RESEARCH BRIEF

Adverse Outcome Pathways (AOPs) and risk assessment – experiences from HBM4EU

KEY MESSAGES

- Adverse outcome pathways (AOPs) describe the **chain of events leading from a molecular perturbation to a detrimental effect on the organism or population**. They are **essential to translate current toxicological data** into health outcomes relevant to risk assessment.
- **New tools were developed for the end-users**, such as risk assessors, to assist them in mapping toxicological data to the AOP knowledge.
- **Within HBM4EU**, several initiatives **significantly contributed to improving AOP knowledge and practical use for risk assessment** by (1) drafting AOPs, (2) creating the AOP-helpFinder web server, and (3) writing a practical guide.
- **Within HBM4EU, the AOP framework was successfully used** to (1) prioritize replacement chemicals, (2) provide mechanistic evidence for chemical-health effect associations in humans, (3) identify or validate biomarkers of effects (BoE), or (4) assist mixture risk assessment.

WHY DO WE NEED AOPs?

We need to know if chemicals present in the environment affect our health.

Testing the chemicals on animals is problematic for ethical, financial, and efficiency reasons, and human relevance is not guaranteed. Therefore, **New Approach Methodologies (NAMs)**, using alternative assays (e.g., *in vitro*) or predictive models, **are increasingly applied**.

In this context, **AOP is a framework that assists the translation of modern toxicological data into predicted adverse health outcomes**.

The AOP framework is also instrumental in organizing the large amount of toxicological information.

WHAT ARE AOPs?

AOPs describe and formalise existing knowledge into **cascades of causally linked events.** An AOP starts from an **initial molecular perturbation** followed by effects at higher biological levels, all the way **to the final adverse outcome** (e.g., disease at either individual or population levels). The biology in AOPs is generic and independent of specific chemicals (stressors) triggering the perturbation.

The Organisation for Economic Co-operation and Development (OECD) is working towards the advancement of the AOP framework in collaboration with its member and partner countries. AOPs are **openly accessible through the** AOP-Wiki, a component of the AOP Knowledge Base (AOP-KB).

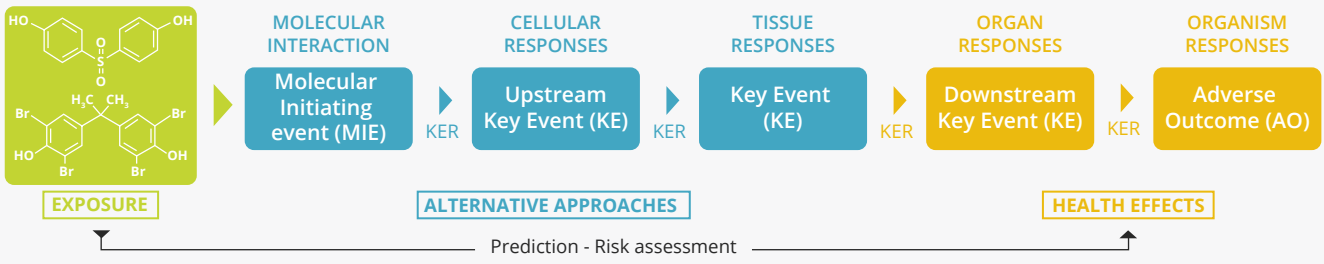

Figure 1: Schematic representation of an AOP and the links with regulatory applications. MIE, molecular initiating event; KE, key event; KER, key event relationship; AO, adverse outcome

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WHY ARE AOPs GOOD TOOLS?

AOPs provide a **reliable framework** for organising biological and toxicological knowledge.

AOPs are **pragmatic tools:** they describe the **key events (KEs)** that are measurable and useful for regulatory applications.

AOPs are **transparent:** the level of confidence and the supporting evidence are described and go through a reviewing process recorded online (AOP-Wiki).

AOPs are **modular,** and this allows to create new connections and AOP networks (e.g., for mixture toxicity).

The **AOP-Wiki** facilitates collective contribution and open access to users.

STRESSORS AND AOPs

Stressors (often chemicals) trigger the initial molecular perturbation, but the **AOP itself is "stressor independent"**. It describes the biology that underlies the chain of events leading to the adverse outcome (AO), regardless of which stressor triggered the molecular initiating event (MIE).

