Regulatory Toxicology and Pharmacology xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Increasing Scientific Confidence in Adverse Outcome Pathways: Application of Tailored Bradford-Hill Considerations for Evaluating Weight of Evidence ☆

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ARTICLE INFO

Article history:
Received 11 March 2015
Available online xxxx

Keywords:
Adverse outcome pathway
Weight of evidence
Mode of action
Bradford-Hill considerations

ABSTRACT

Systematic consideration of scientific support is a critical element in developing and, ultimately, using adverse outcome pathways (AOPs) for various regulatory applications. Though weight of evidence (WoE) analysis has been proposed as a basis for assessment of the maturity and level of confidence in an AOP, methodologies and tools are still being formalized. The Organization for Economic Co-operation and Development (OECD) Users' Handbook Supplement to the Guidance Document for Developing and Assessing AOPs (OECD 2014a; hereafter referred to as the OECD AOP Handbook) provides tailored Bradford-Hill (BH) considerations for systematic assessment of confidence in a given AOP. These considerations include (1) biological plausibility and (2) empirical support (dose-response, temporality, and incidence) for Key Event Relationships (KERs), and (3) essentiality of key events (KEs). Here, we test the application of these tailored BH considerations and the guidance outlined in the OECD AOP Handbook using a number of case examples to increase experience in more transparently documenting rationales for assigned levels of confidence to KEs and KERs, and to promote consistency in evaluation within and across AOPs. The major lessons learned from experience are documented, and taken together with the case examples, should contribute to better common understanding of the nature and form of documentation required to increase confidence in the application of AOPs for specific uses. Based on the tailored BH considerations and defining questions, a prototype quantitative model for assessing the WoE of an AOP using tools of multi-criteria decision analysis (MCDA) is described. The applicability of the approach is also demonstrated using the case example aromatase inhibition leading to reproductive dysfunction in fish. Following the acquisition of additional experience in the development and assessment of AOPs, further refinement of parameterization of the model through expert elicitation is recommended. Overall, the application of quantitative WoE approaches hold promise to enhance the rigor, transparency and reproducibility for AOP WoE determinations and may play an important role in delineating areas where research would have the greatest impact on improving the overall confidence in the AOP.

☆ This manuscript was reviewed in accordance with guidelines of the authors' employers, but does not necessarily reflect policy/positions of the USEPA, JRC, Health Canada or other authors' organizations.

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http://dx.doi.org/10.1016/j.yrtph.2015.04.004
0273-2300/

List of Abbreviations

adverse outcome pathways (AOPs)
weight of evidence (WoE)
Organization for Economic Co-operation and Development (OECD)
modes of action (MoA)
key event (KE)
Key Event Relationship (KER)
molecular initiating event (MIE)
adverse outcome (AO)
(Quantitative) Structure Activity Relationship ((Q)SAR)
Bradford-Hill (BH)
Arylhydrocarbon receptor (AhR)
cytochrome P450 (CYP)
neuropathy target esterase (NTE)
hexavalent chromium (Cr(VI))
testosterone (T)
17β-estradiol (E2)
vitellogenin VTG
messenger RNA (mRNA)
estrogen receptor α (ERα)
estrogen receptor (ER)
high (H)
moderate (M)
low (L)
lines of evidence (LOE)
multi-criteria decision analysis (MCDA)

1. Introduction

A large number of substances in commerce require risk evaluation to protect human health and the environment. A key challenge for the regulatory community is assessing the potential for risks of substances with limited toxicity or toxicology data. Accordingly, various regulatory mandates and related initiatives in Canada, USA, the European Union and, more recently, the Asian Pacific region (see, for example, Council of Labor Affairs, Taiwan, 2012; Dellarco et al., 2010; European Commission, 2006; Hughes et al., 2009; Lowell Center for Sustainable Production, 2012; Meek and Armstrong, 2007, Mitchell et al., 2013) reflect the rapidly growing need for more efficient methods and novel strategies to assess the hazards and risks of a wide array of chemicals. Due to costs and time involved, as well as the desire to reduce animal use in response to ethical considerations, traditional resource-intensive standard *in vivo* toxicology studies are not feasible for the regulatory testing of all chemicals requiring evaluation. Adverse outcome pathways (AOPs) hold great promise as important tools to enhance efficiencies and the future success of risk assessment in the implementation of pathway- and mechanistic-based approaches that are able to accommodate substances and groups of substances with varying amounts and types of toxicological information (e.g., OECD, 2014b; CCA, 2012; Ankley et al., 2010; NRC, 2010; NRC, 2007). However, it is important to note that these promising concepts and approaches supporting the application of pathway-based data and predictive modeling in hazard characterization and risk assessment need further development, evaluation and acceptance before being used routinely.

