

Physiologically-based Pharmacokinetic (PBPK) Modeling of 1,4-Dioxane in Rats, Mice and Humans

Final Report



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Executive Summary

1,4-Dioxane (CAS No. 123-91-1) is used primarily as a solvent or as a stabilizer for solvents. 1,4-Dioxane has been shown to produce liver and nasal tumors in rodents, but the relevance of the nasal tumors is uncertain. Two physiologically-based pharmacokinetic (PBPK) models for 1,4-dioxane and its major metabolite, hydroxyethoxyacetic acid (HEAA), were published in 1990 (Reitz *et al.*, 1990; Leung and Paustenbach, 1990) and were used to derive cancer potency estimates for 1,4-dioxane. Since 1990, new data have been collected for model parameterization and validation. Updated models that incorporate our improved understanding of the uptake, distribution, metabolism, and elimination of 1,4-dioxane and HEAA were developed based on this new data. These models will serve as better tools for uncertainty reduction in future 1,4-dioxane risk assessments.

INTRODUCTION

1,4-Dioxane (CAS No. 123-91-1) is used primarily as a solvent or as a stabilizer for solvents. 1,4-Dioxane has been shown to produce liver and nasal tumors in rodents, but the relevance of the nasal tumors is uncertain (see summary by Stickney et al., 2003). Two physiologically-based pharmacokinetic (PBPK) models for 1,4-dioxane and its major metabolite, hydroxyethoxyacetic acid (HEAA), were published in 1990 (Reitz *et al.*, 1990; Leung and Paustenbach, 1990) and were used to derive improved cancer potency estimates for 1,4-dioxane. These improved potencies were many orders of magnitude less potent than those derived by the USEPA during their last evaluation of 1,4-dioxane carcinogenicity in 1990 using standard default approaches.

The Sapphire Group (2005) previously reviewed the existing 1,4-dioxane PBPK models and made recommendations for filling “data gaps” pertaining to the pharmacokinetics of 1,4-dioxane and HEAA in rats, mice, and humans. Subsequently, studies were performed at Battelle Pacific Northwest Laboratory for the purpose of filling these data gaps (Thrall et al., 2005; Poet et al., 2005, 2006). Three types of studies were performed: partition coefficient measurements, blood time course in mice, and *in vitro* pharmacokinetics. The partition coefficient measurements consisted of new measurements for mouse blood and tissues (liver, kidney, fat, and muscle) and confirmatory measurements for human blood and rat blood and muscle. The blood time course measurements in mice were conducted for gavage administration of nominal single doses (20, 200, or 2000 mg/kg) of 1,4-dioxane administered in water. Vial incubations of 1,4-dioxane with rat liver microsomes failed to produce detectable declines in headspace concentration of 1,4-dioxane or increases in HEAA in buffer. Incubations of 1,4-dioxane with rat and mouse hepatocytes did produce measurable amounts of HEAA, and estimates of rate constants for metabolism of 1,4-dioxane by rat and mouse liver were thus derived.

In the present effort, we have developed PBPK models for the rat, mouse, and human which are consistent with the newly collected data (described above) and previous kinetic studies in rats and human volunteers reported by Young et al. (1977, 1978).

METHODS

Source Data

Mouse pharmacokinetic data were provided in spreadsheet form by Dr. Karla Thrall of Battelle. Some human and rat pharmacokinetic data were available in numerical form from Dr. Dick Reitz (retired, Dow Chemical) and from Young et al. (1976). Additional human and rat pharmacokinetic data were available in graphical form from Young et al. (1977, 1978). Scanned images were converted into numerical data using Plot Digitizer (version

2.4.0), with minor adjustments made to match reported sampling times. Copies of worksheets reporting blood 1,4-dioxane and HEAA concentrations for the four individuals in Young et al. (1977) were graciously provided by Dr. Bill Stott, Dow Chemical Company, Midland, Michigan. A copy of the unpublished detail is included as Appendix A.

Model Description

The model structure was similar to those used by Reitz et al. (1990) and Leung and Paustenbach (1990) and is depicted in **Figure 1**. Model parameter values are summarized in **Table 1**. Tissue volumes and fractional blood flow rates were taken from Brown et al. (1997). Partition coefficients were generally taken from Thrall et al. (2005). The measured mouse kidney:air partition coefficient used for all three species, and muscle:air partition coefficients used for slowly perfused tissues. The rat fat:air value was reported by Reitz et al. (1990). Human liver:air, fat:air and slowly perfused tissue:air partition coefficients were estimated as the average of measured mouse and rat values.

Table 1. PBPK Model Parameter Values for 1,4-Dioxane

Parameter	Units	Rat	Mouse	Human	Source/Comments
Body weight (BW)	kg	0.25	0.025	70	Default; experiment-specific values used when available
Fractional volume of liver (VLC)	(none)	0.034	0.055	0.033	Brown et al. (1997)
Fractional volume of adipose (VFC)	(none)	0.07	0.07	0.214	Brown et al. (1997)
Fractional volume of richly perfused tissues (VRC)	(none)				$VRC = 1 - (VLC + VFC + VSC + VBC + VUC)$
Fractional volume of slowly perfused tissues (VSC)	(none)	0.594	0.549	0.437	Brown et al. (1997)
Fractional volume of blood (VBC)	(none)	0.074	0.049	0.079	Brown et al. (1997)

Parameter	Units	Rat	Mouse	Human	Source/Comments
Fraction of unperfused tissue (VUC)	(none)	0.05	0.054	0.071	Brown et al. (1997)
Normalized alveolar ventilation rate (QPC)	L/hr-kg ^{0.74}	13	20	13	Brown et al. (1997)
Normalized cardiac output (QPC)	L/hr-kg ^{0.74}	13	20	13	Brown et al. (1997)
Fractional blood flow to liver (QLC)	(none)	0.183	0.161	0.227	Brown et al. (1997)
Fractional blood flow to adipose (QFC)	(none)	0.07	0.07	0.052	Brown et al. (1997)
Fractional blood flow to richly perfused tissues (QRC)	(none)				1 - (QLC + QFC + QSC)
Fractional blood flow to slowly perfused tissues (QSC)	(none)	0.336	0.217	0.249	Brown et al. (1997)
Blood/air partition coefficient (PB)	(none)	1861	2002	1666	Thrall et al. (2005)
Liver/air partition coefficient (PLA)	(none)	1862	1143	1500	Rat and mouse: Thrall et al. (2005); human: average of rat and mouse
Adipose/air partition coefficient (PFA)	(none)	851	879	865	Rat: Reitz et al. (1990); mouse: Thrall et al. (2005); human: average of rat and mouse

Parameter	Units	Rat	Mouse	Human	Source/Comments
Richly perfused tissues/air partition coefficient (PRA)	(none)	560	560	560	Mouse kidney, Thrall et al. (2005); rat and human: assumed equal to mouse kidney
Slowly perfused tissues/air partition coefficient (PSA)	(none)	1348	1705	1503	Rat and mouse: Thrall et al. (2005); human: average of rat and mouse
Normalized Maximal rate of metabolism of 1,4-dioxane in liver (VmaxC)	mg/hr-kg ^{0.7}	7.5 or 12.7	39 or 46	54 to 192	Rat (uninduced/induced) and mouse: optimized fit to <i>in vivo</i> data; human: parallelogram approach, based on scaled <i>in vitro</i> data
Michaelis constant for metabolism of 1,4-dioxane in liver (Km)	mg/L	21	21	29 to 147	Rat: optimized fit to <i>in vivo</i> data. Mouse: equality to rat assumed, based on <i>in vitro</i> data; human: scaled from rat <i>in vivo</i> Km using <i>in vitro</i> human:rat ratios
Normalized volume of distribution for metabolite (VDMC)	L/kg	1	0.83	0.83	VDMC not identifiable for rat; value of 1 assumed; mouse: optimized, human: equality to mouse assumed

Parameter	Units	Rat	Mouse	Human	Source/Comments
Elimination rate of metabolite (Kme)	hr ⁻¹	0.48	0.35	0.35	Rat and mouse: optimized based on fit to <i>in vivo</i> data, human: equality to mouse assumed

Estimated/Optimized Parameters

The determination of certain model parameters by estimation/optimization is described in greater detail under “Results”, but described briefly below.

The metabolic rate constants VmaxC (maximum rate of metabolism, normalized to scaled body weight, BW^{0.7}) and Km (Michaelis constant, or apparent enzyme affinity) for rats were derived by fit to the intravenous (iv) data of Young et al. (1978). Young et al. (1978) had noted that administration of a dose of 1000 mg/kg, but not 10 mg/kg 1,4-dioxane appeared to induce metabolism of 1,4-dioxane. Nannelli et al. (2005) also reported the induction of cytochrome P450 2B1/2- and 2E1-dependent metabolic activities in rat liver due to oral exposure to 1,4-dioxane. The appropriateness of dose-specific VmaxC values was tested by optimizing the fit to high or low iv doses separately.

The first-order rate parameter for urinary elimination of HEAA by rats was determined by optimizing the fit to urinary excretion data for iv and oral dosing (Young et al., 1978).

Based on the similarity of Km values derived *in vitro* for metabolism of 1,4-dioxane by rats, mice, and humans, (Poet et al., 2005, 2006), the Km value derived by optimization for rats was also used for the other species. Estimates of the oral absorption rate constant and VmaxC values for mice were made based on fit to blood 1,4-dioxane concentrations reported by Thrall et al. (2005). Because the analytical method measured background/artifactual levels of 1,4-dioxane and HEAA levels in blood of unexposed mice, only values that were >3-fold higher than the background level were used in modeling. The oral absorption rate constant for mice was also applied to simulations of oral dosing in rats.

