TOX/2019/73

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Case studies: applications of physiologically-based pharmacokinetic (PBPK) modelling in human health risk assessment.

Introduction

1. An insufficiency of human pharmacokinetic data is often noted for xenobiotics for which PBPK models are developed and assessed by the Committee. Therefore, a scoping paper on physiologically-based pharmacokinetic (PBPK) modelling used for human health risk assessment (TOX-2019-34) was taken to the COT in July 2019. The discussion of the Committee focussed on ways to assess the reliability of human PBPK models in the absence of human pharmacokinetic data. Approaches that were considered to assess model reliability in this context included use of the read-across approach and conducting interspecies extrapolations to animal species other than humans.

2. The Committee agreed that it would be useful to have further information in the form of case studies, for example where *in vitro* data have been successfully extrapolated to *in vivo*, or cases where risk assessments considered in retrospect may have benefitted from PBPK modelling. It was also noted a workshop on PBPK would be beneficial since the last one hosted by the COT was in 2003. Further information regarding the application of PBPK models in risk assessment is included in this paper.

Role of PBPK modelling in risk assessment

3. Risk assessment is conducted to establish human exposure levels without appreciable risk, and to assess the health risks associated with particular exposure scenarios. Risk assessment requires an understanding of both the hazard characteristics of the chemical and exposure. It is in exposure analysis that PBPK modelling has an important role to play.

4. Chemical risk assessments do not routinely evaluate internal dose. However, internal dose relates more directly (temporally and spatially) to a toxicological response than external dose, and thus provides a better basis for the evaluation of risk. Computational approaches such as the use of PBPK modelling can be used to simulate toxicokinetic data and to estimate internal doses.

Regulatory drivers for the inclusion of toxicokinetic data in risk assessment

5. There has been an international harmonisation of guidelines for the acquisition of toxicokinetic data for pharmaceutical chemicals, which plays a central

role in their safety assessment (Baldrick 2003, ICH 1995). For other xenobiotics however, particularly agrochemicals, the generation of toxicokinetic data in risk assessment is less routine. When toxicokinetic data are generated, it is usually done on a case-by-case basis to refine a risk assessment. Terry *et al.* (2016) provide an overview of the major regulatory initiatives for the integration of toxicokinetic data into the risk assessment process of agrochemicals. These are as follows:

- Guidance documents from REACH outline that the toxicokinetic profile should be considered as part of the human health hazard assessment, for example by deriving a kinetically-derived maximum dose (KMD[1]) for use in repeat dose animal studies (ECHA 2008). However, there is no official requirement for toxicokinetic data due to an insufficient consensus amongst regulators and safety assessors as to how it would be used (ECHA 2008).

- Both ICH and OECD have recognised that toxicokinetic data can improve the assessment of internal exposure and thus the quality of chemical hazard and risk assessment (ICH 1995, OECD 1984). OECD has started to incorporate toxicokinetics into both study design and dose level selection, although the collection of toxicokinetic data is not an official requirement (OECD 2007, 2009, 2010a). The concept of the KMD is acknowledged in various OECD test guidelines including those used for detecting reproductive toxicity (OECD 2011a, OECD 2011b), and for chronic toxicity and carcinogenicity studies (OECD 2010b).

- The Agricultural Chemical Safety Assessment (ACSA) from the Health and Environmental Sciences Institute (HESI) proposes an improved approach to assessing the safety of agrochemicals and advocates the generation and incorporation of ADME and toxicokinetic data in the chemical risk assessment process (Barton *et al.* 2006, Carmichael *et al.* 2006).

- EU regulation 1107/2009 (which replaced 91/414/EEC, the Directive which governs the use of plant protection products within the EU market) states that toxicokinetic data should be used to inform dose selection, and contains significant new requirements for generation and use of toxicokinetic data for most types of toxicology studies (EC 2010).

Instances where PBPK models can be applied in risk assessment

6. The acquisition of toxicokinetic data can improve the quality of chemical risk assessment in various ways (ICH 1995, OECD 1984). There is increasing recognition that collection of toxicokinetic data can assist interpretation of toxicity studies for human risk assessment (Barton *et al.* 2006, Saghir *et al.* 2006). It is important that animal studies are appropriately designed so that the route, frequency

[1] The KMD is based on the premise that the induction of non-linear toxicokinetics can be used as an equivalent indicator of biological stress alongside other traditional toxicity endpoints such as tumour formation. The KMD is defined as the top external dose level for use in toxicology studies and corresponds to the 'inflection point' of non-linear toxicokinetics in repeat-dose studies.

and duration of exposure in rodents are relevant to expected human exposures. Actual or simulated toxicokinetic data can be used to:

- Derive chemical-specific adjustment factors and biomonitoring equivalents, and perform reverse dosimetry and extrapolations across dose, exposure route, and species (described in TOX-2019-34).

- Inform dose selection to derive a KMD and optimal sampling times, or interpret study data where nonlinear toxicokinetics may explain atypical dose responses (Rhomberg *et al.* 2007).

- Give insight into sensitivities to toxicity between individual animals, genders, species or life stages. For example, it is possible to assess whether the observed sensitivities are related to toxicokinetics or toxicodynamics[2].

- Interpret dose-response relationships to support mode-of-action hypotheses about adverse effects seen in animal toxicology studies. For example, to determine that an absent toxicological response is not due to insufficient internal exposure.

- Select appropriate concentrations for *in vitro* assays, or to aid interpretation of the results.

