Towards an MIE Atlas

The roads not taken: avoiding adverse outcome pathways

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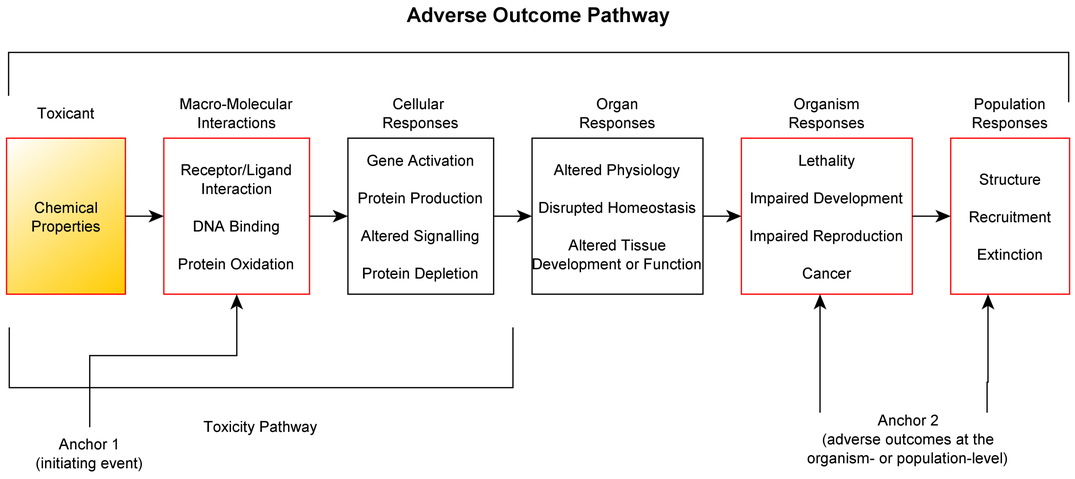
1. Introduction

Consumer and environmental safety decisions are based on exposure and hazard data interpreted using risk assessment approaches. The adverse outcome pathway (AOP) conceptual framework [1] has been presented as a logical sequence of events or processes within biological systems which can be used to understand adverse effects and refine the current risk assessment practice. In-depth understanding of the chemistry and biology behind many currently poorly understood processes will be required to make the approach work. The use of this knowledge in the development of a comprehensive AOP database will be critical research in toxicology. An AOP database will help to identify gaps in knowledge along the pathway that require attention, and target tests and research.



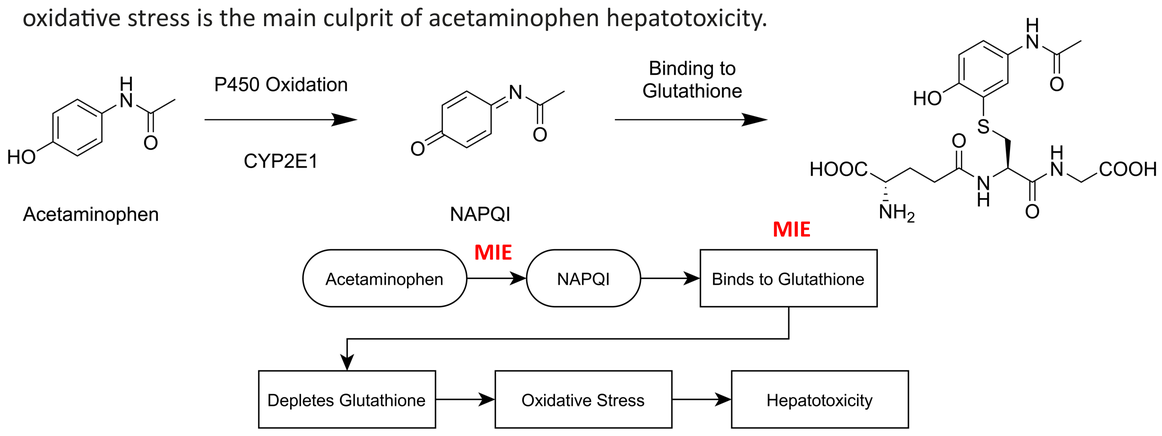
Ankley's conceptual diagram of an AOP, including the MIE. Image adapted from Ankley 2010 [1].

