Mediterranean Journal of Medicine & Medical Sciences
ISSN: 3107-2720
Mediterr J Med Med Sci
doi: 10.5281/zenodo.00000000
https://mmj.org.ly

REVIEW article

Adverse outcome pathways: A mechanistic framework for predictive toxicology and risk assessment

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Received: 04-04-2026, **Accepted:** 05-05-2026, **Published online:** 00-00-2026

HOW TO CITE THIS

Babu et al. Adverse outcome pathways: A mechanistic framework for predictive toxicology and risk assessment. Mediterr J Med Med Sci. 2026; 2(2): 67-77. [Article number: 34]. https://doi.org/10.5281/zenodo.00000000

Keywords: Drug-induced toxicity, quantitative AOP, regulatory toxicology, systems biology

Abstract: The greatest health care and pharmaceutical problems remain adverse drug reactions and chemical toxicities. Because of the extensive use of animal testing, conventional toxicology approaches often do not have the mechanistic insights required to relate molecular perturbations to their adverse biological outcomes. To address this limitation, Adverse Outcome Pathway architecture is a sequence of causally related events beginning with a Molecular Initiating Event, followed by Key Events, and concluding with an Adverse outcome. This paper describes the construction and applications of AOPs in chemical risk assessment, predictive toxicology, and regulatory decision-making. More recent developments, including quantitative AOPs and AOP networks, have enhanced the ability to model biological complexity and dose-response interactions. AOPs provide a solid mechanistic basis of toxicity prediction and allow the transition of modern toxicology to non-animal, human-relevant testing systems, although barriers such as a lack of quantitative data and regulatory acceptability stand.

Introduction

Adverse Drug Reactions (ADRs)are a major clinical and toxicological concern. The WHO defines an ADR as a response of a drug that is not intended and noxious at dosages that are typically administered to humans to prevent, diagnose, or treat an illness [1]. By connecting molecular disruptions to clinical outcomes, AOPs enhance predictive toxicology and lessen the need for animal testing. ADRs are primarily divided into Type A and Type B, but broadly differentiated by various parameters and are illustrated in **Table 1** [2, 3].

Table 1: Classification of ADRs

Type	Name	Description	Examples
A	Augmented	Dose-dependent	-Anticoagulants leading to bleeding
B	Bizarre	Not dose-dependent	-Hypersensitivity to anticonvulsants
C	Chronic (Chemical)	Due to long-term drug use	-Hepatotoxicity led by paracetamol
D	Delayed	Could arise due to accumulation	-Teratogenicity due to phenytoin during pregnancy
E	End-of-use	Withdrawal effects	-Rebound hypertension after stopping clonidine
F	Failure	Unexpected failure of therapy	Antimicrobial resistance, oral contraceptive failure
G	Genetic	Genetic variations in enzymes, transporters, etc.	Stevens-Johnson syndrome due to the HLA-B1502 allele with carbamazepine
H	Hypersensitivity	Immune-mediated responses	Serum sickness
I	Immunological	Hypersensitivity reactions	Anaphylaxis, delayed rash.

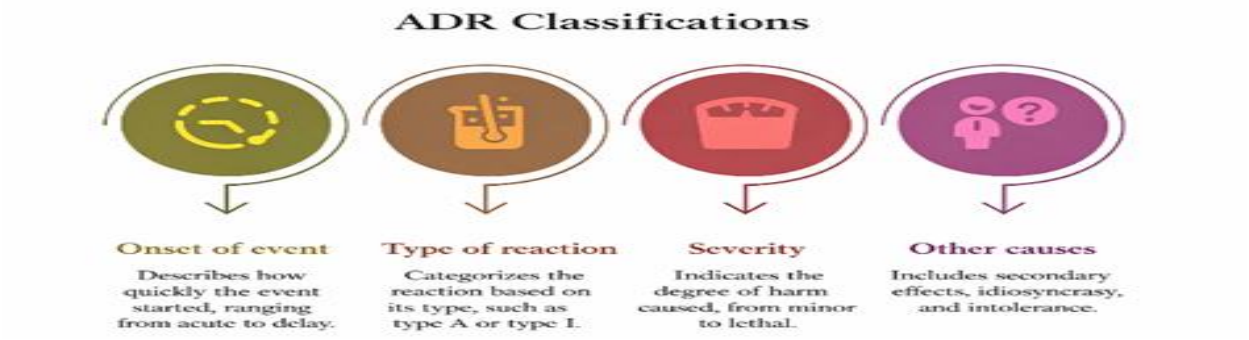

Figure 1: Classification of ADRs

ADRs may also be categorized by severity [4, 5], time of onset [5, 6], and predisposing factors [7, 8] are represented below

