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Evaluation of Oral and Intravenous Route Pharmacokinetics, Plasma Protein Binding, and Uterine Tissue Dose Metrics of Bisphenol A: A Physiologically Based Pharmacokinetic Approach

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Bisphenol A (BPA) is a weakly estrogenic monomer used in the production of polycarbonate plastic and epoxy resins, both of which are used in food contact and other applications. A physiologically based pharmacokinetic (PBPK) model of BPA pharmacokinetics in rats and humans was developed to provide a physiological context in which the processes controlling BPA pharmacokinetics (*e.g.*, plasma protein binding, enterohepatic recirculation of the glucuronide [BPAG]) could be incorporated. A uterine tissue compartment was included to allow the correlation of simulated estrogen receptor (ER) binding of BPA with increases in uterine wet weight (UWW) in rats. Intravenous- and oral-route blood kinetics of BPA in rats and oral-route plasma and urinary elimination kinetics in humans were well described by the model. Simulations of rat oral-route BPAG pharmacokinetics were less exact, most likely the result of oversimplification of the GI tract compartment. Comparison of metabolic clearance rates derived from fitting rat i.v. and oral-route data implied that intestinal glucuronidation of BPA is significant. In rats, but not humans, terminal elimination rates were strongly influenced by enterohepatic recirculation. In the absence of BPA binding to plasma proteins, simulations showed high ER occupancy at doses without uterine effects. Restricting free BPA to the measured unbound amount demonstrated the importance of including plasma binding in BPA kinetic models: the modeled relationship between ER occupancy and UWW increases was consistent with expectations for a receptor-mediated response with low ER occupancy at doses with no response and increasing occupancy with larger increases in UWW.

Key Words: **bisphenol A; PBPK model; endocrine; glucuronide; human; metabolism; pharmacokinetics; physiologically based pharmacokinetics; plasma protein binding; risk assessment.**

INTRODUCTION

Bisphenol A (4,4′-isopropylidene-2-diphenol, BPA) is a monomer used in the production of polycarbonate plastic and epoxy resins, both of which are used in food contact and other applications. The use of these polymers in these applications raises the possibility of oral exposure of humans to trace amounts of the monomer, depending on the level of BPA migration that occurs from the finished and cured polymers (Goodson *et al.*, 2002).

After ingestion of low doses in either rats or humans, the phase II metabolism of BPA to form the glucuronic acid conjugate BPA-monoglucuronide (BPAG) appears to be nearly complete as a result of ''first pass'' metabolism by uridine diphosphate-glucuronosyl transferase (UGT) in the intestine and liver (Inoue *et al.*, 2003; Pottenger *et al.*, 2000; Upmeier *et al.*, 2000; Völkel *et al.*, 2002).

BPAG in rodents is eliminated principally in the bile (Inoue *et al.*, 2001) and subsequently in the feces as BPA (Pottenger *et al.*, 2000), whereas in humans BPAG is eliminated principally in the urine (Völkel *et al.*, 2002). In rats, BPA eliminated via the bile may undergo enterohepatic recirculation, prolonging systemic exposure. Bisphenol A and BPAG pharmacokinetics have been characterized in several strains and sexes of rats, and in male and female humans, but they have been subject only to characterization by classical compartmental and non-compartmental analyses (Pottenger *et al.*, 2000; Sun *et al.*, 2002; Upmeier *et al.*, 2000; Völkel *et al.*, 2002; Yoo *et al.*, 2000).

The predominant concern in the safety assessment of this compound arose from studies that reported weak estrogenic effects of this compound *in vivo* in rodents (European-Commission, 2003; Pottenger *et al.*, 2000). The estrogenicity of BPA is attributed to its weak *in vitro* agonist activity, on the order of 1/10,000 of that of estrogen, and differences in the binding to ERα and ERβ (Matthews *et al.*, 2001). The role of the estrogen receptor in the weak *in vivo* effects implies, but

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does not establish, thresholds for tissue effects and specificity of effects seen in tissues, based on the composition of receptors within various tissues, at least in adults (Andersen and Barton, 1999). BPAG, the principal BPA metabolite, is not estrogenic (Matthews *et al.*, 2001; Snyder *et al.*, 2000; Twomey, 1998). Uterine weight gain, an estrogenic effect attributable to hypertrophy, hyperplasia, and/or the imbibing of water, or some combination of these, has been observed in female rats administered high doses (800 mg/kg) of BPA (Twomey, 1998; Yamasaki *et al.*, 2000).

