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Executive Summary

The published multi-species PBPK models for chloroprene were developed using in vitro to in vivo extrapolation (IVIVE)-based parameterization. In vitro metabolism data collected in species-specific liver, lung, and kidney microsomal incubation studies (Himmelstein et al., 2004a; Yang et al., 2012) were biologically scaled to provide in vivo metabolism parameters in the PBPK model for chloroprene in liver, kidneys, and lungs of mouse, rat and human (Himmelstein et al., 2004b; Yang et al., 2012). In this study, the IVIVE used to parameterize the published model for chloroprene in these three species was reviewed to provide recommendations for any updates to the previously published IVIVE-based parameterization to support the application of the chloroprene PBPK model for risk assessment.

1. Background

In vitro to in vivo extrapolation for parmaeteirzation involves a series of biological scaling steps from in vitro measured metabolic constants, in most cases Vmax and intrinsic clearance (Clint), to corresponding in vivo parameters to use in PBPK models. For Km, consideration of free, i.e., available for metabolism, concentration between in vitro vs. in vivo is important for meaningful IVIVE. In the conversion of Vmax or Clint from in vitro to in vivo, it is critical to select appropriate scaling factors, which are dependent on the in vitro experimental system, the source of tissue, and the species of interest (see the Table 2 below copied from Yoon et al., 2012). For example, an in vitro Vmax of a compound determined in hepatic microsomal incubations, which is expressed in a unit of µmol/min/mg microsomal protein, can be converted to an in vivo liver Vmax that is in a unit of µmol/min/kg $BW^{0.75}$ for use in a PBPK model. In this IVIVE example, a scaling factor referred as mg microsomal protein per gram liver (MPPGL) is used along with liver weight and body weight of the species of concern. Characteristics of the tissue and the donor for the tissue used for in vitro system preparation determine which physiological scaling factors, i.e., liver weight and body weight, to be used for IVIVE. For example, microsomes prepared from an average adult rat(s) can be used to generate in vitro metabolic constants that can be extrapolated to in vivo metabolism parameters for the average adult rat.

Table 2. Summary of QIVIVE calculations for different *in vitro* systems.

System	Typical units for enzyme content in the system	Scaling factors to whole body
Expressed enzyme	pmol/min/pmol enzyme	(ISEF × CYPabundance or RAF) × MPPGL × LW
Microsomes[a]	nmol/min/mg protein	MPPGL × LW
Hepatocytes	nmol/min/10⁶ hepatocytes	HPGL × LW
Liver	nmol/min/g liver	LW
Whole body	nmol/min/whole liver	

LW, liver weight; MPPGL, microsomal protein per gram liver (mg microsomal protein/g liver); HPGL, hepatocellularity per gram liver (number of hepatocytes/g liver).
[a]This scaling based on the protein amount in the subcellular fraction also applies for the cytosol and S9 fraction.

2. IVIVE for parameterization of the chloroprene PBPK model

It is critical to use appropriate scaling factors for accurate IVIVE for confidence in PBPK model outcomes. However, these scaling factors are not routinely measured or reported in publications and it is often hard to track down the sources for those scaling factors used in IVIVE studies (Barter et al., 2007). As such, the scaling factors used in the published chloroprene model were reviewed for their appropriateness for use in parametrization of metabolism in each tissue for mouse, rat and human as well as the sources of the selected values.

2.1   Scaling factors for the average adult of each species

In vitro enzyme kinetic parameters reported in Himmelstein et al. (2004a) and Yang et al., (2012) are considered as representatives of average adult values for each species. Therefore, extrapolation of these in vitro parameters was performed to obtain corresponding in vivo metabolism parameters in the PBPK model for the average adult of mouse, rat or human. Table 1 lists the species and gender specific scaling factors and physiological parameters recommended for use in chloroprene model along with their references. Body weight (BW) values for mouse, rat or human average adults (i.e., parameters for standard organism) are adopted from Brown et al., (1997). Fractional tissue weight values are also from Brown et al. (1997). For mouse and rat, gender specific BW values were used, but fractional tissue weight of the three tissues selected here was assumed to be the same between male and females.

2.1.1   Scaling of metabolic capacity

The capacity of metabolism. i.e., Vmax, is extrapolated from in vitro to in vivo based on the difference in scale between in vitro and in vivo systems. As microsomes was used to generate an in vitro Vmax for chloroprene in a given tissue, the following equation (Eq. 1) is used to scale the in vitro Vmax up to a corresponding in vivo Vmax for whole body.