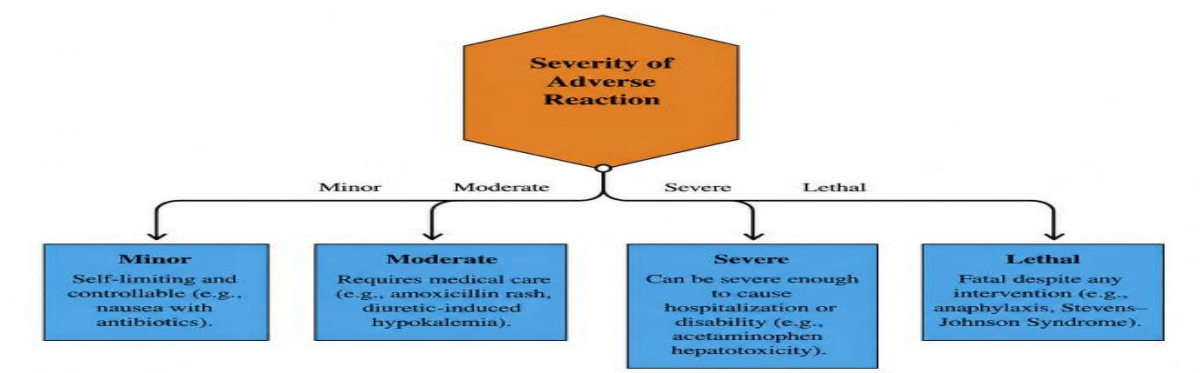

Figure 2: ADRs classified based on severity

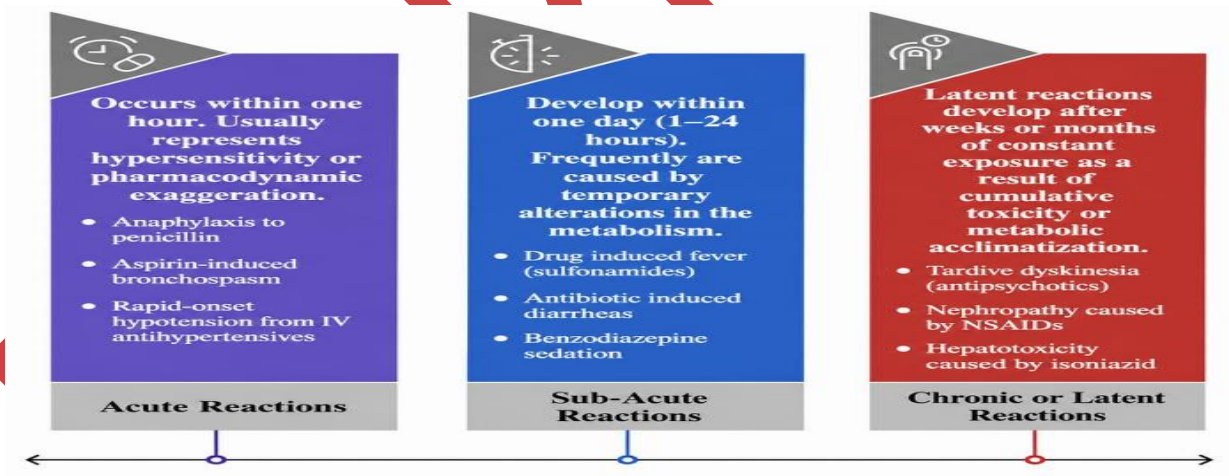

Figure 3: ADRs classified based on time

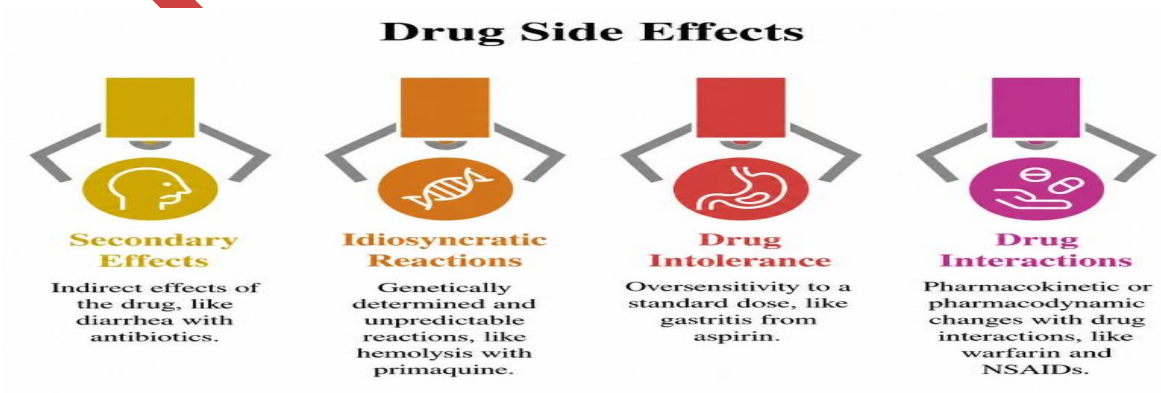

Figure 4: Miscellaneous ADRs

Toxic reactions and mechanistic perceptions: Toxicity is defined as the ability of a substance to be harmful, which is usually dose-dependent and depends on the duration of exposure and biological variability [10]. These reactions can be experienced in three broad situations, namely supratherapeutic doses, therapeutic doses in metabolism of altered conditions, and cumulative exposure [11, 12].

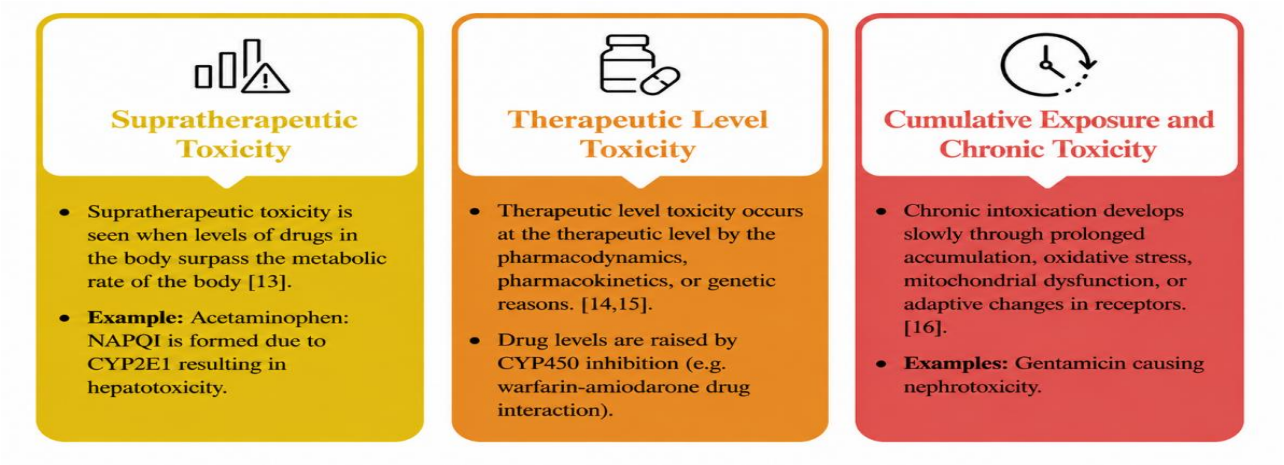

Figure 5: Toxic reactions and types

Drug-induced toxicity: mechanisms: The interactions between the drug, the biological system, and the host factors like the genetics and metabolism are complex and lead to toxicity [2, 12].

