The Adverse Outcome Pathway concept came from the field of ecotoxicology as a way of addressing uncertainty in risk assessment required by new legislation for an increasing number of chemicals and endpoints. This AOP concept builds on the Mechanism and Mode of action concepts and includes the "toxicity pathways" as described in the 2007 NRC report, Toxicity testing in the 21st century: a vision and a strategy.

The AOP framework is a flexible approach allowing the integration of all types of information at different levels of biological organization, from molecular to population level, to provide a rational, biologically based argument (or series of hypotheses) to predict the outcome of an initiating event.

Not all pathways need to be described in detail - rather the pathway is composed of a series of testable biological hypotheses all of which may be more-or-less described, the certainty of the relationships is related to the AOP's applicability to hazard and risk assessments.

The usefulness of the AOP concept in building a predictive toxicological framework manifests in several ways. Depending on the detail and certainty, AOPs can inform:
- chemical grouping or categories and structure activity relationships,
- aid in increasing certainty of interpretation of both existing and new information,
- structure integrated testing strategies
- identify key events for which non-animal tests can be developed,
- ultimately facilitating transparent, mechanism-based, predictive toxicological assessments with low uncertainty and high human relevance

The OECD AOP Development and Reporting Guidance includes three basic elements:
1) MIE (2) intermediate events ⇒ 3) Adverse Outcome Pathway

- MIE: molecular description of how the chemical interacts with the initial biomolecular target
- All specific and well-defined outcomes, associated with OECD TG endpoints
- IE: any one of a number of intermediate steps

Adverse Outcome Pathways

Using AOPs: how certain do you need to be? The AOPs currently under development differ in detail and complexity and are yet incomplete; nevertheless, they all have utility to improve the hazard and risk assessment process. The level of certainty and completion necessary depends on the intended use of the AOP (Figure 4). For example, to use an AOP for building Quantitative Structure-Activity Relationships (QSARs) of MIEs, there must be some solid evidence that the MIE is linked to the AO of interest, but the main focus of certainty would be the chemical and molecular characterization of the MIE itself. To use an AOP for hazard identification or prioritization of chemicals for further testing, strong evidence of the MIE-AO linkage is required, along with substantiation of one or more intermediate events.

Each AOP needs to be developed in sufficient detail, it will also be possible to use them to identify key events for which tests can be developed; the tests would necessarily address a number of critical steps, thereby ensuring that all possible outcomes are adequately covered. Finally, as quantitative information is added to relationships between intermediate events, early events in an AOP can be used directly for risk assessment, without the need to assess the later steps pathway. At this stage, chemical assessment will be streamlined and toxicology transformed from a purely empirical to a predictive science.

Skin sensitization

Skin sensitization involves several cell types and tissues and is a good demonstration of AOP development and use in constructing an integrated testing strategy. Sensitization occurs in two phases: the first, the induction phase, is a result of initial contact with an allergen and primes the system; the second, the elicitation phase, is in response to a subsequent exposure and results in an allergic response. As with the Local Lymph Node Assay, the sensitization AOP focuses on the induction phase.

The induction phase involves initial contact and penetration of the outer dermis of the skin by a potential sensitizer. Metabolism in the skin can either activate or deactivate the chemical (or have no effect), chemicals that are electrophilic after penetrating the skin are more potent sensitizers than non-electrophilic. The electrophile then interacts irreversibly with nucleophilic sites in proteins (e.g. cysteine and lysine residues) to form a hapten-protein complex in the epidermis - this is the sensitization MIE. In both dendritic cells (antigen-processing cells in the skin), the presence of a hapten-protein complex elicits the production of cytokines that in turn stimulate dendritic cells to migrate to regional lymph nodes and activate T cells there. In the human scale, hapten-protein fragments are presented in complex with MHC molecules by dendritic cells to immature T cells, causing the maturation of memory T cells and the acquisition of sensitivity – this is the key physiologic response of the initiation phase.

The MIE is well described and can be used for chemical categories as well as hazard prediction; subsequent intermediate events in keratinocytes, dendritic and T cells are also well described and are being used to design assays to be used in an integrated assessment strategy. Currently, potency assessment is used at the organ level to rank chemicals for further consideration.