Cases where PBPK models have been applied in risk assessment

Perfluorooctanesulfonic acid and perfluorooctanoic acid (PFOS & PFOA)

7. EFSA (2018) documented the PBPK modelling that was performed to estimate the relationships between serum concentrations of PFOA and PFOS and dietary intakes. The resultant BMDLs expressed as PFOS and PFOA levels in plasma, were converted into dietary exposure values, corresponding to lifetime continuous exposure (daily ingested dose).

8. PFOA and/or PFOS are taken up into the plasma (via intravenous, i.v., dosing) or into the gut (via oral dosing). From the gut, PFOA and/or PFOS are transported to the liver by the portal blood. Only the free fractions of PFOA and/or PFOS in plasma are assumed to be available for partitioning into tissues. PFOA and/or PFOS is eliminated through the filtrate compartment to storage into urine, while in the filtrate compartment, PFOA and/or PFOS can be reabsorbed back into the plasma through a saturable process with a transporter maximum constant (Tmc) and affinity constant (Kt).

[2] Toxicodynamics is an assessment of how tissue dose relates to the toxicological response. For example, sulfoxaflor induced reproductive toxicity in rats but not rabbits dosed at a level ≥ 400 ppm in the diet. As both species exhibited similar toxicokinetics, it was possible to determine that this species difference was due to toxicodynamics (Rasoulpour *et al.* 2012).

9. A growth equation was used in the modelling, based on a French survey, describing the increase in weight with age of 4,078 subjects, aged between 3 and 60 years, and 703 subjects of less than 3 years (Loccisano *et al.* 2011).

10. Infants who are breastfed will have an additional exposure during infancy. This varies with length of breastfeeding and the concentration of PFOS/PFOA in breast milk. Breastfeeding increases the plasma concentrations of PFOS/PFOA at the end of infancy and affects the maximum daily ingested dose after infancy that would not lead to a 5-year-old child reaching the $BMDL_5$. The following assumptions were made: the ratio between cord blood and maternal blood PFOS concentration is 1:3 (ATSDR 2015, Manzano-Salgado *et al.* 2015) and the ratio between breast milk and maternal blood PFOS concentration is 1.5:100 (Kärrman *et al.* 2007, Haug *et al.* 2011, Kim *et al.* 2011, Liu *et al.* 2011).

11. Two breastfeeding scenarios were assessed for PFOS:

In scenario 1, modelling was performed assuming a maternal concentration of 7.7 ng/mL (median of medians for adults). The starting serum concentration of PFOS in the newborn was assumed to be 2.6 ng/mL. The PFOS concentration in breast milk was assumed to be 0.12 ng/mL. The constant exposure after breastfeeding was modelled at 1.8 ng/kg bw per day. The resulting serum concentration in the child at 5 years of age was about 7 ng/mL.

In scenario 2, modelling was performed assuming a maternal concentration of 22 ng/mL ($BMDL_5$ in Eriksen *et al.* 2013). The starting serum concentration of PFOS in the newborn was assumed to be 7.3 ng/mL and the PFOS concentration in breast milk was assumed to be 0.33 ng/mL. The constant exposure after breastfeeding was modelled at 1.8 ng/kg per day. The resulting serum concentration in the child at 5 years of age was about 9.6 ng/mL.

12. Comparisons were made between the Loccisano PBPK model, another PBPK model by Worley and a one-compartment steady-state PK model (based on the work of Bartell *et al.* 2010, Olsen *et al.* 2007 and Harada *et al.* 2010) for the chronic daily intake of PFOA that would lead to a plasma concentration of 9.4 ng/ml which is the $BMDL_5$ for an increase of total cholesterol from Steenland *et al.* (2009). All models gave values of around the same order of magnitude (0.85, 1.2 and 2.3 ng/kg bw/day, respectively).

13. Comparisons were also made between the Loccisano PBPK model and the one-compartment steady-state PK model for the chronic daily intake of PFOS that would lead to a plasma concentration of 22 ng/ml which is the $BMDL_5$ for an increase of total cholesterol from Eriksen *et al.* (2013). Again, both models gave values of around the same order of magnitude (1.8 and 2.3 ng/kg bw/day, respectively).

Dioxins

14. To estimate the intake leading to the critical serum levels or body burden, the EFSA CONTAM Panel (2018) considered two models that had been developed that consider accumulation in body fat, induction of liver CYP enzymes, liver sequestration and growth. They evaluated both the model developed by Emond *et al.* (2005, without the breastfeeding period that was included in a later model, Emond

et al. 2016) and a Concentration- and Age-Dependent Model (CADM) as developed by Carrier *et al.* (1995) and optimised by Aylward *et al.* (2005).

Emond model

15. Using the Emond (2005) model, a daily intake of 2 pg/kg bw in a woman of 35 years of age was estimated to result in serum, adipose tissue and liver levels of 51, 19 and 919 pg/g fat, respectively. Thus, there was a ratio of 2.7 between lipid-based levels in serum and adipose tissue, and of 4.1:1 between liver and adipose tissue levels in wet weight (ww) terms. In this model, sequestration increased at relatively low body burdens, with a half-maximal increase in the liver-to-adipose tissue ratio at a body burden around 50 ng/kg ww.

16. EFSA regarded the dose-independent ratio of 2.7 between the lipid-based level in serum and adipose tissue to be critical. Various studies had found this ratio to be around 1:1. The Estimated Daily Intake (EDI) used for establishing the HBGV would be smaller by a factor of 2.7 when basing it on serum levels rather than adipose tissue levels.