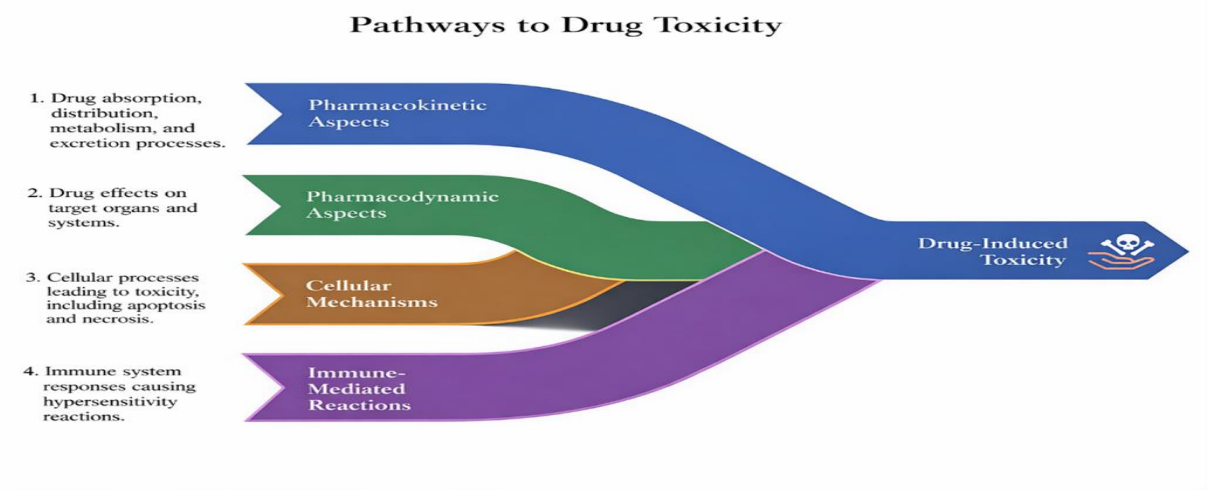

Figure 6: Mechanisms of drug-induced toxicity

Pharmacokinetic-based toxicity: The alterations in ADME increase tissue concentrations and enhance toxic potential as presented in **Figure 3**. Examples: Drug interactions in the inhibition of CYP450, e.g., Ketoconazole, lead to a high concentration of warfarin, and hence, it will cause bleeding complications [14].

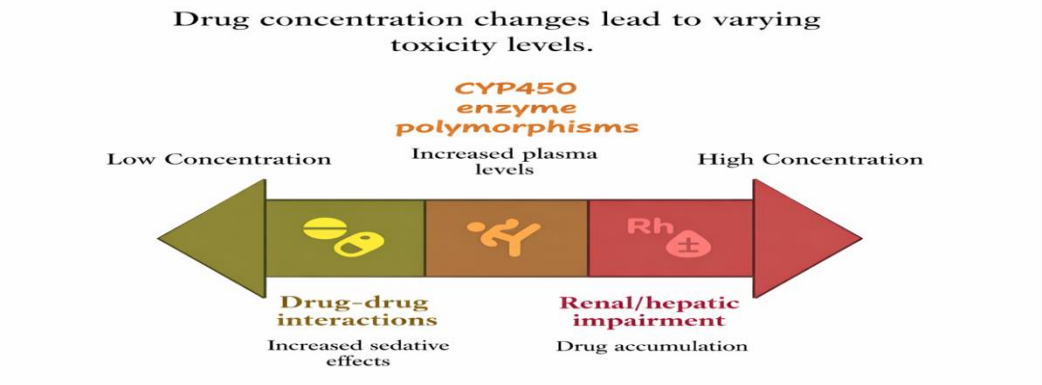

Figure 7: Toxicity based on pharmacokinetic parameters

Toxicity based on pharmacodynamics: due to overstimulation of the receptor at the intended receptor sites or even in the transmission of the signal pathway, provided the drug exists at its therapeutic range level, as illustrated in **Figure 4** [18]. Examples: Bradycardia caused by the action of 0-blockers, respiratory depression caused by opioids [2, 18].

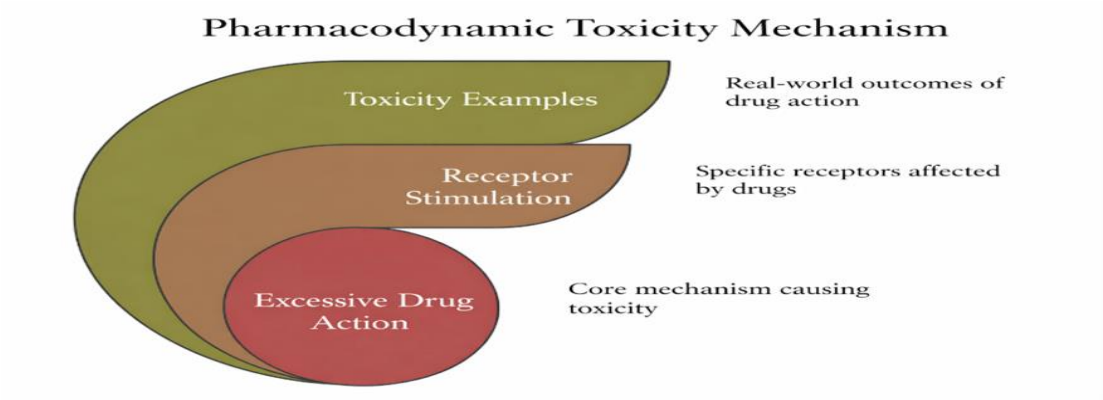

Figure 8: Toxicity based on pharmacodynamic parameters

Cellular mechanisms of toxicity: Apoptosis: Doxorubicin cardiotoxicity by mitochondrial means. Necrosis: Membrane rupture and inflammation (e.g., acetaminophen hepatocellular necrosis). Mitochondrial dysfunction: oxidative phosphorylation inhibition (e.g., valproic acid toxicity) [19].

