Quantitative Evaluation of Alternative Mechanisms of Blood Disposition of Di(\(n\)-butyl) Phthalate and Mono(\(n\)-butyl) Phthalate in Rats

Deborah A. Keys,* † Duncan G. Wallace, † Thomas B. Kepler,* and Rory B. Conolly†,1

*Biomathematics Graduate Program, Department of Statistics, North Carolina State University, Raleigh, North Carolina 27695; and †Chemical Industry Institute of Toxicology Research Triangle Park, North Carolina 27709

Received February 2, 1999; accepted August 26, 1999

Phthalate esters are ubiquitous, low-level environmental contaminants that induce testicular toxicity in laboratory animals. The diester is rapidly metabolized in the gut to the monoester, which causes the testicular toxicity. Several physiologically based pharmacokinetic (PBPK) model structures have been evaluated for di(2-ethylhexyl) phthalate (DEHP) and mono(2-ethylhexyl) phthalate (MEHP). The objective of this study was to test these PBPK models for a less lipophilic phthalate diester, di(\(n\)-butyl) phthalate (DBP), and monoester, mono(\(n\)-butyl) phthalate (MBP). Alternate models describing enterohepatic circulation, diffusion-limited, tissue pH gradients (pH trapping), and a simpler, flow-limited model were evaluated. A combined diffusion-limited and pH trapping model was also tested. MBP tissue: blood partition coefficients were similar when determined either experimentally by a nonvolatile, vial equilibration technique or algorithmically. All other parameters were obtained from the literature or estimated from MBP blood concentrations following intravenous or oral exposure to DBP or MBP. A flow-limited model was unable to predict MBP blood levels, whereas each alternative model had statistically better predictions. The combined diffusion-limited and pH trapping model was the best overall, having the highest log-likelihood function value. This result is consistent with a previous finding that the pH trapping model was the best model for describing DEHP and MEHP blood dosimetry, though it was necessary to extend the model to include diffusion-limitation. The application of the pH trapping model is a step toward developing a generic model structure for all phthalate esters, though more work is required before a generic structure can be identified with confidence. Development of a PBPK model structure applicable to all phthalate esters would support more realistic assessments of risk to human health from exposure to one or more members of this class of compounds.

Key Words: phthalates; DBP; MBP; PBPK; model; flow-limited; diffusion-limited; pH trapping; enterohepatic circulation; log-likelihood ratio test.

Phthalate esters are plasticizers that are ubiquitous low-level environmental contaminants. Di(\(n\)-butyl) phthalate (DBP) is used mainly in polyvinyl chloride and to a lesser degree in paints, glue, and cosmetics. Approximately 49,000 tons of DBP were produced in Western Europe in 1994 (Long and Meek, 1997) and 11,400 tons in the United States in 1987 (ATSDR, 1990). Most nonoccupational human exposure occurs through contaminated food. DBP may migrate into food from plastic wrap or may enter food from general environmental contamination. The estimated total average daily human intake from air, drinking water, and food is 7.4 μg/kg/day (Long and Meek, 1997).

Several phthalate esters, including DBP, are testicular toxicants in rodents at high doses. Oral exposure of adult or juvenile male rats for 4 to 90 days to 500–2600 mg/kg/day DBP resulted in decreased testicular weight, atrophy of the seminiferous tubules, and decreased sperm counts (Cater et al., 1977; Fukuoka et al., 1993; Fukuoka et al., 1989; Fukuoka et al., 1990; Gray et al., 1982; Srivastava et al., 1990). These testicular effects have been shown to be partly reversible (Tanino et al., 1987) and are species sensitive, with rat and guinea pigs more sensitive than mice and hamsters (Gray et al., 1982; NTP, 1995; Oishi and Hiraga, 1980). Pubertal rats are also more sensitive than adult rats to the toxicity of phthalate esters (Sjöberg et al., 1986). Dietary administration of doses equivalent to 66 mg/kg/day throughout gestation to adulthood resulted in malformation of the male reproductive tract (NTP, 1991; Wine et al., 1997), as did gestational and lactational exposure alone to oral doses of 250 mg/kg/day DBP and higher (Mylchreest et al., 1998). The presence of malformations at lower doses in perinatal rats than in adult males indicates that the period of time encompassing gestation and lactation is the most sensitive exposure window for developmental effects due to phthalate ester exposure.

Orally administered phthalate diesters are rapidly metabolized in the gut to monooesters by pancreatic lipase and gut flora. DBP is metabolized to mono(\(n\)-butyl) phthalate (MBP). At doses up to 857 mg/kg DBP, no intact DBP was measured in the blood of exposed rats (NIEHS, 1994), indicating that virtually no intact DBP was absorbed. MBP is further metabolized by ω and ω − 1 oxidation in the rat liver (Albro and Moore, 1974; Williams and Blanchfield, 1975). MBP also

1 To whom correspondence should be addressed at Chemical Industry Institute of Toxicology, 6 Davis Drive, P.O. Box 12137, Research Triangle Park, NC 27709-2137. Fax: (919) 558-1300. E-mail: rconolly@ciit.org.
undergoes glucuronide conjugation in the rat, and both free and conjugate MBP are excreted in the urine (Foster et al., 1983). There is no evidence of tissue accumulation of DBP or its metabolites. Radioactivity was detected in liver, kidney, blood, muscle, adipose tissue, and the intestine 24 h after administration of a single oral dose of 60 mg/kg DBP; however, 90% of the administered radioactivity was excreted in the urine by 48 h (Tanaka et al., 1978).

The phthalate monoester appears to be the active toxicologic compound for the induction of reproductive toxicity (Gray and Gangolli, 1986; Oishi and Hiraga, 1980). MBP induces testicular atrophy after similar oral doses ≥ 400 mg/kg MBP as DBP with the same spectrum of effects (Cater et al., 1977; Foster et al., 1981; Gray et al., 1982; Oishi and Hiraga, 1980).

