TOXICOLOGICAL SCIENCES **91(1)**, 29–41 (2006)
doi:10.1093/toxsci/kfj133
Advance Access publication February 15, 2006

An Updated Physiologically Based Pharmacokinetic Model for Hexachlorobenzene: Incorporation of Pathophysiological States following Partial Hepatectomy and Hexachlorobenzene Treatment

Yasong Lu,* Manupat Lohitnavy,* Micaela B. Reddy,† Ornrat Lohitnavy,* Amanda Ashley,‡ and Raymond S. H. Yang*,[1]

*Quantitative and Computational Toxicology Group, Department of Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, Colorado 80523; †DMPK Group, Preclinical Sciences, Roche Palo Alto LLC, Palo Alto, California 94304; and ‡Department of Cell and Molecular Biology, Colorado State University, Fort Collins, Colorado 80523

Received October 9, 2005; accepted January 27, 2006

Physiologically based pharmacokinetic (PBPK) modeling is generally used for describing xenobiotic disposition in animals and humans with normal physiological conditions. We describe here an updated PBPK model for hexachlorobenzene (HCB) in male F344 rats with the incorporation of pathophysiological conditions. Two more features contribute to the distinctness of this model from the earlier published versions. This model took erythrocyte binding into account, and a particular elimination process of HCB, the plasma-to-gastrointestinal (GI) lumen passive diffusion (i.e., exsorption), was incorporated. Our PBPK model was developed using data mined from multiple pharmacokinetic studies in the literature, and then modified to simulate HCB disposition under the conditions of our integrated pharmacokinetics/liver foci bioassay. This model included plasma, erythrocytes, liver, fat, rapidly and slowly perfused compartments, and GI lumen. To account for the distinct characteristics of HCB absorption, the GI lumen was split into an upper and a lower part. HCB was eliminated through liver metabolism and the exsorption process. The pathophysiological changes after partial hepatectomy, such as alterations in the liver and body weights and fat volume, were incorporated in our model. With adjustment of the transluminal diffusion–related parameters, the model adequately described the data from the literature and our bioassay. Our PBPK model simulation suggests that HCB absorption and exsorption processes depend on exposure conditions; different exposure conditions dictate different absorption and exsorption rates. This model forms a foundation for our further exploration of the quantitative relationship between HCB exposure and development of preneoplastic liver foci.

Key Words: **PBPK model; hexachlorobenzene; medium-term liver foci bioassay.**

Physiologically based pharmacokinetic (PBPK) modeling is generally used to describe and predict chemical pharmacokinetic profiles in animals or humans under normal physiological conditions. The performance of a PBPK model is usually based on *a priori* information, e.g., tissue/organ volumes, blood flow rates, partition coefficients, metabolism rates. Such information is either available in the literature or obtainable through experimentation. Changes in some of those parameters are expected under pathophysiological and/or toxicological conditions, and these changes should be incorporated into the PBPK model. PBPK modeling is an excellent tool for quantitatively analyzing chemical pharmacokinetics in pathophysiological and/or toxicological states (Roth *et al.*, 1993b; Thomas, 1998), although such applications are rare.

Hexachlorobenzene (HCB) is a persistent organic pollutant. Although its commercial production and use were banned, HCB still exists in the environment due to its chemical stability and high lipophilicity. HCB causes various toxic effects in laboratory animals and humans (Alvarez *et al.*, 1999; Koss *et al.*, 1978; Ralph *et al.*, 2003; Schielen *et al.*, 1995; Smith *et al.*, 1987). Despite lack of genotoxicity (Siekel *et al.*, 1991), HCB-induced carcinogenicity was observed in laboratory animals, with the liver being a main target organ (Erturk *et al.*, 1986; Smith *et al.*, 1985).

The pharmacokinetics of HCB have been intensively studied (Koss and Koransky, 1975; Scheufler and Rozman, 1984a,b; Yang *et al.*, 1975, 1978). HCB primarily accumulates in the adipose tissue in the body. Some investigators (Gomez-Catalan *et al.*, 1991; Scheufler and Rozman, 1984a; Yang *et al.*, 1975) reported that HCB binds to erythrocytes. Since this binding influences the disposition of HCB, it is important to take such a process into consideration in PBPK modeling. Furthermore, in addition to a low level of metabolism in the liver, an important elimination pathway for HCB is passive diffusion from blood into the gastrointestinal (GI) lumen (Rozman *et al.*, 1985), a process known as ''exsorption'' (Arimori and Nakano, 1998; Israili and Dayton, 1984). In updating PBPK modeling for HCB, therefore, this important exsorption process should also be incorporated.

[1] To whom correspondence should be addressed at 137A Physiology Building, Quantitative and Computational Toxicology Group, Department of Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, CO 80523. Fax: (970) 491-7569. E-mail: raymond.yang@colostate.edu.

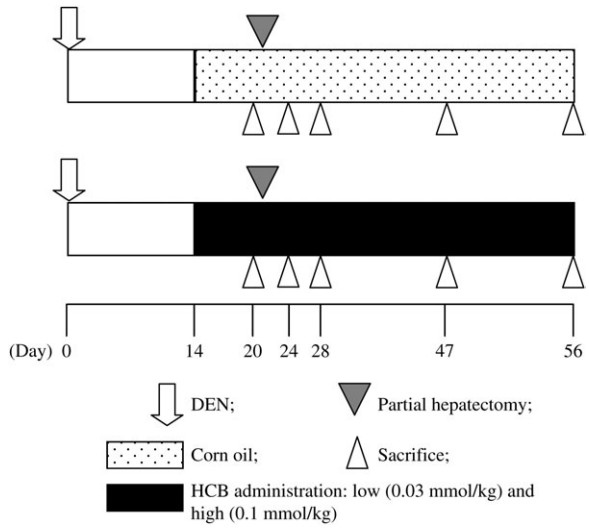

FIG. 1. Experimental design of the HCB pharmacokinetic study integrated in a time-course liver foci bioassay. A single ip injection of DEN was given on day 0. Daily oral gavage of corn oil (control) or HCB was started from day 14. On day 21, a two-thirds PH was performed on the rats. On the surgery day and the following 3 days, HCB was not administered to reduce the stress to the animals. Six rats from each treatment group were sacrificed on days 20, 24, 28, 47, and 56. The liver, kidney, blood, thigh muscle, and abdominal fat were collected for HCB analysis and PBPK modeling.

There are three published PBPK models for HCB hitherto. Yesair *et al.* (1986) first developed a PBPK model for female rats and humans to describe HCB disposition after continuous dietary exposure. Freeman *et al.* (1989) constructed another PBPK model simulating HCB disposition following an iv dose in the rat. Roth *et al.* (1993a) reported a more complicated version for HCB, specially emphasizing the absorption and excretion processes. All these models were constructed for normal rats; no pathophysiological conditions were involved. Moreover, the erythrocyte binding and exsorption processes, important for HCB pharmacokinetics, were not incorporated in one or more of these models. Although exsorption was considered in the Freeman *et al.* (1989) model, their work was limited to iv dosing.

The medium-term liver foci bioassay by Ito *et al.* (2003) is a widely used alternative animal model for evaluating carcinogenicity. As shown in Figure 1, the 8-week protocol involves administration of a potent initiator (diethylnitrosamine [DEN]) followed by test chemical treatment and hepatocyte mitogenic stimulation (partial hepatectomy [PH]) in male F344 rats. The evaluation of carcinogenic potential is achieved by identification and analysis of hepatic glutathione S-transferase placental form (GST-P) preneoplastic foci at the end of the bioassay. In our laboratory, this protocol was modified by incorporating several sacrifice points to collect time-course data on test chemical pharmacokinetics and GST-P foci development (Ou *et al.*, 2001, 2003; Thomas, 1998; Yang *et al.*, 1998).

In the medium-term liver foci bioassay, repeated oral gavage of HCB in a corn oil vehicle significantly promoted GST-P foci development (Gustafson *et al.*, 2000). The computational analyses (clonal growth modeling) in our laboratory successfully simulated the data of HCB-promoted foci development (Ou *et al.*, 2001, 2003). We are interested in determining the relationship between oral exposure to HCB and liver GST-P foci development in the rat by coupling PBPK and clonal growth modeling. Given the limitations of the currently available PBPK models, an HCB PBPK model with the incorporation of pathophysiological conditions is needed.

The purposes of this study were twofold: (1) to modify the existing PBPK models of HCB by incorporating oral exposure route and, more importantly, the erythrocyte binding and exsorption processes, such that the model structure becomes more biologically realistic and (2) to incorporate pathophysiological changes following PH and HCB treatment into the model to describe HCB pharmacokinetics in our medium-term liver foci bioassay. Thus, this study will form a foundation for our further exploration on the quantitative relationship between HCB exposure and GST-P foci development.

MATERIALS AND METHODS

This study consisted of two parts: (1) development of a PBPK model for HCB in the rat under normal physiological conditions with the incorporation of erythrocyte binding of HCB (Gomez-Catalan *et al.*, 1991; Yang *et al.*, 1975) and the exsorption process using pharmacokinetic data mined from the literature and (2) experimental pharmacokinetic study of HCB under the time-course medium-term liver foci bioassay protocol, and simulation of this data set by incorporation of the pathophysiological conditions.

Development of a PBPK Model under Normal Physiological Conditions with Incorporation of Erythrocyte Binding and the Exsorption Process

Literature Data Sets

Three previously reported time-course data sets from independent studies with different dosing regimens were collected from the literature for model development.

