chemical degradation due to gut wall metabolism or efflux transport back to the intestinal lumen or presystemic metabolism in the liver, among other factors [13]. Bioavailability of the toxic component (parent compound or a metabolite) is a critical parameter in human risk assessment (high-to-low dose extrapolation, route-to-route extrapolation) for derivation of an internal value from the external NOAEL or BMD (applied dose). For liver effects upon oral administration, it is the oral absorption that suffices. However, for every effect other than at the portal of entry, it is the bioavailability that is in general a more reliable parameter for further use in risk assessment, not the absorption [14].

**Biochemical pathway**

A series of reactions, typically enzyme-catalysed that are associated with a specific physiological event in a living organism [6].
**Bioinformatics**

Use of information science to integrate diverse, complex data generated by life sciences and organise it in an understandable context [4].

**Biomarker**

A biochemical, physiological, or histological change or aberration in an organism that can be used to estimate either exposure to stressors or resultant effects [4].

A biomarker is a characteristic that can be objectively measured and evaluated as an indicator of physiological as well as pathological process or pharmacological response to a therapeutic intervention [15].

An indicator signalling an event or condition in a biological system or sample and giving a measure of exposure, effect, or susceptibility.

Note: Such an indicator may be a measurable chemical, biochemical, physiological, behavioural, or other alteration within an organism [16].

A change in a biological response (ranging from molecular through cellular and physiological responses) that can be related to exposure to, or toxic effects of, environmental chemicals [17].

**Chemical category**

A group of chemicals whose physico-chemical and human health and/or environmental toxicological properties and/or environmental fate properties are likely to be similar or follow a regular pattern as a result of structural similarity (or other similarity characteristic) [18].
**Endpoint**

The recorded observation coming from an *in chemico* method, an *in vitro* assay or an *in vivo* assay [1].

The measurement of a biological effect, e.g. LC$_{50}$ or EC$_{50}$. A large number of endpoints are used in regulatory assessments of chemicals. These include lethality, carcinogenicity, immunological responses, organ effects, developmental and reproductive effects, etc. In QSAR analysis, it is important to develop models for individual toxic endpoints [9].

**Key Events**

Key events are intermediate events (ones between the molecular initiating event and the apical outcome) that are toxicologically relevant to the apical outcome and experimentally quantifiable [19].

Key events are additional events further along the pathway that lead to, and are experimentally or toxicologically associated with the adverse outcome [1].

Key events are empirically observable precursor steps that are a necessary element of the mode of action or are a biological marker for such an element [20, 21].

**Levels of biological organisation**

The organelle, cellular, tissue/organ and organism (and when required) population [1, 3].

Atom, molecule, cell, tissue, organ, organ system, organism (individual), population, community, ecosystem, biosphere (see Figure 1) [4].

**Mechanism of action**

Denotes the sequence of events leading from the absorption of an effective dose of a chemical to the production of a specific biological response in the target organ. Understanding a chemical’s mechanism requires appreciation of the causality and temporal relationships between the steps leading to a particular toxic endpoint, as well as the steps that lead to an effective dose of the chemical at the relevant biological target(s) [3].
Mechanism of action for toxicity is the detailed molecular description of key events in the induction of cancer or other health endpoints. Mechanism of action represents a more detailed understanding and description of events than is meant by mode of action [9].

A complete and detailed understanding of each and every step in the sequence of events that leads to a toxic outcome, underlying the MOA [22].

**Metabolomics**

The study of chemical processes involving metabolism. Metabolomics is different from transcriptomics and proteomics because it is not related to the transcription-translation paradigm. It is based on the idea that the chemical composition of biological fluids reflects the health of an organism [19].

Metabolomics deals with metabolite profiles of tissues or organs derived from mass spectrometry or nuclear magnetic resonance spectrometry analyses of plasma or homogenates. Metabolic profiling can give an immediate picture of the physiological state of tissue [23].

Global analysis of small molecule metabolites and their relative abundance, generally through nuclear magnetic resonance and mass spectroscopy [4].

The study of the products of biological processes. Such products change in response to such things as nutrition, stress, and disease states [24].

