PHYSIOLOGICALLY BASED PHARMACOKINETICS MODEL OF PRIMIDONE AND ITS METABOLITES PHENOBARBITAL AND PHENYLETHYLMALONAMIDE IN HUMANS, RATS, AND MICE

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(Received November 10, 1997; accepted February 20, 1998)

This paper is available online at http://www.dmd.org

ABSTRACT:
Physiologically based pharmacokinetic modeling of the parent chemical primidone and its two metabolites phenobarbital and phenylethylmalonamide (PEMA) was applied to investigate the differences of primidone metabolism among humans, rats, and mice. The model simulated previously published pharmacokinetic data of the parent chemical and its metabolites in plasma and brain tissues from separate studies of the three species. Metabolism of primidone and its metabolites varied widely among a sample of three human subjects from two separate studies. Estimated primidone metabolism, as expressed by the maximal velocity $V_{\text{max}}$, ranged from 0 to 0.24 mg min$^{-1}$ kg$^{-1}$ for the production of phenobarbital and from 0.003 to 0.02 mg min$^{-1}$ kg$^{-1}$ for the production of PEMA among three human subjects. Further model simulations indicated that rats were more efficient at producing and clearing phenobarbital and PEMA than mice. However, the overall metabolism profile of primidone and its metabolites in mice indicated that mice were at higher risk of toxicity owing to higher residence of phenobarbital in their tissues and owing to the carcinogenic potential of phenobarbital as illustrated in long-term bioassays. This result was in agreement with a recently finished National Toxicology Program (NTP) carcinogenicity study of primidone in rats and mice.

Primidone is a desoxybarbitururate anticonvulsant used for the treatment of partial seizures in humans. It is one of the major drugs used for the treatment of psychomotor epilepsy. Fig. 1 is an illustration of the metabolic pathways of primidone. Oxidation of the second carbon on primidone converts it to phenobarbital in vivo in both animals and humans. Alternatively, ring cleavage of the drug at the second carbon position converts primidone to phenylethylmalonamide (PEMA)$^{1}$ (Baumel et al., 1973). Phenobarbital is a well known anticonvulsant, whereas PEMA has been shown to have anticonvulsant effects in rats. Because of its extensive use by humans, the pharmacokinetics of primidone was extensively investigated in humans, rats, and mice. Plasma concentrations of primidone and its metabolites were measured in two human subjects who individually received a single gavage dose of 500 mg of primidone (Baumel et al., 1972). In another study, application of more sensitive methods was used to investigate the pharmacokinetics of primidone and its metabolites in a single human subject given a single primidone gavage dose of 600 mg (Sato et al., 1986). Pharmacokinetics of primidone was also examined in Swiss-Webster mice where plasma levels of primidone, phenobarbital, and PEMA were determined after the animals received a single gavage dose of 50 mg/kg of the parent drug (Leal et al., 1979). In a different study, plasma levels of the three chemicals were investigated simultaneously in male albino rats after administering single gavage doses of 125, 250, and 500 mg/kg of primidone (Baumel et al., 1973). The toxicity of primidone is compounded by evidence of the carcinogenic potential of phenobarbital in rodents (McClain, 1995) and the mitogenic activity of PEMA in Salmonella (Zeiger et al., 1988). These findings, in addition to its high volume use by humans, stimulated the National Toxicology Program (NTP) to conduct a 2-year chronic carcinogenicity study to fully characterize the chronic toxicity of primidone in Fischer 334 rats and B6C3F1 mice (NTP, in press). The NTP study indicated that primidone was a more potent carcinogen to mice than it was to rats.

The complexities of primidone metabolism complicates the contribution of each metabolite to its potential toxic effect because of exposure to primidone and could impair extrapolations of risk across different species.

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1 Abbreviations used are: PEMA, phenylethylmalonamide; PBPK, physiologically based pharmacokinetic; GI, gastrointestinal.

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Fig. 1. Metabolic pathways of primidone (redrawn from Baumel et al., 1973).
species. Mechanistic modeling in the form of physiologically based pharmacokinetic (PBPK) models offers an approach to analyze the variability of metabolic profiles among species whenever adequate data exist. PBPK models characterize the deposition and metabolism of chemicals in tissues based on established or hypothesized mechanisms. They consist of tissue compartments that are linked by sets of mass balance equations that realistically describe the disposition and metabolism of modeled xenobiotics. Although PBPK models are deterministic in nature, the data are not, so the model parameters are generally estimated using routine statistical methods (e.g., least squares). Hence, whenever distinct kinetic data are available, PBPK models can be used to estimate kinetic constants for individuals of the same species.