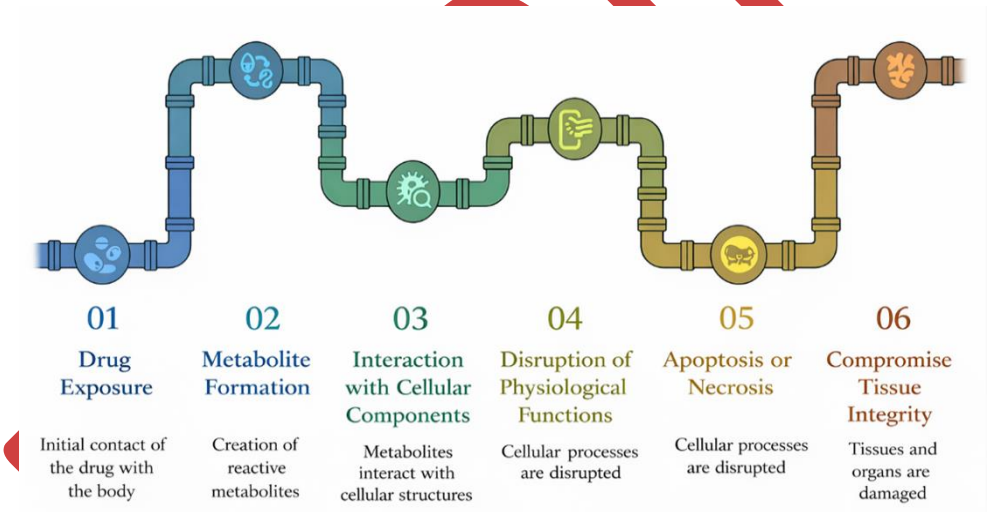

Figure 9: Illustration of the drug-induced cellular damage process

Immunotoxicity and drug-induced hypersensitivity reactions: Drugs may act as haptens, triggering immune-mediated hypersensitivity reactions [20, 21].

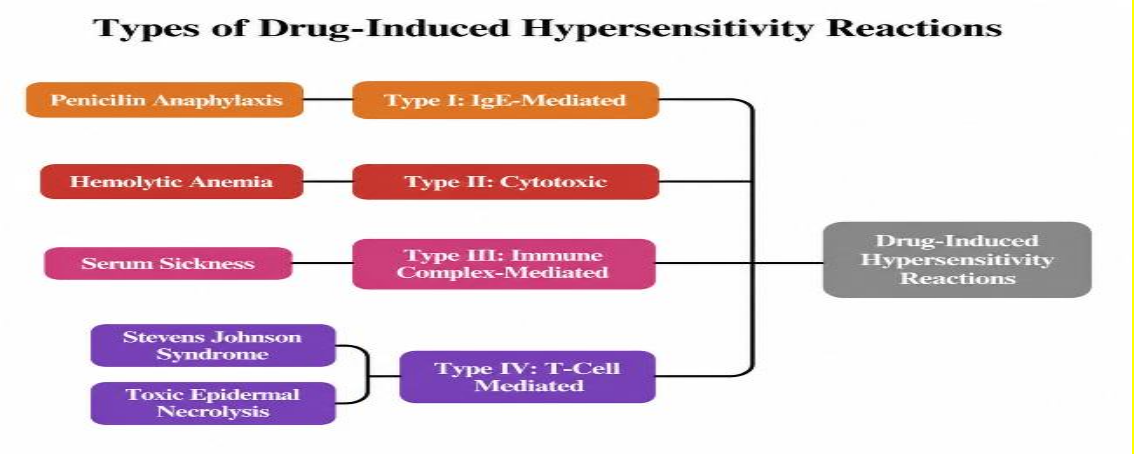

Figure 10: Drug-induced hypersensitivity reactions

Antidotes and mechanisms of action: An antidote overcomes the influence of a poison or a toxic substance. Numerous processes of antidotes have been illustrated in **Figure 7** [23,24].

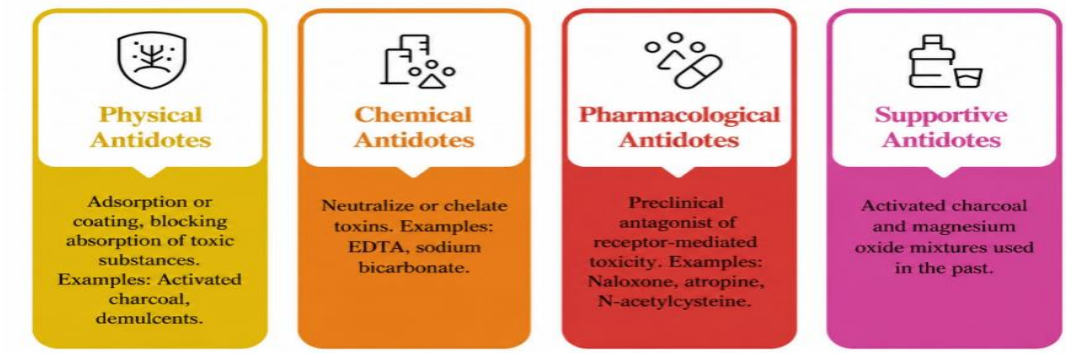

Figure 11: Mechanisms of different types of antidotes

Adverse Outcome Pathways (AOPs): An AOP is an analytical construct that characterizes a causally related chain of events of varying degrees of biological organization that results in an ecotoxicological effect. The key factor in a toxicological knowledge framework under construction to aid in the chemical risk assessment using mechanistic reasoning is AOPs, and the sequential flow of AOP, using multiple components, is illustrated in Figure 8 [25, 26].

