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The Utility of PBPK in the Safety Assessment of Chloroform and Carbon Tetrachloride[1]

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Occupational exposure limits (OELs) for individual substances are established on the basis of the available toxicological information at the time of their promulgation, expert interpretation of these data in light of industrial use, and the framework in which they sit. In the United Kingdom, the establishment of specific OELs includes the application of uncertainty factors to a defined starting point, usually the NOAEL from a suitable animal study. The magnitude of the uncertainty factors is generally determined through expert judgment including a knowledge of workplace conditions and management of exposure. PBPK modeling may help in this process by informing on issues relating to extrapolation between and within species. This study was therefore designed to consider how PBPK modeling could contribute to the establishment of 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 exercised 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 OES. Predictions obtained from the PBPK models indicated that average 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. This study highlights the value that the use of PBPK modeling may add to help inform and improve toxicological aspects of a regulatory process. © 2000 Academic Press

INTRODUCTION

In the United Kingdom (UK) there are two types of occupational exposure limits (OELs) for hazardous substances defined under the Control of Substances Hazardous to Health (COSHH) regulations: occupational exposure standards (OESs) and maximum exposure limits (MELs) (Ogden and Topping, 1997). These exposure limits fit into COSHH as definitions of when control is adequate. As with other OELs both limits are expressed as airborne concentrations of substance, averaged over specified periods of time (time-weighted average; TWA). Two reference periods can be used for each type of limit: long-term (8 h) and short-term (15 min). The latter period is known as a short-term exposure limit (STEL) and is generally set to help control effects such as eye irritation which may occur rapidly. Hence one can have an 8-h TWA OES, a STEL (15-min TWA) OES, an 8-h TWA MEL, and a STEL (15-min TWA) MEL.

An OES is set at a concentration with associated reference period that is judged will not be injurious to workers exposed daily via inhalation (Ogden and Topping, 1997). MELs are set where it is judged that such an airborne concentration cannot be reliably identified or where one can, although control of exposure to this level cannot be secured using reasonably practicable approaches (Ogden and Topping, 1997). OESs and MELs are set on the recommendation of the Health and Safety Commission's (HSC) Advisory Committee on Toxic Substances (ACTS) and its scientific subcom-

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mittee, the Working Group on the Assessment of Toxic Chemicals (WATCH), based on documentation prepared by the Health and Safety Executive (HSE), the regulatory body responsible for occupational health and safety in the UK. Further details of these procedures have been described elsewhere (Ogden and Topping, 1997).

The setting of an OES begins with a requirement for the identification of the critical health effect(s) and whether this/these effects are threshold phenomena. If a threshold exists, a no observed adverse effect level (NOAEL) and/or low observed adverse effect level (LOAEL) for each critical effect is determined from the available toxicological data. Data from exposed humans (in this case by inhalation) are preferable although animal data from exposure by a different route are more often the only data available. In common with many other risk assessment or standard setting situations, the absence of ideal data leads to the use of uncertainty or safety factors (UF or SF) applied to the NOAEL or LOAEL to allow, for example, for the uncertainties involved in extrapolation between and within species (Dourson and Stara, 1983; IPCS, 1994; Renwick, 1993). However, the size of the UF, in the context of promulgating OESs, is influenced not only by the usual considerations in trying to ensure that such extrapolation errs on the side of caution but also by risk management considerations specific to the workplace. Therefore, values used for UFs are often smaller than the "standard extrapolation defaults" associated with the development of standards in other forums (Fairhurst, 1995).

In the absence of ideal data, one approach to making more scientifically based extrapolations is to employ physiologically based pharmacokinetic (PBPK) models. PBPK models can be used to predict the tissue concentrations of chemicals in different species under various conditions based on independent anatomical, physiological, and biochemical parameters (Andersen *et al.*, 1992; Andersen and Krishnan, 1994; Clewell *et al.*, 1995; Krishnan *et al.*, 1994). They are based on knowledge of the anatomy of the organism in question and rules determining movement of drug or substance between tissues based on accepted physiological and physicochemical processes (Ramsey and Andersen, 1984). PBPK models have the potential to aid risk assessment activities and help and inform risk management decisions by offering more reliable predictions of the toxicokinetic consequences at the level of a specific tissue in a specified species, including humans, under particular exposure circumstances (Andersen *et al.*, 1992; Andersen and Krishnan, 1994; Clewell *et al.*, 1995; Krishnan *et al.*, 1994; Loizou *et al.*, 1999). A particular interest to HSE is the potential role of PBPK models in the occupational risk assessment and exposure limit setting process, where PBPK models may help to explore and reduce the inherent uncertainties. In this context it was decided to undertake a retrospective examination of example substances, which had been through the OES setting process some time ago, to see how PBPK modeling could have been applied in that process and, if so, the impact that it might have had. The two solvents, carbon tetrachloride (CCl_4) and chloroform ($CHCl_3$), were considered ideal for this exercise due to their relatively well-developed databases and the prior existence of well-established PBPK models.

$CHCl_3$ and CCl_4 are carcinogenic in animals (Gardner *et al.*, 1994; Delic *et al.*, 1994). It is generally accepted that the toxic effects of $CHCl_3$ and CCl_4 are associated with metabolic activation primarily by the mixed-function oxidase enzyme cytochrome P450 2E1 (CYP2E1) (Butterworth *et al.*, 1997; Nakajima *et al.*, 1995; Wang *et al.*, 1997; Wong *et al.*, 1998). It is well established that $CHCl_3$ acts via a nongenotoxic mode of action (Templin *et al.*, 1996). Studies on the metabolism of $CHCl_3$ indicate that the formation of one or more reactive metabolites, presumably including phosgene (Pohl *et al.*, 1977), leads to binding and damage to cellular macromolecules and ultimately cell death (Illet *et al.*, 1973; Klaassen and Plaa, 1967). Indeed, the peak rate of hepatic and renal $CHCl_3$ metabolism has been proposed as the dose metric that can be correlated with toxicity (Butterworth *et al.*, 1997). Briefly, depending on the dose rate, the production and binding of toxic metabolites to cellular macromolecules leads to cytotoxicity and consequent regenerative cell proliferation (Reitz *et al.*, 1990a,b). Sustained cell proliferation has been hypothesized as the driving force for hepatic and renal tumorigenesis (Larson *et al.*, 1994a,b; Reitz *et al.*, 1990a,b). Therefore, the determination of an OES on the basis of a NOAEL for regenerative cell proliferation should also prevent carcinogenesis (Larson *et al.*, 1994a,b, 1995a,Larson *et al.*, 1996; Thomas *et al.*, 1996a,b,Thomas *et al.*, 1996c, Thomas *et al.*, 1998). Although much of the supporting evidence for this has been generated relatively recently, this is the underpinning rationale on which the UK OES was based (Gardner *et al.*, 1994).