Single iv-dose study. Scheufler and Rozman (1984b) exposed male Sprague-Dawley rats, iv, to 1.5 mg ^{14}C-labeled HCB/kg body weight. HCB time-course tissue concentrations, converted from radioactivity in the tissues determined by liquid scintillation counting, were measured after exposure. The unit conversion was based on the observation that HCB was stored mainly unmetabolized in all tissues examined (Scheufler and Rozman, 1984b). The concentration data were retrieved from the graphs in the paper (Scheufler and Rozman, 1984b) using digiMatic (Windows version 2.2c, FEB Software, Chesterfield, VA). In another study by the same group (Scheufler and Rozman, 1984a), male Sprague-Dawley rats were exposed to the same regimen. Cumulative excretion of HCB and its metabolites, expressed as percentage of the dose, in feces and urine was quantified in a time-course manner up to 56 days. The related data were collected from Scheufler and Rozman (1984a) and two other papers (Freeman *et al.*, 1989; Roth *et al.*, 1993a). As the experimental conditions of the two studies were almost identical, the two data sets were pooled.

Single oral gavage study. Male Wistar rats were orally administered 0.2 mg HCB in 1 ml corn oil after an overnight fast (Yamaguchi *et al.*, 1986). At various time points after exposure, the HCB concentration was measured in adipose tissue, blood, and liver using gas chromatography.

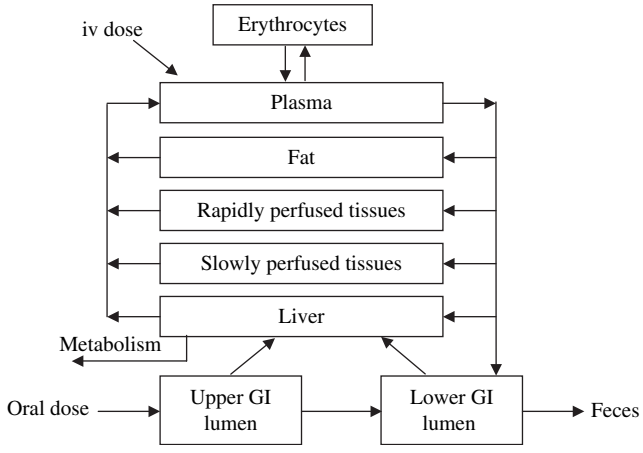

FIG. 2. Diagram of the PBPK model for HCB following iv or oral exposure. For an iv exposure, the upper GI lumen was turned off, and the excretion of metabolites was tracked. For oral exposures, the reverse was true.

Repeated oral gavage study. Koss *et al.* (1978) treated female Wistar rats orally with 50 mg/kg HCB in olive oil every other day up to 15 weeks. HCB concentrations in adipose tissue, liver, and blood were determined at weeks 3, 6, 9, 12, and 15. Time-course daily excretion of HCB in feces was recorded. The amounts of daily excreted main metabolites, i.e., pentachlorophenol (PCP), tetrachlorohydroquinone, and pentachlorothiophenol (PCThP) in the urine and PCP and PCThP in the feces, were also measured at the five time points. We calculated the daily sum of these metabolites in molar unit, which was an estimate of the amount of HCB metabolized per day; the metabolites retained in the tissues were neglected because they only accounted for a very small portion of the total metabolites.

Model Development under Normal Physiological Conditions

The model development under normal physiological conditions followed a two-step process:

First, the development was based on the single iv dose data set (Scheufler and Rozman, 1984a,b) because (1) iv dosing studies, which bypassed the absorption phase, provided ''cleaner'' pharmacokinetic data and (2) the data were from the most detailed studies, including both time-course tissue concentrations and excretion data.

Second, the model, once developed, was modified to describe the single oral exposure data (Yamaguchi *et al.*, 1986) and the repeated oral exposure data (Koss *et al.*, 1978) by incorporating the GI absorption process.

This two-step process was expected to progressively minimize the uncertainties in the model parameterization.

Model structure. The model structure included the liver, blood, fat, rapidly and slowly perfused compartments, and GI lumen (Fig. 2). Blood was divided into two subcompartments, plasma and erythrocytes, based on the observations that in rats the HCB concentration in erythrocytes was 5.6-fold higher than in plasma *in vitro* (Yang *et al.*, 1975) and about 10-fold higher *in vivo* (Gomez-Catalan *et al.*, 1991; Scheufler and Rozman, 1984a). Because the association and dissociation constants between HCB and erythrocytes are not available, a linear distribution of HCB between plasma and erythrocytes was assumed (see the Supplemental Data for the equation). We feel that, in the absence of data, this is the simplest and least troublesome assumption to follow. HCB in plasma was available for tissue uptake, which was assumed flow limited. To examine whether the incorporation of HCB binding with erythrocytes improves model simulation, we simulated the iv dosing data (Scheufler and Rozman, 1984a,b) with and without the binding to erythrocytes as shown in Figure 3D.

Following an iv exposure, the amount was assumed to be immediately introduced into plasma at the start of simulation, from where it partitioned into erythrocytes and other compartments. Metabolism of HCB in the liver was very low and was assumed to be a first-order process. Once produced, the metabolites were rapidly excreted: 42% via urine and 58% via feces (Koss and Koransky, 1975). As HCB is poorly metabolized, the metabolites in any tissue are negligible, which is experimentally supported (Koss and Koransky, 1975). With this simplification, the tissue radioactivity, including in plasma, was considered the parent compound HCB. The excretion of the metabolites was traced only during simulation of the single iv dose data. The induction of HCB metabolism was not considered due to lack of information.

Our initial attempt with a single–GI lumen structure could not simulate the shapes of the tissue concentration data following an oral dose (Yamaguchi *et al.*, 1986). Since a two–GI lumen structure could adequately describe tissue concentrations following the oral exposure of some chemicals in corn oil (Staats *et al.*, 1991) and HCB was administered in oily vehicles in the collected studies (Koss *et al.*, 1978; Yamaguchi *et al.*, 1986), the GI lumen was divided into an upper and a lower part (Fig. 2). For an oral administration, both parts played their respective roles, i.e., HCB was absorbed from both sites, meanwhile the chemical moved downward through the whole lumen. The absorption and downward movement were described with first-order equations.

The blood-to-lumen exsorption process occurs in both small and large intestines (Richter and Schafer, 1981), but only the large intestine determines the net excretion of HCB in the feces (Rozman *et al.*, 1985). Thus, in our model, HCB was exsorbed only into the lower GI lumen, from where it may be reabsorbed into the liver or excreted via feces. HCB exsorption, (re)absorption, and excretion via feces were first-order processes. The absorption of orally dosed HCB and reabsorption of exsorbed HCB in the lower GI lumen may follow the same mechanism and thus were controlled by the same rate constant. The model equations are presented in the Supplemental Data.

Parameterization. Since the rats in the reported studies were in a fast growth phase and the simulation durations were longer than 1 month, changes in the body weights and tissue/organ volumes were incorporated. The age-dependent body weights were obtained from the corresponding study (Koss *et al.*, 1978) or other papers (Hida *et al.*, 1999; Schoeffner *et al.*, 1999). The growth curves were interpolated with a TABLE function (Advanced Continuous Simulation Language [ACSL] programming, AEgis Technologies Group, Huntsville, AL) in the model codes. The volume of each compartment was defined with body weight–dependent functions unless otherwise indicated. The cardiac output of blood (QBld) was proportional to (body weight)$^{0.75}$ (Brown *et al.*, 1997). The cardiac output of plasma (QPl, l/h), i.e., the flow rate of plasma through the heart, was then determined by

$$\mathrm{QPl} = \mathrm{QBld} \times (1.0 - \mathrm{HMTC}), \quad (1)$$

where HMTC is rat hematocrit. The plasma flow rate to each compartment was governed by the respective flow fraction of QPl. All physiological parameters used are summarized in Table 1.

Defined as the ratios of tissue concentrations over the plasma concentration, the partition coefficients (Table 2) of fat, liver, and rapidly perfused compartment were estimated from the repeated oral gavage data (Koss *et al.*, 1978) where steady state was reached. As Freeman *et al.* (1989) had done in their study, we plotted the fat, liver, or kidney concentration as a function of the blood concentration at all five time points from Koss *et al.* (1978) data, and determined the slope (i.e., $\Delta y/\Delta x$ or tissue concentration/blood concentration) using linear regression analysis with the function going through the origin. The slopes were then recognized as tissue partition coefficients (all correlation coefficients $r^2 > 0.96$). The partition coefficient of the rapidly perfused compartment was set the same as that of kidney. While it was not available in the repeated oral gavage study (Koss *et al.*, 1978), the plasma concentration was estimated to be 4.8-fold lower than the blood concentration based on Gomez-Catalan *et al.* (1991). The partition coefficient of the slowly perfused compartment (represented by muscle) was from Freeman *et al.* (1989).

Other parameters with no information were optimized by model fitting to the data sets; they were considered as ''adjustable parameters.'' For simulating the iv dose data (Scheufler and Rozman, 1984a,b), there were four adjustable parameters: rate constants of metabolism, exsorption, reabsorption, and fecal

