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The Adverse Outcome Pathway: A Multifaceted Framework Supporting 21st Century Toxicology

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Abstract

The adverse outcome pathway (AOP) framework serves as a knowledge assembly, interpretation, and communication tool designed to support the translation of pathway-specific mechanistic data into responses relevant to assessing and managing risks of chemicals to human health and the environment. As such, AOPs facilitate the use of data streams often not employed by risk assessors, including information from *in silico* models, *in vitro* assays and short-term *in vivo* tests with molecular/biochemical endpoints. This translational capability can increase the capacity and efficiency of safety assessments both for single chemicals and chemical mixtures. Our mini-review describes the conceptual basis of the AOP framework and aspects of its current status relative to use by toxicologists and risk assessors, including four illustrative applications of the framework to diverse assessment scenarios.

Keywords

Adverse Outcome Pathway; Chemical Assessment; Human Health; Environment

1.1 Changing Face of Regulatory Toxicology: A Brief Synopsis

The past decade has witnessed an unprecedented expansion in the variety and volume of molecular and biochemical data available for taxa ranging from bacteria to humans. This has fueled significant advances in fields such as evolutionary biology, agricultural sciences, and biomedical diagnostics and technology. Other disciplines also have started to seize the opportunities provided by new data streams and tools to address past and present challenges.

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AUTHOR DECLARATION TEMPLATE

We wish to confirm that there are no known conflicts of interest ass ociated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

Toxicology is one of the disciplines that stands to significantly benefit from these new sources of biological knowledge.

Toxicology is among the most applied of the biological sciences, driven largely by mandates to assess specific aspects of chemical safety relative to human health and the environment. Historically this has been achieved mostly through the generation of data for a relative handful of high priority/visibility chemicals using well-defined animal models and apical endpoints. However, increasing societal awareness of, and concern for the number of chemicals with limited or no hazard/risk information has resulted in legislative mandates such as the REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) program in Europe and recent revisions to the Toxic Substances Control Act (TSCA) in the US, which require consideration of the possible health and ecological effects of a much larger chemical universe than in the past [1, 2]. There also is an increasing emphasis on understanding the effects of a wide variety of chemical mixtures on human health and the environment; for example, in North America the Great Lakes Restoration Initiative has identified complex mixtures of “chemicals of emerging concern” as one of the highest priority stressors in the lakes [3].

These types of newer regulatory programs and monitoring initiatives highlight the necessity of identifying and developing rapid, cost-effective approaches for predicting the potential toxicity of substances to augment (or replace) the *in vivo* test methods that traditionally have supported chemical risk assessments [4]. These approaches may include *in silico* models, *in vitro* assays (including those conducted in a high-throughput [HPT] format), and short-term *in vivo* tests with molecular/biochemical endpoints (including ‘omics) indicative of perturbation of biological pathways.

A critical challenge to using alternative tools and data types for chemical safety assessment involves translation of this information into apical responses applicable to risk assessment, such as impacts on survival, reproduction, induction of cancer, etc., in individuals and, in the case of ecological effects, populations. The adverse outcome pathway (AOP) framework was developed to address this translation challenge [5]. Herein we describe the conceptual basis and current status of the AOP framework, provide examples of its use in different types of chemical assessments, and touch on several recent developments relevant to the future of the framework.

1.2 Definition and Attributes of the AOP Framework

The AOP framework reflects an evolution of prior pathway-based concepts, most notably mechanism or mode of action, for assembling and depicting toxicological data across biological levels of organization [5–7]. An AOP consists of a series of measurable key events (KEs) linked to one another by key event relationships (Figure 1). The first KE generally is a molecular initiating event (MIE), which captures the interaction of a chemical with a biological macromolecule, that triggers subsequent KEs which could result in an adverse outcome (AO) at the individual or population level [8, 9]. Explicit in an AOP is that the KEs are causally linked to one-another, an attribute that can be formally assessed using weight-of-evidence analyses [10, 11]. An important property of AOPs is that they are

chemically-agnostic, capturing response-response relationships that result from a given perturbation of a MIE that could be caused by any of a number of chemical (or even non-chemical) stressors [8].

The AOP framework provides a connection between mechanism-based effects measurements and apical outcomes on two levels. First, in assembling evidence supporting the proposed mechanism, AOPs provide the understanding needed to interpret data from measurements of KEs as they relate to an apical endpoint of regulatory concern (Figure 1-top). Having a structured framework for this purpose is important because the predictive utility of early KEs for the eventual AO can be limited for a variety of reasons. For example, homeostatic mechanisms will serve to obviate AOs in many cases, but modulating factors such as genetic differences, pre-existing disease, and alterations from other environmental stressors can magnify responses in some situations. Because of this, it is important to capture and organize existing knowledge and make it available for interpretation of these new data streams. In fact, once the factors influencing the propagation of the signal from the early KEs to the final AOs are sufficiently described, using early KEs as the primary assay for toxicity has the advantage of them being measurable in either *in vitro* or *in vivo* systems within hours to days, as opposed to the weeks, months or even years it can take for apical AOs to manifest.