Human VmaxC estimates were made using the parallelogram approach, relying on the “best fit” *in vivo* values derived for rats and mice and the *in vitro* rates determined using rat, mouse, and human hepatocytes (Poet et al., 2005, 2006). Hepatocyte yields of 128, 110, or 137 × 10⁶ hepatocytes per gram of mouse, rat, and human liver (Seglen, 1978, Arias et al.,

1982, and Carlile et al., 1997), respectively, and the default tissue volumes and body weights in Table 1 were used to scale *in vitro* data.

The first order elimination rate for metabolite in urine (K_{me}) of rats was estimated by best fit to amounts excreted when rats were dosed by single iv or gavage (Young et al., 1978). K_{me} and the volume of distribution of the metabolite (VDMC) of mice was estimated by best fit to blood concentrations of HEAA measured in mice dosed by gavage (Thrall et al., 2005).

Model Validation

The model was further tested against additional data of Young et al. (1976, 1977, 1978) and Thrall et al. (2005) as described under “Results.”

Software and Algorithms

All simulations and parameter fitting were conducted using ACSL Sim 11.4 and ACSL Math, Version 2.5.4 (Aegis Technologies, Hunstville, Alabama) on a Dell Optiplex GX260 computer with a Pentium 4 processor. The Gear algorithm was used for integration of double precision variables. Parameter fitting was performed using the relative error model (variance is assumed to be proportional to the measured value across the range of measured values, or heteroscedasticity = 2) and the Nelder-Mead algorithm. The fitting criterion was maximization of the log likelihood function. Starting values for parameter fitting in ACSL Math were determined from parameter estimates derived by visual best fit in ACSL Sim. Goodness of fit is described as the “percentage of variation explained”, which is similar to the r^2 value derived for linear regression.

RESULTS

Determination of V_{maxC} and K_m for the Rat

Preliminary values of V_{maxC} and K_m in the rat were derived by optimizing the fit to the 1000 mg/kg iv data (Young et al., 1978) (“induced” rat V_{maxC}) and iv doses of 3, 10, 30, and 100 mg/kg (“uninduced” rat V_{maxC}). The 300 mg/kg iv data were initially omitted as a likely border-line case which could distort the optimization of fit to “high” and “low” data. Preliminary best-fit values of $V_{maxC} = 12.8 \pm 0.036$ mg/hr-kg^{0.7} and $K_m = 22.0 \pm 1$ mg/L were derived for the induced rat, and values of $V_{maxC} = 7.4 \pm 0.05$ mg/hr-kg^{0.7} and $K_m = 20.5 \pm 1.4$ mg/L for the uninduced rat. Because of the similarity of the K_m s for the uninduced and induced rats, an average value of $K_m = 21$ mg/L was selected as being applicable to all doses. With the K_m value set at 21 mg/L, the best-fit value of V_{maxC} for induced rats, 12.7 ± 0.08 mg/hr-kg^{0.7} (80.4% of variation explained) was determined from fit of the 1000 mg/kg data. Likewise, a best fit value of $V_{maxC} = 7.5 \pm 0.2$ mg/hr-kg^{0.7} was derived for uninduced rats (66.6 % of variation explained). The best-fit V_{maxC} for the 300

mg/kg iv data was found to be $10.8 \pm 0.2 \text{ mg/hr-kg}^{0.7}$, indicating that these data would be more appropriately described by the “induced” V_{maxC} rather than the uninduced V_{maxC} . The model fit to the iv data is shown in **Figure 2**.

Determination of V_{maxC} , K_m , and K_A for the Mouse

The *in vivo* K_m value for the mouse was estimated as being equal to the best-fit rat value of 21 mg/L. The basis for this selection was that the *in vitro* K_m s for production of HEAA from 1,4 dioxane from incubated rat and mouse hepatocytes (2.51 ± 0.88 and 2.63 ± 0.68 mg/ml) are statistically indistinguishable. Thus it is expected that the *in vivo* K_m s will also be similar. The *in vivo* mouse data (Thrall et al., 2005) have insufficient samples where the blood concentration of 1,4-dioxane was at or below the likely K_m , so it was not possible to identify the *in vivo* K_m on the basis of fit to the *in vivo* data.

Mouse V_{maxC} and K_A values were derived by optimizing fit to the blood 1,4-dioxane concentrations in mice administered nominal doses of 200 and 2000 mg/kg 1,4-dioxane by gavage in a water vehicle. 1,4-Dioxane measurements in blood of the animals in the 20 mg/kg group were indistinguishable from the background for the analytical method, and thus could not be used for pharmacokinetic analysis. Because doses >300 mg/kg have been found to induce 1,4-dioxane metabolism in rats, the possibility of dose-dependency of V_{maxC} was also assumed for mice. Preliminary V_{maxC} and K_A values for potentially induced mice (2000 mg/kg dose) were $46.6 \pm 1.1 \text{ mg/hr-kg}^{0.7}$ and $0.73 \pm 0.09/\text{hr}$, while the preliminary values for uninduced mice (200 mg/kg) were $39.1 \pm 0.3 \text{ mg/hr-kg}^{0.7}$ and $0.94 \pm 0.009/\text{hr}$. Because the absorption rate would be expected to be similar across doses, a single value of 0.8/hr was assumed for both doses. With K_A fixed, dose-dependent V_{maxC} values were then optimized as 46 ± 1 and $39 \pm 1 \text{ mg/hr-kg}^{0.7}$ for 2000 mg/kg and 200 mg/kg mice, respectively (91.8 and 91.5 % of variation explained, respectively). The model fit to the mouse oral data is shown in **Figure 3**.

Scaling of *in vitro* Metabolism Data/Estimation of Human V_{maxC} and K_m

The *in vitro* V_{max} values for rats and mice (Poet et al., 2005) were scaled to estimated *in vivo* rates, which were compared to the optimized values. The scaled and optimized rat V_{maxC} s were very similar. The discrepancy between the scaled and optimized mouse values was larger, which was attributed to possible induction in mice at the lowest dose tested (200 mg/kg). The ratio of optimized/scaled values for the rat was used to adjust the scaled human V_{maxC} values to projected *in vivo* values.

Table 2. Scaling of 1,4-Dioxane Metabolism in Hepatocytes

	<i>In vitro</i> rate ($\mu\text{g/hr} \cdot 10^6$ cells) ^a	Scaled rate ($\text{mg/hr} \cdot$ $\text{kg}^{0.7}$)	Optimized <i>in vivo</i> rate ($\text{mg/hr} \cdot$ $\text{kg}^{0.7}$) ^b	Ratio of <i>in vivo</i> /scaled rates	Estimated <i>in vivo</i> rate ($\text{mg/hr} \cdot$ $\text{kg}^{0.7}$)
Rat	1.9	5.5	7.5	1.4	Not applicable
Mouse	3.7	7.5	39	5.2	Not applicable
Human (representative) ^c	3.4	55	Not applicable	1.4 ^d	75
Human (minimum)	2.4	39	Not applicable	1.4 ^d	54
Human (maximum)	8.7	141	Not applicable	1.4 ^d	192

^aPoet et al. (2005, 2006)

^bLowest tested dose

^cAverage of three similar individual values (Poet et al., 2006)

^dAssumed equal to rat ratio

The K_m value derived for the rat *in vitro* (2,510 mg/L) differs substantially from the K_m estimated from the *in vivo* data (21 mg/L). This difference may be related to unexpected difficulty with measuring 1,4-dioxane metabolism *in vitro* (i.e., the inability to detect 1,4-dioxane disappearance or HEAA appearance using microsomes). Human *in vivo* K_m s were estimated by multiplying the *in vitro* values by the *in vivo/in vitro* ratio for the rat. K_m s for representative, minimum, and maximum cases were 32, 29, and 147 mg/L.

Estimation of K_{me} for the Rat

The first order rate constant for the urinary elimination of the 1,4-dioxane metabolite HEAA by rats was estimated based on fit to the time course for total amount of HEAA eliminated in urine by rats dosed with 1,4-dioxane by iv (10 or 1,000 mg/kg) or gavage administration (10, 100, or 1,000 mg/kg) (Young et al., 1978). Dose-specific $V_{max}C_s$ (derived as described above) were used. The oral absorption rate constant for the rat was assumed to be equal to the best-fit value derived for the mouse ($K_A = 0.8$). The optimized value of K_{me} for the rat was $0.48 \pm 0.049/\text{hr}$ (93.0 % of variation explained). The model fit to the rat urinary metabolite data is shown in **Figure 4**.

K_{me} values were also estimated for each of data set individually. The optimal K_{me} values ± the standard deviations generally encompassed the optimal values for all five data sets considered together. The single exception was the low dose (10 mg/kg) iv data, where an optimal fit was found with K_{me} = 0.16 ± 0.02/hr. Because the optimal K_{me} for an equal oral dose was more in line with the group K_{me} value (0.62 ± 0.11/hr), a dose-dependence in K_{me} did not seem to be indicated. The K_{me} value derived for the rat using all five data sets was used in the modeling.

Estimation of K_{me} and VDMC for the Mouse.

