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The Adverse Outcome Pathway approach in nanotoxicology

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ABSTRACT

An Adverse Outcome Pathway (AOP) is a conceptual construct that describes existing knowledge on the link between a molecular initiating event and an adverse outcome. A sequential chain of causally related events is portrayed at different levels of biological organisation. AOPs are considered to be useful mechanistic blueprints for the development of novel tools for human and environmental risk assessment. Following OECD guidance, an increasing number of AOPs for chemically-induced adverse effects in humans and environmental species are being proposed. Due to their unique properties, the toxicity of nanomaterials (NMs) and chemicals is often difficult to directly compare since their mechanisms usually differ. While there are still many knowledge gaps in our understanding of NM toxicity, an ever increasing number of mechanistic studies are shedding light on their toxicokinetic and toxicodynamic properties. In this paper, we introduce the concept of AOPs and analyse its possible implementation for nanotoxicology. We illustrate how the AOP framework can be used to rationally combine mechanistic knowledge relating to both NM- and chemically-induced liver toxicity to fill information gaps and guide the development of toxicity testing strategies. The differences between NM and chemically-induced adversity are proposed to be primarily related to differences in toxicokinetics and the nature of the initial Key Events in the AOP. Consequently, much of the mechanistic knowledge captured by AOPs that have been developed from consideration of chemically-induced toxicity is also relevant to describe AOPs applicable to NMs, at least in qualitative terms, and thus can be used to inform predictive modelling and risk assessment of NM toxicity.

Contents

Abbreviations: AO, adverse outcome; AOP, Adverse Outcome Pathway; CNTs, carbon nanotubes; H_2O_2, hydrogen peroxide; IATA, Integrated Approaches to Testing and Assessment; ILSI-RSI, International Life Sciences Risk Sciences Institute; IPCS, International Programme on Chemical Safety; ITS, Integrated Testing Strategies; KE, Key Event; KER, Key Event Relationship; MIE, molecular initiating event; MoA, Mode of Action; MWCNTs, multi-walled carbon nanotubes; NADPH, nicotinamide adenine dinucleotide phosphate; NM, nanomaterial; NLRP-3, NOD-like receptor family, pyrin domain containing 3; $^1O_2$, singlet oxygen; $O_2^{\cdot-}$, superoxide; OECD, Organisation for Economic Co-operation and Development; QSAR, quantitative structure–activity relationship; REACH, Registration, Evaluation, Authorisation and Restriction of Chemicals; ROS, reactive oxygen species; SAS, synthetic amorphous SiO_2; SCCS, Scientific Committee on Consumer Safety; SiO_2, silicon dioxide; TGF-β1, transforming growth factor beta 1; TiO_2, titanium dioxide; WHO, World Health Organization.

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1. Introduction

For the regulatory assessment of chemicals, *in vivo* testing is still used extensively to fulfil information requirements, even though animal tests are typically very time-consuming, costly and questionable from an ethical perspective. Moreover, standard guideline tests offer sparse information on the mechanism of toxicity of a substance and thus provide little help in explaining why a substance might cause an adverse effect of regulatory concern. More than a decade ago, recommendations already emerged to focus on intelligent testing strategies [1] that move away from a "generalized, checklist approach" to cover data gaps by acquiring only essential information [2]. This has led to the development of Integrated Testing Strategies (ITS) to support the implementation of legislation such as REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) in the European Union [3,4], and to more recent efforts within the Organisation for Economic Co-operation and Development (OECD) to develop Integrated Approaches to Testing and Assessment (IATA) which optimally combine and exploit existing information, *in vitro* assay data and computational predictions to satisfy specific information requirements [5].

The International Programme on Chemical Safety (IPCS) of the World Health Organization (WHO) and the International Life Sciences Risk Sciences Institute (ILSI-RSI) initiated the Mode of Action (MoA) human relevance framework [6] for a better evaluation and harmonisation of the assessment of chemical risks. Following this, in 2012, a programme for the development of Adverse Outcome Pathways (AOPs) was launched by the OECD which has taken up many of the aspects of the WHO/IPCS work on MoA [7]. Initially described in the context of ecotoxicological risk assessment, an AOP was defined as "a conceptual construct that portrays existing knowledge concerning the linkage between a direct molecular initiating event (MIE) and an adverse outcome (AO)", by capturing the sequential chain of causally-linked Key Events (KEs) at different levels of biological organisation [8]. Subsequently, the AOP concept was extended to support the assessment of human health effects. AOPs aim to support regulatory decision-making by providing the knowledge base to support the development of novel test methods and (OECD) Test Guidelines, QSAR tools and IATA.