The generation and consideration of mechanistic data has the potential to increase our understanding of the modes of action (MoA) underlying the toxicity of various individual chemicals and groups of chemicals. It is anticipated that MoA information will lead to improved estimation of potential risk to human health and the environment. Investigators continue to elucidate the modes and mechanisms underlying toxicity-related adverse effects by applying emerging and increasingly more sophisticated computational, molecular and *in vitro* technologies. Such approaches have the potential to be used qualitatively and/or quantitatively in a predictive manner to identify potential toxicities in the absence of definitive data on adverse effects. A major challenge faced by both research and regulatory scientists is the integration of data and information being generated from diverse sources at many different levels of biological organization in a manner that is transparent, informative and suitable for regulatory decision-making.

Conceptually, an AOP is similar to a MoA (OECD, 2013) with the MoA representing a chemical and species specific application of the more general AOP. The AOP construct (Fig. 1) portrays a MoA in a structured framework that organizes and links knowledge of Key Events (KEs; a change in biological state that is both measurable and essential to the progression of a defined biological perturbation) in a sequence that commences with the molecular initiating event (MIE; the initial point of chemical-biological interaction within the organism that starts the pathway) and proceeds through a series of higher order biological events, culminating with the *in vivo* adverse outcome (AO) of interest to risk assessment. The series of biological events, or KEs, are connected to one another via linkages defined as Key Event Relationships (KERs). An AOP that is anchored to both a MIE and an AO provides a consistent structure that facilitates effective application and integration of diverse information on MoAs for various hazard and risk assessment uses, and provides a tool for the identification of key uncertainties and research needs (Ankley et al., 2010; OECD, 2013). Villenueve et al. (2015a, 2015b) provide detailed discussion of definitions of MIEs, KEs and KERs as well as strategies, principles and best practices to use when developing AOPs, and refer to work reported here with respect to conduct of WoE evaluations; other products from the 2014 workshop "Advancing AOPs for Integrated Toxicology and Regulatory Applications" can be found at https://aopkb.org/saop/workshops/somma.html#manuscripts.

Under the auspices of the Organisation for Economic Cooperation and Development (OECD), scientists across the world and from all sectors have an opportunity to develop AOPs which will be peer- reviewed and publically accessible through a wiki-based tool (AOP-Wiki; aopwiki.org). Using the wiki format, contributions to improving the science basis and range of applications of AOPs can be made by experts from all sectors and regions. When fully actualized, the AOP-Wiki will serve as a knowledge base of AOPs, KEs and KERs for a wide spectrum of toxicologically-relevant pathways. This organized and integrated information is envisioned to address or inform a number of analytical domains in the decision-making process including: (1) efficient grouping of chemicals based on common pathways of toxicity and potential consideration of non-test methods, such as read-across and (quantitative) structure-activity relationship ((Q)SAR) modeling or targeted testing to fill data needs; (2) identification of research priorities relevant to data gaps in regulatory test batteries; (3) providing a framework for priority setting; and, (4) hazard characterization and risk assessment that incorporate qualitative and quantitative determinations of human and/or ecological relevance and

Please cite this article in press as: Becker, R.A., et al. Increasing Scientific Confidence in Adverse Outcome Pathways: Application of Tailored Bradford-Hill Considerations for Evaluating Weight of Evidence. Regul. Toxicol. Pharmacol. (2015), http://dx.doi.org/10.1016/j.yrtph.2015.04.004