The volume of distribution of the 1,4-dioxane metabolite HEAA (VDMC) and the rate constant for urinary elimination of HEAA were optimized based on the fit to the time course of HEAA in blood of mice dosed with 200 or 2,000 mg/kg 1,4-dioxane by gavage (Thrall et al., 2005). The resulting values were VDMC = 0.83 ± 0.12 L/kg and K_{me} = 0.35 ± 0.02/hr (56.7 % of variation explained). If the low-dose HEAA data were included, a similar K_{me} value resulted (0.40/hr), but VDMC was significantly reduced (0.56 L/kg), and the fit deteriorated substantially (41.7% of variation explained). The VDMC and K_{me} values from the mid- and high-doses (with the low dose omitted) were used in modeling (**Figure 3**).

Model Validation/Fit to Other Rodent Data

Model outputs were compared to other data not used in fitting model parameters. The model predictions gave an excellent match to the 1,4-dioxane exhalation data after a 1,000 mg/kg iv dose. 1,4-Dioxane exhalation was overpredicted by a factor of ~3 for 10 mg/kg iv dose. Similarly, the simulations of exhaled 1,4-dioxane after oral dosing were excellent at 1000 mg/kg, very good at 100 mg/kg (within 50%), but poor at 10 mg/kg (model overpredicts by a factor of five). The prediction of the 1,4-dioxane exhalation data (Young et al., 1978) is shown in **Figure 5**.

The simulation of blood 1,4-dioxane concentrations in rats exposed to 50 ppm 1,4-dioxane (Young et al., 1978) was excellent (**Figure 6**), but total excretion in urine was under predicted by a factor of 3 (data not shown). In order to match the model prediction to the data for HEAA excretion, the inhalation rate had to be increased by factor of almost 4, and blood concentrations were no longer accurately predicted. While restraint in a head-only chamber (Young et al., 1978) might be expected to cause some stress, a four fold increase in ventilation rate seems unlikely.

Predictions of blood concentrations of 1,4-dioxane and HEAA were made for mice exposed to a low dose (20 mg/kg) of 1,4-dioxane by gavage (Thrall et al., 2005). Predictions were consistent with the measured levels of 1,4-dioxane in blood not being distinguishable from the background of the method (~1.6 mg/L). The model dramatically underpredicted the blood concentrations of HEAA 0.5 and 1 hr after dosing, while overpredicting at 2 hrs (**Figure 7**). The model predicted that HEAA levels would be above the background of the method (~1.1 mg/L) at the 3 and 6 hr sample points, but they were not.

Fit of the Model to Human Volunteer Data

The fit of the model to the human data (Young et al., 1977) (**Figures 8 and 9**) was problematic. Using physiological parameters of Brown et al. (1997) and measured partitioning parameters (Thrall et al., 2005; Reitz et al., 1990) with no metabolism, measured blood 1,4-dioxane concentrations reported by Young et al. could not be achieved unless the estimated exposure concentration was increased from 53 to 100 ppm. Inclusion of any metabolism necessarily decreased predicted blood concentrations. If estimated metabolism rates were used (**Table 1 and 2**) with the reported exposure concentration, urinary metabolite excretion was underpredicted. Urinary metabolite excretion rates could be matched if either exposure concentration was increased to 62 ppm, or alveolar ventilation (QPC) was increased to 17 L/hr-kg^{0.74}. Both of these adjustments are plausible. Because the volunteers were given “bottled water, coffee, and a sandwich on demand” (Young et al., 1977) it is possible that additional 1,4-dioxane partitioned into food and beverages, increasing the total dose. The QPC estimate taken from Brown et al. (1997) (QPC assumed equal to cardiac output), 13 L/hr-kg^{0.74} is on the low side; the average value reported by Price et al. (2003) is 18 L/hr-kg^{0.74}. The ventilation rate used by Reitz et al. (1990) equates to a QPC of 30 L/hr-kg^{0.74}, which seems inconsistent with the low activity levels (volunteers were seated in an exposure chamber, Young et al., 1977). With the ventilation rate or concentration adjusted to match urinary excretion, the human model predicts significantly lower blood concentrations of 1,4-dioxane (~6 fold) than reported by Young et al. (1977). Conversely, if the estimated exposure concentration is increased by a factor of ~6, model predictions are consistent with measured blood 1,4-dioxane concentrations of individuals P, T, and G, but urinary excretion of HEAA is overestimated by a factor of ~6.

To increase the predicted level of 1,4-dioxane in human blood, both Reitz et al. (1990) and Leung and Paustenbach (1990) decreased the effective volume of distribution for the parent compound. The effective volume of distribution is the sum of the blood volume and the sum of the tissue volume multiplied by the ratio of the tissue:air and blood:air partition coefficient for all the tissues. Reitz et al. (1990) decreased the effective volume of distribution by doubling the blood:air partition coefficient, while Leung and Paustenbach (1990) reduced the tissue:air partition coefficient of the largest compartment, the slowly perfused tissues, by a factor of 2.5. The validity of these adjustments need to be considered. The original human blood:air partition coefficient (Reitz et al., 1990; Leung and Paustenbach, 1990) was confirmed by Thrall et al. (2005). The measured rat muscle:air partition coefficient was 997 ± 254 (Leung and Paustenbach), but Reitz et al. (1990) used the liver:air partition coefficient (1557) in place of the measured muscle:air value. The measurements of Thrall et al. (2005) (PSA= 1348 for rat, 1705 for mouse) confirm that the originally measured value of the rat muscle:air partition coefficient was too low. Thus the manipulation of the slowly perfused tissue partitioning by Leung and Paustenbach does not seem justified. 1,4-Dioxane appears to be rapidly distributed into tissues (brain, liver, kidney, and testes), with peak concentrations of radiolabel achieved within 15 minutes of ip injection (Mikheev et al., 1990). The tissue/blood ratios of radiolabel (Mikheev et al., 1990) were consistent with the PRA/PB ratio of 1,4-dioxane. Overall, the information on 1,4-dioxane partitioning does not

support the alterations Reitz et al. (1990) and Leung and Paustenbach (1990) made in their attempts to fit the human data of Young et al. (1977).

Fit of the Model to Human Occupational Exposure Data

In contrast to the fit to the volunteer blood concentrations, the fit to the urinary concentrations of 1,4-dioxane and HEAA in occupationally exposed workers (Young et al., 1976), the fit was excellent (**Table 3**). Because there is no “urine compartment” per se, some assumptions were made to convert the Young et al. (1976) urinary concentration data into estimated body burden. It was assumed the urinary concentration \times urine production rate = body burden \times elimination rate into urine. The urine production rate was assumed to be 1 ml/min (Young et al., 1977). The elimination rate of 1,4-dioxane into urine by humans (0.0033/hr) was taken from Young et al. (1977). The elimination rate of HEAA into urine was the value derived from the mouse model (0.35/hr). The group average values of estimated body burden of 1,4-dioxane and HEAA are within 10% of the modeled group average value.

Table 3. Comparison of Model Predictions and Experimental Data for Concentrations of 1,4-Dioxane and HEAA in Urine of Workers

Employee (Body Weight, kg; workdays)	1,4-Dioxane in air (ppm) ^a	Estimated 1,4-dioxane in body (mg)		Estimated HEAA in body (1,4-dioxane mg equivalent) ^a	
		Estimated ^a (mean \pm SD)	Model prediction	Estimated ^a (mean \pm SD)	Model prediction
A (74.8, 1)	1	6.88	3.42	0.91	3.49
B (110.7, 5)	1.6 \pm 0.5	5.28 \pm 3.2	8.04	3.47 \pm 1.46	7.22
C (74.4, 4)	2.0 \pm 1.0	5.76 \pm 0.64	6.81	9.38 \pm 2.32	6.95
D (79.4, 5)	1.8 \pm 0.4	5.92 \pm 2.24	6.54	7.09 \pm 3.45	6.53
E (78.5, 5)	1.1 \pm 0.6	4.80 \pm 1.12	3.95	6.73 \pm 2.14	3.96
Average (83.56, 4)	1.6 \pm 0.7	5.60 \pm 1.92	6.11	6.25 \pm 3.26	6.0

^aBody burden after 7.5 hrs exposure, based Young et al. (1976), estimated as described in text

DISCUSSION

Comparison with Previous PBPK Models

The partition coefficients used in this work (Thrall et al., 2005), have previously been compared to those used in previous PBPK models. Perhaps the most important comparison is that the results of Thrall et al. (2005) confirm the measured human blood:air partition coefficient values reported by Reitz et al. (1990), but not used in the modeling in that paper.

The V_{maxC} , K_m , and K_{me} derivations for the rat for this modeling effort and the previous efforts (Reitz et al., 1990; Leung and Paustenbach, 1990) drew on the same experimental data sets (Young et al., 1978). The rat V_{maxC} s derived in this effort (7.5 and 12.7 mg/hr-kg^{0.7}, for uninduced and induced rats, respectively) were intermediate between the values determined by Leung and Paustenbach (1990) (normalized values of 5.0 and 9.2 mg/hr-kg^{0.7} calculated from reported V_{max} values) and Reitz et al. (1990) (13.7 mg/hr-kg^{0.7}) and were similar to the value derived from scaling the *in vitro* data (Poet et al., 2005). The ratio of induced V_{maxC} to uninduced V_{maxC} determined by Leung and Paustenbach (1990) was similar to the ratio from the current effort (current: 1.7, previous: 1.8). The *in vivo* rat K_m for the current effort (21 mg/L) was intermediate between the Reitz et al. (1990) and Leung and Paustenbach (1990) values of 7.5 and 29.4 mg/L, respectively. The V_{maxC}/K_m ratios for the current effort (0.36 and 0.60 L/hr-kg^{0.7}, uninduced and induced) were closer to the V_{maxC}/K_m ratio of Reitz et al. (1990) (0.47 L/hr-kg^{0.7}) than Leung and Paustenbach (0.67 and 0.12 L/hr-kg^{0.7}, uninduced and induced). The K_{me} value of 0.28/hr used by Reitz et al. (1990) appeared to have been derived only from the iv data. In contrast, the current evaluation ($K_{me} = 0.48$ /hr) used both iv and oral data, and one of the iv data sets was found to best fit a much lower K_{me} than the other data sets, as discussed above.

