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Editorial

Quantitative in vitro to in vivo extrapolation (QIVIVE): An essential element for in vitro-based risk assessment

There is increasing recognition of the need to use efficient approaches to perform risk assessment of high numbers of chemicals in a short time. The reliance on approaches consisting of live animal experimentation has its drawbacks in terms of ethical, economical and – not least – scientific limitations in assessing risks in a high-throughput mode. The quantitative interpretation of toxic effects of compounds in in vitro studies, using in silico approaches such as systems biological descriptions of toxicity pathways and physiologically based biokinetic modeling (PBBK), is a necessary component of the National Academy of Sciences vision on toxicity testing in the 21st century (NRC, 2007). The limited studies performed with this approach to date have shown that good predictions for the risk of the use of chemicals can be made. However, a number of limitations have also become clear and more standardization of methods is needed before quantitative in vitro to in vivo extrapolations (QIVIVE) can be fully implemented in risk assessments

The first article in this special issue (Blaauboer, 2015) is an introduction to QIVIVE for the readers. It gives an overview of the progress in using in vitro data for risk assessment purposes as well as current issues in implementing in vitro toxicology in toxicological risk assessment. Key elements of using in vitro experimental assays, the majority of which have largely been developed to study the effects of chemicals at cellular or tissue levels and to understand their mechanism of actions, to quantitatively access risks of chemical exposure in humans are discussed. The importance of integrating in vitro experimental and computational modeling approaches is emphasized with regard to addressing the complexity of biokinetics both in vitro and in vivo, solely based on in vitro or in silico information. In this line of thought, the following elements of the QIVIVE process are covered in this special issue:

- How can we effectively and efficiently integrate information on the metabolism of compounds, to estimate in vivo clearance as well to characterize the potential for bioactivation?
- How can we improve the accuracy of in vitro toxicity assays by determining the free concentrations of chemical compounds that come into contact with the cells?
- How can we provide a flexible and yet robust scheme for integrating these different elements in a high throughput environment?
- How can we use in silico modeling approaches to support animal-free testing?

We believe that each of the next 10 articles in this issue describe both focused and integrated research maturing in the field to address these elements to support assessing human risks from chemical exposure on the basis of in vitro toxicity data.

The relevance of estimated safe human exposure conditions based on in vitro concentration-response relationship for toxicodynamic effects is critically dependent on how properly kinetics are considered. Incorporation of kinetics in QIVIVE has two aspects. One is to characterize the true effective concentration of a chemical in the in vitro assay system in order to link it to the relevant counterpart at the site of action in the in vivo target tissue or cells. The other is accounting for in vivo absorption, distribution, metabolism, and excretion, which in vitro cell-based assays inherently lack. Kinetic modeling approaches are required along with appropriate in vitro experimental data for kinetics to achieve these two goals. The first 6 articles are devoted to describe the essential steps for incorporating kinetics in QIVIVE (understanding the need for considering kinetics, obtaining kinetic data from in vitro and in silico experiments, performing biologically relevant extrapolation of in vitro-derived values based on free concentration), and provide case studies on predicting the equivalent human exposure to the effective concentration in vitro (Wilk-Zasadna et al., 2015; Tolonen and Pelkonen, 2015; Groothuis et al., 2015; Gülden et al., 2015; Yoon et al., 2015; Campbell et al., 2015).

Wilk-Zasadna and colleagues, 2015 discuss the importance of incorporating biokinetic information into integrated risk assessment based on non-animal approaches. Understanding in vitro kinetics in in vitro toxicity testing systems is imperative for a proper in vitro–in vivo extrapolation of toxic responses. Although metabolism is considered an essential part of kinetic evaluation, predicting metabolism in vitro or in silico is still regarded as a bottleneck in implementing QIVIVE in practice. This article discusses the current status of in vitro metabolism studies for QIVIVE extrapolation to support non-animal based hazard and risk assessment approaches. A short overview of the methodologies for in vitro metabolism studies is provided along with recommendations for priority research and other activities to ensure further widespread acceptance of in vitro-based metabolism prediction. Tolonen and Pelkonen, 2015 provide a focused discussion on analytical challenges for conducting rapid metabolism characterization for QIVIVE. A precise and robust analytical technique for identification and measurement of a chemical and its metabolites is an absolute prerequisite in predicting

