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TREND EDITORIAL

Editorial trend: adverse outcome pathway (AOP) and computational strategy — towards new perspectives in ecotoxicology

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Abstract

The adverse outcome pathway (AOP) has been conceptualized in 2010 as an analytical construct to describe a sequential chain of causal links between key events, from a molecular initiating event leading to an adverse outcome (AO), considering several levels of biological organization. An AOP aims to identify and organize available knowledge about toxic effects of chemicals and drugs, either in ecotoxicology or toxicology, and it can be helpful in both basic and applied research and serve as a decision-making tool in support of regulatory risk assessment. The AOP concept has evolved since its introduction, and recent research in toxicology, based on integrative systems biology and artificial intelligence, gave it a new dimension. This innovative *in silico* strategy can help to decipher mechanisms of action and AOP and offers new perspectives in AOP development. However, to date, this strategy has not yet been applied to ecotoxicology. In this context, the main objective of this short article is to discuss the relevance and feasibility of transferring this strategy to ecotoxicology. One of the challenges to be discussed is the level of organisation that is relevant to address for the AO (population/community). This strategy also offers many advantages that could be fruitful in ecotoxicology and overcome the lack of time, such as the rapid identification of data available at a time *t*, or the identification of "data gaps". Finally, this article proposes a step forward with suggested priority topics in ecotoxicology that could benefit from this strategy.

Keywords Adverse outcome pathway · Ecotoxicology · Toxicology · in silico methodologies · Computational biology · AOP Help-Finder

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Abbreviations

AO	Adverse Outcome
AOP	Adverse Outcome Pathway
AOP-KB	AOP knowledgebase
AOPN	Adverse Outcome Pathway Network
BPR	Biocide Product Regulation
FASSET	Framework for ASSessment of Environmental impacT
ECHA	European CHemicals Agency
IATA	Integrated Approaches to Testing and Assessment
KE	Key Event
KER	Key Event Relationship
MoA	Modes of Action
MIE	Molecular Initiating Event
NAMs	New Approach Methodologies
OECD	Organization for Economic Co-operation and Development
qAOP	quantitative AOP
PARC	Partnership for the Assessment of Risks from Chemicals
PBPK-TD	Physiologically Based Kinetic and Dynamic
QSAR	Quantitative Structure Activity Relationship
REACH	Registration, Evaluation, Authorisation and Restriction of CHemicals
WoE	Weight of Evidence

Context and objectives

The adverse outcome pathway (AOP) has been conceptualized in ecotoxicology in 2010 by Ankley and Villeneuve from the US EPA (Ankley et al. 2010) as an analytical construct to describe a sequential chain of causal links between key events (KE), from a molecular initiating event (MIE) leading to an adverse effect, considering several levels of biological organization. It was initially proposed for uses in ecotoxicology. The use of this conceptual framework was then extended to the field of toxicology in which it has proven its relevance as well as its efficiency to help *in fine* deciphering and underlying mechanisms at each level of biological organization. Recent research in toxicology, based on integrative system biology and artificial intelligence, gave it a new dimension. To initiate discussion and a more effective knowledge transfer from toxicology to ecotoxicology, the evertéa Foundation (https://fondationevertea.org/; ex. Rovaltain Foundation), a French non-profit organization in the field of health and environment, and more precisely environmental toxicology and ecotoxicology, organized a workshop on AOP and new *in silico* perspectives in ecotoxicology that brought together researchers from several French universities and institutes, namely, CNRS, IFREMER, INERIS, INRAE, INSERM and IRSN. This paper proposes a synthesis of fruitful ideas and future directions for research identified by this expert group with widely diverse skills.

Contributions of adverse outcome pathways (AOP)

The succession of biological processes used to build an AOP starts with a specific KE called MIE, e.g. inhibition of enzymatic activity or DNA fragmentation, which leads to a series of biological KE linked by KE relationships (KER). Then adverse outcomes (AO) at the apical level are identified, e.g. impaired reproduction, abnormal individual growth or declined population size.