The toxicity of CCl_4 is believed to be associated with the binding of metabolites to tissue adducts which have a half-life of about 24 h (Paustenbach *et al.*, 1988; Wang *et al.*, 1997; Wong *et al.*, 1998). Therefore, the amount of CCl_4 metabolized in 24 h, including an 8-h inhalation exposure period, was chosen as the dose surrogate.

In the early 1990s, the UK OESs for $CHCl_3$ (Gardner *et al.*, 1994) and CCl_4 (Delic *et al.*, 1994) were both set at 2 ppm, 8-hr TWA. For $CHCl_3$ this value was derived from a repeat exposure inhalation NOAEL of 25 ppm, 4 h/day in the rat, with liver and kidney damage being the key toxicological effects. This NOAEL was taken as equivalent to 10 ppm for a full shift (8-h) exposure. The OES of 2 ppm allowed for a factor of 5 to accommodate

TABLE 1
Parameters Used in the PBPK Models

Parameter	Mouse	Rat	Human
Weights (kg)			
Body	0.03	0.23	70
	Percentage body weight		
Liver	5.86	3.4	3.14
Kidney	1.7	0.7	0.44
Fat	6	7	23.1
Rapidly perfused	3.3	5	3.27
Slowly perfused	74.14	74.9	61.05
Flows (liters h^{-1})			
Alveolar ventilation	2.01	4.6	339
Cardiac output	2.01	4.6	339
	Percentage cardiac output		
Liver	25	25	25
Kidney	25	25	25
Fat	2	5	5
Rapidly perfused	29	26	26
Slowly perfused	19	19	19

interspecies extrapolation to humans; the documentation does not refer to consideration of or a specified factor being introduced for intraspecies (i.e., human population) variability. For CCl_4, the OES of 2 ppm 8-h TWA was based on the data from repeat exposure inhalation studies in animals in which liver damage was observed (Delic *et al.*, 1994). NOAELs were available for a number of species (rats to primates) ranging from 5 to 50 ppm with effects only being seen within a species at an exposure concentration at least 2-fold higher than the NOAEL. The OES of 2 ppm 8-h TWA was therefore established at a value 2.5-fold lower than the lowest NOAEL (5 ppm) across all species and five times lower than the LOAEL (10 ppm) in the most sensitive species (rat) while also taking into account that the primate, with a much higher NOAEL of 50 ppm, is likely to be a more suitable model for the human. However, again the documentation does not refer to the consideration or specification of a factor to allow for intraspecies variability.

Existing PBPK models for both substances were used to describe the metabolic variation between the experimental species and humans and to investigate to what extent the UFs applied in the promulgation of the OESs accommodated any differences in the predicted toxicokinetic behavior between species. Furthermore, since variability in the human population was not overtly mentioned as a consideration in the derivation of the OESs, it was decided to investigate this aspect by the use of Monte Carlo techniques to predict variation both in the human population and also in the experimental animal models. Since the setting of the OESs (early 1990s) the database on $CHCl_3$, in particular, has expanded considerably. Thus, in this study, we used a NOAEL determined in the female $B6C3F_1$ mouse, which is reported to be the most sensitive species to $CHCl_3$ toxicity (Larson *et al.*, 1996; Templin *et al.*, 1998) rather than data from the rat, as used previously (see above); for CCl_4 the NOAEL determined in rat of unspecified strain as used in the original OES derivation (Delic *et al.*, 1994) was employed.

MATERIALS AND METHODS

Mouse PBPK model for chloroform. Recent studies have shown that the female $B6C3F_1$ mouse is the most sensitive species to $CHCl_3$ hepatotoxicity and nephrotoxicity, respectively, with NOAEL of 10 ppm by the inhalation route (Larson *et al.*, 1996; Templin *et al.*, 1998). Therefore, the PBPK model was based on the mouse so that the most conservative estimates for tissue dosimetry and peak rates of metabolism could be predicted.

The PBPK model for $CHCl_3$ used in this study was based on the model developed by Corley *et al.* (1990). Organ volumes and blood flows are listed in Table 1 and partition coefficients and metabolic constants for $CHCl_3$ are listed in Table 2. The model was modified to simulate an exposure regime of 6 h per day for 5 consecutive days. The 6-h inhalation exposure period was modeled as a constant concentration. The *peak* rate of liver metabolism was reported to reflect the rate of formation of toxic metabolites in the cell (Corley *et al.*, 1990; Larson *et al.*, 1994a,b; Reitz *et al.*, 1990) and was, therefore, used as the target organ dose surrogate. However, the peak rate of metabolism was achieved at about 3.7 h which implied that simulation of 8-h (the OES TWA reference period) exposure periods was not necessary. The loss of metabolic capacity (presumably

TABLE 2
Partition Coefficients and Metabolic and Macromolecular Binding Constants for Chloroform Models

Partition coefficients	Mouse	Human
Blood/air	21.3	7.43
Liver/air	19.1	17
Kidney/air	11	11
Fat/air	242	280
Rapidly perfused/air	19.1	17
Slowly perfused/air	13	12
Metabolic and macromolecular binding constants		
V_{maxc} (mg h^{-1} kg^{-1} body wt)	22.8	15.7
K_m (mg $liter^{-1}$)	0.35	0.45
k_{loss} (liters h^{-1})	5.72×10^{-4}	0
k_{resyn} (liters h^{-1})	0.13	0
A (kidney/liver)	0.15	3.3×10^{-2}
fMMB (h^{-1}), liver	3×10^{-3}	2.02×10^{-3}
fMMB (h^{-1}), kidney	1.0×10^{-2}	9.31×10^{-3}

