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

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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-limitation, 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 with 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., 1987; 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 monoesters 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
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 test the applicability of the DEHP and MEHP PBPK model structure to the less lipophilic phthalate diester DBP and its monoester MBP. The parameterization of the DEHP/MEHP model for DBP/MBP included laboratory measurement of MBP tissue:blood partition coefficients. A combined diffusion-limited and pH trapping model that had not been examined in the context of previous work on DEHP/MEHP was also examined in the present study.

**METHODS**

**Chemicals.** [C14] Mono(n-butyl) phthalate (MBP) (CAS No. 131-70-4) with a radiochemical purity of 98.3% was obtained from Wizard Laboratories, Inc. (West Sacramento, CA). Propylene carbonate (PC) (CAS No. 108-32-7) was obtained from Aldrich Chemical Co. (Milwaukee, WI).

**Animals.** This study was conducted under federal guidelines for the care and use of laboratory animals (NRC, 1996) and was approved by the CIFT Institutional Animal Care and Use Committee. Ten-week-old male CD rats (Sprague-Dawley) were obtained from Charles River Breeding Laboratories (Raleigh, NC). Animals were housed individually in solid-bottom plastic cages with Alpha Dri bedding under standard conditions of temperature, humidity, and light. Rodent feed (NIH-07) and deionized water via water bottles were available ad libitum. Rats were euthanized with CO2, and whole blood was collected from the vena cava using a heparinized syringe. Liver, muscle, fat, and testes were then removed and stored at -80°C until needed for measurement of tissue:blood partition coefficients.

**Measured partition coefficients.** Tissue:propylene carbonate (PC) partition coefficients were determined for MBP using a vial equilibration method developed for nonvolatile chemicals (Murphy et al., 1995). Radiolabeled MBP was equilibrated between tissue homogenates, saline or whole blood and saline-saturated PC at 37°C. Aliquots of PC were taken at 2 time points to ensure equilibrium had been reached. All procedures of Murphy and colleagues were followed except that aliquots of 1.2 ml of 1:2 tissue weight:saline volume ratio rather than undiluted fat were used. Furthermore, each PC sample was counted once for 10 min and recounted the following day. Less than a 1% error in counts was achieved counting aliquots of approximately 10,000 dpm per sample. The quantity of MBP in each equilibration vial was 0.3 μg (~62,000 dpm). Tissue:PC and saline:PC partition coefficients were calculated using the methods of Murphy and colleagues. For blood, the amount of MBP in reference and test vials, A1 and A2, respectively, were expressed as

\[ A_k = C_k \cdot V_{PC} + C_k \cdot V_s \cdot \lambda_s \]

\[ A_k = C_k \cdot V_{PC} + C_k \cdot V_s \cdot \lambda_s \]

where \( C_k \) is the MBP concentration (dpm) in the reference vial PC, \( V_{PC} \) is the volume (ml) of the PC layer, \( V_s \) is the volume (ml) of saline, \( \lambda_s \) is the saline:PC partition coefficient, \( C_T \) is the MBP concentration (dpm) in the test vial PC layer, \( V_T \) is the volume (ml) of blood, and \( \lambda_{PC} \) is the blood:PC partition coefficient.

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 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 Poluin 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 (\( K_{ow} \)). A \( K_{ow} \) for nonionized MBP of 3.03 was estimated based on its chemical structure using ProLogD 2.0 software (Compudrug Chemistry Ltd., Burlingame, CA) and used as an input to the Poluin and Krishnan algorithm. A testes water fraction of tissue weight of 0.80, a total lipids fraction of tissue weight of 0.023 (Fiserova-Bergerova, 1983), a phospholipid fraction (total 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 Poluin 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 Poluin and Krishnan 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 considering the potential enterohepatic circulation (NIEHS, 1994; NIEHS, 1995).

In the pH trapping model, each compartment was divided into intracellular (blood) and extracellular compartments with a flow controlled by the permeation coefficient-surface area-cross product. The Henderson-Hasselbalch equation was used to calculate equilibrium concentrations of ionized and nonionized MBP in each extracellular and intracellular compartiment. 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 intracellular and extracellular 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 compartments. Each of these compartments was further divided into extracellular (blood) and intracellular compartments, 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.

**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.

**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.
intracellular compartment. MBP was predicted to reach equilibrium again where it was mostly ionized MBP. The permeation coefficient-surface area-cross product combined with the nonionized MBP tissue:blood partition coefficients (Table 2) controlled the rate at which nonionized MBP was predicted to leave the intracellular compartment.

Model simulation. The resulting systems of mass-balance differential equations were solved by numerical integration using the Gear Algorithm for stiff systems by ACSLTOX (Advanced Continuous Simulation Language) (Pharsight, Mountain View, CA) on a Pentium II 300/mHz desktop PC. More details concerning the model equations can be found in the Appendix.