CADM

17. The CADM was developed for TCDD and estimates the levels in the fat compartment (lipid based), liver (wet weight) and total body (wet weight), the latter based on relative fractions of 25% and 3% of the body weight for the fat compartment and the liver. The original CADM took liver sequestration into account and included lipid partitioning of TCDD across the intestinal epithelium from the blood into faecal contents, based on studies by Moser & McLachlan (1999). This model was further adapted by Ruiz *et al.* (2014) to include a growth curve and a breastfeeding period. Several discrepancies were noted, and the model was modified by the CONTAM Panel.

18. Since the CADM was developed for TCDD, which contributes only 3.4% to the current exposure, there was considerable uncertainty with respect to other congeners. Nor do the calculations take historical exposure into account, which may have been higher, but this would have caused higher levels than those predicted. However, there are indications that the levels in food and also exposure did stabilise during the last decade.

19. The data showed that breastfeeding contributes considerably to the serum levels of TCDD at 9 years of age. The milk levels were not linear with the exposure of mothers, due to sequestration and higher elimination at higher exposure. As a result, the impact of breastfeeding decreased at higher exposure of mothers.

Bisphenol A

20. The toxicokinetic models evaluated by EFSA (2015) were the rat and human models previously published by Emond and colleagues (Emond *et al.* 2006, 2017) and the so-called concentration- and age-dependant model (CADM), developed by Carrier *et al.* (1995), further optimised by Aylward *et al.* (2005) and adapted by Ruiz *et al.* (2014) to include a growth curve and a breastfeeding period.

21. No currently available toxicokinetic study in humans covers the time course of unconjugated BPA concentrations in plasma (serum) so internal dosimetrics (e.g., AUC) for unconjugated BPA in humans to support a Human Equivalent Dose (HED) approach are lacking. An HED of 0.1 means that humans require 1/10 of the dose given to an animal, on a mg/kg b.w. basis, to produce an equivalent AUC value. EFSA reviewed the current PBPK models on serum BPA levels for adults and newborns, which included internal dosimetrics for the HED approach.

22. Unknown/uncertain parameter values for gastrointestinal absorption, metabolism, excretion, and enterohepatic recycling in rats were estimated by fitting to oral gavage data in rats. Other parameter values such as the volume of distribution for the conjugated BPA were fitted to human toxicokinetic data and the Michaelis constant for glucuronidation was taken from *in vitro* studies with rat liver microsomes or hepatocytes. Extending the model to humans required (apart from adjusting the physiological parameters) scaling the parameters for metabolism and elimination to human toxicokinetic data (Völkel *et al.* 2002).

23. To assess the aggregated oral and dermal exposure to BPA, information was needed on the dermal absorption and penetration of BPA, to quantify how an external dermal dose translates into an internal dose for unconjugated BPA. This information would allow the conversion of the external dermal dose into an equivalent oral dose to provide an aggregated exposure estimate that could be compared to a HBGV but no suitable human TK study currently exists. However, several *in vitro* studies on cutaneous penetration using pig skin and human skin samples and an *in vivo* study in rats with dermal BPA can simulate the fate of BPA taken up dermally. The CEF Panel used the PBPK model of Mielke *et al.* (2011) to simulate the aggregated oral and dermal exposure, as well as the PBPK model of Fisher *et al.* (2011), to derive the HED. The CEF Panel used a skin absorption of 10% for exposure scenarios with dermal contact to thermal paper, assuming that a steady state concentration gradient for BPA existed across the stratum corneum.

24. PBPK model predictions were performed for adult males and children (1.5 – 4.5 years), using the model parameters given by Mielke *et al.* (2011) and Mielke and Gundert-Remy (2009). Compared to the published model version (Mielke et al., 2011), the PBPK model was slightly modified in respect to the simulation of dermal exposure. For the uptake of BPA from cosmetics, a constant uptake rate was assumed, leading to 50% absorption of the external dermal dose within 24 h. For the uptake of BPA from thermal paper, it was assumed that thermal paper is touched once a day and that BPA migrates into a depot in the moisture film on the skin surface within a short duration of 5 min. The amount migrating into this skin-surface depot is 100% of the external dermal exposure. During each day (= 24 h), 10% of the initial depot content on the skin surface is assumed to diffuse across the skin barrier into the skin compartment according to a first-order process.

25. The exposure to and uptake of BPA from the various sources was implemented as a continuous and constant dermal uptake of BPA in cosmetics, a single dermal contact to thermal paper per day, and a dietary exposure involving 3 meals per day and the oral uptake via dust was assumed to be synchronised with dietary uptake. Dermal uptake via dust was regarded as negligible. PBPK modelling

was used to simulate the serum concentration of unconjugated BPA. The simulation was run for 10 days to reach a steady state.

26. The predicted serum concentration-time profile for the last day was used to determine the area under the curve (AUC). Since only a reference dose for external oral exposure was available EFSA expressed both oral and dermal exposures as external oral equivalents and summed them to give an aggregated exposure.