TABLE 3
Physiological Parameter Distributions Used in Monte Carlo Analysis

	Mouse			Rat			Human		
Parameter	Mean	SD	Distribution	Mean	SD	Distribution	Mean	SD	Distribution
Body weight (kg)	.025	0.005	Normal	0.225	0.045	Normal	70	9	Normal
Organs (% body wt)									
Liver	0.058	0.012	—	0.034	0.007	—	3.14	1	—
Kidney	0.017	0.003	—	0.007	0.001	—	0.44	0.1	—
Fat	0.06	0.012	—	0.07	0.014	—	0.23	0.07	—
Rapidly perfused	—	—	—	—	—	—	6	1.5	—
Slowly perfused	—	—	—	—	—	—	61	12.2	—
Flows (liters h^{-1})									
Alveolar ventilation	1.76	0.35	Lognormal	4.57	0.91	Lognormal	347.9	85.4	Lognormal
Cardiac output	1.76	0.35	—	4.57	0.91	—	347.9	85.4	—
Liver	0.25	0.05	Normal	0.25	0.05	Normal	25	5.5	Normal
Kidney	0.25	0.05	—	0.25	0.05	—	25	5.5	—
Fat	0.02	0.004	—	0.05	0.01	—	5	0.8	—
Rapidly perfused	—	—	—	—	—	—	n/d[a]	n/d	n/d
Slowly perfused	—	—	—	—	—	—	19	8.6	Normal

[a] n/d, not determined.

due to binding of toxic metabolites to cytochrome P450 2E1) observed only in the mouse was described by *kloss* (liters mg^{-1}) and the regeneration of metabolic capacity was described by *kresyn* (h^{-1}). The proportion of kidney to liver metabolic activity was represented by the proportionality constant, *A*, and the fraction of total metabolites that covalently bind to biological macromolecules *in vivo* in liver and kidney was represented by the constants, fMMBL and fMMBK (h^{-1}), respectively (Corley *et al.*, 1990). Therefore, the peak rate of hepatic $CHCl_3$ metabolism, expressed as nmol h^{-1} g^{-1} (wet weight liver tissue), corresponding to exposure at the NOAEL level of 10 ppm (Larson *et al.*, 1996) for induced hepatic cell proliferation in the female $B6C3F_1$ mouse, was estimated.

The peak rate of hepatic $CHCl_3$ metabolism corresponding to exposure at the OES of 2 ppm 8-h TWA was also calculated for comparison to the human value.

Human PBPK model for chloroform. Mouse physiological and anatomical parameters and partition coeffcients were replaced with human values taken from the literature (Corley *et al.*, 1990; Loizou and Cocker, 1997) and are listed in Tables 1 and 2.

The human PBPK model was used to (1) calculate the peak rate of hepatic $CHCl_3$ metabolism corresponding to inhalation exposure at 2 ppm, 8-h TWA (i.e., the UK 8-h TWA OES), (2) "back calculate" the corresponding inhalation exposure concentration required to generate the same peak rate of metabolism in humans as that obtained in the mouse exposed to the NOAEL (10 ppm), and (3) calculate the dose–response curve of peak rate of hepatic $CHCl_3$ metabolism versus inhalation exposure concentration.

Human and mouse Monte Carlo analysis for chloroform exposure. The Monte Carlo method is a form of uncertainty analysis that allows the propagation of uncertainty through a model which results in an estimate of the variance in model output. This is achieved by randomly sampling model parameters from defined distributions (e.g., normal, lognormal) with defined central tendencies and variability (Thomas *et al.*, 1996a,b,c). Among the parameters sampled the distributions of cardiac output, partition coefficients and metabolic parameters were assumed to have lognormal distributions while the remaining parameters were assumed to have normal distributions. The distributions for the mouse parameters were set at 20% of mean values (Tables 3–5) (Thomas *et al.*, 1996a,b,c). The proportionality constant *A*, representing the proportion of kidney to liver metabolic activity, was not included in the Monte Carlo analysis because no distributions for the human constant were available. This was considered acceptable due to the small amount of kidney metabolism relative to the liver. The interindividual variability in the peak rate of $CHCl_3$ metabolism after inhalation exposure to 2 ppm in humans and 2 and 10 ppm in the mouse was assessed by randomly sampling the various parameters of the PBPK model 1000 times. The peak rate of metabolism output from the Monte Carlo analysis was presented as a histogram. Cumulative frequency distributions of peak metabolic rate of $CHCl_3$ in humans and the mouse were also determined. Median values were compared. The 95th percentile was estimated for humans and compared to the 5th percentiles for the mouse after exposure at 2 and 10 ppm, respectively.

TABLE 4
Partition Coefficients and Metabolic Constant Distributions Used in Monte Carlo Analysis of Chloroform Model

	Mouse			Human		
Parameter	Mean	SD	Distribution	Mean	SD	Distribution
Partition coefficients						
Blood/air	24.1	4.82	Lognormal	7.43	1.4	Lognormal
Liver/air	16.9	3.38	—	17	3.2	—
Kidney/air	12.2	2.44	—			
Fat/air	242	48.4	—	280	28	—
Rapidly perfused/air	16.9	3.38	—	26	2.5	—
Slowly perfused/air	13	2.26	—	12	2.4	—
Metabolic constants						
V_{maxc} (mg h^{-1} kg^{-1} body wt)	11.3	2.26	—	7	1.4	Lognormal
K_m (mg $liter^{-1}$)	0.76	0.15	—	0.25	0.05	—

Rat PBPK model for carbon tetrachloride. There are no reports in the literature describing which species is the most sensitive to CCl_4 toxicity. The overwhelming majority of studies used various strains of rat with no indication of relative sensitivity. Therefore, a PBPK model for the rat was used to investigate CCl_4 metabolism and biokinetics.

The model for $CHCl_3$ was modified for CCl_4 by replacing the $CHCl_3$ partition coefficients and metabolic rate constants with those of CCl_4. Rat anatomical and physiological parameters for the CCl_4 model were obtained from the literature (Gargas *et al.*, 1989; Paustenbach *et al.*, 1988). The 24-h cumulative amount of hepatic CCl_4 metabolite levels corresponding to exposure at the NOAEL of 5 ppm (Delic *et al.*, 1994) was expressed as nmol g^{-1} (wet weight liver tissue) 24 h^{-1}.

The 24-h cumulative amount of hepatic CCl_4 metabolism corresponding to exposure at the OES of 2 ppm, 8-h TWA was also calculated for comparison to the human value.

