Allen, T. E., et al. (2014). "Defining molecular initiating events in the adverse outcome pathway framework for risk assessment." Chem Res Toxicol 27(12): 2100-2112. Consumer and environmental safety decisions are based on exposure and hazard data, interpreted using risk assessment approaches. The adverse outcome pathway (AOP) conceptual framework has been presented as a logical sequence of events or processes within biological systems which can be used to understand adverse effects and refine current risk assessment practices in ecotoxicology. This framework can also be applied to human toxicology and is explored on the basis of investigating the molecular initiating events (MIEs) of compounds. The precise definition of the MIE has yet to reach general acceptance. In this work we present a unified MIE definition: an MIE is the initial interaction between a molecule and a biomolecule or biosystem that can be causally linked to an outcome via a pathway. Case studies are presented, and issues with current definitions are addressed. With the development of a unified MIE definition, the field can look toward defining, classifying, and characterizing more MIEs and using knowledge of the chemistry of these processes to aid AOP research and toxicity risk assessment. We also present the role of MIE research in the development of in vitro and in silico toxicology and suggest how, by using a combination of biological and chemical approaches, MIEs can be identified and characterized despite a lack of detailed reports, even for some of the most studied molecules in toxicology.

Angrish, M. M., et al. (2015). "Probe molecule (PrM) approach in adverse outcome pathway (AOP) based high-throughput screening (HTS): in vivo discovery for developing in vitro target methods." Chem Res Toxicol 28(4): 551-559. Efficient and accurate adverse outcome pathway (AOP) based high-throughput screening (HTS) methods use a systems biology based approach to computationally model in vitro cellular and molecular data for rapid chemical prioritization; however, not all HTS assays are grounded by relevant in vivo exposure data. The challenge is to develop HTS assays with unambiguous quantitative links between in vitro responses and corresponding in vivo effects, which is complicated by metabolically insufficient systems, in vitro to in vivo extrapolation (IVIVE), cross-species comparisons, and other inherent issues correlating IVIVE findings. This article introduces the concept of ultrasensitive gas phase probe molecules (PrMs) to help bridge the current HTS assay IVIVE gap. The PrM concept assesses metabolic pathways that have already been well-defined from intact human or mammalian models. Specifically, the idea is to introduce a gas phase probe molecule into a system, observe normal steady state, add chemicals of interest, and quantitatively measure (from headspace gas) effects on PrM metabolism that can be directly linked back to a well-defined and corresponding in vivo effect. As an example, we developed the pharmacokinetic (PK) parameters and differential equations to estimate methyl tertiary butyl ether (MTBE) metabolism to tertiary butyl alcohol (TBA) via cytochrome (CYP) 2A6 in the liver from human empirical data. Because MTBE metabolic pathways are well characterized from in vivo data, we can use it as a PrM to explore direct and indirect chemical effects on CYP pathways. The PrM concept could be easily applied to in vitro and alternative models of disease and
phenotype, and even test for volatile chemicals while avoiding liquid handling robotics. Furthermore, a PrM can be designed for any chemical with known empirical human exposure data and used to assess chemicals for which no information exists. Herein, we propose an elegant gas phase probe molecule-based approach to in vitro toxicity testing.


Cytokines, low-molecular-weight messenger proteins that act as intercellular immunomodulatory signals, have become a mainstream preclinical marker for assessing the systemic inflammatory response to external stressors. The challenge is to quantitate from healthy subjects cytokine levels that are below or at baseline and relate those dynamic and complex cytokine signatures of exposures with the inflammatory and repair pathways. Thus, highly sensitive, specific, and precise analytical and statistical methods are critically important. Investigators at the U.S. Environmental Protection Agency (EPA) have implemented advanced technologies and developed statistics for evaluating panels of inflammatory cytokines in human blood, exhaled breath condensate, urine samples, and murine biological media. Advanced multiplex, bead-based, and automated analytical platforms provided sufficient sensitivity, precision, and accuracy over the traditional enzyme-linked immunosorbent assay (ELISA). Thus, baseline cytokine levels can be quantified from healthy human subjects and animals and compared to an in vivo exposure response from an environmental chemical. Specifically, patterns of cytokine responses in humans exposed to environmental levels of ozone and diesel exhaust, and in rodents exposed to selected pesticides (such as fipronil and carbaryl), were used as case studies to generally assess the taxonomic applicability of cytokine responses. The findings in this study may aid in the application of measureable cytokine markers in future adverse outcome pathway (AOP)-based toxicity testing. Data from human and animal studies were coalesced and the possibility of using cytokines as key events (KE) to bridge species responses to external stressors in an AOP-based framework was explored.


Ecological risk assessors face increasing demands to assess more chemicals, with greater speed and accuracy, and to do so using fewer resources and experimental animals. New approaches in biological and computational sciences may be able to generate mechanistic information that could help in meeting these challenges. However, to use mechanistic data to support chemical assessments, there is a need for effective translation of this information into endpoints meaningful to ecological risk-effects on survival, development, and reproduction in individual organisms and, by extension, impacts on populations. Here we discuss a framework designed for this purpose, the adverse outcome pathway (AOP). An AOP is a conceptual construct that portrays existing knowledge
concerning the linkage between a direct molecular initiating event and an adverse outcome at a biological level of organization relevant to risk assessment. The practical utility of AOPs for ecological risk assessment of chemicals is illustrated using five case examples. The examples demonstrate how the AOP concept can focus toxicity testing in terms of species and endpoint selection, enhance across-chemical extrapolation, and support prediction of mixture effects. The examples also show how AOPs facilitate use of molecular or biochemical endpoints (sometimes referred to as biomarkers) for forecasting chemical impacts on individuals and populations. In the concluding sections of the paper, we discuss how AOPs can help to guide research that supports chemical risk assessments and advocate for the incorporation of this approach into a broader systems biology framework.


The Adverse Outcome Pathway (AOP) framework provides a template that facilitates understanding of complex biological systems and the pathways of toxicity that result in adverse outcomes (AOs). The AOP starts with a molecular initiating event (MIE) in which a chemical interacts with a biological target(s), followed by a sequential series of KEs, which are cellular, anatomical, and/or functional changes in biological processes, that ultimately result in an AO manifest in individual organisms and populations. It has been developed as a tool for a knowledge-based safety assessment that relies on understanding mechanisms of toxicity, rather than simply observing its adverse outcome. A large number of cellular and molecular processes are known to be crucial to proper development and function of the central (CNS) and peripheral nervous systems (PNS). However, there are relatively few examples of well-documented pathways that include causally linked MIEs and KEs that result in adverse outcomes in the CNS or PNS. As a first step in applying the AOP framework to adverse health outcomes associated with exposure to exogenous neurotoxic substances, the EU Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) organized a workshop (March 2013, Ispra, Italy) to identify potential AOPs relevant to neurotoxic and developmental neurotoxic outcomes. Although the AOPs outlined during the workshop are not fully described, they could serve as a basis for further, more detailed AOP development and evaluation that could be useful to support human health risk assessment in a variety of ways.


The Adverse Outcome Pathway (AOP) concept has recently been proposed to support a paradigm shift in regulatory toxicology testing and risk assessment. This concept is similar to the Mode of Action (MOA), in that it describes a sequence of measurable key events triggered by a molecular initiating event in which a stressor interacts with a biological target. The resulting cascade of key events includes molecular, cellular, structural and functional changes in biological systems, resulting in a measurable adverse outcome. Thereby, an AOP ideally provides information relevant to chemical structure-activity relationships as a
basis for predicting effects of structurally similar compounds. AOPs could potentially also form the basis for qualitative and quantitative predictive modeling of the human adverse outcome resulting from molecular initiating or other key events for which higher-throughput testing methods are available or can be developed. A variety of cellular and molecular processes are known to be critical for normal function of the central (CNS) and peripheral nervous systems (PNS). Because of the biological and functional complexity of the CNS and PNS, it has been challenging to establish causative links and quantitative relationships between key events that comprise the pathways leading from chemical exposure to an adverse outcome in the nervous system. Following introduction of the principles of MOA and AOPs, examples of potential or putative adverse outcome pathways specific for developmental or adult neurotoxicity are summarized and aspects of their assessment considered. Their possible application in developing mechanistically informed Integrated Approaches to Testing and Assessment (IATA) is also discussed.


For toxicologists who are in any way associated with skin sensitisation, the last two decades have seen a series of fundamental changes. We have migrated from old-style guinea-pig assays, via the refined and reduced Local Lymph Node Assay (LLNA), to witness the imminent dominance of in vitro and in silico methods. Yet, over the same period, the use of the output data for human safety assurance has evolved from 'black box' risk assessment, via the quantitative risk assessment enabled by the LLNA measurement of potency, to a new period of relative uncertainty. This short review will endeavour to address these topics, all the while keeping a focus on three essential principles: a) that skin sensitisation potential is intrinsic in the molecular structure of the chemical; b) that test methods should have a mechanistic foundation; and finally c) that the only reason for undertaking any skin sensitisation work has to be the protection of human health.


Systematic consideration of scientific support is a critical element in developing and, ultimately, using adverse outcome pathways (AOPs) for various regulatory applications. Though weight of evidence (WoE) analysis has been proposed as a basis for assessment of the maturity and level of confidence in an AOP, methodologies and tools are still being formalized. The Organization for Economic Co-operation and Development (OECD) Users’ Handbook Supplement to the Guidance Document for Developing and Assessing AOPs (OECD 2014a; hereafter referred to as the OECD AOP Handbook) provides tailored Bradford-Hill (BH) considerations for systematic assessment of confidence in a given AOP. These considerations include (1) biological plausibility and (2) empirical support (dose-response, temporality, and incidence) for Key Event Relationships (KERs), and (3) essentiality of key events (KEs). Here, we test the application of these
tailored BH considerations and the guidance outlined in the OECD AOP Handbook using a number of case examples to increase experience in more transparently documenting rationales for assigned levels of confidence to KEs and KERs, and to promote consistency in evaluation within and across AOPs. The major lessons learned from experience are documented, and taken together with the case examples, should contribute to better common understanding of the nature and form of documentation required to increase confidence in the application of AOPs for specific uses. Based on the tailored BH considerations and defining questions, a prototype quantitative model for assessing the WoE of an AOP using tools of multi-criteria decision analysis (MCDA) is described. The applicability of the approach is also demonstrated using the case example aromatase inhibition leading to reproductive dysfunction in fish. Following the acquisition of additional experience in the development and assessment of AOPs, further refinement of parameterization of the model through expert elicitation is recommended. Overall, the application of quantitative WoE approaches hold promise to enhance the rigor, transparency and reproducibility for AOP WoE determinations and may play an important role in delineating areas where research would have the greatest impact on improving the overall confidence in the AOP.


An Adverse Outcome Pathway (AOP) represents the existing knowledge of a biological pathway leading from initial molecular interactions of a toxicant and progressing through a series of key events (KEs), culminating with an apical adverse outcome (AO) that has to be of regulatory relevance. An AOP based on the mode of action (MOA) of rodent liver tumor promotion by dioxin-like compounds (DLCs) has been developed and the weight of evidence (WoE) of key event relationships (KERs) evaluated using evolved Bradford Hill considerations. Dioxins and DLCs are potent aryl hydrocarbon receptor (AHR) ligands that cause a range of species-specific adverse outcomes. The occurrence of KEs is necessary for inducing downstream biological responses and KEs may occur at the molecular, cellular, tissue and organ levels. The common convention is that an AOP begins with the toxicant interaction with a biological response element; for this AOP, this initial event is binding of a DLC ligand to the AHR. Data from mechanistic studies, lifetime bioassays and approximately thirty initiation-promotion studies have established dioxin and DLCs as rat liver tumor promoters. Such studies clearly show that sustained AHR activation, weeks or months in duration, is necessary to induce rodent liver tumor promotion--hence, sustained AHR activation is deemed the molecular initiating event (MIE). After this MIE, subsequent KEs are 1) changes in cellular growth homeostasis likely associated with expression changes in a number of genes and observed as development of hepatic foci and decreases in apoptosis within foci; 2) extensive liver toxicity observed as the constellation of effects called toxic hepatopathy; 3) cellular proliferation and hyperplasia in several hepatic cell types. This progression of KEs culminates in the AO, the development of
hepatocellular adenomas and carcinomas and cholangiolar carcinomas. A rich data set provides both qualitative and quantitative knowledge of the progression of this AOP through KEs and the KERs. Thus, the WoE for this AOP is judged to be strong. Species-specific effects of dioxins and DLCs are well known—humans are less responsive than rodents and rodent species differ in sensitivity between strains. Consequently, application of this AOP to evaluate potential human health risks must take these differences into account.


New in vitro testing strategies make it possible to design testing batteries for large numbers of environmental chemicals. Full utilization of the results requires knowledge of the underlying biological networks and the adverse outcome pathways (AOPs) that describe the route from early molecular perturbations to an adverse outcome. Curation of a formal AOP is a time-intensive process and a rate-limiting step to designing these test batteries. Here, we describe a method for integrating publicly available data in order to generate computationally predicted AOP (cpAOP) scaffolds, which can be leveraged by domain experts to shorten the time for formal AOP development. A network-based workflow was used to facilitate the integration of multiple data types to generate cpAOPs. Edges between graph entities were identified through direct experimental or literature information, or computationally inferred using frequent itemset mining. Data from the TG-GATEs and ToxCast programs were used to channel large-scale toxicogenomics information into a cpAOP network (cpAOPnet) of over 20,000 relationships describing connections between chemical treatments, phenotypes, and perturbed pathways as measured by differential gene expression and high-throughput screening targets. The resulting fatty liver cpAOPnet is available as a resource to the community. Subnetworks of cpAOPs for a reference chemical (carbon tetrachloride, CCl4) and outcome (fatty liver) were compared with published mechanistic descriptions. In both cases, the computational approaches approximated the manually curated AOPs. The cpAOPnet can be used for accelerating expert-curated AOP development and to identify pathway targets that lack genomic markers or high-throughput screening tests. It can also facilitate identification of key events for designing test batteries and for classification and grouping of chemicals for follow up testing.


This article studies alternative toxicological approaches, with new (skin sensitization, ToxCast) and previous (carcinogenicity) analyses. Quantitative modeling of rate-limiting steps in skin sensitization and carcinogenicity predicts the majority of toxicants. Similarly, successful (Quantitative) Structure-Activity Relationships models exploit the quantification of only one, or few rate-limiting steps. High-throughput assays within ToxCast point to promising associations
with endocrine disruption, whereas markers for pathways intermediate events have limited correlation with most endpoints. Since the pathways may be very different (often not simple linear chains of events), quantitative analysis is necessary to identify the type of mechanism and build the appropriate model.