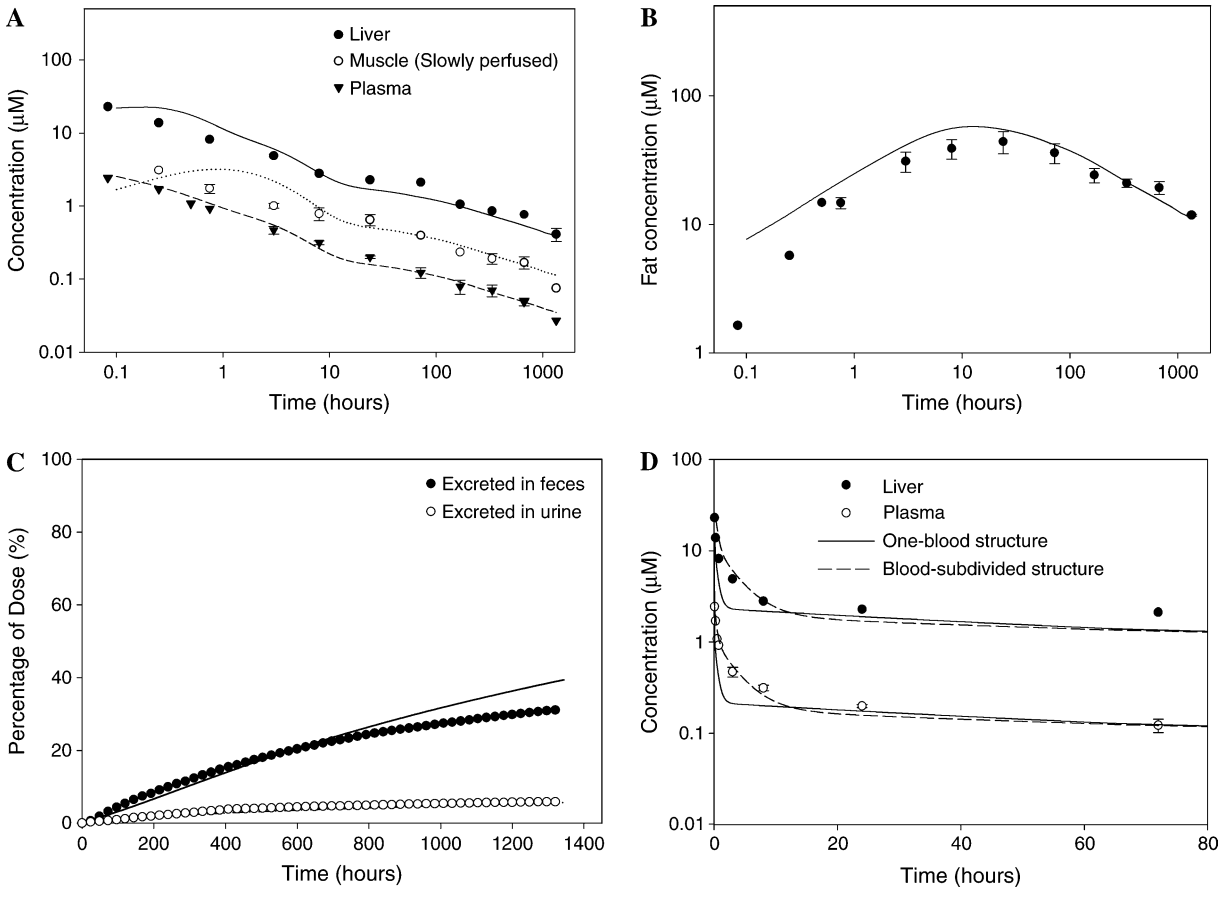

FIG. 3. For a single iv dose of 1.5 mg/kg in the rat, model simulations and data of (A) HCB concentrations in the liver, muscle (slowly perfused compartment), and plasma; (B) HCB concentration in the fat; (C) percentages of the dose excreted in the urine and feces; and (D) the liver and plasma concentrations in the first 80 h simulated with and without the incorporation of HCB binding to erythrocytes. The lines are simulations and the symbols are experimental data (Scheufler and Rozman, 1984a,b).

excretion. Manual adjustment was initially used to get a sense of the effects of these parameters on the simulation results. Then with the best-visual-fit values as start values, ACSL MATH-based statistical optimization was executed. This optimization process included the following: (1) Local optimization, i.e., each individual parameter was adjusted to give the best fit to the piece of data to which this parameter had the most influence, whereas the other adjustable parameters were held fixed. For example, the rate constants of exsorption and reabsorption were adjusted against the tissue concentrations, the metabolism rate constant against the amount of metabolites in urine, and the fecal excretion rate constant against the amount in feces. (2) Global optimization, i.e., all the adjustable parameters were varied simultaneously against the whole data set. The locally optimized values were inputs for the global optimization, and they were constrained in narrow ranges to avoid statistically best but biologically implausible values. The optimization was performed using the generalized reduced gradient method, with the heteroscedasticity value set to 2 to minimize the relative error between the calculated results and experimental data.

For simulating the single and repeated oral gavage data, there were six adjustable parameters: the rate constants of metabolism, exsorption, absorption from the upper GI lumen and absorption from the lower GI lumen, mass transfer from the upper to the lower GI lumen, and excretion in feces. According to the absorption and exsorption characteristics of HCB (see the ''Discussion'' section), we did not expect that a single set of the adjustable parameters would fit both oral data sets, which was supported by our initial trials. Hence, holding other adjustable parameters as constants in the two scenarios, we allowed the exsorption rate constant to vary with exposure conditions. Intensive manual adjustment was made to achieve the best visual fit to these two data sets. All adjustable parameters are summarized in Table 2.

Model validation. A separate study, different from the data sets used to build the model, where female Wistar rats were given a single oral dosing of ^{14}C-HCB at 20 or 180 mg/kg in olive oil (Koss and Koransky, 1975) was used to validate the model. The tissue concentrations were determined by radioactivity. Koss and Koransky (1975) found that even at the level of 180 mg/kg, the radioactivity in the liver and fat was nearly completely attributed to the parent compound; this result supports the appropriateness of using this data set for model validation.

Sensitivity analysis. Sensitivity analysis is a valuable method for identifying key parameters affecting a pharmacokinetic measurement. Clewell *et al.* (1994) defined log-normalized sensitivity parameters (LSPs) as

$$\mathrm{LSP} = \frac{\partial \ln R}{\partial \ln x}, \tag{2}$$

where R is a model output and x is the parameter for which the sensitivity is being examined. This definition quantifies the percentage change in an output value due to the percentage change in a parameter. In this study, the liver concentration was an output of most concern. Thus, we determined the sensitivity of the liver concentration to the fat and liver volume fractions, tissue partition coefficients, and the adjustable parameters following a single oral

TABLE 1
Physiological Parameters for the HCB PBPK Model

Parameters	Single iv dose (Scheufler and Rozman, 1984a,b)	Single oral gavage (Yamaguchi *et al.*, 1986)	Repeated oral gavage (Koss *et al.*, 1978)
Body weight (BW) at start of experiment (kg)	0.234[a]	0.125[b]	0.135[c]
Tissue volumes (or volume fractions)			
Fat volume fraction (VFC)		$0.199 \times BW + 0.01664$[d,e]	0.05[d,f]
Liver volume (VL) (l)		$0.0321 \times BW + 0.00197$[e,g]	N/A
Liver volume fraction (VLC)		N/A	0.037[h]
Blood volume (VB) (l)		$0.062 \times BW + 0.0012$[e,i]	
Rapidly perfused (VR) (l)		$0.0333 \times BW + 0.01203$[e,g]	
Slowly perfused (VS) (l)		$0.91 \times BW - VF - VL - VB - VR$[e]	
Hematocrit (HMTC)		0.367[i]	
Cardiac output constant (QCC) (l/h/kg$^{0.75}$)		14.1[d]	
Tissue plasma flow fractions			
Fat (QFC)		0.07[d]	
Liver (QLC)		0.18[d]	
Rapidly perfused (QRC)		0.76 − QLC	
Slowly perfused (QSC)		0.24 − QFC	

Note. N/A: not applicable.
[a]Age-dependent bw adopted from Schoeffner *et al.* (1999).
[b]Age-dependent bw adopted from Hida *et al.* (1999).
[c]Age-dependent bw recorded in Koss *et al.* (1978).
[d]Brown *et al.* (1997).
[e]Body weight was in kilograms in all equations.
[f]Assumed to be constant because HCB inhibits fat accumulation in the body (Alvarez *et al.*, 1999; Smith *et al.*, 1987).
[g]Schoeffner *et al.* (1999).
[h]The value at the start of experiment was estimated from Brown *et al.* (1997) but the time-dependent values later in the experiment were from Koss *et al.* (1978).
[i]Lee and Blaufox (1985).

gavage dose. The LSPs were calculated at multiple time points using the central difference method. An LSP greater than 1 indicates that error in a parameter results in amplified error in the related output (Clewell *et al.*, 1994).

Simulation of the Pharmacokinetic Data from the Time-Course Medium-Term Liver Foci Bioassay by Incorporating the Pathophysiological Conditions

Pharmacokinetic Study Under the Time-Course Liver Foci Bioassay Protocol

HCB (99% purity) and 1,2,4,5-tetrabromobenzene (97% purity) were purchased from Aldrich Chemical (Milwaukee, WI). DEN was obtained from Sigma Chemical (St. Louis, MO). Toluene (99.9% purity) and sulfuric acid were supplied by VWR Scientific (Denver, CO). Anhydrous sodium sulfate was purchased from Fisher Scientific (Houston, TX). Florisil was provided by Alltech Associates (Deerfield, IL).

Male F344 rats, 30 days of age, purchased from Harlan Sprague-Dawley (Indianapolis, IL), were housed in the Painter Center, Colorado State University. It is fully accredited by the American Association for Accreditation of Laboratory Animal Care. Animals were given food (Harlan Teklad NIH-07 diet, Madison, WI) and water *ad libitum*, and lighting was set on a 12-h light/dark cycle. The study was conducted in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals.

After 4 weeks of acclimation, the rats were randomized by weight, divided into three groups, and treated according to the time-course liver foci bioassay (Fig. 1). At week 0, the rats were given a single ip injection of DEN (200 mg/kg) dissolved in 0.9% saline. Two weeks later, the rats began receiving a daily oral gavage (5 ml/kg body weight) of corn oil (control), 0.03 mmol/kg HCB (low dose), or 0.1 mmol/kg HCB (high dose) in corn oil until sacrifice. According to the earlier work by our group (Ou *et al.*, 2001), at 0.1 mmol/kg, HCB significantly promoted GST-P foci formation in the medium-term liver foci bioassay. In this study, we used a second dose, 0.03 mmol/kg, in order to examine GST-P foci formation at this lower dose, although GST-P foci data were beyond the scope of this report. At week 3 (day 21), a two-thirds PH was performed on the rats. On the surgery day and the following 3 days, HCB was not administered to reduce the stress to the animals while recovering from surgery. On days 20, 24, 28, 47, and 56, six rats from each group were sacrificed by aortic exsanguination under anesthesia. These time points cover pre- and post-PH periods. The body and liver weights of each rat were recorded at sacrifice. The liver, kidney, blood, thigh muscle, and abdominal fat were collected from each rat for HCB analysis and subsequent PBPK modeling. All tissue samples were frozen with liquid nitrogen and stored at −70°C until analysis.