On the other hand, evidence collected for **prototypical stressors** (typically chemicals that serve as models during the development of AOP) **provides support and confidence in AOPs.** Therefore, this valuable source of information for chemical risk assessment is stored in the AOP-Wiki.

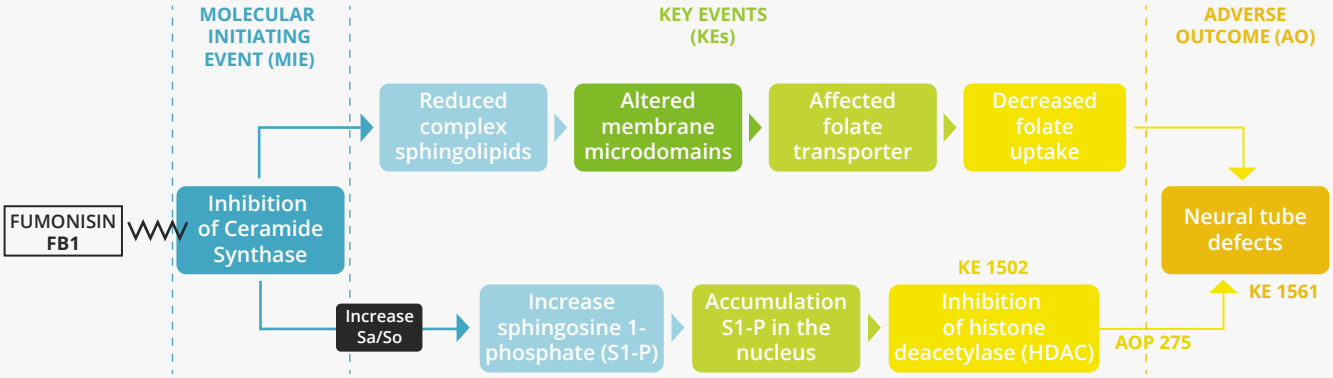
HOW DID HBM4EU CONTRIBUTE TO AOPs?

Drafted AOPs

AOPs are currently being developed within the work package on exposure and health effects.

Using toxicological data from the prototypical stressor **fumonisin FB1**, a neurotoxic mycotoxin, one AOP is being developed that **links inhibition of ceramide synthase (the MIE) to neural tube defects (the AO,** KE1561 in the AOP-wiki) through perturbations to sphingolipid metabolism and subsequent effects on folate signaling (first AOP) and/or inhibition of histone deacetylase (KE1502 in the AOPwiki) (second AOP).

Figure 2: Putative AOPs for CerS inhibition-mediated NTD. Dashed arrows indicate that more KEs are presumably involved. In particular, the AOP 275 in the AOP-Wiki proposes a mechanism for linking HDAC inhibition with neural tube defects. The Increase of sphinganine/sphingosine (Sa/So) ratio is proposed as a biomarker of effect and is expected to result from the MIE but is not a KE per se.



Another AOP has been developed for nephrotoxicity (the AO) induced by exposure to toxic metals, involving the **binding to thiol containing molecules (the MIE)**, and then possibly oxidative stress and extensive apoptosis in the proximal tubule in the kidney.

Two AOPs have also been entered in the AOP-Wiki: the AOP 318 describes a chain of events leading from glucocorticoid receptor activation to hepatic steatosis – which means there is extra fat in the liver- , and the AOP 372 depicts the KEs leading from androgen receptor antagonism to testicular cancer. Both AOPs are still at the initial phase of development.

Finally, an AOP has been drafted as a result of an HBM4EU-funded interdisciplinary project involving several HBM4EU partners. The **AOP was delineated using advanced bioinformatic tools to integrate the results of multi-omics** performed within the project. It describes a mechanism that **links a decrease in glutamate levels to neurodevelopmental defects.**

The AOP-HelpFinder webserver

A new computational tool, named AOP-helpFinder, has been developed and tested within HBM4EU to assist the end-users

in linking stressors and key events from AOPs. AOP-helpFinder is based on artificial intelligence, text mining, and graph theory. It automatically screens abstracts from the published scientific literature **to identify and extract links between data on chemicals (stressors) and biological information that may be involved in AOP (MIE, KE, and AO)**. Suitability of the AOP-helpFinder was demonstrated in studies with bisphenol S (BPS), bisphenol F (BPF), and other prioritized HBM4EU pesticides to examine their potential linkages with the biological perturbations compiled in the AOP-wiki database. Under the HBM4EU and OBERON projects, the tool has been optimized using a set of endocrine disrupting chemicals and metabolic outcomes and is now freely available as an easy-to-use web server.