27. Toxicokinetic studies in various laboratory animal species (Doerge *et al.* 2010a,b,c, 2011a,b, 2012) provide internal dose metrics for neonatal-to-adult stages and for different routes of exposure (oral and intravenous/subcutaneous). PBPK models have also been developed to predict the internal exposures in laboratory animals and humans in a route-specific manner. Overall, this information allows extrapolation to humans and the application of the HED concept for providing Human Equivalent Dose Factors (HEDF) which account for the toxicokinetic portion of the interspecies differences. Multiplying the HEDF by a reference point of a critical toxicity study yielded a human-equivalent oral dose that could be used for risk assessment. For scenarios with aggregated oral and dermal exposures, PBPK modelling was used to estimate the internal dose metrics for unconjugated BPA and to convert external dermal doses into equivalent oral doses.

Acrylamide and glycidamide

28. EFSA (2015) considered several published approaches to PBPK modelling of acrylamide absorption, metabolism and disposition that had the objective of predicting human internal exposures to acrylamide and glycidamide for use in reducing the uncertainty in risk assessment inherent in animal to human extrapolations.

29. HEDs were calculated from the Young *et al.* (2007), Walker *et al.* (2007) and Sweeney *et al.* (2010) PBPK models that predicted rodent AUC/human AUC ratios for a common dose of acrylamide (0.1 mg/kg b.w.) and represented the multiple of the acrylamide dose to a rodent that a human would require to obtain an equivalent AUC for either acrylamide or glycidamide. PBPK modelling predicted that a 1.4 or 2.1-fold higher dose of acrylamide was required in a human to achieve the same glycidamide -AUC as that in the female or male mouse, respectively. Thus, mice appear to be more proficient in converting acyrlamide to glycidamide than humans. The HED for GA-related endpoints derived from the three PBPK models for male rats ranged between 0.69–1.3, whereas the HED from female rats was intermediate between the HEDs from mice.

30. The CONTAM Panel also compared the internal dosimetry for risk assessment human cancer and neurotoxicity as derived from PBPK studies of Doerge *et al.* (2008), Tardiff *et al.* (2010) and DeWoskin *et al*. (2013). In all cases, the reference point from rat dose response data was multiplied by the appropriate HED to determine the human equivalent reference point. The reference points reported were essentially identical to those reported in the US-EPA assessment of acrylamide (US EPA 2010).

31. The Panel concluded that the HEDs derived from equivalent acrylamide-AUCs in rats and mice suggest that endpoints related to acrylamide-mediated effects (e.g. neurotoxicity) require 4- to 6-fold higher doses in rats when compared to humans, based on interspecies differences in toxicokinetics. However, 0.5- to 0.7-fold lower doses of acrylamide would be required in mice to produce equivalent glycidamide-AUCs for genotoxicity-related endpoints when compared to humans. The derivation of these values, however, did not lead to EFSA changing the margin of exposure based on a $BMDL_{10}$ for carcinogenicity in mice that would be considered to be of low concern to the consumer (10,000).

Chloroform and carbon tetrachloride: assessment of interspecies differences in metabolism

32. This study was designed to consider how PBPK modelling could contribute to the establishment of occupational exposure limits (OELs). PBPK models were developed for chloroform (mouse and human) and carbon tetrachloride (rat and human). These substances were chosen for examination because of the extent of their toxicological databases and availability of existing PBPK models. The models were used to predict the rate (chloroform) or extent (carbon tetrachloride) of metabolism of these substances, in both rodents and humans. Monte Carlo analysis was used to investigate the influence of variability within the human and animal model populations. The ratio of the rates/extent of metabolism predicted for humans compared to animals was compared to the uncertainty factors involved in setting the occupational exposure standards. Predictions obtained from the PBPK models indicated that average rates of rat and mouse metabolism of carbon tetrachloride and chloroform, respectively, are much greater than that of the average human. Application of Monte Carlo analysis indicated that even those people who have the fastest rates or most extensive amounts of metabolism in the population are unlikely to generate the levels of metabolite of these substances necessary to produce overt toxicity in rodents. (Delic *et al.* 2000).

Vinyl acetate: interspecies extrapolation of a rat PBPK model to assess human health risk

33. To assess the health risk of inhalation exposure to vinyl acetate, a PBPK model was developed for rats and extrapolated to humans (Bogdanffy *et al*. 1999). The cytotoxicity of vinyl acetate is hypothesised to be associated with a reduction in intracellular pH caused by acetic acid, one of its metabolites (Bogdanffy *et al*. 2001). However, experimental data on the dose metric relevant to risk assessment (pH changes in nasal tissues) were not available in rats or humans due to technical difficulties in its measurement. The PBPK model reproduced the pharmacokinetics of vinyl acetate and its metabolites in the upper respiratory tract of rats. However, the closeness of the model simulations to human pharmacokinetic data could not be assessed because human data were not available. Despite this deficiency, the level of confidence in this PBPK model for the intended purpose was characterised as “medium” on the basis of biological basis, performance and reliability of the simulated dose metric (the latter, in this case, being supported by a sensitivity analysis to identify key parameters determining the dose metric in rats and humans) (WHO 2010).