Based on published papers involving AOP approaches, some general characteristics appear: (1) an AOP is not specific to a chemical. It starts with the MIE, meaning that an AOP is "stressor-agnostic"; (2) an AOP is modular, made up of KE linked together by KER; (3) several AOP that share at least one common KE can constitute an adverse outcome pathways network (AOPN); (4) a single AOP can be considered as a unique assessment entity; and (5) AOP are scalable and can evolve over time. Figure 1, inspired from the conceptualization by Villeneuve et al. (2014), provides a schematic representation of an AOP.

An AOP aims to identify and organize available knowledge about toxic effects of chemicals and drugs, either in ecotoxicology or toxicology. It can be helpful in both fundamental and applied research, as well as to serve as a decision-making tool in support of regulatory risk assessment. Since the concept has been defined, the Organization for Economic Co-operation and Development (OECD) supports the development of AOP leading to scientific publications and producing guidance documents. If suspected to be triggered by a given prototypical stressor, e.g. pesticides, pharmaceuticals or other environmental stressors, the MIE is a crucial trigger that will be under in-depth scrutiny for its potential to lead to AO through a particular sequence of events (Allen et al. 2014). However, the conceptual AOP framework remains stressor-agnostic, meaning that the prototypical stressors themselves are not explicitly included within the AOP general scheme. As a regulatory helping tool, AOP makes it possible to circumvent certain limitations inherent to experiments performed with chemical substances. In particular, the number of molecules released within the environment due to human activities is so huge (only 500 over 100,000 chemicals on the market benefit of a well-characterized toxicity (ECHA 2017)) that several adverse effects, e.g. pathogenicity, metabolic diseases or disrupted neurodevelopment, but also numerous levels of organization and endpoints which could be affected by the contaminants, are poorly addressed. Moreover, the reduction of animal testing required by the OECD is a challenging requirement to fulfil, including the need for extrapolation of effects from one

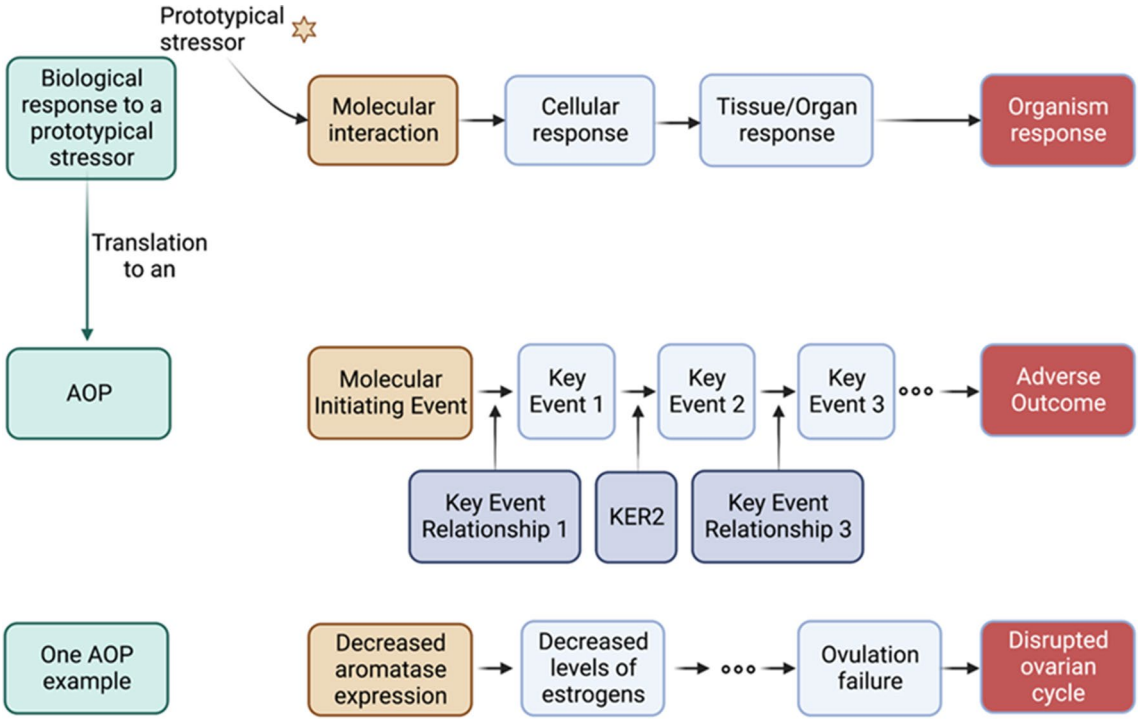

Fig. 1 Schematic representation of an adverse outcome pathway (AOP) with a molecular initiating event (MIE) and several key events (KE) linked together with key event relationships (KER) leading to an adverse outcome (AO). The final line is an example based on the inhibition of aromatase activity: the MIE (decreased aromatase expression) leads to a sequence of KE which ultimately leads to an AO (disrupted ovarian cycle)