Human PBPK model for carbon tetrachloride. Rat physiological and anatomical parameters and partition coeffcients were replaced with human values taken from the literature (Corley *et al.*, 1990; Loizou and Cocker, 1997) and are listed in Tables 1 and 6.

The human PBPK model was used to (1) calculate the 24-h cumulative amount of hepatic CCl_4 metabolism corresponding to inhalation exposure at 2 ppm, 8-h TWA (i.e., the UK 8-h TWA OES), (2) back calculate the corresponding inhalation exposure concentration required to generate the same 24-h cumulative amount of hepatic CCl_4 metabolism in humans as that obtained in the rat exposed to the NOAEL (5 ppm), and (3) calculate the dose–response curve of 24-h cumulative amount of hepatic CCl_4 metabolism versus inhalation exposure concentration (μmol g^{-1} 24 h^{-1}).

Human and rat Monte Carlo analysis for carbon tetrachloride. The model for Monte Carlo analysis of human interindividual variability in $CHCl_3$ metabolism was modified to describe the variation in partition coefficients and metabolic rate constants for CCl_4 and are listed in Tables 3 and 5. The distributions for the rat parameters were set at 20% of mean values (Thomas *et al.*, 1996a,b,c).

TABLE 5
Partition Coefficients and Metabolic Constant Distributions Used in Monte Carlo Analysis of Carbon Tetrachloride Model

	Rat			Human		
Parameter	Mean	SD	Distribution	Mean	SD	Distribution
Partition coefficients						
Blood/air	4.52	0.35	Lognormal	2.64	1.4	Lognormal
Liver/air	14.2	1.2	—	14.2	3.2	—
Kidney/air	14.2	1.2	—	14.2	3.2	
Fat/air	359	11	—	359	28	—
Rapidly perfused/air	14.2	1.2	—	14.2	2.5	—
Slowly perfused/air	4.57	0.59	—	4.57	2.4	—
Metabolic constants						
V_{maxc} (mg h^{-1} kg^{-1} body wt)	0.99	0.2	—	0.53	0.11	Lognormal
K_m (mg $liter^{-1}$)	0.25	0.05	—	0.25	0.05	—

TABLE 6
Partition Coefficients and Metabolic Constants for Carbon Tetrachloride Models

Partition coefficients	Rat	Human
Blood/air	4.52	2.64
Liver/air	14.2	14.2
Kidney/air	14.2	14.2
Fat/air	359	359
Rapidly perfused/air	14.2	14.2
Slowly perfused/air	4.57	4.57
Metabolic constants		
V_{maxc} (mg h^{-1} kg^{-1} body wt)	0.99	0.53
K_m (mg $liter^{-1}$)	0.25	0.25

The interindividual variability in the 24-h cumulative amount of hepatic CCl_4 metabolism after inhalation exposure to 2 ppm in humans and 2 and 5 ppm in the male rat was assessed by randomly sampling the various parameters of the PBPK model 1000 times. The 24-h cumulative amount of hepatic CCl_4 metabolism was output from the Monte Carlo analysis and presented as a histogram. Cumulative frequency distributions of the 24-h cumulative amount of hepatic CCl_4 metabolism in humans and the rat were also determined. Median values were compared. The 95th percentile was estimated for humans and compared to the 5th percentiles for the rat after exposure at 2 and 5 ppm, respectively.

Software packages. Parameter sampling routines, Monte Carlo uncertainty analysis, and modeling exercises were performed using both ACSL Tox software (Pharsight Corp., Mountain View, CA) and MATLAB (The Math Works Inc., Natick, MA).

RESULTS

Calculations using the mouse chloroform model. The predicted mean peak rate of hepatic $CHCl_3$ metabolism in the female $B6C3F_1$ mouse after exposure to the NOAEL of 10 ppm by inhalation was calculated to be 391 nmol h^{-1} g^{-1} (Fig. 1). The mean peak rate of hepatic $CHCl_3$ metabolism after exposure at the 8-h TWA OES of 2 ppm was calculated to be 78 nmol h^{-1}, representing a fivefold difference.

Calculations using the human chloroform model. As shown in Fig. 1, a simulated inhalation exposure concentration of 130 ppm was required to generate a mean peak rate of hepatic $CHCl_3$ metabolism of 391 nmol h^{-1} g^{-1} in humans. This was a 13-fold higher airborne concentration than the mouse NOAEL of 10 ppm predicted to produce this rate of metabolism in that species. Also, an exposure concentration of 23 ppm was required to generate a mean peak rate of hepatic $CHCl_3$ metabolism in humans of 78 nmol h^{-1} g^{-1}, the mean peak rate predicted to occur in the mouse at the 8-h TWA OES of 2 ppm. The mean peak rate of hepatic $CHCl_3$ metabolism in humans after exposure at the OES of 2 ppm was calculated to be 5 nmol h^{-1} g^{-1}. Therefore, the ratio of the mean peak rates of hepatic $CHCl_3$ metabolism in the mouse at the NOAEL of 10 ppm, compared with the human OES of 2 ppm (i.e., 391/5), was 78. The ratio of mouse to human mean peak rate of hepatic $CHCl_3$ metabolism after exposure of both species at the 8-h TWA OES of 2 ppm (i.e., 78/5) was 15. The results are summarized in Table 7.

The relationship of mean peak rate of metabolism of chloroform versus exposure concentration showed that saturation of metabolism is predicted to occur

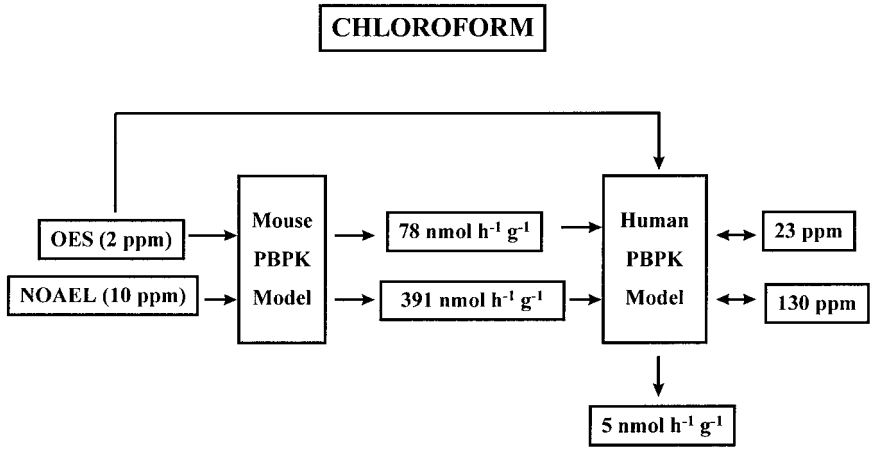

FIG. 1. Schematic diagram summarising the input (airborne concentration in ppm) and outputs (peak rate of metabolism in $nmol^{-1}$ h^{-1} g^{-1}) of the mouse and human PBPK models for chloroform (OES, occupational exposure standard as 8-h TWA; NOAEL, no observed adverse effect level).