Methylene chloride: interspecies extrapolation of a mouse PBPK model to assess human health risk

34. A PBPK model was developed to investigate why methylene chloride caused liver and lung tumours in mice by inhalation at 2000 and 4000 ppm, but was not carcinogenic via the oral route of exposure (Andersen *et al.* 1987). Methylene chloride is metabolised to carbon dioxide by an GSH-conjugation pathway, and to carbon monoxide by an oxidation pathway. Either of these two pathways can produce reactive intermediates. The PBPK model was used to calculate the rate of metabolism through each pathway per volume of tissue per unit time. The carcinogenic responses in both tissues were closely correlated with the GSH-pathway, and not with the oxidation pathway. The PBPK model indicated that ingestion of methylene chloride via drinking water leads to very low tissue exposures to the GSH-pathway metabolites due to slower intake and first-pass metabolism in the liver. This provided an explanation as to why methylene chloride is not carcinogenic by the oral dose route. The estimated health risk from this analysis was two-orders of magnitude lower than estimated by the 1985 US EPA default procedures (Andersen 2003). The extrapolation assumed that mouse and human tissues would be equally responsive to equivalent tissue exposures to the reactive GSH-pathway intermediates. This PBPK model has been used in risk assessments by Health Canada in their assessment for the general population under the Canadian Environmental Protection Act (Health Canada 1993), the US Occupational Safety and Health Administration (OSHA) for establishing the permissible exposure limit (OSHA 1997), and the US EPA in the Integrated Risk Information System (IRIS) assessment for inhalation cancer risk (US EPA 1987).

Vinyl chloride: derivation of an inhalation reference concentration

35. A PBPK model was developed for vinyl chloride to support tissue dosimetry for cancer risk assessment (Clewell *et al.* 2001). Vinyl chloride causes liver angiosarcoma in various species including humans. It is metabolised to a DNA-reactive epoxide which can react with DNA to form promutagenic adducts. Inhalation studies in rats were used to correlate tumour outcomes with tissue concentrations of the epoxide (the dose metric). The PBPK model was then used to estimate the external exposures associated with this dose metric in humans. The health risk for lifetime human exposure to 1 ppb vinyl chloride daily was estimated using the linearised multistage cancer model (cases of angiosarcoma/million exposed individuals/ppb vinyl chloride). Animal-based PBPK risk estimates for human inhalation exposure to vinyl chloride were consistent with risk estimates based on human epidemiological data, and were lower than those used in environmental decision-making by a factor of 80. Subsequently this study was used to assist the establishment of an inhalation reference concentration (RfC) for vinyl chloride (US EPA 2000).

Methods for conducting interspecies extrapolation using PBPK models

36. The first use of a PBPK model to perform an interspecies extrapolation (rodents to humans) for a regulatory risk assessment was by the US EPA with methylene chloride in 1987 (Andersen *et al.* 1987; see paragraph 34). The methods

of extrapolation involved 1) replacing fixed rodent parameter values with human values obtained from the literature or experimental data, and 2) use of allometry[3]. These methods have subsequently been used in a number of literary publications, for example, to extrapolate a rat model to humans for the assessment of toluene exposure (Tardif *et al.* 1997). De Buck *et al.* (2007) showed these approaches are reasonably successful in predicting human pharmacokinetics for 26 drugs from calibrated rat models.

37. Parameters describing xenobiotic metabolism (namely, the Michaelis-Menten parameter Vmax[4]) can be difficult to extrapolate across species because kinetic constants do not necessarily follow a predictable pattern, and there may be unknown interspecies differences in metabolism. There are however some approaches that have been used:

- The 'parallelogram' approach. This may be used for predicting metabolic rate constants for the human PBPK model from 1) *in vivo* rodent studies, and 2) *in vitro* studies using rodent and human tissues. Subsequently, a ratio between rodent *in vitro* and *in vivo* measures is calculated and used to derive the 'missing' human *in vivo* parameter (Knudsen *et al.* 2016).

- Allometry. For example, Vmax has been scaled in an allometric manner using an allometric exponent value of 0.75 (West *et al.* 2002).

- *In vitro* to *in vivo* extrapolation (IVIVE), described below.

IVIVE

38. From an ethical standpoint there are limited opportunities to collect *in vivo* toxicokinetic data from human subjects. Subsequently, *in vitro* data that can be used to estimate *in vivo* concentration or activity are becoming increasingly popular, particularly in respect of estimating rates of metabolism in humans (Lipscomb & Poet 2008). This process is called IVIVE.

39. IVIVE is, unlike allometry, based on an understanding of the experimental and biological factors that influence *in vivo* estimation using *in vitro* data. PBPK modelling provides an effective framework for conducting IVIVE because their physiological structure facilitates the incorporation of *in vitro*-derived chemical-specific parameters in order to predict *in vivo* ADME processes.

[3] Allometric scaling is the use of a regression equation involving body weight to predict a parameter of interest in a different animal species (Lindstedt & Schaeffer 2002). This is applied in PBPK modelling to scale animal parameters to humans (Ings 1990). The general allometric equation takes the form $y = a(\text{body weight})^b$, where y is the model parameter of interest. These parameters typically include absorption and metabolism rates. However the inherent uncertainty/ variability of body weight and the allometric coefficient (a) and exponent (b) is often not taken into account (Bois 1999, Bois 2000).

[4] The other Michaelis-Menten parameter (Km) does not require inter-species scaling, since it describes the affinity between enzyme and substrate (Ward *et al.* 1988, Chiu *et al.* 2009). Although in most cases of enzyme-catalysed biotransformation reactions follow Michaelis-Menten kinetics, non-Michaelis-Menten kinetics can also accommodated by IVIVE (Kedderis 2007, Kramer & Tracy 2008).