species to another. AOP today forms one of the preferred tools to be used in integrated approaches to testing and assessment (IATA), thus constituting a "weight of evidence" (WoE) support in the perspective of mechanism-based risk assessment (Piva et al. 2011). The WoE is an approach that consists of using a combination of information from several independent sources to give sufficient evidence to fulfil an information requirement. In our case, WoE can help to determine the robustness and relevance of constitutive elements of an AOP, notably KER (Becker et al. 2015). Indeed, AOP can influence regulatory decisions (OECD 2018) when a chemical is identified as an effective prototypical stressor with a given AOP. As a proof of this potential support, the OECD launched the AOP knowledgebase (AOP-KB; https://aopkb.oecd.org/) in 2014, which includes the AOP-Wiki database (https://aopwiki.org), a repository of all AOP (under development or reviewed for endorsement). Similarly, AOP are dynamic documents that depend on new scientific data and methods, allowing them to be updated through collaborative efforts to consolidate available ecotoxicological/toxicological knowledge relevant for a given regulatory challenge.

Besides their regulatory potentialities, AOP could tackle another great environmental challenge. Indeed, the development of AOP through the use of artificial intelligence (text mining in PubMed) can identify unanticipated links between AO and certain KE (Jornod et al. 2022; Jaylet et al. 2023, see below the focus on an *in silico* strategy).

Focus on *in silico* strategy for the development of AOP

The research team INSERM T3S recently developed an innovative *in silico* strategy named AOP-helpFinder (https://aop-helpfinder.u-paris-sciences.fr/) combining both text mining and computational biology to build, respectively, pre-AOP in a first step and validate them as AOP in a second step (Carvaillo et al. 2019; Rugard et al. 2020; Jornod et al. 2020). This strategy offers new perspectives in AOP development and endorsement, notably for AOPN. It is based on a text mining approach of the scientific literature in toxicology allowing the identification of potential causal links between prototypical stressors and AO, thus characterizing KE and then KER. This process helps the development of AOP (from MIE to AO, using initially prototypical stressors) which can be deposited on AOP-Wiki (https://aopwiki.org/). This strategy has been applied to bisphenols (Carvaillo et al. 2019; Rugard et al. 2020) pesticides (Jornod et al. 2020) and more recently ionizing radiation (Jaylet et al. 2022). Bisphenols S and F

have been, respectively, linked to obesity and breast and thyroid malignancies, while ionizing radiation was associated with microcephaly in humans. To date, this *in silico* strategy has not been applied to ecotoxicology yet.

The *in silico* strategy described here allows to automatically extract existing information from a large set of data, e.g. co-occurrence of groups of terms (biological events and stressors), in PubMed abstracts, benefiting of high-performance methods based on artificial intelligence (such as machine learning). Combined with manual curation, the related information can be integrated to build AOP frameworks that require further validation through experimental studies. Therefore, this strategy helps to formulate new hypotheses for experimental science.

Description of the in silico strategy

Numerous experimental data exist but can be dispersed across various data sources, mainly the scientific literature, e.g. PubMed, and databases, e.g. ToxCast® or CompTox®. One possibility to gather information from the published scientific literature is to manually query specific repositories such as the PubMed database and to combine results with complementary data from other databases such as ToxCast®. A study combining information on toxic effects from manual literature searches with information from, for example, the ToxCast® and AOP-Wiki databases, to establish connections between environmental chemicals and toxic effects can be time consuming (Bajard et al. 2019), especially if many biological events (MIE, KE, AO) are under investigations, and if the number of prototypical stressors is high. To overcome this problem, the use of artificial intelligence (text mining in PubMed) can facilitate the development of AOP and help identify unanticipated links between AO and certain KE.

