SRA Dose-Response Specialty Group
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In vitro-in vivo extrapolation of the dose-response relationship for cellular perturbations by toluene using a cellular dosimetry model

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There is increasing interest and focus on animal replacement methods and perturbation of toxicity pathways in risk assessment. The report on toxicity testing in 21st century by the National Academy of Sciences has raised the awareness and triggered further development of animal-alternative methods and models to replace traditional toxicity tests. One of the bottlenecks in this regard relates to the lack of tools to interpret the results of in vitro and high throughput assays for risk assessment purposes, and particularly to extrapolate to in vivo conditions. In vitro to in vivo extrapolations have already been conducted to estimate LOAEL using physiologically based pharmacokinetic (PBPK) models (Blaauboer, B. J., et al. 2000. in Progress in the reduction refinement and replacement of animal experimentation. Balls, M. van Zeller, A.M and Halder, M. E. Eds. pp 525-536). However no tool is available to facilitate the calculation of dose to cells both in vitro and in vivo. Such cellular dosimetry tools would be useful for the conduct of in vitro to in vivo extrapolations. In this study, multicompartmental models describing the in vitro and in vivo systems were developed and evaluated using toluene as the model substrate. Specifically, the objective of the study was to use cellular level PBPK modeling to predict in vivo dose-response relationship from in vitro concentration-response relationship. The in vitro and in vivo models have been described as two and four compartments, respectively. The in vitro model contained two compartments: the cell and the culture medium (CM). The in vivo model of the tissues consisted of four compartments: the cell and interstitial fluid (IF) as the extravascular components; the erythrocyte and plasma for the vascular (blood) component. Cellular level human PBPK model was adapted from the model previously developed by Tardif et al. (1997; Toxicol appl pharmacol 144:120-134). The original model consisted of four compartments: liver fat richly perfused and poorly perfused tissues. A brain compartment was added for the purpose of the present study. Each compartment was then subdivided into the cell, the interstitial fluid and the blood (plasma + erythrocytes) which were interconnected by diffusion constants and tissue blood flow rates. The distribution of toluene between the compartments of both in vitro and in vivo models was calculated by associating together the partition coefficients relating those compartments (Cell:CM, cell:IF, IF:blood and tissue:blood partition coefficients (PCs)). The free concentration of toluene in neutral lipids, neutral phospholipids and water was calculated on the basis of its solubility (vegetable oil:water PC = 603.4). In turn, the toluene binding to haemoglobin was calculated by multiplying the free concentration in the microenvironment and a haemoglobin:microenvironment PC. The in vitro toluene concentration in the neuroblastoma culture system was calculated by multiplying the CM concentration (McDermott et al., 2007. Toxicol Appl Pharmacol, 219:85-94) by the cell:CM PC. The in vivo concentration within the human PBPK model was calculated based on the consideration that the substance crosses the capillary wall and diffuses into the interstitial subcompartment, and from the IF, diffuses into the cell across the cellular membrane. Thus, the simulation of the in vivo brain cell concentration of toluene by the human PBPK model was facilitated by the inclusion of cell:IF PC. The PBPK simulations were carried out to extrapolate

the toluene concentration-response relationship from in vitro SH-SY5Y cell toxicity study (McDermott et al., 2007. Toxicol Appl Pharmacol, 219:85-94) to in vivo steady state conditions (20 days exposure). The toxicity endpoints chosen were the LDH leakage and the intracellular Ca^{2+} concentration. The EMEM:air PC predicted in the present study was 78 % of the value measured by McDermott et al. (2007. Toxicology in Vitro 21:116-124). For LDH leakage and intracellular Ca^{2+} effects, the in vitro concentrations of 5.64 (NOAEL), 13.1 (LOAEL), 22.6, 47.0, and 76.5 µM corresponded to the human exposure concentrations of 154.4, 256.8, 376.2, 678.3, and 1043 ppm based on identical brain cell concentrations at steady-state. In comparison, the less serious LOAELs for neurological effects have been reported to range from 35 ppm (17 yrs average; increased color confusion index) to 200 ppm (>18mo 2-8 h/d; mild intoxication) for humans (ATSDR, 2000). Overall, the cellular dosimetry model developed in this study represents a potentially useful tool for conducting in vitro-in vivo extrapolation of the cellular-level effects and perturbations evaluated in high throughput assays. (Supported by AFSSET).