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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

Table 1
Responses given by the food panel on what they consider the most important drivers and/or barriers and number of points assigned to the different factors.

Factor	Cluster[a]	Level[b]	Barrier (<) or driver (>)	Related responses in food panel (quotes)	Total points assigned by the food panel
1	Uncertain predictability 3R methods/lack of validation	micro	<	- "Validation of testing strategies" (2) - "Lack of predictive methods for chronic exposure scenarios" (2) - "Lack of confidence that the results using alternative are adequately protective of human health. How should such be 'validated' and against what if they are based on human systems? What might be missed?" (2.5) - "*In vitro* methods are perceived as variable and not robust" (1) - "Lack of quantitative data from *in vitro* tests for risk assessment" (0.4) - "Alternative tests are not yet available to cover relevant toxicological effects, such as on the reproductive system" (2) - "Lack of cohesive approaches, accepted batteries of *in vitro* tests" (combined responses: 0.5 + 0.5+1.7) - "Lack of fitness for purpose" (0.5) - "Limitations in standardisation, validation, proficiency testing, transferability, reproducibility (across labs)" (0.5) - "I think the animal model is still considered as golden standard. Even if *in vitro* methods can provide better information than *in vivo* methods in certain situations, this information is still not accepted if it does not match with the golden standard" (2) - "Limitations in the number of testing labs" (0.5) - "Complex endpoints/systemic toxicity cannot be captured with one or a few alternative as such. Capturing these endpoints requires combining various types of methods (0.5)."	16.6
2	3R methods provide more mechanistic information	micro	>	- "Possibility to generate data that are more relevant for humans" (1.3) - "Better predictability for the human situation as a result of the use of human cell and tissues" (1.5) - "Possibility to assess mixture effects" (1) - "Provide information when there is a lack of *in vivo* tox data" (combined responses:0.5 + 0.5) - "Needed to set priorities" (combined responses:1 + 0.5) - "Rapid screening of chemicals for decision-making" (0.7)	7
3	Insufficient guidance for regulators/industry[c]	meso	< , >	- "Level of expertise and habits of current risk assessors in regulatory bodies prevent them from changing their way of working" (2) - "From a regulators perspective, there is a need for guidance and acceptance of how to utilize the information" (1) - "Resistance of decision making without historical *in vitro* data (lack of experience & understanding). Unlike *in vivo* tox, there is not a big database for *in vitro* data yet" (0.5) - "Industry submissions depend directly on the regulatory guidance, which is telling the applicant and reviewer what to look for. Although it will take some time for both industry and regulators to learn how to work with non-animal safety data, I would say there is a positive relationship. Legislation and guidance are definitely more a driver of change than a barrier" (2)	5.5
4	Insufficient harmonization of legislation[c]	meso	< , >	- "Legislative requirements, the time it takes to change them and lack of harmonisation across countries and chemical sectors, so that animal testing may still be required despite the acceptance of alternative in some places" (0.8) - "Legislation/policy makers: legislation holds on to the golden standard. Requests to include weight of evidence and read across are being made, but in practise these approaches are hardly accepted " (1) - "Legislation and especially guidance are evolving in response to new non-animal data, and so will help to implement alternatives" (3)[d]	4.8
5	Risk-averse society	macro	<	- "Lack of trust in the large amount of research data generated in this field by industry" (1) - "Strict regime in acceptance of uncertainties" (0.4) - "We have to be confident that we do not put consumer health at greater risk than when using data from conventional testing procedures" (2) - "Expectations more demanding than for *in vivo* tests" (0.5)	3.9
6	Challenging *in vitro-in vivo* extrapolation	micro	<	- "Alternative tests do not readily allow incorporation of information on absorption, distribution, metabolism and excretion – data for PBTK modelling are rarely available" (1) - "Translation into risk assessment (e.g. exposure assessment, kinetics predictions)" (combined responses:1 + 0.3+0.5) - "Methods provide no quantitative outcomes. Many methods only provide insights in the type of effects/ mechanisms and not a dose-response relationship that can be linked to the human situation" (1)	3.8

(*continued on next page*)

Table 1 (*continued*)

Factor	Cluster[a]	Level[b]	Barrier (<) or driver (>)	Related responses in food panel (quotes)	Total points assigned by the food panel
7	Technological innovations	micro	>	- "Advances in biological knowledge (e.g. iPS cells, signalling pathways), technology (e.g. robotic screening, DNA sequencing) and computational power" (1.7) - "Technological innovations" (0.8) - "Exposure based risk assessments are increasingly accepted, particularly the Threshold of Toxicological Concern principle" (1)[e]	3.5
8	Societal concerns animal testing	macro	>	- "No need for animals" (0.4) - "Reluctance to use animals (3R awareness) " (combined responses:1 + 1.5) - "Less use of experimental animals. In addition, there is increasing within industry to avoid animal experimentation as well as regulation that prohibits animal experimentation (e.g. cosmetics directive)" (0.5)	3.4
9	Difficult accessibility regulators to discuss acceptance criteria	micro/meso	<	- "The distance between developers of *in vitro* methods and the final users is large and I think there is also little communication between both groups" (2) - "Lack of cohesion between *in vitro* suppliers/users and regulatory bodies" (1.3)	3.3
10	Lack of concrete policy goals to stimulate the 3R/Lack of funding	meso	<	- "Lack of funding for proper validation and development of alternative techniques" (2) - "The standing committee SCOPAFF has mandate, and is represented by policy makers. Scientists in EFSA panels "only" have an advisory role. For 3R methods to be implemented, there needs to be good communication between scientists and risk assessors in EFSA panels and policy makers in SCOPAFF." (1.3)	3.3
				TOTAL	55