Step 1: identification and integration of existing experimental and field data from scientific literature using text mining. Building of a pre-AOP

In this context, a data mining tool, named AOP-helpFinder (https://aop-helpfinder.u-paris-sciences.fr/), was recently developed (Carvaillo et al. 2019; Jornod et al. 2022; Jornod et al. 2022 ; Jaylet et al. 2023). It performs a comprehensive analysis of the literature using artificial intelligence and machine learning techniques. It combines two approaches, "text mining" and "graph theory" (to find the shorter pathway between two points in a network), to explore the content of abstracts of scientific articles, leading to identification of possible links (and their strength though a confidence score) between prototypical stressors and events, or event-event linkage, which constitute an AOP, i.e. MIE, KE, KER and AO. Concomitantly, co-occurred prototypical stressors and biological events are automatically identified and extracted from published abstracts in the PubMed database. This tool provided fast and effective results for environmental substances such as bisphenol S, bisphenol F and a set of pesticides, proving its efficiency to propose new AOP or to optimize existing ones (Fig. 2). Following this step of extraction of biological key events and the identification of causal links, a manual inspection of the scientific articles is performed to check the reliability of data.

Step 2: integration of data from other sources using system biology. Building of an AOP

Following the "text mining" step, the second one consists in integrating data from multiple and diverse existing databases, from *in vitro* to *in vivo*, e.g. CompTox (https://comptox.epa.gov/), ToxCast (https://www.epa.gov/chemical-research/exploring-toxcast-data-downloadable-data) or AOP Wiki (https://aopwiki.org/). This step allows to identify new KE and KER to integrate to the pre-AOP built from STEP 1 (Fig. 2). Integration of *in vitro* and *in vivo* data is also used to predict the relationship between prototypical stressors and MIE in an AOP, i.e. which prototypical stressors may elicit a molecular target that can initiate an AOP. Besides, the development of an AOP is chemically based and relies on two different methods: predictions based on chemical structural properties, e.g. quantitative structure activity relationships (QSAR) for MIE identifications and predictions based on experimental data/observed effects, e.g. multi-omics responses, for KE and KER identifications.

Integrative system biology-based predictions

Prediction based on structural properties

Many tools and methods based on chemical structural properties are used to determine potential chemical functions and can be useful to identify elements of an AOP, notably the MIE:

One of the first tools developed is databases gathering QSAR for both toxicology and ecotoxicology. Such databases provide information about both acute and chronic toxicity (Table 1).

Systems biology also includes the use of the "read-across" method that consists in using relevant information from structurally "analogous" substances to predict the properties, e.g. acute toxicity or solubility, of "target" substances. The read-across method is commonly used for filling data gaps in the regulation, e.g. Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) or Biocide Products Regulation (BPR), by allowing to reduce