The second role for the AOP is to serve as a scaffold for assembling data associated with a given outcome in an organized manner (Figure 1-bottom). By assembling these data in the context of an AOP, it allows for different measures of pathway perturbation to be compared with one another with regard to their predictive capacity for an AO, rather than an *a priori* selection of one measurement as the “gold standard”. Having all information in a structured framework also enables defined approaches for integrated toxicity assessments that incorporate information from multiple endpoints to improve the prediction of an AO.

AOPs are deliberate simplifications of normal biological pathways intended to facilitate depiction and ready communication of what can be very complex processes. A common misconception about AOPs is that they can depict KEs along a given pathway only in a linear manner, thus ignoring potentially important interactions between pathways [12]. Linear AOPs, however, can be assembled to produce AOP networks that capture shared nodes and interactions among pathways [8, 13, 14]. Furthermore, it is possible to assemble quantitative AOPs (qAOPs) that consider quantitative relationships between KEs, including feedback models designed to reflect system regulation, to predict AOs [15]. For example, Conolly et al. [16] recently described a qAOP that utilizes a feedback-controlled hypothalamic-pituitary-gonadal axis model to enable predictions of reproductive capacity in fish exposed to chemicals that inhibit sex steroid synthesis. Basically, the AOP framework is capable of capturing sufficient complexity to ensure application to a variety of assessment scenarios/challenges.

AOPs have received substantial attention as an organizing framework for toxicologically-relevant biological information, for example, in the extant scientific literature (Figure 2). A pragmatic example of interest in the AOP framework involves the Organisation for Economic Cooperation and Development (OECD) which, starting in 2012, has supported

activities of a workgroup of international experts to publish harmonized guidance for the description, evaluation, and technical review of the scientific robustness of AOPs [6, 17, 18]. The AOP framework is envisioned by the OECD as a critical tool supporting the mutual acceptance of toxicological data by diverse regulatory authorities. To further enhance harmonization, the OECD also has helped facilitate development of an internationally accessible and searchable source of AOP information [19], that includes the AOP Wiki [20], an interactive knowledgebase for describing, displaying, and archiving AOPs and AOP networks. The AOP Wiki currently contains more than 200 AOPs at different stages of development, which describe processes/endpoints relevant both to human health and the environment.

1.3 Examples of AOP Uses

The AOP framework is intended to be flexible relative to potential research and regulatory applications. Below we provide four comparatively brief, illustrative examples of application of the concept to diverse assessment scenarios.

1.3.1 Case Example 1: Predicting Skin Sensitization

Skin sensitization involves covalent modification of cellular proteins in skin by electrophilic compounds, which subsequently can enhance reactions to allergens. As such, this is an important endpoint for safety assessments involving personal care products, which historically has been evaluated using *in vivo* assays. However, legislation in the European Union dictated moving away from whole animal tests for evaluating sensitization, resulting in the need for an alternative assessment approach [21]. This has been addressed by developing an AOP for skin sensitization that includes description of several intermediate KEs related to induction of inflammatory cytokines and proliferation of T-cells [20 (AOP 40), 22]. This AOP, which has been supported by extensive technical review [23, 24], provides the basis for identifying and validating a suite of *in vitro* assays reflecting these intermediate KEs. Data from this assay suite for test chemicals of interest can be assessed using modeling approaches such as Bayesian network analysis to combine/weight data from different biological levels of organization, captured in the AOP, to produce categorical predictions of the potential for skin sensitization [25, 26]. This effort shows how capturing pathway-based data in an AOP can facilitate the use of alternative data streams as a replacement for conventional test methods [27].

