Omics-based input and output in the development and use of adverse outcome pathways
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Abstract

Adverse outcome pathways (AOPs) are pragmatic tools in toxicology and risk assessment with broad potential. AOPs are designed to provide a clear-cut mechanistic representation of toxicological effects that span over different layers of biological organization. AOPs share a common structure consisting of a molecular initiating event, a series of key events connected by key event relationships and an adverse outcome. AOPs can serve a number of purposes pertinent to safety assessment of chemicals, such as the establishment of quantitative structure−activity relationships, the development of novel *in vitro* toxicity screening tests, and the elaboration of prioritization strategies. Development of AOPs ideally complies with guidelines issued by the Organization for Economic Cooperation and Development. Omics, in particular transcriptomics, plays a major role in the establishment and application of AOPs by defining key events and by providing biomarkers for toxicity screening, respectively.

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Prediction of chemical-induced insults in humans still remains a huge challenge. This particularly holds true for the pharmaceutical sector, where preclinical animal models pick up merely 50% of all human drug−induced liver injuries [1,2]. For this reason, as well as because of ethical constraints, increasing attention is paid to the use of human liver−based *in vitro* models for predicting toxicity triggered by chemicals. Although promising, most of these nonanimal systems predict only half of clinical cases of chemical adversity [1,3]. Among the many reasons, to explain this shortcoming, are gaps in our current understanding of the mechanisms that underlie chemical-induced toxicity. In this respect, a number of pragmatic tools to rationally and visually capture existing knowledge regarding the mechanistic basis of toxicity have been introduced in the last decades. A major step in this direction came with the establishment of the mode-of-action concept in 2001, which encompasses a series of key events (KEs) along a biological pathway from the initial chemical interaction to the adverse outcome (AO) [4−7]. Another tool was adopted in 2010, namely, the AO pathway (AOP), which refers to a conceptual construct that portrays existing knowledge concerning the linkage between a direct molecular initiating event (MIE) and AO at a biological level of organization relevant to risk assessment (*i.e.*, safety evaluation of chemicals) [7]. The scope of an AOP is broader compared with the mode of action as it considers effects at the population level. Furthermore, although the mode of action tends to be chemical-specific and considers kinetic aspects, AOPs are chemical agnostic and describe toxicological processes from a purely dynamic angle [8−13].

AOPs are intended to support regulatory decision-making on the basis of desire to make effective use of mechanistic data, particularly novel data that can be generated more rapidly and cost effectively in a high-throughput format, such as omics-based information, rather than relying only on apical outcome data traditionally measured in whole organism toxicity tests [9,10,12−15]. AOPs primarily support hazard identification, which is a critical step in the chemical risk assessment paradigm. The specific application of an AOP is, however, dictated by the available data and by the degree of maturity of the AOP. AOPs can serve as the basis for generating integrated approaches to testing and assessment (IATAs). The IATA is a pragmatic approach that exploits and weighs all existing information for the purpose of chemical risk assessment [16,17]. Although IATAs provide a platform for data integration and a means for targeted testing for a specific purpose, it is not necessarily framed by a mechanistic rationale. AOPs can provide this mechanistic basis and identify data gaps or contextualize a diverse universe of existing data [14,17]. AOPs can help to develop chemical categories on the

basis of biological responses. A chemical category is defined as a group of chemicals whose physicochemical and human health properties are likely to be similar or to follow a regular pattern. The next step is to substantiate the chemical category with experimental data and nontesting approaches, including quantitative structure–activity relationship methodologies. Once the chemical category is fully established, it can be used for data gap–filling strategies, such as read-across techniques that apply relevant information from analogous substances to predict the toxicological properties of a target substance [14,18]. AOPs may also facilitate prioritization of chemicals for further testing. In this process, substances are screened for their potential to trigger a specific and measurable biological response, in this case related to one or more MIEs and/or KEs. Substances identified as presenting a risk to induce a specific toxicological effect are subsequently ranked according to potency, whereby the most potent substances receive the highest priority to undergo more detailed testing [9,16,18]. Other applications for AOPs include the labeling of chemicals on the basis of their potency to activate an AOP and the development of *in vitro*, *in silico*, and/or *in chemico* methods to test specific MIEs and/or KEs [9,18].

Because of the increasing use of AOPs, the Organization for Economic Cooperation and Development (OECD) initiated an international AOP development program in 2012, followed by the publication of a guidance document on the development and assessment of AOPs [18], which was recently updated [19], and the establishment of the AOP knowledge base [20] in collaboration with a number of other agencies. The AOP knowledge base

consists of five modules, of which the AOP Wiki provides an open source interface that serves as a central repository for AOPs. Furthermore, the OECD published a user's handbook that provides information on the AOP development, including instructions on how to build and assess AOPs [21]. An AOP developed within a project of the AOP program at the OECD undergoes a rigorous internal and external review process. If successfully completed, this ends with endorsement and publication in the OECD series on AOPs [14]. At present, the AOP Wiki contains about 280 AOPs for a wide variety of human and ecotoxicological endpoints [21].