[a] Clusters are obtained from Schiffelers et al. (2014) with two newly added clusters (i.e. guidance for regulators/industry and technological innovations).

[b] Factors were attributed to different levels according to the multilevel perspective on Technology Transition (Geels, 2002). Micro level means: niche level in which novelties are developed; meso level means sociotechnical regime level (including the patchwork of rules and regulations, available expertise, current practices, and connected institutions; and the macro level corresponds to the sociotechnical landscape level (Schiffelers et al., 2012).

[c] The responses related to guidance for regulators/industry and harmonization of legislation (Factors 3 and 4) were overall considered to represent a barrier, meaning there is currently a lack of these. However, from some of the responses it is clear that sufficient guidance and legislation can also be seen as a driver.

[d] Related to factor 3, but with a main focus on legislation.

[e] The Threshold of Toxicological Concern (TTC) principle was discussed to best fit the "Technological innovation" category as it can be considered an innovation in risk assessment paradigms based on exposure waiving.

Table 2
Factors influencing regulatory acceptance and use of 3R methods in order of perceived dominance within the food sector compared with previous results obtained by Schiffelers et al. (2014) for the pharmaceutical and chemical sector.

Factor	Food panel	Barrier (<) or driver (>)	Pharmaceutical panel	Barrier (<) or driver (>)	Chemical panel	Barrier (<) or driver (>)
1	Uncertain predictability 3R methods/ lack of validation	<	Insufficient harmonization of legislation	<	Challenging *in vitro-in vivo* extrapolation	<
2	3R methods provide more mechanistic information	>	Uncertain predictability 3R methods/lack of validation	<	Lack of global harmonization & Mutual acceptance of Data	<
3	Insufficient guidance regulators/ industry	<	Cooperation (including data sharing) & communication between stakeholders	>	Lack of concrete policy goals to stimulate the 3Rs	<
4	Insufficient harmonization of legislation	<	3R methods provide more mechanistic information	>	Insufficient attention for probabilistic design in entire chain	<
5	Risk-averse society	<	Early involvement regulators to discuss acceptance criteria	>	Current thinking in terms of hazard instead of risk	<
6	Challenging *in vitro-in vivo* extrapolation	<	Implementation of directive 2010/63/EU-on the protection of animals used for scientific purposes situation"	>	Cooperation & communication between stakeholders (including data sharing)	>
7	Technological innovations	>	Risk-averse society	<	Difficult accessibility regulators to discuss acceptance criteria	<

these results with *in silico* PBPK methods. Although the stakeholders were asked to provide their ideas on the major factors that influence acceptance of 3R methods in general, the mentioned factors were assumed to be applicable to 3R methods for kinetics as well.

3.2. Creating a "window of opportunity" for the adoption of 3R methods for kinetics in safety evaluations

According to the multilevel perspective on technology transitions (Geels, 2002), alignment of the three levels (i.e. micro, meso, and macro, as described in 2.1) is required for innovations to break through. This can be achieved when a development of an innovation (micro level) meets with a change or request for change in the regulatory regime (meso level) and/or within the broader context of society (macro level) (Schiffelers et al., 2014). The large-scale testing program EU-REACH (EU, 2006) as well as the ban on animal testing for cosmetics within the EU (EU, 2006) and the Directive 2010/63/EU on the protection of animals used for scientific purposes (EU, 2010), provide clear examples of how changes in regulation (meso level), can lead to increasing efforts in the development and inclusion of 3R methods (micro level). These changes in regulation were introduced to meet with a societal concern about the safety of chemicals (macro level) and/or ethical concerns about animal testing (macro level).

Both a "risk averse society" and "societal concerns about animal testing" were identified in the current survey to influence the development and acceptance of 3R methods in safety evaluations of food chemicals (Factor 5 and 8 in Table 1, respectively). The possibility of kinetic data to reduce uncertainties in risk evaluations (meeting a risk averse society) may provide a good opportunity for 3R methods for kinetics to be implemented on a short term in regulatory safety evaluations (EFSA, 2012; Meek and Lipscomb, 2015). For most chemicals of concern within food, human studies cannot be performed and 3R methods for kinetics could allow to translate effective doses and resulting blood and tissue concentrations in animals to equivalent oral doses in humans (EFSA, 2012; Meek and Lipscomb, 2015). The use of such methods would thus allow a better prediction of levels leading to potential effects in humans (including human variability). Although this will not lead to a direct reduction in animal usage, the same kinetic data can effectively be used on a longer term within alternative testing strategies for *in vitro-in vivo* extrapolations (meeting societal concerns about animal testing).