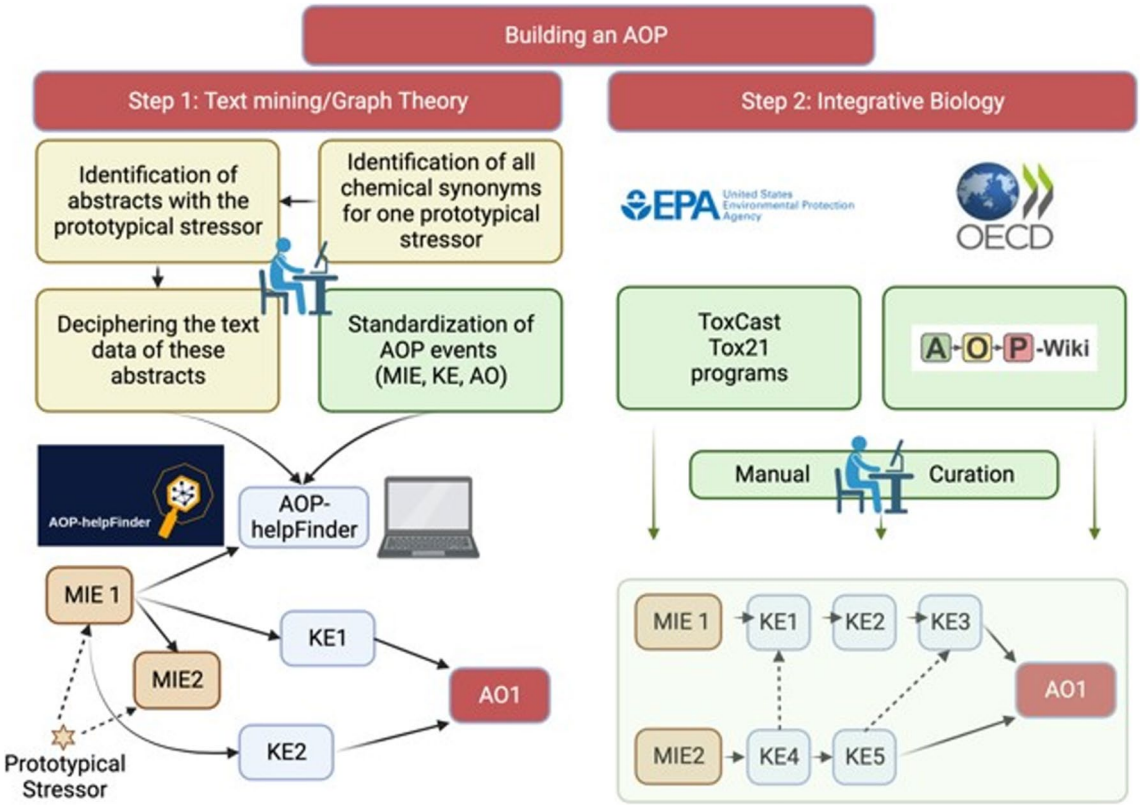

Fig. 2 Overview of the global experimental approach used for the *in silico* strategy

Table 1 Example of chosen databases

Name	Description	Website
OECD QSAR Toolbox	A tool for category formation and read-across that uses "profilers" to enable grouping	https://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm
VEGA	A tool that provides access to a series of QSAR models for predicting toxicity for a range of species (including acute toxicity *for Daphnia*) integrating interspecies and intra-species seasonal variations	www.vegahub.eu/
Danish QSAR database	The Danish QSAR Database includes more than 200 QSAR models covering a wide range of hazardous properties relevant for human health and the environment such as acute toxicity to rat, mouse, fish, *Daphnia* and algae, as well as many physical–chemical and environmental fate properties, skin irritation, sensitization, mutagenicity, cancer and reproductive toxicity including potential for endocrine disruption	https://qsar.food.dtu.dk/
ADMET	Predictor: a tool under private licence that estimates many properties related to ADME (absorption, distribution, metabolism, elimination) and toxicity (including acute toxicity for fish and *Daphnia*)	https://www.simulations-plus.com/software/admetpredictor/

experimental testing. It is also interesting to mention docking methodology, i.e. prediction of substrate/receptor interaction for identification of MIE based on a basic search algorithm tool and an energy scoring function for generating and evaluating ligand fixation.

Functional approaches based on observed effect/experimental data like multi-omics

Complementary to structural approaches, tools based on biological process description, like multi-omics, are also

available to reinforce the construction of the predictive AOP, notably regarding the identification of new KE and KER:

Beside databases presented before and predictive tools, longitudinal analysis, i.e. from foetal life to adult, tools based on multi-omics, on functional validation, are powerful to investigate and build the AOP chain elements, in particular in exposome-wide association studies. The integration of data coming from different omics approaches is a key for a system biology approach to gain a full understanding of how stressors impact a biological system (Brockmeier et al. 2017; Azimzadeh et al. 2022). One of the main reasons for that is the high sensitivity and precocity of multi-omics data through developmental stages, allowing the inference of the AOP chain elements. Multi-omics integration, e.g. proteomic, transcriptomic, metabolomic or epigenomic approaches, enables (i) to find interactions between molecular compounds involved in multiple cell functions and signalling pathways, (ii) to rely on WoE (Azimzadeh et al. 2022) and (iii) to identify new biomarkers allowing the robust identification of novel KE, KER and even AO. For example, transcriptomic and proteomic approaches were used to enhance the AOP framework related to oestrogen interference effect induced by triphenyl phosphate, highlighting the link between a MIE (activation of G protein-coupled oestrogen receptor) and several AO such as abnormal immune function and cancer through the activation of several pathways, including PI3 kinase-Akt signalling pathway or MAPK signalling pathways (Guan et al. 2022). In another example, transcriptome and epigenome modifications (DNA methylation) collectively pointing at developmental defects in neurogenesis and muscle development (Murat El Houdigui et al. 2020) supporting the effects of ionizing radiation on zebrafish behaviour demonstrated during embryonic development (Murat El Houdigui et al. 2019). In *Caenorhabditis elegans*, omics, histological and apical data enabled to show that radiation-induced reproductive toxicity was associated with multi-scale effects, i.e. germ line, defence systems, mitochondrial function and lipid metabolism (Guédon et al. 2021 ; Dubois et al. 2019 ; Dufourcq-Sekatcheff et al. 2021), probably coming from multiple origins (decrease in the number of spermatocytes and egg-laying rate) and starting from embryogenesis (Dufourcq-Sekatcheff et al. 2021). These data were used to initiate the construction of an AOP "decrease in progeny", consolidated by the addition of the results acquired by European partners, recorded in the dedicated OECD database (AOP wiki#396) with the prospects of becoming quantitative (dose–response modelling, functional validation). Importantly, systems toxicology approaches are also emerging in other relevant sentinel species, such as the freshwater crustacean *Gammarus fossarum* and contributing in an improved knowledge of the molecular physiology and mode of action of contaminants in aquatic ecosystems (Degli Esposti et al. 2019, Koenig et al. 2021).

For more reliability, the use of dose–response modelling is highly recommended, e.g. using DRomics (Larras et al. 2018) or BMDexpress (Yang et al. 2007; Phillips et al. 2019) to find the most sensitive biological pathways triggered at the lowest dose. In this approach, the use of relationship between dose and multi-omics response enables to reinforce the biological pathways identified (Larras et al. 2020 ; Song et al. 2023).

Together, by combining text mining and system biology, the *in silico* strategy offers many advantages with regards to classical building of AOP. First, the abundance and diversity of the scientific literature used for data mining and specific databases, e.g. *in silico*, *in vitro* or *in vivo*, reinforce the power of the methods and its robustness. Furthermore, *in silico* methods allow a rapid exploration and integration of these diverse sources, which can become very time-consuming otherwise. The abundance of sources and confronted data also favour the efficient identification of data gaps necessary to focus on.

Quantitative AOP: a complementary method

A complementary method would consist in building quantitative AOP (qAOP), which is defined by Conolly et al. (2017) as "an AOP for which the quantitative understanding of the relationship that underlie transitions from one KE to the next, and critical factors that modulate those relationships, are sufficiently well defined to allow quantitative prediction of the probability or severity of the AO for a given level of perturbation of the MIE". For example, a qAOP would be constructed combining physiologically based kinetic and dynamic (PBPK-TD) models (Tebby et al. 2019 ; Mit et al. 2021) with an AOP; the information provided by the PBPK-TD models would permit to anticipate the distribution/concentration of a particular contaminant, which could be compared to the affinity of a molecular target identified in one MIE; subsequently, this will permit to anticipate when the contaminant triggers the MIE (and the cascade of KE in the AOP) and leads in the time frame to the AO. Nevertheless, there is a crucial need for raw data to create qAOP. Some recent studies successfully applied qAOP, such as Conolly et al. (2017), to describe the linkage between inhibition of cytochrome P450 19A aromatase (the MIE) and population-level decreases in the fathead minnow, or Perkins et al. (2019) to illustrate the need for toxicokinetic models to provide linkages between exposure and qAOP, to extrapolate from *in vitro* to *in vivo* and to extrapolate across species. Beside these limits, with the integration of the temporal and spatial dimension, qAOP models are increasingly considered as predictive computational tools,

Table 2 Toxicology vs ecotoxicology (non-exhaustive table)

Similarities with toxicology
The use of interspecies data is possible, even if the development of an AOP is more relevant with a single species
The same frame, methodology and terminology should be used and enables data/knowledge structuration
The identification of data gaps is possible in ecotoxicology as well in toxicology with this strategy
Common intracellular AOP components, e.g. KE or KER, can be found and share in both toxicology and ecotoxicology, such as for oxidative stress and DNA damage
Need of harmonization on the ontology used for KE and AO to develop AOPN
Specificities of ecotoxicology
The scarcity of reliable and exploitable data at environmental scale (population and ecosystem): ecological (predation, density-dependent process, …) and environmental factors (e.g. temperature for aquatic organisms) must be considered at the different biological levels, as they can mask any chemical effects
Integrate ecotoxicological data from (sentinel) organisms living in their natural habitat

gaining more and more interest due to their potential regulatory applications for chemical risk assessment (Spinu et al. 2020).