A physiologically based pharmacokinetic (PBPK) model was developed previously for di(2-ethylhexyl) phthalate (DEHP) and its monoester, MEHP (Keys et al., 1999). In that study, several relatively complicated PBPK model structures (enterohepatic circulation, diffusion-limitation, and pH trapping) were compared to a simpler flow-limited model. The pH trapping model provided the best description of DEHP and MEHP dosimetry. The objective of the present study was to develop a PBPK model for DBP/MBP and to simultaneously evaluate the predictive ability of the Poulin and Krishnan algorithm from the MBP partition coefficients were also estimated using the methods of Murphy and colleagues. For blood, the amount of MBP in reference and test vials, $A_r$ and $A_t$, respectively, were expressed as

$$A_r = C_r \cdot V_{PC} + C_e \cdot V_t \cdot \lambda,$$

$$A_t = C_t \cdot V_{PC} + C_e \cdot V_s \cdot \lambda_{PC},$$

where $C_r$ is the MBP concentration (dpm) in the reference vial, $V_{PC}$ is the volume (ml) of the PC layer, $V_t$ is the volume (ml) of saline, $\lambda$ is the saline:PC partition coefficient, $C_t$ is the MBP concentration (dpm) in the test vial PC layer, $V_s$ is the volume (ml) of blood, and $\lambda_{PC}$ is the blood:PC partition coefficient.

Setting $A_x = A_r$ and rearranging terms yielded the following equation for calculation of the blood:PC partition coefficient:

$$\lambda_{PC} = \frac{(C_x - C_t) \cdot V_{PC} + C_e \cdot V_t \cdot \lambda}{C_t \cdot V_s}.$$  

Pairing of test and reference vials did not result in a significant reduction in variance in a randomized experimental block analysis of variance (ANOVA). Thus test and reference vials were not paired in further analysis. To determine if equilibrium had been reached, test vial concentrations as dpm from each time point were compared using a one-sided t-test to test the null hypothesis that the test vial concentrations from the second time point were greater or equal to the test vial concentrations at the first time point. A decrease in test vial concentration indicates that chemical was continuing to partition into the tissue. If equilibrium was reached, test vial concentrations were pooled, and reference vial concentrations were pooled. An average test vial concentration and an average reference vial concentration from both time points were used in the calculation of tissue:PC partition coefficients. Tissue:blood partition coefficients were calculated by dividing the calculated tissue:PC partition coefficients by the calculated blood:PC partition coefficient. Approximate standard errors of the tissue: blood partition coefficients were calculated to sum the propagation of error from the measured quantities (i.e., standard errors mean test and reference vial concentrations) to the calculated tissue: blood partition coefficients (See Appendix).

**Estimated partition coefficients.** The algorithm of Poulin and Krishnan (1995) was used to estimate the nonionized MBP fat:blood, testes:blood, and muscle:blood partition coefficients from the n-octanol:water partition coefficient ($K_{ow}$). $K_{ow}$ for nonionized MBP of 3.03 was estimated based on its chemical structure using ProLogP 2.0 software (CompuDrug Chemistry Ltd., Burlingame, CA) and used as an input to the Poulin and Krishnan algorithm. A tests water fraction of tissue weight of 0.80, a total lipids fraction of tissue weight of 0.023 (Fiserova-Bergrova, 1983), a phospholipid (fraction of total lipids) of 0.64 (Williams et al., 1945), and a neutral lipids fraction of total lipids of 0.36 were used to calculate the testes:blood partition coefficient. The slowly perfused tissues: blood partition coefficient was set equal to the predicted muscle:blood partition coefficient, and the rapidly perfused tissues: blood partition coefficient was set equal to the predicted liver:blood partition coefficient.

For comparison to the measured partition coefficients, MBP tissue: blood partition coefficients were also estimated using the Poulin and Krishnan algorithm from the MBP $K_{ow}$ predicted at physiological pH (i.e., for ionized MBP) as calculated by ProLogD 2.0. This comparison was conducted to evaluate the predictive ability of the Poulin and Krishna algorithm.

**Flow-limited PBPK model.** A flow-limited model structure for nonvolatile chemicals was adapted from Ramsey and Andersen (1984) to describe the dosimetry of DBP and MBP (Fig. 1A). In a flow-limited model, the rate of
transport of chemical into the tissue is limited by blood flow to that particular tissue, whereas diffusion is assumed to be very rapid. Because intact DBP was measured in the blood after oral doses up to 857 mg/kg (NIEHS, 1994; NIEHS, 1995), the first-order oral absorption rate of DBP was set to zero, effectively removing all DBP compartments from the model except the small intestine. Because MBP is nonvolatile, the concentration of MBP in venous blood was assumed to be equal to the concentration of chemical in arterial blood; therefore no exchange of blood MBP with inhaled air was described. A testes compartment was separated from other rapidly perfused tissues because the testes are the target organ for reproductive toxicity of MBP in males. The blood was represented as a compartment as DBP, if given intravenously, is metabolized in the blood. The small intestine was included as a compartment rather than the whole gastrointestinal tract because pancreatic lipase accounts for most of the gut hydrolysis of DBP to MBP and is released directly into the small intestine via the pancreatic duct.

Hydrolysis of DBP to MBP in the small intestine was described by a first-order rate constant. Michaelis-Menten kinetics were examined but did little to improve the model fit. First-order kinetics were thus used for reasons of model parsimony. Further metabolism of MBP in the liver, which includes several metabolic pathways, was lumped into a single Michaelis-Menten pathway. First-order MBP metabolism was explored but found to be unable to predict MBP blood levels following exposure to both a low intravenous dose of DBP and to a higher oral dose of DBP. Unlike MEHP, MBP goes through glucuronide conjugation in the rat, and conjugated MBP is the major urinary metabolite (Foster et al., 1983). Conjugation was not represented explicitly in the PBPK model, although the rate of conjugation contributes to the lumped Michaelis-Menten hepatic rate parameter. The absorption of MBP following oral administration (gavage) was simulated as a first-order process for uptake into the liver from the small intestine. Intravenous administration was modeled as an infusion directly into the blood compartment.