Evaluation of cells, tissues, or biological fluids for changes in metabolite levels that follow exposure to a given substance, in order to determine the metabolic processes involved, to evaluate the disruption in intermediary metabolic processes that results from exposure to that substance, or to determine the part of the genome that is responsible for the changes [16].

**Mode of action**

The Mode of Action (MoA) can be defined in the context of Figure 1. As such, it relates to the events including, and downstream of, the toxicity pathway. These could lead to an adverse effect in an individual. The MoA starts with the molecular initiating event. Unlike AOP, it does not (usually) include consideration of exposure or effects at high levels than the individual.
The sequence of key events and cellular and biochemical events (measurable parameters), starting with the interaction of an agent with the target cell, through functional and anatomical changes, resulting in cancer or other adverse health effects [20, 21, 25].

Mode of action differs from mechanism, in that the mode of action requires a less detailed understanding of the molecular basis of the toxic effect [26].

Mode of action for toxicity is the description of 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 [9].

A common set of physiological and behavioural signs that characterise a type of adverse biological response, where the major (but not all) biochemical steps are understood [22].

*Molecular Initiating Event*

The initial point of chemical-biological interaction within the organism that starts the pathway [1].

Direct interaction of a chemical with specific biomolecules [4].

The molecular level, chemical-induced perturbation of a biological system [19].

Chemical interaction at a molecular target leading to a particular adverse outcome [19].

The seminal interaction (e.g. DNA-binding, protein oxidation, or receptor/ligand interaction) of a chemical with a biological target [19].
**Molecular screening**

Molecular screening combines rapid screening methods with toxicogenomics with the objective of applying biochemical and cellular genomic methods to category analysis. The premise of molecular screening of toxicity is driven by interactions with cellular targets of one form or another so to initially assess toxicity, one needs to identify the proper target of concern and an appropriate assay is needed to assess the likelihood of interaction with the chemical(s) of concern [23].

**Non-apical endpoint**

Alternative, suborganism-level, *in vitro* responses, biomarkers, QSARs, genomics [4].

Intermediate event or step at a level of biological organization below that of the apical endpoint [19].

**Pathway perturbation**

Critical alteration of a toxicity pathway by an environmental agent or its metabolites that can impair normal biological function to such an extent that an adverse health effect may occur [10].

**Proteomics**

Proteomics deal with cell and tissue-wide expression of proteins encoded by a genome. After transcriptomics, proteomics is the next step in omics studies. It is more complicated than genomics because while a particular genome is more or less constant, the proteins that are produced differs from one cell type to another and from time to time in the same cell type [23].

Proteomics confirms the presence and quantifies the protein. Merrick and Bruno have termed a distinct set of expressed proteins that distinguish between health, toxicity or disease as “toxicity signature” [27].

Global analysis of proteins in a sample and their relative abundance or modifications [4].

The study of proteomes, which are collections of proteins. Proteins carry out the functions encoded by genes [24].
Rapid screening methods

Rapid screening methods include techniques which assess molecular properties or \textit{in vitro} responses. They range from simple structure-activity analyses to high-throughput \textit{in chemico} and cellular assays, to mid-level throughput \textit{in vitro} and \textit{ex vivo} assays [23].

Pharmacological or toxicological Screening

Pharmacological or toxicological screening consists of a specified set of procedures to which a series of compounds is subjected to characterize pharmacological and toxicological properties and to establish dose–effect and dose–response relationships [8].

Source to Outcome Pathway

The Source to Outcome Pathway can be defined in the context of Figure 1. As such it relates to the complete understanding of the effects of a chemical substance from environmental contamination through to effects at the community level. It incorporates the AOP concept and hence toxicity pathways and MoA.

The continuum or cascade of measurable events starting from release into the environment and ending at an adverse outcome [20].

Structural alerts

Structural alerts are atom-based fragments which, when present in a molecule, are an indication that a compound can be placed into a particular category [3].

System biology

Study of the mechanisms underlying complex biological processes as integrated systems of many diverse, interacting components.

\textit{Note}: It involves (1) collection of large sets of experimental data (by high-throughput technologies and/or by mining the literature of reductionist molecular biology and biochemistry), (2) proposal of mathematical models that might account for at least some significant aspects of this data set, (3) accurate computer
solution of the mathematical equations to obtain numerical predictions, and (4) assessment of the quality of the model by comparing numerical simulations with the experimental data [8].