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metabolism and biokinetics for QIVIVE. High-resolution mass spectrometry is considered as the best tool at the moment for the purpose. However, it is clear that improvements in techniques for separation and detection, identification and quantification of chemicals are required for QIVIVE in order to cover the wide spectrum of chemicals. This article covers current challenges in this area focusing on LC–MS techniques and also highlights key factors associated with sample preparation, testing conditions, and strengths and weaknesses of a particular technique available for each of the relevant tasks. It is of note that the reliability and robustness of quantification of a chemical and/or its metabolites is the most important prerequisite for QIVIVE to achieve the most reliable in vivo prediction, which cannot be overlooked due to the need for high throughput.

Consideration of biologically effective, i.e., free, concentration is one of the most important factors in successful QIVIVE and yet is a major source of uncertainty. Groothuis and colleagues 2013 provided a thorough review on dose metric consideration issues in in vitro assays with an aim to reduce current uncertainties associated using incorrect dose metrics in conducting QIVIVE in regulatory toxicology. Major factors affecting the free concentration in vitro are summarized along with suggestions for experimental or modeling methods to measure or predict this free concentration. Recommendations are given when and how to consider alternative dose metrics instead of nominal concentrations in in vitro assay systems with which one can expect to reduce effect concentration variability between in vitro assays and to better relate effect concentrations in vitro to their equivalent biologically effective concentrations in vivo. The study conducted by Gülden and colleagues 2013 is a good case study demonstrating the importance of considering in vitro experimental system-related factors that affect chemical bioavailability in vitro and consequently, the apparent concentration-effect relationship. They illustrate how cell binding affects the bioavailability of a chemical in an in vitro toxicity assay using the observed apparent dependency of EC50 on cell density in the incubation vessel. The free concentration is suggested as the most applicable dose metric for concentration-effect relationship analysis. To estimate free concentration as well as factors affecting bioavailability of a chemical in cellular assays such as cell binding, a combination of modeling approaches is recommended. Characterizing in vitro dose-metrics is the first and the most critical step in QIVIVE as obtaining correct dose-response curves in vitro determines the reliability of using QIVIVE for risk or safety assessment purposes.

Kinetic modeling, especially PBBK modeling, is a key component in QIVIVE. It provides a way to incorporate kinetics into consideration in animal-free, in vitro-based safety/risk assessment and to relate in vitro toxicity assay findings to human safe exposure estimates. To be truly animal-free testing, these models also need to be developed based on in vitro or in silico data. A case study on carbaryl by Yoon and colleagues 2014 shows the process of parameterizing a PBBK model using in vitro kinetic data. It guides the readers through steps for biological scaling of in vitro-derived metabolic constants to corresponding in vivo parameters and also for defining free concentrations that are available for metabolism or other interactions with enzymes, e.g., binding to cholinesterases, both in in vitro and in vivo. Although current limitations are discussed as well, the proposed in vitro-based parameterization approach for developing PBBK models will contribute to reducing the need for in vivo human data for model development as well as uncertainties associated with using animal based parameters in predicting human safety. It will also promote the use of QIVIVE for safety or risk assessment purposes by increasing the availability of PBBK models for the process. Campbell and colleagues 2015 used PBBK models parameterized using both in vitro and in silico information to conduct QIVIVE for parabens. In their study, human biomonitoring data for parabens were used to calculate margins of safety for the potential estrogenicity of parabens. This study shows that human biomonitoring data can be used to support animal-free in vitro-based safety assessment through QIVIVE. Effective concentrations for potential biological effects, the estrogenicity of parabens in this case, are measured in vitro. Using biokinetic modeling and reverse dosimetry, the daily dose that would produce the equivalent paraben concentrations in blood can be predicted. Similarly, urinary concentrations of parabens and their metabolites that correspond to the estimated daily dose can be predicted by the PBBK models for comparison with biomonitoring data. The article by McNally and colleagues, 2013 focuses on the process of bringing the estimation of safe human exposure through QIVIVE to the population level using PBBK modeling. They introduce a virtual human population generator, PopGen which is a publically available web-based application that simulates realistic human variability in anatomical, physiological, and phase I metabolism parameters in healthy populations. They demonstrate how this modeling platform can be used for QIVIVE by providing population parameters for PBBK models. In addition, case studies for exposure reconstruction from human biomonitoring data measured in blood are presented as analogs of the process of exposure or dose reconstruction from concentration-response measurements from in vitro assays.

