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Expert opinions on the acceptance of alternative methods in food safety evaluations: Formulating recommendations to increase acceptance of non-animal methods for kinetics

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ABSTRACT

Inclusion of alternative methods that replace, reduce, or refine (3R) animal testing within regulatory safety evaluations of chemicals generally faces many hurdles. The goal of the current work is to i) collect responses from key stakeholders involved in food safety evaluations on what they consider the most relevant factors that influence the acceptance and use of 3R methods and to ii) use these responses to formulate activities needed to increase the acceptance and use of 3R methods, particularly for kinetics. The stakeholders were contacted by e-mail for their opinions, asking the respondents to write down three barriers and/or drivers and scoring these by distributing 5 points over the three factors. The main barriers that obtained the highest aggregated scores were i) uncertain predictability 3R methods/lack of validation, ii) insufficient guidance regulators/industry and iii) insufficient harmonization of legislation. The major driver identified was the possibility of 3R methods to provide more mechanistic information. Based on the results, recommendations are given to enhance the acceptance and application of 3R toxicokinetic methods in food safety evaluations. These include steering of regulatory data requirements as well as creating (funding) opportunities for development and validation of alternative methods for kinetics and development of guidances.

1. Introduction

The development of alternative methods that replace, reduce, or refine (3R) animal testing for regulatory safety evaluations primary targets at decreasing the reliance on animal experimental results. In addition, by doing so, toxicologists also aim to increase the human relevance of their studies and reduce costs and time for testing. Nevertheless, to date, the regulatory use of 3R methods is still limited. This indicates the importance of understanding the hurdles in the adoption of 3R methods as well as the drivers that could enhance the process. Recently, Schiffelers et al., 2014 identified various factors influencing regulatory acceptance and use of 3R methods in the pharmaceutical and chemical sector based on expert panel interviews with relevant stakeholders from academia, regulatory authorities and industry. Cross-sectorial barriers that were observed in that study included i) the existing uncertainties of 3R methods, ii) the lack of harmonization of legislation and test requirements, and iii) the striving for risk minimization (resulting in avoidance of the use of novel methods with unknown uncertainties). Differences between the sectors were also identified. For example, the most important barriers reported within the pharmaceutical panel included the "insufficient harmonization of legislation" and "uncertain predictability/lack of validated 3R methods", whereas the most important barriers reported by the chemical panel included the "challenging of *in vitro-in vivo* extrapolation" and "lack of global harmonization & mutual acceptance of data". Cross-sectorial drivers identified were the i) informative and mechanism-based character of 3R methods, ii) ethical concern about animal testing, and iii) concrete policy goals/legislation to stimulate the 3Rs (Schiffelers et al., 2014).

The study of Schiffelers et al. (2014) specifically focused on the pharmaceutical and chemical sector. It is unclear to what extent the development and acceptation of 3R methods within safety evaluations of food chemicals (including food contaminants, additives and food-contact materials) is influenced by similar factors. The goal of the

Abbreviations: 3R, replace, reduce, or refine; EFSA, European Food Safety Authority; EU, European Union; PBPK, physiologically based pharmacokinetic; QIVIVE, Quantitative *in vitro-in vivo* extrapolations; REACH, Registration, Evaluation, Authorisation and Restriction of Chemicals

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current work was to i) collect responses from key stakeholders involved in food safety on what they consider the most relevant barriers and drivers in the acceptance and use of 3R methods for evaluations and ii) to use these responses to formulate activities needed to increase the acceptance and use of 3R methods, particularly for addressing kinetic characteristics of food chemicals as case study. 3R methods for toxicokinetics gain increasing attention with respect to the development of alternatives to *in vivo* testing, as these are effective tools for extrapolating *in vitro* toxicity effect concentrations to equivalent human oral doses (Louisse et al., 2017; Bessems et al., 2014; Coecke et al., 2013; Wilk-Zasadna et al., 2015; Yoon et al., 2012; Zhang et al., 2012; Rietjens et al., 2011). Given this crucial role within quantitative *in vitro-in vivo* extrapolations (QIVIVE), there is a need for increased acceptance and use of 3R methods for kinetics within regulatory safety evaluations.

2. Stakeholder responses

2.1. Collection of the stakeholder responses

Stakeholders actively working in the field of food safety evaluations were approached by e-mail for their opinions on the factors that influence the acceptance and use of 3R methods within safety evaluations of food chemicals (See Acknowledgements for the list of responders and their affiliations). A similar approach to Schiffelers et al., 2014 was taken, asking the respondents to write down three factors (either barriers or drivers), which they perceived to be most influential on the acceptance and use of 3R methods, and to score these by distributing 5 points over the three mentioned factors. The approach was different to the one followed by Schiffelers et al. (2014) with respect to the clustering of the factors. In the present survey, the factors were not clustered according to similar response before the respondents assigned their points. A total of 9 stakeholders were approached of which 8 (89%) responded. Two responders shared the survey with other colleagues, resulting in a final number of 11 respondents. It should be noted that 4 stakeholder responses were returned in a format that was different from the requested format, resulting in more than three factors and more than 5 points. In this case, all factors mentioned were included in the survey, but the number of points divided over the responses were corrected proportionally to obtain a total of 5 points. The total number of points distributed over the different factors therefore added up to 55.