Samples (0.5 ml blood, 0.1 g fat, and 0.2 g other tissues) of the HCB-treated rats, spiked with 1,2,4,5-tetrabromobenzene as an internal standard, were digested with 3 ml of 60% sulfuric acid overnight, and then extracted with 5 ml of toluene three times. The resulting extracts were concentrated to about 2 ml and cleaned through a column containing 1 g anhydrous sodium sulfate and 1 g Florisil. Each column was washed five times with 2 ml of pentane per time after the passage of the concentrated extract. The eluant was concentrated and then analyzed on an HP-5890 II Plus gas chromatograph (Hewlett Packard, Wilmington, DE) equipped with an electron capture detector. An EQUITY-5 fused silica capillary column (Supelco, PA) was employed. Helium and nitrogen were used as the carrier and makeup gases with flow rates of 4 and 60 ml/min, respectively. The oven temperature was initially 150°C for 1 min, increased to 175°C at 10°C/min, where it remained for 10 min. The temperatures of the inlet and detector were 250 and 300°C, respectively.

TABLE 2
Partition Coefficients and Rate Constants for the HCB PBPK Model

	Single iv dose (Scheufler and Rozman, 1984a,b)	Single oral gavage (Yamaguchi *et al.*, 1986)	Repeated oral gavage (Koss *et al.*, 1978)	Medium-term bioassay (low dose)[a]	Medium-term bioassay (high dose)[a]
Partition coefficients					
Fat (PF)	315.0[b]	315.0[b]	315.0[b]	200.0[c]	200.0[c]
Liver (PL)	10.7[b]	10.7[b]	10.7[b]	10.7[b]	10.7[b]
Rapidly perfused (PR)	7.0[b]	7.0[b]	7.0[b]	7.0[b]	7.0[b]
Slowly perfused (PS)	3.2[d]	3.2[d]	3.2[d]	3.2[d]	3.2[d]
Rate constants					
Metabolism (KMET) (l/h)	0.00195[e]	0.00195[f]	0.00195[f]	0.00195[f]	0.00195[f]
Exsorption (KBGI) (l/h)	0.03[e]	0.045[g]	0.02[g]	0.1[h]	0.1[h]
Upper GI absorption (KGILV1) (h^{-1})	N/A	0.082[g]	0.082[g]	0.012[h]	0.012[h]
Lower GI absorption (KGILV2) or reabsorption (KGILV) (h^{-1})[j]	0.003[e]	0.007[g]	0.007[g]	0.0007[i]	0.0007[i]
Upper-to-lower transfer (KUpLow) (h^{-1})	N/A	0.004[g]	0.004[g]	0.004[i]	0.004[i]
Fecal excretion (KFEC) (h^{-1})	0.00065[e]	0.0014[g]	0.0014[g]	0.0014[i]	0.0014[i]

Note. N/A: not applicable.
[a]Our pharmacokinetic study integrated in the liver foci bioassay.
[b]Estimated from Koss *et al.* (1978).
[c]Estimated from our bioassay data.
[d]From Freeman *et al.* (1989).
[e]ACSL MATH-based optimization against the iv dose data (Scheufler and Rozman, 1984a,b).
[f]Metabolism rate constant was assumed to be the same in all exposure scenarios.
[g]Visual fitting to two data sets (Koss *et al.*, 1978; Yamaguchi *et al.*, 1986).
[h]Visual fitting to the low- and high-dose data from our bioassay.
[i]Adopted from the single and repeated oral gavage studies.
[j]The lower GI absorption and reabsorption processes were controlled by the same rate constant.

The recoveries of HCB and the internal standard of this method were in the range of 70–100%.

Differences in the body weight, liver weight, and liver/body weight ratio among the control and treated groups were analyzed for significance with and without the factor of time using ANOVA at $\alpha = 0.05$. All statistical analyses were performed using SAS (version 8.02; SAS Institute Inc., Cary, NC).

Simulation of the Pharmacokinetic Data by Incorporating the Pathophysiological Conditions

The PBPK model, developed by us earlier, was employed to describe the data of HCB pharmacokinetic study integrated in the time-course liver foci bioassay. The DEN treatment and surgery on the liver distinguished the rats in this bioassay from those in other studies. As there was a 2-week recovery interval after DEN injection, we assumed that the physiological conditions of the rats were back to normal at the beginning of HCB administration, and hence the DEN-related alterations were not considered in the model simulation. Following PH, drastic physiological responses occur in the liver and the whole body to signal and facilitate liver regeneration (Katagiri, 1988; Michalopoulos and DeFrances, 1997; Sabugal *et al.*, 1996). Since it is impractical and unnecessary to capture every pathophysiological change mathematically, in our model simulation we chose to incorporate the changes in the body and liver weights and fat volume because of their major impacts on HCB disposition. HCB treatment may also contribute to those changes (Alvarez *et al.*, 1999; Koss *et al.*, 1978; Smith *et al.*, 1987). After the PH, the liver weight immediately decreased by 70%; the body weight and fat volume also reduced (Katagiri, 1988; Sabugal *et al.*, 1996). The body and liver weights were recorded at several time points prior to and after the surgery, but the fat volume change was modeled based on the following rationale.

The fat volume fraction (VFC) prior to PH was calculated according to the equation derived from historical data by Brown *et al.* (1997):

$$\mathrm{VFC}\,(\%\,\mathrm{BW}) = 35 \times \mathrm{BW} + 0.205, \tag{3}$$

where BW is body weight in kg. After the surgery, to describe the fat mobilization and redeposit, we expressed the VFC with a two-term function as suggested by Thomas (1998):

$$\mathrm{VFC} = a \times \exp[-b \times (t - 336)] + c \times (t - 336), \tag{4}$$

where t is the time (h) following DEN injection; the number 336 refers to the time (h) when HCB dosing began; a, b, and c are constants controlling the shape of the function. The constants a, b, and c were assigned the values 1.1, 0.017, and 6.0×10^{-5}, respectively, such that the VFC reduced to 2.38% from 7.6% (low-dose group) or 7.4% (high-dose group) in the first 7 days after PH and then gradually increased to approximately 6% at the end of simulation (1344 h). This function was in line with our qualitative understanding of fat mobilization and redeposit after PH and it has helped us in the PBPK model simulation of pharmacokinetics of pentachlorobenzene in partially hepatectomized rats in our laboratory.

The partition coefficients of the liver and rapidly (set the same as kidney partition coefficient) and slowly perfused compartments (set the same as muscle partition coefficient) estimated from our data were similar to those from the repeated oral gavage data (Koss *et al.*, 1978); hence, the latter were used in the model simulation. The fat partition coefficient, however, was about 200.0, lower than that estimated from Koss *et al.* (1978). This discrepancy may result from the change in fat composition due to the mobilization of nonpolar lipids

after PH (Katagiri, 1988) in the bioassay. The value 200.0 was used and remained constant during the simulation.

Software. The model was coded and the simulations and optimizations were performed using ACSL Tox 11.8.4 (AEgis Technologies Group). The sensitivity analysis was executed using acslXtreme 2.0.1.2 (Xcellon, Austin, TX).

RESULTS

Model Performance under Normal Physiological Conditions

Description of the iv dose study data. The model simulations of HCB concentrations in the liver, plasma, and muscle (slowly perfused compartments) (Fig. 3A) and in fat (Fig. 3B) were consistent with the experimental data reported by Scheufler and Rozman (1984a,b). The percentages of the dose excreted in the urine and feces were also well described by the model simulation (Fig. 3C). According to the model simulation, HCB appeared in the lower GI lumen shortly after administration, which was observed by Scheufler and Rozman (1984b). The percentage of the dose in the GI lumen increased from 0 at the time of exposure to about 45.6 in 312 h; thereafter the percentage gradually decreased to 24 at the end of simulation (1344 h). Since biliary excretion is known to be a minor route of excretion (Ingebrigtsen *et al.*, 1981), these results implied the importance of exsorption for HCB elimination.

The performance of the present model was compared with that of a previously employed model which did not consider HCB binding with erythrocytes as employed previously (Freeman *et al.*, 1989; Roth *et al.*, 1993a; Yesair *et al.*, 1986). As shown in Figure 3D, the simulations of the two PBPK models were different during the first 10 h: the present model traced the liver and plasma concentrations very well, whereas the model without HCB binding incorporated underpredicted the data.

Description of the single and repeated oral gavage study data. Comparisons between the model simulations and the single oral gavage data (Yamaguchi *et al.*, 1986) are shown in Figure 4 for the fat, liver, and blood HCB concentrations. The plot indicated that the model simulations were in good agreement with the data. The model also simulated the tissue HCB concentrations consistently with the repeated oral gavage data (Koss *et al.*, 1978) (Fig. 5A). The estimates of HCB excreted in the feces per day and metabolized per day at five time points were generally comparable to the experimental data (Figs. 5B and 5C). An approximately twofold difference between the exsorption rate constants of the two exposure scenarios was necessary for a good description of both data sets (Table 2).

Model validation. Using Koss and Koransky (1975) time-course concentration data from rats, we validated our model under the condition of single oral gavage dosing. Since the value of one adjustable parameter, exsorption rate constant, had to be varied when fitting the single (Yamaguchi *et al.*, 1986) and repeated (Koss *et al.*, 1978) oral gavage data (0.045 vs. 0.02 l/h, Table 2; see the ''Discussion'' section for the reasons), both values were tested during the validation. When the exsorption rate constant was set at 0.02 l/h, the model predictions agreed with the data (Figs. 6A and 6B) well. However, when the value was 0.045 l/h, the model notably underpredicted the second and third time points (data not shown).