**Enterohepatic circulation PBPK model.** Enterohepatic circulation was considered a viable hypothesis because MBP is a charged molecule and conjugated MBP has a molecular weight above 325, which is considered a lower bound for molecular weight for enterohepatic circulation in the rat (Guthrie and Hodgson, 1987). Because MBP is conjugated in the rat and MEHP is not, MBP would be even more likely than MEHP to undergo enterohepatic circulation. Secondary peaks in MBP blood concentrations following intravenous exposure to MBP and oral exposure to DBP also indicated potential enterohepatic circulation (NIEHS, 1994; NIEHS, 1995). In addition, 10% of radiolabeled, intravenously administered [14C]DBP was recovered in the bile at 5 h, and 4.5% of an oral dose after 6 h, with both free and conjugated MBP identified in the bile (Kaneshima et al., 1978). In another study, 44% of an orally administered DBP dose was recovered in the bile after 3 days (Tanaka et al., 1978). Enterohepatic circulation of MBP was described in the model by adding a flow of MBP from the liver back to the small intestine with an adjustable time delay (Fig. 1B). The time delay accounted for the time required for bile to flow from the liver to the intestinal lumen.

**Diffusion-limited PBPK model.** Diffusion-limitation is also likely, as MBP is a relatively large charged molecule with a molecular weight of 222. In the diffusion-limited model, the rate of diffusion from the tissue blood into the tissue rather than blood flow limited the transport of MBP into the liver, fat, testes, and slowly and rapidly perfused tissues. Each of these compartments was further divided into extracellular (blood) and intracellular compartments (tissue), each with its own volume (Fig. 1C). The volume of the extracellular compartment was set to the tissue blood volume (Table 1), and the volume of the intracellular compartment was set to the difference between the total tissue volume and the tissue blood volume. The permeation coefficient-surface area-cross product controlled the flow from the extracellular compartment to the intracellular compartment (See Appendix).

**pH trapping PBPK model.** The pH trapping model was based on the model developed by O’Flaherty et al. (1992). In the pH trapping model, each compartment was divided into extracellular and intracellular compartments with the same extracellular and intracellular volumes as the diffusion-limited model (Fig. 1D). Only nonionized MBP was free to move between the blood and tissue. In the tissue, MBP was predicted to reach a new equilibrium of mostly ionized MBP and was trapped there until it was deionized and released. Ionization and deionization are assumed to be instantaneous. The Henderson-Hasselbalch equation was used to calculate equilibrium concentrations of ionized and nonionized MBP in each extracellular and intracellular compartment based on a extracellular pH set to the pH of arterial blood (7.4) and an average intracellular pH of 7.0 (Guyton, 1991). A pKa of 3.79 was estimated based on chemical structure using pKalc 3.2 (Compudrug Chemistry Ltd., Burlingame, CA). Nonionized MBP tissue-blood partition coefficients (Table 2) were used in this model. The complexity of the pH trapping model may be necessary to describe MBP blood dosimetry, as MBP is mostly ionized at physiological pH.

**Diffusion-limited, pH trapping PBPK model.** In the pH trapping model with diffusion-limitation, MBP entered the extracellular compartment of the tissue where it was predicted to reach equilibrium between ionized and nonionized MBP according to the Henderson-Hasselbalch equation. The permeation coefficient-surface area-cross product then controlled the rate at which nonionized MBP partitioned into the intracellular compartment. Once in the
in the nested approach described below. For the flow-limited model, MBP absorbance rate of MBP ($k_{a,w}^n$), holding metabolic parameters constant. Next, holding MBP metabolism and absorption parameters constant, MBP blood concentrations following oral exposure to 43, 50, 100, 200, or 857 mg/kg DBP were used to estimate the gut metabolism rate parameter ($k_{m}^n$). Finally, an overall optimization that included all the data and parameters was conducted using the nested approach estimates as initial values. The heteroscedasticity parameter $\gamma$ was allowed to vary from 2.0 for this final total optimization. Individual animal rather than mean concentrations were used to more accurately estimate $\gamma$. This resulted in an optimal set of parameter estimates with an associated log-likelihood function value. Log-likelihood function plots were examined at each step and after the total optimization to validate that local optimal parameter estimates were found and to investigate covariance between parameters.

For each alternative model structure, the same approach to parameterization was followed. All adjustable parameters not found in the flow-limited model (e.g., the permeation surface area-cross product for the diffusion-limited model) were also initially estimated from the intravenous MBP blood data simultaneously with MBP metabolism parameters. Again, a full optimization of all parameters to all data was performed.

**MBP time course data.** MBP plasma concentration time courses were obtained from two studies (NIEHS, 1994; NIEHS, 1995). These studies included concentrations following an intravenous exposure of 11-week-old Wistar Furth rats to 8 mg/kg MBP, or an oral exposure of Wistar Furth rats to 34 mg/kg MBP, 43 mg/kg DBP, or 857.1 mg/kg DBP, with one rat at each time point (NIEHS, 1994). Concentrations were also measured following oral exposure of 11-week-old Sprague-Dawley rats to 50, 100, or 200 mg/kg DBP with three rats at each time point (NIEHS, 1995). Additional details of the experimental design and analytical techniques can be obtained from the respective study reports. The data used in the present study were taken directly from the study reports for each individual animal. DBP was not detected in any sample. The ratio of plasma MBP concentrations to whole blood MBP concentrations was predicted to be 1.1 at physiologic pH by the Poulin and Krishnan $K_{o,w}$ algorithm; hence, we thought it reasonable to use plasma concentration time courses to fit model parameters for describing whole blood concentrations.

**PBPK model discrimination.** The ability of each alternative model structure to fit the experimental data was compared directly to the flow-limited