Using *in silico* strategy described in ecotoxicology

The similarities with toxicology and the challenging questions in ecotoxicology

One of the main goals of this opinion paper is to look at the feasibility of knowledge transfer from toxicology to ecotoxicology using the presented *in silico* strategy. In other words, can we apply this strategy to ecotoxicology and if yes, how?

When compared to toxicology, the use of the *in silico* strategy to build AOP in ecotoxicology raises its own specific challenges. For example, in toxicology, most studies focus on one specific organism, while ecotoxicological studies mostly focused on ecosystems and several integrated populations. Therefore, the challenges appear to be more inherent to the nature of the questions related to the field itself than to the limitations of the *in silico* approach. Both similarities and potential caveats are summarized in Table 2, which is not exhaustive.

The most burning questions we identified are:

- What level of organization shall the AO target? Indeed, ecotoxicology is a field that address individual, population, community and ecosystem levels, while toxicology focuses mainly on individual scale. Thus, the regulatory questions can be asked at these different organizational levels. How to precisely define the AO and at which level is an additional difficulty add a layer of complexity compared to toxicology. Furthermore, to assess population effects, species-specific ethology and ecology traits must also be considered, e.g. reproductive and escape behaviour or life traits of species.
- Which database should we use for the construction of an AOP in ecotoxicology? Databases, e.g. *in vitro* databases, in ecotoxicology, are less developed than in toxicology, and numerous databases in ecotoxicology are intern to laboratory and institute.
- How to access to these databases? The less abundant databases regarding system biology in comparison to toxicology are mostly due to the important number of model species studied in ecotoxicology. Regarding specific databases in ecotoxicology that could be used to implement data in a pre-AOP, i.e. Step 2 of the *in silico* strategy, FREDERICA (2008) is a demonstrative example. FREDERICA is an online database collecting available literature on the biological effects of chronic exposure to ionizing radiation. Fish, mammals and terrestrial plants are the wildlife groups most widely reported, representing all together 70.5% of the FREDERICA data for chronic irradiation. Furthermore, the Framework for Assessment of Environmental Impact (FASSET) project, funded by the European Commission (Contract No. FIGE-CT-2000-00102), produces other databases than the one for radiation effects. Furthermore, recent technologies have drastically enriched the current ecotoxicological data pool available:
  - The assessment of genotoxicity by DNA seq (mutation rates, copy number variation)X;
  - The study of genetic variation and epigenetic modification using nanopore sequencing coupling;
  - Nanopore sequencing for environmental DNA to study biodiversity.

To meet the challenge of this translation between the two disciplines, it is still useful to illustrate its feasibility through concrete examples, which is what we discussed during evertéa Foundation's workshop.

Suggested priority topic, i.e. AO to focus on, in ecotoxicology that could benefit from data mining

The workshop organized by the evertéa Foundation highlighted several topics of interest, starting from the AO, for which this *in silico* strategy could be applied:

- Reproduction decline
- Metabolic (energetic …) disorders
- Immune system disorders
- Neurotoxicity and behaviour disorders
- Growth disorders

Most of these endpoints refer to outcomes that are highly prioritized by international consortiums and ongoing Horizon 2020 (H2020) European projects related, for example, to endocrine or neuroendocrine disruptions (ED) (https://eurion-cluster.eu; Chauhan et al. 2022). Recently, the Horizon Europe has funded a huge initiative, involving more than 200 partners, called "the European Partnership for the Assessment of Risks from Chemicals (PARC)". The PARC project aims to develop next-generation chemical risk assessment to protect human health and the environment. One of the PARC priorities is to work on data gaps and new approach methodologies (NAMs) development on bisphenol A (BPA) alternatives and associated mixtures. They are also highly relevant at the ecotoxicological level, e.g. population dynamics (Tollefsen et al. 2022), allowing strengthening of the links between KE in both disciplines (Jaylet et al. 2022). Moreover, the huge number of abstracts available on PubMed related to these ED-linked pathologies made them highly relevant for the *in silico* strategy. Indeed, this strategy was applied in toxicology to describe and decipher networks of AOP linked to bisphenols (Carvaillo et al. 2019; Rugard et al. 2020) and pesticides (Jornod et al. 2020) in particular. In theory, there are no methodological obstacles to apply this *in silico* strategy to ecotoxicology.