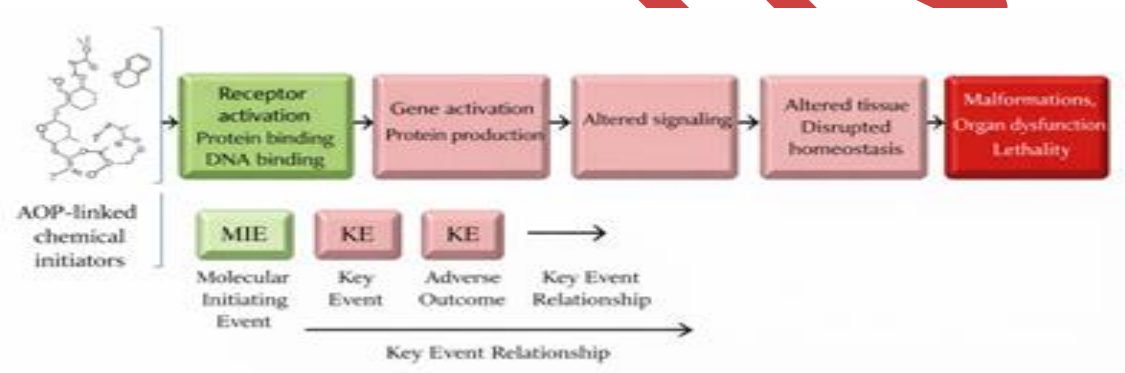

Figure 12: Flow of AOP

AOPs provide a mechanistic framework linking molecular interactions to adverse biological outcomes, thereby improving toxicity understanding and risk assessment [22, 25, 26]. *Mechanistic insight:* Replaces black-box models by identifying causal biological pathways of toxicity [22, 26]. *Alternative to animal testing:* Supports New Approach Methodologies (NAMs) for non-animal toxicology [22, 25]. *Risk assessment:* Enhances regulatory decision-making through Integrated Approaches to Testing and Assessment (IATA) [25, 26]. *Hazard identification:* Enables early detection of key events for prioritization and regulation of chemicals [22, 25]. *Evolution and global efforts:* The OECD's AOP Development Programme (2012-2025) has standardized AOP construction. The AOP-Wiki and eAOP Portal are world databases on collaborative development and provide the transparency and reproducibility of mechanistic toxicology [22, 25]. *Quantitative AOPs **(qAOPs)**:* They can even be used to predict dose-response relationships in combination with exposure data. Recent advances have pushed AOPs to a quantitative domain, including dose response information and probabilistic modelling as a predictor of the probability of certain outcomes [22, 26]. The OECD AOP Knowledge Base, including AOP-Wiki and eAOP Portal, supports data sharing, pathway development [25, 26], eAOP Portal [25, 27], and AOP Wiki standardization (**Figure** 13-15) [22, 25, 27].