The purpose of this effort is to apply PBPK models to determine the metabolic profile of primidone among three human subjects, rats, and mice partially by simulating published data with optimized pharmacokinetic constants and partially by using existing model structures on the distribution and metabolism of its major metabolites. The derived metabolic profiles of primidone and its metabolites will be used to assess the role of pharmacokinetics in the difference of species response to the parent chemical between rats and mice as was illustrated by the NTP study.

**Materials and Methods**

**PBPK Model Compartments and Construct.** A central PBPK model of primidone was connected, by way of the liver compartment, to a PBPK model for each metabolite (fig. 2). This was done so that plasma levels of primidone and its metabolites can be simulated. Each PBPK model includes the following tissues: lung, fat, muscle, skin, heart, kidney, gastrointestinal (GI) tract (represented by small intestine), brain, and arterial and venous blood compartments. The following detailed description of the mathematical equations used in the model reveals all assumptions embedded in the overall model.

The usual approach for primidone application is by oral administration, especially in experimental settings where an initial gavage dose is introduced into the lumen and is then absorbed through the GI tissue to be distributed via the portal vein to the liver. To accommodate this mechanism, a compartment for the GI tract was included that consisted of a lumen and tissue subcompartments for the primidone model only. Absorption of the chemical from the GI lumen into the GI tissue is modeled by a first-order rate equation as follows:

\[
\frac{dAMT_{\text{lmn}}}{dt} = -K_{\text{abs}} AMT_{\text{lmn}}
\]

where \( AMT_{\text{lmn}} \) is the amount (mg) of primidone in the GI lumen, and \( K_{\text{abs}} \) is the first-order absorption rate constant (min\(^{-1}\)) for transfer from GI lumen into the GI tissue. Using simple, equilibrium partitioning between arterial blood and GI tissue, the rate of change in the GI tissue subcompartment is defined as follows:

\[
\frac{dAMT_{\text{gi}}}{dt} = Q_{\text{gi}} \left( C_{\text{art}} - C_{\text{gi}} \right) P_{\text{gi}} + K_{\text{abs}} AMT_{\text{lmn}}
\]

where \( AMT_{\text{gi}} \) is the amount of primidone (mg) in the GI tissue, \( Q_{\text{gi}} \) is the portal vein blood flow (ml/min), \( P_{\text{gi}} \) is the GI tissue/blood partition coefficient, \( C_{\text{art}} \) is the concentration (mg/ml) of primidone in arterial blood, and \( C_{\text{gi}} \) is the
concentration of primidone (mg/ml) in GI tissue. For this tissue and all other
tissues, concentration is defined as the ratio of amount (AMT) to tissue
volume. Following absorption through the GI tissue, primidone is distributed
to the liver where it is either metabolized (assuming Michaelis-Menten kinet-
ics) or further distributed to other tissues as follows:

\[
\frac{dAMT_{lvr}}{dt} = Q_{lvr} C_{lvr} + Q_{lvr} C_{art} - (Q_{lvr} + Q_{lvr} C_{lvr} V_{max}_{lvr} C_{lvr} + C_{lvr} V_{max}_{lvr} C_{lvr} + C_{lvr})
\]

where \( AMT_{lvr} \) is the amount (mg) of primidone in the liver, \( Q_{lvr} \) is the hepatic
arterial blood flow (ml/min), \( C_{lvr} \) is the concentration (mg/ml) of primidone in
tissue, \( P_{lvr} \) is the liver tissue/blood partition coefficient of primidone,
\( V_{max}_{lvr} \) and \( K_{lvr} \) are the Michaelis-Menten metabolism constants for
primidone oxidation to phenobarbital, and \( V_{max}_{lvr} \) and \( K_{lvr} \) are the metabolism rate
constants for primidone cleavage to PEMA. Both \( V_{max}_{lvr} \) and \( V_{max}_{lvr} \) are scaled to the 0.7 power of body weight.

Distribution of primidone and its metabolites to brain tissue is governed by
a diffusion limited process through the blood-brain barrier. For this reason,
the brain compartment was divided into separate blood and tissue compart-
ments similar to a previous phenobarbital model construct (Igari et al., 1982).
Assuming simple diffusion kinetics, the following equation describes the mass
balance of the brain blood capillary subcompartment:

\[
\frac{dAMT_{brnc}}{dt} = V_{brnc} DR \left( C_{brnc} FR - \frac{C_{brnc}}{(1 + B_{plasma})} \right) + Q_{brnc} (C_{art} - C_{brnc})
\]

where \( AMT_{brnc} \) is the amount (mg) of primidone in brain capillary, \( V_{brnc} \) is the volume (ml) of brain tissue, \( DR \) is the diffusion rate constant (min \(^{-1}\))
of primidone between tissue and blood, \( C_{brnc} \) is the concentration (mg/ml) of
chemical in brain tissue, \( FR \) is the ratio of free to tissue concentrations of
the chemical, \( C_{brnc} \) is the concentration (mg/ml) of primidone in brain capillary,
\( B_{plasma} \) is the binding fraction of primidone to red blood cells, and \( Q_{brnc} \) is the
brain blood flow (ml/min).