Reitz et al. (1990) estimated V_{maxC} and K_m values for mice by averaging the values derived for rat and humans, but had no data against which to validate these parameters. In the current effort, *in vitro* data indicated that the mouse K_m was similar to the rat value (Poet et al., 2005). The *in vivo* rat K_m was identified as ~21 mg/L by optimization. This value is similar to the value of 16.2 mg/L previously estimated by Reitz et al. (1990). The V_{maxC} estimated by Reitz et al. (10 mg/hr-kg^{0.7}) is significantly lower than the value estimated using fits to the 200 and 2000 mg/kg dosing data (39 and 45 mg/hr-kg^{0.7}, respectively). It is possible that the V_{maxC} identified for 200 mg/kg does not represent an “uninduced” value, but rather a value that is not induced to the same extent as the 2000 mg/kg dose. In rats, the transition from doses that do not induce 1,4-dioxane metabolism to doses that do induce metabolism is between 100 and 300 mg/kg. The larger discrepancy in mice, as compared to rats, between the *in vivo* best-fit value and scaled *in vitro* V_{maxC} also supports the theory that the 200 mg/kg dose induced 1,4-dioxane metabolism.

Fit of the Model to Rat and Mouse Experimental Data

The optimized model parameters provide a good fit to the blood measurements of 1,4-dioxane in mice and rats (**Figure 2, 3, and 6**) and exhaled breath 1,4-dioxane at mid- to high-doses (**Figure 5**). The poorer fit to the low-dose exhaled breath 1,4-dioxane may reflect limited metabolism in the upper respiratory tract which does not contribute significantly to whole body metabolism, but scrubs some 1,4-dioxane from exhaled breath. The fit to and prediction of the HEAA data was somewhat less successful than the prediction of the 1,4-dioxane data. The lack of fit to some of the HEAA data is likely due to an overly simplistic description of its distribution and elimination (single compartment, first order elimination).

Application of the Human Model in Risk Assessment

Clearly there is sufficient data to support the use of a PBPK model rather than generic scaling factors for interspecies scaling of dosimetry. Since there is limited human data on which to validate the model, the most appropriate use of the model needs to address uncertainties associated with the limited *in vivo* data and the uncertainties in the *in vitro* data (*i.e.*, discrepancies between rat *in vitro* and *in vivo* Kms). An issue that deserves consideration is that the unadjusted human model predicts significantly lower blood concentrations of 1,4-dioxane (~6 fold). If blood or tissue 1,4-dioxane level were to be used as a dose metric in risk assessment, the unadjusted model would result in a less conservative assessment. We can see three options that might be pursued: (1) manipulate the human model parameters to match the available human *in vivo* data, (2) use the unadjusted human model as-is, or (3) use the unadjusted human model, but multiply 1,4-dioxane dose metrics by the 6-fold discrepancy with the available experimental data. The first and third options assume that the Young et al. (1977) human data are “right”, and the model predictions are adjusted, while under the second option, the model is “right” and the data are “wrong”. We recommend option (3) as the impact of this discrepancy is clearly and consistently accounted for in the risk assessment. Under option (1), the parameter adjustments that are made could have a different impact under low-dose or route-to-route extrapolation that would be complicated to identify. We cannot recommend option (2) in isolation because of the potential skewing of the risk assessment towards inadequate health protection, but it may be worthwhile to use option (2) in combination with option (1) or (3) as a possible lower-bound estimate. Despite the limitations of the human model, the use of a validated mouse model and a refined rat model, combined with a better understanding of the validity of the human model provide the tools for more scientifically credible risk assessments than could be done in the absence of these models, or the previously available PBPK models for 1,4-dioxane.

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Appendix A

Personal communication from Dr. Bill Stott, Dow Chemical Company, Midland, Michigan

1059 - 14

9.22.76

Dioxane: Human Inhalation Study

WORK SHEET

Purpose: assay for 1,4-Dioxane in plasma
and urine of inhalation exposed humans

method: see publication Braun (1976)

results:

Plasma (ppm) Dioxane

subjects →

Time ↓	P	T	C	G	avg
1 0.5 hrs	1.0 1.5 1.5	0.3 0.3	1.7 1.7	0.5 0.8	1.0
2 1.0 hrs	4.1 4.3 4.6	1.7 2.5 2.1	5.7 8.9 5.5	3.7 2.5 2.6	4.4
3 2.0 hrs	4.0 7.4 8.7	3.7 6.9 6.9	14.2 13.5 14.1	4.7 8.0 8.0	9.4
4 3.0 hrs	9.5 9.4 13.9 10.5	8.7 10.7 9.2	16.3 19.6 17.2	10.2 10.6 10.6	10.6
5 4.0 hrs	10.7 10.0 10.1	13.0 9.2 11.6	19.7 20.1 19.6	12.5 12.1 12.3	13.4
6 5.0 hrs	10.1 (25)	11.6 (28)	19.6 (22)	12.3 (26)	13.0
7 6.0 hrs	9.0	10.1	20.7	12.2	13.0
8 10 hr	5.2 19. 2.3	7.1 2.3 1.5	10.1 5.2 4.7	6.9 3.3 3.1	5.0
9 2.0 hr	2.1 0.9 0.7	1.9 0.5 1.7	5.2 0.7 2.1	3.2 1.7 1.3	2.1
10 3.0 hr	0.8 0.1 0.9	1.1 0.2 0.7	1.9 0.6 1.9	1.5 0.7 0.7	1.4
11 4.0 hr	0.5 (0.6)	0.4 (0.1)	1.2 (0.1)	0.7 (0.4)	0.7
12 5.0 hr	0.3 (0.3)	0.1 (0.1)	0.5 (0.2)	0.2 (0.2)	0.3
13 6.0 hr	<0.1	<0.1	0.2 0.1	<0.1	

50 ppm

READ BY _____ DATE _____

1059 - 14

Urices (ppm) Dioxene 9-28

WORK SHEET

#	Time	P	T	C	G	A
1	0-6	3.7 2.2 3.1	3.0 3.5 5.2	2.0 2.4 3.2	2.7 1.7 2.2	
2	6-8	3.57 1.03 0.77	0.91 0.57 0.71	0.53 2.35 0.79	0.21 0.25 0.66	0.5
3	8-10	0.67 0.01 0.23 0.01	0.19 0.35 0.27	0.24 0.12 0.18	0.23 0.22 0.22	2.6
4	10-12	0.02 ⁺	0.03 ⁺	0.10 ⁺	0.03 ⁺	10.5
5	12-14	nil	nil	nil	nil	
+ S/N= 15	14-16	nil	nil	nil	nil	
7	16-24	nil	nil	nil	nil	

Urine (ppm) HEPA

Sample Interval	P	T	C	G	average conc.	average am ⁴
(ml) mg/ml	vol. conc.	vol. conc.	vol. conc.	vol. conc.		
0-6	(220) 340 128	(405) 484 114	(242) 273 109	(170) 300 121		
6-8	(592) 214 83	(138) 509 82	(130) 585 122	(630) 193 128		
8-10	(174) 487 48	(222) 674 48	(200) 491 28	(314) 321 51		
10-12	(107) 436 39	(230) 218 26	(172) 277 16	(100) 475 53		
12-14	(280) 138 18	(107) 341 15	(122) 224 10	(600) 132 50		
14-16	(312) 51 6.7	(141) 95 1.8	(160) 15	(674) 60 6.7		
16-24	(311) 6.8	(403) 4.5	(203) nil	(378) 8		
24-36	nil	nil	nil	nil		
36-48	nil	nil	nil	nil		
Total mg of HEPA	690mg	522mg	570mg	791mg		

READ BY _____ DATE _____

Plasma (ppm) HEATH
Diagra 9.30.76

#	Time	P	T	C	G	1059-14 WORK SHEET
1	0.5				nil	
2	1				nil	
3	2				nil	
4	3				nil	
5	4	nil	nil	nil	nil	
6	5	2.81	nil? nil?	1.67? ^{2.31}	nil	
7	6	4.17	3.58	6.46	nil?	
8	1 hr	nil? nil?	8.86	7.53	nil?	
9	2	nil? nil?	5.44	7.05	nil?	
10	3	nil	7.15	7.71	nil	
11	4	nil	nil	2.11	nil	
12	5	nil		nil		

nil? present but unmeasurable.
nil = 5 ppm
nil = 10 ppm

- N.B. - an interference peak of extremely variable size was encountered during the HEATH plasma assay. Several columns (OV-1 thru OV-225, Poropak, Carbowax 101, 102, an LSP-2100) were investigated to eliminate the problem. OV-17 ^{at 130°C} performed best but did not completely solve the problem.

scan #1

scan #3

(int.)
a 10ppm sample with good resolution.
(int.)
a 10ppm sample with poor resolution.