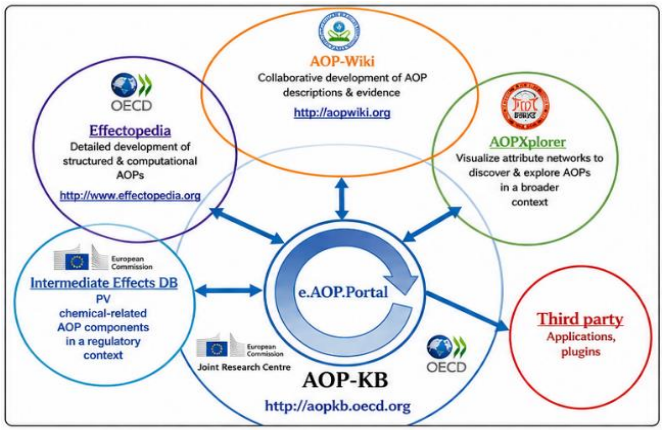

Figure 13: AOP-Knowledge base

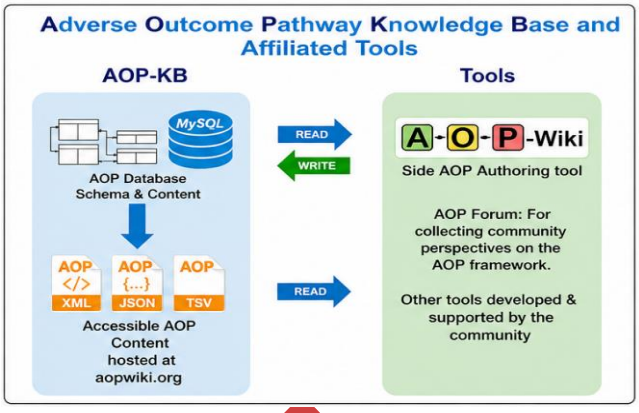

Figure 14: AOP-KB in association with certain tools

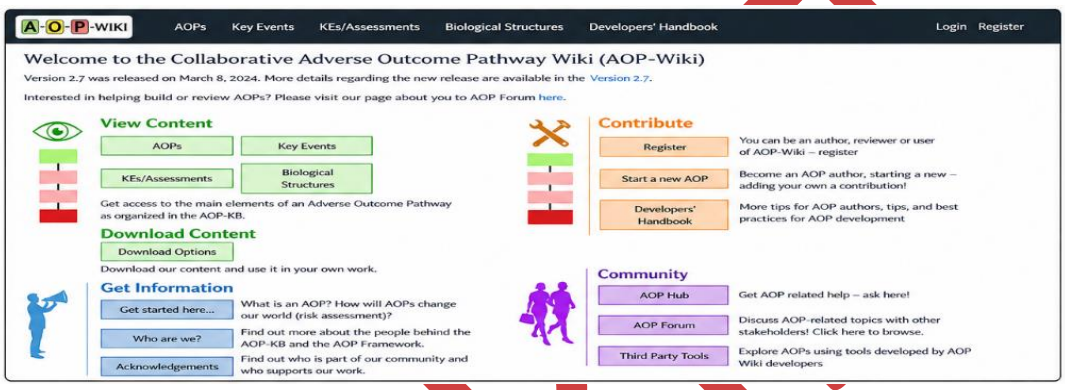

Figure 15: AOP WIKI portal

Regulatory authorities: *International cooperation and collaboration with OECD (Organisation for Economic Cooperation and Development):* The OECD and Effectopedia are to facilitate mechanistic toxicology and chemical risk assessment to promote interoperability, enabling the sharing and interoperability of data among researchers (**Figure 12**) [25, 27].

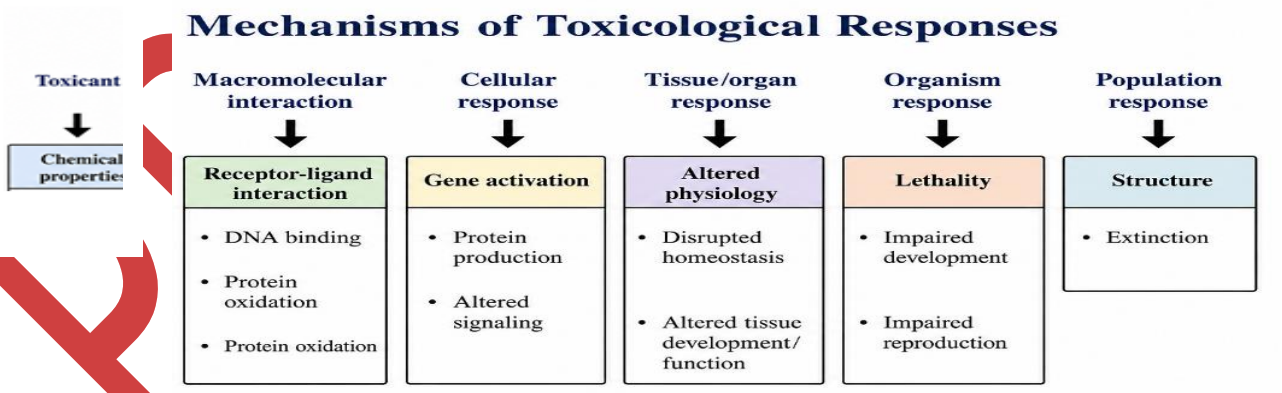

Figure 16: Actions of components in the AOP framework

U.S. Environmental Protection Agency (EPA): The EPA has been developing more and more the use of Adverse Outcome Pathway (AOP), which seeks to enhance environmental and chemical risk assessment using mechanistic data and high-throughput screening tools (**Figure 14**) [28]. These consequences can be human health effects or effects on human survival, growth, and reproduction of wildlife species [26, 28].

European Food Safety Authority (EFSA) and European Chemicals Agency (ECHA): The European agencies (EFSA) and (ECHA)use AOP-based frameworks, endocrine disruption and carcinogenicity [29] are some of the key mechanistic events resulting in toxic effects and are collected as a database at the EFSA website, where supportive tools like ML accompany the systematic review and critical examination of *in vivo* and *in vitro* studies [29].

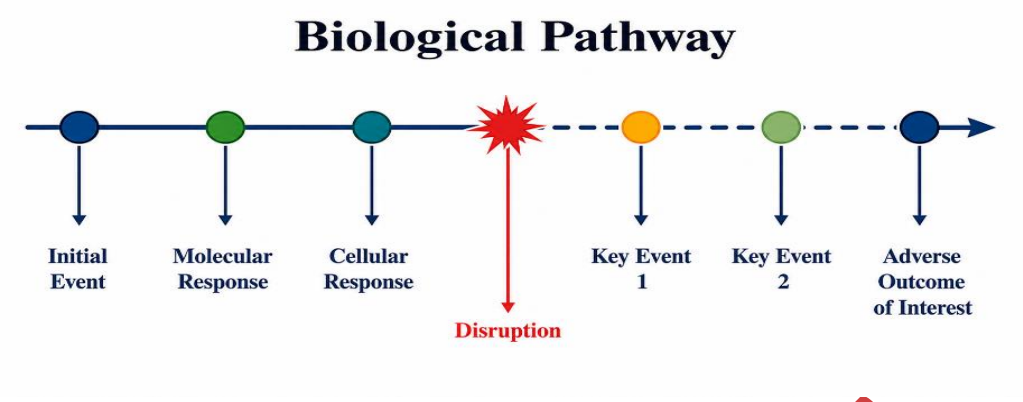

Figure 17: Representation of AOP as a biological pathway by EPA

Figure 18: EFSA website

Case studies are represented for illustrating an AOP Application: Aromatase Inhibition that results in Reproductive Dysfunction in Fish: This Adverse Outcome Pathways was officially accepted as AOP #23 by the OECD [5, 26] and is demonstrated in the **Figure.** Title of AOP: Female fish inhibition of Aromatase decoys fecundity. Chemical Stressor: Fadrozole, a non-steroidal aromatase inhibitor that is used in the treatment of breast cancer. AOP Sequence (**Figure 19**):