Table 1 provides the overview of the responses given by the stakeholders on what they consider the most important drivers and/or barriers in the development and acceptance of alternative methods in the risk evaluation of food chemicals. The responses were clustered according to the categories previously defined by Schiffelers et al. (2014). Compared with Schiffelers et al., 2014, two new categories were defined: i) insufficient guidance regulators/industry and ii) technological innovations. Given that the current survey with the food panel has been performed in a different setting than the surveys with the pharmaceutical and chemical panel, it cannot be concluded that these new defined categories are specific for food safety evaluations.

To provide relevant information on the possibility to control a certain driver or barrier each of the categories were classified on a “micro”, “meso”, or “macro” scale, as previously done by Schiffelers et al. (2014) according to the multi-level perspective theory (Geels, 2002). Micro level relates to the level at which new tests are developed and tested. The meso level applies to rules, regulations, expertise, practices and instructions and the macro level relates to the broader societal features. In general, barriers at micro level provide more control possibilities (for policy makers and developers) than factors at meso - or macro level (Schiffelers et al., 2014).

All respondents were given the opportunity to respond to the clustering. Where relevant, the outcomes of the discussion on the clustering are provided as footnote in Table 1. Overall, from Table 1 it can be concluded that the four factors that obtained the highest aggregated scores included responses that relate to i) uncertain predictability 3R methods/lack of validation (barrier), ii) the possibility of 3R methods to provide more mechanistic information (driver), iii) insufficient guidance for regulators/industry (barrier) and iv) insufficient harmonization of legislation (barrier). This top 4 factors represented 58% of the available points, indicating a consensus between responders in factors influencing regulatory acceptance.

2.2. Comparison with previous findings by Schiffelers et al. (2014)

In Table 2 the seven highest ranked responses obtained from the food panel are compared with the previous results from Schiffelers et al. (2014) obtained through interviews with a panel of experts in the field of pharmaceuticals and a panel of experts in the field of chemicals. This comparison particularly shows an overlap between the current survey and responses from the pharmaceutical panel. Though the ranking is different, 4 out of 7 factors overlap with the pharmaceutical panel (i.e. uncertain predictability 3R methods/lack of validation, 3R methods provide more mechanistic information, insufficient harmonization of legislation, risk-averse society), whereas 1 factor overlaps with the chemical panel (i.e. challenging *in vitro-in vivo* extrapolation).

3. Application of the results from the stakeholder survey to formulate a policy strategy to enhance the implementation of 3R methods for kinetics in food chemical safety evaluations

3.1. 3R methods for kinetics

Kinetics deals with the absorption, distribution, metabolism and excretion (ADME) of compounds in an organism. Within regulatory risk evaluations, kinetic data provide valuable insights in e.g. bioavailability, bioaccumulation potential and the formation of metabolites. Information on kinetics allows to better understand the toxicity, intra- and interspecies differences as well as dose-dependent effects regarding the fate of a chemical or its metabolite(s) in organisms (Bessems et al., 2014; Punt et al., 2011; Rietjens et al., 2011). Moreover, there is an increasing scientific interest in the use of kinetic data in the development of alternatives to animal testing as *in vitro* toxicokinetic data can effectively be used to extrapolate *in vitro* toxicity results to the *in vivo* situation (Louisse et al., 2017; Bessems et al., 2014; Coecke et al., 2013; Wilk-Zasadna et al., 2015; Yoon et al., 2012; Zhang et al., 2012; Punt et al., 2011).

We recently reviewed the predictive value and current use/acceptance of *in vivo* and alternative approaches for kinetics in regulatory risk evaluations of foodborne chemicals (Punt et al., 2017). To identify best practices in different regulatory domains we compared the use of kinetic data in risk evaluations of food chemicals based on scientific opinions of the European Food Safety Authority (EFSA) to that of pesticides and pharmaceuticals as published within *EFSA Conclusions on Pesticides* and *EMA Public Assessment Reports*, respectively. We revealed a poor correlation between the *in vivo* bioavailability in rats and dogs vs that in humans. In contrast, *in vitro* (human) kinetic data have been demonstrated to provide adequate predictions of the fate of compounds in humans, using appropriate *in vitro-in vivo* scalers and by integrating *in vitro* kinetic data with *in silico* kinetic modelling. Even though *in vitro* kinetic data were found to be occasionally included within risk evaluations of food chemicals, in particular results from Caco-2 absorption experiments and *in vitro* data on gut-microbial conversions, only a minor use of *in vitro* methods for metabolism and quantitative *in vitro-in vivo* extrapolation methods was identified. Yet, such quantitative predictions are essential in the development of alternatives to animal testing as well as to increase human relevance of toxicological risk evaluations (Punt et al., 2017).

The stakeholder opinions of the food panel can be used to enhance the acceptance of 3R methods for kinetics within food safety evaluations, including quantitative *in vitro* kinetic data and the integration of