The protection from endocrine disruptors is a high regulatory priority. Key issues are the characterization of in vivo assays, and the identification of reference chemicals to validate alternative methods. In this exploration, publicly available databases for in vivo assays for endocrine disruption were collected and compared: Rodent Uterotrophic, Rodent Repeated Dose 28-day Oral Toxicity, 21-Day Fish, and Daphnia magna reproduction assays. Only the Uterotrophic and 21-Day Fish assays results correlated with each other. The in vivo assays data were viewed in relation to the Adverse Outcome Pathway, using as a probe 18 ToxCast in vitro assays for the ER pathway. These are the same data at the basis of the EPA agonist ToxERscore model, whose good predictivity was confirmed. The multivariate comparison of the in vitro/in vivo assays suggests that the interaction with receptors is a major determinant of in vivo results, and is the critical basis for building predictive computational models. In agreement with the above, this work also shows that it is possible to build predictive models for the Uterotrophic and 21-Day Fish assays using a limited selection of Toxcast assays.


This paper presents new data-based analyses on the ability of alternative methods to predict the skin sensitization potential of chemicals. It appears that skin sensitization, as shown in humans and rodents, can be predicted with good accuracy both with in vitro assays and QSAR approaches. The accuracy is about the same: 85-90%. Given that every biological measure has inherent uncertainty, this performance is quite remarkable. Overall, there is a good correlation between human data and experimental in vivo systems, except for sensitizers of intermediate potency. This uncertainty/variability is probably the reason why alternative methods are quite efficient in predicting both strong and non-sensitizers, but not the intermediate potency sensitizers. A detailed analysis of the predictivity of the individual approaches shows that the biological in vitro assays have limited added value in respect to the in chemico/QSAR ones, and suggests that the primary interaction with proteins is the rate-limiting step of the entire process. This confirms evidence from other fields (e.g., carcinogenicity, QSAR) indicating that successful predictive models are based on the parameterization of a few mechanistic features/events, whereas the consideration of all events supposedly involved in a toxicity pathway contributes to increase the uncertainty of the predictions.

adverse outcome pathway analysis." Environ Toxicol Chem.
Current ecological risk assessment and water quality regulations for nickel (Ni) use mechanistically based, predictive tools such as biotic ligand models (BLMs). However, despite many detailed studies, the precise mechanism(s) of Ni toxicity to aquatic organisms remains elusive. This uncertainty in the mechanism(s) of action for Ni has led to concern over the use of tools like the BLM in some regulatory settings. To address this knowledge gap, the authors used an adverse outcome pathway (AOP) analysis, the first AOP for a metal, to identify multiple potential mechanisms of Ni toxicity and their interactions with freshwater aquatic organisms. The analysis considered potential mechanisms of action based on data from a wide range of organisms in aquatic and terrestrial environments on the premise that molecular initiating events for an essential metal would potentially be conserved across taxa. Through this analysis the authors identified 5 potential molecular initiating events by which Ni may exert toxicity on aquatic organisms: disruption of Ca2+ homeostasis, disruption of Mg2+ homeostasis, disruption of Fe2+/3+ homeostasis, reactive oxygen species-induced oxidative damage, and an allergic-type response of respiratory epithelia. At the organ level of biological organization, these 5 potential molecular initiating events collapse into 3 potential pathways: reduced Ca2+ availability to support formation of exoskeleton, shell, and bone for growth; impaired respiration; and cytotoxicity and tumor formation. At the level of the whole organism, the organ-level responses contribute to potential reductions in growth and reproduction and/or alterations in energy metabolism, with several potential feedback loops between each of the pathways. Overall, the present AOP analysis provides a robust framework for future directed studies on the mechanisms of Ni toxicity and for developing AOPs for other metals. Environ Toxicol Chem 2017;9999:1-10. (c) 2016 SETAC.


Air pollution consists of a complex mixture of particulate and gaseous components. Individual criteria and other hazardous air pollutants have been linked to adverse respiratory and cardiovascular health outcomes. However, assessing risk of air pollutant mixtures is difficult since components are present in different combinations and concentrations in ambient air. Recent mechanistic studies have limited utility because of the inability to link measured changes to adverse outcomes that are relevant to risk assessment. New approaches are needed to address this challenge. The purpose of this manuscript is to describe a conceptual model, based on the adverse outcome pathway approach, which connects initiating events at the cellular and molecular level to population-wide impacts. This may facilitate hazard assessment of air pollution mixtures. In the case reports presented here, airway hyperresponsiveness and endothelial dysfunction are measurable endpoints that serve to integrate the effects of individual criteria air pollutants found in inhaled mixtures. This approach incorporates information from experimental and observational studies into a sequential series of higher order effects. The proposed model has the potential to
facilitate multipollutant risk assessment by providing a framework that can be used to converge the effects of air pollutants in light of common underlying mechanisms. This approach may provide a ready-to-use tool to facilitate evaluation of health effects resulting from exposure to air pollution mixtures.


Adverse Outcome Pathways (AOPs) provide an opportunity to develop new and more accurate safety assessment processes for drugs and other chemicals, and may ultimately play an important role in regulatory decision making. Not only can the development and application of AOPs pave the way for the development of improved evidence-based approaches for hazard and risk assessment, there is also the promise of a significant impact on animal welfare, with a reduced reliance on animal-based methods. The establishment of a useable and coherent knowledge framework under which AOPs will be developed and applied has been a first critical step towards realizing this opportunity. This article explores how the development of AOPs under this framework, and their application in practice, could benefit the science and practice of safety assessment, while in parallel stimulating a move away from traditional methods towards an increased acceptance of non-animal approaches. We discuss here the key areas where current, and future initiatives should be focused to enable the translation of AOPs into routine chemical safety assessment, and lasting 3Rs benefits.


Reliable quantification of gene and protein expression has potential to contribute significantly to the characterization of hypothesized modes of action (MOA) or adverse outcome pathways for critical effects of toxicants. Quantitative analysis of gene expression by benchmark dose (BMD) modeling has been facilitated by the development of effective software tools. In contrast, protein expression is still generally quantified by a less robust effect level (no or lowest [adverse] effect levels) approach, which minimizes its potential utility in the consideration of dose-response and temporal concordance for key events in hypothesized MOAs. BMD modeling is applied here to toxicological data on testicular toxicity to investigate its potential utility in analyzing protein expression relevant to the proposed MOA to inform human health risk assessment. The results illustrate how the BMD analysis of protein expression in animal tissues in response to toxicant exposure: (1) complements other toxicity data, and (2) contributes to consideration of the empirical concordance of dose-response relationships, as part of the weight of evidence for hypothesized MOAs to facilitate consideration and application in regulatory risk assessment. Lack of BMD analysis in proteomics has likely limited its use for these purposes. This paper illustrates the added value of BMD modeling to support and strengthen hypothetical MOAs as a basis to facilitate the translation and uptake of the results of proteomic research into risk assessment.


The adverse outcome pathway (AOP) is a framework to mechanistically link molecular initiating events to adverse biological outcomes. From a regulatory perspective, it is of crucial importance to determine the confidence for the AOP in question as well as the quality of data available in supporting this evaluation. A weight of evidence approach has been proposed for this task, but many of the existing frameworks for weight of evidence evaluation are qualitative and there is not clear guidance regarding how weight of evidence should be calculated for an AOP. In this paper we advocate the use of a subject matter expertise driven approach for weight of evidence evaluation based on criteria and metrics related to data quality and the strength of causal linkages between key events. As a demonstration, we notionally determine weight of evidence scores for two AOPs: Non-competitive ionotropic GABA receptor antagonism leading to epileptic seizures, and Antagonist-binding and stabilization of a co-repressor to the peroxisome proliferator-activated receptor alpha (PPARalpha) signaling complex ultimately causing starvation-like weight loss.


This study determined the effects of the estrogen receptor agonist ethinylestradiol (EE2) and the phospholipase A2 inhibitor quinacrine (QUIN) on the pathways controlling follicular development, steroidogenesis, oocyte maturation, ovulation and spawning success in adult zebrafish. Both EE2 and QUIN inhibited spawning but did so through different mechanisms. EE2 affected follicular development (reduced ovarian size and reduction in the proportion of cortical alveolus, vitellogenic and mature follicle stages), steroidogenesis (reduced expression of aromatase), maturation (reduced luteinizing hormone receptor expression) and ovulation (reduced expression of cytosolic phospholipase A2 and the nuclear progesterone receptor). Although EE2 alters the proportion of follicle stages within the ovary, the downregulation of gene expression as a consequence of EE2 exposure was primarily due to a decline in expression of the genes of interest in vitellogenic and mature ovarian follicles. QUIN targeted ovulation via a reduction of the steroid 17alpha,20beta dihydroxy-4-prenen-3-one (17alpha,20beta-P) and decreased expression of the prostaglandin metabolizing enzyme cyclooxygenase 2. This study demonstrates the usefulness in defining the impacts of toxicants at the molecular and cellular, organ and whole organism level and how connections between these impacts can be used to describe the adverse outcome pathways (AOPs) that mediate toxicant action. Histological analysis and gene expression were effective tools in defining the AOPs of QUIN and EE2 while the measurement of reproductive hormones level did not provide much valuable information regarding the toxicant's mode of action.

One objective in developing adverse outcome pathways (AOPs) is to connect biological changes that are relevant to risk assessors (i.e., fecundity) to molecular and cellular-level alterations that might be detectable at earlier stages of a chemical exposure. Here, we examined biochemical responses of fathead minnows (Pimephales promelas) to inform an AOP relevant to spironolactone's activation of the androgen receptor, as well as explore other biological impacts possibly unrelated to this receptor. Liquid chromatography with high resolution mass spectrometry (LC-MS) was used to measure changes in endogenous polar metabolites in livers of male and female fish that were exposed to five water concentrations of spironolactone (0, 0.05, 0.5, 5, or 50 \mu g L^{-1}) for 21 days. Metabolite profiles were affected at the two highest concentrations (5 and 50 \mu g L^{-1}), but not in the lower-level exposures, which agreed with earlier reported results of reduced female fecundity and plasma vitellogenin (VTG) levels. We then applied partial least squares regression to assess whether metabolite alterations covaried with changes in fecundity, VTG gene expression and protein concentrations, and plasma 17beta-estradiol and testosterone concentrations. Metabolite profiles significantly covaried with all measured endpoints in females, but only with plasma testosterone in males. Fecundity reductions occurred in parallel with changes in metabolites important in osmoregulation (e.g., betaine), membrane transport (e.g., l-carnitine), and biosynthesis of carnitine (e.g., methionine) and VTG (e.g., glutamate). Based on a network analysis program (i.e., mummichog), spironolactone also affected amino acid, tryptophan, and fatty acid metabolism. Thus, by identifying possible key events related to changes in biochemical pathways, this approach built upon an established AOP describing spironolactone's androgenic properties and highlighted broader implications potentially unrelated to androgen receptor activation, which could form a basis for the development of an AOP network.


The Adverse Outcome Pathway (AOP) concept is expected to guide risk assessors in their work to use all existing information on the effects of chemicals on humans and wildlife, and to target the generation of additional information to the regulatory objective. AOPs will therefore be used in the Organisation for Economic Co-operation and Development (OECD) chemical safety programme, as underlying scientific rationales for the development of alternative methods for hazard assessment, such as read-across, in vitro test methods or the development of integrated testing strategies that have the potential to replace animal tests. As a proof-of-concept, the OECD has developed an AOP for skin sensitisation, and as a follow-up has: a) implemented the AOP into the OECD QSAR Toolbox, so that information related to the Key Events (KEs) in the AOP can be used to group chemicals that are expected to act by the same mechanism and hence have the same skin sensitisation potential; b) developed alternative test methods for the KEs, so that ultimately chemicals can be tested for skin sensitisation without the use of animal tests. The development of integrated testing strategies based on the AOP is ongoing. Building on this proof-of-concept, the OECD has launched an AOP development programme with a first batch of
AOPs published in 2016. A number of IT tools, which together form an AOP Knowledge Base, are at various stages of development, and support the construction of AOPs and their use in the development of integrated approaches for testing and assessment. Following the publication of the first batch of AOPs, OECD member countries will decide on priorities for their use in supporting the development of tools for regulatory use.

Driscoll, J. J., et al. (2010). "The sumoylation pathway is dysregulated in multiple myeloma and is associated with adverse patient outcome." Blood 115(14): 2827-2834. Multiple myeloma (MM) is a plasma cell neoplasm that proceeds through a premalignant state of monoclonal gammopathy of unknown significance; however, the molecular events responsible for myelomagenesis remain uncharacterized. To identify cellular pathways deregulated in MM, we addressed that sumoylation is homologous to ubiquitination and results in the attachment of the ubiquitin-like protein Sumo onto target proteins. Sumoylation was markedly enhanced in MM patient lysates compared with normal plasma cells and expression profiling indicated a relative induction of sumoylation pathway genes. The Sumo-conjugating enzyme Ube2I, the Sumo-ligase PIAS1, and the Sumo-inducer ARF were elevated in MM patient samples and cell lines. Survival correlated with expression because 80% of patients with low UBE2I and PIAS1 were living 6 years after transplantation, whereas only 45% of patients with high expression survived 6 years. UBE2I encodes the sole Sumo-conjugating enzyme in mammalian cells and cells transfected with a dominant-negative sumoylation-deficient UBE2I mutant exhibited decreased survival after radiation exposure, impaired adhesion to bone marrow stroma cell and decreased bone marrow stroma cell-induced proliferation. UBE2I confers cells with multiple advantages to promote tumorigenesis and predicts decreased survival when combined with PIAS1. The sumoylation pathway is a novel therapeutic target with implications for existing proteasomal-based treatment strategies.

Edwards, S. W., et al. (2016). "Adverse Outcome Pathways-Organizing Toxicological Information to Improve Decision Making." J Pharmacol Exp Ther 356(1): 170-181. The number of chemicals for which environmental regulatory decisions are required far exceeds the current capacity for toxicity testing. High-throughput screening commonly used for drug discovery has the potential to increase this capacity. The adverse outcome pathway (AOP) concept has emerged as a framework for connecting high-throughput toxicity testing (HTT) and other results to potential impacts on human and wildlife populations. As a result of international efforts, the AOP development process is now well-defined and efforts are underway to broaden the participation through outreach and training. One key principle is that AOPs represent the chemical-agnostic portions of pathways to increase the generalizability of their application from early key events to overt toxicity. The closely related mode of action framework extends the AOP as needed when evaluating the potential risk of a specific chemical. This in turn enables integrated approaches to testing and assessment (IATA), which incorporate results of assays at various levels of biologic organization such as in silico; HTT; chemical-specific aspects including absorption, distribution,
metabolism, and excretion (ADME); and an AOP describing the biologic basis of toxicity. Thus, it is envisaged that provision of limited information regarding both the AOP for critical effects and the ADME for any chemical associated with any adverse outcome would allow for the development of IATA and permit more detailed AOP and ADME research, where higher precision is needed based on the decision context.