### TABLE 1

<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (BW) (kg)</td>
<td>0.265</td>
<td>Extrapolated from age (11 weeks)</td>
</tr>
<tr>
<td>Cardiac output (L/h)</td>
<td>5.66</td>
<td>Calculated based on ILSI, 1994</td>
</tr>
<tr>
<td>Blood flow ($Q_f$) fraction of cardiac output</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver ($Q_l$)</td>
<td>0.183</td>
<td>ILSI, 1994</td>
</tr>
<tr>
<td>Fat ($Q_f$)</td>
<td>0.07</td>
<td>ILSI, 1994</td>
</tr>
<tr>
<td>Testes ($Q_t$)</td>
<td>0.013</td>
<td>Tabrizchi and Pang, 1993</td>
</tr>
<tr>
<td>Slowly perfused tissues ($Q_s$)</td>
<td>0.157</td>
<td>0.24 – $Q_l$ – $Q_f$</td>
</tr>
<tr>
<td>Rapidly perfused tissues ($Q_r$)</td>
<td>0.577</td>
<td>0.76 – $Q_l$ – $Q_f$</td>
</tr>
<tr>
<td>Compartment volumes ($V_i$) fraction of body weight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver ($V_l$)</td>
<td>0.0366</td>
<td>ILSI, 1994</td>
</tr>
<tr>
<td>Fat ($V_f$)</td>
<td>0.069</td>
<td>Bailey et al., 1980</td>
</tr>
<tr>
<td>Testes ($V_t$)</td>
<td>0.01</td>
<td>ILSI, 1994</td>
</tr>
<tr>
<td>Blood ($V_b$)</td>
<td>0.07</td>
<td>ILSI, 1994</td>
</tr>
<tr>
<td>Small intestine ($V_{si}$)</td>
<td>0.0139</td>
<td>ILSI, 1994</td>
</tr>
<tr>
<td>Slowly perfused tissues ($V_s$)</td>
<td>0.741</td>
<td>0.82 – $V_l$ – $V_b$</td>
</tr>
<tr>
<td>Rapidly perfused tissues ($V_r$)</td>
<td>0.0534</td>
<td>0.09 – $V_l$ – $V_b$</td>
</tr>
<tr>
<td>Compartment blood volume fraction of tissue volume</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver ($V_l$)</td>
<td>0.21</td>
<td>ILSI, 1994</td>
</tr>
<tr>
<td>Fat ($V_f$)</td>
<td>0.05</td>
<td>Anderson et al., 1993</td>
</tr>
<tr>
<td>Testes ($V_t$)</td>
<td>0.03</td>
<td>Chubb and Desjardins, 1982</td>
</tr>
<tr>
<td>Slowly perfused tissues ($V_s$)</td>
<td>0.04</td>
<td>ILSI, 1994 (muscle)</td>
</tr>
<tr>
<td>Rapidly perfused tissues ($V_r$)</td>
<td>0.021</td>
<td>Liver value used</td>
</tr>
</tbody>
</table>

**Table 2**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Vial equilibration</th>
<th>$K_{o,w}$ estimation ($\text{pH } 7.0$)</th>
<th>$K_{o,w}$ estimation (nonionized)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>1.22 ± 0.25</td>
<td>0.9</td>
<td>15.8</td>
</tr>
<tr>
<td>Fat</td>
<td>0.05 ± 0.5</td>
<td>0.9</td>
<td>313</td>
</tr>
<tr>
<td>Muscle</td>
<td>Negative</td>
<td>0.9</td>
<td>4.6</td>
</tr>
<tr>
<td>Testes</td>
<td>1.9 ± 0.21</td>
<td>1.0</td>
<td>4.9</td>
</tr>
</tbody>
</table>

* Mean ± approximate standard error (See Appendix).

**Adaptable parameters.** Metabolism and absorption rates, permeation coefficient-surface area-cross product, enterohepatic circulation rate, and time delay were estimated from pharmacokinetic data in adult male rats by maximum likelihood parameter estimation as implemented in the ACSLtox software. The Nelder-Mead algorithm was used for likelihood maximization, and the heteroscedasticity parameter ($\gamma$) was fixed at 2.0 for consistency between data sets. The heteroscedasticity parameter is a parameter in the power-of-the-mean error model used in ACSLtox. A heteroscedasticity parameter of 2.0 means that the standard deviation is proportional to the value of the mean (i.e., relative error). This value of 2.0 was appropriate since the variance in concentration measurements was usually proportional to the concentration value.

There were 4–6 adjustable parameters in each model. Initially, the adjustable parameters were optimized no more than three at a time to different data sets in the nested approach described below. For the flow-limited model, MBP blood concentrations following MBP intravenous exposure were used to estimate liver MBP metabolism parameters ($V_{l,m}^n$ and $K_{m}^n$). Blood concentrations following MBP oral exposure were then used to estimate the first-order absorption rate of MBP ($k_{a,w}^n$), holding metabolic parameters constant. Next, holding MBP metabolism and absorption parameters constant, MBP blood concentrations following oral exposure to 43, 50, 100, 200, or 857 mg/kg DBP were used to estimate the gut metabolism rate parameter ($k_{m}^n$). Finally, an overall optimization that included all the data and parameters was conducted using the nested approach estimates as initial values. The heteroscedasticity parameter $\gamma$ was allowed to vary from 2.0 for this final total optimization. Individual animal rather than mean concentrations were used to more accurately estimate $\gamma$. This resulted in an optimal set of parameter estimates with an associated log-likelihood function value. Log-likelihood function plots were examined at each step and after the total optimization to validate that local optimal parameter estimates were found and to investigate covariance between parameters.

For each alternative model structure, the same approach to parameterization was followed. All adjustable parameters not found in the flow-limited model (e.g., the permeation surface area-cross product for the diffusion-limited model) were also initially estimated from the intravenous MBP blood data simultaneously with MBP metabolism parameters. Again, a full optimization of all parameters to all data was performed.

**PBPK model discrimination.** The ability of each alternative model structure to fit the experimental data was compared directly to the flow-limited model.
PBPK model using the log-likelihood ratio test. The log-likelihood ratio test could be used to discriminate between models because each alternative model could be reduced to the flow-limited model by removing 1–2 additional adjustable parameters and reidentifying some model parameters. The test statistic L was calculated as

\[ L = -2 \cdot (LLF_{reduced} - LLF_{full}) \]

where \( LLF_{reduced} \) is the optimal value of the log likelihood of the flow-limited or reduced parameter model and \( LLF_{full} \) is the optimal value of the log-likelihood function of the alternative or full parameter model. The degrees of freedom (df) for each test was the number of additional adjustable parameters in each alternative model (enterohepatic circulation, \( df = 2 \); diffusion-limited, \( df = 1 \); pH trapping, \( df = 1 \); diffusion-limited, pH trapping, \( df = 1 \)). Although there are formally no additional adjustable parameters in the pH trapping model, the effective \( df \) for the log-likelihood ratio test equals 1. Even though there are additional parameters in the pH trapping model, the parameters are not adjusted but rather fixed independent of the data. However, the pH trapping model can be mathematically reduced to the flow-limited model by setting one additional parameter equal to zero. The nonionized tissue:blood partition coefficients are multiplied by a proportionality factor \( \theta \), which if set to zero reduces the pH trapping model to the flow-limited model. \( \theta \) is not an adjustable parameter but rather set to 1. However, adjustment of \( \theta \) would only result in an even higher log-likelihood ratio value than the current value at \( \theta = 1 \); hence, if the pH trapping model is statistically superior at \( \theta = 1 \), this is sufficient for statistical significance using the log-likelihood ratio test.