TABLE 7
Summary of Chloroform and Carbon Tetrachloride Data

Inhalation exposure concentration (ppm)	Peak rate of metabolism (nmol h^{-1} g^{-1})		Rodent:human ratio of peak rate of metabolism
	Mouse	Human	
OES (2 ppm)	78	5	(OES/OES) 15.6
Mouse NOAEL (10 ppm)	391	—	(NOAEL/OES) 78.2
23 ppm	—	78	—
130 ppm	—	391	—
	24-h cumulative metabolism (μmol h^{-1} g^{-1})		
	Rat	Human	
OES (2 ppm)	0.14	0.01	(OES/OES) 8.8
Rat NOAEL (5 ppm)	0.33	—	(NOAEL/OES) 21.4
21	—	0.14	—
150	—	0.33	—

TABLE 8
Comparative Cumulative Frequency Distributions of Chloroform and Carbon Tetrachloride Metabolism

Peak rate of metabolism (nmol h^{-1} g^{-1})	Inhalation exposure to chloroform		
	Human (2 ppm)	Mouse (2 ppm)	Mouse (10 ppm)
Median	1.6	76.2	379
95%	3.1	—	—
5%	—	53.5	267
24-h cumulative metabolism (μmol h^{-1} g^{-1})	Inhalation exposure to carbon tetrachloride		
	Human (2 ppm)	Rat (2 ppm)	Rat (5 ppm)
Median	0.01	0.14	0.33
95%	0.03	—	—
5%	—	0.08	0.22

in both species at a similar level of about 200 ppm (Fig. 2).

Monte Carlo analysis for chloroform. The range and frequencies of the predicted peak rate of hepatic $CHCl_3$ metabolism for 1000 individual humans and mice are shown in Table 8 and Fig. 3; cumulative frequency distribution estimates for the populations are shown in Fig. 4. At exposure to the 8-h TWA OES (2 ppm), the overwhelming majority of humans (839 from 1000) from the population in the histogram (Fig. 3) are predicted to have a peak rate of metabolism no greater than 5 nmol h^{-1} g^{-1}, with the highest rate ~9 nmol h^{-1} g^{-1}. In contrast, the slowest peak metabolic rates predicted in the mouse population in the histograms were ~50 nmol h^{-1} g^{-1} at the 8-h TWA OES of 2 ppm and ~250 nmol h^{-1} g^{-1} at the NOAEL of 10 ppm. From Fig. 4 (and Table 8) it can be seen that the peak rate of metabolism at the 8-h TWA OES of 2 ppm is estimated to be ~3 $nmol^{-1}$ $h^{-}1$ g^{-1} or less for 95% of the human population. In contrast, for the mouse population exposed to the NOAEL of 10 ppm, the peak rate of metabolism is 267 $nmol^{-1}$ h^{-1} g^{-1} for 5% or more of the population.

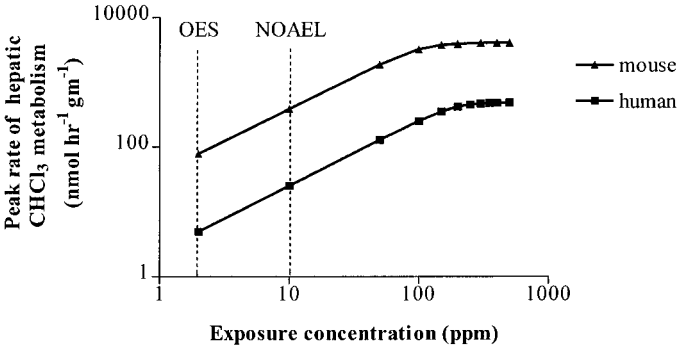

FIG. 2. Dose–response curves for hepatic chloroform metabolism in the mouse and humans. Hypothetical inhalation exposure to the UK 8-h TWA OES (2 ppm), $B6C3F_1$ female mouse NOAEL (10 ppm), and a range of concentrations from 50 to 500 ppm in serial increments of 50 ppm chloroform were input into the PBPK models. The corresponding peak rates of hepatic chloroform metabolism were generated by the models and plotted against the hypothetical inhalation exposure concentrations.

Calculations using the rat carbon tetrachloride model. The median amount of metabolite formation in the male rat liver after exposure to the NOAEL of 5 ppm for 8 h by inhalation was calculated to be 0.33 μmol g^{-1} 24 h^{-1} (Fig. 5). The median amount of hepatic metabolite formation per 24 h after exposure at the 8-h TWA OES of 2 ppm was calculated to be 0.14 μmol g^{-1} 24 h^{-1}, representing an approximate threefold difference (Fig. 5; Table 7).

Calculations using the human carbon tetrachloride model. A simulated inhalation exposure concentration of 150 ppm 8-h TWA was needed to generate a median cumulative amount of hepatic metabolism of 0.33 μmol g^{-1} 24 h^{-1} in humans, the median amount predicted for the rat at the NOAEL of 5 ppm (Fig. 5). The median cumulative metabolite level for the human after exposure to the 8-h TWA OES of 2 ppm for 8 h was calculated to be ~0.016 μmol g^{-1} 24 h^{-1} liver tissue (Fig. 5). Therefore, the ratio of the median 24-h cumulative metabolite level in the rat at the NOAEL of 5 ppm for 8 h compared with the human 8-h TWA OES of 2 ppm (i.e., 0.33/0.016) was 21.4. The ratio of rat to human predicted median 24-h cumulative metabolite levels when both species were exposed to the 8-h TWA OES of 2 ppm for 8 h (i.e., 0.14/0.016) was 8.8.