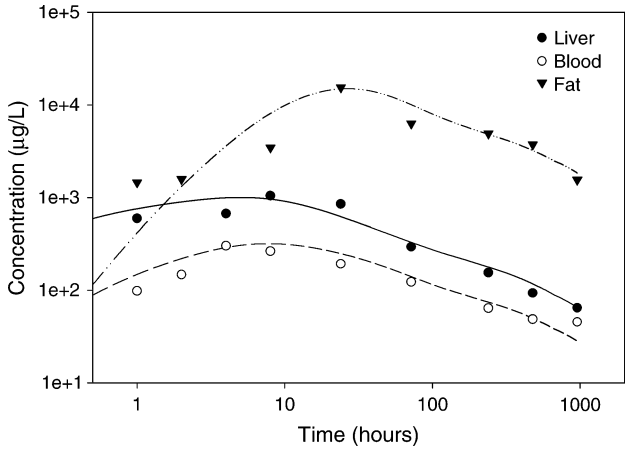

FIG. 4. For a single oral gavage of 0.2 mg HCB in the rat, model simulations and data of HCB concentrations in the fat, liver, and blood are shown. The lines are simulations and the symbols are experimental data (Yamaguchi *et al.*, 1986).

Sensitivity analysis. The sensitivity of the liver HCB concentration to the liver and fat volume fractions, tissue partition coefficients, and the adjustable parameters at multiple time points following an oral dose is shown in Table 3. The liver partition coefficient had the largest effect on the liver concentration, the fat volume fraction and partition coefficient had moderate effects, and the other parameters had only mild to little effect.

Pharmacokinetic Data from the Time-Course Bioassay

Effects of HCB treatment on the liver and body weights of the rats. All the rats in the study survived, except one in the control group for an unknown reason. The body and liver weights of each rat were recorded at five sacrifice times (Table 4). The HCB treatment did not significantly change the body weight at any time point. The liver weight was significantly increased only on day 47 in the high-dose group. The low-dose HCB treatment increased the liver/body weight ratio on day 28, whereas the high dose increased this index significantly on days 20, 28, and 47. At the end of the experiment (day 56), the livers accounted for approximately 3.0–3.6% of the body weights.

HCB disposition in the tissues. Time-course HCB tissue concentrations are shown in Figures 6A and 6B (low dose) and

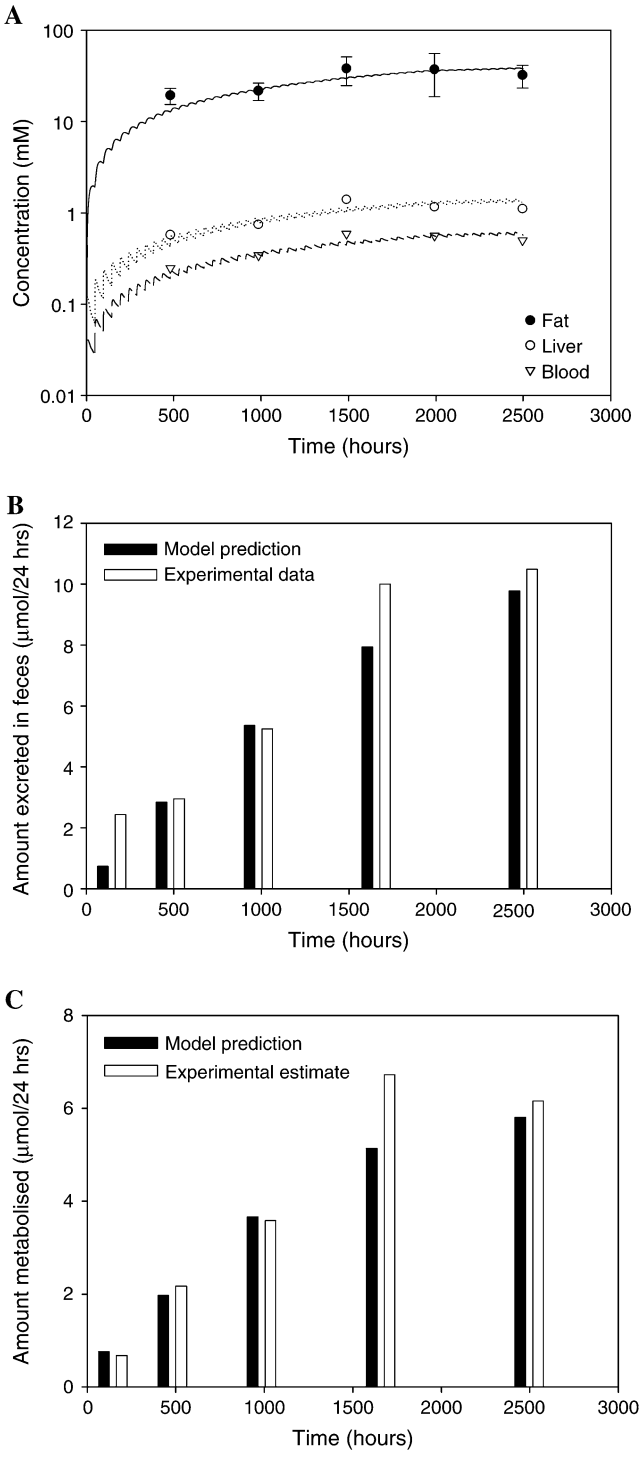

FIG. 5. For repeated oral gavage of 50 mg/kg HCB every other day in the rat, model simulations and data of HCB (A) concentrations in the fat, liver, and blood, (B) the amount excreted in feces per 24 h at five time points, and (C) the amount metabolized per 24 h at five time points are shown. In A, the lines are simulations and the symbols are experimental data (Koss *et al.*, 1978).

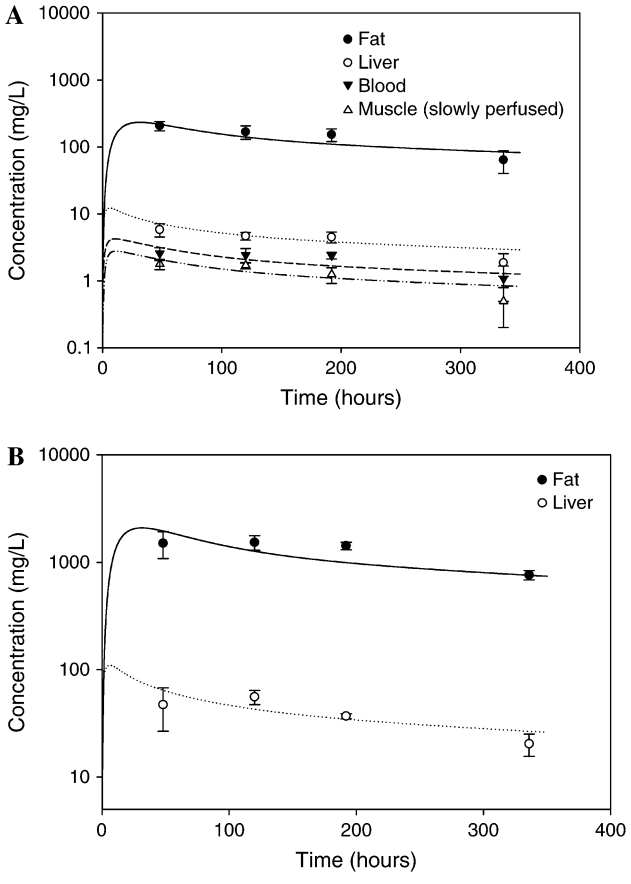

FIG. 6. For a single oral gavage dose of HCB in the rat, model simulations and data of HCB concentrations in the (A) fat, liver, blood, and muscle (slowly perfused compartment) at 20 mg/kg and (B) fat and liver at 180 mg/kg are shown.

Figures 7A and 7B (high dose). Similar disposition patterns were observed in both treated groups: the highest concentration in fat, followed by the liver, kidney, blood, and muscle. With a threefold difference in the exposure levels, there was approximately a two- to threefold difference in the corresponding tissue concentrations of the two treated groups.

Simulation of the Pharmacokinetic Data in the Time-Course Bioassay by Incorporating Pathophysiological Conditions

The model simulations and experimental data are shown in Figures 7A and 7B (low dose) and Figures 8A and 8B (high dose). The concentrations in various tissues/organs started to increase immediately after 336 h, when HCB administration began. Corresponding to the PH at 504 h and, more importantly, the treatment suspension during 504 through 600 h, there was concentration reduction in the interval. The model well predicted the late time points; yet the first time point was generally overpredicted.

TABLE 3
Log-Normalized Sensitivity Parameter Values for HCB Liver Concentration

	Hours after a single oral dose			
Parameters	1	24	72	336
Volume fractions				
Liver	0	0	0	0
Fat	0.001	−0.4982	−0.338	−0.238
Partition coefficients				
Liver	1.5103	1.8236	1.8241	1.8176
Fat	−0.0037	−0.8275	−0.5628	−0.3540
Rapidly perfused	−0.1702	−0.0311	−0.0137	−0.0078
Slowly perfused	−0.0817	−0.0813	−0.0461	−0.0619
Rate constants				
Metabolism	0.0053	0.0042	0.004	0.004
Exsorption	−0.0918	0.1024	0.0966	0.0973
Upper GI absorption	0.1509	−0.0464	1.2297	−0.0373
Lower GI absorption	0.0008	0.0127	0.0137	0.0139
Upper-to-lower transfer	−0.0131	0.0089	0.0096	−0.0063
Fecal excretion	0	−0.0076	0.0031	0.0028

DISCUSSION

This study reported an updated PBPK model for HCB in the rat with incorporation of erythrocyte binding, exsorption, and pathophysiological conditions following HCB treatment and PH. The model simulated well the pharmacokinetic data from a number of studies reported in the literature, as well as our own integrated pharmacokinetics/liver foci bioassay.