READ BY _____ DATE _____

Figure 1. Structure of 1,4-Dioxane PBPK Model

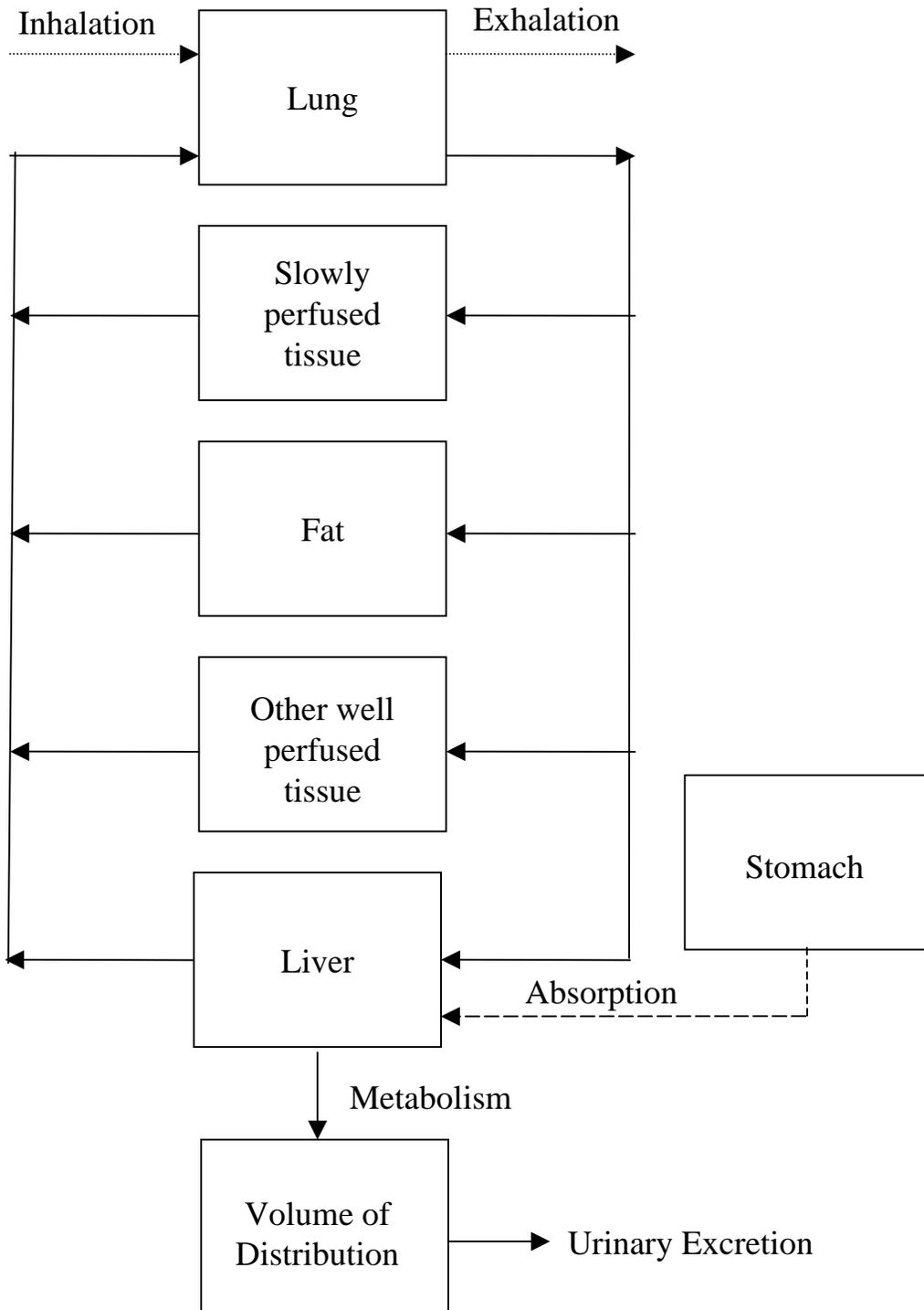


Figure 2. Fit to rat iv data (Young et al., 1978)

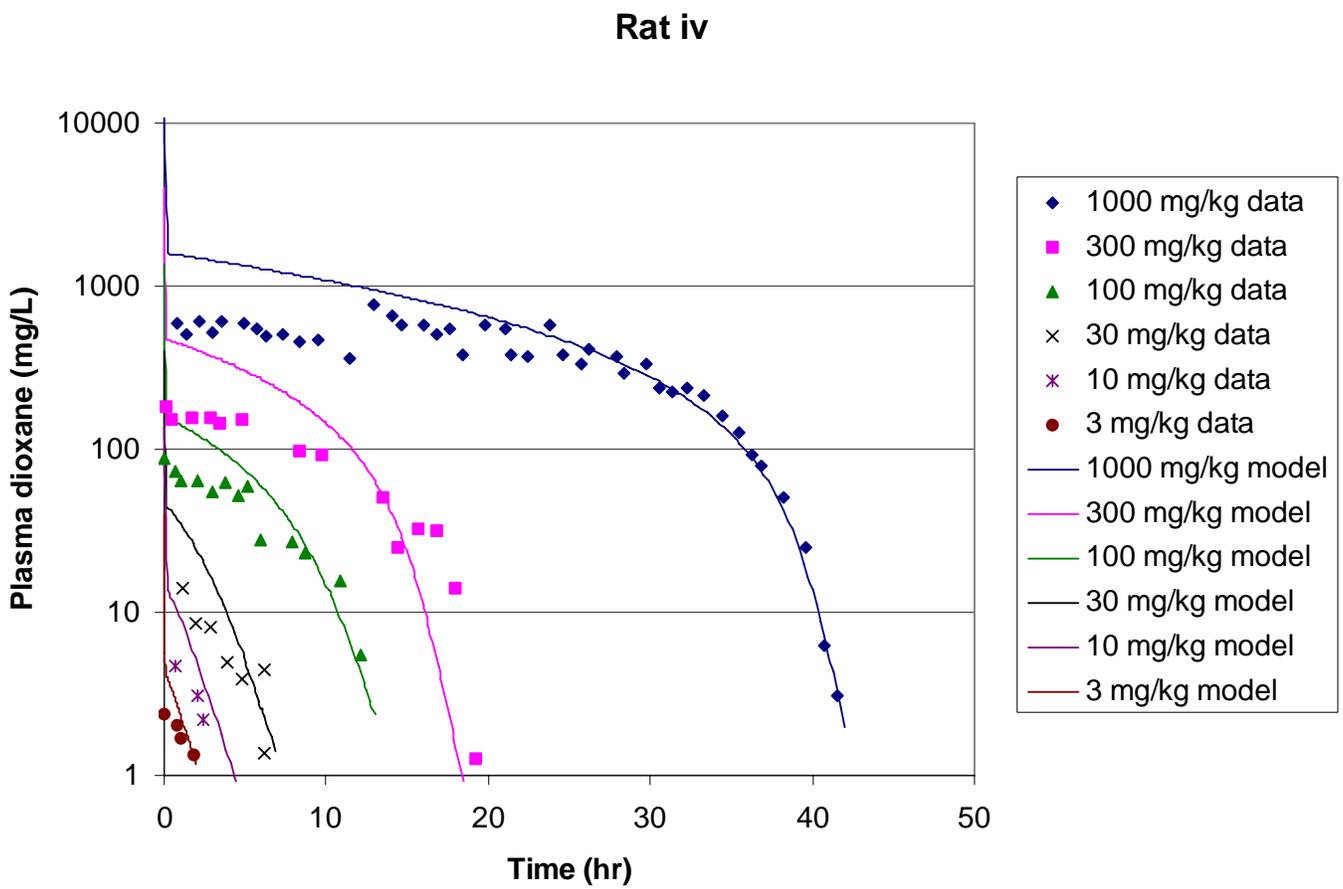
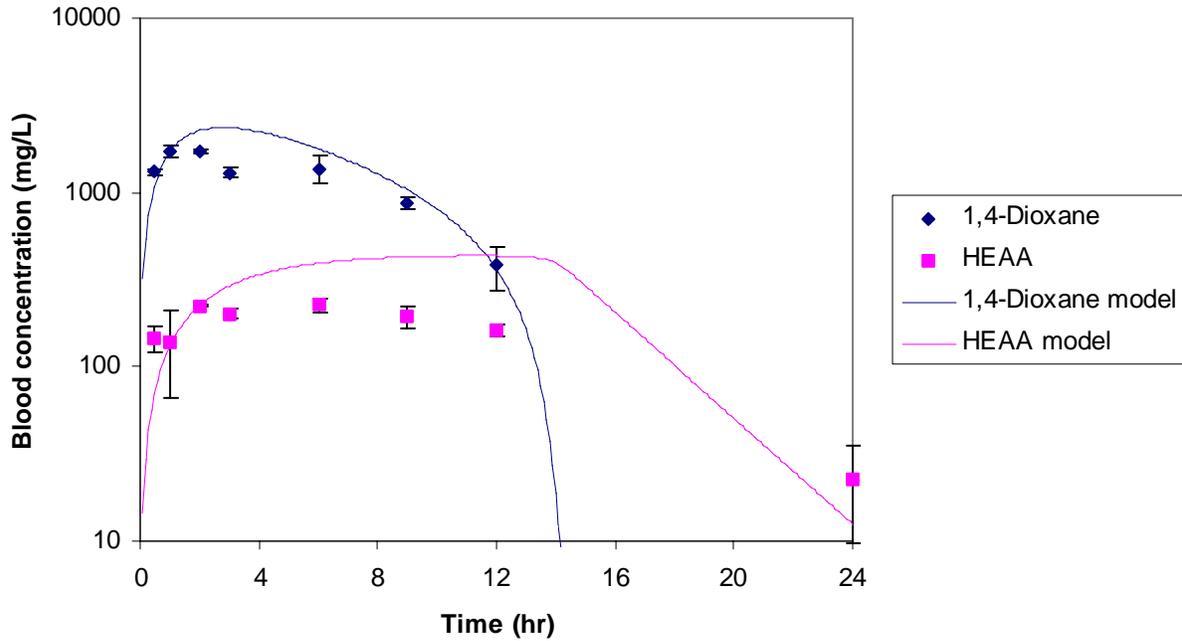