A computational framework was developed to assist in screening and prioritizing chemicals based on their dosimetry, toxicity, and potential exposures. The overall strategy started with contextualizing chemical activity observed in high-throughput toxicity screening (HTS) by mapping these assays to biological events described in Adverse Outcome Pathways (AOPs). Next, in vitro to in vivo (IVIVE) extrapolation was used to convert an in vitro dose to an external exposure level, which was compared with potential exposure levels to derive an AOP-based margins of exposure (MOE). In this study, the framework was applied to estimate MOEs for chemicals that can potentially cause developmental toxicity following a putative AOP for fetal vasculogenesis/angiogenesis. A physiologically based pharmacokinetic (PBPK) model was developed to describe chemical disposition during pregnancy, fetal, neonatal, and infant to adulthood stages. Using this life-stage PBPK model, maternal exposures were estimated that would yield fetal blood levels equivalent to the chemical concentration that altered in vitro activity of selected HTS assays related to the most sensitive vasculogenesis/angiogenesis putative AOP. The resulting maternal exposure estimates were then compared with potential exposure levels using literature data or exposure models to derive AOP-based MOEs.


Alternative approaches have been promoted to reduce the number of vertebrate and invertebrate animals required for the assessment of the potential of compounds to cause harm to the aquatic environment. A key philosophy in the development of alternatives is a greater understanding of the relevant adverse outcome pathway (AOP). One alternative method is the fish embryo toxicity (FET) assay. Although the trends in potency have been shown to be equivalent in embryo and adult assays, a detailed mechanistic analysis of the toxicity data has yet to be performed; such analysis is vital for a full understanding of the AOP. The research presented herein used an updated implementation of the Verhaar scheme to categorize compounds into AOP-informed categories. These were then used in mechanistic (quantitative) structure-activity relationship ((Q)SAR) analysis to show that the descriptors governing the distinct mechanisms of acute fish toxicity are capable of modeling data from the FET assay. The results show that compounds do appear to exhibit the same mechanisms of toxicity across life stages. Thus, this mechanistic analysis supports the argument that the FET
assay is a suitable alternative testing strategy for the specified mechanisms and that understanding the AOPs is useful for toxicity prediction across test systems.


Adverse Outcome Pathways (AOPs) describe toxicant effects as a sequential chain of causally linked events beginning with a molecular perturbation and culminating in an adverse outcome at an individual or population level. Strategies for developing AOPs are still evolving and depend largely on the intended use or motivation for development and data availability. Four ecotoxicological AOP case studies, developed for different purposes, are described herein. In each situation, creation of the AOP began in a manner determined by the initial motivation for its creation, and expanded either to include additional components of the pathway, or to address the domains of applicability in terms of chemical initiators, susceptible species, life stages, etc. From these case studies, some general strategies can be gleaned which a developer may find useful for supporting an existing AOP or creating a new one. Several web-based tools which can aid in AOP assembly and evaluation of weight of evidence for scientific robustness of AOP components are highlighted. This article is protected by copyright. All rights reserved.


Bisphenol A (BPA) is one of the most widely used and extensively studied chemicals. Numerous studies have reported in vitro effects or animal adverse findings at BPA doses lower than the no observed adverse effect levels (NOAELs) established in regulatory toxicity studies and used for human health risk assessment. Intensive discussions on the adequacy and relevance of test systems have not satisfactorily resolved whether positive or negative animal and/or in vitro findings are more relevant for human health risk assessment purposes. BPA imperfectly mimics endogenous estrogens at membrane-bound estrogen receptors in the fM-nM concentration range, and may have downstream pleiotropic effects such as human seminoma proliferation and mammary gland hyperplasia after in utero exposure which are not detectable in regulatory toxicology studies. We argue that a structured approach like the OECD Adverse Outcome Pathway (AOP) framework is needed to help researchers in designing relevant studies, and risk assessors in evaluating them. The huge amount of experimental data generated for BPA has highlighted data gaps in basic biology and the shortcomings of current approaches to hazard characterization and risk assessment. Establishing AOPs for BPA, and other endocrine active chemicals, will require major scientific as well as training investments by all responsible stakeholders.

This study was designed to develop and validate a short-term in vivo protocol termed the Fetal Phthalate Screen (FPS) to detect phthalate esters (PEs) and other chemicals that disrupt fetal testosterone synthesis and testis gene expression in rats. We propose that the FPS can be used to screen chemicals that produce adverse developmental outcomes via disruption of the androgen synthesis pathway more rapidly and efficiently, and with fewer animals than a postnatal one-generation study. Pregnant rats were dosed from gestational day (GD) 14 to 18 at one dose level with one of 27 chemicals including PEs, PE alternatives, pesticides known to inhibit steroidogenesis, an estrogen and a potent PPARalpha agonist and ex vivo testis testosterone production (T Prod) was measured on GD 18. We also included some chemicals with "unknown" activity including DMEP, DHeP, DHEH, DPHCH, DAP, TOTM, tetrabromo-diethyl hexyl phthalate (BrDEHP), and a relatively potent environmental estrogen BPAF. Dose-response studies also were conducted with this protocol with 11 of the above chemicals to determine their relative potencies. CD-1 mice also were exposed to varying dose levels of DPeP from GD 13 to 17 to determine if DPeP reduced T Prod in this species since there is a discrepancy among the results of in utero studies of PEs in mice. Compared to the known male reproductive effects of the PEs in rats the FPS correctly identified all known "positives" and "negatives" tested. Seven of eight "unknowns" tested were "negatives", they did not reduce T Prod, whereas DAP produced an "equivocal" response. Finally, a dose-response study with DPeP in CD-1 mice revealed that fetal T Prod can be inhibited by exposure to a PE in utero in this species, but at a higher dose level than required in rats. Key words. Phthalate Syndrome, Fetal endocrine biomarkers, Phthalate adverse outcome pathway, testosterone production, fetal rat testis.


Social pressure to minimize the use of animal testing and the ever-increasing concern on animal welfare, together with the need for more human-relevant and more predictive toxicity tests, are some of the drivers for new approaches to chemical screening. These approaches must also be able to accelerate the screening and assessment of the thousands of chemicals that are currently in use and in development for potential hazards to human and ecological health. Ideally, approaches are needed that decrease (or eliminate) animal testing while increasing predictivity. Efforts in many countries have focused on a toxicological pathway-based vision for human health assessments relying on in vitro systems and predictive models,1 vision equally applicable to ecological risk assessment.2 A pathway-based analysis of chemical effects opens numerous opportunities to apply nontraditional approaches for understanding the risks of chemical exposure. Conservation of molecular initiating and key events leading to adverse outcomes of regulatory concern provide a defensible framework for extrapolating chemical effects across species, even if the specific adverse outcomes differ between them.3.


Antagonism of ionotropic GABA receptors (iGABARs) can occur at three distinct types of receptor binding sites causing chemically induced epileptic seizures. Here we review three adverse outcome pathways, each characterized by a specific molecular initiating event where an antagonist competitively binds to active sites, negatively modulates allosteric sites or noncompetitively blocks ion channel on the iGABAR. This leads to decreased chloride conductance, followed by depolarization of affected neurons, epilepsy-related death and ultimately decreased population. Supporting evidence for causal linkages from the molecular to population levels is presented and differential sensitivity to iGABAR antagonists in different GABA receptors and organisms discussed. Adverse outcome pathways are poised to become important tools for linking mechanism-based biomarkers to regulated outcomes in next-generation risk assessment.


Adverse outcome pathways (AOPs) organize knowledge on the progression of toxicity through levels of biological organization. By determining the linkages between toxicity events at different levels, AOPs lay the foundation for mechanism-based alternative testing approaches to hazard assessment. Here, we focus on growth impairment in fish to illustrate the initial stages in the process of AOP development for chronic toxicity outcomes. Growth is an apical endpoint commonly assessed in chronic toxicity tests for which a replacement is desirable. Based on several criteria, we identified reduction in food intake to be a suitable key event for initiation of middle-out AOP development. To start exploring the upstream and downstream links of this key event, we developed three AOP case studies, for pyrethroids, selective serotonin reuptake inhibitors (SSRIs) and cadmium. Our analysis showed that the effect of pyrethroids and SSRIs on food intake is strongly linked to growth impairment, while cadmium causes a reduction in growth due to increased metabolic demands rather than changes in food intake. Locomotion impairment by pyrethroids is strongly linked to their effects on food intake and growth, while for SSRIs their direct influence on appetite may play a more important role. We further discuss which alternative tests could be used to inform on the predictive key events identified in the case studies. In conclusion, our work demonstrates how the AOP concept can be used in practice to assess critically the knowledge available for specific chronic toxicity cases and to identify existing knowledge gaps and potential alternative tests.


To elucidate the effects of chemicals on populations of different species in the environment, efficient testing and modeling approaches are needed that consider multiple stressors and allow reliable extrapolation of responses across species. An adverse outcome pathway (AOP) is a concept that provides a framework for
organizing knowledge about the progression of toxicity events across scales of biological organization that lead to adverse outcomes relevant for risk assessment. In this paper, we focus on exploring how the AOP concept can be used to guide research aimed at improving both our understanding of chronic toxicity, including delayed toxicity as well as epigenetic and transgenerational effects of chemicals, and our ability to predict adverse outcomes. A better understanding of the influence of subtle toxicity on individual and population fitness would support a broader integration of sublethal endpoints into risk assessment frameworks. Detailed mechanistic knowledge would facilitate the development of alternative testing methods as well as help prioritize higher tier toxicity testing. We argue that targeted development of AOPs supports both of these aspects by promoting the elucidation of molecular mechanisms and their contribution to relevant toxicity outcomes across biological scales. We further discuss information requirements and challenges in application of AOPs for chemical- and site-specific risk assessment and for extrapolation across species. We provide recommendations for potential extension of the AOP framework to incorporate information on exposure, toxicokinetics and situation-specific ecological contexts, and discuss common interfaces that can be employed to couple AOPs with computational modeling approaches and with evolutionary life history theory. The extended AOP framework can serve as a venue for integration of knowledge derived from various sources, including empirical data as well as molecular, quantitative and evolutionary-based models describing species responses to toxicants. This will allow a more efficient application of AOP knowledge for quantitative chemical- and site-specific risk assessment as well as for extrapolation across species in the future.


Gust, K. A., et al. (2016). "Limitations of toxicity characterization in life cycle assessment: Can adverse outcome pathways provide a new foundation?" Integr Environ Assess Manag 12(3): 580-590. Life cycle assessment (LCA) has considerable merit for holistic evaluation of product planning, development, production, and disposal, with the inherent benefit of providing a forecast of potential health and environmental impacts. However, a technical review of current life cycle impact assessment (LCIA) methods revealed limitations within the biological effects assessment protocols, including: simplistic assessment approaches and models; an inability to integrate emerging types of toxicity data; a reliance on linear impact assessment models; a lack of methods to mitigate uncertainty; and no explicit consideration of effects in species of concern. The purpose of the current study is to demonstrate that a new concept in toxicological and regulatory assessment, the adverse outcome pathway (AOP), has many useful attributes of potential use to ameliorate many of these problems, to expand data utility and model robustness, and to enable more accurate and defensible biological effects assessments within LCIA. Background, context, and examples have been provided to demonstrate these potential
benefits. We additionally propose that these benefits can be most effectively realized through development of quantitative AOPs (qAOPs) crafted to meet the needs of the LCIA framework. As a means to stimulate qAOP research and development in support of LCIA, we propose 3 conceptual classes of qAOP, each with unique inherent attributes for supporting LCIA: 1) mechanistic, including computational toxicology models; 2) probabilistic, including Bayesian networks and supervised machine learning models; and 3) weight of evidence, including models built using decision-analytic methods. Overall, we have highlighted a number of potential applications of qAOPs that can refine and add value to LCIA. As the AOP concept and support framework matures, we see the potential for qAOPs to serve a foundational role for next-generation effects characterization within LCIA. Integr Environ Assess Manag 2016;12:580-590. Published 2015. This article is a US Government work and is in the public domain in the USA.


Although activation of the B-cell receptor (BCR) signaling pathway is implicated in the pathogenesis of chronic lymphocytic leukemia (CLL), its clinical impact and the molecular correlates of such response are not clearly defined. T-cell leukemia 1 (TCL1), the AKT modulator and proto-oncogene, is differentially expressed in CLL and linked to its pathogenesis based on CD5(+) B-cell expansions arising in TCL1-transgenic mice. We studied here the association of TCL1 levels and its intracellular dynamics with the in vitro responses to BCR stimulation in 70 CLL cases. The growth kinetics after BCR engagement correlated strongly with the degree and timing of induced AKT phospho-activation. This signaling intensity was best predicted by TCL1 levels and the kinetics of TCL1-AKT corecruitment to BCR membrane activation complexes, which further included the kinases LYN, SYK, ZAP70, and PKC. High TCL1 levels were also strongly associated with aggressive disease features, such as advanced clinical stage, higher white blood cell counts, and shorter lymphocyte doubling time. Higher TCL1 levels independently predicted an inferior clinical outcome (ie, shorter progression-free survival, P < .001), regardless of therapy regimen, especially for ZAP70(+) tumors. We propose TCL1 as a marker of the BCR-responsive CLL subset identifying poor prognostic cases where targeting BCR-associated kinases may be therapeutically useful.