Using a similar set of assumptions, the mass balance equation for the brain
tissue compartment is as follows:

\[
\frac{dAMT_{t} \ k_{1}}{dt} = V_{t} FR \left( C_{brnc} - \frac{C_{t}}{P_{t}} \right)
\]

where the parameters are defined analogously to those for the brain capillary.

The remaining model compartments are all assumed to follow first order,
flow-limited tissue distribution leading to equations of the following form:

\[
\frac{dAMT_{t} \ k_{2}}{dt} = Q_{t} (C_{t} - C_{t} P_{t})
\]

where \( AMT_{t} \) is the amount (mg) of primidone in tissue, \( Q_{t} \) is the blood flow
(ml/min) through tissue, \( C_{t} \) and \( P_{t} \) are the tissue concentration (mg/ml) and
tissue/blood partition coefficient of the chemical, respectively.

Additional compartments for arterial and venous blood were also included in the model as follows (respectively):

\[
\frac{dAMT_{ Venous} \ k_{3}}{dt} = Q_{total} \left( C_{total} \ - C_{tart} \right)
\]

where \( AMT_{ Venous} \) is the amount (mg) in arterial blood, \( Q_{total} \) is the total cardiac output (ml/min), and \( C_{total} \) and \( P_{total} \) are the lung tissue concentration (mg/ml) and
tissue/blood partition coefficient, respectively.

\[
\frac{dAMT_{ Venous} \ k_{4}}{dt} = Q_{venous} C_{venous} + \sum_{all \ tissue \ except \ GI \ and \ brain} Q_{t} \ C_{t} - Q_{total} C_{venous}
\]

where \( AMT_{Venous} \) and \( C_{venous} \) are the amount (mg) and concentration of (mg/ml) of
the chemical in venous blood, respectively. The remaining parameters are as
defined above.

The PBPK submodels for phenobarbital and PEMA (see fig. 2) are con-
structed in a similar fashion to the one for primidone except for the GI and liver
compartments. In the GI tissue compartment, the lumen subcompartment is
eliminated, as these two metabolites are not administered orally for this
analysis, and the liver compartment is modeled as follows:

\[
\frac{dAMT_{liver} \ k_{5}}{dt} = Q_{liver} C_{liver} + Q_{liver} C_{art} + Q_{liver} C_{liver} + C_{liver} V_{max}_{liver} C_{liver} + C_{liver} V_{max}_{liver} C_{liver} + C_{liver})
\]

where \( AMT_{liver} \) is the amount (mg) of phenobarbital or PEMA in liver as
they are produced from primidone, \( C_{liver} \) or \( C_{art} \) is the arterial concentration
(mg/ml) of phenobarbital or PEMA, \( C_{liver} \) or \( C_{liver} \) is the concentration (mg/ml) of
phenobarbital or PEMA in liver, \( C_{liver} \) or \( C_{liver} \) is the GI tissue concentration
(mg/ml) of phenobarbital or PEMA, \( C_{liver} \) or \( C_{liver} \) is the liver tissue/blood
partition coefficient for phenobarbital or PEMA, \( C_{liver} \) or \( C_{liver} \) is the GI tissue/ blood
partition coefficient of phenobarbital or PEMA, and where \( dProd_{liver} \)
represents the rate of production of either metabolite from primidone and
\( dmetab_{liver} \) is the rate of elimination of either phenobarbital or PEMA.

For phenobarbital, the rate of production is given as follows:

\[
\frac{dProd_{liver} \ k_{6}}{dt} = V_{max}_{liver} C_{liver} + C_{liver} V_{max}_{liver} C_{liver} + C_{liver} V_{max}_{liver} C_{liver} + C_{liver})
\]

whereas for PEMA it is as follows:

\[
\frac{dProd_{liver} \ k_{7}}{dt} = V_{max}_{liver} C_{liver} + C_{liver} V_{max}_{liver} C_{liver} + C_{liver} V_{max}_{liver} C_{liver} + C_{liver})
\]

The \( dmetab_{liver} \) rate of metabolism for phenobarbital is given as follows:

\[
\frac{dmetab_{liver} \ k_{8}}{dt} = V_{liver} C_{liver} + C_{liver} V_{liver} C_{liver} + C_{liver} V_{liver} C_{liver} + C_{liver})
\]

and for PEMA it is as follows:

\[
\frac{dmetab_{liver} \ k_{9}}{dt} = V_{liver} C_{liver} + C_{liver} V_{liver} C_{liver} + C_{liver} V_{liver} C_{liver} + C_{liver})
\]

where \( K_{ind} \) is the first order rate constant (min \(^{-1}\)) of phenobarbital metabolism
and \( V_{liver} \) (mg/min) and \( K_{liver} \) (mg/ml) are the Michaelis-Menten constants for
PEMA metabolism. \( V_{liver} \) is also scaled to the 0.7 power of body weight.
Documented slow metabolic rate of phenobarbital was better assumed by a
first order kinetic rate, which is a modification of the saturable Michaelis-
Menten equation when affinity is low (Igari et al., 1982).