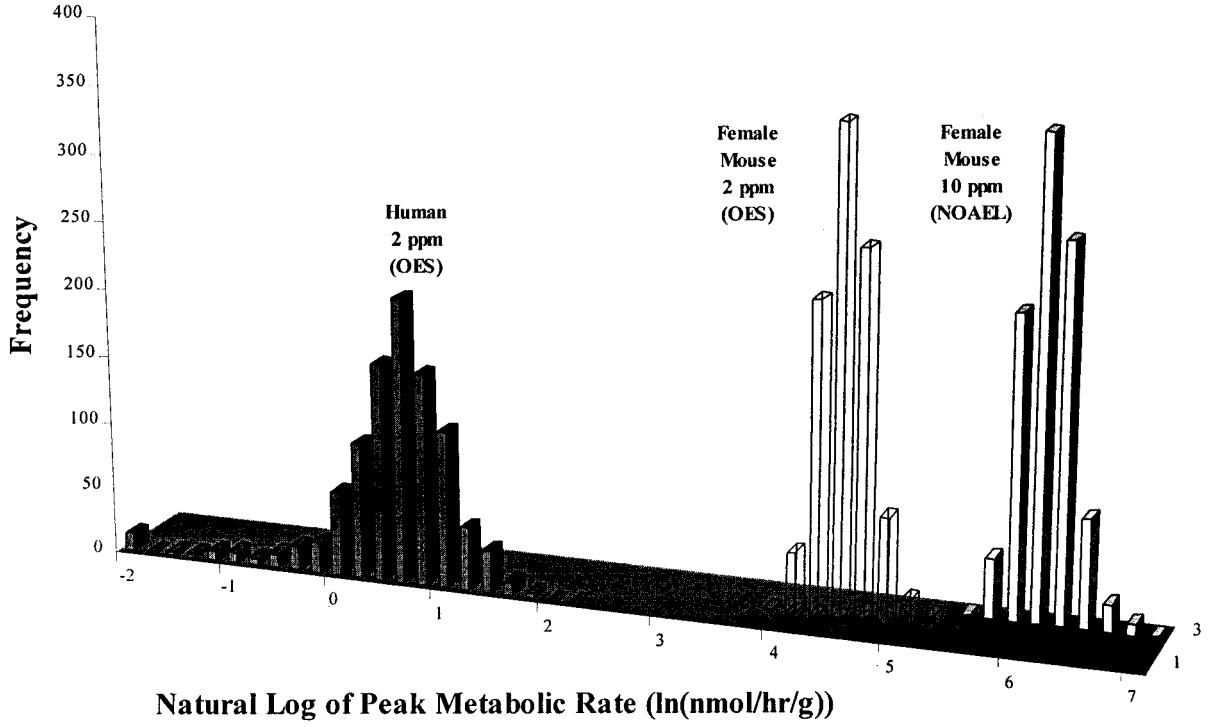

FIG. 3. Interindividual variability in chloroform metabolism. Peak rates of chloroform metabolism after inhalation exposure to 2 ppm in humans and 2 and 10 ppm in the mouse was assessed by randomly sampling various parameters of the PBPK model 1000 times as described under Materials and Methods. The frequencies of the natural logarithm of peak rate of metabolism are presented as histograms. The data ranges were 0.02–7.97 nmol h^{-1} g^{-1} for the human exposed to 2 ppm and 42.1–195.3 and 209.9–974.8 nmol h^{-1} g^{-1} for the rat exposed to 2 and 10 ppm, respectively.

The relationship between metabolism of CCl_4 and exposure concentration showed that a transition from first-order to mixed-order kinetics occurs at about 50 ppm (Fig. 6).

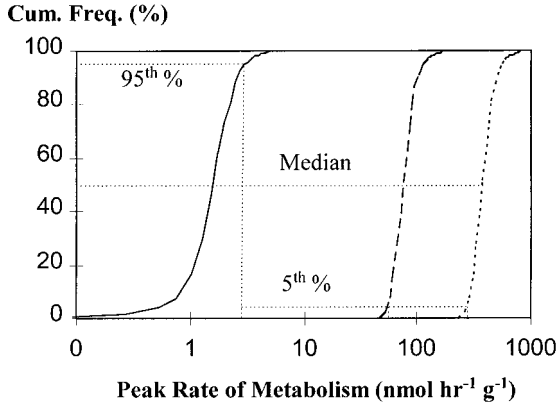

FIG. 4. Estimated cumulative frequency distributions for peak rate of metabolism of chloroform in humans and the $B6C3F_1$ mouse. The distributions were determined from Monte Carlo simulation data. The median for the human data (solid line) obtained from a 2 ppm exposure simulation was 1.62 nmol h^{-1} g^{-1}. This was approximately 47 and 234 times smaller than the median values (76.23 and 378.97 nmol h^{-1} g^{-1}) obtained from mouse simulations at 2 (long dashed line) and 10 ppm (short dashed line), respectively.

Monte Carlo analysis for carbon tetrachloride. The range and frequencies of the predicted 24-h cumulative hepatic metabolite levels for 1000 individual human and rats are shown in Table 8 and Fig. 7; cumulative frequency distribution estimates are shown in Fig. 8. For a constant 8-h exposure to the 8-h TWA OES of 2 ppm it was predicted that 95% of the human population described by the model would have cumulative hepatic metabolite levels of 0.035 μmol g^{-1} 24 h^{-1} or less. By comparison the 5th percentile cumulative hepatic metabolite level predicted for the rat population described by the model was ~0.087 μmol g^{-1} 24 h^{-1} at the 8-h TWA OES of 2 ppm for 8 h and ~ 0.22 μmol g^{-1} 24 h^{-1} at the NOAEL of 5 ppm for 8 h.

DISCUSSION

The use of physiologically based pharmacokinetic modeling to retrospectively examine the choice of uncertainty factors in the setting of OESs for $CHCl_3$ and CCl_4 has demonstrated that the current OESs are reassuring, at least with respect to toxicokinetics.