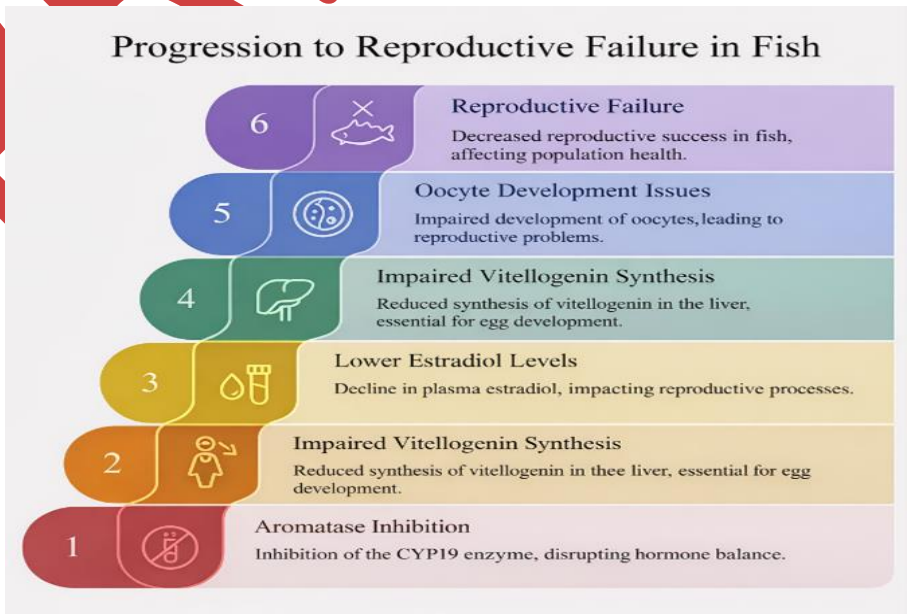

Figure 19: Flowchart of aromatase inhibition leading to reproductive dysfunction in fish

Significance: The regulatory bodies, like the USEPA and OECD, have extensively applied it in the screening of environmental chemicals and identification of hazards [22, 25, 31]. Covalent Protein Binding and Resulting Liver Injury (Drug-Induced Liver Injury -DILI): The acetaminophen overdose is a classic example used in mechanistic toxicology and development of AOP [14, 30]. AOP Title: Covalent Binding of Reactive Metabolite Hepatocellular Death Liver Failure Chemical Stressor: Acetaminophen (paracetamol) at overdose levels, AOP sequence.

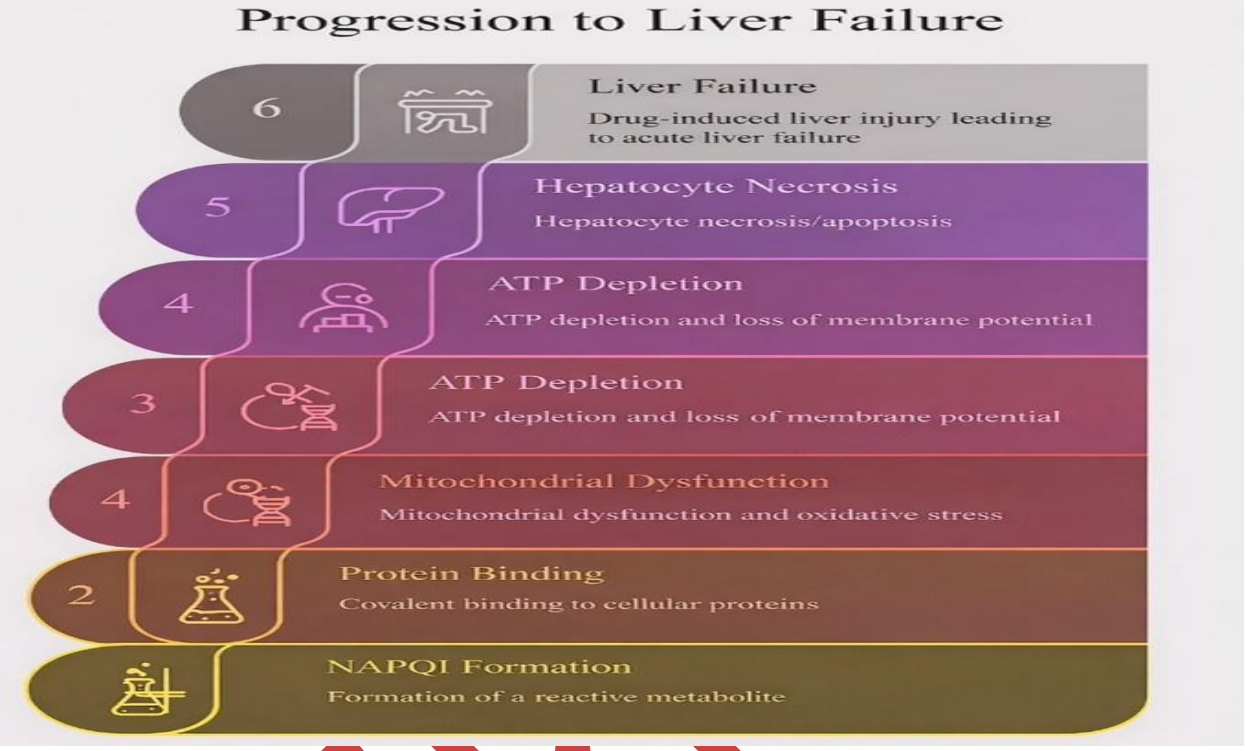

Figure 20: Flowchart of covalent protein binding leading to liver injury -DILI

Significance: AOP can be applied here as it allows the prediction of hepatotoxic potential at an early stage during the preclinical development of drugs [14, 30].