Additionally, the model accounts for enzyme induction resulting from the
accumulation of phenobarbital in the liver. This induction only affects the
metabolism rates of primidone and PEMA and no other portion of the model.
The induction of phenobarbital metabolism by phenobarbital itself has been
shown to be insignificant (Maniara et al., 1988). Therefore, when applicable,
the induced rates were multiplied by a factor (1 + \( K_{ind} \)) where \( K_{ind} \) is
calculated as follows:

\[
K_{ind} = \frac{V_{ind} AMT_{ph}}{K_{min} + AMT_{ph}}
\]

Finally, the value of \( K_{ind} \) was lagged for 2 hr in all cases to allow for the
induction process to take course.

**Model Parameters.** Body weights were averaged as 70, 0.133, and 0.035
kg for humans, rats, and mice, respectively. The remaining physiological
parameters were obtained from a report prepared by the international Life
Sciences Institute as shown in table 1 (Risk Science Institute, 1994).

The concentrations of all chemicals in the brain tissues of rats and mice were
sensitive to the values for \( FR \) and \( DR \). These parameters were optimized
to the available brain tissues data, as shown under Results. The value of $B_{\text{plasma}}$ was set at 0.438 in all model simulations of brain data (Igari et al., 1982).

Volume of blood ($V_{\text{blood}}$) was calculated based on plasma volume ($V_{\text{plasma}}$) as follows:

$$V_{\text{plasma}} = 44 \left( \frac{\text{Body weight (kg)}}{100} \right)^{0.7}$$

$$V_{\text{blood}} = \frac{V_{\text{plasma}}}{(1 - \text{hematocrit})}$$

where hematocrit was set at 0.45 for all species (Igari et al., 1982).

The tissue/blood partition coefficients for the primidone and PEMA sub-models were calculated from their respective n-octanol/water partition coefficients $K_{\text{OW}}$ for all species according to a previously published procedure (Poulin and Krishnan, 1995a). Their procedure depends on profiles for water and lipid contents (neutral and phospholipids) of all tissues in each species. Hence, tissue composition data were collected from available literature for humans, rats, and mice. For human tissues, water and lipid contents were collected from literature for blood, liver, lung, muscle, kidney, brain, and adipose tissues (Poulin and Krishnan, 1995b). Water content of human GI

### TABLE 1

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Human</th>
<th>Rat</th>
<th>Mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Blood Flow</td>
<td>% Volume</td>
<td>% Blood Flow</td>
</tr>
<tr>
<td>Brain</td>
<td>11.4</td>
<td>3.3</td>
<td>5.7</td>
</tr>
<tr>
<td>Brain capillary</td>
<td>18</td>
<td>1.7</td>
<td>3.3</td>
</tr>
<tr>
<td>GI tissue</td>
<td>4</td>
<td>0.47</td>
<td>6.6</td>
</tr>
<tr>
<td>Lung</td>
<td>100</td>
<td>0.76</td>
<td>0.7</td>
</tr>
<tr>
<td>Liver</td>
<td>22.7</td>
<td>2.75</td>
<td>16.1</td>
</tr>
<tr>
<td>Muscle</td>
<td>33.4</td>
<td>40</td>
<td>52.1</td>
</tr>
<tr>
<td>Kidney</td>
<td>17.5</td>
<td>0.44</td>
<td>11.1</td>
</tr>
<tr>
<td>Skin</td>
<td>5.8</td>
<td>3.71</td>
<td>5.8</td>
</tr>
<tr>
<td>Fat</td>
<td>5.2</td>
<td>23.42</td>
<td>7</td>
</tr>
<tr>
<td>Venous blood</td>
<td>—</td>
<td>71</td>
<td>—</td>
</tr>
<tr>
<td>Arterial blood</td>
<td>—</td>
<td>71</td>
<td>—</td>
</tr>
</tbody>
</table>

* Percent volume of brain tissue.
* Source of data.
* As represented by small intestinal tissue.
* Assumed similar to mice.