In this study an inhalation exposure concentration of 130 ppm $CHCl_3$ (65-fold higher than the current OES) was predicted as being needed to generate the same mean peak rate of metabolism in humans as that ob-

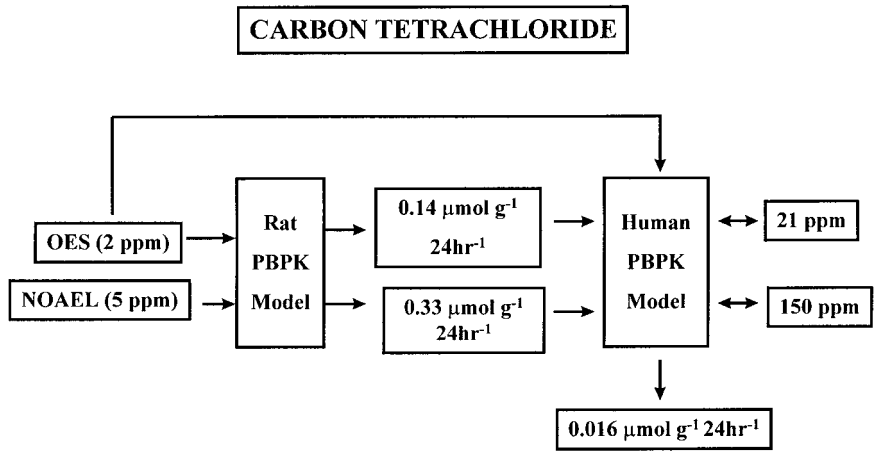

FIG. 5. Schematic diagram summarizing the input (airbone concentration in ppm) and outputs (24-h cumulative hepatic metabolite level in μmol g^{-1} 24 h^{-1}) of the rat and human PBPK models for carbon tetrachloride (OES, occupational exposure standard as 8-h TWA; NOAEL, no observed adverse effect level).

tained in female $B6C3F_1$ mice exposed by inhalation to the corresponding mouse NOAEL (10 ppm). Conversely the mean peak rate of metabolism at 10 ppm (the mouse NOAEL) is predicted to be approximately 78-fold lower in humans than in mice. Therefore, the current OES of 2 ppm is predicted to be far below the concentration required to cause the generation of toxic metabolites in humans at the rate that is necessary to produce cytotoxicity and sustained cell proliferation in mice.

These comparisons, however, are based on the average predicted peak rates in the respective populations and do not take into account variation away from these mean values. It is possible that there may be individuals in the human population who metabolize $CHCl_3$ at a rate more like that seen in the mouse. In order to address this possibility, Monte Carlo analysis was conducted, which showed that the majority of humans in the population described by the model have a predicted peak rate of $CHCl_3$ metabolism at the OES of 2 ppm that does not exceed 5 nmol h^{-1} g^{-1}. Five per 1000 (0.5%) individuals are predicted to have peak rates of metabolism of 10 nmol h^{-1} g^{-1} at the OES. This compares with the predicted value of ~50 nmol h^{-1} g^{-1} for the predicted slowest peak metabolic rate in the mouse at 2 ppm. The more appropriate comparison is with a predicted value for the slowest peak rate in the mouse of ~250 nmol h^{-1} g^{-1} at the NOAEL of 10 ppm. Thus comparing the rate of the fastest (representing 0.5%) peak metabolisers in the described human population exposed to 2 ppm with the slowest in the mouse at the NOAEL in this species gives a reassuring ratio of 25 (i.e., 250 nmol h^{-1} g^{-1}/10 nmol h^{-1} g^{-1}). In the majority of the described human population (e.g., 95%) the ratio is even higher (250/5 = 50).

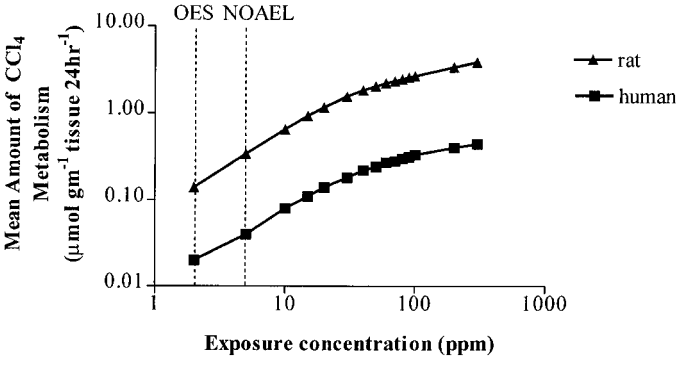

FIG. 6. Dose–response curves for hepatic carbon tetrachloride metabolism in the rat and humans. Hypothetical inhalation exposure to the UK 8-h TWA OES (2 ppm), the male rat NOAEL (5 ppm), and a range of concentrations from 10 to 1000 ppm carbon tetrachloride were input into the PBPK models. The corresponding 24-h cumulative hepatic metabolite levels were generated by the models and plotted against the hypothetical inhalation exposure concentrations.

The toxicity of CCl_4 is less well defined than of $CHCl_3$ although it is thought that CCl_4 metabolites bind to tissue macromolecules which have long clearance half-lives (Paustenbach *et al.*, 1988). The PBPK model used in this study described CCl_4 metabolism as occurring through a single, saturable oxidative pathway. The metabolic rate constants indicate that this pathway is of low affinity and low capacity. The median 24-h cumulative amount of metabolite at the NOAEL in the rat is predicted to be 0.33 μmol g^{-1} 24 h^{-1}, approximately 21-fold higher than the median (0.016 μmol g^{-1} 24 h^{-1}) predicted for the human at the OES. As for $CHCl_3$, this comparison is based on average (median) values. Outputs from the Monte Carlo analysis for CCl_4 indicated that the predicted greatest metabolizers (95th percentile) in the described human population would have 24-h cumulative amounts of metabolism of