Communication and dissemination

Because the risk assessment is typically focused on chemicals, in HBM4EU, we contributed to **improving the quality of information on prototypical stressors (HBM4EU priority compounds) in AOP-Wiki and the communication about the practical use of AOPs**. For instance, the additional deliverable AD13.7 provides a practical guide for AOP end-users, such as risk assessors, with case studies and concrete examples.

WHICH AOPs FOR WHICH APPLICATION?

During the development process, AOPs go from the initial stages, where the chain of key events is described briefly and qualitatively, to the advanced stages, where deeper and possibly quantitative information is added and reviewed.

For certain uses, such as risk assessment, more advanced AOPs are required. However, AOPs at earlier stages of development are in larger amounts and may also be helpful for other applications, such as the prioritization of chemicals.

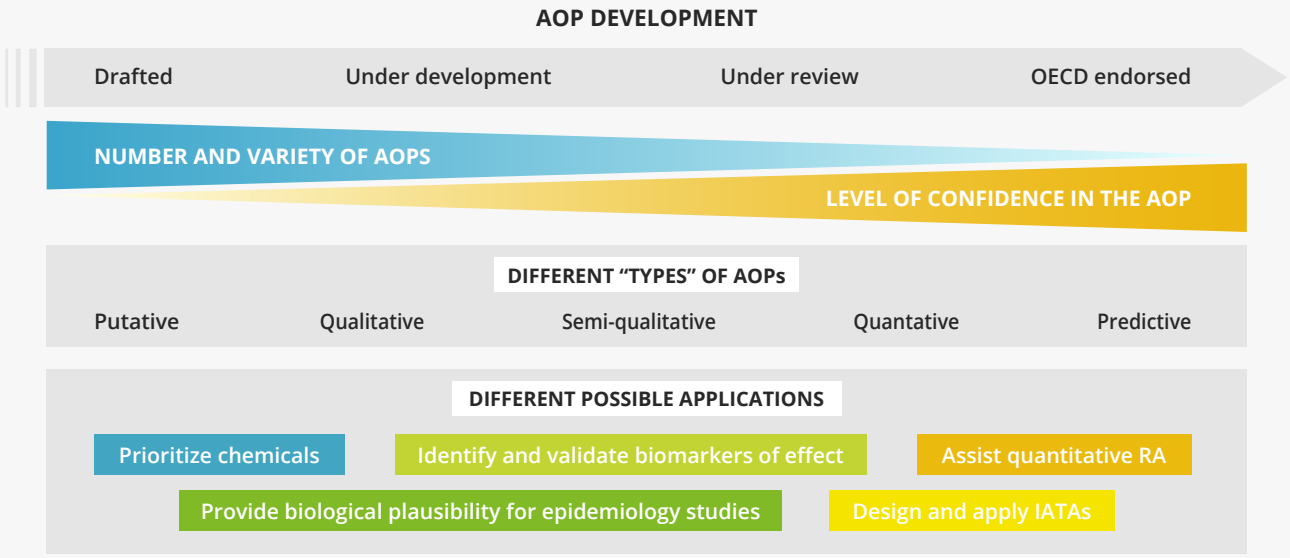


Figure 3: Different types of AOPs with diverse levels of development may be appropriate, depending on the application.

HOW TO USE AOP INFORMATION FOR CHEMICAL RISK ASSESSMENT?

Essential questions to be asked by risk assessors

For what? Because they are living documents, AOPs can have several levels of confidence, complexity, reviewing, and quantitative information. Whether an AOP can be applied for regulatory purposes depends on the regulatory approach and the policy context (problem formulation).