Figure 3. Fit to mouse gavage data (Thrall et al., 2005)

2000 mg/kg dose



200 mg/kg dose

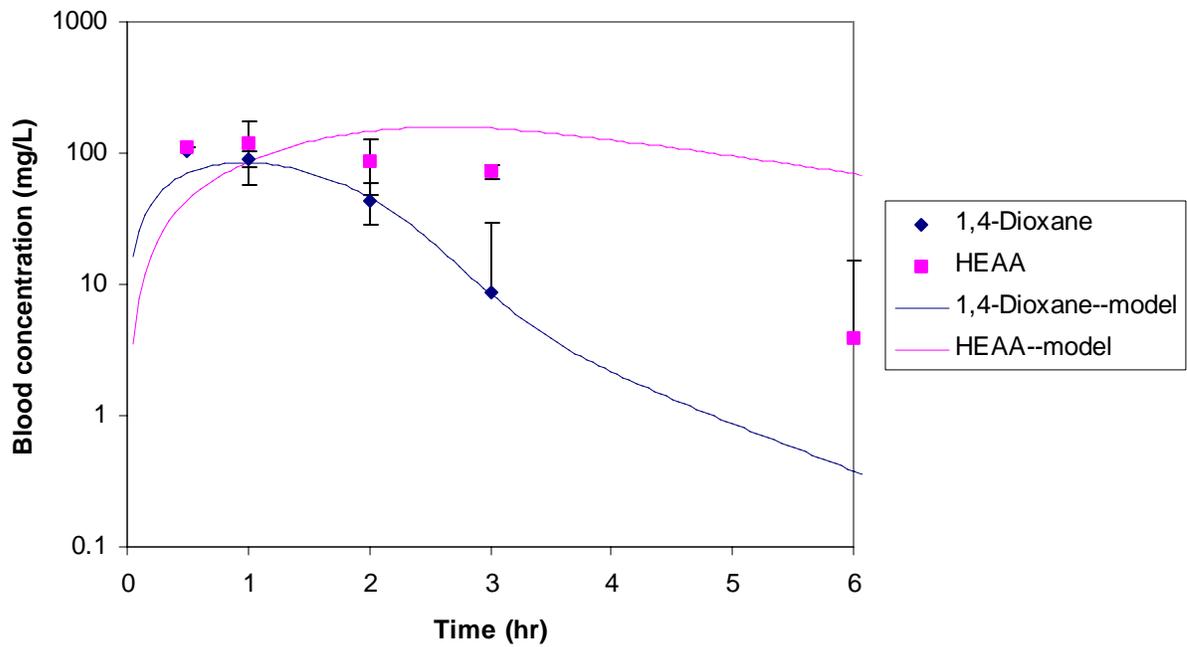
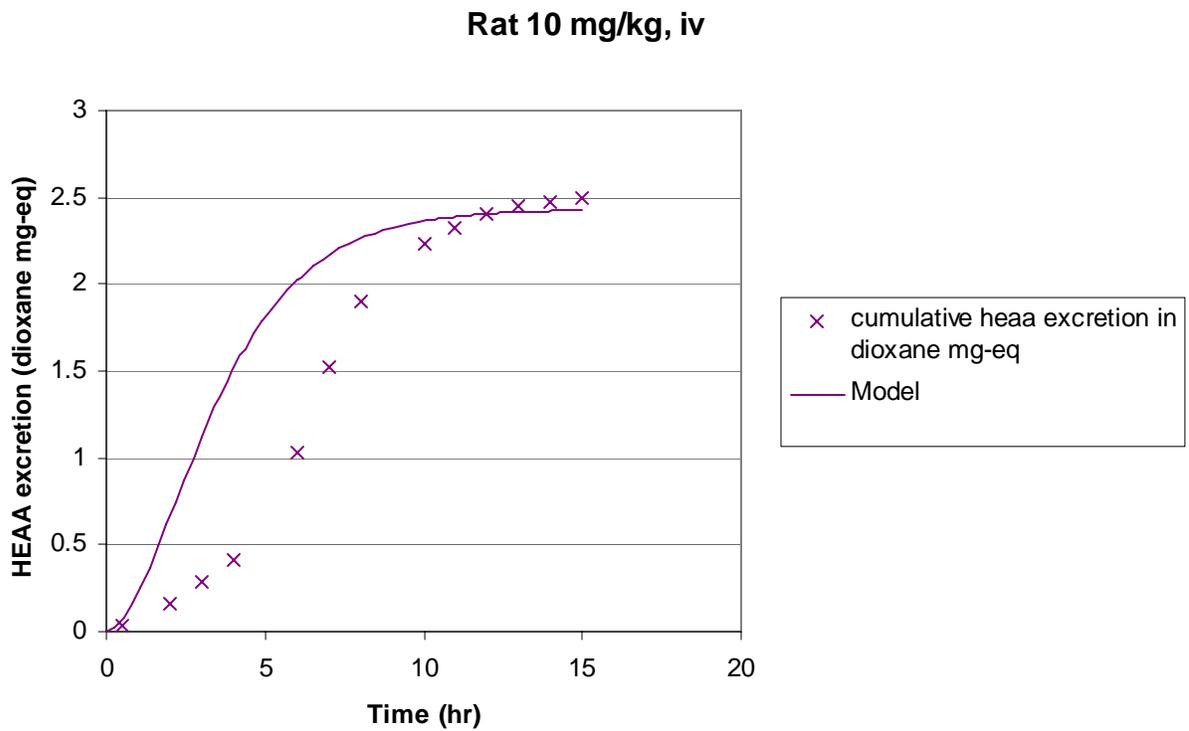
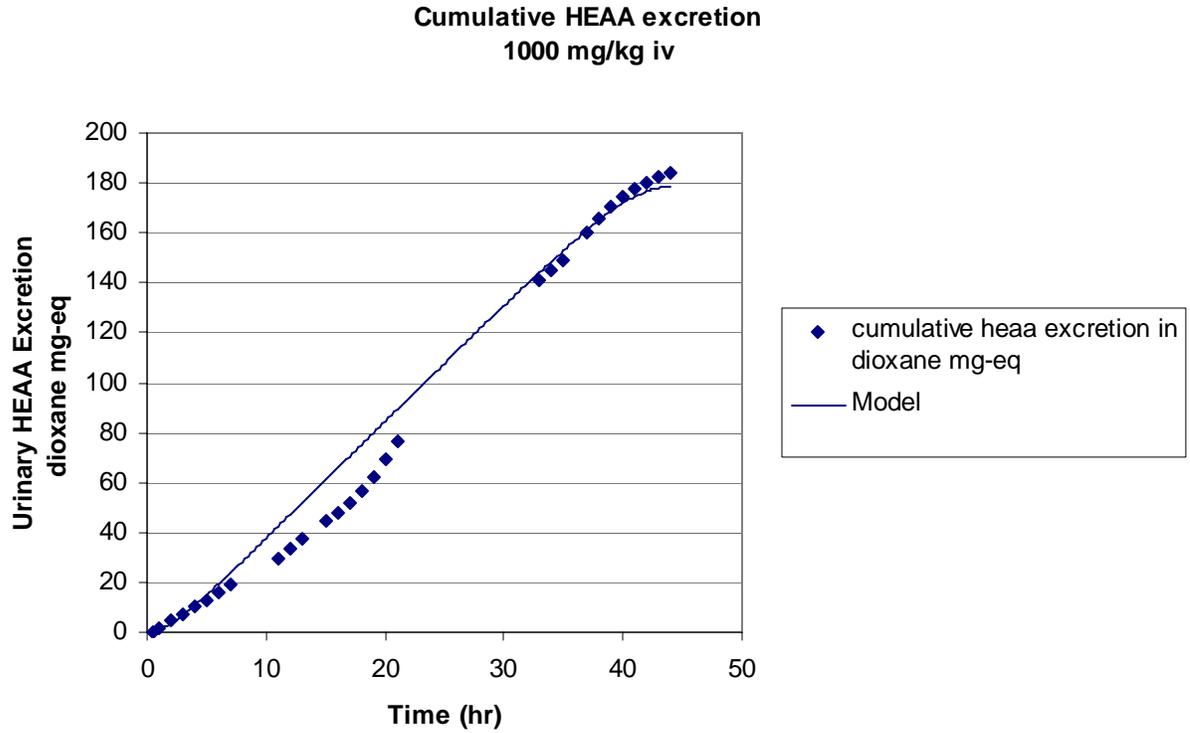