40. IVIVE commonly refers to the estimation of metabolic clearance rates *in vivo* and is based on the premise that the overall rate of enzyme-catalysed reactions is directly proportional to the total enzyme present in the system. For example, a value of Vmax derived from *in vitro* assays using isolated hepatocytes is sometimes expressed as metabolism rate per million cells (mg/ sec/ 10^6 cells). Subsequently a scaling factor, hepatocellularity per gram of liver (HPGL), is required to estimate the rate for the whole liver (Kenyon 2012):

> Whole liver Vmax (mg/ sec) = Vmax (mg/ sec/ 10^6 cells) x HPGL (cells/ g-liver) x liver weight (g) [1]

41. The use of IVIVE with PBPK models has the potential to reduce the need for *in vivo* studies in animals or humans, but there are limitations to this approach. For example, although suspension cultures of primary human hepatocytes have the capacity for phase II metabolism and *de novo* cofactor synthesis, they often:

- do not include physiologically relevant levels of plasma proteins.
- can only be maintained for a few hours in detached form before metabolic activity and cell viability wane.
- lack canalicular efflux transport capability, and in general do not contain all the relevant transporters and other physiological machinery existing in animals.
- have limited ability to assess complex xenobiotic-transport interactions such as non-competitive mechanisms and metabolite interactions.

These limitations may lead to inaccurate estimates of metabolic clearance *in vivo* (Bell *et al.* 2018). Indeed, there appears to be a systematic bias in the estimation of intrinsic liver clearance (Clint) from *in vitro* versus *in vivo* data, with *in vitro* based estimates underestimating *in vivo* clearance for small values of Clint but with the opposite relationship at large values of Clint (Yoon *et al.* 2014).

Case studies: use of IVIVE in PBPK modelling for risk assessment

PFOS, triclosan, pyridaben and fluazinam: derivation of AOP-based margins of exposure

42. El-Masri *et al.* (2016) applied life-stage specific PBPK models to estimate maternal exposures which correspond to the foetal blood concentrations that were shown to induce *in vitro* activity in foetal vasculogenesis/angiogenesis disruption AOP assays. The estimates of maternal exposure were then compared to realistic exposure estimates derived from biomonitoring data in order to derive AOP-based margins of exposure. The life-stage PBPK model was applied by assuming initial blood levels of the chemicals to be negligible for a neonate at birth. The model simulation starts by increasing chemical oral intake rate based on increasing bodyweight of a neonate and continues until adulthood and during pregnancy (conception was assumed to occur at age 25 years). During infant growth to

adulthood, temporal *in vivo* hepatic clearance rates were estimated by linear scaling of *in vitro* clearance rates using hepatocyte content as a function of liver weight.

Estragole: assessment of interspecies differences in metabolism

43. The extent of bioactivation of the herbal constituent estragole to its ultimate carcinogenic metabolite 1'-sulfooxyestragole depends on the relative levels of bioactivation and detoxification pathways. The present study investigated the kinetics of the metabolic reactions of both estragole and its proximate carcinogenic metabolite 1'-hydroxyestragole in humans in incubations with relevant tissue fractions. Based on the kinetic data obtained, a PBPK model for estragole in human was defined to predict the relative extent of bioactivation and detoxification at different dose levels of estragole. The outcomes of the model were subsequently compared with those previously predicted by a PBPK model for estragole in male rat to evaluate the occurrence of species differences in metabolic activation. The results obtained reveal that formation of 1'- oxoestragole (which represents a minor metabolic route for 1'-hydroxyestragole in rat) is the main detoxification pathway of 1'-hydroxyestragole in humans. Due to a high level of this 1'- hydroxyestragole oxidation pathway in human liver, the predicted species differences in formation of 1'-sulfooxyestragole remain relatively low, with the predicted formation of 1'-sulfooxyestragole being twofold higher in human compared with male rat, even though the formation of its precursor 1'-hydroxyestragole was predicted to be fourfold higher in human. Overall, it is concluded that in spite of significant differences in the relative extent of different metabolic pathways between human and male rat there is a minor influence of species differences on the ultimate overall bioactivation of estragole to 1'-sulfooxyestragole. (Punt *et al.* 2009)

Estragole: derivation of a CSAF

44. This study investigated interindividual variation in liver levels of the proximate carcinogenic metabolite of estragole, 1'-hydroxyestragole, due to variation in two key metabolic reactions involved in the formation and detoxification of this metabolite, namely 1'-hydroxylation of estragole and oxidation of 1'-hydroxyestragole. Formation of 1'-hydroxyestragole is predominantly catalysed by P450 1A2, 2A6, and 2E1, and results of the present study support that oxidation of 1'-hydroxyestragole is catalysed by 17b-hydroxysteroid dehydrogenase type 2 (17b- HSD2). In a first approach, the study defined PBPK models for 14 individual human subjects, revealing a 1.8-fold interindividual variation in the area under the liver concentration-time curve (AUC) for 1'-hydroxyestragole within this group of human subjects. Variation in oxidation of 1'-hydroxyestragole by 17b-HSD2 was shown to result in larger effects than those caused by variation in P450 enzyme activity. In a second approach, a Monte Carlo simulation was performed to evaluate the extent of variation in liver levels of 1'-hydroxyestragole that could occur in the population as a whole. This analysis could be used to derive a chemical-specific adjustment factor (CSAF), which the authors defined as the 99th percentile divided by the 50th percentile of the predicted distribution of the AUC of 1'- hydroxyestragole in the liver. The CSAF was estimated to range between 1.6 and 4.0, depending on the level of variation that was taken into account for oxidation of 1'-hydroxyestragole. Comparison of the CSAF to the default uncertainty factor of 3.16 for human variability in biokinetics reveals that the default uncertainty factor adequately protects 99% of the population. (Punt *et al.* 2010).

