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Proposing a scientific confidence framework to help support the application of adverse outcome pathways for regulatory purposes

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ABSTRACT

An adverse outcome pathway (AOP) describes the causal linkage between initial molecular events and an adverse outcome at individual or population levels. Whilst there has been considerable momentum in AOP development, far less attention has been paid to how AOPs might be practically applied for different regulatory purposes. This paper proposes a scientific confidence framework (SCF) for evaluating and applying a given AOP for different regulatory purposes ranging from prioritizing chemicals for further evaluation, to hazard prediction, and ultimately, risk assessment. The framework is illustrated using three different AOPs for several typical regulatory applications. The AOPs chosen are ones that have been recently developed and/or published, namely those for estrogenic effects, skin sensitisation, and rodent liver tumor promotion. The examples confirm how critical the data-richness of an AOP is for driving its practical application. In terms of performing risk assessment, human dosimetry methods are necessary to inform meaningful comparisons with human exposures; dosimetry is applied to effect levels based on non-testing approaches and *in vitro* data. Such a comparison is presented in the form of an exposure:activity ratio (EAR) to interpret biological activity in the context of exposure and to provide a basis for product stewardship and regulatory decision making.

1. Introduction

Societal demands for safer and more sustainable chemical products are stimulating changes in toxicity testing and assessment frameworks. Chemical safety assessments are expected to be conducted faster and with fewer animals, and at the same time, the number of chemicals that require assessment is also rising with the number of different regulatory programmes increasing worldwide. These considerations have stimulated a shift in thinking about how toxicity testing and their evaluations need be conducted in the future-moving away from extensive toxicity testing based on phenotypic responses in animals towards pathway approaches based on (quantitative) structure–activity relationships ((Q)SAR), toxicokinetics, physiological mechanisms and dose-dependent biological changes underlying toxicity in exposed organisms. Since "safety," by definition, includes both the inherent hazards of the substances that make up a product and exposures that occur as a result of use of the product, improvements are needed in both approaches for evaluating intrinsic hazards and approaches for determining exposures. These visions were articulated to a large extent in the 2007 NRC report "Toxicity Testing in the 21st Century: A Vision and a Strategy" (NRC, 2007) and the 2012 NRC report "Exposure Science in the 21st Century: A Vision and A Strategy" (NRC, 2012; Cohen Hubal et al., 2010).

A move towards more mechanistically based risk assessments implies with it the use of *in vitro* tests, including high throughput and high content (HT/HC) screening methods, coupled with the application of a range of computational methods for data analysis and predictive modeling. Thus achieving the visions of Tox21 and EXPO21 relies on 4 key components:

- The generation of *in vitro* data.
- The derivation of models from these biological activity assays that predict downstream biological responses of toxicological relevance.

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- Exposure modeling to relate predicted downstream biological responses of toxicological relevance to exposures from uses of chemical products.
- A tiered framework for proceeding to more complex assessment procedures when greater precision is warranted to support a specific regulatory or product stewardship decision.

A key, overarching component is a biological construct for appropriate interpretation of these data so that prediction models can guide regulatory uses and decision making. An adverse outcome pathway (AOP) could serve as such a construct.

2. Adverse outcome pathway

An AOP is a sequence of events from the first critical molecular event (known as the molecular initiating event or MIE) to an *in vivo* adverse outcome (AO) (Ankley et al., 2010). Although the molecular initiating event has been defined as the first key event (KE) in the AOP causally linked to an adverse outcome, in practice the MIE is being used to characterize the first molecular interaction which itself might not be causal. The term "initial molecular event" (IME) was coined by Patlewicz et al. (2013a) to replace the molecular initiating event in an effort to represent this important distinction[1]. Subsequent to the molecular initiating event, additional key events will contribute to and culminate in the occurrence of the adverse outcome. The Organization for Economic Co-operation and Development (OECD) has developed guidance on developing and assessing AOPs that is in alignment with guidance from the World Health Organization (WHO), International Program on Chemical Safety (WHO-IPCS) and ILSI Health and Environmental Science Institute (HESI) on mode of action (MoA), Human Relevance (HRF) and Key Event Dose Response (KEDRF) (Julien et al., 2009; Meek et al., 2003; Meek, 2008; Meek et al., 2014a,b; OECD, 2013).