Necessity of Building an Updated PBPK Model for HCB in the Context of the Medium-Term Liver Foci Bioassay

The conventional long-term carcinogenesis protocol is strikingly time and resource intensive. With the large number of chemicals in commerce, it is virtually impossible to obtain carcinogenesis data on each chemical with the conventional method, let alone countless chemical mixtures to which humans are actually exposed. Therefore, alternative methods are in urgent need. Yang *et al.* (1998) proposed approaches integrating computational modeling with *in vitro* biological systems and *in vivo* bioassays. In this regard, the integration of biologically based computational modeling with a well-recognized shorter-term bioassay for evaluating carcinogenesis would provide opportunity for the creation of predictive tools. In our laboratory, we have modified the Ito's medium-term liver foci bioassay into an integrated time-course pharmacokinetics/liver foci bioassay, and studied several chlorobenzenes (Ou *et al.*, 2003; Thomas, 1998). The GST-P foci development promoted by HCB, pentachlorobenzene, 1,2,4,5-tetrachlorobenzene, and 1,4-dichlorobenzene has been successfully simulated with clonal growth models (Ou *et al.*, 2001, 2003; Thomas, 1998). To build dose-response (foci development) relationships, chemical target doses (i.e., liver concentrations of test chemicals in this case) should be achieved, which entails the application of PBPK modeling. It is possible that by studying a series of congeners such as chlorobenzenes, quantitative structure-activity correlation can be established and coupled with PBPK and biologically based pharmacodynamic modeling (e.g., clonal growth modeling) for predictive purposes.

This reported study is a part of a larger ongoing project in our laboratory which aims at developing a predictive tool for carcinogenesis of chemical mixtures by integrating *in vitro* biological systems, medium-term pharmacokinetics/liver foci bioassays, and PBPK and clonal growth modeling. The PBPK model described here will be applied with a clonal growth model in the future to form a biologically based dose-response model for HCB that will shed light on the dose-response (GST-P foci formation) curve in the low-dose region and facilitate risk assessment.

Although there are three published PBPK models for HCB thus far (Freeman *et al.*, 1989; Roth *et al.*, 1993a; Yesair *et al.*, 1986), we developed the present model because (1) Simulating

TABLE 4
Body and Liver Weights and Liver/Body Weight Ratios of the Rats in the Liver Foci Bioassay

	Body weight (g)			Liver weight (g)			Liver/body weight ratio (%)		
Days after DEN initiation	Control	Low dose	High dose	Control	Low dose	High dose	Control	Low dose	High dose
20	204.6 ± 5.9	212.1 ± 13.0	205.0 ± 9.1	7.3 ± 0.2	7.5 ± 0.7	7.6 ± 0.4	3.56 ± 0.09	3.53 ± 0.13	3.71 ± 0.07*!
24	190.5 ± 9.0	191.6 ± 11.3	195.6 ± 13.4	5.3 ± 0.7	5.0 ± 0.4	5.3 ± 0.6	2.79 ± 0.33	2.63 ± 0.17	2.71 ± 0.17
28	204.9 ± 21.1	208.5 ± 14.9	207.4 ± 6.5	6.2 ± 0.6	7.0 ± 0.8	7.0 ± 0.6	3.04 ± 0.16	3.37 ± 0.24*	3.38 ± 0.21*
47	271.1 ± 15.4	262.7 ± 16.9	270.3 ± 20.5	8.4 ± 0.4	8.3 ± 0.6	9.3 ± 0.9*	3.08 ± 0.10	3.17 ± 0.07	3.45 ± 0.13*!
56	291.1 ± 25.1	284.9 ± 9.3	305.7 ± 21.1	9.5 ± 1.0	9.1 ± 0.8	10.5 ± 0.9!	3.28 ± 0.12	3.19 ± 0.24	3.44 ± 0.10

Note. The days 20, 24, 28, 47, and 56 after DEN initiation are equivalent to the days 6, 10, 14, 34, and 42 after the commencement of the oral administration with corn oil or 0.03 or 0.1 mmol/kg HCB.

*$p < 0.05$, compared to the concurrent control group.

!$p < 0.05$, compared to the concurrent low-dose group.

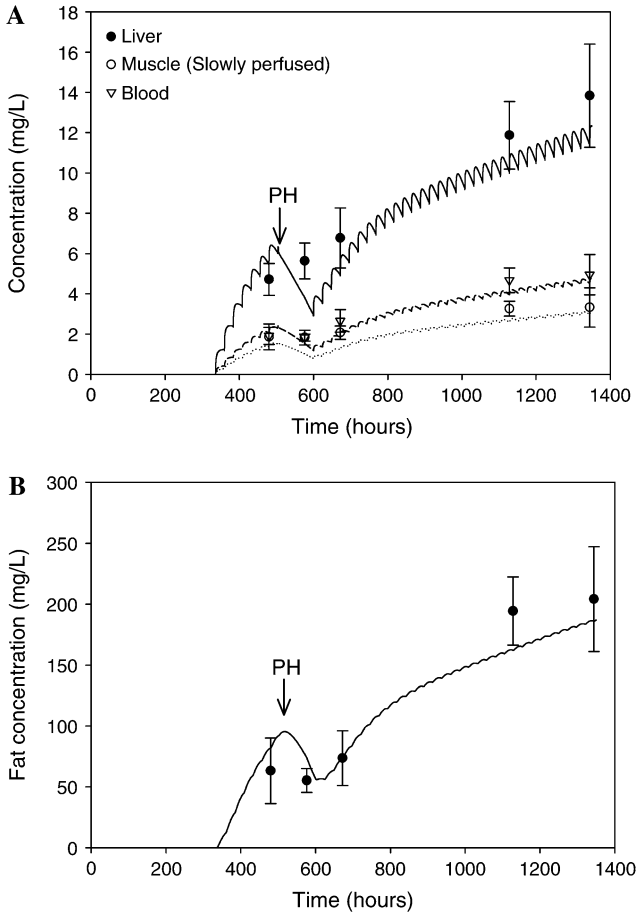

FIG. 7. Model simulations and experimental data of HCB concentrations in the (A) liver, muscle (slowly perfused compartment), and blood and (B) fat in the rat in the pharmacokinetic study integrated in the liver foci bioassay where the dose of HCB was 0.03 mmol/kg are shown. The lines are simulations and the symbols are experimental data. The arrows indicate when the PH was performed.

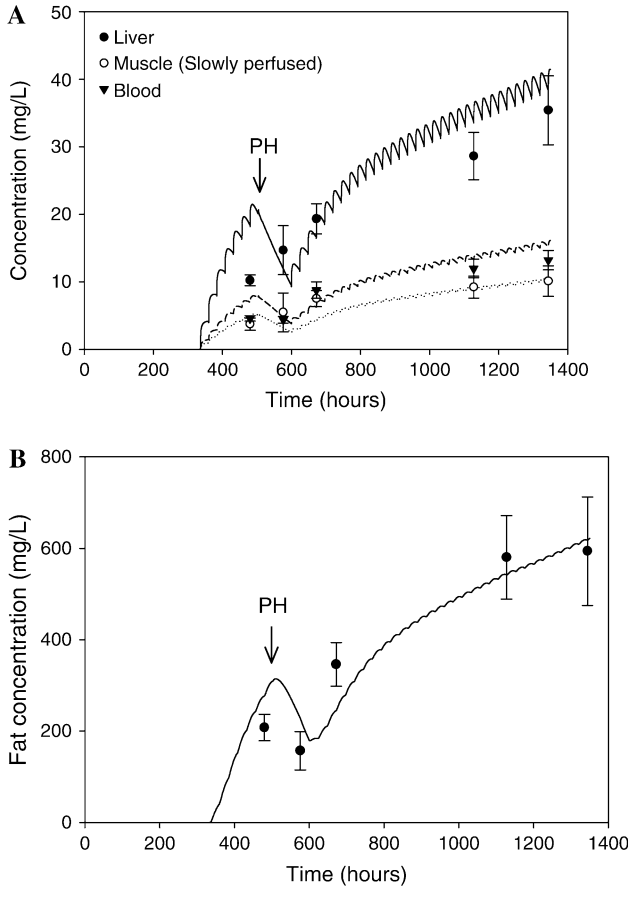

FIG. 8. Model simulations and experimental data of HCB concentrations in the (A) liver, muscle (slowly perfused compartment), and blood; and (B) fat in the rat in the pharmacokinetic study integrated in the liver foci bioassay where the dose of HCB was 0.1 mmol/kg. The lines are simulations and the symbols are experimental data. The arrows indicate when the PH was performed.

the liver concentration in the rat following HCB oral dosing and PH was of our most concern, but the previous models do not fit our purpose well. The Freeman *et al.* model intended to simulate and explain the distribution pattern of HCB in the body following an iv dose, and did not include an oral exposure route; the Yesair *et al.* model focused on female rats and humans with inclusion of a breast compartment and pregnancy state; and the Roth *et al.* model specifically emphasized GI absorption and excretion processes. Furthermore, these models have structures, corresponding to their respective goals, that are unnecessarily complicated for our purpose. (2) We wanted to incorporate in the model the two features of HCB pharmacokinetics, exsorption (Rozman *et al.*, 1985) and erythrocyte binding (Gomez-Catalan *et al.*, 1991; Yang *et al.*, 1975). The consideration of exsorption prevents assigning unreasonable high values to the parameters related to the minor elimination pathways (e.g., biliary excretion). The binding of HCB to erythrocytes hampers tissue uptake and reduces the declination of the concentrations in the plasma and tissues; incorporation of this binding process improved model simulation as illustrated in Figure 3D. (3) We needed to take into account the pathophysiological state that resulted from the experimental conditions of the liver foci bioassay. In order to build a dose–foci formation relationship, the prediction of liver concentration under the pathophysiological state is essential.

HCB Pharmacokinetics

It is already clear that HCB distribution in the body is predominantly affected by the fat contents of tissues (Koss and Koransky, 1975; Koss *et al.*, 1978; Scheufler and Rozman, 1984b) due to its high lipophilicity. Even in our liver foci bioassay, the fat still had the highest HCB concentration (Figs. 7 and 8) regardless of the unusual experimental conditions. Our model analysis indicated that the liver HCB concentration was moderately sensitive to the fat volume and fat partition coefficient as shown in Table 3. In our time-course liver foci

bioassay, PH was involved. Although the liver concentration was insensitive to the liver volume, the change in the liver volume (through PH) causes dramatic pathophysiological responses, including fat mobilization and redeposit. The sensitivity of liver concentration to fat volume warranted the inclusion of a change in the fat volume fraction (Equation 4) during simulation of the pharmacokinetic data from our bioassay.