Each OECD-compliant AOP comprises two fundamental components, namely, KEs and KE relationships (KERs), which link together in a causal chain that spans from the molecular domain up to the level of the organism or population (Figure 1). A KE represents a measurable change in a biological state that is essential, but not necessarily sufficient, for progression from the MIE to the AO. The MIE and AO are two specialized KE types [12,18,21–23].

AOPs can be developed by the following six strategies. In (i) top-down, (ii) middle-out, and (iii) bottom-up approaches, AOP development starts from an MIE, KE, or AO. (iv) Case study strategies begin with an AOP on the basis of one or more model chemicals, which are subsequently generalized to other chemicals. (v) AOP development by analogy defines an AOP in one organism that is extrapolated to other species. (vi) AOP development from data mining uses high-throughput and high-content data, including omics-based information, to identify KEs [12,24–26]. These six different AOP

Figure 1
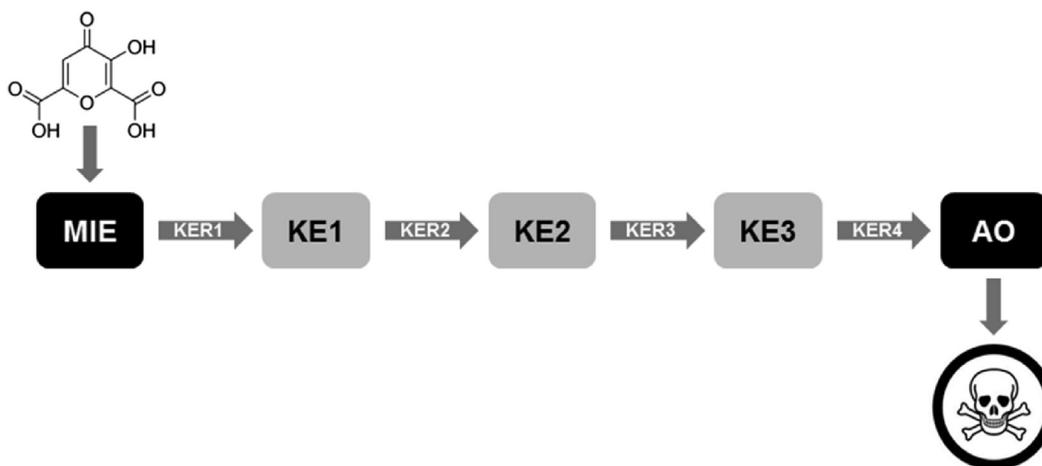
Generic structure of an AOP. Each AOP consists of two anchors, namely, the molecular initiating event (MIE), which refers to the interaction of a chemical with a biological system at the molecular level, and the adverse outcome (AO), which is the actual apical toxicological endpoint. The entire response matrix between the MIE and AO is filled with key events (KEs), which represent changes in the biological state that are both measurable and essential to the progression of a defined biological perturbation leading to a specific AO. Subsequent KEs are connected by key event relationships (KERs), defining a link between both KEs and that facilitate inference or extrapolation of the state of the downstream KE from the known, measured, or predicted state of the upstream KE. AOP, adverse outcome pathway.

development strategies can be combined, and their selection is determined by the intended AOP application and by the availability of existing data [12].

The AOP development process is a continuous and dynamic activity and involves gradually building an AOP from a hypothesized set of KEs and KERs by providing empirical evidence [10–12]. As such, three stages can be distinguished in this process. (i) The putative stage relies on pooling of general toxicological knowledge or statistical inference derived from (omics-based) data sets. (ii) In the formal stage, the endeavor shifts to refinement of KEs and KERs by including a more systematic and rigorous assembly of evidence underpinning the AOP. Such evidence fuses consideration of biological plausibility with supporting empirical data and can be retrieved from the scientific literature. (iii) In the quantitative stage, descriptions are made on how to quantify KEs and KERs, such as by establishing dose–effect relationships [12]. The vast majority of currently available AOPs does not reach this final quantitative stage [13].

