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Lessons learned from read-across case studies for repeated-dose toxicity

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ABSTRACT

A series of case studies designed to further acceptance of read-across predictions, especially for chronic health-related endpoints, have been evaluated with regard to the knowledge and insight they provide. A common aim of these case studies was to examine sources of uncertainty associated with read-across. While uncertainty is related to the quality and quantity of the read across endpoint data, uncertainty also includes a variety of other factors, the foremost of which is uncertainty associated with the justification of similarity and quantity and quality of data for the source chemical(s). This investigation has demonstrated that the assessment of uncertainty associated with a similarity justification includes consideration of the information supporting the scientific arguments and the data associated with the chemical, toxicokinetic and toxicodynamic similarity. Similarity in chemistry is often not enough to justify fully a read-across prediction, thus, for chronic health endpoints, toxicokinetic and/or toxicodynamic similarity is essential. Data from New Approach Methodology(ies) including high throughput screening, *in vitro* and *in chemico* assay and *in silico* tools, may provide critical information needed to strengthen the toxicodynamic similarity rationale. In addition, it was shown that toxicokinetic (i.e., ADME) similarity, especially metabolism, is often the driver of the overall uncertainty.

1. Introduction

Legislative requirements for the registration and safety assessment of chemicals, along with the need to obtain toxicological information without resorting to animal testing, have stimulated a more critical examination of read-across (RA). The concept of category formation, chemical grouping and RA is used to support chemical safety assessment by filling data gaps without the need for further *in vivo* testing (ECHA, 2014; OECD, 2014a; Stanton and Kruszewski, 2016). Historically, the fundamental assumptions of RA are that chemicals, which are similar in their structure, will have similar chemical properties and, thereby, have similar toxicokinetic and toxicodynamic properties (Cronin et al., 2013). A group of substances with similar toxicokinetic and toxicodynamic properties can be considered a toxicological meaningful category or a group of chemicals whose human health and/or environmental toxicological properties are likely to be similar or follow a regular pattern for a particular hazard. RA of toxic potencies based on such a category is a valuable approach to data gap filling, thus having a number of regulatory applications. Briefly, experimentally-derived toxicological properties from one or more source chemicals may be read across to fill the data gap for a target chemical, which is "similar" and for which an experimentally derived toxicological value is wanting and such prediction can be used for screening, priority setting, hazard assessment or risk assessment (Patlewicz and Fitzpatrick, 2016).

1.1. Background

Since the review of Cronin et al. (2013), a number of papers have appeared that focus on modern-day RA. Many of these, including Blackburn and Stuard (2014), European Chemicals Agency (ECHA) (2015), Organisation for Economic Co-operation and Development (OECD) (2015) and Schultz et al. (2015), have put forward efforts to improve RA arguments and improve and innovate approaches (Batke et al., 2016; de Abrew et al., 2016; Shah et al., 2016; van Ravenzwaay et al., 2016). More recently, Ball et al. (2016) summarised the state-of-the-art surrounding read-across, along with

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reasons relating to regulatory non-acceptance, and compiled relevant guidance under the heading of "Good Read-Across Practice"; Hartung (2016) described the concept of linking different types of data and tools under the umbrella of good read-across practices.

It is acknowledged RA is not a new concept (cf. Hanway and Evans, 2000), despite this, a number of challenges continue to impede its wider use. When applying RA to fill a toxicological data gap, a number of fundamental questions repeatedly arise (Schultz, 2014), including:

- Is it possible to form a robust group of chemicals (often referred to as a chemical category) which includes the target chemical?
- Is the category relevant to fill a data gap considering the toxicology of the endpoint under assessment?
- Are there appropriate toxicology studies of sufficiently high quality for the source chemical(s) to allow a meaningful RA?
- Are the uncertainties defined and are they acceptable in order to use the read across prediction(s) to fill the data gap(s) for a specific regulatory purpose?

To address these questions and others, a flexible strategy for developing and reporting a repeated-dose RA prediction was devised and applied in the case studies (Schultz et al., 2015). Briefly, this strategy focuses on the two main elements of a RA, namely:

- assessment of the similarity between source and target substance(s) and,
- assessment of the uncertainties in the RA process and ultimate prediction.