Figure 4. Fit to rat urinary excretion data



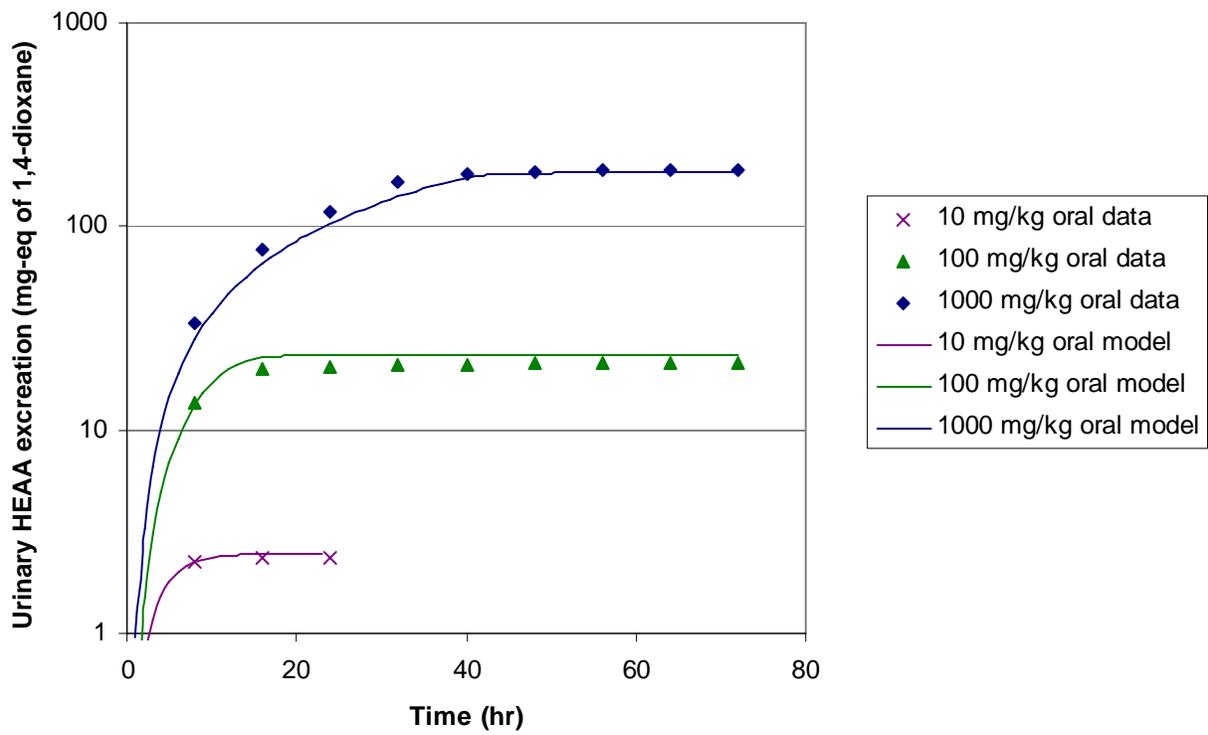
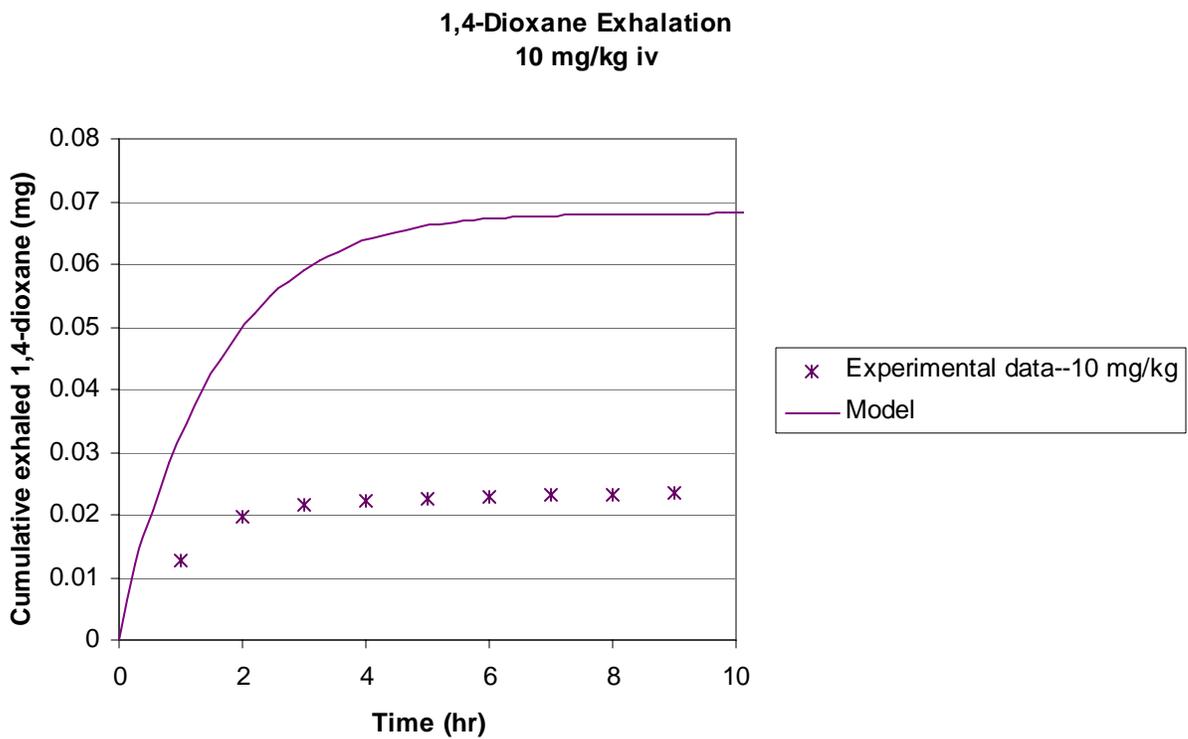
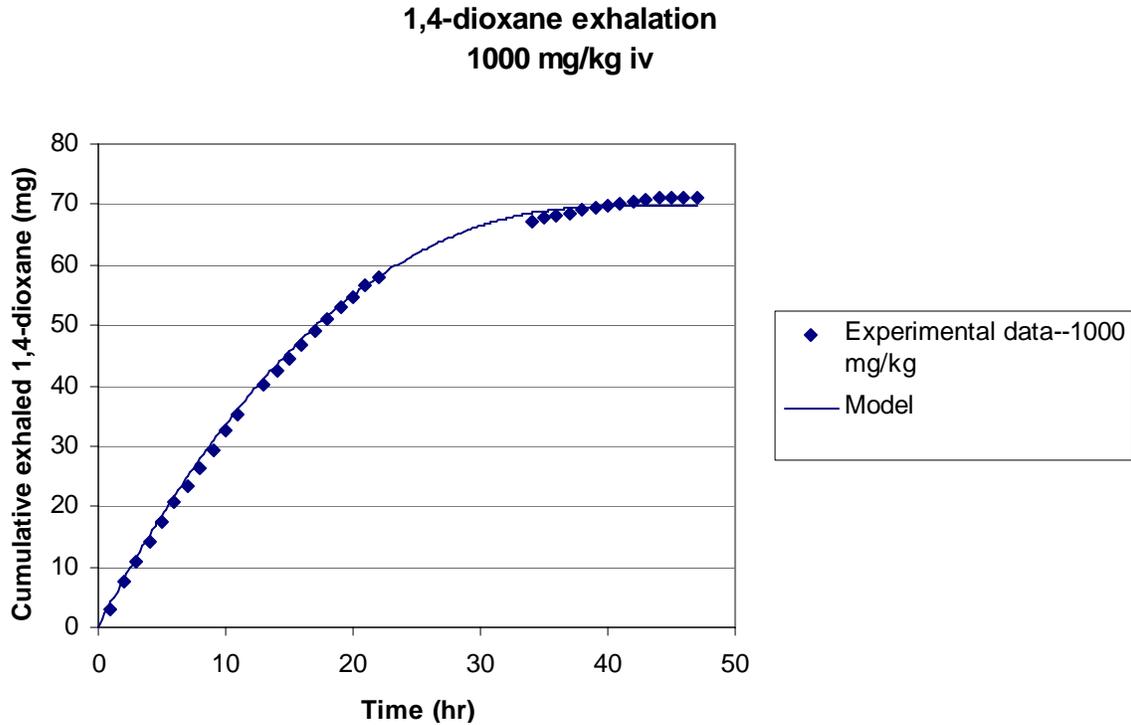


