A framework for improving the quality of in vitro toxicity data for quantitative *in vitro* to *in vivo* extrapolation of 'difficult' chemicals

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The nominal concentration, i.e. the theoretical concentration based on amount of test chemical added to culture medium, is generally used to express concentration-effect relationships in *in vitro* toxicity tests. However, for instable, volatile, highly plastic- and plasma protein-bound chemicals, the nominal concentration does not represent the concentration responsible for the observed effect at the target site in cells. The aim of this study is to develop a framework to better dose and assess chemical exposures in *in vitro* assays to improve quantitative *in vitro-in vivo* extrapolations (QIVIVE), and thus the regulatory acceptance of *in vitro* toxicity assays. The "Better *in vitro* Dosing" framework consists of a decision tree to help toxicologists decide how best to dose and control the exposure of their test chemical in *in vitro* toxicity assays. Firstly, physicochemical property cut-offs, including for log D, plasma protein binding and Henry's Law Constant, are to be proposed to indicate when alternative dosing technologies are beneficial. Secondly, three tools are developed to dose *in vitro* toxicity assays that account and control for the degradation, evaporation and binding of chemicals to the *in vitro* system setup. These tools include 1. exposing cells in capped glass vials that can be aligned to analytical instruments to confirm exposure concentrations, 2. partition-controlled dosing where polymers sheets are loaded with test chemicals and added to *in vitro* wells to continuously dose the exposure medium, and 3. a custom-made closed chamber system for dosing (semi)volatile chemicals through the headspace. As a proof-of-principle, the tools are tested on the ToxTracker and ToxProfiler assays, a series of reporter gene assays for genotoxicity and cellular stress. First results with alkylbenzenes, phthalates and bisphenols indicate that for a select number of chemicals using these tools indeed maintain chemical concentrations in exposure medium constant and result in reproducible concentration-effect relationships. Experimental results, together with public physicochemical and bioassay data, are integrated into a database, to be used in the decision tree as well as in physiologically based pharmacokinetic (PBK) and QVIVE models. Translating these effect concentrations using PBK models yields *in vivo* inhaled doses known to cause acute toxicity in animal tests.