Trichloroethylene (TCE): assessment of interindividual variability of metabolism rates in humans

45. The hepatotoxicity of TCE is mediated by acid metabolites formed by cytochrome P450 2E1 (CYP2E1) oxidation, and differences in the CYP2E1 expression are hypothesised to affect susceptibility to TCE's liver injury. This study was designed specifically to examine the contribution of statistically quantified variance in enzyme content and activity on the risk of hepatotoxic injury among adult humans. Lipscomb *et al.* 2003 combined data sets describing (1) the microsomal protein content of human liver, (2) the CYP2E1 content of human liver microsomal protein, and (3) the *in vitro* Vmax for TCE oxidation by humans. The 5th and 95th percentiles of the resulting distribution (TCE oxidised per minute per gram liver) differed by approximately six-fold. These values were converted to mg TCE oxidised/h/kg body weight and incorporated in a human PBPK model. Simulations of 8-hour inhalation exposure to 50 ppm and oral exposure to 5 µg TCE/L in 2 L drinking water showed that the amount of TCE oxidised in the liver differs by 2% or less under extreme values of CYP2E1 expression and activity (here, selected as the 5th and 95th percentiles of the resulting distribution). This indicates that differences in enzyme expression and TCE oxidation among the central 90% of the adult human population account for approximately 2% of the difference in production of the risk-relevant PK outcome for TCE-mediated liver injury. Integration of *in vitro* metabolism information into physiological models may reduce the uncertainties associated with risk contributions of differences in enzyme expression and the uncertainty factors that represent PK variability. (Lipscomb *et al.* 2003).

Risk assessments that may retrospectively benefit from PBPK modelling

2-butoxyethanol (2-BE): adjustment to its biological monitoring guidance value

46. 2-BE is a glycol ether used in a number of commercial and domestic products due to its hydrophilic and lipophilic properties. Human exposure is most likely to occur from inhalation and dermal absorption during the use of such products and the UK occupational exposure standards (OES) set by HSE are 25 ppm (8 h, time-weighted average) and 50 ppm (15 min, short-term exposure limit). The UK biological monitoring health guidance value for urinary (free) butoxyacetic acid (BAA) measured post-shift is 240 mmol/mol creatinine.

47. Franks *et al.* (2006) developed a PBPK model describing the disposition of 2-BE in order to predict the urinary concentration of its major metabolite, butoxyacetic acid (BAA) under a range of exposure scenarios. The model included such features as multiple entry routes into the body, varying workload conditions, metabolism in the liver and elimination of free BAA in urine by glomerular filtration and acid transport. Good agreement between model predictions and existing experimental data of total BAA levels in the blood and urine over various exposure conditions were observed. The PBPK model allowed comparison of disparate studies and also enabled the prediction of urinary concentrations of BAA post-shift.

48. By calculating the total amount of BAA, any interindividual variability in conjugation is taken into account. This led Franks *et al.* (2006) to conclude that a biological monitoring guidance value should be proposed for total rather than free

BAA with a value of 250 mmol/mol of creatinine (post-shift), based on an 8 h exposure to 25 ppm 2-BE at resting working conditions.

POPs: assessment of interspecies differences in ADME to characterise human health risks (particularly in respect of infant exposure), and derivation of CSAFs for POPs with different half-lives

49. While PBPK modelling has been used recently in risk assessments of dioxins and PFOA and PFOS by EFSA, it may also be useful for other persistent organic pollutants (POPs) to allow for interspecies differences in toxicokinetics and the fact that exposure may be significantly higher for limited periods of life (e.g. during breastfeeding than later in life) but toxicity may depend on body burdens reached later in life.

50. Half-lives of elimination often differ substantially for POPs between humans and laboratory animal species used in toxicological testing, yet the default uncertainty factor of 100 has traditionally been used to establish health-based guidance values, which might not adequately allow for the toxicokinetic differences between species. For example, the half-life of elimination of DDT in humans has been estimated to be 2.1-2.2 years (Ritter *et al.* 2009), but 19 days in rats (WHO 2011). The Provisional Tolerable Daily Intake (PTDI) established by the Joint FAO/WHO Meeting on Pesticide Residues in 2000 was based on a NOAEL for developmental toxicity in rats and the default uncertainty factor of 100. The half-life of elimination of the DDT metabolite DDE, which is the primary form of total DDT found in the diet, has been estimated to be 6.2-7.6 years in humans (Ritter *et al.* 2009) but 16 weeks in rats (WHO 2011).

51. In addition, for many POPs exposure via food has decreased to low levels over time but exposure can be higher in breastfed infants due to existing maternal body burdens and thus elevated dietary intakes occur for a limited period of life. In the 2014 COT Statement on hexachlorocyclohexanes (HCHs) in the infant diet, margins of exposure (MOEs) were calculated for β-HCH based on a LOAEL for centrilobular hypertrophy in a 13-week study in rats. The estimated MOEs were >100 and not considered a concern, except for exclusively breast-fed infants if based on the highest concentration reported in breast milk. However, the distribution of concentrations of β-HCH in breast milk was highly skewed, with the maximum concentration being 35 times higher than the next highest measured concentration, and the statement noted that the toxicological effects of β-HCH relate to long term exposures and lower MOEs during the limited period of breastfeeding may not be problematic. The statement reported that half-lives of elimination of β-HCH in rats are around 1 month but were estimated to be 7.2 to 7.6 years in humans in a study of formerly exposed workers (COT 2014).