Bisphenol A binds to plasma proteins in rodents, monkeys, and humans. The bound form represents ~90–95% and the free form ~5–10% of the total (Csanady *et al.*, 2002; Kurebayashi *et al.*, 2002; Mayersohn, 2003). Humans and monkeys appear to have modestly lower free fractions (5%) (Csanady *et al*., 2002) than rats (~5–10%) (Mayersohn, 2003). The impact of this protein binding on uptake into potential target tissues, especially those with low metabolic capacity where metabolism is not expected to affect uptake (*e.g.*, uterus, brain), should be considered an important part of the pharmacokinetic (PK) characterizations of BPA, because binding may restrict access to the tissue and the target receptor population within the tissue (Mendel, 1992).

The pharmacokinetics of BPA are complicated by interspecies differences in the biliary elimination of BPAG, the involvement of the gastrointestinal (GI) tract in glucuronidation, and enterohepatic recirculation. Although these processes may be expected to affect BPA pharmacokinetics, the extent to which the pharmacokinetics of BPAG influences those of BPA has not been evaluated. In addition to describing the bulk kinetics of BPA and BPAG, dose–response models of weak estrogen receptor (ER) agonists should incorporate a description of the relationship between blood concentrations (free or total) and tissue ER binding (Plowchalk and Teeguarden, 2002). Such analyses are not easily approached with standard compartmental and non-compartmental models, but they may be more effectively addressed with physiologically based pharmacokinetic (PBPK) models. Physiologically based pharmacokinetic models describe the relationship between the ''external'' dose administered and the internal or target tissue concentrations and thus improve predictions of the dose–response relationship. Several PBPK models have been used successfully in this capacity (Andersen *et al.*, 1987; Clewell *et al.*, 1997) because of their flexibility in incorporating information about the physicochemical characteristics of a chemical, as well as the physiological and biochemical processes (*e.g.*, absorption, distribution, metabolism, elimination, protein and receptor binding) that control target tissue concentrations (Andersen *et al.*, 1987; Plowchalk and Teeguarden, 2002). Such models can also leverage the available information about other key events (such as protein and receptor binding) that are related to either pharmacologic effects or adverse effects.

The principal objective of this work was to develop an oral-route pharmacokinetic model for BPA in rats and humans that (1) incorporated restrictions on the concentrations of unbound (free) BPA in the plasma that result from plasma protein binding and (2) predicts the degree of ER binding that may occur in the uterus. The model incorporates information on BPA pharmacokinetics after intravenous (i.v.) dosing and explores the reasons for route-specific differences in BPA kinetics. We also sought to assess the ability of a simple BPAG sub-model to describe BPAG pharmacokinetics and evaluate the extent to which BPAG pharmacokinetics may influence BPA pharmacokinetics.

METHODS

In vivo *pharmacokinetic data.* Two published manuscripts contain the pharmacokinetic data that were considered for the rat BPA/BPAG model, one describing oral-route pharmacokinetics in male and female Fischer rats (Pottenger *et al.* 2000) and one describing both i.v. and oral-route pharmacokinetics in female DA/Han rats (Upmeier *et al.*, 2000). Two additional i.v. data sets in male rats (Sun *et al.*, 2002; Yoo *et al.*, 2000) showed considerably different pharmacokinetic (PK) characteristics than the other published studies and were determined to be less reliable for use in developing the rat model (see *Discussion*). A single study describes the pharmacokinetics of BPA/BPAG in human plasma after low-dose oral exposure (Völkel *et al.*, 2002), and these data were used to parameterize the human PBPK model. The methods and results in the published studies used in the modeling are described briefly here.