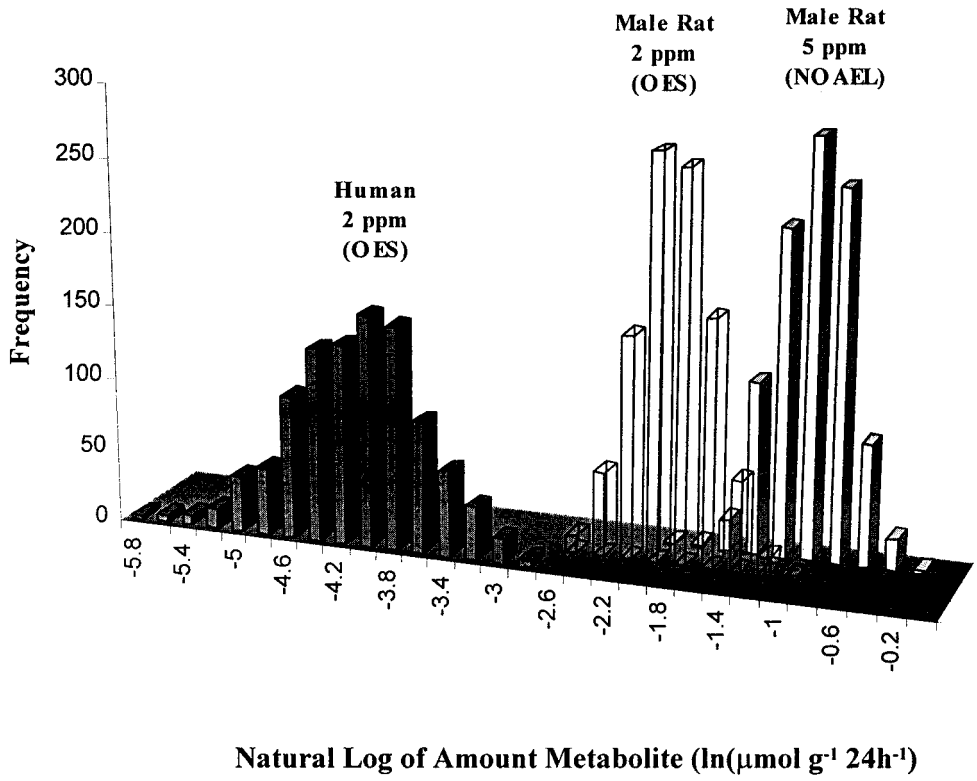

FIG. 7. Interindividual variability in carbon tetrachloride metabolism. The 24-h cumulative amount of carbon tetrachloride metabolism after inhalation exposure to 2 ppm in humans and 2 and 5 ppm in the rat was assessed by randomly sampling various parameters of the PBPK model 1000 times as described under Materials and Methods. The frequencies of the natural logarithm of 24-h cumulative hepatic metabolite are presented as histograms. The data ranges were 0.002–0.061 μmol g^{-1} 24 h^{-1} for the human exposed to 2 ppm and 0.064–0.316 and 0.117–0.799 μmol g^{-1} 24 h^{-1} for the rat exposed to 2 and 5 ppm, respectively.

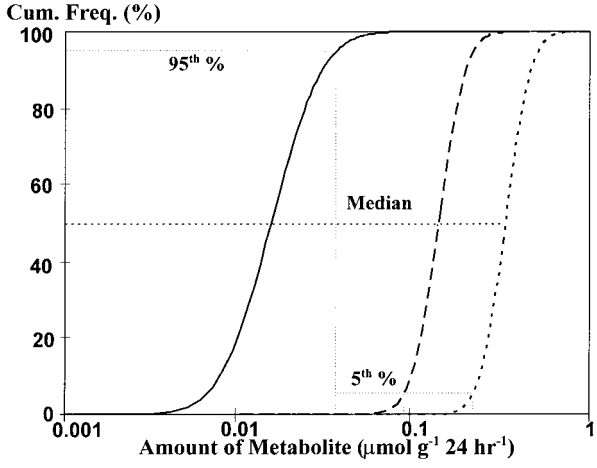

FIG. 8. Estimated cumulative frequency distributions for the 24-h cumulative amount of carbon tetrachloride metabolism in humans and the rat. The distributions were determined from Monte Carlo simulation data. The median for the human data (solid line) obtained from a 2 ppm exposure simulation was 0.016 μmol g^{-1} 24 h^{-1}. This was approximately 8.7 and 21.4 times smaller than the median values (0.14 and 0.33 μmol g^{-1} 24 h^{-1}) obtained from rat simulations at 2 (long dashed line) and 5 ppm (short dashed line), respectively.

~0.03 μmol g^{-1} 24 h^{-1} at the 8-h TWA OES of 2 ppm. In comparison, the least metabolizers (5th percentile) in the described rat population at the NOAEL of 5 ppm were predicted to be ~0.08 μmol g^{-1} 24 h^{-1}. This gives a ratio (rat NOAEL/human OES) for the least rat to greatest human metabolisers of around 2.7.

This analysis is based on a comparison of differences in toxicokinetics. To extend the conclusions to cover resultant toxicity consequences would require an assumption that the toxicodynamics of $CHCl_3$ and CCl_4 in rodents and humans are qualitatively and quantitatively similar. The assumption of a qualitative similarity would seem reasonable based upon the knowledge of metabolism in these species and the generally similar spectrum of toxic responses observed in both animals and humans (Gardner *et al.*, 1994; Delic *et al.*, 1994). Whether or not there are quantitative similarities or differences in toxicodynamics is less clear although this cannot be discounted. Nevertheless, the predicted ratios between the fastest metabolizers in the described human population at the OES and the slowest metabolizers in the described animal model populations at their respective NOAELs are greater than the uncertainty factors used originally in the establishment of the OESs and even greater for most of

the population, in the direction of reassurance. Thus assuming that similar modes of action are acting in both the human and the animal models then human liver and kidney (the primary target tissues) would need to be substantially more intrinsically sensitive to the toxic effects of $CHCl_3$ and CCl_4 than those of the mouse or rat respectively, for there to be any concern about the possibility of toxicity at the OES values.

CONCLUSIONS

The establishment in the UK of the OESs for $CHCl_3$ and CCl_4 during the early 1990s was undertaken with little or no detailed knowledge of inter- and intraspecies differences and variability in toxicokinetics and toxicodynamics. The subsequent availability of more detailed toxicokinetic information and PBPK modeling has enabled the current analysis to be performed. This analysis is reassuring in that it indicates that workers exposed to $CHCl_3$ and CCl_4 at the current UK OESs will not generate metabolites to levels in target tissues associated with toxicity, even allowing for variation in the population. In general, worldwide, PBPK modeling has found limited use in a formal regulatory context and has not yet been used in this context within the UK. Although it is not a technique that currently is likely to be used routinely in the regulation of industrial chemicals, this study has demonstrated the value that may be gained from using such an approach. Where possible in the future, use of PBPK modeling and associated approaches will be made in the UK occupational regulatory setting in order to help inform and improve the toxicological aspects of the regulatory process.

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