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What level of confidence in the AOP/ what level of evidence? Even AOPs with lower confidence and levels of complexity may be helpful for prioritization/categorization and designing research to address gaps in risk assessment (especially for data-poor chemicals, as shown within HBM4EU for novel flame retardants (FRs), bisphenol S (BPS), and bisphenol F (BPF)). For quantitative risk assessment, AOPs or AOP networks with a high degree of confidence and some level of quantitative data are required.

What is the biological applicability domain? Another consideration for determining whether an AOP is fit-for-purpose for regulatory application is its biological domain of applicability. The AOP-Wiki includes taxonomic, sex, and life-stage applicability, which have dedicated sections on the page of each AOP (for example, it can be found here).

Connecting toxicological data with AOPs

The AOP-Wiki does not aim at providing comprehensive lists of stressors reported to trigger all key events (or alter key event relationships). It is recommended that end-users (such as riks assessors) directly link toxicological data from different sources with the information captured in the AOP-Wiki.

Specific tools have been developed that assist the end-users in linking data with the AOP-Wiki content, such as the already mentioned AOP-helpFinder elaborated within HBM4EU, or the Abstract sifter available in the "comptox dashboard" of the U.S. Environmental Protection Agency (EPA).

Detailed information on assays for MIE/KE can be found in the "How It is Measured or Detected" section on the page of each specific key event in AOP-wiki.

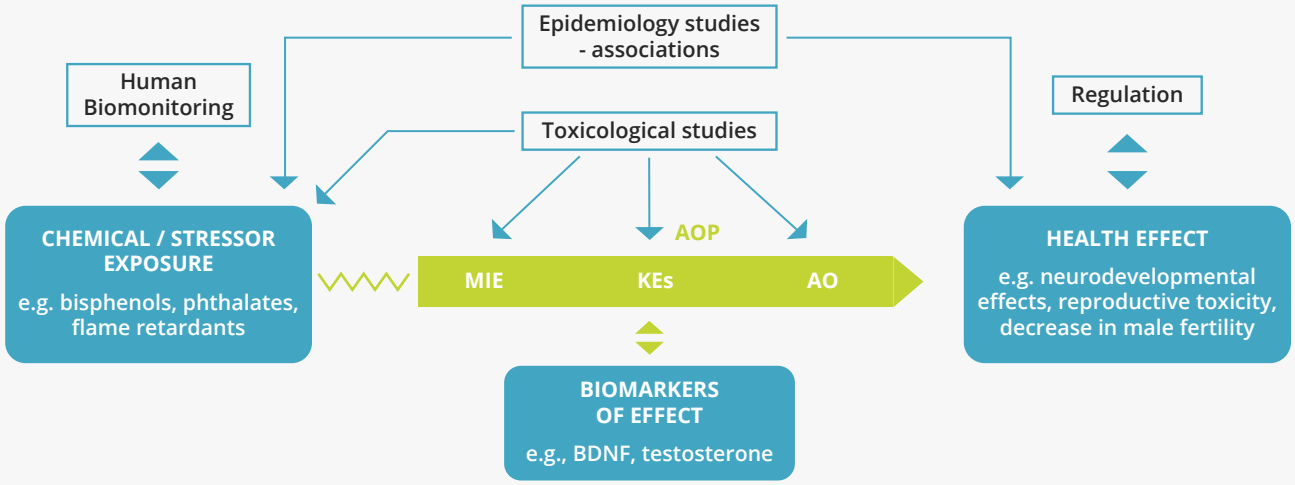

Figure 4: Connections between the AOP knowledge and the different aspects of chemical risk management

HOW DID HBM4EU USE AOPs?

For prioritization

Within HBM4EU, the AOP-Wiki was very helpful to **optimize and potentiate the scarce data available for substitute chemicals**, such as novel FRs, used as substitutes for the restricted brominated FRs, and BPS and BPF isomers, used as substitutes for BPA. Establishing connections between toxicological data and KEs from the AOP-Wiki **highlighted health outcomes of highest concern** that helped to prioritise future research directions: hepatotoxicity, neurotoxicity, and reproduction toxicity for novel FRs, obesity, and metabolic disruptions for BPS, and thyroid cancer for BPF isomers.