Pottenger and co-workers reported the time course of BPA in whole blood and the total radioactivity in plasma in adult male and female Fischer 344 (F344) rats after oral gavage dosing of 10 or 100 mg/kg of radiolabeled ^{14}C-BPA. Male rats did not have detectable levels of BPA in blood after the lower dose, and peak concentrations in the females were only 2–4 times the limit of detection (~0.04 μM) (Pottenger *et al.*, 2000). The data on the concentrations of BPA in the blood after the 100 mg/kg dose were the most complete and were therefore used for development of the model. Gender differences in the pharmacokinetics of BPA after oral administration were observed in the study by Pottenger and co-workers. The percentage of dose excreted in the urine and feces as a function of time was not published, but was obtained from the original study report prepared for good laboratory practice (GLP) purposes. Bisphenol A concentrations were measured in samples of whole blood, and radioactivity derived from the radiolabeled test material was measured in the blood plasma, necessitating the following additional calculations to arrive at blood BPAG concentrations: (1) the concentration of BPA in the blood was divided by 0.83 (rat blood:plasma partition coefficient (Mayersohn, 2003) to calculate the BPA concentration in the plasma and converted to nmoles per gram of plasma (assuming a density equal to water); (2) total plasma radioactivity reported in μg-BPA-equivalents/g plasma was converted to nmol BPA-equivalents/g plasma; (3) the calculated nmol/g BPA in plasma (step 1) was subtracted from the nmol BPA-equivalents/g plasma (step 2) to estimate the nmol/g plasma of metabolites; (4) metabolites were assumed to be entirely BPAG because the metabolite characterization of the plasma at selected times demonstrated that nearly all of the radioactivity in the plasma after oral dosing was BPAG, with only minor contributions from other metabolites. The glucuronide represented >90% of total metabolites for all doses, sexes, and times, except the 100 mg/kg males 15 min after exposure, where the glucuronide was ~70% of the total; (5) BPAG was assumed not to distribute to red blood cells, so the blood concentrations of BPAG were estimated as the plasma concentration × (1.0-hematocrit). The rat hematocrit was assumed to be 0.45 (Waynford and Flecknell 1992).

Upmeier reported the plasma pharmacokinetics of BPA after oral (10 or 100 mg/kg) and i.v. (10 mg/kg) administration of BPA in female DA/Han rats (Upmeier *et al.*, 2000). The authors refer to the unconjugated form of BPA measured in plasma as ''free'' BPA. This naming convention should not

be confused with the one used here, and elsewhere, in which ''free BPA'' refers to the fraction of unconjugated BPA not bound to plasma proteins or tissue receptors, and the glucuronidated form is referred to directly (*i.e.*, BPAG). The oral data from the Upmeier *et al.* study were not useful for confirming/validating parameterization of the model made using the data from Pottenger *et al.* (2000) because of extremely high variability in the measured plasma BPA concentrations (standard deviations approximately equal to mean values). The authors note the variability and present individual animal plasma BPA concentrations 10 min after oral dosing that range from ~5 to ~90 ng/ml, and that rapidly fall or rapidly rise afterward, presenting no consistent pattern. The ''composite design'' used for this study, where samples were taken from each rat at several times, but not at all time points, may have contributed to the variability.

The pharmacokinetics of BPA and BPAG in humans after a low oral dose of BPA was recently described by Völkel and co-workers (2002). Three groups consisting of either three adult females, three adult males, or four adult males were administered 5 mg of d_{16}-BPA in a hard gelatin capsule, and the concentrations of BPA and BPAG were determined by gas chromatography/mass spectometry (GC/MS) and liquid chromatography/mass spectrometry (LC-MS)/MS in plasma and urine up to 32 h after administration. The doses correspond to 54–90 μg/kg body weight. To avoid confounding measured plasma BPA with observable background contamination of BPA, d_{16}-BPA was used to characterize the pharmacokinetics of BPA. BPAG was the only compound that could be detected in the blood of the study volunteers. Bisphenol A was below the limit of detection in both urine (6 nM) and plasma (9 nM). There were no observable differences in the blood concentrations of BPAG in males and females, suggesting that there were no significant differences between males and females in BPA metabolism, although the sample size is small. Völkel reports a clearance rate of 0.13 l/min, which approximates renal clearance rates of creatinine in humans.