It is worth noting the publication of this strategy predates ECHA's Read-Across Assessment Framework (RAAF) (ECHA, 2015). Regardless of process, the standards for accepting a RA prediction are likely to vary little, as the aim of a RA is to provide a prediction(s) that is (more or less) equivalent to that which would be obtained from the standard animal study.

In order to address at least some of the above questions, and to determine the suitability of RA to fill data gaps for repeated-dose toxicity (focussing on the oral route of exposure to the rat), Berggren et al. (2015) recommended that a series of case studies be conducted for the most likely RA scenarios. An additional recommendation was that each case study be evaluated in a two-step process. The initial step was to be a "traditional" RA using established *in vivo* data supplemented, as applicable, with conventional *in vitro* and classic structure-activity relationship information. The second iteration was to be a RA with the initial information and data supplemented with "New Approach Methodology" (NAM) data from high-throughput screening (HTS), novel *in vitro* methods and/or toxicogenomic assays.

Following an external review process, the findings of four case studies for the filling of data gaps for repeated-dose toxicity using RA have been published, covering a variety of RA scenarios. The RA case studies were all for 90 day rat repeated-dose toxicity and explored:

i) The suitability of 2-propen-1-ol as a read-across analogue for other short chain primary and secondary β-olefinic alcohols on the basis of similarity in metabolic transformation (Przybylak et al., 2017).
ii) The use of data for short-chain mono-alkylphenols to fill data gaps for other mono-alkylphenols on the basis of similarity in toxicokinetics and toxicodynamics (Mellor et al., 2017).
iii) An investigation of saturated 1-alkanols presumed to be of low toxicity and varying in toxicokinetics as a results of alkyl chain (assuming no branching on the alkyl chain) (Schultz et al., 2017a).
iv) Consideration of 2-alkyl-1-alkanols where branching of the alkyl chain may affect RA for low toxicity chemicals (Schultz et al., 2017b).

Whilst the reader is encouraged to examine the case studies (Przybylak et al., 2017; Mellor et al., 2017; Schultz et al., 2017a, 2017b), a summary of the findings is presented in Table 1. As summarised in Table 1, the four RA case studies were evaluated in terms of the robustness of arguments and the uncertainty associated with the different elements of the category formation. It is important to note that these case studies were not performed for the purpose of regulatory submission, but to investigate the process of RA and how it could be improved. As such they provide a rich source of potential knowledge and learning for the development and direction of future RA studies. It is also acknowledged that various other RA case studies have been published (Blackburn et al., 2011; de Abrew et al., 2016; van Ravenzwaay et al., 2016) and, whilst they have not been evaluated explicitly in this investigation as they are based on different endpoints and approaches, there has been implicit learning from these.

1.2. The aim

The present paper recapitulates with examples the lessons learned from the recent series of case studies which illustrate specific issues associated with modern-day toxicological RA of repeated dose toxicity. The case studies cited have the advantage of having undergone external review prior to publication. We believe this summary of lessons has the potential of furthering the acceptance of RA predictions, especially for predictions of NOAELs from repeated-dose toxicity studies.

2. Methods and materials

The findings reported here build on previous analyses, starting with guidance (ECETOC, 2012; ECHA, 2009; 2011; OECD, 2007; 2011, 2014a, 2015) on grouping of chemicals and RA as well as other publications in this area (Ball et al., 2014, 2016; Cronin et al. (2013); Blackburn and Stuard, 2014; Patlewicz et al., 2013a, 2013b, 2014, 2015; Patlewicz and Fitzpatrick, 2016).

As stated in the introduction, the case studies from which the findings in this paper were arrived at were Przybylak et al. (2017), Mellor et al. (2017) and Schultz et al. (2017a, 2017b). Each case study is consistent with RA principles previously described (e.g., ECHA, 2013a; 2013b; OECD, 2015; Schultz et al., 2015). These four case studies, while developed by an iterative effort of their authors, were extensively reviewed by various independent experts.

3. Lessons learned

RA case studies have crucial evidentiary value in regulatory toxicology. Amongst other things, they provide a means of illustrating how it may be possible to move from chemical-by-chemical assessments based on animal testing to assessments by interpolation within a toxicologically-relevant and mechanistically plausible chemical category. In the context of this paper, case studies provided an opportunity to benchmark some of the lessons learned or confirmed to use RA in a regulatory context.

3.1. Today's read-across

Early approaches to RA, e.g. identification of analogues with varying chain lengths (Hanway and Evans, 2000; Patlewicz and