System biology is defined as the biology of dynamic interacting networks. It requires the use of variety of analytical platforms as well as bioinformatics, data integration, and modelling [15].

Study of relationships and flow of biological information between elements of biological systems, with the goal of understanding and predicting emergent properties of those systems [28].

**Toxicity Pathway**

The toxicity pathway can be defined in the context of Figure 1. As such it relates to the perturbation of a normal biochemical pathway from the molecular initiating event to the cellular effect. It is at the heart of the MoA and AOP concepts, however it is not linked directly to an apical effect.

Cellular response pathways that, when sufficiently perturbed, are expected to result in adverse health effects are termed *toxicity pathways* [29].

After the toxic chemical reaches a target tissue, a molecular initiating event occurs that results in a cellular response, which has been called a toxicity pathway [5].

**Toxicogenetics**

Study of the influence of hereditary factors on the effects of potentially toxic substances on individual organisms [8].

**Toxicogenomics**

Toxicogenomics is an integration of conventional toxicology, bioinformatics methods and genomics and is defined as the study of the response of a genome to hazardous chemicals. [23].

Toxicogenomics uses the three major -omics technologies: transcriptomics, proteomics and metabolomics. [23].
Toxicogenomics is defined as the application of genomic technologies (for example, genetics, genome sequence analysis, gene expression profiling, proteomics, metabolomics, and related approaches) to study the adverse effects of environmental and pharmaceutical chemicals on human health and the environment. Toxicogenomics combines toxicology with information-dense genomic technologies to integrate toxicant-specific alterations in gene, protein, and metabolite expression patterns with phenotypic responses of cells, tissues, and organisms. Toxicogenomics can provide insight into gene environment interactions and the response of biologic pathways and networks to perturbations. Toxicogenomics may lead to information that is more discriminating, predictive, and sensitive than that currently used to evaluate toxic exposure or predict effects on human health [24].

Scientific subdiscipline that combines toxicology with genomics to determine how an organism’s genetic make-up influences its response to a toxic substance [8].

Transcriptomics

Transcriptomics deals with genome wide scale mRNA expression using DNA microarray and other high through put technologies that can estimate quantity of mRNA. [23].

The study of transcriptomics examines the expression level of mRNAs in a given tissue, organ or other cell population, using DNA microarray and other high-throughput technologies that can estimate the quantities of mRNAs [24].

Transcriptomics (or gene expression profiling) is the study of mRNA—the intermediary step between genes and proteins that indicates genes that are active (as opposed to dormant or silent) [24].

Transcriptomics (also referred to as expression profiling) uses DNA microarrays (commercially available arrays or custom ones) and a DNA copy of RNA is made using reverse transcriptase. In expression profiling gene profiles are clustered into a gene expression signature. The rational is such signatures are more sensitive and accurate methods than outcomes (e.g. histopathology) from traditional test guidelines [27].
4. IDENTIFICATION OF MISSING DEFINITIONS

There are a number of terms, which are often used in the literature and are well understood. However, there are no concise definitions to describe these terms. Therefore, it would be useful to create such definitions and complete the glossary.

Below is the list of such terms:

*Cellular response*

*Cellular signalling pathway*

*Effectopedia*

*Molecular site of action*

*Computational toxicology*

5. CONCLUSIONS

21st Century (eco)toxicology is based on Mode of Action (MoA) or Adverse Outcome Pathways (AOPs) approaches, which describe the biochemical-physiological basis for the toxicological effect. A multitude of disciplines from system biology and computational toxicology will contribute to developing and elucidating AOPs. This results in creating and using a variety of terms and definitions describing the processes on the different level of biological organization. Because the final use of the AOPs is hazard or risk assessment, it is necessary to standardise the terminology used in developing and recording the AOP. Therefore, a number of definitions have been collected from the literature. It is hope that this document may be a starting point for the harmonisation of the terminology relevant to AOP.
6. REFERENCES


http://www.oecd-library.org/docserver/download/fulltext/9741701e.pdf?expires=1331235086&id=id&accname=freecContent&checksum=B58CD7A86BA6334FE77E7DBF241DF31F


19. Personal comment of Terry Schultz.