OECD's work programme for developing AOPs stemmed from the desire to enhance read-across within chemical categories. AOPs should thus facilitate the transition from categories that have been largely structurally based to categories that are informed by the inclusion of additional biological information (van Leeuwen et al., 2009). In 2010, the OECD held a workshop entitled "Using Mechanistic Information in Forming Chemical Categories." This workshop discussed the types of activities that could form the basis of an OECD AOP work programme including the development of a library of AOPs and MIEs which could subsequently be included in the OECD QSAR Toolbox (OECD, 2011). A complementary driver was the 7th Amendment to the Cosmetics Directive which established a ban on animal testing for repeated dose toxicity endpoints for cosmetics by 2013 (EC, 2009; Hartung et al., 2011). Indeed an ongoing joint research effort between Cosmetics Europe and the European Commission known as SEURAT-1 is investigating approaches to replace the types of repeated dose toxicity testing that would be necessary to assure the safety of cosmetic substances by exploiting an AOP framework (http://www.seurat-1.eu/).

Whilst there is a wealth of activity on the development of AOPs in particular within the OECD programme, far less attention has been placed on their evaluation and practical application in a regulatory context. The purpose of this paper is to propose a scientific confidence framework (SCF) to outline the types of considerations pertinent when applying and evaluating AOPs for different regulatory purposes and to highlight its utility with a few illustrative examples. The SCF incorporates established thinking regarding Mode of Action (MoA), the notion of "fit-for-purpose" as a necessary aspect of problem formulation, and the need to consider human dosimetry (Becker et al., 2012, 2014b,c).

[1] Drewe et al. (2014) coined the term pre-MIE as an alternative to IME to make the same distinction. This was in an effort to ensure that MIEs that were not truly causally linked were not being used as direct predictors of the adverse outcome.

3. Challenges in applying AOPs in regulatory decision making: a framework to document scientific confidence

The OECD AOP work programme foresees AOPs as addressing several different regulatory purposes. These include (1) development of chemical categories based on biological responses (2) informing test method refinement/development and (3) developing integrated approaches to testing and assessment (IATA) for hazard and risk assessment. Although not explicitly stated in the OECD work programme, AOPs can also be used for prioritization purposes, which may be viewed as a distinct application stemming from chemical categorization based on biological responses. In addition, an AOP can be used as the central organizing conceptual approach for a chemical risk assessment, in a manner analogous to the use of MoA in the ILSI KEDRF and HESI Q-KEDRF (Julien et al., 2009; Simon et al., 2014).

The current OECD AOP work programme falls under the direction of the Extended Advisory Group for Molecular Screening and Toxicogenomics (EAGMST), and is focused on the development of AOPs, associated guidance and knowledge management tools such as the AOP Wiki. Although there is a workflow described to outline the steps of AOP development, the endorsement and regulatory application, as noted by Vinken (2013), has not yet been considered in any great detail by the OECD. In an idealized case, an AOP would include a description of all key events, delineation of methods which can be used to measure each key event, descriptions of each key event relationship (KER), and quantitative models for each KER to permit statistical prediction of a downstream key event from an upstream key event. If all of this information were available, quantitative predictions of the adverse outcome (AO) could be made from an upstream key event. However, for almost all AOPs, our current state of understanding does not allow for a quantitative prediction of a downstream key event or the ultimate adverse outcome from an upstream key event. Typically, quantitative prediction models are lacking, and thus predicting quantitative hazards falls short of achieving the desired degree of scientific confidence. Therefore, the use of AOPs to quantitatively predict human toxicity or risks may not become routine for some time to come. Nonetheless, depending upon the degree of understanding, AOPs can still be practically used in a number of ways for regulatory purposes. The extent to which an AOP can be used in any of the applications delineated above will depend on the maturity or completeness of the AOP itself. The application of a given AOP to a specific regulatory challenge will depend in a large part on how the scientific basis of the AOP has been justified and documented.

Cox et al. (2014) put forward a scientific confidence framework designed to aid in the development, evaluation and communication of the scientific confidence in Tox21 assays and their prediction models. Specifically the framework was designed as a means of documenting the performance and robustness of assays and their prediction models within the context of a biological pathway that culminated in an adverse effect (*i.e.*, an AOP) and was aimed at a given regulatory purpose, whether it be for priority setting, read-across, screening level hazard identification, *etc.* The framework was derived using the OECD QSAR validation principles (OECD, 2004) and the Institute of Medicine (IOM) biomarkers guidance (IOM, 2010). It is composed of three inter-related core elements, (1) analytical validation, (2) qualification and (3) utilization which can be readily adapted for AOPs. These three core elements have been integrated in an extended scientific confidence framework for AOPs in a stepwise manner (Table 1).