Model physiologic parameters. The rat physiologic parameters, including tissue blood volumes, were taken from Model Parameters for PBPK Models (1994) with the exception of the testes blood flow rate (Tabrizchi and Pang, 1993), fat blood volume (Andersen et al., 1993), and testes blood volume (Chubb and Desjardins, 1982). Body weight was estimated to be 265 kg for an 11-week-old rat using an age-related growth equation for F-344 rats (ILSI, 1994). Parameter values are summarized in Table 1. Exposure-related parameters were set up according to conditions in each individual experiment.

Adjustable parameters. Metabolism and absorption rates, permeation coefficient-surface area-cross product, enterohpatic 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 was used. The ability of each alternative model structure to fit the experimental data was compared directly to the flow-limited model, MBP blood concentrations following MBP intravenous exposure were used to estimate liver MBP metabolism parameters (\(V_m^\text{in} \) and \(K_m^\text{in} \)). Blood concentrations following MBP oral exposure were then used to estimate the first-order absorption rate of MBP (\(k_a^\text{in} \)), holding metabolic parameters constant. Next, holding MBP metabolism and absorption parameters constant, MBP blood concentrations following oral exposure to 43, 50, 100, 200, or 857.1 mg/kg DBP were used to estimate the gut metabolism rate parameter (\(k_m^\text{gut} \)). 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 \(K_{ow}\) 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 model, MBP blood concentrations following MBP intravenous exposure were used to estimate liver MBP metabolism parameters (\(V_m^\text{in} \) and \(K_m^\text{in} \)). Blood concentrations following MBP oral exposure were then used to estimate the first-order absorption rate of MBP (\(k_a^\text{in} \)), holding metabolic parameters constant. Next, holding MBP metabolism and absorption parameters constant, MBP blood concentrations following oral exposure to 43, 50, 100, 200, or 857.1 mg/kg DBP were used to estimate the gut metabolism rate parameter (\(k_m^\text{gut} \)). 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.

<table>
<thead>
<tr>
<th>Table 1: Physiological Parameter Values</th>
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<tbody>
<tr>
<td>Description</td>
</tr>
<tr>
<td>Body weight (BW) (kg)</td>
</tr>
<tr>
<td>Cardiac output (L/h)</td>
</tr>
<tr>
<td>Blood flow (Qf) fraction of cardiac output</td>
</tr>
<tr>
<td>Liver (Qi)</td>
</tr>
<tr>
<td>Fat (Qi)</td>
</tr>
<tr>
<td>Slowly perfused tissues (Qi)</td>
</tr>
<tr>
<td>Rapidly perfused tissues (Qi)</td>
</tr>
<tr>
<td>Compartments volumes (Vi) fraction of body weight</td>
</tr>
<tr>
<td>Liver (Vi)</td>
</tr>
<tr>
<td>Fat (Vi)</td>
</tr>
<tr>
<td>Testes (Vi)</td>
</tr>
<tr>
<td>Blood (Vi)</td>
</tr>
<tr>
<td>Small intestine (Vi)</td>
</tr>
<tr>
<td>Slowly perfused tissues (Vi)</td>
</tr>
<tr>
<td>Rapidly perfused tissues (Vi)</td>
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<tr>
<td>Compartments blood volume fraction of tissue volume</td>
</tr>
<tr>
<td>Liver</td>
</tr>
<tr>
<td>Fat</td>
</tr>
<tr>
<td>Testes</td>
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<tr>
<td>Slowly perfused tissues</td>
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<tr>
<td>Rapidly perfused tissues</td>
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**Table 2: Mono(n-butyl) Phthalate Tissue:Blood Partition Coefficients Determined by Vial Equilibration vs. Estimated from Log n-Octanol:water Partition Coefficient (Kow)**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Vial equilibration</th>
<th>(K_{ow}) estimation</th>
<th>(K_{ow}) estimation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(tissue pH = 7.0)</td>
<td>(nominonized)</td>
<td>(nominonized)</td>
</tr>
<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).

\(^{\dagger}\) Poulin and Krishnan (1995). Estimated using the \(K_{ow}\) partition coefficient for MBP at physiologic pH where it is largely ionized and thus has minimal solubility in octanol (used for the flow limited, enterohpatic circulation and diffusion limited models).

\(^{\ddagger}\) Poulin and Krishnan (1995). Estimated using the \(K_{ow}\) partition coefficient for unionized MBP (used for the pH trapping and combined diffusion limited-pH trapping models).
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 $K_{w/o}$ 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 $K_{w/o}$ algorithm-predicted values (Table 2) except for the measured fat:blood partition coefficient, which was an order of magnitude lower than the $K_{w/o}$ algorithm-predicted value. This discrepancy is likely due to the fact that $K_{w/o}$ 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-limited 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 enterohpatic 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 enterohpatic circulation nor the diffusion-limited models made statistically improved predictions of MBP blood concentrations over the flow-limited model (Table 3). The enterohpatic 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 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
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 \(V_{\text{max}}\) and \(K_{\text{M}}\), where a ridge indicating covariance between parameters was found. Thus estimates for \(V_{\text{max}}\) and \(K_{\text{M}}\) 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 ± 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 were 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.
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 extended to predict fetal concentrations of diester and monoester and thereby aid in decreasing uncertainty in 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., 1998; NTP, 1991; Wine 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 i) are measured in mg, and all concentrations (C i) 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

\[
\frac{dA_{i}^{D}}{dt} = R_{0}^{D} - R_{met,si}^{D} \\
\frac{dA_{i}^{M}}{dt} = R_{0}^{M} + mc \cdot R_{met,si}^{D} - k_{a}^{M} \cdot A_{si}^{M} \text{ and} \\
R_{met,si}^{D} = k_{si}^{D} \cdot A_{si}^{D},
\]