1.3.2 Case Example 2: Prioritizing Endocrine Disrupting Chemicals

Chemicals that exert adverse effects in humans and wildlife through their ability to alter endocrine function have been a topic of scientific and regulatory concern for almost 25 years [28]. The US Environmental Protection Agency (USEPA) has a legislated mandate to develop a screening and testing program to identify potential adverse endocrine-mediated effects of more than 10,000 chemicals [29], a task that cannot plausibly be accomplished in a reasonable timeframe solely through *in vivo* testing [30, 31]. To address this challenge, *in vitro* HTP data and models are being used to prioritize the list of target chemicals for those likely to act via endocrine MIEs of regulatory concern, such as activation or antagonism of estrogen or androgen receptors and inhibition of specific enzymes involved in sex steroid or

thyroid hormone synthesis. Browne et al. [30] recently described this approach using estrogen receptor activation as an example. In this context the AOP framework provides demonstrable linkages between *in silico* or *in vitro* measures of bioactivity and potential adverse effects *in vivo* [31], thus supporting both identification of assays suitable for detecting MIEs of concern, and providing conceptual "phenotypic anchoring" supporting their use in the prioritization process.

1.3.3 Case Example 3: Evaluating Pesticide Toxicity to Pollinators

Key pollinator species, such as honeybees, have experienced significant worldwide declines, resulting in concerns for possible effects on global food production. In the US, for example, a national strategy has been developed to assess the significance and causes of pollinator declines [32]. A number of chemical and non-chemical stressors have been proposed as contributing to declines, one of the more prominent of which are neonicotinoid pesticides [33, 34]. However, significant uncertainties exist as to the biological plausibility of a link between the molecular action (MIE) of neonicotinoids—activation of the nicotinic acetylcholine receptor—and impacts on honeybee colonies. To help assess the veracity of hypothesized effects of neonicotinoids on honeybees, LaLone et al. [14] assembled an AOP network based on molecular, biochemical, physiological, behavioral, and population data from more than 220 papers in the open literature. Not only were they able to demonstrate a plausible linkage between perturbation of nicotinic acetylcholine receptor signaling and adverse effects in honeybees, but the analysis highlighted areas of uncertainty that would benefit from focused research and/or monitoring [14]. In this example, the AOP framework supported integration of a complex, biologically-diverse dataset in the context of evaluating causal relationships among endpoints at different levels of organization, and served as a basis for generating hypotheses to test these interactions.

1.3.4 Case Example 4: Evaluating Hazards of Complex Chemical Mixtures

Newer approaches being advocated/used in predictive toxicology for single chemicals can, in conjunction with the AOP framework as a translator, also be employed to help assess risks of complex mixtures of chemicals. A significant challenge in assessing complex mixtures is predicting the possible biological effects of hundreds or even thousands of contaminants, many of which may be unknown. Schroeder et al. [35] recently described how suites of HTP assays can be used to measure diverse bioactivities of complex contaminant mixtures in surface waters to effectively augment more targeted instrumental analyses. Measurements from HTP assays often correspond to MIEs or early KEs (e.g., receptor activation, enzyme inhibition, etc.), so it is possible to query/cross-reference knowledgebases such as the AOP Wiki to translate bioactivity data generated from complex mixtures into potential hazards in exposed organisms, such as fish [35, 36]. The AOP framework also can serve as the basis for translating molecular/biochemical data from field-collected animals exposed to complex mixtures into endpoints useful for inferring hazard/risk [37]. Miller et al. [38] recently described a study in which an AOP construct was linked to a population model for white suckers, a large cyprinid species indigenous to the Great Lakes, to predict population status based on observed changes in sex steroid synthesis in fish exposed to a complex pulp and paper mill effluent. In this application, it was not necessary to know the identity of the chemicals responsible for decreasing steroid synthesis, only that they consistently affected

an early KE in an AOP relating depressed steroid synthesis to decreased egg production and, hence, population status [20 (AOP 25); 38].

1.4 Concluding Thoughts

The AOP concept has matured from a largely conceptual construct to an increasingly practical and sophisticated knowledge-assembly/communication tool with multiple applications. In addition to the types of uses illustrated above, the AOP framework is being applied to more novel scenarios, such as consideration of contaminant interactions with environmental variables associated with climate change [39], evaluation of environmental and/or human health effects of nanomaterials and ionizing radiation [40–43], chemical life-cycle assessment and alternatives analysis [44, 45], and the design of hypothesis-driven environmental monitoring programs [46]. Furthermore, AOPs are being considered by the biomedical community as a means to support drug discovery/development and understand disease initiation/progression [e.g., 47–50].

In addition to novel applications, innovative scientific approaches are being identified/employed in support of basic AOP development. For example, recent efforts have focused on the identification, development and evaluation of new AOPs based on ‘omic and/or HTP data, systems/network modeling, and/or repositories of curated toxicity information [52–55]. Increasing the “library” of available AOPs is critically important to supporting the varied applications of the framework in the future.