Recommendation 1: Enhance the use of *in vitro* and *in silico* kinetic approaches within risk evaluations to reduce uncertainties in the extrapolation of animal experimental results to humans. On a long run these approaches can then be used in alternative testing strategies for in vitro-in vivo extrapolations.

Legal data requirements will play an important role in the ultimate submission of non-animal kinetic data. No barriers seem to exist in submitting 3R methods for kinetics. For example, EFSA described in a recent guidance that toxicokinetic data can be derived from a suite of studies covering ADME, including *in vitro*, *in silico* and *in vivo* studies, and single and repeated dose kinetics (Adler et al., 2011; EFSA, 2012). Nonetheless, offering the opportunity to submit non-animal kinetic data is not the same as a legal requirement to do so. Indeed, a recent change in Regulation (EU) No 283/2013 (laying down the data requirements for pesticide active substance evaluations) shows the importance of legislation in enhancing 3R kinetic data submission. According to this new regulation *in vitro* metabolism studies with animal and human material (microsomes or intact cell systems) must be submitted for new pesticide active substances to evaluate possible species difference (EU, 2013). Because of this regulation, 60% of pesticide active substance evaluations contain *in vitro* metabolism data upon first submission (Punt et al., 2017). These results indicate the importance of establishing harmonized regulatory policy on the inclusion of 3R kinetic methods in risk evaluations of food chemicals. The need for harmonized legislation was also indicated as an important factor affecting the acceptation of

3R methods in safety evaluations of food chemicals in the current study (Table 1, Factor 4).

Recommendation 2: Make *in vitro* kinetic data a regulatory data requirement as is the case within the current EU Regulation for pesticide active substances.

Stimulating adoption in (food) safety evaluation also requires validation of 3R methods for kinetics. The food panel clearly identified a limited predictability of 3R methods/lack of validation as the major barrier in the implementation of 3R methods (Factor 1, Table 1). In addition, insufficient guidance is a defined barrier (Factor 3, Table 1). These two barriers may be very much related. Concerns about predictability, lack of validated methods and guidance for regulators also apply to 3R methods for kinetics (Punt et al., 2017; Coecke et al., 2013; Bessems et al., 2014). *In vitro* methods for kinetics capture individual aspects of kinetic processes, including for example absorption and metabolic rates of a compound. For most *in vitro* kinetic methods (e.g. Caco-2 permeability, metabolism measurements with microsomes or hepatocytes), reported experimental conditions within scientific literature are manifold and no uniform protocols have been developed. No standardized procedures are also available for the integration of *in vitro* kinetic data with *in silico* physiologically based pharmacokinetic (PBPK) models (Paini et al., 2017). Given that from a scientific point of view, the predictability of these 3R methods for kinetics is generally good (Punt et al., 2017), there is a need to develop standardized protocols and validation of these protocols, specifying also the boundaries of the different assays (e.g. type of chemicals that fall in a specific applicability domain) and acceptance criteria for risk assessors (e.g. time-range experiments, checks for linearity) (Brooks et al., 2004). These results indicate the importance of policy strategies to steer opportunities, not only for the development of new methods, but especially for adequate validation of existing 3R methods. The involvement of risk assessors in the development and validation of 3R methods for kinetics is essential to meet the need for guidance for regulators/industry. The need for funding is supported by a comment of one of the stakeholders, who indicated that there is currently a “Lack of funding for proper validation and development of alternative techniques” (Table 1, factor 10).

Recommendation 3: Creating (funding) opportunities for validation of non-animal methods for kinetics and the development of guidances.

4. Conclusion

In conclusion, the stakeholder opinions revealed various critical factors that influence the development and regulatory acceptance of 3R methods within food safety evaluations. These include i) uncertain predictability of 3R methods/lack of validation (barrier), ii) the possibility of 3R methods to provide more mechanistic information (driver), iii) insufficient guidance regulators/industry (barrier) and iv) insufficient harmonization of legislation (barrier). Given that the expert panel consisted of toxicologists from academia, industry, and regulatory authorities, it should be noted that the identified factors predominantly presents a scientists’ perspective. Inclusion of stakeholders from the public domain, such as NGOs, was beyond the scope of the present study, but could provide relevant additional insights, e.g. on moral issues. Using the stakeholder opinions to define a policy strategy towards the inclusion of 3R methods for kinetics in regulatory safety evaluations of food chemicals, indicate the importance of steering regulatory data requirements as well as creating (funding) opportunities for the development and validation of 3R kinetic methods and the development of guidances.

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