Figure 5. Prediction of 1,4-dioxane exhalation by rats



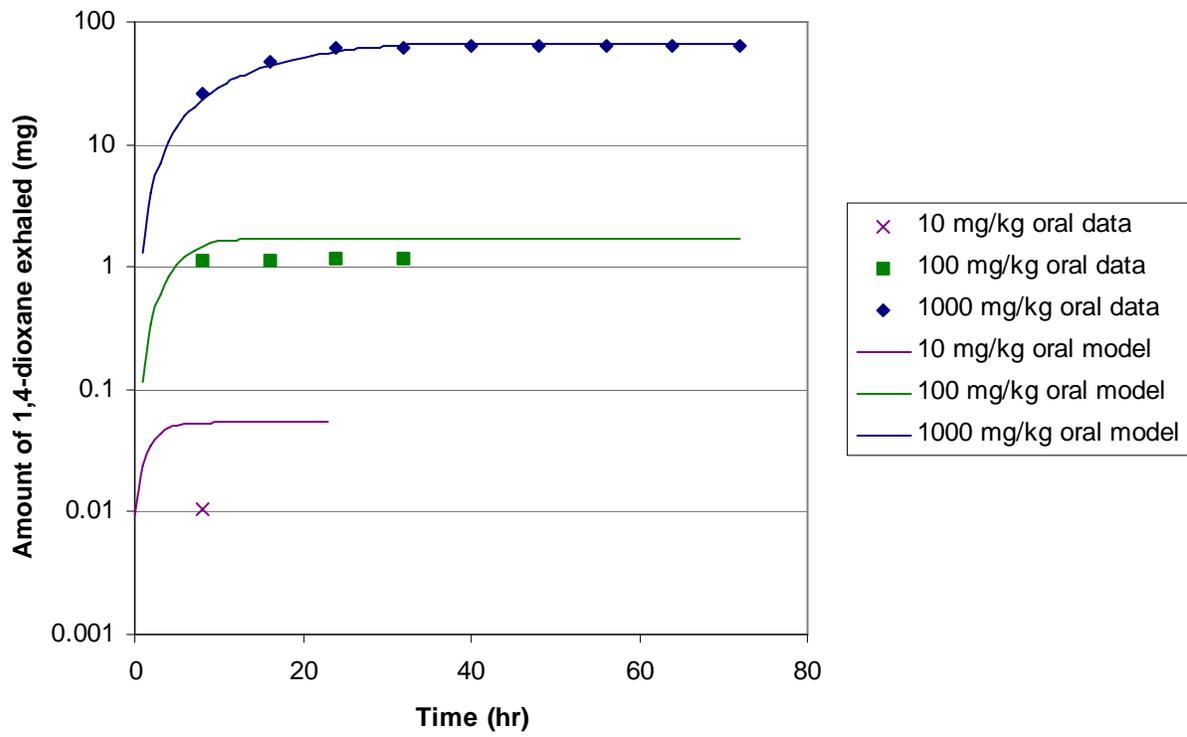


Figure 6. Prediction of 1,4-dioxane in blood of rats exposed by inhalation

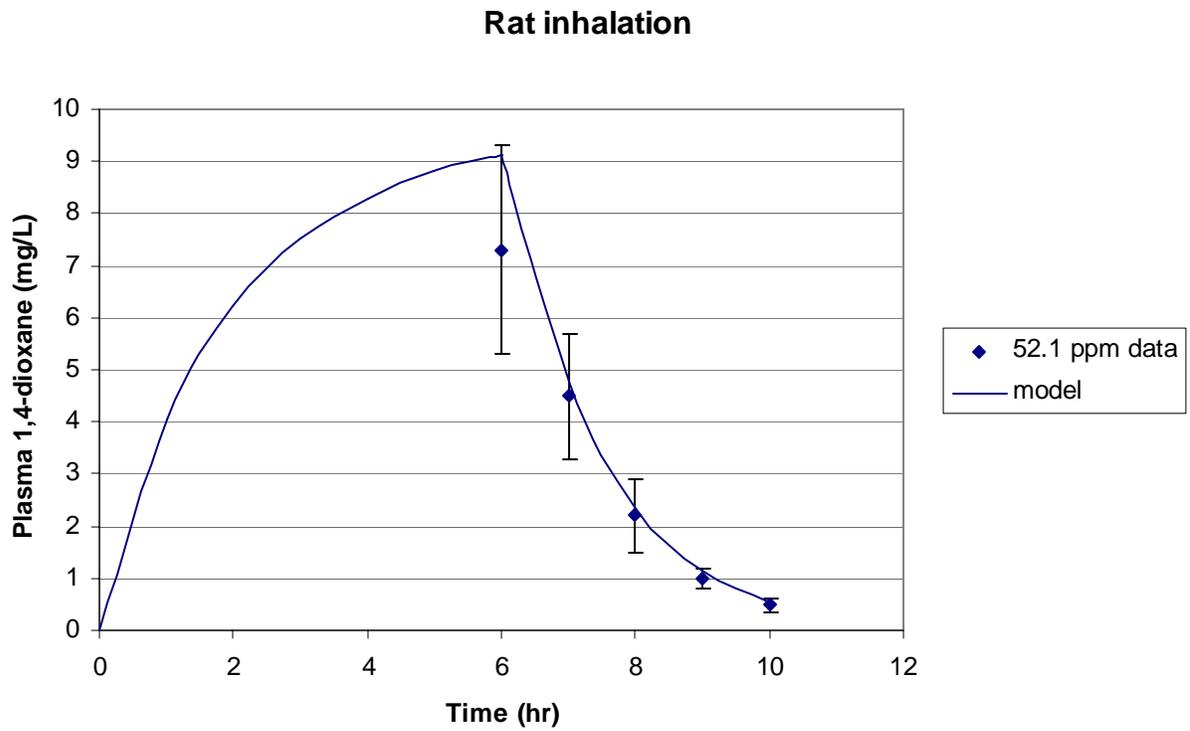


Figure 7. Prediction of mouse low-dose

20 mg/kg dose

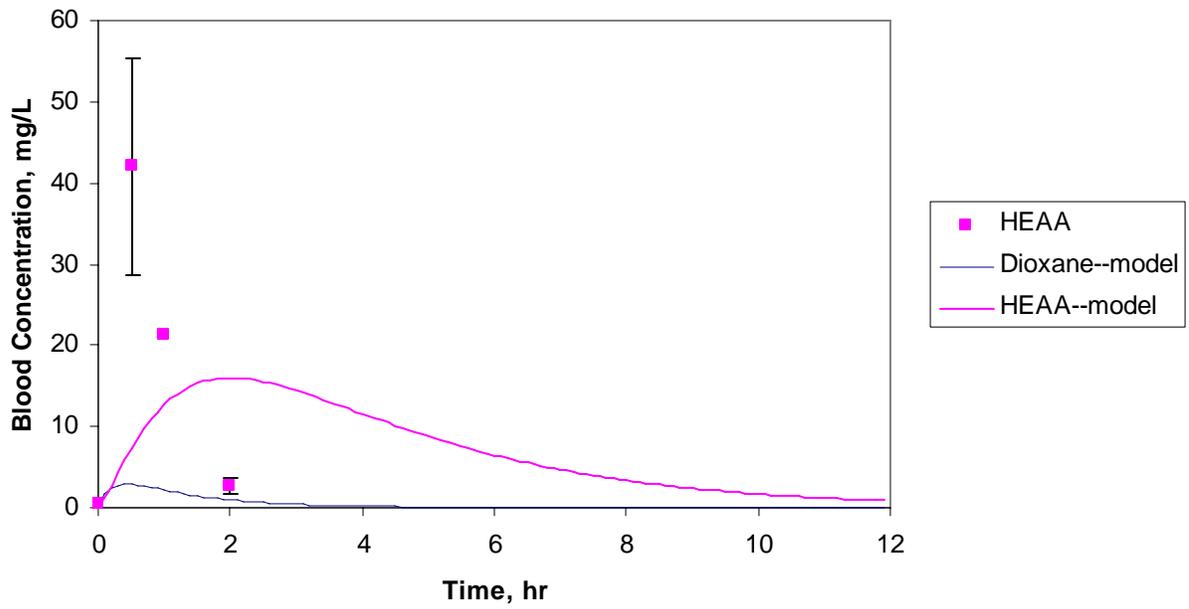


Figure 8. Prediction of human volunteer data (1,4-Dioxane) (Young et al., 1977)

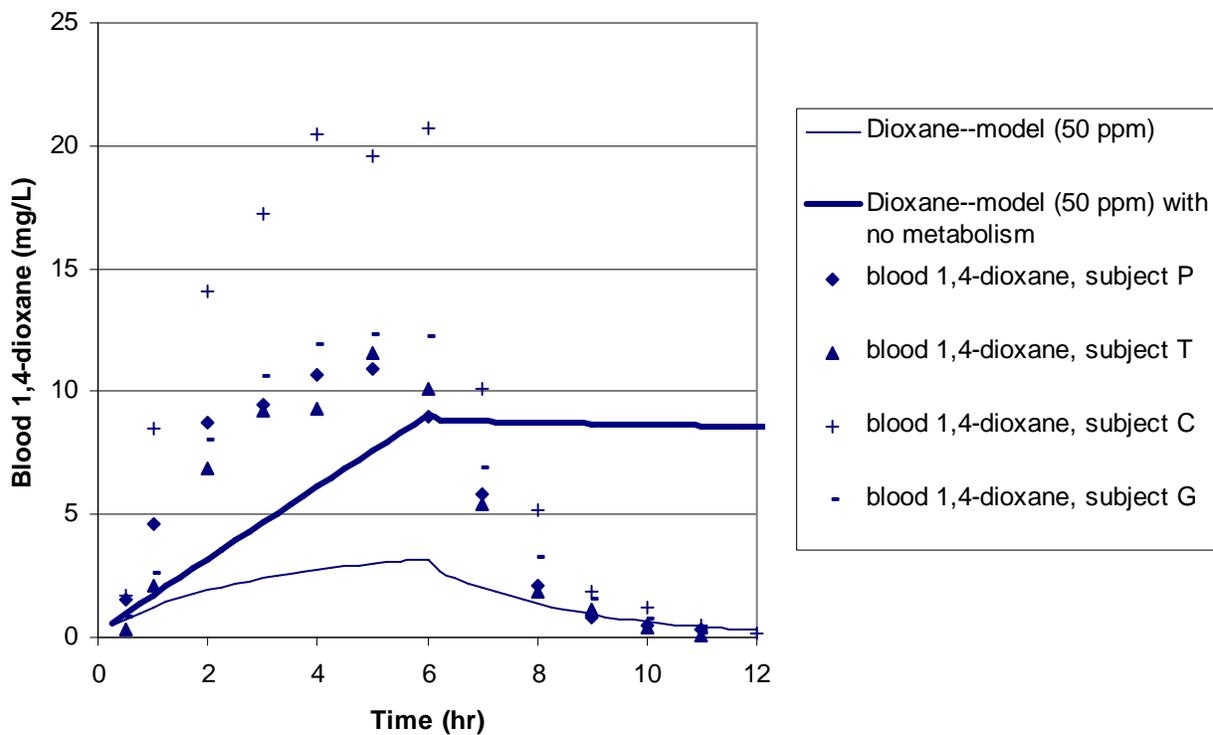


Figure 9. Prediction of human volunteer data (HEAA) (Young et al., 1977)