Many of the technical and practical advancements in the AOP framework have occurred as a result of recommendations from different international fora [e.g., 15, 53, 56, 57], including a recent SETAC Pellston meeting in Cornwall, ON, Canada (April 2017). This meeting utilized a novel “horizon scanning” approach to identify upcoming/priority issues for AOP development and use based on input from the broader scientific and regulatory communities [12]. Topics included development and practical implementation of AOP networks and qAOPs, case examples of regulatory use of AOPs, and development of a roadmap for a long-term, sustainable model supporting AOP development and use [58].

Evolution of the AOP concept thus far has been facilitated through the individual and joint efforts of a variety of research and regulatory organizations around the world, representing governmental, business, and academic interests. It is this multi-sectorial interest that has advanced the AOP concept and hopefully will continue to support its development as a flexible and practical tool supporting 21st century toxicology.

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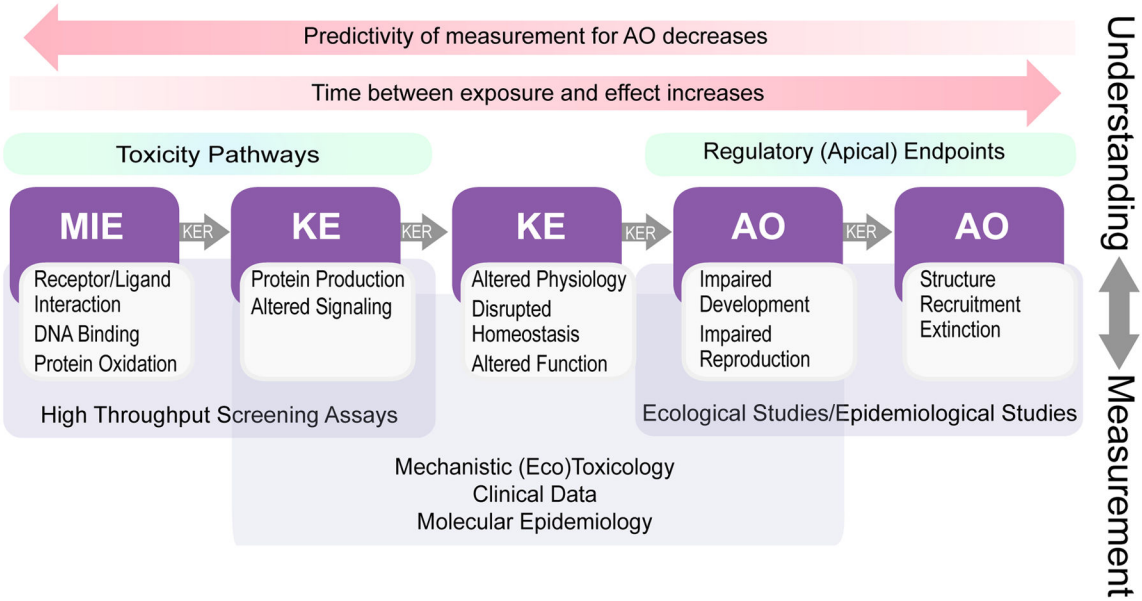

Figure 1.

Depiction of the role of the adverse outcome pathway (AOP) framework in linking various data streams to outcomes relevant to regulatory decision-making for chemicals. MIE – Molecular Initiating Event, KE – Key Event, KER – Key Event Relationship, AO – Adverse Outcome

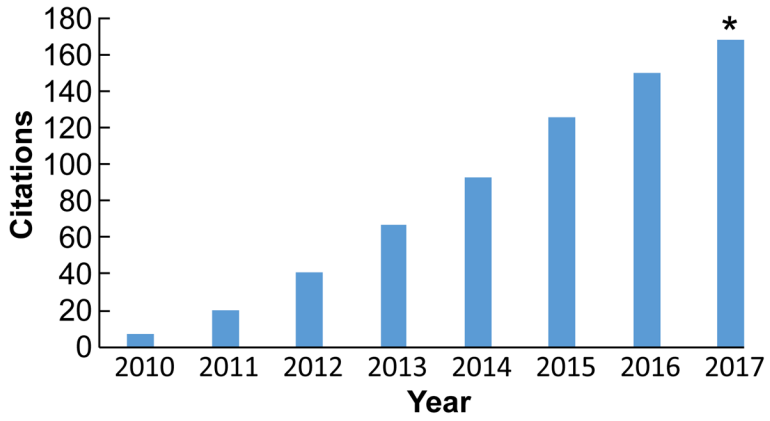

Figure 2.

Temporal citation analysis of an initial paper describing the adverse outcome pathway (AOP) framework (Ankley et al. 2010). “Final” data for 2017 are extrapolated from 6-month values. Analysis was conducted using the Web of Science (Clarivate Analytics).