Plasma protein binding. Blood was collected from adult (76–83 d) male and female F344 rats (Harlan Sprague Dawley) via the hepatic vein and mixed with heparin (150 U/10 ml blood) (Mayersohn, 2003) under ether anesthesia. Plasma was obtained by centrifugation of blood pooled from 2–8 animals at 3000 × *g* for 10 min. The pH was adjusted to 7.4 with the addition of H_3PO_4. Bisphenol A was added to plasma at final concentrations of 1.33, 11.26, 26.16, 50.99, 75.82, 100.65, 150.30, and 199.96 μg/ml, each concentration containing a constant amount of radioactive BPA. After 15 min incubation at 37°C, two aliquots were removed and radioactivity was quantified (Beckman, liquid scintillation system) before (plasma) and after (filtrate) ultrafiltration centrifugation (Amicon MW cutoff 30,000, 2000 × *g*, 15 min). Nonspecific binding in the test system was characterized and found not to interfere with the accuracy of the binding assay. The amount of free BPA was calculated as 100× (filtrate counts/plasma counts) (see Table 1 in the Supplementary Material online).

Standard Scatchard analysis was used to estimate the BPA equilibrium binding association constant (K_a, and K_d [as $1/K_a$]) and maximum binding (B_{max}) for binding to rat plasma proteins. The ratio bound BPA/free BPA was plotted as a function of bound BPA (mM) (Fig. 1). Equation (1) describes the relationship of the plotted data:

$$\frac{BPA_{Bound}}{BPA_{Free}} = K_a{*}B_{max} - Ka{*}BPA_{Bound}, \quad (1)$$

where K_a has units of μM^{-1}, and B_{max}, BPA_{Bound} and BPA_{Free} have units of μM. The slope of the line is $-K_a$, and the x-intercept is B_{max}. A linear regression of these data was conducted and the K_a (slope) and B_{max} (intercept) were determined separately for males and females, the results of which are presented in Figure 1. The B_{max} and K_d for adult female F344 rats were 3200 μM and 190 μM, respectively. The B_{max} and Kd for adult male F344 rats were 3000 μM and 200 μM, respectively. Average values of 3100 μM (B_{max}) and 195 μM (K_d) were used for adult F344 rats. These values result in a modeled free fraction of BPA in plasma of ~0.06 (6%) over the range of concentrations tested.

Uterine wet weight. Uterotrophic response to natural estrogens or xenoestrogens appears to arise principally from the occupancy of the uterine estrogen receptors. Although there is some evidence for androgen receptor–mediated uterine response or other non-ER–mediated modes of action (Owens and Ashby, 2002), a recent study has indicated that BPA does not act through the non-receptor–mediated mechanism of extracellular-regulated kinases (Bulayeva *et al.*, 2004). The dose metrics for estrogen receptor occupancy by an ER agonist are likely a more accurate measure for predicting uterine responses than are the blood concentrations of the agonist. This is certainly true for estradiol (Barton and Andersen, 1997; Clark and Peck, 1979; Jensen, 1965). To provide a dose metric relevant to ER-mediated effects, a uterine compartment containing ER was incorporated into the PBPK model for BPA.