Electronic nicotine (and non-nicotine) delivery systems (E(N)NDS): derivation of a pharmacokinetic endpoint for use in risk characterisation

52. A tabulation of user exposure to the constituent ingredients present within E(N)NDS was presented to COT in October 2019 (TOX/2019/61). The Committee agreed that there needs to be better quantification of E(N)NDS user exposure to the normal ingredients in order to better assess the health risk. The inhalational exposures calculated from E(N)NDS use generally exceeded the available guideline

values for the constituent ingredients. The relevance and limitations of comparing E(N)NDS user exposures that will occur over a long-term as short duration individual peak exposures to the guideline values which are based on continuous exposure were discussed. It was suggested that a pharmacokinetic approach could be used to calculate the maximum plasma concentration (Cmax) and the area under the concentration-time curve (AUC) to assess which approach best reflects the exposure and predicts potential health-based outcomes. It was noted that PBPK models are available through the US EPA for pesticide crop sprayers that have been developed for assessing short-term intermittent human exposures, which may be helpful for assessing exposure of E(N)NDS users. It was suggested that these matters could be further discussed at the potency/PBPK workshop scheduled for March 2020, and additionally what the key data requirements might be for existing models.

Amphetamine analogues: application of PBPK modelling and the read-across approach to assess toxicity

53. 1,3-Dimethylamylamine (DMAA) is an amphetamine derivative which is considered to be medicinal. However, DMAA analogues (such as DMHA and DMBA) have been detected in various sports and weight-loss supplements (Cohen *et al.* 2017). Although there is little toxicological information on these novel compounds, it is considered that the use of the read-across approach to extrapolate hazard information from DMAA, in addition to PBPK modelling for exposure assessment, may be applied for their risk assessment.

Summary

54. These case studies demonstrate that PBPK modelling can been used to evaluate internal dose and can be applied in chemical risk assessment in various ways. PBPK modelling enables the evaluation of health risk based on internal dose instead of the traditional approach whereby the toxic response is usually related to the external dose administered in animal toxicology studies. Although this approach is gaining momentum throughout Europe and North America, there is generally a lack of scientific consensus in terms of how PBPK models are evaluated, due to their perceived complexity and diverse applications.

Questions for the Committee:

55. Members are asked to consider the following:

i). Do Members of the Committee have any comments on the cases where:

a) PBPK models have been applied in risk assessment?
b) IVIVE has been in PBPK modelling for risk assessment?
c) previous risk assessments may have benefitted from PBPK modelling?

ii) Do Members consider that future risk assessments would benefit from PBPK modelling?

Secretariat
December 2019

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List of abbreviations

ACSA - Agricultural chemical safety assessment

ADME - Absorption, distribution, metabolism and excretion

AUC - Area under the concentration-time curve

AOP - Adverse outcome pathway

BAA - butoxyacetic acid

2-BE - 2-butoxyethanol

$BMDL_5$ - Benchmark dose level, where change in response is likely to be < 5%

BPA - Bisphenol A

Bw - bodyweight

CADM - Concentration- and age-dependent model

CEF Panel - Panel on food contact materials, enzymes and processing aids

Clint - intrinsic liver clearance

Cmax - maximum plasma concentration

CONTAM Panel - Panel on contaminants in the food chain

CSAF - Chemical-specific adjustment factor

DDT - Dichlorodiphenyltrichloroethane

DMAA - 1,3-Dimethylamylamine

DMBA - 1,3-Dimethylbutylamine

DMHA - Dimethylhexylamine

EDI - Estimated daily intake

EFSA - European food safety authority

E(N)NDS - Electronic nicotine (and non-nicotine) delivery systems

GA – Glycidamide

GSH - Glutathione

H - hour

HBGV - Health-based guidance value

HCHs - hexachlorocyclohexanes

HED - Human equivalent dose

HEDF - Human equivalent dose factors

HESI - Health and environmental sciences institute

HSE - Health and safety executive

ICH - International council for harmonisation of technical requirements for pharmaceuticals for human use

IRIS - Integrated risk information system

IVIVE - *In vitro* to *in vivo* extrapolation

Kg - kilogram

KMD - Kinetically-derived maximum dose

Km - concentration of substrate which permits the enzyme to achieve half Vmax

Kt - Transporter affinity constant

LOAEL - lowest observable adverse effect level

MOE - Margin of exposure

NOAEL - No observable adverse effect level

OECD - Organisation for economic co-operation and development

OELs - occupational exposure limits

OES - occupational exposure standards

PBPK - Physiologically-based pharmacokinetic

PFOA - Perfluorooctanoic acid

PFOS - Perfluorooctanesulfonic acid

PK - pharmacokinetic

POPs - Persistent organic pollutants

ppm - Parts per million

PTDI - Provisional tolerable daily intake

REACH - Registration, evaluation, authorisation and restriction of chemicals

RfC - Inhalation reference concetration

TCDD - 2,3,7,8-Tetrachlorodibenzodioxin

TCE - Trichloroethylene

Tk - toxicokinetic

Tmc - Transporter maximum constant

US EPA - United states environmental protection agency

US OSHA - United states occupational safety and health administration

Vmax - reaction rate when enzyme is fully saturated by substrate