### TABLE 2

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Water Content (fraction of tissue weight)</th>
<th>Total Lipid Content (fraction of tissue weight)</th>
<th>Phospholipids (fraction of total lipids)</th>
<th>Neutral Lipids (fraction of total lipids)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>0.96 (Poulin and Krishnan, 1995a)</td>
<td>0.0023 (Poulin and Krishnan, 1995a)</td>
<td>0.361 (Poulin and Krishnan, 1995a)</td>
<td>0.631</td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>0.63 (Poulin and Krishnan, 1995a)</td>
<td>0.0051 (Poulin and Krishnan, 1995a)</td>
<td>0.77 (Poulin and Krishnan, 1995a)</td>
<td>0.23</td>
</tr>
<tr>
<td>Adipose</td>
<td>0.12 (Poulin and Krishnan, 1995a)</td>
<td>0.855 (Poulin and Krishnan, 1995a)</td>
<td>0.003 (Poulin and Krishnan, 1995a)</td>
<td>0.11</td>
</tr>
<tr>
<td>Liver</td>
<td>0.7 (Poulin and Krishnan, 1995a)</td>
<td>0.06 (Poulin and Krishnan, 1995a)</td>
<td>0.42 (Poulin and Krishnan, 1995a)</td>
<td>0.58</td>
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<tr>
<td>Muscle</td>
<td>0.743 (Poulin and Krishnan, 1995a)</td>
<td>0.0111 (Poulin and Krishnan, 1995a)</td>
<td>0.541 (Poulin and Krishnan, 1995a)</td>
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</tr>
<tr>
<td>Brain</td>
<td>0.75 (Wang et al., 1995)</td>
<td>0.24 (Gospe and Calaban, 1988)</td>
<td>0.65 (Cabezas et al., 1991)</td>
<td>0.35</td>
</tr>
<tr>
<td>GI</td>
<td>0.7 (Wang et al., 1995)</td>
<td>0.1711 (Dudeja and Mahwood, 1982)</td>
<td>0.411 (Dudeja and Mahwood, 1982)</td>
<td>0.51</td>
</tr>
<tr>
<td>Skin</td>
<td>0.7 (Cameron et al., 1983)</td>
<td>0.2 (Gray and Yardley, 1975)</td>
<td>0.35 (Gray and Yardley, 1975)</td>
<td>0.65</td>
</tr>
<tr>
<td>Heart</td>
<td>0.75 (Wang et al., 1995)</td>
<td>0.0111 (Grollman and Costello, 1972)</td>
<td>0.16 (Kalen et al., 1989)</td>
<td>0.84</td>
</tr>
<tr>
<td>Lung</td>
<td>0.75 (Wang et al., 1995)</td>
<td>0.06 (Kornburst and Hatch, 1984)</td>
<td>0.23 (Kornburst and Hatch, 1984)</td>
<td>0.77</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.65 (Wang et al., 1995)</td>
<td>0.04 (Lemieux et al., 1984)</td>
<td>0.65 (Lemieux et al., 1984)</td>
<td>0.35</td>
</tr>
</tbody>
</table>

* Calculated as 1-phospholipid fraction.
* Source of data.
* As represented by small intestinal tissue.
* Assumed similar to mice.

### TABLE 3

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Water Content (fraction of tissue weight)</th>
<th>Total Lipid Content (fraction of tissue weight)</th>
<th>Phospholipids (fraction of total lipids)</th>
<th>Neutral Lipids (fraction of total lipids)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>0.96 (Poulin and Krishnan, 1995a)</td>
<td>0.0058 (Stockier et al., 1987)</td>
<td>0.55 (Stockier et al., 1987)</td>
<td>0.45</td>
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<tr>
<td>Erythrocytes</td>
<td>0.63 (Cameron et al., 1983)</td>
<td>0.0053 (Stockier et al., 1987)</td>
<td>0.711 (Stockier et al., 1987)</td>
<td>0.23</td>
</tr>
<tr>
<td>Adipose</td>
<td>0.12 (Cunnane et al., 1986)</td>
<td>0.855 (Poulin and Krishnan, 1995a)</td>
<td>0.77 (Poulin and Krishnan, 1995a)</td>
<td>0.23</td>
</tr>
<tr>
<td>Liver</td>
<td>0.711 (Durbin et al., 1992)</td>
<td>0.16 (Cunnane et al., 1986)</td>
<td>0.32 (Cunnane et al., 1986)</td>
<td>0.111</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.72 (Durbin et al., 1992)</td>
<td>0.28 (Schophoerst et al., 1985)</td>
<td>0.62 (Schophoerst et al., 1985)</td>
<td>0.38</td>
</tr>
<tr>
<td>Brain</td>
<td>0.72 (Durbin et al., 1992)</td>
<td>0.17 (Schophoerst et al., 1985)</td>
<td>0.32 (Schophoerst et al., 1985)</td>
<td>0.68</td>
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<tr>
<td>GI</td>
<td>0.7 (Chatterjee et al., 1990)</td>
<td>0.12 (Schophoerst et al., 1985)</td>
<td>0.43 (Schophoerst et al., 1985)</td>
<td>0.57</td>
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<tr>
<td>Skin</td>
<td>0.73 (Kircuta et al., 1975)</td>
<td>0.12 (Schophoerst et al., 1985)</td>
<td>0.63 (Schophoerst et al., 1985)</td>
<td>0.37</td>
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<tr>
<td>Heart</td>
<td>0.75 (Durbun et al., 1992)</td>
<td>0.16 (Schophoerst et al., 1985)</td>
<td>0.56 (Schophoerst et al., 1985)</td>
<td>0.44</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.75 (Durbun et al., 1992)</td>
<td>0.17 (Schophoerst et al., 1985)</td>
<td>0.35 (Schophoerst et al., 1985)</td>
<td>0.65</td>
</tr>
</tbody>
</table>