For epidemiology studies

There are several examples within HBM4EU where mechanistic information from **AOPs provided empirical evidence to support associations** between chemical exposure and health outcomes reported in **human epidemiology studies**. These include, for example, associations between phthalate exposure and reproductive toxicity, BPA exposure and neurodevelopmental outcomes, nFRs exposure and decrease in male fertility, metal exposure and impairment of motor and cognitive development, and fumonisin exposure and neural tube defects.

For biomarkers of effect (BoEs)

A systematic literature search focusing on reproductive toxicity and BoEs associated with phthalate exposure was combined with information from the AOP-Wiki. This provided **mechanistic evidence** for most of the BoEs previously implemented in human observational studies and identified molecular initiating and key events for developing **new BoEs**. With a similar approach, AOP information provided **support for several BoEs associated with BPA** exposure. It highlighted a promising novel BoE for BPA-induced neurodevelopmental toxicity: alteration of brain derived neurotrophic factor (BDNF), as a readout of a central KE within an AOP network.

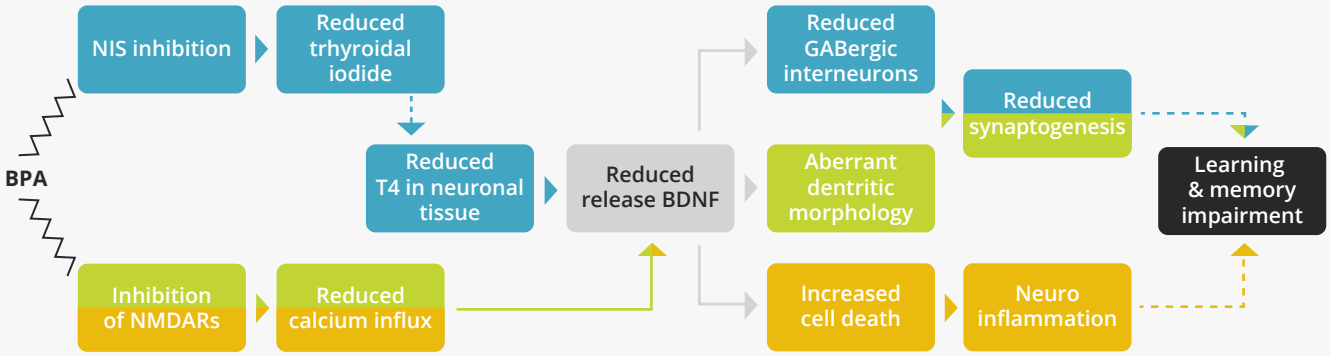

Figure 5: Simplified scheme of AOPs from the AOP-Wiki (**AOP 52 in blue**, **AOP 13 in green**, and **AOP 12 in orange**) that may underly BPA-induced neurodevelopmental defects. Several studies report the effects of BPA exposure on the MIEs and KEs of the AOP network. Dashed arrows indicate that some KEs are not shown in the scheme. Adapted from Mustieles et al, 2020.

For mixture risk assessment

The AOP developed within HBM4EU for toxic metal-induced nephrotoxicity was used (work in progress) for the derivation of equivalence factors to **refine the risk assessment of combinations of toxic metals**.

CONCLUSIONS

As the number of new chemicals is continually growing, regulators need new ways to increase the efficiency of hazard and risk assessment of chemicals. AOPs help establish the relevance of data from alternative approaches to animal testing, thus facilitating hazard identification and characterization. This makes AOPs (and the AOP-Wiki) an essential tool that should be integrated with other relevant aspects in regulatory decision-making, such as the context of the decision, the human biomonitoring (HBM) data, or information on toxicokinetic properties.

It is also important to note that the current information captured in the AOP-Wiki does not cover all toxicity pathways which may be relevant, and some AOPs may be of insufficient quality from a regulatory perspective. Further expanding AOP knowledge by proposing new AOPs, adding data to existing AOPs, or implementing tools and case studies will therefore be essential to broaden the use of AOPs in the risk assessment. In this context, several initiatives within HBM4EU have contributed to increased AOP knowledge and usability for risk assessment and demonstrated how the AOP framework can be instrumental within several aspects of toxicological and HBM research.

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