Developing In Vitro Metabolic Clearance Data to Support Development of the Internal Threshold of Toxicological Concern (iTTC)

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Background: Regulatory agencies across the world are facing the challenge of performing risk-based prioritizations and evaluations of thousands of chemicals in commerce. Rather than perform extensive in vivo testing based on toxicological responses in animals, which, in terms of time and resources, is not practical for thousands of substances, there has been a concerted effort within the scientific and regulatory communities towards development and utilization of alternative approaches. The Threshold of Toxicological Concern (TTC) is an approach that can be integrated with knowledge of exposure to enable efficient risk-based prioritization and screening of thousands of chemicals (Patlewicz et al., 2018). The TTC is used as a surrogate for chemical specific toxicity criteria. TTC values were derived from the evaluation of empirical curated toxicity dose response data from hundreds of chemicals with diverse structures, with different TTC values assigned based on structural considerations. If human exposure to a substance is below the relevant TTC value, it can be judged "with reasonable confidence, to present a low probability of a risk" (Munro et al., 1996).

As noted by Ellison et al. 2019, "An 'internal TTC' (iTTC; i.e., a TTC based on plasma concentration) has been suggested by several groups (Bessems, 2009; Coecke et al., 2013; Hartung, 2017; Partosch et al., 2015) as a possible evolution of the externally-based TTC that could be a useful approach in the development of non-animal methods and as a tool to further refine the use of TTC and expand its applicability. If an iTTC database could be established to derive iTTC thresholds, then human plasma concentrations (estimated or measured) for a given exposure scenario could be compared to these iTTC thresholds." Potential applications of the iTTC thresholds could include: risk-based safety assessments for dermal and inhalation exposures; development of metabolism-based structure-activity relationship assessments; risk-based screening of aggregate exposures of a given substance from multiple routes of exposures; in vitro safety evaluation by comparing the iTTC to concentrations producing bioactivity in in vitro biological assays.

Ellison et al. 2019 have proposed an approach for deriving iTTCs (Figure 1.). The steps in this approach are:

1. Identifying existing TTC datasets which contain chemical specific NOAELs expressed as an external dose in mg/kg/day.

2. For each chemical, identify chemical-specific ADME data through a multi-tiered approach of literature searching, using in silico estimation tools and generating empirical data. This ADME data will be used as input parameters to support PBPK modeling.

3. Chemical-specific PBPK modeling will then be conducted to convert the chemical-specific external exposures from the TTC dataset of chemicals to an internal blood concentration (Css, AUC, or Cmax) for each chemical. PBPK modeling will be conducted using the appropriate species, dose, and route from the toxicity study. This will provide an estimate of the chemical-specific internal exposure associated with a given NOAEL from that toxicity study.

4. The distribution of chemical-specific Css values will then be evaluated and an appropriately low (e.g. 5th percentile) Css threshold will be identified for the group of chemicals and by Cramer Class.

5. These Css thresholds will then be used to derive iTTC values. Appropriate adjustment factors will need to be considered and applied to the Css values.

Research Objectives and Project Scope:

Laboratory studies twill generate empirical animal model in vitro hepatic metabolic clearance data for a specific set of chemicals that comprise the TTC database. This research project includes: chemical procurement, bioanalytical method development and generating data for approximately 180 chemicals. The in vitro intrinsic clearance of the test chemicals will be determined using a substrate depletion approach (Obach et al., 1999). Decrease in parent chemical concentration over time is measured with a validated analytical method. Incubations using enzymatically inactive cryohepatocytes are carried out as negative controls to distinguish between enzymatic biotransformation and biotic decease. In vitro intrinsic clearance is evaluated using cryopreserved hepatocytes. Assays will include test chemical and pooled cryopreserved hepatocytes (e.g. 0.5 million viable cells/mL and 0.2 mL reaction volume), in suspension, from preclinical species (primarily rat, but in some instances may also include mouse, dog or rabbit). The species will be the same as the species used in the in vivo chemical toxicity study used in developing the TTC database. Six time points (e.g. 10, 20, 30, 40, 120 240 min) will be evaluated.

The chemical selection process for the metabolism studies will be conducted by the ACC LRI iTTC project team in consultation with the Cosmetics Europe iTTC project team. The chemical selection process follows these principles: (1) Only non-fragrance materials will be tested. Note: it is expected that fragrance materials will evaluated in one or more separate research projects that will be supported by RIFM; (2) Cramer Class I and III chemicals will be tested. These Cramer Classes are most readily accepted and cover the majority of industry chemicals. Cramer Class II chemicals will be supported by RIFM sponsored studies, since Cramer Class II is important for fragrance materials; (3) 83 chemicals which had in vivo PK data but lacked in vitro metabolism data (based on the literature search) will be tested. The new metabolism data will allow in vitro to in vivo extrapolation comparisons; (4) 100 chemicals lacking any existing in vivo PK data and in vitro metabolism data will be tested. Chemicals have been grouped using a clustering approach (k-means cluster) that accounts for structure and physiochemical properties and assigns the chemical to one of twenty-five possible bins. Four chemicals will be tested for each bin, two chemicals (a high and low NOAEL) for Cramer Class I and 2 chemicals (a high and low NOAEL) from Cramer Class III will be included. When there are ‘ties’ within a given cluster-Cramer Class grouping (i.e. two chemicals with the same high NOAEL), preference will be given to the non-cosmetic chemicals. Applying the above principles to the iTTC chemical list results in ~180 chemicals for in vitro metabolism studies. The chemical space for the test chemicals will span across Cramer Class I and III and will represent a variety of industrial chemicals.

Implications: An ‘internal TTC’ (iTTC; i.e., a TTC based on plasma concentration) represents an extension and evolution of the externally-based TTC. The iTTC is envisioned to be a useful approach in the development of non-animal methods and as a tool to further refine the use of the TTC concept and expand its applicability.

Collaborations: Overall, this research project will be complimentary to projects supported by Cosmetics Europe and RIFM

Key words: TTC, iTTC, hepatic metabolism, PBPK, in vitro clearance

Project start and end dates: June 2019 – December 2020

Peer-reviewed publication(s): None to date.

Other publication(s): None to date.

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