* Calculated as 1-phospholipid fraction.
* Assumed similar to rat’s values.
* Source of data.
* As represented by small intestinal tissue.
tissue was assumed to be 0.7 of tissue weight, whereas total lipid and phospholipids contents were calculated to be 0.012 of total tissue weight and 0.39 of total lipid contents, respectively (Nakazawa et al., 1977). Water content of human skin tissue was assumed to be 0.7, whereas total and phospholipid contents were obtained as 0.1 of total tissue weight and 0.53 of total lipids contents (Gray and Yardley, 1975). Human heart tissue contents and profile were assumed to be similar to muscle. Tables 2 and 3 are listings of the water and lipid contents in rats and mice tissues, respectively. Table 4 lists the partition coefficient used in the model for all three chemicals in human and rodent tissues.

The partition coefficients for phenobarbital in rat tissues were available in the literature (Igari et al., 1982). However, for verification purposes, phenobarbital partition coefficients were also calculated based on its $K_{ow}$ value and compared with corresponding values for rats (table 4). The parameters agreed in all cases except for the fat tissue; the calculated partition coefficient was 10 times higher than the experimentally observed one. For this reason, all calcu-

### TABLE 4

Partition coefficients for primidone, PEMA, and phenobarbital in human, rat, and mouse tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Primidone ($\log K_{ow} = 0.111$)</th>
<th>PEMA ($\log K_{ow} = 0.15$)</th>
<th>Phenobarbital ($\log K_{ow} = 1.47$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>Lung 0.12</td>
<td>1.4</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>Heart 1.26</td>
<td>1.7</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>Liver 1.33</td>
<td>2.3</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>GI 0.11</td>
<td>2.1</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>Kidney 1.31</td>
<td>1.05</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>Muscle 1.26</td>
<td>1.03</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Skin 1.54</td>
<td>2.5</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>Fat 0.32</td>
<td>0.27</td>
<td>0.3</td>
</tr>
</tbody>
</table>

*Values were obtained from literature (Igari et al., 1982).

GI as represented by small intestine.

### TABLE 5

Optimized absorption and metabolism parameters in three human subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Human (subject A)</th>
<th>Human (subject B)</th>
<th>Human (subject C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_{abs}$ (min$^{-1}$)</td>
<td>0.004</td>
<td>0.08</td>
<td>0.003</td>
</tr>
<tr>
<td>$V_{maxph}$ (mg/min/kg)</td>
<td>0.003</td>
<td>0</td>
<td>0.24</td>
</tr>
<tr>
<td>$K_{mph}$ (mg/ml)</td>
<td>0.1</td>
<td>—</td>
<td>0.1</td>
</tr>
<tr>
<td>$V_{maxpm}$ (mg/min/kg)</td>
<td>0.0026</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>$K_{mpm}$ (mg/ml)</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>$V_{ind}$ (unitless)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>$K_{mind}$ (mg)</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>$K_{met}$ (ml/min$^{-1}$)</td>
<td>3.14</td>
<td>—</td>
<td>(NA)*</td>
</tr>
<tr>
<td>$V_{pema}$ (mg/min/kg)</td>
<td>0.11</td>
<td>0.51</td>
<td>0.22</td>
</tr>
<tr>
<td>$K_{pema}$ (mg/ml)</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>

*Could not be determined.
FIG. 4. Model simulations of primidone (solid lines) and PEMA (dashed lines) for human subject B (a) and human subject C (b). Phenobarbital was not detected in either case. Data were obtained from Baumel et al., 1972.