The combined pH trapping and diffusion-limited model was compared to the pH trapping model with \( df = 1 \). The test statistic \( L \) was compared to the critical value of the \( \chi^2 \) statistic at the \( \alpha = 0.05 \) significance level.

RESULTS

Partition Coefficients

There were no significant decreases in MBP propylene carbonate concentrations between propylene carbonate MBP time points. Thus equilibrium was assumed, and means and standard errors for pooled test and pooled reference vials were calculated. The mean calculated MBP saline:propylene carbonate partition coefficient was 1.43 ± 0.02 SEM. The experimentally determined partition coefficients were compared to those predicted by the \( K_{ow} \) algorithm at a tissue pH of 7.0 and a blood pH of 7.4 for further validation of the nonvolatile vial equilibration technique (Table 2). The mean muscle:PC partition coefficient was slightly negative in value and is not presented because this is biologically unrealistic. This result suggests that the technique employed was not sensitive enough to pick up the minimal solubility of DBP in muscle. The negative result is interpreted as a random variation that is not inconsistent with this minimal solubility. There is reasonably good agreement between the other experimental and \( K_{ow} \) algorithm-predicted values (Table 2) except for the measured fat:blood partition coefficient, which was an order of magnitude lower than the \( K_{ow} \) algorithm-predicted value. This discrepancy is likely due to the fact that the \( K_{ow} \) is not a reliable surrogate for biologic lipids for relatively hydrophilic organics like MBP. N-octanol appears to solubilize hydrophilic compounds to a greater degree than do biologic lipids (Poulin and Krishnan, 1996).

The measured partition coefficients were used in simulations with the flow-limited, enterohepatic circulation, and diffusion-limited models. The partition coefficients for unionized MBP obtained with the Poulin and Krishnan algorithm were used for the pH trapping model and for the combined pH trapping-diffusion limitation model. The algorithm-derived values were used for these latter models, as MBP is largely ionized under the laboratory conditions used for partition coefficient determinations. Because laboratory measurement of the MBP muscle:blood partition coefficient did not produce a meaningful value (Table 2), this partition coefficient was set equal to the MEHP muscle:blood partition coefficient (0.38), which was measured using the same vial equilibration technique (Keys et al., 1999). The actual MBP muscle:blood partition coefficient would be expected to be less than the MEHP muscle:blood partition coefficient, as MBP is more hydrophilic than MEHP. Multiple optimizations were run at lower values of the muscle:blood partition coefficient (as low as 0.2) to test if the results of the model discrimination process were sensitive to this parameter. Order and significant differences of alternative models were unaffected by the choice of the muscle:blood partition coefficient within these ranges. The rapidly perfused tissue:blood partition coefficient was set equal to the liver:blood partition coefficient.

Simulations of Rat Blood Concentrations after Intravenous MBP Exposure

The flow-limited model provided a poor fit to the blood concentrations following intravenous exposure to 8 mg/kg MBP. The enterohepatic circulation model was statistically a better fit than the flow-limited model (Table 3). The enterohepatic circulation model underpredicted early time points. However, it accurately simulated the secondary peak concentration of MBP. The diffusion-limited model was also a statistically better fit than the flow-limited model (Table 3) and successfully predicted the early MBP time points. The pH trapping model was also a statistically better fit than the flow-limited model; however, all MBP concentrations were underpredicted (Fig. 2). A diffusion-limited, pH trapping model was a statistically better fit than the pH trapping model (Table 3) and successfully predicted both early and late time points due to the addition of diffusion-limitation (Fig. 2).

Simulations of Rat Blood Concentrations after Oral MBP Exposure

The flow-limited model was again a poor fit to the blood concentrations following oral exposure to 34 mg/kg MBP. Neither the enterohepatic circulation nor the diffusion-limited models made statistically improved predictions of MBP blood concentrations over the flow-limited model (Table 3). The enterohepatic circulation model underpredicted early time points and slightly overpredicted later time points, whereas the diffusion-limited model clearly overpredicted most MBP concentration values. The pH trapping model also did not make
statistically improved predictions, underpredicting most time points (Fig. 3). However, the diffusion-limited, pH trapping model predicted all but the last time point well (Fig. 3) and had a statistically improved fit to the data over both the flow-limited and pH trapping models (Table 3).

Simulations of Rat Blood Concentrations after Oral DBP Exposure

The flow-limited model provided a poor fit to MBP blood concentrations following oral exposure to DBP. The enterohepatic circulation model made statistically better predictions of MBP blood concentrations over the flow-limited model (Table 3). However, the diffusion-limited, pH trapping model did not provide the correct shape for the concentration-time curve, with a peak that was smooth and low rather than sharp and high. The pH trapping model made statistically better predictions of MBP blood concentrations also (Table 3) compared to the flow-limited model. This tracked the decline of MBP over time well, but peak MBP concentrations were again underpredicted. The diffusion-limited, pH trapping model made statistically better predictions of MBP blood concentrations than the pH trapping model (Table 3) and predicted the peak MBP concentrations well at all five dose levels (Fig. 5). However, the rapid dip in MBP concentrations followed by a secondary peak was not explained by this model and may be indicative of enterohepatic circulation.