In modern toxicology, substantial efforts are undertaken to develop alternative solutions for in vivo toxicity testing. The adverse outcome pathway (AOP) concept could facilitate knowledge-based safety assessment of chemicals that does not rely exclusively on in vivo toxicity testing. The construction of an AOP is based on understanding toxicological processes at different levels of biological
organisation. Here, we present the developed AOP for liver fibrosis and demonstrate a linkage between hepatic injury caused by chemical protein alkylation and the formation of liver fibrosis, supported by coherent and consistent scientific data. This long-term process, in which inflammation, tissue destruction, and repair occur simultaneously, results from the complex interplay between various hepatic cell types, receptors, and signalling pathways. Due to the complexity of the process, an adequate liver fibrosis cell model for in vitro evaluation of a chemical's fibrogenic potential is not yet available. Liver fibrosis poses an important human health issue that is also relevant for regulatory purposes. An AOP described with enough mechanistic detail might support chemical risk assessment by indicating early markers for downstream events and thus facilitating the development of an in vitro testing strategy. With this work, we demonstrate how the AOP framework can support the assembly and coherent display of distributed mechanistic information from the literature to support the use of alternative approaches for prediction of toxicity. This AOP was developed according to the guidance document on developing and assessing AOPs and its supplement, the users' handbook, issued by the Organisation for Economic Co-operation and Development.


Natural and synthetic chemicals are essential to our daily lives, food supplies, health care, industries and safe sanitation. At the same time protecting marine ecosystems and seafood resources from the adverse effects of chemical contaminants remains an important issue. Since the 1970s, monitoring of persistent, bioaccumulative and toxic (PBT) chemicals using analytical chemistry has provided important spatial and temporal trend data in three important contexts; relating to human health protection from seafood contamination, addressing threats to marine top predators and finally providing essential evidence to better protect the biodiversity of commercial and non-commercial marine species. A number of regional conventions have led to controls on certain PBT chemicals over several years (termed 'legacy contaminants'; e.g. cadmium, lindane, polycyclic aromatic hydrocarbons [PAHs] and polychlorinated biphenyls [PCBs]). Analytical chemistry plays a key role in evaluating to what extent such regulatory steps have been effective in leading to reduced emissions of these legacy contaminants into marine environments. In parallel, the application of biomarkers (e.g. DNA adducts, CYP1A-EROD, vitellogenin) and bioassays integrated with analytical chemistry has strengthened the evidence base to support an ecosystem approach to manage marine pollution problems. In recent years, however, the increased sensitivity of analytical chemistry, toxicity alerts and wider environmental awareness has led to a focus on emerging chemical contaminants (defined as chemicals that have been detected in the environment, but which are currently not included in regulatory monitoring programmes and whose fate and biological impacts are poorly understood). It is also known that natural chemicals (e.g. algal biotoxins) may also pose a threat to marine species and seafood quality. Hence complex mixtures of legacy contaminants, emerging
chemicals and natural biotoxins in marine ecosystems represent important scientific, economic and health challenges. In order to meet these challenges and pursue cost-effective scientific approaches that can provide evidence necessary to support policy needs (e.g. the European Marine Strategy Framework Directive), it is widely recognised that there is a need to (i) provide marine exposure assessments for priority contaminants using a range of validated models, passive samplers and biomarkers; (ii) integrate chemical monitoring data with biological effects data across spatial and temporal scales (including quality controls); and (iii) strengthen the evidence base to understand the relationship between exposure to complex chemical mixtures, biological and ecological impacts through integrated approaches and molecular data (e.g. genomics, proteomics and metabolomics). Additionally, we support the widely held view that (iv) that rather than increasing the analytical chemistry monitoring of large number of emerging contaminants, it will be important to target analytical chemistry towards key groups of chemicals of concern using effects-directed analysis. It is also important to evaluate to what extent existing biomarkers and bioassays can address various classes of emerging chemicals using the adverse outcome pathway (AOP) approach now being developed by the Organization for Economic Cooperation and Development (OECD) with respect to human toxicology and ecotoxicology.


The developing fish heart is vulnerable to a diverse array of toxic chemical contaminants in freshwater, estuarine, and marine habitats. Globally occurring examples of cardiotoxic agents include dioxins, polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs). The disruption of cardiac function during the process of heart morphogenesis can lead to adverse outcome pathways (AOPs) that can negatively affect fish survival at hatching as well as later life stages. Proximal impacts include cardiogenic fluid accumulation (edema) and defects of the body axis and jaw that preclude larval feeding. More subtle changes in heart development can produce permanent structural defects in the heart that reduce cardiac output and swimming performance in older fish. In recent decades, the presence of edema in fish embryos and larvae has been a very common bioindicator of cardiotoxicity. However, the different ways that edema forms in fish from different habitats (i.e., freshwater vs. marine, pelagic vs. demersal) has not been rigorously examined. Oil spills are an important source of PAHs in fish spawning areas worldwide, and research is revealing how patterns of cardiogenic edema are shaped by species-specific differences in developmental anatomy and ionoregulatory physiology. Here we review the visible evidence for circulatory disruption across nine freshwater and marine fish species, exposed to crude oils from different parts of the world. We focus on the close interconnectedness of the cardiovascular and osmoregulatory systems during early development, and corresponding implications for fish in hyperosmotic and hyposmotic habitats. Finally, we suggest there may be poorly understood adverse outcomes pathways related to osmotic gradients and water
movement within embryos, the latter causing extreme shifts in tissue osmolality.


Allergic sensitisation of the respiratory tract by chemicals is associated with rhinitis and asthma and remains an important occupational health issue. Although less than 80 chemicals have been confirmed as respiratory allergens the adverse health effects can be serious, and in rare instances can be fatal, and there are, in addition, related socioeconomic issues. The challenges that chemical respiratory allergy pose for toxicologists are substantial. No validated methods are available for hazard identification and characterisation, and this is due in large part to the fact that there remains considerable uncertainty and debate about the mechanisms through which sensitisation of the respiratory tract is acquired. Despite that uncertainty, there is a need to establish some common understanding of the key events and processes that are involved in respiratory sensitisation to chemicals and that might in turn provide the foundations for novel approaches to safety assessment. In recent years the concept of adverse outcome pathways (AOP) has gained some considerable interest among the toxicology community as a basis for outlining the key steps leading to an adverse health outcome, while also providing a framework for focusing future research, and for developing alternative paradigms for hazard characterisation. Here we explore application of the same general principles to an examination of the induction by chemicals of respiratory sensitisation. In this instance, however, we have chosen to adopt a reverse engineering approach and to model a possible AOP for chemical respiratory allergy working backwards from the elicitation of adverse health effects to the cellular and molecular mechanisms that are implicated in the acquisition of sensitisation.


An adverse outcome pathway (AOP) helps to organize existing knowledge on chemical mode of action, starting with a molecular initiating event such as receptor binding, continuing through key events, and ending with an adverse outcome such as reproductive impairment. AOPs can help identify knowledge gaps where more research is needed to understand the underlying mechanisms, aid in chemical hazard characterization, and guide the development of new testing approaches that use fewer or no animals. A September 2014 workshop in Bethesda, Maryland considered how the AOP concept could improve regulatory assessments of chemical toxicity. Scientists from 21 countries, representing industry, academia, regulatory agencies, and special interest groups, attended the workshop, titled Adverse Outcome Pathways: From Research to Regulation. Workshop plenary presentations were followed by breakout sessions that considered regulatory acceptance of AOPs and AOP-based tools, criteria for building confidence in an AOP for regulatory use, and requirements to build
quantitative AOPs and AOP networks. Discussions during the closing session emphasized a need to increase transparent and inclusive collaboration, especially with disciplines outside of toxicology. Additionally, to increase impact, working groups should be established to systematically prioritize and develop AOPs. Multiple collaborative projects and follow-up activities resulted from the workshop.


Maintaining the viability of populations of plants and animals is a key focus for environmental regulation. Population-level responses integrate the cumulative effects of chemical stressors on individuals as those individuals interact with and are affected by their conspecifics, competitors, predators, prey, habitat, and other biotic and abiotic factors. Models of population-level effects of contaminants can integrate information from lower levels of biological organization and feed that information into higher-level community and ecosystem models. As individual-level endpoints are used to predict population responses, this requires that biological responses at lower levels of organization be translated into a form that is usable by the population modeler. In the current study, we describe how mechanistic data, as captured in adverse outcome pathways (AOPs), can be translated into modeling focused on population-level risk assessments. First, we describe the regulatory context surrounding population modeling, risk assessment and the emerging role of AOPs. Then we present a succinct overview of different approaches to population modeling and discuss the types of data needed for these models. We describe how different key biological processes measured at the level of the individual serve as the linkage, or bridge, between AOPs and predictions of population status, including consideration of community-level interactions and genetic adaptation. Several case examples illustrate the potential for use of AOPs in population modeling and predictive ecotoxicology. Finally, we make recommendations for focusing toxicity studies to produce the quantitative data needed to define AOPs and to facilitate their incorporation into population modeling.


BACKGROUND: A diverse class of engineered nanomaterials (ENMs) exhibiting a wide array of physical-chemical properties that are associated with toxicological effects in experimental animals is in commercial use. However, an integrated framework for human health risk assessment (HHRA) of ENMs has yet to be established. Rodent 2-year cancer bioassays, clinical chemistry, and histopathological endpoints are still considered the 'gold standard' for detecting substance-induced toxicity in animal models. However, the use of data derived from alternative toxicological tools, such as genome-wide expression profiling and in vitro high-throughput assays, are gaining acceptance by the regulatory community for hazard identification and for understanding the underlying mode-of-action. Here, we conducted a case study to evaluate the application of global
gene expression data in deriving pathway-based points of departure (PODs) for multi-walled carbon nanotube (MWCNT)-induced lung fibrosis, a non-cancer endpoint of regulatory importance. METHODS: Gene expression profiles from the lungs of mice exposed to three individual MWCNTs with different physical-chemical properties were used within the framework of an adverse outcome pathway (AOP) for lung fibrosis to identify key biological events linking MWCNT exposure to lung fibrosis. Significantly perturbed pathways were categorized along the key events described in the AOP. Benchmark doses (BMDs) were calculated for each perturbed pathway and were used to derive transcriptional BMDs for each MWCNT. RESULTS: Similar biological pathways were perturbed by the different MWCNT types across the doses and post-exposure time points studied. The pathway BMD values showed a time-dependent trend, with lower BMDs for pathways perturbed at the earlier post-exposure time points (24 h, 3d). The transcriptional BMDs were compared to the apical BMDs derived by the National Institute for Occupational Safety and Health (NIOSH) using alveolar septal thickness and fibrotic lesions endpoints. We found that regardless of the type of MWCNT, the BMD values for pathways associated with fibrosis were 14.0-30.4 mug/mouse, which are comparable to the BMDs derived by NIOSH for MWCNT-induced lung fibrotic lesions (21.0-27.1 mug/mouse). CONCLUSIONS: The results demonstrate that transcriptomic data can be used to as an effective mechanism-based method to derive acceptable levels of exposure to nanomaterials in product development when epidemiological data are unavailable.


Ongoing honey bee (Apis mellifera) colony losses are of significant international concern because of the essential role these insects play in pollinating crops. Both chemical and non-chemical stressors have been implicated as possible contributors to colony failure; however, the potential role(s) of commonly-used neonicotinoid insecticides has emerged as particularly concerning. Neonicotinoids act on the nicotinic acetylcholine receptors (nAChRs) in the central nervous system to eliminate pest insects. However, mounting evidence indicates that neonicotinoids also may adversely affect beneficial pollinators, such as the honey bee, via impairments on learning and memory, and ultimately foraging success. The specific mechanisms linking activation of the nAChR to adverse effects on learning and memory are uncertain. Additionally, clear connections between observed impacts on individual bees and colony level effects are lacking. The objective of this review was to develop adverse outcome pathways (AOPs) as a means to evaluate the biological plausibility and empirical evidence supporting (or refuting) the linkage between activation of the physiological target site, the nAChR, and colony level consequences. Potential for exposure was not a consideration in AOP development and therefore this effort should not be considered a risk assessment. Nonetheless, development of the AOPs described herein has led to the identification of research gaps which, for example, may be of high priority in understanding how perturbation of
pathways involved in neurotransmission can adversely affect normal colony functions, causing colony instability and subsequent bee population failure. A putative AOP network was developed, laying the foundation for further insights as to the role of combined chemical and non-chemical stressors in impacting bee populations. Insights gained from the AOP network assembly, which more realistically represents multi-stressor impacts on honey bee colonies, are promising toward understanding common sensitive nodes in key biological pathways and identifying where mitigation strategies may be focused to reduce colony losses.

Landesmann, B., et al. (2013). "Adverse outcome pathway-based screening strategies for an animal-free safety assessment of chemicals." Altern Lab Anim 41(6): 461-471. Currently, the assessment of risk to human health from exposure to manufactured chemicals is mainly based on experiments performed on living animals (in vivo). Substantial efforts are being undertaken to develop alternative solutions to in vivo toxicity testing. This new paradigm, based on the Mode-of-Action (MoA) framework, postulates that any adverse human health effect caused by exposure to an exogenous substance can be described by a series of causally-linked biochemical or biological key events with measurable parameters. The elaboration of mechanistic knowledge through literature research is necessary for a MoA-driven design of integrated testing strategies using in vitro methods for in vivo predictions. The objective of our ongoing research is to demonstrate the feasibility of an integrated approach to predict human toxicity following the Adverse Outcome Pathway (AOP) framework. In our previous work on MoA with the HepaRG cell model, we developed a strategy to identify chemicals that were hepatotoxic. This pioneered an innovative way of using data from in vitro experiments to group chemicals based on their MoA, which is likely to be an important step in a toxicity testing strategy.

Lee, J. W., et al. (2015). "Significance of adverse outcome pathways in biomarker-based environmental risk assessment in aquatic organisms." J Environ Sci (China) 35: 115-127. In environmental risk assessments (ERA), biomarkers have been widely used as an early warning signal of environmental contamination. However, biomarker responses have limitation due to its low relevance to adverse outcomes (e.g., fluctuations in community structure, decreases in population size, and other similar ecobiologically relevant indicators of community structure and function). To mitigate these limitations, the concept of adverse outcome pathways (AOPs) was developed. An AOP is an analytical, sequentially progressive pathway that links a molecular initiating event (MIE) to an adverse outcome. Recently, AOPs have been recognized as a potential informational tool by which the implications of molecular biomarkers in ERA can be better understood. To demonstrate the utility of AOPs in biomarker-based ERA, here we discuss a series of three different biological repercussions caused by exposure to benzo(a)pyrene (BaP), silver nanoparticles (AgNPs), and selenium (Se). Using mainly aquatic invertebrates and selected vertebrates as model species, we focus on the development of the AOP concept. Aquatic organisms are suitable bioindicator
species whose entire lifespans can be observed over a short period; moreover, these species can be studied on the molecular and population levels. Also, interspecific differences between aquatic organisms are important to consider in an AOP framework, since these differences are an integral part of the natural environment. The development of an environmental pollutant-mediated AOP may enable a better understanding of the effects of environmental pollutants in different scenarios in the diverse community of an ecosystem.