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 mc is the ratio of the molecular weight of MBP to DBP (0.799). The rate of MBP absorption (h⁻¹) is represented by \( k_{a}^{M} \) and \( k_{a}^{D} \) 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

\[
\frac{dA_{t}^{M}}{dt} = Q_{t} \cdot (C_{b} - C_{si}) \text{ and} \\
C_{si} = \frac{C_{t}}{P_{t}};
\]

where \( Q_{t} \) is the blood flow rate to the testes (L/h), \( C_{b} \) is the concentration of MBP in the systematic blood, \( C_{si} \) is the concentration of MBP in the venous blood of the testes, and \( P_{t} \) is the 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: \( k_{m} \), 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

\[
\frac{dA_{i}^{M}}{dt} = R_{0}^{M} + mc \cdot R_{met,si}^{D} - k_{a}^{M} \cdot A_{si}^{M} + k_{en} \cdot A_{i}(t - \tau),
\]

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

\[
\frac{dA_{l}^{M}}{dt} = Q_{l} \cdot (C_{b} - C_{v,l}) - R_{met,l}^{M} - k_{en} \cdot A_{l}
\]

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
\[
\frac{dA_{i,E}}{dt} = Q_i \cdot (C_b - C_{i,E}) + PA \cdot \left( \frac{C_{i,l}}{P_i} - C_{i,E} \right) \quad \text{and}
\]
\[
\frac{dA_{i,l}}{dt} = PA \cdot \left( C_{i,l} - \frac{C_{i,l}}{P_i} \right),
\]
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 \( i = 1, f, t, sp, \text{ or } r \). 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 \( (C_{i,I}) \) in extracellular tissue \( i \) \( (i = 1, f, t, sp, \text{ or } r) \) are derived from the Henderson-Hasselbalch equations and are expressed as

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

\[
C_{i,I} = C_{i,E} \cdot (1 + 10^{pH_I - pK_a}),
\]
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 \( P_{i} \) is the nonionized MBP tissue:blood partition coefficient for tissue \( i \). \( A_{i,E}^M \), 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

\[
C_{i,E} = \frac{A_{i,E}}{V_{i,E} \cdot C_{i}} \cdot (1 - 10^{pH_I - pK_a}).
\]

**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 \( i \) \( (i = 1, f, t, sp, \text{ or } r) \) are given by

\[
\frac{dA_{i,E}}{dt} = Q_i \cdot \left( C_b - \frac{A_{i,E}}{V_{i,E}} \right) - PA \cdot \left( \frac{A_{i,E}}{V_{i,E}} - \frac{A_{i,l}}{V_{i,l} \cdot P_i} \right) \quad \text{and}
\]
\[
\frac{dA_{i,l}}{dt} = PA \cdot \left( \frac{A_{i,E}}{V_{i,E}} - \frac{A_{i,l}}{V_{i,l} \cdot P_i} \right),
\]
and the amount of nonionized MBP in the extracellular and intracellular compartments of tissue \( i \) is given by

\[
A_{i,E} = \frac{A_{i,E}}{1 + 10^{(pH_I - pK_a)}} \quad \text{and}
\]
\[
A_{i,l} = \frac{A_{i,E}}{1 + 10^{(pH_I - pK_a)}}.
\]

**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:propylene partition coefficients \( s_{i,pc} \).

\[
s_{i,pc} = \frac{(V_{pc} + V_i \cdot \lambda_i)}{V_i \cdot C_i} \cdot \sqrt{\frac{\sigma_i^2}{C_i^2} + \frac{C_{i}^2}{C_i} \cdot s_i^2},
\]
where \( s_{i,pc}^2 \) is the sample variance of the mean reference vial concentration (dpm) and \( s_i^2 \) is the sample variance of the mean test vial concentration (dpm). The approximate standard error for blood:propylene partition coefficients \( s_{i,pc} \) is calculated as

\[
s_{i,pc} = \frac{V_{pc} \cdot C_i}{V_i} \cdot \sqrt{s_i^2 + \frac{C_{i}^2}{C_i} \cdot s_i^2},
\]
Finally the approximate standard error of the tissue:blood partition coefficient \( s_{i,pc} \) is calculated as

\[
s_{i,pc} = \frac{1}{\lambda_{pc}} \cdot \sqrt{s_i^2 + \frac{\lambda_i^2 \cdot s_{i,pc}^2}{\lambda_{pc}^2}}.
\]

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