FIG. 5. Model simulations of plasma concentrations of primidone (a), plasma concentrations phenobarbital (b), plasma concentrations PEMA for Wistar rats (c), and brain tissue concentrations of primidone (d) of three different single primidone gavage doses. Solid, dashed, and dotted lines are model simulations of the 500, 250, and 125 mg/kg primidone gavage doses, respectively. Triangles, squares, and circles depict data corresponding to initial gavage doses of 500, 250, and 125 mg/kg of primidone, respectively. Data were obtained from Baumel et al., 1973.
lated fat tissue/blood partition coefficients were divided by 10 for all species. In this manner, order of lipophilicity of the chemicals (PEMA, primidone, phenobarbital) is also maintained. The discrepancy between experimental partition coefficients and those predicted in the adipose tissue may be attributed to the physical and chemical differences between octanol and lipids, which constitute most of the adipose tissue. The deviation is less obvious in other tissues where lipids is not a major component.

Simulation Software. The model was constructed using SCoP Simulation Control Program version 3.51 (Simulation Resources, Inc.) on a Silicon Graphics workstation. The SCoP program uses an optimization algorithm called PRAXIS, for “Principal axis method.” The optimization method is included in the simulation package as a SCoPfit program.

Results

Estimation of Metabolic Parameters. The purpose of this work was to estimate the metabolic constants for primidone and its two metabolites by applying PBPK modeling. Derived metabolic constants are related to the metabolism of primidone to phenobarbital and PEMA, metabolism of phenobarbital and PEMA themselves, and phenobarbital enzyme induction constants ($V_{\text{ind}}$ and $K_{\text{mind}}$) for each species. The interdependencies of these constants is a reflection of the relationships between the parent chemical and its metabolites. Therefore, estimation of these metabolic constants can only be achieved for data where the levels of the three chemicals are measured simultaneously. Once data were available, the metabolic constants were estimated simultaneously by least square optimizing methods of the model’s simulations to data.

Metabolic Constants of Primidone and Its Metabolites in Humans. Primidone and its metabolite plasma levels were determined in two studies. In the first study, a human subject (subject A) received a
single oral dose of 600 mg (Sato et al., 1986). Two other human subjects (subject B and subject C) were given a single primidone oral dose of 500 mg in the second study (Baumel et al., 1972). Analysis of subject A’s plasma showed the presence of the three chemicals in contrast to subjects B and C, who only produced levels of primidone and PEMA in their plasma. The model-derived metabolic constant for all three human subjects is given in table 5. Fig. 3 is an illustration of the model simulation with optimized metabolic constants for subject A’s data. Fig. 4 depicts the model simulations for subjects B and C. Comparing all human parameter values indicated that all subjects differed in their abilities to absorb primidone. Although absorption rates changed among subjects, extended comparisons of their metabolic profiles are still adequate because these profiles are controlled by the descending parts of the pertinent plasma concentration curves. Hence, further analysis of the parameters in table 5 implies that subject B was not able to produce phenobarbital in contrast to subjects A and C. Although subject C did not show plasma levels of phenobarbital, the model best fit to the plasma levels of both primidone and PEMA in this case had to yield a value for the metabolism of primidone to phenobarbital. This best fit resulted in a least square value of 0.327 compared with 0.513 when the production of phenobarbital was set to zero. The fact that phenobarbital was not detected in the plasma of subjects B and C may be attributed to the deficiency of subject B to produce it (V_{maxph} = 0) and the efficiency of subject C to clear it (high K_{met}). Furthermore, as shown in table 5, the apparent maximum velocities of primidone metabolism to PEMA were 0.0026, 0.02, and 0.01 mg/min kg^{-1} for subjects A, B, and C, respectively. Once more, these values indicate a wide range of variability among these three individuals.

**Metabolic Constants of Primidone and Its Metabolites in Rats and Mice.** Primidone and its metabolite plasma concentrations were determined in groups of albino rats at single gavage doses of 125, 250, and 500 mg/kg (Baumel et al., 1973). Fig. 5 depicts the model simulations in comparison with data when optimization of the metabolic constant is exercised. Additional optimization of FR and DR resulted in the model simulations to the brain tissue data as shown in fig. 5d. The overall optimized constants in rats are given in table 6.

Simultaneous measurements of plasma levels of the three chemicals were also determined in Swiss-Webster mice (Leal et al., 1979). The animals in these experiments received a single gavage dose of 50 mg/kg. Fig. 6 represents the model simulations of the plasma levels of the three chemicals in comparison with the published data for mice. Additional simulations of the brain tissue of primidone levels from the same study are also provided in fig. 6d. Optimized parameters derived from these simulations are given in table 7.

**Parameter Sensitivity Analysis.** Sensitivity analysis reflected the regions along the simulated curves where the parameter variability is of significant effect. Hence, absorption first order constant (K_{abs}) and the GI partition coefficient are most sensitive at the beginning of each simulation. As simulations proceeded, metabolic constants increased and absorption-related ones decreased in sensitivity.