There is pharmacokinetic difference for HCB in male and female rats (Kuiper-Goodman *et al.*, 1977), which can largely be attributed to the different metabolism patterns in the two genders (Renner, 1988). However, the fact that we were able to simulate all the data sets well without considering gender differences in the PBPK model suggests that the gender differences are not a sufficiently sensitive issue to affect our simulation outcomes. HCB can induce its own metabolism upon repeated dosing (Clark *et al.*, 1981). However, HCB is relatively resistant to metabolism (Koss and Koransky, 1975). Our sensitivity analysis suggested that the liver concentration of HCB is insensitive to the metabolism rate constant. Therefore, during our model development, the assumptions that the pharmacokinetic difference between male and female rats was negligible and that the metabolism rate constant was not changed by repeated dosing were reasonable and did not affect the model simulations significantly.

Model Parameter Adjustment

Although the model structure in this study remained almost unchanged during the development and simulation processes, some transluminal diffusion (i.e., absorption and exsorption)–related parameters had to be varied to fit multiple data sets. Given the specific absorption and excretion characteristics of HCB as described below, this adjustment is justifiable.

The absorption of HCB has been proposed as a passive diffusion process (Gobas *et al.*, 1993). In the GI lumen, HCB is transported in micelles to the intestinal wall, where it separates from the lipid and bile salts to diffuse as a single molecule through the wall. At the other side of the intestinal wall, HCB is reassociated with lipids or lipoproteins for further transport. Essentially, the diffusion is governed by equilibrium partitioning between the blood and the lumen. However, the whole absorption process (from the GI lumen to blood) is also affected by the availability of bile salts, digestibility of lipids in the lumen, and the amount of lipids in the intestinal tissue. These lipid-related factors in the GI tract are often varied with exposure conditions, such as the kind of oil vehicle, oral gavage volume, and gavage frequency. If the animals are not fasted prior to treatment, the amount and the types of foods being given would also play a role in the absorption process.

It was reported that even if the lipid-based dietary HCB concentration was lower than the blood concentration, net absorption still occurred (Schlummer *et al.*, 1998). To explain this observation, Schlummer *et al.* (1998) suggested a fat-flush hypothesis. In the small intestine, the absorbed dietary lipid elevates the lipid content in the intestinal tissue, diluting the local chemical concentration; meanwhile, the lipid-based luminal concentration is increased due to the reduction in the lipid volume. These two changes, in combination, amplify the transluminal diffusion gradient and greatly facilitate absorption. This hypothesis illustrates the involvement of lipid in HCB absorption. It also points out the complexity of the absorption process in the GI tract and the inadequacy of relying on one absorption rate constant to simulate such a complicated phenomenon.

As a highly lipophilic and metabolically resistant chemical, HCB is predominantly excreted by blood-to-lumen passive diffusion (Rozman *et al.*, 1985), i.e., exsorption, which has been documented for hydrophobic drugs (Arimori and Nakano, 1998; Israili and Dayton, 1984). Although exsorption occurs at both small and large intestines (Richter and Schafer, 1981), the latter is the major site for HCB net excretion (Rozman *et al.*, 1985). The outward diffusion gradient, and thus the exsorption, relies on the fat content in the large intestinal lumen, which comes from unabsorbed lipid, sloughed epithelial cells, or bacterial activity. Therefore, the exsorption rate constant is expected to vary in different situations. Indeed, in this study, adjustment of this parameter is necessary to fit all data sets.

The absorption and exsorption of HCB are two inverse processes, lumen-to-blood (absorption) versus blood-to-lumen (exsorption) diffusion, which result in an enteroenteric recirculation (Israili and Dayton, 1984). Due to the microenvironmental changes along the GI lumen, the absorption decreases and the exsorption increases gradually from the proximal to the distal end. The fate of the chemical relies on the relative magnitude of the two processes.

The absorption and exsorption processes of HCB, and probably other metabolically resistant and highly lipophilic chemicals as well, are very complicated. Given the above discussions, it is not surprising that a single set of parameters would not be adequate for simulation of multiple sets of experimental data. Variations in those processes across dose regimens are hitherto rarely studied. Wang *et al.* (2000) examined the dispositions of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) across experimental conditions (oral gavage in female, iv injections in female and male Sprague-Dawley rats at comparable doses) using PBPK modeling. To fit the respective data sets, the rate constants of elimination from the kidney and liver had to vary among the studies. The Wang *et al.* (2000) study and the present study, on the one hand, reveal the utility of PBPK modeling for studying the complex absorption and excretion kinetics of lipophilic chemicals through conduction of *in silico* experimentations via computer simulation of different dosing scenarios. On the other hand, they form a challenge to the well-known capability of PBPK modeling in extrapolation across dosing scenarios and species. Although successful examples of dosimetry extrapolation for volatile chemicals using PBPK modeling have been published (Reitz *et al.*, 1988), little has been reported for nonvolatile

lipophilic chemicals, e.g., HCB, dioxins. Therefore, further study is warranted in the future to answer the following questions: What are the factors affecting the absorption and elimination processes of nonvolatile lipophilic chemicals? How do the factors vary across experimental conditions? How do we describe these variations quantitatively?

Incorporation of Pathophysiological Conditions and Uncertainties in the Model Simulation in the Context of the Liver Foci Bioassay

A notable treatment on the rats in our pharmacokinetic study integrated in the liver foci bioassay is PH. In addition to the desirable stimulated hepatocyte growth, many pathophysiological alterations ensue. The body and liver weights temporarily decrease, the latter more dramatically. The fat is mobilized to provide substrates and energy for liver regeneration, causing decrease in fat volume and increase in blood lipid level (Katagiri, 1988). In the liver, lipoprotein lipase expression (Sabugal *et al.*, 1996) and lipid contents (Katagiri, 1988) are elevated; various cell proliferation–related cytokines and hormones are imported or/and expressed (Michalopoulos and DeFrances, 1997). Some, if not all, of these alterations will influence HCB kinetics.

From the modeling perspective, the incorporation of any pathophysiological condition is exercised by adjustment of parameters according to the underlying mechanism(s) related to the condition. In our study, the two-thirds PH resulted in the loss of body weight and mobilization of fat due to the trauma and the needed energy for growing back the liver mass. Thus, the pathophysiological conditions were reflected by the changes in the body and liver weights and fat volume and were incorporated into our modeling. Theoretically, other pathophysiological changes, such as those in the liver partition coefficient (probably increased due to fat accumulation in the liver) and in other partition coefficients (plasma concentration would be increased due to hyperlipidemia after PH), can also occur and they should be included in the modeling. However, we currently do not have sufficient experimental information on these potential changes and these aspects are beyond the scope of the present paper.

There are uncertainties in simulating our HCB pharmacokinetic data in the bioassay using the established PBPK model: (1) Fat mobilization and redeposit following PH are qualitatively known; but to our knowledge, no accurate time-course fat volume data under such conditions are available. (2) In both normal and partially hepatectomized rats, HCB is likely to have the same absorption and exsorption mechanisms. However, the rate constants could be altered due to PH, dose level, dose duration, or continuous application of corn oil. In this study, the rate constants were fixed throughout the simulation durations. The first time point of the HCB concentration in this bioassay was generally overpredicted, which implied that lower absorption rate constants might be valid prior to PH.

In summary, an updated PBPK model for HCB was developed based on our current knowledge on HCB, the data sets from the literature, as well as from our experiment. The model development followed a sequential process from simulating the single iv-dose study data to the single and repeated oral gavage study data. The model successfully described the data sets. The established PBPK model was then employed to simulate HCB disposition by incorporating the conditions as well as pathophysiological changes in the medium-term liver foci bioassay. With necessary adjustment of the rate constants of absorption and exsorption, the simulations were consistent with our data. The necessity of variation of the absorption and exsorption parameters for different exposure scenarios reflected the complex kinetics of HCB in the GI tract.

SUPPLEMENTAL DATA

Supplementary data are available online at www.toxsci.oxfordjournals.org.

ACKNOWLEDGMENTS

This study is supported by the NIOSH/CDC grant 1 RO1 OH07556, NIEHS Training Grant 1 T32 ES 07321, and scholarships from U.S. Fulbright Foundation and Naresuan University, Thailand. We thank Ms. Laura Chubb, Ms. Tracy Nichols, Drs. Todd Painter and Charles Dean, and other colleagues in the Quantitative and Computational Toxicology Group for their excellent technical assistance. The valuable suggestions from Dr. Melvin Andersen on model development are greatly appreciated. We also thank Mr. Xiaohui Xu for supporting statistical analysis of experimental data.

REFERENCES

Alvarez, L., Randi, A., Alvarez, P., Kolliker Frers, R., and Kleiman de Pisarev, D. L. (1999). Effect of hexachlorobenzene on NADPH-generating lipogenic enzymes and L-glycerol-3-phosphate dehydrogenase in brown adipose tissue. *J. Endocrinol. Investig.* **22,** 436–445.

Arimori, K., and Nakano, M. (1998). Drug exsorption from blood into the gastrointestinal tract. *Pharmacol. Res.* **15,** 371–376.

Brown, R. P., Delp, M. D., Lindstedt, S. L., Rhomberg, L. R., and Beliles, R. P. (1997). Physiological parameter values for physiologically based pharmacokinetic models. *Toxicol. Ind. Health* **13,** 407–484.

Clark, D. E., Ivie, G. W., and Camp, B. J. (1981). Effects of dietary hexachlorobenzene on in vivo biotransformation, residue deposition, and elimination of certain xenobiotics by rats. *J. Agric. Food Chem.* **29,** 600–608.

Clewell, H. J., III, Lee, T. S., and Carpenter, R. L. (1994). Sensitivity of physiologically based pharmacokinetic models to variation in model parameters: Methylene chloride. *Risk Anal.* **14,** 521–531.