In vivo Vmax (mg/h/kg $BW^{0.75}$) = in vitro Vmax (µmol/h/mg microsomal protein_tissue) x MPPGL (mg microsomal protein_tissue/g tissue) x BW (kg) x VtissueC x 1000 (g/kg) ÷ $BW^{0.75}$ x MW (µg/umol)÷1000 (µg/mg)   *(Eq. 1)*

, where MPPGL represents milligram microsomal protein content per gram of tissue, MW molecular weight, and VtissueC fractional weight of the given tissue to BW.

2.1.2   Selection of scaling factors for liver, lung and kidney of each species

Microsomes were used to determine in vitro enzyme kinetic parameters for chloroprene (Himmelstein et al., 2004a). Therefore, the biological scaling factor to use is the mg microsomal protein content per gram tissue (MPPGL, MPPGLU, or MPPGK for liver, lung, and kidney, respectively). In the original Himmelstein model, the values for the MPPG_tissue scaling factors for each species are 35, 49, and 56.9 mg microsomal protein/g

liver for mouse, rats, and humans, respectively, whereas for lung microsomes, 23 mg microsomal protein/g tissue was used for all animal species (Himmelstein et al., 2004b). This report reviews the appropriateness of the scaling factors selected for use in the original model.

Barter et al. (2007 and 2008) provided a comprehensive review of the liver microsomal content (MPPGL) for human and rat. Based on their meta-analysis and consensus report of the human data (Barter et al., 2007), 40 mg/g liver is recommended for human adults for chloroprene IVIVE-PBPK modeling. Lab to lab differences in microsomal preparation techniques and tissue sources are considered as the main factors for the variability in reported MPPGL values (Barter et al., 2007; Medinsky et al., 1994). Inter-species difference in microsomal protein per gram tissue appears to small in general, and is much smaller than the variability within species resulting from the experimental factors (Houston and Galetin, 2008; Barter et al., 2007; Csanády et al., 1992; Litterst et al., 1975). A MPPGL value of 35 mg/g liver was reported by Medinsky et al. (1994) for both rat and mouse. For rat, another value was available recommended by Houston and Galetin at 45 mg/g liver. We recommend to use the average of the two, 40 mg/g liver for rat for the chloroprene IVIVE-PBPK modeling. The values for mouse in Litterst et al. or Csanády et al. studies were not recommended for IVIVE directly as their results appear not corrected for experimental loss of microsomal proteins during preparation. It is important to use the scaling factors that were corrected for loss and thus, close to their in vivo values to reduce the uncertainty in IVIVE to the extent possible. Although we recommend these MPPGL values (Table 1), it should be noted that they fall in a similar range with those used by Himmelstein et al. (2004b).

For kidney microsomal content, additional caution was made. Kidney cortex is frequently used rather than using the whole tissue to prepare kidney microsomes because cytochrome P450 enzyme expression is known to be much higher in cortex compared to the medullar region. However, such details are often not reported in publications. In general, MPPGK based on kidney cortex microsomes is about two times higher than that based on whole kidney tissue microsomes (Scotcher et al., 2017). As the chloroprene model describes kidney metabolism in the whole tissue, MPPGK based on whole kidney tissue needs to be used. One half of the reported cortex-based MPPGK reported by Scotcher et al. (2017) is recommended for use as the MPPGK for whole kidney microsomes data-based extrapolation for chloroprene IVIVE in humans. For rat, 18 mg/g kidney determined by Yoon et al. (2007) is recommended as the study reported a rat specific MPPGK value for whole kidney.

It was challenging to find MPPGLU (mg lung microsomal protein per gram tissue) values, in particular for humans. Himmelstein et al. estimated MPPGLU as 23 mg per g tissue based on a few available studies and the assumed microsomal recovery. This value is in line with other studies reporting MPPGLU values about 30 - 50% of the MPPGL within species for rat and mouse (Litterst et al., 1975; Yoon et al., 2006 and 2007). It was challenging to find any data for human MPPGLU. While Boogard et al. (2000) reported the lung microsomal content in three different species, their microsomal protein recovery seems to be much lower than other studies for both liver and lung (Litterst et al., 1975 and Yoon et al., 2006 and 2007) raising a concern for the correction for protein recovery. The

measured microsomal protein content in lung in this study however, appears to be similar among the three species. As they were all determined under the same experimental condition, it would be reasonable to use the same value for all three species. Medinsky et al. (1994) reported 20 mg/g lung for mouse. As such, it is considered reasonable to keep the 20 mg per g tissue as MPPGLU based on the mouse value reported by Medinsky et al. (1994).