According to previous survey, about two million of people were expected to suffer from toxic effects due to humidifier disinfectant (HD), regardless of healing or not. Extremely small group are recognized as HDs' victims. Up to now, previous research tried to focus on interstitial fibrosis on terminal bronchiole because it is specific finding, compared with other diseases. To figure out overall effects from HDs, we recommend adverse outcome pathways (AOPs) as new approach. Reactive oxygen species (ROS) generation, decreased T-cell and pro-inflammatory cytokine release from macrophage could be key events between the exposure to HDs and diseases. ROS generation, decreased cell and pro-inflammatory cytokine release from macrophage could be cause of interstitial fibrosis, pneumonia and many other diseases such as asthma, allergic rhinitis, allergic dermatitis, fetal death, premature baby, autoimmune disease, hepatic toxicity, renal toxicity, cancer, and so on. We predict potential disease candidate by AOPs. We can validate the real risk of the adverse outcome by epidemiologic and toxicologic study using big data such as National Health Insurance data and AOPs knowledge base. Application of these kinds of new methods can find the potential disease list from the exposure to HD.


BACKGROUND: Dysregulation of the mammalian target of rapamycin (mTOR) pathway has been shown to contribute to tumorigenesis. This study explored protein expression profiles of mTOR pathway and the relationship with prognosis in patients with nonsmall cell lung carcinoma (NSCLC). METHODS: The protein expression profiles of mTOR/phosphorylated (p-)mTOR, phosphoinositide-dependent kinase 1 (PDK1)/p-PDK1, p-Akt1, and P70 ribosomal protein S6 kinase (P70S6K)/p-P70S6K were determined via immunohistochemical staining assay. The clinical prognostic values of both single and combined protein expression were investigated with univariate and multivariate survival analysis. RESULTS: Compared with normal lung tissues, the protein levels of mTOR/p-mTOR, p-Akt1 Ser473/Thr308, and P70S6K/p-P70S6K were higher (all P < .05), whereas p-PDK1 was lower (P < .05) in tumor tissues. p-mTOR expression was associated with histological differentiation, histological type, lymph node invasion, and stage (all P < .05). Overall survival in NSCLC patients was significantly shorter in cases with positive phenotype for p-mTOR, p-PDK1, and p-P70S6K (all P < .05). Subjects with coexpression of any 2 of p-mTOR, p-PDK1, p-Akt1
Ser473, and p-P70S6K demonstrated worse prognosis than those expressing no biomarker or any 1 biomarker alone (all P < .05). Multivariate analysis showed that the combination of p-mTOR/p-P70S6K is an independent prognostic factor in addition to tumor stage. CONCLUSIONS: This study provides clinical evidence that activated components of mTOR pathway, not total protein, are predictors of poor prognosis in NSCLC. Moreover, evaluating protein-expression profiles of these molecules might be a new strategy for individual therapy in subjects with NSCLC.


The development of in vitro testing strategies may achieve a cost-effective generation of comprehensive datasets on a large number of chemicals, according to the requirements of the European Regulation REACH. Much emphasis is placed on in vitro methods based on subcellular mechanisms (e.g., nuclear receptor interaction), but it is necessary to define the predictive value of molecular or biochemical changes within an adverse outcome pathway (AOP). AOP pivots on the description of the flow from a molecular initiating event through a cascade of intermediate events needed to produce a specific adverse effect at organism level: downstream responses at cell level are, therefore, essential to define an AOP. Several in vitro assays are based on human cell lines representative of endocrine-targeted tissues (e.g., prostate) and on functional biomarkers of clinical relevance (e.g., PSA secretion in human prostate epithelial cells). We discuss the implementation of such functional biomarkers in the AOP context.


Consumer safety risk assessment of skin sensitization requires information on both consumer exposure to the ingredient through product use and the hazardous properties of the ingredient. Significant progress has been made in determining the hazard potential of ingredients without animal testing. However, hazard identification is insufficient for risk assessment, and an understanding of the dose-response is needed. Obtaining such knowledge without animal testing is challenging and requires applying available mechanistic knowledge to both assay development and the integration of these data. The recent OECD report "The Adverse Outcome Pathway for Skin Sensitization Initiated by Covalent Binding to Proteins" presents the available mechanistic knowledge of the sensitization response within an adverse outcome pathway (AOP). We propose to use this AOP as the mechanistic basis for physiologically- and mechanistically-based toxicokinetic-toxicodynamic models of the sensitization response. The approach would be informed by non-animal data, provide predictions of the dose-response required for risk assessment, and would be evaluated against human clinical data.

There is a long history of using both in silico and in vitro methods to predict adverse effects in humans and environmental species where toxicity data are lacking. Currently, there is a great deal of interest in applying these methods to the development of so-called 'adverse outcome pathway' (AOP) constructs. The AOP approach provides a framework for organizing information at the chemical and biological level, allowing evidence from both in silico and in vitro studies to be rationally combined to fill gaps in knowledge concerning toxicological events. Fundamental to this new paradigm is a greater understanding of the mechanisms of toxicity and, in particular, where these mechanisms may be conserved across taxa, such as between model animals and related wild species. This presents an opportunity to make predictions across diverse species, where empirical data are unlikely to become available as is the case for most species of wildlife.


The Organisation for Economic Co-operation and Development (OECD) has launched the Adverse Outcome Pathway (AOP) Programme to advance knowledge of pathways of toxicity and improve the use of mechanistic information in risk assessment. An AOP links a molecular initiating event (MIE) to an adverse outcome (AO) through intermediate key events (KE). Here, we present the scientific evidence in support of an AOP whereby chemicals that bind to tubulin cause microtubule depolymerization resulting in spindle disorganization followed by altered chromosome alignment and segregation and the generation of aneuploidy in female germ cells, ultimately leading to aneuploidy in the offspring. Aneuploidy, an abnormal number of chromosomes that is not an exact multiple of the haploid number, is a well-known cause of human disease and represents a major cause of infertility, pregnancy failure, and serious genetic disorders in the offspring. Among chemicals that induce aneuploidy in female germ cells, a large majority impairs microtubule dynamics and spindle function. Colchicine, a prototypical chemical that binds to tubulin and causes microtubule depolymerization, is used here to illustrate the AOP. This AOP is specific to female germ cells exposed during the periovulation period. Although the majority of the data come from rodent studies, the available evidence suggests that the MIE and KEs are conserved across species and would occur in human oocytes. The development of AOPs related to mutagenicity in germ cells is expected to aid the identification of potential hazards to germ cell genomic integrity and support regulatory efforts to protect population health.


The Adverse Outcome Pathway (AOP) framework represents a valuable conceptual tool to systematically integrate existing toxicological knowledge from
a mechanistic perspective to facilitate predictions of chemical-induced effects across species. However, its application for decision-making requires the transition from qualitative to quantitative AOP (qAOP). Here we used a fish model and the synthetic glucocorticoid beclomethasone dipropionate (BDP) to investigate the role of chemical-specific properties, pharmacokinetics, and internal exposure dynamics in the development of qAOPs. We generated a qAOP network based on drug plasma concentrations and focused on immunodepression, skin androgenisation, disruption of gluconeogenesis and reproductive performance. We showed that internal exposure dynamics and chemical-specific properties influence the development of qAOPs and their predictive power. Comparing the effects of two different glucocorticoids, we highlight how relatively similar in vitro hazard-based indicators can lead to different in vivo risk. This discrepancy can be predicted by their different uptake potential, pharmacokinetic (PK) and pharmacodynamic (PD) profiles. We recommend that the development phase of qAOPs should include the application of species-species uptake and physiologically-based PK/PD models. This integration will significantly enhance the predictive power, enabling a more accurate assessment of the risk and the reliable transferability of qAOPs across chemicals.


As documented in the recent OECD report 'the adverse outcome pathway for skin sensitisation initiated by covalent binding to proteins' (OECD, 2012), the chemical and biological events driving the induction of human skin sensitisation have been investigated for many years and are now well understood. Several non-animal test methods have been developed to predict sensitiser potential by measuring the impact of chemical sensitisers on these key events (Adler et al., 2011; Maxwell et al., 2011); however our ability to use these non-animal datasets for risk assessment decision-making (i.e. to establish a safe level of human exposure for a sensitising chemical) remains limited and a more mechanistic approach to data integration is required to address this challenge. Informed by our previous efforts to model the induction of skin sensitisation (Maxwell and MacKay, 2008) we are now developing two mathematical models ('total haptenated protein' model and 'CD8(+) T cell response' model) that will be linked to provide predictions of the human CD8(+) T cell response for a defined skin exposure to a sensitising chemical. Mathematical model development is underpinned by focussed clinical or human-relevant research activities designed to inform/challenge model predictions whilst also increasing our fundamental understanding of human skin sensitisation. With this approach, we aim to quantify the relationship between the dose of sensitiser applied to the skin and the extent of the hapten-specific T cell response that would result. Furthermore, by benchmarking our mathematical model predictions against clinical datasets
(e.g. human diagnostic patch test data), instead of animal test data, we propose that this approach could represent a new paradigm for mechanistic toxicology.


This paper summarizes recent developments in the continuing evolution of Human Relevance Frameworks to systematically consider the weight of evidence of hypothesized modes of action in animals and their potential human relevance for both cancer and non-cancer effects. These frameworks have been developed in initiatives of the International Life Sciences Institute Risk Sciences Institute and the International Programme on Chemical Safety engaging large numbers of scientists internationally. They are analytical tools designed to organize information in hazard characterization as a basis to clarify the extent of the weight of evidence for mode of action in animals and human relevance and subsequent implications for dose-response. They are also extremely helpful in identifying critical data gaps. These frameworks which are illustrated by an increasing number of case studies, have been widely adopted into international and national guidance and assessments and continue to evolve, as experience increases in their application.


The World Health Organization/International Programme on Chemical Safety mode of action/human relevance framework has been updated to reflect the experience acquired in its application and extend its utility to emerging areas in toxicity testing and non-testing methods. The underlying principles have not changed, but the framework’s scope has been extended to enable integration of information at different levels of biological organization and reflect evolving experience in a much broader range of potential applications. Mode of action/species concordance analysis can also inform hypothesis-based data generation and research priorities in support of risk assessment. The modified framework is incorporated within a roadmap, with feedback loops encouraging continuous refinement of fit-for-purpose testing strategies and risk assessment. Important in this construct is consideration of dose-response relationships and species concordance analysis in weight of evidence. The modified Bradford Hill considerations have been updated and additionally articulated to reflect increasing experience in application for cases where the toxicological outcome of chemical exposure is known. The modified framework can be used as originally intended, where the toxicological effects of chemical exposure are known, or in hypothesizing effects resulting from chemical exposure, using information on putative key events in established modes of action from appropriate in vitro or in silico systems and other lines of evidence. This modified mode of action framework and accompanying roadmap and case examples are expected to contribute to improving transparency in explicitly addressing weight of evidence considerations in mode of action/species concordance analysis based on both
conventional data sources and evolving methods.


Mode of action is defined as a series of key biological events leading to an observed toxicological effect (for example, metabolism to a toxic entity, cell death, regenerative repair and tumors). It contrasts with mechanism of action, which generally involves a detailed understanding of the molecular basis for an effect. A framework to consider the weight of evidence for hypothesized modes of action in animals and their relevance to humans, has been widely adopted and used by government agencies and international organizations. The framework, developed and refined through its application in case studies for principally non-DNA-reactive carcinogens, has more recently been extended to DNA-reactive carcinogens, non-cancer endpoints and different life stages. In addition to increasing transparency, use of the framework promotes consistency in decision-making concerning adequacy of weight of evidence, facilitates peer input and review and identifies critical research needs. The framework provides an effective tool to facilitate discussion between the research and risk assessment communities on critical data gaps, which if filled, would permit more refined estimates of risk. As a basis for additionally coordinating and focusing research on critical data gaps in a risk assessment context, five key events in the mode of action for benzene-induced leukemia are proposed: (1) benzene metabolism via Cytochrome P450, (2) the interaction of benzene metabolites with target cells in the bone marrow, (3) formation of initiated, mutated target cells, (4) selective proliferation of the mutated cells and (5) production of leukemia. These key events are considered in a framework analysis of human relevance as a basis to consider appropriate next steps in developing research strategies.


The mode of action human relevance (MOA/HR) framework increases transparency in systematically considering data on MOA for end (adverse) effects and their relevance to humans. This framework continues to evolve as experience increases in its application. Though the MOA/HR framework is not designed to address the question of "how much information is enough" to support a hypothesized MOA in animals or its relevance to humans, its organizing construct has potential value in considering relative weight of evidence (WOE) among different cases and hypothesized MOA(s). This context is explored based on MOA analyses in published assessments to illustrate the relative extent of supporting data and their implications for dose-response analysis and involved comparisons for chemical assessments on trichloropropane, and carbon tetrachloride with several hypothesized MOA(s) for cancer. The WOE for each hypothesized MOA was summarized in narrative tables based on comparison and contrast of the extent and nature of the supporting database versus potentially inconsistent or missing information. The comparison was based on
evolved Bradford Hill considerations rank ordered to reflect their relative contribution to WOE determinations of MOA taking into account increasing experience in their application internationally. This clarification of considerations for WOE determinations as a basis for comparative analysis is anticipated to contribute to increasing consistency in the application of MOA/HR analysis and potentially, transparency in separating science judgment from public policy considerations in regulatory risk assessment.


The development of adverse outcome pathways (AOPs) is becoming a key component of twenty-first century toxicology. AOPs provide a conceptual framework that links the molecular initiating event to an adverse outcome through organized toxicological knowledge, bridging the gap from chemistry to toxicological effect. As nuclear receptors (NRs) play essential roles for many physiological processes within the body, they are used regularly as drug targets for therapies to treat many diseases including diabetes, cancer and neurodegenerative diseases. Due to the heightened development of NR ligands, there is increased need for the identification of related AOPs to facilitate their risk assessment. Many NR ligands have been linked specifically to steatosis. This article reviews and summarizes the role of NR and their importance with links between NR examined to identify plausible putative AOPs. The following NRs are shown to induce hepatic steatosis upon ligand binding: aryl hydrocarbon receptor, constitutive androstane receptor, oestrogen receptor, glucocorticoid receptor, farnesoid X receptor, liver X receptor, peroxisome proliferator-activated receptor, pregnane X receptor and the retinoic acid receptor. A preliminary, putative AOP was formed for NR binding linked to hepatic steatosis as the adverse outcome.