**Discussion**

Metabolism of primidone varied widely among three human subjects, rats, and mice as predicted by PBPK modeling. In all cases presented in this study, rats were the most capable species of producing phenobarbital from primidone. This is illustrated by computing their apparent metabolic rate (V_{max}/K_{met}) so that the mathematical interdependence of V_{max} and K_{met} is avoided. Rats had an apparent metabolic rate of 2.5 compared with 0.94 ml/min kg^{-1} for mice and close to the highest one computed for humans (subject C at 2.4 ml/min kg^{-1}). Moreover, the model simulations suggested that mice had the lower rate of phenobarbital clearance among all the investigated cases. This lower rate of phenobarbital clearance results in a steady state level of phenobarbital in plasma at a higher value in mice than rats when primidone is administered chronically. Fig. 7 shows the simulations of the steady state levels of primidone and its metabolites in rats and mice when a daily gavage dose of 50 mg/kg of primidone is administered for a week. The figure shows about a 2-fold increase of the plasma levels of phenobarbital for mice than the predicted levels in rats. The continuous presence of this level of phenobarbital in mice in a chronic carcinogenicity study of primidone may partly explain the higher potency of the drug to mice than rats.

![FIG. 7. Model simulations of primidone (dotted line) and phenobarbital and PEMA (solid lines) for 1 week.](image-url)

(a) A daily gavage dose of 50 mg/kg of primidone in mice; (b) a daily gavage dose of 50 mg/kg of primidone in rats.
This conclusion is in agreement with a recent NTP study on the carcinogenicity of primidone, which found clear evidence that the chemical was a hepatic carcinogen in B6C3F1 mice (at dietary levels of 30, 65, or 150 mg/kg) but not in Fischer 344 rats (at dietary levels of 25, 50, or 100 mg/kg) (NTP, In Press).

The metabolic profile of primidone varies widely among humans, which may indicate the presence of sensitive human populations that may produce greater amounts of primidone metabolites. To illustrate this point, the levels of the chemicals in human plasma are depicted in fig. 8 in the case if subjects A, B, and C received a daily primidone gavage dose of 500 mg, respectively. The simulated levels of primidone and phenobarbital in subject A follows a sustained increase in blood. This finding results from the slower rates of primidone and phenobarbital metabolic clearances from the blood as indicated by their model-derived values (see table 5). In the other subjects, primidone levels sustain a constant level, and phenobarbital was not detected. Therefore, extrapolating the NTP carcinogenicity study of primidone, fig. 8 simulations imply that subject A may be at a higher risk of phenobarbital-induced cancer than the other two subjects.

In this example, PBPK modeling provided a tool to derive the metabolic constants of primidone and its metabolites when all chemicals were measured in plasma. The model parameterization was partly computed (partition coefficients) and partly optimized to statistically best fit published data. As in any modeling effort, the issue of identifiability is of concern. Is there enough data to allow the accurate estimation of parameters? In general, different parameters are sensitive to specific portions of the data curves. For example, parameters related to absorption (e.g. $K_{abs}$) has to fit the rising portion of primidone plasma levels. Metabolism parameters of primidone to phenobarbital (e.g. $V_{m,ph}$, $K_{mph}$) has to fit the descending portions of primidone plasma levels in addition to the rising portion of the phenobarbital ones. Similarly, the metabolism parameters for PEMA (e.g. $V_{m,pema}$, $K_{mpm}$) has to fit, along with the phenobarbital ones, the declining portions of primidone levels and their rising ones for PEMA. Once the metabolic constants for primidone to phenobarbital and PEMA are fixed, the metabolic constants for the later chemicals (e.g. $K_{met}$, $V_{pema}$, and $K_{pema}$) have to fit their plasma-declining portions of each chemical individually. The necessary condition that the model had to fit the plasma levels of all three chemicals simultaneously alleviated some of the concerns regarding this question.
Additional simulations at different levels of the parent chemical in rats and humans, and in brain tissues of rats and mice, added more confidence to the model-derived parameter estimations. To further examine the model-derived metabolic estimates, a search of available literature values yielded a range of 3 to 3.8 ml/hr-1kg-1 for the metabolic rates of phenobarbital in humans (Yukawa et al., 1992). This range is close to the model-derived rate in humans (3.14 ml/min -1 for subject A), which corresponds to a value of 2.69 ml/hr-1kg-1 assuming subject A weighs 70 kg.

References

Cunnane SC, Manku MS and Horrobin DF (1986) n-3 essential fatty acids decrease weight gain and metabolic rates of phenobarbital in humans (Yukawa et al., 1992).
Durbin PW, Jeung N, Kullgren B and Clemons GK (1992) Gross composition and plasma and
Grollman S and Costello L (1972) Effect of age and exercise on lipid content of various tissues