Due to a lack of time or funding, the research teams in ecotoxicology dealing with MoA and/or the study of the effects of stressors on main physiological functions (AO) do not systematically include the construction of AOP in their projects. The integration of this strategy into research projects, with the support of the INSERM T3S unit and the evertéa Foundation, could overcome this lack of time and provide new elements to existing AOP or enable the development of new AOP. At the end, the AOP can be useful for regulatory decision-making.

Complementary to mechanistic studies, the transposition of this approach to ecotoxicology would open new perspectives, such as (1) the construction of AOP literature and networks of AOP, (2) the rapid identification of ecotoxicological data available on the effects of a xenobiotic or the alteration of a biological pathway, (3) the identification of "data gaps", (4) the identification of new biomarkers, (5) the determination of complex and/or controversial mechanisms and (6) the identification of common KE that will serve as a health indicator at both the toxicological and ecotoxicological level.

General discussion/perspectives

The last COVID-19 crisis has shed the light on how anthropic activities affecting ecosystems have dramatic consequences on the health of human populations. The term "global health" or "planetary health" is now used, highlighting the need to consider the intricate interaction between ecological and human health. For example, one of the underlying risks identified by the public is the decrease in pollinator populations, which is already an issue regarding pollinator and associated community diversity and could also lead to a dramatic decrease in plant diversity and quantity. This decrease in pollinators has multiple causes, including the use of pesticides that are lethal for them. People are also becoming aware of soil and water pollution (potentially drinking water) with a reflection on the loss of biodiversity.

Predictive toxicology, which was initially designed to anticipate the risks associated with chemical molecules (many of which being insufficiently characterized in terms of toxicity), is a powerful tool, and the development of AOP represents a promising tool for this field involving several methodologies, e.g. experimental or *in silico* methods.

The transposition of such methods to the ecotoxicology field faces several issues: although those methods will face the same problem of research resources as in health research, specific challenges inherent to the ecosystem's study research field will be encountered. For instance, one of the questions raised in this article is the level of organization targeted for AO to be relevant in ecotoxicology. We can also anticipate that prioritization of AO will be a key issue in the future due to the multiplicity of ecosystem constituents (microbiota, plants, animals, fungi).

The temporality will also be a major factor to consider. In this context, the *in silico* strategy opens up the prospect of combining two essential approaches to environmental chemical stress over the next 10 years: chemical risk and long-term observation. Prioritization of AO in a close future to target major concerns in terms of environmental risk will be a challenge as we could miss important long-term consequences that do not appear to be of concern in the

short-term. AO may also need to consider the whole exposome, which is the sum of the exposures of one organism over her/his lifetime. For an ecosystem, this temporal dimension will be difficult to define, for example, what period of exposure should be addressed?

Moreover, one will not have to consider only chemicals prototypical stressors, but physical ones as well. If the loss of biodiversity is classically presented in the media being associated with the chemical exposome (e.g. pesticide exposure), physical prototypical stressors such as temperature (global warming), ionizing and non-ionizing radiation will also play an essential role in the prediction of environmental risks notwithstanding the global rising temperature. It is also possible that "social" prototypical stressors will play a key role as well, e.g. migration of populations to escape a danger. While being non-specific to the AOP, such factors will probably need to be considered for the development in a near future.

Thus, it appears that together with complementary methods, the AOP *in silico* methods could be a great tool to address and answer broad questions in the field of ecotoxicology, considering the challenges raised above: the available databases, the scale of the study and the temporality. As for the global health approach, the development of AOP *in silico* is ambitious. However, the AOP appears today as an applicable tool in predictive toxicology for environmental chemical risk assessment or ecotoxicology for ecosystem health assessment.

Declarations

Conflict of interest The authors declare no competing interests.

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