Evaluation of newly developed AOPs includes consideration of the so-called three tailored Bradford Hill criteria. The Bradford Hill criteria have been initially introduced to determine causality of associations observed in epidemiological studies [27]. They have been adopted to assess AOPs, albeit in a more tailored format. In rank order, these tailored Bradford Hill considerations include (i) biological plausibility, (ii) essentiality, and (iii) empirical support. The former and the latter are considered for each KER individually, whereas essentiality of the KEs is scrutinized in the context of the overall AOP. Each of these tailored Bradford Hill considerations is subjected to weight-of-evidence analysis, whereby confidence is judged as strong, moderate, or weak for each of the KEs, KERs, and the AOP as such, on the basis of the availability of documentation and/or empirical support [8]. The purpose of this weight-of-evidence analysis is to transparently document the certainty and uncertainty existing in specific lines of evidence that support the AOP [28].

Different types of information can be used during AOP development. Characterization of the MIE at the molecular level relies on *in silico* (*i.e.*, computational) or *in vitro* (*i.e.*, cell culture) data. KEs at the organelle and cellular levels are typically supported by *in vitro* or, to a lesser extent, by *in vivo* (*i.e.*, animal experimentation and clinical information) testing outcomes, whereas KEs at the tissue, organ, and organism levels routinely depend on *in vivo* experimentation results [10,13,14]. Because of their scope, omics-based data can be used to feed virtually all information blocks in AOPs. In this respect, omics-based disciplines, such as AOPs, address a wide repertoire of levels of biological organization. Undoubtedly, however, most focus in the field of toxicology

and risk assessment has been and is still on the basis of transcriptomics. Indeed, compared with other omics areas, such as proteomics and metabonomics, transcriptomics enables to gather a wealth of data in a high-throughput, user-friendly, and relatively cheap way. Transcriptomics, basically, is a holistic approach that intends to study the entire transcriptome (*i.e.*, the collection of all mRNAs in a cell or tissue). The transcriptomics area is a rapidly evolving field with new technologies being developed at a high pace. At present, two main transcriptomic approaches are followed, using (i) microarrays and (ii) RNA sequencing. The former quantifies a set of predetermined sequences, whereas RNA sequencing applies high-throughput sequencing to capture all sequences [29,30]. Bioinformatics has emerged as an integral part of omics sciences to cope with the vast amount of data that are typically produced in these areas. In the specific case of transcriptomics, bioinformatics strategies, such as differential gene expression analysis and pathway analysis, allow to characterize specific changes in the transcriptome in different conditions or in response to different treatments. Such 'gene blueprints' are extremely valuable for toxicologists as they allow to pinpoint 'transcriptomic signatures' for specific types of chemical-induced toxicities. Obviously, this is highly beneficial for AOP development. In fact, transcriptomic data can be used in at least two ways in the AOP field. (i) Transcriptomic information can form the solid basis for defining MIEs and/or KEs. Transcriptomics can hereby be used either as a set of standalone data or may complement other types of information as AOP input. AOPs are known to be living documents partly because of the fact that they should be iteratively refined every time new relevant data become available [12,13]. This particularly holds true for transcriptomic data because of their high-throughput nature. (ii) Transcriptomic data, as AOP output, can provide a set of biomarkers eligible for toxicity testing and hazard identification. Indeed, AOPs can be used as the conceptual basis for establishing batteries of *in vitro* methods, whether or not combined with *in silico* and/or *in chemico* assays, mechanistically anchored in AOPs to accurately test and predict chemical-induced toxicity. Transcriptomic signatures hereby serve as first-tier identifiers of chemical adversity and may be followed up by more specific single-target methods at the transcriptional, translational, or activity level serving as second-tier identifiers of toxicity. In this context, an integrated omics-based verification of an AOP of cholestatic liver injury was recently performed [31]. This study intended to experimentally validate a previously introduced AOP from bile salt export pump inhibition to cholestasis [32] using HepaRG cell cultures exposed to the prototypical cholestatic drug bosentan. The outcome was evaluated by means of transcriptomics, proteomics, and metabonomics, each that provided a set of novel mechanistically anchored

biomarkers linked to the proposed KEs. These may be more early markers compared with current indicators of cholestasis and, thus, are eligible candidates for hazard identification purposes [31].

Taken together, transcriptomic information thus plays a ubiquitous and versatile role in the AOP field. Future efforts should be focused on quantifying transcriptomic signatures fit for risk assessment purposes by linking them to tools and parameters currently used in daily regulatory practice for setting safety limits, such as no-observed-adverse-effect levels, margins of safety, and acceptable daily intake values. In addition, other established omics sciences, such as proteomics and metabonomics, would greatly benefit from a technological boost for more user-friendly and high-throughput application in toxicology and risk assessment. This should be paralleled by further exploration of more recently introduced omics sciences, including cytomics, which uses promising and powerful techniques, such as high-content analysis. When combined with transcriptomics testing, this will certainly enable even more accurate prediction of chemical-induced toxicity in humans.

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