The molecular initiating event (MIE) can be thought of as a gateway to the AOP. In the purest sense an MIE is a chemical interaction leading to a downstream outcome pathway [2]. It is accepted that one MIE can lead to a number of AOPs, or a single AOP may be the result of a number of different MIEs. Some examples of MIEs would be a compound binding to a protein, or inhibiting an enzyme. Chemistry is key to understanding the MIE. What is it about these compounds that allow them to do this?

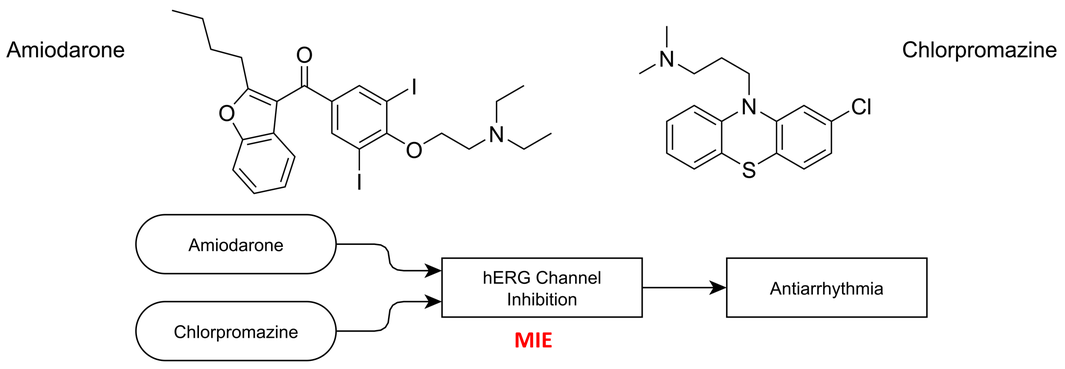
Using chemical knowledge of the structural features and reactivity patterns that govern these chemical interactions, a greater understanding of why chemicals lead to toxicological effects can be gained. Compounds have been investigated using toxicological databases and literature searches. Using chemical knowledge and what was found in the literature, a picture of each compound's toxicity has been built. These can be seen as an effort to compile the data on each compound, to give greater insight by being able to observe the bigger picture. MIEs have been identified for each compound based on the definition that the MIE is the first molecular interaction between a molecule and a biomolecule leading to an outcome.

2. The Construction of MIE/AOP Maps

Acetaminophen (Paracetamol) is a widely used, mild analgesic. Acetaminophen overdoses can cause potentially fatal liver failure, via an oxidised metabolite - *N*-acetyl-*p*-benzoquinone imine (NAPQI). Acetaminophen is converted to NAPQI by a P450 oxidation process in the liver, mediated by the enzyme CYP2E1 [3]. This activation is the MIE. NAPQI then binds to the reactive oxygen species (ROS) scavenger glutathione, depleting this vital protection against oxidative stress [3]. This processes may be thought of as the MIE for NAPQI. When the glutathione defence is depleted the excess NAPQI binds to cellular proteins, lipids and nucleic acids, activates calpains, and generates ROS [4,5]. The resulting oxidative stress is the main culprit of acetaminophen hepatotoxicity.

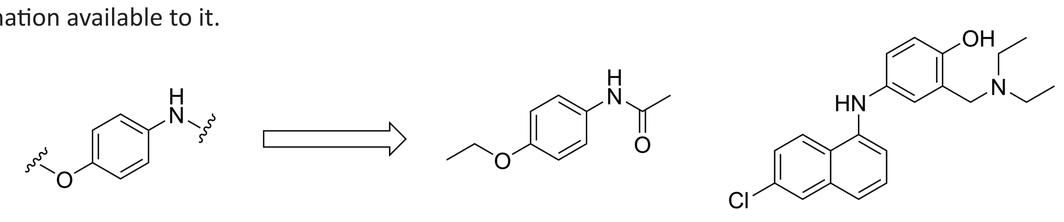


Amiodarone, a class III antiarrhythmic, and Chlorpromazine, a dopamine antagonist and antipsychotic, have both been found to inhibit human ether-a-go-go-related gene (hERG) channels [6,7]. These MIEs lead to antiarrhythmic properties. The key mechanistic structure for this activity in amiodarone is a basic nitrogen flexibly attached to an aromatic ring [7]. This substructure is repeated in chlorpromazine, and thus similar molecular interactions within the channel may be inferred.