Model Discrimination

The log-likelihood ratio value obtained from simultaneously optimizing all parameters to all data was used. The

![FIG. 2. Comparison of pH trapping models predictions with rat blood concentrations following intravenous exposure to 8 mg/kg MBP. pH trapping model predictions with and without diffusion-limitation are shown. Each measured value is from a different rat.](image)

![FIG. 3. Comparison of pH trapping models predictions with rat blood concentrations of MBP following oral exposure to 34 mg/kg MBP. Model predictions from the pH trapping model with and without diffusion-limitation are shown. Each measured value is from a different rat.](image)
The diffusion-limited, pH trapping model was statistically better than either the flow-limited or pH trapping models by 85 log units ($p < 0.005$) and 32 log units ($p < 0.005$), respectively. The diffusion-limited, pH trapping model was log 19 units better than the next best model, which was the enterohepatic circulation model. In addition, the enterohepatic circulation model underestimated peak MBP concentrations, which is undesirable, as peak MBP concentrations may be the key pharmacokinetic factor driving reproductive toxicity as a result of acute oral DBP exposure. Thus, the diffusion-limited, pH trapping model was concluded to be the best and an adequate model for describing MBP blood levels following intravenous exposure to MBP or oral exposure to DBP or MBP.

Adjustable Parameter Estimates

The estimated values of the adjustable parameters in the diffusion-limited, pH trapping model are presented in Table 4. Two dimensional log-likelihood function surface plots were examined for all 10 combinations of pairs of parameters. A distinct optimum was found for each pair with the exception of $V_{\text{max},I}$ and $K_{M,I}$, where a ridge indicating covariance between parameters was found. Thus estimates for $V_{\text{max},I}$ and $K_{M,I}$ must be considered to be preliminary. Blood concentrations following various doses of MBP intravenously would result in unique estimates of these two parameters.

The value of the heteroscedasticity parameter $\gamma$ was estimated to be 1.8, close to the expected relative error ($\gamma = 2$). Separate estimation of $\gamma$ from the slope of a linear regression of mean versus variance of MBP concentrations also resulted in an estimation of 1.8 for $\gamma$. This lends confidence to the

**FIG. 4.** (A) Comparisons of enterohepatic circulation model predictions with rat MBP blood concentrations following oral exposure to 50, 200 and 857 mg/kg DBP (NIEHS, 1994; NIEHS, 1995). (B) Comparisons of enterohepatic circulation model predictions with rat MBP blood concentrations following oral exposure to 43 and 100 mg/kg DBP (NIEHS, 1994; NIEHS, 1995). Measured values after 43 and 857 mg/kg are each from a different rat, and measured values after 50, 100, or 200 mg/kg are mean concentrations $\pm$ SE ($n = 3$) for different rats at each time point.

**FIG. 5.** (A) Comparisons of diffusion-limited, pH trapping model predictions with rat MBP blood concentrations following oral exposure to 50, 200, and 857 mg/kg DBP (NIEHS, 1994; NIEHS, 1995). (B) Comparisons of diffusion-limited, pH trapping model predictions with rat MBP blood concentrations following oral exposure to 43 or 100 mg/kg DBP (NIEHS, 1994; NIEHS, 1995). Data are the same as in Figure 4.
maximum likelihood method employed in ACSLopt for the estimation of $\gamma$.

**DISCUSSION**

The major conclusion of this work is that a diffusion-limited, pH trapping model, which incorporates intracellular and extracellular pH differences and tracks concentrations of both unionized and ionized MBP, is the best PBPK model of those tested for prediction of MBP blood dosimetry. This conclusion is consistent with the earlier finding that a pH trapping PBPK model was the best model of those tested for describing DEHP and MEHP blood and testes dosimetry (Keys et al., 1999). However, inclusion of diffusion-limitation was necessary to adequately predict MBP blood concentrations but not necessary to predict DEHP and MEHP blood and tissue levels. Theoretically, MEHP transport into tissues should be also diffusion-limited if MBP transport is diffusion-limited, as MEHP is a larger molecule than MBP with molecular weights 278 and 222, respectively. However, the MBP plasma concentration data were obtained from iv and oral exposures that were an order of magnitude lower than exposures producing the DEHP and MEHP plasma and tissue data. Thus, it seems that these lower concentration pharmacokinetic data are more sensitive for discerning diffusion-limitation than the higher-dose data. The addition of diffusion-limitation to the current DEHP and MEHP PBPK model would only improve the DEHP and MEHP PBPK model predictions by adding one new adjustable parameter.

*In vitro* tissue:blood partition coefficients for MBP were smaller than those measured for MEHP in all tissues except the testes, whereas MBP was an order of magnitude more soluble in saline than MEHP. Tissue:blood partition coefficients were expected to be smaller for MBP than MEHP, as MBP has a shorter side/chain length and is thus less lipophilic than MEHP. Gut metabolism of DBP to MBP was modeled as a linear process, whereas saturable metabolism was necessary to model DEHP gut metabolism (Keys et al., 1999). This difference is probably due to the higher doses of DEHP than DBP in the oral pharmacokinetic study, which was used for estimation of gut metabolism parameters (2000 mg/kg DEHP vs. 43–857 mg/kg DBP). The fact that DBP metabolism could be modeled as a linear process does indicate, however, that the gut metabolism of phthalate diesters is not saturated until very high concentrations in the rat. Maximum likelihood estimates of a MEHP $V_{\text{max}}$ value in the liver was similar to those for MBP, 12.0 mg/h and 11.6 mg/h, respectively, whereas estimated MEHP $K_m$ value in the liver was 1.5 orders of magnitude lower than MBP, 0.5 mg/L and 18.2 mg/L, respectively. The fact that the MEHP $K_m$ was estimated to be lower than the MBP $K_m$ may indicate phthalate ester metabolizing P450s have a higher affinity for MEHP than MBP. However, as covariance of $V_{\text{max}}$ and $K_m$ in the liver prevented the unique estimation of their values, more *in vivo* or *in vitro* data are necessary before this can be confirmed. Also, the metabolism of MBP in the liver, which is currently represented as one saturable rate, is really a composite of at least two metabolic pathways and urinary excretion. The estimated rate of MBP absorption was slightly higher than for MEHP, 9.9 h$^{-1}$ versus 7 h$^{-1}$, which agrees with the results of a previous study in which investigators found that the amount of phthalate ester flux was 9.6 $\mu$mol/h for MBP and 7.7 $\mu$mol/h for MEHP in an everted gut-sac preparation (White et al., 1980).