In practical terms, the description of an AOP is highly structured and follows well-defined principles and conventions, as described in OECD guidance, a supplementary 'User handbook' [9], and in the scientific literature [10–12]. For example, KEs have to be both measurable and essential (but not necessarily sufficient) for the AO in question, and the evidence presented to support the causal linkages between individual KEs, termed Key Event Relationships (KERs), should be based on both biological plausibility and empirical data. Evidence can be derived from various sources including *in vivo* and *in vitro* studies, or from computational modelling [8]. An AO can be defined at various levels: for human health effects, an AO seldom relates to whole population level, but rather to individual organ damage (e.g. liver fibrosis), which has consequences on the individual, whereas in environmental toxicology the AO usually relates to growth inhibition, reduced survival or reproductive impairment of an individual (e.g. a fish) and the consequences on the whole population. The MIE describes the interaction of a material (e.g. chemical) with a biological target, and can be either specific, such as ligand–receptor interaction, or non-specific (e.g. a toxicant physically residing in a bio-membrane) [9]. By definition, an AOP consists of a single MIE and a single AO, but can have multiple causally-linked KEs (Fig. 1). This leads to a simplified and "linear" representation of an individual AOP, which may be an adequate basis for prediction in certain cases. However, since KEs can be shared by different AOPs, and one MIE can lead to multiple AOs and vice versa, AOP networks generally represent a more relevant basis for toxicity prediction [12]. To facilitate the development of AOPs within a network context and to provide a practical collaborative platform for AOP developers to systematically capture, share and integrate their AOP knowledge, the AOP Knowledge Base, including the AOP-Wiki, has been launched in 2014 as publicly accessible tool [13].

Building of networks can be further supported by the emerging concept of Aggregate Exposure Pathways (AEPs), which has been recently introduced to integrate also complex exposure scenarios

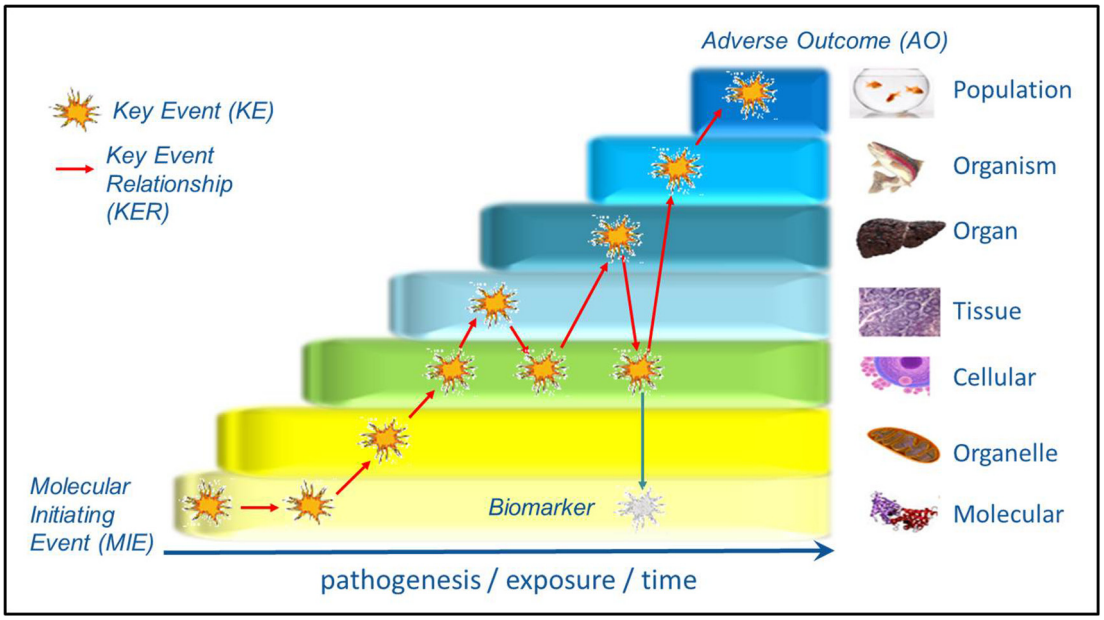

Fig. 1. Exemplary flow scheme of a typical AOP, starting from the molecular initiating event (MIE), inducing a variety of Key Events (KEs) connected by Key Event Relationships (KERs, red arrows) and resulting in a single specific Adverse Outcome (AO).