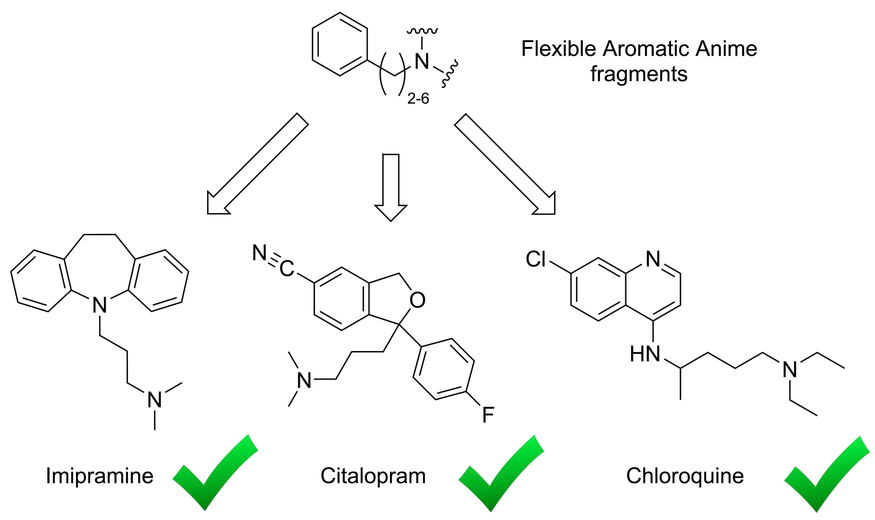


3. Structure Activity Relationships

Once fragments were identified they were matched to other example compounds using the Derek Nexus [8], a knowledge-based expert system that predicts toxicity based on reasoning with the information available to it.



The para-aminophenol fragment was based on the formation of NAPQI from acetaminophen leading to oxidative stress and hepatic damage. As well as acetaminophen, Derek identified phenacetin and amiodiaquine as examples containing the fragment. Phenacetin is known to cause hepatotoxicity in rats and mice, even when not metabolised to acetaminophen [9]. Its toxicity in humans however seems to be minimal. Amiodiaquine has several reported cases of hepatotoxicity in humans accompanying agranulocytosis, including some fatalities [10].



The flexible aromatic amine fragment was based on hERG inhibition of amiodarone and chlorpromazine. Derek did not associate the fragments with cardiac toxicity, but did provide several structures which were further investigated - imipramine, citalopram, and chloroquine.

Literature that details experiments in which hERG channels were found to be inhibited by these compounds was subsequently found for imipramine [11], citalopram [12] and chloroquine [13], strengthening claims for this structure-activity relationship.

4. The MIE/AOP Database

Our MIE/AOP database, compiled from the literature, illustrates the power of this approach as a simplification of toxicological space. The total number of MIEs currently in the database is 74, while the number of adverse outcomes (AOs) is 214. Numbers of MIEs and AOs by compound are shown below.

Compound	Acetaminophen	Amiodarone	Chlorpromazine	Kojic acid	Methotrexate
No. of MIEs	10	11	6	8	12
No. of AOs	22	47	34	26	38

The number of MIEs is less than the number of AOs for all compounds. Coupled with overlap between compounds at the MIE level, such as hERG inhibition being associated with more than one compound in the database, this shows our strategy represents a great simplification in toxicology.

5. Conclusions

- **Literature searches have been used to investigate the MIEs of drug compounds.**
- **Knowledge gained from the literature and chemical intuition were combined to elucidate structural fragments responsible.**
- **Fragments have been investigated to find further examples of matching structure and toxicological endpoint, they are expected to have the same MIE.**
- **This work provides support for an SAR based approach to toxicology built around MIEs, rather than apical endpoints.**
- **Work completed so far only represents a tiny part of toxicological and chemical space, further work will allow the MIE approach to reach its full potential as a valuable tool for toxicologists.**

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