NIEHS (1994) and NIEHS (1995) used Wistar-Furth and Sprague-Dawley rats, respectively. Our analyses of blood MBP after oral dosing with DBP examined data from both of these studies without attempting to develop rat strain-specific PBPK models. No data are currently available that would support such model development. Moreover, reasonable fits to all of the data were obtained with a single model, suggesting that intrastrain differences in the pharmacokinetic behavior of DBP and MBP are not large.

The results of this work show that a diffusion-limited, pH trapping PBPK model can accurately describe the *in vivo* pharmacokinetics of di(n-butyl) phthalate and its metabolite mono(n-butyl phthalate). Consideration of tissue pH gradients and ionized monoester along with diffusion-limitation is critical for description of MBP blood and thus target organ dosimetry. The enterohepatic circulation model underestimated MBP peak blood concentrations following oral exposure to DBP, but this does not mean that enterohepatic circulation is not occurring. In fact, the existence of secondary peaks in both the iv and oral concentration time courses and the measurement of MBP in the bile suggests that enterohepatic circulation most likely is occurring. The results of this work do, however, indicate that enterohepatic circulation is not critical for a reasonable overall description of MBP blood dosimetry. Enterohepatic circulation may affect only the variance in the concentration-time curves (nonmonotonic elimination phase) but not the AUC and peak concentration values. The AUC and peak concentrations are the most crucial to estimate, as they are the most likely candidates for a dose metric for phthalate monoester.

---

**TABLE 4**

**Diffusion-Limited pH Trapping Model Estimated Parameter Values**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Estimated value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{\text{max}}$ &amp; $K_m$ &amp; $k_a$ &amp; $K_m$ &amp; $k_g$ &amp; $P_A$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_{\text{max}}$</td>
<td>Maximum rate of MBP liver metabolism</td>
<td>4.3</td>
<td>mg/h</td>
</tr>
<tr>
<td>$K_m$</td>
<td>Michaelis-Menten constant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_a$</td>
<td>Absorption rate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_g$</td>
<td>First-order DBP gut metabolism rate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$P_A$</td>
<td>Permeation coefficient-surface area-cross product</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note.* All data used for estimation are from NIEHS (1994) and NIEHS (1995).
The adequate and superior fit of the diffusion-limited, pH trapping model for DBP and MBP combined with the adequate and superior fit of the pH trapping model for DEHP and MEHP are initial steps toward developing a generic phthalate ester PBPK model. The next steps toward developing a generic model are validation of the DBP and MBP PBPK model and application of the current model structure to other phthalate esters. Development of a generic phthalate ester PBPK model would allow for more biologically based human risk assessments of male reproductive toxicity for exposure to one or more phthalate esters.

Once parameterized for phthalate-specific parameters, target tissue doses in testes could be predicted to help interpret rat toxicological studies. Also, the model could be scaled to humans by incorporating human data on absorption and urinary excretion that will allow predictions of target tissue doses in humans. This would decrease the level of uncertainty in the current risk assessments for phthalate esters.

In addition, concern for the effects of phthalates following in utero exposure has been raised as recent work that shows this to be the most sensitive end point (Mylchreest et al., 1997). A generic phthalate ester PBPK model could be extended to predict fetal concentrations of diester and monoester and thereby aid in decreasing uncertainty in risk assessments for pregnant women exposed to one or more phthalate esters.

**APPENDIX**

**Model Equations**

The following is a description of the equations used in the PBPK model simulations. All amounts (A') are measured in mg, and all concentrations (C') are measured in mg/L for i = b (blood), si (small intestine), l (liver), sp (slowly perfused), f (fat), t (testes), and r (rapidly perfused), and x = D (DBP) and M (MBP).

**Flow-Limited Model**

The flow-limited model equations for DBP and MBP were identical to those used by Ramsey and Anderson (1984) with the following exceptions.

The rates of change of the amount of DBP and MBP in the small intestine are given by

\[
\frac{dA_{si}^D}{dt} = R_0^D - R_{met,si}^D
\]

\[
\frac{dA_{si}^M}{dt} = R_0^M + m_c \cdot R_{met,si}^D - k_a^M \cdot A_{si}^M
\]

\[
R_{met,si}^D = k_{si}^D \cdot A_{si}^D
\]

where \( R_0^x \) is rate of oral dosing of phthalate (mg/h) for \( x = D \) or \( M \), \( R_{met,si}^D \) is the rate of metabolism of DBP to MBP in the small intestine, and \( m_c \) is the ratio of the molecular weight of MBP to DBP (.799). The rate of MBP absorption (h\(^{-1}\)) is represented by \( k_a^M \) and \( k_{en}^M \) is the first-order rate of DBP metabolism to MBP in the small intestine (h\(^{-1}\)).

The rate of change of MBP in the testes is given by

\[
\frac{dA_t^M}{dt} = Q_t \cdot (C_b - C_{at}) + C_{at} \cdot \frac{C_t}{P_t}
\]

where \( Q_t \) is the blood flow rate to the testes (L/h), \( C_b \) is the concentration of MBP in the systemic blood, \( C_{at} \) is the concentration of MBP in the venous blood of the testes, and \( P_t \) is the MBP testes:blood partition coefficient.

The rate of change of MBP in the blood is given by

\[
\frac{dA_b^M}{dt} = \sum Q_i \cdot C_{v,i} - Q_c \cdot C_b + R_{iv}^M,
\]

where \( Q_i \) is the blood flow rate (L/h), and \( C_{v,i} \) is the concentration of MBP in the venous blood for \( i = f, t, l, sp, \) and \( r \); \( Q_c \) is total cardiac output (L/h); and \( R_{iv}^M \) is the rate of MBP intravenous dosing (mg/h).

**Enterohepatic Circulation Model**

The enterohepatic circulation model included two additional parameters: \( k_{en} \), the first-order rate of transfer of MBP from the liver to the small intestine via the bile (h\(^{-1}\)) and \( \tau \), a time delay (h). The rate of change of the concentration of MBP in the small intestine is given by

\[
\frac{dA_{si}^M}{dt} = R_0^M + m_c \cdot R_{met,si}^D - k_a^M \cdot A_{si}^M + k_{en} \cdot A_i(t - \tau),
\]

and the rate of change of the concentration of MBP in the liver is given by

\[
\frac{dA_l^M}{dt} = Q_t \cdot (C_b - C_{at}) - R_{met,l}^M - k_{en} \cdot A_l,
\]

where \( R_{met,l}^M \) is the rate of MBP metabolism in the liver.