Table 1. Average adult parameters recommended for in vitro to in vivo extrapolation

Parameter	B6C3F1 Mouse (Female)	B6C3F1 Mouse (Male)	F344 Rat (Female)	F344 Rat (Male)	Average Human	Reference
BW (kg)	0.035	0.04	0.33	0.45	70	Brown et al, 1997 (page 415 in text)
Liver fractional weight (VLC)	0.0549	0.0549	0.0366	0.0366	0.0257	Brown et al, 1997 (Tables 4, 5, & 7)
Lung fractional weight (VLUC)	0.00730	0.00730	0.005	0.005	0.0076	Brown et al, 1997 (Tables 4, 5, & 7)
Kidney fractional weight (VKC)	0.01670	0.01670	0.0073	0.0073	0.0044	Brown et al, 1997 (Tables 4, 5, & 7)
Liver mg microsomal protein per g liver (MPPGL)	35	35	40[a]	40 [a]	40	Medinsky et al., 1994 for mouse; Medinsky et al., 1994 and Houston and Galetin, 2008 for rat; Barter et al., 2007 for human
Lung mg microsomal protein per g lung (MPPGLU)	20	20	20	20	20	Medinsky et al., 1994 and Boogard et al., 2000 for all species
Kidney mg microsomal protein per g kidney (MPPGK)[b]	18	18	18	18	11	Yoon et al., 2007 for mouse and rat; Scotcher et al., 2017 for human

[a] Average of the two values reported in Medinsky et al., 1994 and Houston and Galetin, 2008.

[b] Values from these references are rounded.

2.1.3   IVIVE for first order metabolic clearance in rat and human lung

Yang et al. (2012) reported both Vmax and Km for chloroprene metabolism in all three tissue microsomes for mouse. However, for rat and human, only intrinsic clearance (as Vmax/Km) was reported for lung as the available in vitro data was only informative to estimate Clint in rat and human, but not informative to estimate Vmax and Km separately. The slopes of chloroprene disappearance in lung microsomes showed linear kinetics for the range of substrate concentrations used in the in vitro lung metabolism studies (Himmelstein et al., 2004a). Among those concentrations, four out of the five concentrations used in lung microsomal incubation were overlapped with the ones used for liver microsomal incubation studies. This could be suggesting a higher Km and/or much smaller Vmax for chloroprene oxidation in lung microsomes than that in liver microsomes. Whichever the case was, the measured first order clearance was similar to the level of non-biological loss observed during incubation indicating that the metabolic clearance in the lung of rat or human would be expected to be significantly lower than mouse. To be conservative, the observed first order loss in lung microsomes for rat and human was considered as metabolic clearance and was extrapolated to in vivo as first order clearance in the lung (KFLUC) in the published model. Using this first order clearance presents an issue however, when applying the model to a dose range in which the tissue concentration becomes higher than the Km in the lung. In such high dose conditions, the relative risk estimate for the lung vs. liver would shift as the current lung description cannot capture the saturable nature of chloroprene metabolism leading to an overestimation of lung metabolism and therefore risk estimate, at higher exposure conditions.

To avoid an overestimation of lung metabolism at high dose, it is recommended to estimate or infer a Km for lung metabolism in rat and human. Then estimate a Vmax from the observed first order clearance in vitro using the relationship of Clint = Vmax/Km, which holds true at low substrate concentrations, e.g., below Km. It is likely that the relative contribution of individual cytochrome (CYP) P450 enzymes toward chloroprene metabolism is tissue-dependent as the expression level of each CYP enzyme is tissue-dependent. In addition, affinity of the metabolism, i.e., Km, is different among different CYP enzymes contributing to tissue-dependent changes in relative contribution of CYP enzymes to a compound metabolism. For example, butadiene, a structural analog of chloroprene, is a substrate for at least two CYP enzymes including CYP 2El and CYP 2A6 (Csanady et al., 1992; Duescher and Elfarra, 1994). These CYPs have different affinities to butadiene as suggested by the different Km values observed for each tissue (Csanady et al., 1992). The lung Km values appear to be similar or higher in general than those for liver in all three species, this study results suggest. For mouse and rat, lung values were about 2 fold higher than the liver values. This is consistent with the in vitro observation by Himmelstein et al. (2004a), which implies a higher Km in lung than liver microsomes in rat and mice, i.e., at the overlapping substrate concentrations, no saturation was observed in lung unlike the liver. It is also consistent with the mouse results showing the lung Km being greater than that of the liver by a 1.5 – 5.3 fold, depending on the gender of the animals. Therefore, it would be reasonable to use a 2-fold higher Km for lung than the liver in each species for mouse and rat.

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