This study develops and evaluates a mechanistic model of the hatching of zebrafish eggs that were exposed to CuO engineered nanoparticles (ENP) in a high-throughput screening system and places this model in an adverse outcome pathway (AOP) that also includes CuO ENP dissolution and Cu bioaccumulation. Cu(2+) inhibits the proteolytic activity of Zebrafish Hatching Enzyme 1 and thereby delay or impair hatching success. This study demonstrates that noncompetitive inhibition kinetics describe the impact of dissolved Cu on hatching; it is estimated that indefinitely long exposure to 1.88 μM dissolved Cu in the environment reduces hatching enzyme activity by 50%. The complexity arising from CuO ENP dissolution and CuO ENP assisted bioaccumulation of Cu has led to apparently contradictory findings about ion versus "nano" effects on hatching. Model-mediated data analyses indicate that, relative to copper salts, CuO ENPs increase the uptake rates of Cu into the perivitelline space up to 8 times. The toxicity assessment framework in this study can be adapted to
accommodate other types of toxicant, environmental samples and other aquatic oviparous species.


The Adverse Outcome Pathway (AOP) framework is a tool for making biological connections and summarizing key information across different levels of biological organization to connect biological perturbations at the molecular level to adverse outcomes for an individual or population. Computational approaches to explore and determine these connections can accelerate the assembly of AOPs. By leveraging the wealth of publicly available data covering chemical effects on biological systems, computationally-predicted AOPs (cpAOPs) were assembled via data mining of high-throughput screening (HTS) in vitro data, in vivo data and other disease phenotype information. Frequent Itemset Mining (FIM) was used to find associations between the gene targets of ToxCast HTS assays and disease data from Comparative Toxicogenomics Database (CTD) by using the chemicals as the common aggregators between datasets. The method was also used to map gene expression data to disease data from CTD. A cpAOP network was defined by considering genes and diseases as nodes and FIM associations as edges. This network contained 18,283 gene to disease associations for the ToxCast data and 110,253 for CTD gene expression. Two case studies show the value of the cpAOP network by extracting subnetworks focused either on fatty liver disease or the Aryl Hydrocarbon Receptor (AHR). The subnetwork surrounding fatty liver disease included many genes known to play a role in this disease. When querying the cpAOP network with the AHR gene, an interesting subnetwork including glaucoma was identified. While substantial literature exists to support the potential for AHR ligands to elicit glaucoma, it was not explicitly captured in the public annotation information in CTD. The subnetwork from this analysis suggests a cpAOP that includes changes in CYP1B1 expression, which has been previously established in the literature as a primary cause of glaucoma.

These case studies highlight the value in integrating multiple data sources when defining cpAOPs for HTS data.


The adverse outcome pathway (AOP) concept links molecular perturbations with organism and population-level outcomes to support high-throughput toxicity (HTT) testing. International efforts are underway to define AOPs and store the information supporting these AOPs in a central knowledge base; however, this process is currently labor-intensive and time-consuming. Publicly available data sources provide a wealth of information that could be used to define computationally predicted AOPs (cpAOPs), which could serve as a basis for creating expert-derived AOPs in a much more efficient way. Computational tools for mining large datasets provide the means for extracting and organizing the information captured in these public data sources. Using cpAOPs as a starting point for expert-derived AOPs should accelerate AOP development. Coupling this with tools to coordinate and facilitate the expert development efforts will increase
the number and quality of AOPs produced, which should play a key role in advancing the adoption of HTT testing, thereby reducing the use of animals in toxicity testing and greatly increasing the number of chemicals that can be tested.


An adverse outcome pathway (AOP) describes the causal linkage between initial molecular events and an adverse outcome at individual or population levels. Whilst there has been considerable momentum in AOP development, far less attention has been paid to how AOPs might be practically applied for different regulatory purposes. This paper proposes a scientific confidence framework (SCF) for evaluating and applying a given AOP for different regulatory purposes ranging from prioritizing chemicals for further evaluation, to hazard prediction, and ultimately, risk assessment. The framework is illustrated using three different AOPs for several typical regulatory applications. The AOPs chosen are ones that have been recently developed and/or published, namely those for estrogenic effects, skin sensitisation, and rodent liver tumor promotion. The examples confirm how critical the data-richness of an AOP is for driving its practical application. In terms of performing risk assessment, human dosimetry methods are necessary to inform meaningful comparisons with human exposures; dosimetry is applied to effect levels based on non-testing approaches and in vitro data. Such a comparison is presented in the form of an exposure:activity ratio (EAR) to interpret biological activity in the context of exposure and to provide a basis for product stewardship and regulatory decision making.


Adverse outcome pathways (AOPs) offer a pathway-based toxicological framework to support hazard assessment and regulatory decision-making. However, little has been discussed about the scientific confidence needed, or how complete a pathway should be, before use in a specific regulatory application. Here we review four case studies to explore the degree of scientific confidence and extent of completeness (in terms of causal events) that is required for an AOP to be useful for a specific purpose in a regulatory application: (i) Membrane disruption (Narcosis) leading to respiratory failure (low confidence), (ii) Hepatocellular proliferation leading to cancer (partial pathway, moderate confidence), (iii) Covalent binding to proteins leading to skin sensitization (high confidence), and (iv) Aromatase inhibition leading to reproductive dysfunction in fish (high confidence). Partially complete AOPs with unknown molecular initiating events, such as 'Hepatocellular proliferation leading to cancer', were found to be valuable. We demonstrate that scientific confidence in these pathways can be increased through the use of unconventional information (eg, computational identification of potential initiators). AOPs at all levels of confidence can contribute to specific uses. A significant statistical or
quantitative relationship between events and/or the adverse outcome relationships is a common characteristic of AOPs, both incomplete and complete, that have specific regulatory uses. For AOPs to be useful in a regulatory context they must be at least as useful as the tools that regulators currently possess, or the techniques currently employed by regulators.


The toxicological effects of many stressors are mediated through unknown, or incompletely characterized, mechanisms of action. The application of reverse engineering complex interaction networks from high dimensional omics data (gene, protein, metabolic, signaling) can be used to overcome these limitations. This approach was used to characterize adverse outcome pathways (AOPs) for chemicals that disrupt the hypothalamus-pituitary-gonadal endocrine axis in fathead minnows (FHM, Pimephales promelas). Gene expression changes in FHM ovaries in response to seven different chemicals, over different times, doses, and in vivo versus in vitro conditions, were captured in a large data set of 868 arrays. Potential AOPs of the antiandrogen flutamide were examined using two mutual information-based methods to infer gene regulatory networks and potential AOPs. Representative networks from these studies were used to predict network paths from stressor to adverse outcome as candidate AOPs. The relationship of individual chemicals to an adverse outcome can be determined by following perturbations through the network in response to chemical treatment, thus leading to the nodes associated with the adverse outcome. Identification of candidate pathways allows for formation of testable hypotheses about key biological processes, biomarkers, or alternative endpoints that can be used to monitor an AOP. Finally, the unique challenges facing the application of this approach in ecotoxicology were identified and a road map for the utilization of these tools presented.


BACKGROUND: Adverse outcome pathways (AOPs) link adverse effects in individuals or populations to a molecular initiating event (MIE) that can be quantified using in vitro methods. Practical application of AOPs in chemical-specific risk assessment requires incorporation of knowledge on exposure, along with absorption, distribution, metabolism, and excretion (ADME) properties of chemicals. OBJECTIVES: We developed a conceptual workflow to examine exposure and ADME properties in relation to an MIE. The utility of this workflow was evaluated using a previously established AOP, acetylcholinesterase (AChE) inhibition. METHODS: Thirty chemicals found to inhibit human AChE in the ToxCast assay were examined with respect to their exposure, absorption potential, and ability to cross the blood-brain barrier (BBB). Structures of active chemicals were compared against structures of 1,029 inactive chemicals to detect possible parent compounds that might have active metabolites. RESULTS: Application of the workflow screened 10 "low-priority" chemicals of 30
active chemicals. Fifty-two of the 1,029 inactive chemicals exhibited a similarity threshold of \( \geq 75\% \) with their nearest active neighbors. Of these 52 compounds, 30 were excluded due to poor absorption or distribution. The remaining 22 compounds may inhibit AChE in vivo either directly or as a result of metabolic activation. CONCLUSIONS: The incorporation of exposure and ADME properties into the conceptual workflow eliminated 10 "low-priority" chemicals that may otherwise have undergone additional, resource-consuming analyses. Our workflow also increased confidence in interpretation of in vitro results by identifying possible "false negatives." CITATION: Phillips MB, Leonard JA, Grulke CM, Chang DT, Edwards SW, Brooks R, Goldsmith MR, El-Masri H, Tan YM. 2016. A workflow to investigate exposure and pharmacokinetic influences on high-throughput in vitro chemical screening based on adverse outcome pathways. Environ Health Perspect 124:53-60; http://dx.doi.org/10.1289/ehp.1409450.


The U-SENS assay, formerly known as MUSST (Myeloid U937 Skin Sensitization Test), is an in vitro method to assess skin sensitization. Dendritic cell activation following exposure to sensitizers was modelled in the U937 human myeloid cell line by measuring the induction of the expression of CD86 by flow cytometry. The predictive performance of U-SENS was assessed via a comprehensive comparison analysis with the available human and LLNA data of 175 substances. U-SENS showed 79% specificity, 90% sensitivity and 88% accuracy. A four laboratory ring study demonstrated the transferability, reliability and reproducibility of U-SENS, with a reproducibility of 95% within laboratories and 79% between-laboratories, showing that the U-SENS assay is a promising tool in a skin sensitization risk assessment testing strategy.


Despite a long history of successful use, routine application of some anticoagulant rodenticides (ARs) may be at a crossroad due to new regulatory guidelines intended to mitigate risk. An adverse outcome pathway for ARs was developed to identify information gaps and end points to assess the effectiveness of regulations. This framework describes chemical properties of ARs, established macromolecular interactions by inhibition of vitamin K epoxide reductase, cellular responses including altered clotting factor processing and coagulopathy, organ level effects such as hemorrhage, organism responses with linkages to reduced fitness and mortality, and potential consequences to predator populations. Risk assessments have led to restrictions affecting use of some second-generation ARs (SGARs) in North America. While the European regulatory community
highlighted significant or unacceptable risk of ARs to nontarget wildlife, use of SGARs in most EU member states remains authorized due to public health concerns and the absence of safe alternatives. For purposes of conservation and restoration of island habitats, SGARs remain a mainstay for eradication of invasive species. There are significant data gaps related to exposure pathways, comparative species sensitivity, consequences of sublethal effects, potential hazards of greater AR residues in genetically resistant prey, effects of low-level exposure to multiple rodenticides, and quantitative data on the magnitude of nontarget wildlife mortality.


Non-communicable diseases (NCDs) are a major cause of premature mortality. Recent studies show that predispositions for NCDs may arise from early-life exposure to low concentrations of environmental contaminants. This developmental origins of health and disease (DOHaD) paradigm suggests that programming of an embryo can be disrupted, changing the homeostatic set point of biological functions. Epigenetic alterations are a possible underlying mechanism. Here, we investigated the DOHaD paradigm by exposing zebrafish to subtoxic concentrations of the ubiquitous contaminant cadmium during embryogenesis, followed by growth under normal conditions. Prolonged behavioral responses to physical stress and altered antioxidative physiology were observed approximately ten weeks after termination of embryonal exposure, at concentrations that were 50-3200-fold below the direct embryotoxic concentration, and interpreted as altered developmental programming. Literature was explored for possible mechanistic pathways that link embryonic subtoxic cadmium to the observed apical phenotypes, more specifically, the probability of molecular mechanisms induced by cadmium exposure leading to altered DNA methylation and subsequently to the observed apical phenotypes. This was done using the adverse outcome pathway model framework, and assessing key event relationship plausibility by tailored Bradford-Hill analysis. Thus, cadmium interaction with thiols appeared to be the major contributor to late-life effects. Cadmium-thiol interactions may lead to depletion of the methyl donor S-adenosyl-methionine, resulting in methylome alterations, and may, additionally, result in oxidative stress, which may lead to DNA oxidation, and subsequently altered DNA methyltransferase activity. In this way, DNA methylation may be affected at a critical developmental stage, causing the observed apical phenotypes.


Adverse outcome pathways (AOPs) are designed to describe linkages of key events within a biological pathway that result in an adverse outcome associated with chemical perturbation of a well-defined molecular initiating event. Risk assessors have traditionally relied on data from apical endpoints (e.g., mortality,
growth, reproduction) to derive benchmark values for use in determining the potential adverse impacts of chemicals. One goal in building reliable and well-characterized AOPs is to identify relevant in vitro assays and/or in vivo biomarkers that could be used in screening the potential hazard of substances, thereby reducing costs and increasing the number of chemicals that can be evaluated in a timely fashion. The purpose of this review article is to build an AOP for substances with a molecular initiating event of acetylcholinesterase inhibition leading to acute mortality following guidance developed by the Organisation for Economic Cooperation and Development. In contrast to most other AOPs developed to date, in which coverage is for a relatively limited taxonomic group or life stage, this AOP is applicable to a wide range of species at multiple life stages. Furthermore, while development of most AOPs has relied on data for a few model chemicals, the AOP described in the present review captures information from a large number of studies with a diversity of organophosphate and carbamate insecticides.

Sakuratani, Y., et al. (2013). "Categorization of nitrobenzenes for repeated dose toxicity based on adverse outcome pathways." SAR QSAR Environ Res 24(1): 35-46. Adoption of the data-gap filling method for complex endpoints such as repeated dose toxicity (RDT) and reproductive/developmental toxicity is one of the most important issues affecting international chemical management at present. A categorization method based on adverse outcome pathways (AOPs) has recently been investigated for such complex endpoints. In this paper, we report results of the categorization of nitrobenzenes for RDT based on the AOPs obtained by analysing the detailed RDT test reports for 24 different nitrobenzenes already evaluated. In most RDT testing of nitrobenzenes without hydroxyl groups or acid groups, findings related to haemolytic anaemia and liver effects were observed at low dosages. It was, therefore, possible to assume common AOPs for haemolytic anaemia and liver effects induced by these nitrobenzenes. As a result, a group of nitrobenzenes was defined as a single category for both haemolytic anaemia and liver effects, respectively, based on these AOPs.