**Diffusion-Limited Model**

The diffusion-limited model included one additional parameter not found in the flow-limited model: \( PA \), the permeation coefficient-surface area-cross product (h\(^{-1}\)). The rate of change of the amount of MBP in the blood and tissue fractions is expressed as
\[ \frac{dA_{i,E}}{dt} = Q_i \cdot (C_b - C_{i,E}) + PA \cdot \left( \frac{C_{i,l}}{P_i} - C_{i,E} \right) \]

and

\[ \frac{dA_{i,l}}{dt} = PA \cdot \left( C_{i,l} - \frac{C_{i,l}}{P_i} \right), \]

where \( A_{i,E} \) is the amount of MBP in the extracellular portion of tissue \( i \), \( A_{i,l} \) is the amount of MBP in the intracellular portion of tissue \( i \), and \( P_i \) is the MBP tissue:blood partition coefficient for \( i = 1, f, t, sp, \) and \( r \). In the liver compartment, the same Michaelis-Menten metabolism and first-order absorption terms are used as in the flow-limited model.

**pH Trapping Model**

The pH trapping model introduces three additional parameters: \( pH_T \) is the extracellular \( pH \); \( pH_I \), the intracellular \( pH \); and the MBP \( pK_a \). The concentrations of nonionized (\( C_{i,E} \)) and total MBP (\( C_{i,E} \)) in extracellular tissue \( i \) are derived from the Henderson-Hasselbalch equations and are expressed as

\[ C_{i,E} = \frac{A_i^M}{P_i \cdot V_{i,l} \cdot (1 + 10^{(pH_T - pK_a)}) + V_{i,E} \cdot (1 + 10^{(pH_I - pK_a)})}, \]

and

\[ C_{i,E} = C_{i,E} \cdot (1 + 10^{(pH_T - pK_a)}), \]

where \( V_{i,l} \) is the volume of the intracellular compartment, \( V_{i,E} \) is the volume of the extracellular compartment (i.e., tissue blood volume), and \( A_i^M \) is the nonionized MBP tissue:blood partition coefficient for tissue \( i \). \( A_i^M \), the total amount of MBP in tissue \( i \), is calculated by solving the ordinary differential equation

\[ \frac{dA_{i,E}^M}{dt} = Q_i \cdot (C_b - C_{i,E}). \]

The concentration of nonionized (\( C_{i,E} \)) is reached by instantaneous partitioning between unionized MBP in tissue and blood and is given by

\[ C_{i,E} = C_{i,E} \cdot P_{i,l}. \]

The concentration of total MBP in the intracellular tissues (\( C_{i,l} \)) is also derived from the Henderson-Hasselbalch equation and is expressed as

\[ C_{i,l} = C_{i,l} \cdot (1 + 10^{(pH_T - pK_a)}). \]

**Diffusion-Limited pH Trapping Model**

The diffusion-limited, pH trapping model combines the equations and parameters of the diffusion-limited and pH trapping models. The rates of change of the total amount of MBP in the extracellular and intracellular compartments of tissue \( i \) are given by

\[ \frac{dA_{i,E}}{dt} = Q_i \cdot \left( C_b - \frac{A_{i,E}}{V_{i,E}} \right) + PA \cdot \left( \frac{A_{i,E}}{V_{i,E}} - \frac{A_{i,l}}{V_{i,l} \cdot P_i} \right) \]

and

\[ \frac{dA_{i,l}}{dt} = PA \cdot \left( \frac{A_{i,E}}{V_{i,E}} - \frac{A_{i,l}}{V_{i,l} \cdot P_i} \right), \]

and the amount of nonionized MBP in the extracellular and intracellular compartments of tissue \( i \) is given by

\[ A_{i,E} = \frac{A_{i,E}}{1 + 10^{(pH_T - pK_a)}}, \]

and

\[ A_{i,l} = \frac{A_{i,l}}{1 + 10^{(pH_T - pK_a)}}. \]

**Approximate Partition Coefficient Standard Error Calculation**

The following equations were derived based on Taylor’s series expansions to calculate approximate standard error of mean tissue:proplylene partition coefficients (\( s_{i,PC} \))

\[ s_{i,PC} = \frac{(V_{i,E} + V_{i,l} + \lambda_i)}{V_{i,E} \cdot C_t} \cdot \sqrt{s_{i,E}^2 + \frac{C_t}{C_p} \cdot s_{i,E}^2}, \]

where \( s_{i,E}^2 \) is the sample variance of the mean reference vial concentration (dpm) and \( s_{i,E}^2 \) is the sample variance of the mean test vial concentration (dpm). The approximate standard error for blood:proplylene partition coefficients (\( s_{i,PC} \)) is calculated as

\[ s_{i,PC} = \frac{V_{i,E}}{V_{i,l} \cdot C_t} \cdot \sqrt{s_{i,E}^2 + \frac{C_t}{C_p} \cdot s_{i,E}^2}. \]

Finally the approximate standard error of the tissue:blood partition coefficient (\( s_{i,E} \)) is calculated as

\[ s_{i,E} = \frac{1}{\lambda_{i,PC}} \cdot \sqrt{s_{i,E}^2 + \frac{\lambda_{i,PC} \cdot s_{i,PC}^2}{\lambda_{i,PC}^2}}, \]

**ACKNOWLEDGMENTS**

We are grateful to Mr. Brad Collins and Dr. Mike Cunningham (NIEHS) for providing copies of the study reports. We thank Drs. Kannan Krishnan (University of Montreal) and Patrick Poulin (Hoffman-La Roche) for help with the partition coefficient algorithm, and Mr. John Murphy for technical support.
assistance. The software ACSLtox was generously provided by MGA software for use by DAK during her predoctoral studies. We thank Dr. Paul Schlosser for advice about optimization and critical review, Drs. Julia Kimbell for critical review, and Barbara Kuyper for editorial assistance with this manuscript. Dr. Deborah Keys was supported by a NSF graduate traineeship in scientific computation. We also acknowledge the support of CITO member companies.

REFERENCES