The second part of this special issue is devoted to implementation of QIVIVE for today's risk or safety assessment (Wetmore, 2015; Adeleye et al., 2015; Meek and Lipscomb, 2015). The review provided by Wetmore, 2015 describes QIVIVE in a high-throughput environment. In addition to providing a way to conduct animal-free testing with more human relevance, high-throughput in vitro toxicity testing strategies present an opportunity to rapidly screen a large number of chemicals. However, effective concentration information from high-throughput in vitro toxicity screening assays can be misleading if kinetics are not considered. QIVIVE was conducted using the data from high-throughput in vitro hepatocyte clearance and protein binding assays, so called high-throughput kinetics, to predict in vivo oral exposures needed to achieve blood concentrations equivalent to those eliciting bioactivity in vitro. In conjunction with human exposure estimates, this high-throughput QIVIVE provided a means to prioritize chemicals based on the in vitro assay-based margin of exposure, demonstrating an example of applying QIVIVE for risk assessment in a high-throughput manner. Although in vitro assays are developed for mechanistic studies for potential biological effects of chemical exposure, it would require an innovative approach to define a point of departure for human safety based on in vitro findings. Adeleye and colleagues 2014 present their progress in a prototype toxicity pathway-based risk assessment that aims to implement the Toxicity Testing in the 21st century vision. They guide the readers through the process of examining a prototype toxicity pathway, DNA damage responses via the p53 network, and constructing a strategy for the development of a pathway based risk assessment using a case study approach. The goal was to evaluate if the in vitro dose-response for quercetin could be sufficiently understood to construct a pathway-based risk assessment without performing in vivo carcinogenicity studies. High content dose-response data was used to determine point of departure concentrations in vitro that are then related to blood concentrations in vivo. This study presents the current progress in an ongoing research effort aimed at providing a pathways-based, proof-of-concept, in vitro-only safety assessment for consumer use products. Finally, Meek and Lipscomb, 2015 provide a discussion on the current challenges and strategies regarding gaining acceptance for the use of in vitro toxicity assays and QIVIVE in regulatory risk assessment. They provide current experience in the incorporation of mechanistic and in vitro data in risk assessment as case studies in the context of

identified principles to increase the potential for timely acceptance of more progressive and tailored in vitro testing strategies by the regulatory community. They propose a pragmatic, tiered data driven framework which includes increasing reliance on in vitro data and QIVIVE in consideration of these principles.

In this special issue, each of the articles highlights the current status, challenges, and future directions to promote the use of in vitro data in risk and safety assessment, with emphases on different aspects of QIVIVE. However, one thing that is recognized and emphasized in common in all of the articles is the need for concerted efforts in order to move forward on integrated testing strategies for animal-free toxicity testing that are based on in vitro information and modeling approaches. We note that some of the topics introduced in this issue might already be outdated by the time they are published, as we are witnessing remarkably rapid advances in the in vitro toxicology as well as computational modeling fields. Our hope is that this is indeed the case and the next special issue will come out soon with more case examples of QIVIVE implementations in regulatory toxicology.

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