Interventions to Prevent ADRs and Toxic Outcomes: Various interventional strategies are usually adopted to minimize the frequency of ADRs and toxic consequences, as represented in **Figure 18**, as follows: Pharmacovigilance and ADR reporting systems, Dose optimization and therapeutic monitoring, Rational drug design and toxicological screening, and Electronic prescribing and decision-support tools

Evaluation of Key Event Relationships (KERs) in the AOP Framework: The KERs link molecular perturbations to adverse outcomes [22,26], which include: Biological plausibility: It indicates the logical and scientific plausibility of the connection between two important events, which are consistent to the current body of biological knowledge and on molecular biological, toxicological and physiological evidence to see that the downstream occurrence is a reasonable outcome of the upstream change. Essentially, it determines the degree of importance of a particular event to the occurrence of other outcomes. The essentiality is highly supported with experimental studies involving genetic knockouts, enzyme/pathway blockers that inhibit the subsequent key event. Empirical support is supported by empirical data, which shows a consistent, measurable, time-dependent relationship between events in studies, species, or dose ranges. Together, these three elements-plausibility, essentiality, and empirical evidence-determine how confident researchers and regulators can be in an AOP's validity, guiding its acceptance in predictive toxicology and risk assessment [25, 26].

Limitations and Doing Business with AOP-Based Toxicology: Although the Adverse Outcome Pathway (AOP) paradigm represents a promising move towards mechanistic toxicology and predictive modelling, there

are scientific, technical, and regulatory challenges to it and elimination of these challenges is key to the wider application of AOP-based methods in toxicological studies and regulatory decision-making [22, 25, 26]. Incomplete Biological information: Incomplete biological understanding limits linkage between molecular events and outcomes [22]. Example: Unknown downstream consequences of some disruptions of metabolic enzymes [22].

Lack of Quantitative Data (qAOPs): Quantitative AOPs (qAOPs), which link dose–response and time-course data, are still in early development and require extensive experimental and modelling efforts [25].

Single Chemical and Pathway Focus: Many AOPs characterize highly simplistic, linear pathways that are in relation to a single chemical stressor and a distinct endpoint. Thus, it needs to develop AOP networks that can capture biological complexity [26].

Validation and Regulatory Acceptance: Regulatory agencies may be reluctant to conduct risk evaluations based on untested or developing AOPs. So, Formal validation procedures, increased standardization, and additional real-life case studies are required [25, 26].

Problems of Data Sharing and Harmonization: The lack of centralized, FAIR (Findable, Accessible, Interoperable, Reusable) data sets is a disadvantage for AOP development [25].

Resource-Intensive Development: AOPs development and validation involve the use of interdisciplinary effort, skills in molecular biology, toxicology, computational modelling, and risk assessment. Formal endorsement, as conducted by international regulatory bodies, might be time-consuming and lead to delays in the use of AOP-based tools [26].

Future preferences and future research: The future directions will entail better scientific depth, technical usefulness, and regulatory integration [22, 25, 26].

AOP Networks (AOPNs) development: Replacement of linear AOPs with networked AOPs to increase their ability to model the complexity of biological systems [22].

Advancements in Quantitative AOPs (qAOPs): qAOPs will assist the regulators in establishing chemical safety thresholds with more precision. Introduction Objective: The objectives are to close the divide between mechanistic science and regulatory dose-setting [25].

Combination with Omics and Artificial Intelligence Technologies: The new technologies, which include genomics, proteomics, and metabolomics, will be of great benefit to AOP development as they can deliver high-throughput data that can point out new key events and biomarkers of toxicity. [22, 25]

The regulatory Acceptance and Standardisation: Ongoing standardisation attempts by OECD, US EPA, and EU officials, AOP documentation (via AOP-Wiki). Integrated Approaches to Testing and Assessment (IATA) Use. Prolonged use of AOPs in risk-based decision-making. Training, Outreach, and Interdisciplinary Collaboration: Future success will rely on learning and competence development in the field of toxicogenomics, systems biology, and computational modelling.

Global Collaboration and Open Science: *Cooperation in the World and Free Science:* International harmonization of AOPs with shared databases (AOP-KB, Effectopedia) and shared development of AOPs. *Initiative:* AOP-Wiki, community-based and collaborative tools of OECD [25].

Conclusion: The paradigm shift from conventional, observational toxicology to a mechanistic and predictive science is shown by the AOP concept. AOPs offer a clear and organized method of comprehending toxicity at the molecular, cellular, and organismal levels by outlining the sequential connections between Molecular Initiating Events (MIEs), Key Events (KEs), and Adverse Outcomes (AOs). In line with international ethical standards and the 3Rs-Replacement, Reduction, and Refinement, this systems-based approach not only

increases the biological relevance of safety testing but also dramatically lowers reliance on animal experiments. With the active participation of international regulatory bodies, including the U.S. EPA, ECHA, and OECD, AOPs are becoming more widely acknowledged as a fundamental component of next-generation chemical and pharmaceutical assessment frameworks. AOPs represent a shift toward mechanistic and predictive toxicology by linking molecular events to adverse outcomes. Integration with qAOPs, AI, and regulatory frameworks will enhance their application in drug safety and risk assessment." In the end, industry, academics, and regulators must work together to standardize procedures, improve data exchange, and advance mechanistic validation if AOP implementation is to be successful. AOPs have the potential to transform evidence-based toxicology through these collaborative developments, guaranteeing safer treatments, better environmental protection, and better public health outcomes in the future.

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Author contribution: PVB conceived and designed the study. CS collected data and contributed to drafting the manuscript. NSL revised it for intellectual context. All authors approved the final version and agreed to be accountable for its contents.
Conflict of interest: The authors declare the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
Ethical issues: The authors observed the ethical issues, including plagiarism, informed consent, data fabrication or falsification, and double publication or submission.
Data availability statement: All data supporting the findings of this study are included within the article and its referenced sources.
Generative AI disclosure: No generative AI was used in the preparation of this manuscript.