Erturk, E., Lambrecht, R. W., Peters, H. A., Cripps, D. J., Gocmen, A., Morris, C. R., and Bryan, G. T. (1986). Oncogenicity of hexachlorobenzene. *IARC Sci. Publ.* **77,** 417–423.

Freeman, R. A., Rozman, K. K., and Wilson, A. G. E. (1989). Physiological pharmacokinetic model of hexachlorobenzene in the rat. *Health Phys.* **57**(Suppl. 1), 139–147.

Gobas, F. A., McCorquodale, J. R., and Haffner, G. G. (1993). Intestinal absorption and biomagnification of organochlorines. *Environ. Toxicol. Chem.* **12,** 567–576.

Gomez-Catalan, J., To-Figueras, J., Rodamilans, M., and Corbella, J. (1991). Transport of organo-chlorine residues in rat and human blood. *Arch. Environ. Contam. Toxicol.* **20,** 61–66.

Gustafson, D. L., Long, M. E., Thomas, R. S., Benjamin, S. A., and Yang, R. S. (2000). Comparative hepatocarcinogenicity of hexachlorobenzene, pentachlorobenzene, 1,2,4,5-tetrachlorobenzene, and 1,4-dichlorobenzene: Application of a medium-term liver focus bioassay and molecular and cellular indices. *Toxicol. Sci.* **53,** 245–252.

Hida, Y., Matsui, N., Kawada, T., and Fushiki, T. (1999). Ultrasonography evaluation of abdominal fat in live rats. *J. Nutr. Sci. Vitaminol.* **45,** 609–619.

Ingebrigtsen, K., Skaare, J. U., Nafstad, I., Forde, M. (1981). Studies on the biliary excretion and metabolism of hexachlorobenzene in the rat. *Xenobiotica* **11,** 795–800.

Israili, Z. H., and Dayton, P. G. (1984). Enhancement of xenobiotic elimination: Role of intestinal excretion. *Drug Metab. Rev.* **15,** 1123–1159.

Ito, N., Tamano, S., and Shirai, T. (2003). A medium-term rat liver bioassay for rapid in vivo detection of carcinogenic potential of chemicals. *Cancer Sci.* **94,** 3–8.

Katagiri, S. (1988). Experimental study on lipid metabolism after partial hepatectomy—With reference to mitochondrial function of the regenerating liver. *Nippon Ganka Gakkai Zasshi* **89,** 694–702 (In Japanese).

Koss, G., and Koransky, W. (1975). Studies on the toxicology of hexachlorobenzene I. Pharmacokinetics. *Arch. Toxicol.* **34,** 203–212.

Koss, G., Seubert, S., Seubert, A., Koransky, W., and Ippen, H. (1978). Studies on the toxicology of hexachlorobenzene. III. Observations in a long-term experiment. *Arch. Toxicol.* **40,** 285–294.

Kuiper-Goodman, T., Grant, D. L., Moodie, A., Korsrud, G. O., Munro, I. C. (1977). Subacute toxicity of hexachlorobenzene in the rat. *Toxicol. Appl. Pharmacol.* **40,** 529–579.

Lee, H. B., and Blaufox, M. D. (1985). Blood volume in the rat. *J. Nucl. Med.* **25,** 72–76.

Michalopoulos, G. K., and DeFrances, M. C. (1997). Liver regeneration. *Science* **276,** 60–66.

Ou, Y. C., Conolly, R. B., Thomas, R., Gustafson, D. L., Long, M. E., Dovrev, I. D., Chubb, L. S., Xu, Y., Lapidot, S., Andersen, M. E., *et al.* (2003). Stochastic simulation of hepatic preneoplasic foci development for four chlorobenzene congeners in a medium-term bioassay. *Toxicol. Sci.* **73,** 301–314.

Ou, Y. C., Conolly, R. B., Thomas, R. S., Xu, Y., Andersen, M. E., Chubb, L. S., Pitot, H. C., and Yang, R. S. H. (2001). A clonal growth model: Time-course simulations of liver foci growth following penta- or hexachlorobenzene treatment in a medium-term bioassay. *Cancer Res.* **61,** 1879–1889.

Ralph, J. L., Orgebin-Crist, M. C., Lareyre, J. J., and Nelson, C. C. (2003). Disruption of androgen regulation in the prostate by the environmental contaminant hexachlorobenzene. *Environ. Health Perspect.* **111,** 461–466.

Reitz, R. H., McDougal, J. N., Himmelstein, M. W., Nolan, R. J., and Schumann, A. M. (1988). Physiologically based pharmacokinetic modeling with methylchloroform: Implications for interspecies, high dose/low dose, and dose route extrapolations. *Toxicol. Appl. Pharmacol.* **95,** 185–199.

Renner, G. (1988). Hexachlorobenzene and its metabolism. *Toxicol. Environ. Chem.* **18,** 51–78.

Richter, E., and Schafer, S. G. (1981). Intestinal excretion of hexachlorobenzene. *Arch. Toxicol.* **47,** 233–239.

Roth, W. L., Freeman, R. A., and Wilson, A. G. E. (1993a). A physiologically based model for gastrointestinal absorption and excretion of chemicals carried by lipids. *Risk Anal.* **13,** 531–543.

Roth, W. L., Weber, L. W. D., Stahl, B. U., and Rozman, K. (1993b). A pharmacodynamic model of triglyceride transport and deposition during feed deprivation or following treatment with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the rat. *Toxicol. Appl. Pharmacol.* **120,** 126–137.

Rozman, T., Scheufler, E., and Rozman, K. (1985). Effect of partial jejunectomy and colectomy on the disposition of hexachlorobenzene in rats treated or not treated with hexadecane. *Toxicol. Appl. Pharmacol.* **78,** 421–427.

Sabugal, R., Robert, M. Q., Julve, J., Auwerx, J., Llobera, M., and Peinado-Onsurbe, J. (1996). Hepatic regeneration induces changes in lipoprotein lipase activity in several tissues and its re-expression in the liver. *Biochem. J.* **318**(Pt. 2), 597–602.

Scheufler, E., and Rozman, K. (1984a). Comparative decontamination of hexachlorobenzene-exposed rats and rabbits by hexadecane. *J. Toxicol. Environ. Health* **14,** 353–362.

Scheufler, E., and Rozman, K. (1984b). Effect of hexadecane on the pharmacokinetics of hexachlorobenzene. *Toxicol. Appl. Pharmacol.* **75,** 190–197.

Schielen, P., Den Besten, C., Vos, J. G., Van Bladeren, P. J., Seinen, W., and Bloksma, N. (1995). Immune effects of hexachlorobenzene in the rat: Role of metabolism in a 13-week feeding study. *Toxicol. Appl. Pharmacol.* **131,** 37–43.

Schlummer, M., Andreas Moser, G., and McLachlan, M. S. (1998). Digestive tract absorption of PCDD/Fs, PCBs, and HCB in humans: Mass balances and mechanistic considerations. *Toxicol. Appl. Pharmacol.* **152,** 128–137.

Schoeffner, D. J., Warren, D. A., Muralidara, S., Bruckner, J. V., and Simmons, J. E. (1999). Organ weights and fat volume in rats as a function of strain and age. *J. Toxicol. Environ. Health A* **56,** 449–462.

Siekel, P., Chalupa, I., Beno, J., Blasko, M., Novotny, J., and Burian, J. (1991). A genotoxicological study of hexachlorobenzene and pentachloroanisole. *Teratog. Carcinog. Mutagen.* **11,** 55–60.

Smith, A. G., Dinsdale, D., Cabral, J. R., and Wright, A. L. (1987). Goitre and wasting induced in hamsters by hexachlorobenzene. *Arch. Toxicol.* **60,** 343–349.

Smith, A. G., Francis, J. E., Dinsdale, D., Manson, M. M., and Cabral, J. R. (1985). Hepatocarcinogenicity of hexachlorobenzene in rats and the sex difference in hepatic iron status and development of porphyria. *Carcinogenesis* **6,** 631–636.

Staats, D. A., Fisher, J. W., and Connolly, R. B. (1991). Gastrointestinal absorption of xenobiotics in physiologically based pharmacokinetic models. A two-compartment description. *Drug. Metab. Dispos.* **19,** 144–148.

Thomas, R. S. (1998). The use of biologically-based models for integrating short-term cancer bioassays, mechanisms of action, and target tissue dosimetry: Application to pentachlorobenzene. Ph. D. Dissertation, Department of Environmental Health, Colorado State University, Fort Collins, CO.

Wang, X., Santostefano, M. J., DeVito, M. J., and Birnbaum, L. S. (2000). Extrapolation of a PBPK model for dioxins across dosage regimen, gender, strain, and species. *Toxicol. Sci.* **56,** 49–60.

Yamaguchi, Y., Kawano, M., and Tatsukawa, R. (1986). Tissue distribution and excretion of hexabromobenzene and hexachlorobenzene administered to rats. *Chemosphere* **15,** 453–459.

Yang, R. S. H., Coulston, F., and Golberg, L. (1975). Binding of hexachlorobenzene to erythrocytes: Species variation. *Life Sci.* **17,** 545–550.

Yang, R. S. H., Pittman, K. A., Rourke, D. R., and Stein, V. B. (1978). Pharmacokinetics and metabolism of hexachlorobenzene in the rat and rhesus monkey. *J. Agric. Food Chem.* **26,** 1076–1083.

Yang, R. S. H., Thomas, R. S., Gustafson, D. L., Campain, J., Benjamin, S. A., Verhaar, H. J., and Mumtaz, M. M. (1998). Approaches to developing alternative and predictive toxicology based on PBPK/PD and QSAR modeling. *Environ. Health Perspect.* **106**(Suppl. 6), 1385–1393.

Yesair, D. W., Feder, P. I., Chin, A. E., Naber, S. J., Kuiper-Goodman, T., Scott, C. S., and Robinson, P. E. (1986). Development, evaluation and use of a pharmacokinetic model for hexachlorobenzene. *IARC Sci. Publ.* 297–318.