Schultz, T. W., et al. (2016). "The adverse outcome pathway for skin sensitisation: Moving closer to replacing animal testing." Altern Lab Anim 44(5): 453-460. This article outlines the work of the Organisation for Economic Co-operation and Development (OECD) that led to being jointly awarded the 2015 Lush Black Box Prize. The award-winning work centred on the development of 'The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins'. This Adverse Outcome Pathway (AOP) has provided the mechanistic basis for the integration of skin sensitisation-related information. Recent developments in integrated approaches to testing and assessment, based on the AOP, are summarised. The impact of the AOP on regulatory policy and on the Three Rs are discussed. An overview of the next generation of the skin sensitisation AOP module in the OECD QSAR Toolbox, based on more-recent
work at the Laboratory of Mathematical Chemistry, is also presented.


Crude oil spills are a worldwide ocean conservation threat. Fish are particularly vulnerable to the oiling of spawning habitats, and crude oil causes severe abnormalities in embryos and larvae. However, the underlying mechanisms for these developmental defects are not well understood. Here, we explore the transcriptional basis for four discrete crude oil injury phenotypes in the early life stages of the commercially important Atlantic haddock (Melanogrammus aeglefinus). These include defects in (1) cardiac form and function, (2) craniofacial development, (3) ionoregulation and fluid balance, and (4) cholesterol synthesis and homeostasis. Our findings suggest a key role for intracellular calcium cycling and excitation-transcription coupling in the dysregulation of heart and jaw morphogenesis. Moreover, the disruption of ionoregulatory pathways sheds new light on buoyancy control in marine fish embryos. Overall, our chemical-genetic approach identifies initiating events for distinct adverse outcome pathways and novel roles for individual genes in fundamental developmental processes.


Chemical regulation is challenged by the large number of chemicals requiring assessment for potential human health and environmental impacts. Current approaches are too resource intensive in terms of time, money and animal use to evaluate all chemicals under development or already on the market. The need for timely and robust decision making demands that regulatory toxicity testing becomes more cost-effective and efficient. One way to realize this goal is by being more strategic in directing testing resources; focusing on chemicals of highest concern, limiting testing to the most probable hazards, or targeting the most vulnerable species. Hypothesis driven Integrated Approaches to Testing and Assessment (IATA) have been proposed as practical solutions to such strategic testing. In parallel, the development of the Adverse Outcome Pathway (AOP) framework, which provides information on the causal links between a molecular initiating event (MIE), intermediate key events (KEs) and an adverse outcome (AO) of regulatory concern, offers the biological context to facilitate development of IATA for regulatory decision making. This manuscript summarizes discussions at the Workshop entitled "Advancing AOPs for Integrated Toxicology and Regulatory Applications" with particular focus on the role AOPs play in informing the development of IATA for different regulatory purposes.


Developmental toxicity can be caused through a multitude of mechanisms and
can therefore not be captured through a single simple mechanistic paradigm. However, it may be possible to define a selected group of overarching mechanisms that might allow detection of the vast majority of developmental toxicants. Against this background, we have explored the usefulness of retinoic acid mediated regulation of neural tube and axial patterning as a general mechanism that, when perturbed, may result in manifestations of developmental toxicity that may cover a large part of malformations known to occur in experimental animals and in man. Through a literature survey, we have identified key genes in the regulation of retinoic acid homeostasis, as well as marker genes of neural tube and axial patterning, that may be used to detect developmental toxicants in in vitro systems. A retinoic acid-neural tube/axial patterning adverse outcome pathway (RA-NTA AOP) framework was designed. The framework was tested against existing data of flusilazole exposure in the rat whole embryo culture, the zebrafish embryotoxicity test, and the embryonic stem cell test. Flusilazole is known to interact with retinoic acid homeostasis, and induced common and unique NTA marker gene changes in the three test systems. Flusilazole-induced changes were similar in directionality to gene expression responses after retinoic acid exposure. It is suggested that the RA-NTA framework may provide a general tool to define mechanistic pathways and biomarkers of developmental toxicity that may be used in alternative in vitro assays for the detection of embryotoxic compounds.


The comprehensive understanding of the precise mode of action and/or adverse outcome pathway (MoA/AOP) of chemicals has become a key step toward the development of a new generation of predictive toxicology tools. One of the challenges of this process is to test the feasibility of the molecular modelling approaches to explore key molecular initiating events (MIE) within the integrated strategy of MoA/AOP characterisation. The description of MoAs leading to toxicity and liver damage has been the focus of much interest. Growing evidence underlines liver PPARgamma ligand-dependent activation as a key MIE in the elicitation of liver steatosis. Synthetic PPARgamma full agonists are of special concern, since they may trigger a number of adverse effects not observed with partial agonists. In this study, molecular modelling was performed based on the PPARgamma complexes with full agonists extracted from the Protein Data Bank. The receptor binding pocket was analysed, and the specific ligand-receptor interactions were identified for the most active ligands. A pharmacophore model was derived, and the most important pharmacophore features were outlined and characterised in relation to their specific role for PPARgamma activation. The results are useful for the characterisation of the chemical space of PPARgamma full agonists and could facilitate the development of preliminary filtering rules for the effective virtual ligand screening of compounds with PPARgamma full agonistic activity.

van der Veen, J. W., et al. (2014). "Anchororing molecular mechanisms to the adverse

Allergic contact dermatitis (ACD) is a hypersensitivity immune response induced by small protein-reactive chemicals. Currently, the murine local lymph node assay (LLNA) provides hazard identification and quantitative estimation of sensitizing potency. Given the complexity of ACD, a single alternative method cannot replace the LLNA, but it is necessary to combine methods through an integrated testing strategy (ITS). In the development of an ITS, information regarding mechanisms and molecular processes involved in skin sensitization is crucial. The recently published adverse outcome pathway (AOP) for skin sensitization captures mechanistic knowledge into key events that lead to ACD. To understand the molecular processes in ACD, a systematic review of murine in vivo studies was performed and an ACD molecular map was constructed. In addition, comparing the molecular map to the limited human in vivo toxicogenomic data available suggests that certain processes are similarly triggered in mice and humans, but additional human data will be needed to confirm these findings and identify differences. To gain insight in the molecular mechanisms represented by various human in vitro systems, the map was compared to in vitro toxicogenomic data. This analysis allows for comparison of emerging in vitro methods on a molecular basis, in addition to mathematical predictive value. Finally, a survey of the current in silico, in chemico, and in vitro methods was used to indicate which AOP key event is modeled by each method. By anchoring emerging classification methods to the AOP and the ACD molecular map, complementing methods can be identified, which provides a cornerstone for the development of a testing strategy that accurately reflects the key events in skin sensitization.


Several experimental studies have shown that carbon nanotubes (CNT) can induce respiratory effects, including lung fibrosis. The cellular and molecular events through which these effects develop are, however, not clearly elucidated. The purpose of the present review was to analyze the key events involved in the lung fibrotic reaction induced by CNT and to assess their relationships. We thus address current knowledge and gaps with a view to draft an Adverse Outcome Pathway (AOP) concerning the fibrotic potential of CNT. As for many inhaled particles, CNT can indirectly activate fibroblasts through the release of pro-inflammatory (IL-1beta) and pro-fibrotic (PDGF and TGF-beta) mediators by inflammatory cells (macrophages and epithelial cells) via the induction of oxidative stress, inflammasome or NF-kB. We also highlight here direct effects of CNT on fibroblasts, which appear as a new mode of toxicity relatively specific for CNT. Direct effects of CNT on fibroblasts include the induction of fibroblast proliferation, differentiation and collagen production via ERK 1/2 or Smad signaling. We also point out the physico-chemical properties of CNT important for their toxicity and the relationship between in vitro and in vivo effects. This knowledge provides evidence to draft an AOP for the fibrogenic activity of CNT, which allows developing simple in vitro models contributing to predict the CNT
effects in lung fibrosis, and risk assessment tools for regulatory decision.


The fish early-life stage (FELS) test (Organisation for Economic Co-operation and Development [OECD] test guideline 210) is the primary test used internationally to estimate chronic fish toxicity in support of ecological risk assessments and chemical management programs. As part of an ongoing effort to develop efficient and cost-effective alternatives to the FELS test, there is a need to identify and describe potential adverse outcome pathways (AOPs) relevant to FELS toxicity. To support this endeavor, the authors outline and illustrate an overall strategy for the discovery and annotation of FELS AOPs. Key events represented by major developmental landmarks were organized into a preliminary conceptual model of fish development. Using swim bladder inflation as an example, a weight-of-evidence-based approach was used to support linkage of key molecular initiating events to adverse phenotypic outcomes and reduced young-of-year survival. Based on an iterative approach, the feasibility of using key events as the foundation for expanding a network of plausible linkages and AOP knowledge was explored and, in the process, important knowledge gaps were identified. Given the scope and scale of the task, prioritization of AOP development was recommended and key research objectives were defined relative to factors such as current animal-use restrictions in the European Union and increased demands for fish toxicity data in chemical management programs globally. The example and strategy described are intended to guide collective efforts to define FELS-related AOPs and develop resource-efficient predictive assays that address the toxicological domain of the OECD 210 test.


Organization of existing and emerging toxicological knowledge into adverse outcome pathway (AOP) descriptions can facilitate greater application of mechanistic data, including those derived through high-throughput in vitro, high content omics and imaging, and biomarker approaches, in risk-based decision making. The previously ad hoc process of AOP development is being formalized through development of internationally harmonized guidance and principles. The goal of this article was to outline the information content desired for formal AOP description and some rules of thumb and best practices intended to facilitate reuse and connectivity of elements of an AOP description in a knowledgebase and network context. For example, key events (KEs) are measurements of change in biological state that are indicative of progression of a perturbation toward a specified adverse outcome. Best practices for KE description suggest that each KE should be defined as an independent measurement made at a particular level of biological organization. The concept of "functional equivalence"
can help guide both decisions about how many KEs to include in an AOP and the specificity with which they are defined. Likewise, in describing both KEs and evidence that supports a causal linkage or statistical association between them (ie, a key event relationship; KER), best practice is to build from and contribute to existing KE or KER descriptions in the AOP knowledgebase rather than creating redundant descriptions. The best practices proposed address many of the challenges and uncertainties related to AOP development and help promote a consistent and reliable, yet flexible approach.


An adverse outcome pathway (AOP) is a conceptual framework that organizes existing knowledge concerning biologically plausible, and empirically supported, links between molecular-level perturbation of a biological system and an adverse outcome at a level of biological organization of regulatory relevance. Systematic organization of information into AOP frameworks has potential to improve regulatory decision-making through greater integration and more meaningful use of mechanistic data. However, for the scientific community to collectively develop a useful AOP knowledgebase that encompasses toxicological contexts of concern to human health and ecological risk assessment, it is critical that AOPs be developed in accordance with a consistent set of core principles. Based on the experiences and scientific discourse among a group of AOP practitioners, we propose a set of five fundamental principles that guide AOP development: (1) AOPs are not chemical specific; (2) AOPs are modular and composed of reusable components-notably key events (KEs) and key event relationships (KERs); (3) an individual AOP, composed of a single sequence of KEs and KERs, is a pragmatic unit of AOP development and evaluation; (4) networks composed of multiple AOPs that share common KEs and KERs are likely to be the functional unit of prediction for most real-world scenarios; and (5) AOPs are living documents that will evolve over time as new knowledge is generated. The goal of the present article was to introduce some strategies for AOP development and detail the rationale behind these 5 key principles. Consideration of these principles addresses many of the current uncertainties regarding the AOP framework and its application and is intended to foster greater consistency in AOP development.


Adverse outcome pathways (AOPs) are novel tools in toxicology and human risk assessment with broad potential. AOPs are designed to provide a clear-cut mechanistic representation of critical toxicological effects that span over different layers of biological organization. AOPs share a common structure consisting of a molecular initiating event, a series of intermediate steps and key events, and an adverse outcome. Development of AOPs ideally complies with OECD guidelines. This also holds true for AOP evaluation, which includes consideration of the Bradford Hill criteria for weight-of-evidence assessment and meeting a set of key questions defined by the OECD. Elaborate AOP frameworks have yet been
proposed for chemical-induced skin sensitization, cholestasis, liver fibrosis and liver steatosis. These newly postulated AOPs can serve a number of ubiquitous purposes, including the establishment of (quantitative) structure-activity relationships, the development of novel in vitro toxicity screening tests and the elaboration of prioritization strategies.


Drug-induced liver injury is a prominent reason for premarketing and postmarketing drug withdrawal and can be manifested in a number of ways, such as cholestasis, steatosis, and fibrosis. The mechanisms driving these toxicological processes have been well characterized and have been embedded in adverse outcome pathway frameworks in recent years. This review evaluates these constructs and simultaneously illustrates their use in the preclinical testing of drug-induced liver injury.


Adverse outcome pathways (AOPs) are novel tools in toxicology and human 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. Development and evaluation of AOPs ideally complies with guidelines issued by the Organization for Economic Cooperation and Development. AOP frameworks have yet been proposed for major types of drug-induced injury, especially in the liver, including steatosis, fibrosis, and cholestasis. These newly postulated AOPs can serve a number of purposes pertinent to safety assessment of drugs, in particular the establishment of quantitative structure-activity relationships, the development of novel in vitro toxicity screening tests, and the elaboration of prioritization strategies.

Adverse outcome pathways (AOPs) have been recently introduced in human risk assessment as pragmatic tools with multiple applications. As such, AOPs intend to provide a clear-cut mechanistic representation of pertinent toxicological effects. AOPs are typically composed of a molecular initiating event, a series of intermediate steps and key events, and an adverse outcome. In this study, an AOP framework is proposed for cholestasis triggered by drug-mediated inhibition of the bile salt export pump transporter protein. For this purpose, an in-depth survey of relevant scientific literature was carried out in order to identify intermediate steps and key events. The latter include bile accumulation, the induction of oxidative stress and inflammation, and the activation of specific nuclear receptors. Collectively, these mechanisms drive both a deteriorative cellular response, which underlies directly caused cholestatic injury, and an adaptive cellular response, which is aimed at counteracting cholestatic insults. AOP development was performed according to Organisation for Economic Co-operation and Development (OECD) guidance, including critical consideration of the Bradford Hill criteria for weight of evidence assessment and the OECD key questions for evaluating AOP confidence. The postulated AOP is expected to serve as the basis for the development of new in vitro tests and the characterization of novel biomarkers of drug-induced cholestasis.


Chemicals affect unicellular algae as a result of toxicokinetic and toxicodynamic processes. The internal concentration of chemicals in algae cells typically reaches equilibrium within minutes, while damage cumulatively increases over hours. The time gap between the steady state of internal exposure and damage development is thus suspected to span up to hours, mainly due to toxicodynamic processes. The quantification of rate-limited toxicodynamic processes, aggregated as a progressive effect from an initiating molecular event through biological key events toward the adverse outcome on algae growth inhibition, might discriminate between different adverse outcome pathways (AOPs). To support our hypothesis, we selected six chemicals according to different physicochemical properties and three distinctly dissimilar AOPs. The time courses of internal concentrations were linked to the observed affected Scenedesmus vacuolatus growth using toxicokinetic-toxicodynamic modeling. Effects on cell growth were explained by effect progression and not by the time to reach internal equilibrium concentration. Effect progression rates ranged over 6 orders of magnitude for all chemicals but varied by less than 1 order of magnitude within similar AOP (photosystem II inhibitors > reactive chemicals > lipid biosynthesis inhibitors), meaning that inhibitors of photosystem II advance an effect toward algae growth fastest compared to reactive chemicals and inhibitors of lipid biosynthesis.

The fish early life-stage (FELS) test guideline (OECD 210 or OCSPP 850.1400) is the most frequently used bioassay for predicting chronic fish toxicity and supporting aquatic ecological risk assessments around the world. For each chemical, the FELS test requires a minimum of 360 fish and 1 to 3 months from test initiation to termination. Although valuable for predicting fish full life-cycle toxicity, FELS tests are labor and resource intensive and, due to an emphasis on apical endpoints, provide little to no information about chemical mode of action. Therefore, the development and implementation of alternative testing strategies for screening and prioritizing chemicals has the potential to reduce the cost and number of animals required for estimating FELS toxicity and, at the same time, provides insights into mechanisms of toxicity. Using three reference chemicals with well-established yet distinct adverse outcome pathways (AOPs) in early life stages of fish, we proposed FELS-specific AOPs as conceptual frameworks for identifying useful chemical screening and prioritization strategies. The reference chemicals selected as case studies were a cardiotoxic aryl hydrocarbon receptor agonist (2,3,7,8-tetrachlorodibenzo-p-dioxin), neurotoxic acetylcholinesterase inhibitor (chlorpyrifos), and narcotic surfactant (linear alkylbenzene sulfonate). Using qualitative descriptions for each chemical during early fish development, we developed generalized AOPs and, based on these examples, proposed a three-tiered testing strategy for screening and prioritizing chemicals for FELS testing. Linked with biologically based concentration-response models, a tiered testing strategy may help reduce the reliance on long-term and costly FELS tests required for assessing the hazard of thousands of chemicals currently in commerce.


Current risk assessment guidance calls for an individual chemical-by-chemical approach that fails to capture potential interactive effects of exposure to environmental mixtures and genetic variability. We conducted a review of the literature on relationships between prenatal and early life exposure to mixtures of lead (Pb), arsenic (As), cadmium (Cd), and manganese (Mn) with neurodevelopmental outcomes. We then used an adverse outcome pathway (AOP) framework to integrate lines of evidence from multiple disciplines based on evolving guidance developed by the Organization for Economic Cooperation and Development (OECD). Toxicological evidence suggests a greater than additive effect of combined exposures to As-Pb-Cd and to Mn with any other metal, and several epidemiologic studies also suggest synergistic effects from binary combinations of Pb-As, Pb-Cd, and Pb-Mn. The exposure levels reported in these epidemiologic studies largely fall at the high-end (e.g., 95th percentile) of biomonitoring data from the National Health and Nutrition Examination Survey (NHANES), suggesting a small but significant potential for high-end exposures.
This review integrates multiple data sources using an AOP framework and provides an initial application of the OECD guidance in the context of potential neurodevelopmental toxicity of several metals, recognizing the evolving nature of regulatory interpretation and acceptance.

Wang, Y. and H. Duan (2015). "[Development and application of adverse outcome pathway in toxicology research]." Zhonghua Yu Fang Yi Xue Za Zhi 49(12): 1115-1118. Adverse outcome pathway (AOP) was a conceptual construct that integrated existing knowledge concerning the pathway of causal linkages between a molecular initiating event (MIE) and a final adverse effect at individual or population levels. The AOP methodology could be used as a basis for effects extrapolation and was an approach towards providing a framework for collecting and evaluating relevant chemical, biological and toxicological information. The framework would play an important role in risk assessment. We reviewed the concept of AOP, the development and assessment of the framework and the established models in toxicology researches. And the prospects and challenges of its application in toxicology were also introduced.


An adverse outcome pathway (AOP) is a sequence of key events from a molecular-level initiating event and an ensuing cascade of steps to an adverse outcome with population-level significance. To implement a predictive strategy for ecotoxicology, the multiscale nature of an AOP requires computational models to link salient processes (e.g., in chemical uptake, toxicokinetics, toxicodynamics, and population dynamics). A case study with domoic acid was used to demonstrate strategies and enable generic recommendations for developing computational models in an effort to move toward a toxicity testing paradigm focused on toxicity pathway perturbations applicable to ecological risk assessment. Domoic acid, an algal toxin with adverse effects on both wildlife and humans, is a potent agonist for kainate receptors (ionotropic glutamate receptors whose activation leads to the influx of Na(+) and Ca(2)(+)). Increased Ca(2)(+) concentrations result in neuronal excitotoxicity and cell death, primarily in the hippocampus, which produces seizures, impairs learning and memory, and alters behavior in some species. Altered neuronal Ca(2)(+) is a key process in domoic acid toxicity, which can be evaluated in vitro. Furthermore, results of these assays would be amenable to mechanistic modeling for identifying domoic acid concentrations and Ca(2)(+) perturbations that are normal, adaptive, or clearly toxic. In vitro assays with outputs amenable to measurement in exposed populations can link in vitro to in vivo conditions, and toxicokinetic information will aid in linking in vitro results to the individual organism. Development of an AOP required an iterative process with three important outcomes: a critically reviewed, stressor-specific AOP; identification of key processes suitable for evaluation with in vitro assays; and strategies for model development.

TBBPA is a non-genotoxic flame retardant used to improve fire safety in a wide variety of consumer products. Estimated human exposures to TBBPA are very low (<0.000084 mg/kg-day), relative to the doses (500 and 1000 mg/kg-day of TBBPA) administered in a recent bioassay that resulted in uterine tumors in Wistar Han rats following chronic exposure. As part of an effort to characterize the relevance of the uterine tumors to humans, data and biological knowledge relevant to the progression of events associated with TBBPA-induced uterine tumors in female rats were organized in an adverse outcome pathway (AOP) framework. Based on a review of possible MOAs for chemically induced uterine tumors and available TBBPA data sets, a plausible molecular initiating event (MIE) was the ability of TBBPA to bind to and inhibit estrogen sulfotransferases, the enzymes responsible for sulfation of estradiol. Subsequent key events in the AOP, including increased bioavailability of unconjugated estrogens in uterine tissue, would occur as a result of decreased sulfation, leading to a disruption in estrogen homeostasis, increased expression of estrogen responsive genes, cell proliferation, and hyperplasia. Available data support subsequent key events, including generation of reactive quinones from the metabolism of estrogens, followed by DNA damage that could contribute to the development of uterine tumors. Uncertainties associated with human relevance are highlighted by potential strain/species sensitivities to development of uterine tumors, as well as the characterization of a dose-dependent MIE. For the latter, it was determined that the TBBPA metabolic profile is altered at high doses (such as those used in the cancer bioassay), and thus an MIE that is only operative under repeated high dose, administration. The MIE and subsequent key events for the development of TBBPA-induced uterine tumors are not feasible in humans given differences in the kinetic and dynamic factors associated with high dose exposures in rats relative to human exposure levels to TBBPA.


2,4-dinitrotoluene (2,4-DNT) is a nitroaromatic used in industrial dyes and explosives manufacturing processes that is found as a contaminant in the environment. Previous studies have implicated antagonism of PPARalpha signaling as a principal process affected by 2,4-DNT. Here, we test the hypothesis that 2,4-DNT-induced perturbations in PPARalpha signaling and resultant downstream deficits in energy metabolism, especially from lipids, cause organism-level impacts on exercise endurance. PPAR nuclear activation bioassays demonstrated inhibition of PPARalpha signaling by 2,4-DNT whereas PPARgamma signaling increased. PPARalpha (−/−) and wild-type (WT) female mice were exposed for 14 days to vehicle or 2,4-DNT (134 mg/kg/day) and performed a forced swim to exhaustion 1 day after the last dose. 2,4-DNT
significantly decreased body weights and swim times in WTs, but effects were significantly mitigated in PPARalpha (-/-) mice. 2,4-DNT decreased transcript expression for genes downstream in the PPARalpha signaling pathway, principally genes involved in fatty acid transport. Results indicate that PPARgamma signaling increased resulting in enhanced cycling of lipid and carbohydrate substrates into glycolytic/gluconeogenic pathways favoring energy production versus storage in 2,4-DNT-exposed WT and PPARalpha (-/-) mice. PPARalpha (-/-) mice appear to have compensated for the loss of PPARalpha by shifting energy metabolism to PPARalpha-independent pathways resulting in lower sensitivity to 2,4-DNT when compared with WT mice. Our results validate 2,4-DNT-induced perturbation of PPARalpha signaling as the molecular initiating event for impaired energy metabolism, weight loss, and decreased exercise performance.


Efforts are underway to transform regulatory toxicology and chemical safety assessment from a largely empirical science based on direct observation of apical toxicity outcomes in whole organism toxicity tests to a predictive one in which outcomes and risk are inferred from accumulated mechanistic understanding. The adverse outcome pathway (AOP) framework provides a systematic approach for organizing knowledge that may support such inference. Likewise, computational models of biological systems at various scales provide another means and platform to integrate current biological understanding to facilitate inference and extrapolation. We argue that the systematic organization of knowledge into AOP frameworks can inform and help direct the design and development of computational prediction models that can further enhance the utility of mechanistic and in silico data for chemical safety assessment. This concept was explored as part of a workshop on AOP-Informed Predictive Modeling Approaches for Regulatory Toxicology held September 24-25, 2015. Examples of AOP-informed model development and its application to the assessment of chemicals for skin sensitization and multiple modes of endocrine disruption are provided. The role of problem formulation, not only as a critical phase of risk assessment, but also as guide for both AOP and complementary model development is described. Finally, a proposal for actively engaging the modeling community in AOP-informed computational model development is made. The contents serve as a vision for how AOPs can be leveraged to facilitate development of computational prediction models needed to support the next generation of chemical safety assessment.


The adverse outcome pathway (AOP) framework could be helpful for chemical risk assessment and mechanistic research. The aim of the present study was to unravel the mechanism of chlordecone-induced endocrine disruption by illustrating the main molecular initiating event (MIE)/perturbations responsible for
the observed effects. In silico simulations were performed to predict the MIE(s), and the results pointed to agonistic interaction with estrogen receptors (ERalpha, ERbeta), androgen receptor (AR), cytochrome P450 (CYP19A) by chlordecone. In vivo endocrine disruptions were evaluated in rare minnow (Gobiocypris rarus) exposed to 0.01, 0.1, 1 and 10 μg L−1 chlordecone from 2 h post-fertilization until sexually mature. In the females, increases of vitellogenin (vtg) mRNA levels in liver and gonad, plasma estradiol (E2), testosterone (T) and E2/T, and renalsomatic index confirmed the role of agonism of ER and CYP19A as MIEs, but the decreased gonadosomatic index, degenerated ovaries as well as the feed-forward response pointed to other potential but important MIEs and corresponding AOPs. In the males, increased E2/T ratio, increased testis vtg mRNA levels and occurrence of intersex confirmed the roles of agonism of ERalpha and CYP19A as main MIEs in chlordecone-induced endocrine disruptions. Our results also fetches out the limit of AOPs in predicting the adverse outcomes and explaining the mechanism of chemicals at present, thus reflected a critical need for expanding AOPs and AOP network before using it in chemical risk assessment.


The Organisation for Economic Cooperation and Development's (OECD) Adverse Outcome Pathway (AOP) programme aims to develop a knowledgebase of all known pathways of toxicity that lead to adverse effects in humans and ecosystems. A Users' Handbook was recently released to provide supplementary guidance on AOP development. This article describes one AOP-alkylation of DNA in male premeiotic germ cells leading to heritable mutations. This outcome is an important regulatory endpoint. The AOP describes the biological plausibility and empirical evidence supporting that compounds capable of alkylating DNA cause germ cell mutations and subsequent mutations in the offspring of exposed males. Alkyl adducts are subject to DNA repair; however, at high doses the repair machinery becomes saturated. Lack of repair leads to replication of alkylated DNA and ensuing mutations in male premeiotic germ cells. Mutations that do not impair spermatogenesis persist and eventually are present in mature sperm. Thus, the mutations are transmitted to the offspring. Although there are some gaps in empirical support and evidence for essentiality of the key events for certain aspects of this AOP, the overall AOP is generally accepted as dogma and applies broadly to any species that produces sperm. The AOP was developed and used in an iterative process to test and refine the Users' Handbook, and is one of the first publicly available AOPs. It is our hope that this AOP will be leveraged to develop other AOPs in this field to advance method development, computational models to predict germ cell effects, and integrated testing strategies.

Using paraoxon as a reference acetylcholinesterase (AChE) inhibitor, the objective of this study was to develop an adverse outcome pathway (AOP) that provided quantitative linkages across levels of biological organization during zebrafish embryogenesis. Within normal zebrafish embryos, we first demonstrated that ache transcripts and AChE activity increased in a stage-dependent manner following segmentation. We then showed that static exposure of embryos to paraoxon (31.2-500 nM) from 5 to 96 hpf resulted in significant stage- and concentration-dependent AChE inhibition, albeit these effects were fully reversible within 48 h following transfer to clean water. However, even in the presence of significant AChE inhibition, exposure to non-teratogenic paraoxon concentrations (\(\leq 250\) nM) did not adversely impact secondary motoneuron development at 96 hpf. Therefore, we investigated the potential effects of paraoxon exposure on spontaneous tail contractions at 26 hpf - an early locomotor behavior that results from innervation of primary (not secondary) motoneuron axons to target axial muscles. Based on these studies, the frequency of spontaneous tail contractions at 26 hpf - a developmental stage with minimal AChE expression and activity - was significantly higher following exposure to paraoxon concentrations as low as 31.2 nM. Overall, our data suggest that (1) normal AChE activity is not required for secondary motoneuron development and (2) spontaneous tail contractions at 26 hpf are sensitive to paraoxon exposure, an effect that may be independent of AChE inhibition. Using a well-studied reference chemical, this study highlights the potential challenges in developing quantitative AOPs to support chemical screening and prioritization strategies.