Studies of uterine responses to estrogens or xenoestrogens typically involve one or more doses per day for 3 days (Ashby and Tinwell, 1998; Barton, 1999; O'Connor *et al.*, 1996; Odum *et al.*, 1997). The uterine wet weight increase after three daily doses of estradiol has been shown to be much greater than after a single dose, a finding that has led to adoption of this dosing regimen in uterotrophic assay protocols. However, the relationship between receptor occupancy and the uterine weight increase in a 3-day dosing regimen is not clear. The time course events leading to uterine weight gain can be generalized as occurring in two phases: early phase biosynthetic events occurring 0–6 h after exposure and later events culminating in increases in uterine weight gain 6–24 h after exposure. The available data for increases in wet and dry weight after exposure to BPA all use multi-day dosing regimens, so two dose metrics for receptor occupancy were estimated for comparison with the response data. These dose metrics are the percent receptor occupancy at 6 h after dosing (POCCU1) and the 0–24 h area under the curve (AUC) for the occupied ER in the uterus (AUCUB1). The model does not include dimerization of the serum estradiol ER complex or binding of the agonist-occupied ER dimer complex with the nuclear DNA, so neither of these dose metrics attempts to relate the degree of nuclear receptor occupancy to the uterotrophic response following a single dose of BPA.

Estrogen receptor occupancy dose metrics—POCCU1 and AUCUB1—after oral administration of BPA were compared to the increases in uterine wet weights reported by Twomey (1998). Two comparisons were made, one that included the effect of plasma protein binding restrictions on free BPA and one that did not. In the Twomey study, BPA was administered for 3 consecutive days at dose rates of 10, 100, 200, and 800 mg/kg/day, after which uterine weight was assayed.

PBPK model structure. The model was initially developed for adult male and female rats and was later extended to humans. A nested model structure is used, with sub-models for BPA and BPAG comprising the overall model (Fig. 2). The BPA model consists of compartments representing blood, uterus, liver, the lumen of the GI tract, and a body compartment representing the remaining perfused tissues. In this model, BPA distributes to and from a non-metabolizing body compartment. The other tissues are formulated the same as the body compartment, but they have additional terms describing processes that affect tissue concentrations of BPA (metabolism, protein or ER binding, uptake). Tissue compartments are formulated as perfusion limited (well mixed, rapid equilibrium), as described elsewhere (Andersen, 1981). Tissues volumes, flows, and rates of uptake, metabolism, and elimination are scaled allometrically to adjust for differing body weights of the rats used in the PK studies. The body compartment is described by the following equations, where ''1'' refers to BPA, ''2'' to BPAG, ''A'' to amount (*i.e.*, dA/dt), and ''free'' to BPA not bound to proteins or receptors:

$$\frac{dA(BPA)_d}{dt} = ad1 = Qd \times (cb1 - cvd1), \quad (2)$$

$$cd1 = ad1/vd, \quad (3)$$

$$cvd1 = cd1/pd1, \quad (4)$$

where Qd is the blood flow (l/h), cb1 and cd1 are the arterial blood concentration and body compartment concentration of BPA (mg/l), cvd1 is the exiting venous concentration of BPA (mg/l), ad1 is the amount (mg) of BPA

TABLE 1
Rat and Human Model Oral-Route Parameters

Parameter	Symbol	Rat	Human	Reference[b]
Body weight (kg)	BW	Study specific	78.0 ♂ 60.7 ♀	H: Values reported by (Völkel *et al.*, 2002)
Cardiac output (l/h/kg$^{0.75}$)	QCC	14	12.9 ♂ 13.21 ♀	R: (Brown *et al.*, 1997) H: (ICRP 1975)
Blood flows (fraction of cardiac output)				
Body	QD	0.82	0.77 ♂ 0.75 ♀	Calculated as QCC-QLC-QUC
Liver	QLC	0.18	0.23	(Brown *et al.*, 1997)
Uterus	QUC	0.0025	0.02	R: (Kerr *et al.*, 1992; Zhang *et al.*, 1995) H: (Stock and Metcalfe, 1994)
Organ volumes (fraction of body weight)				
Blood	VBC	0.074	0.079	(Brown *et al.*, 1997)
Bone	VBoneC	0.07	0.143	(Brown *et al.*, 1997)
Liver	VLC	0.04	0.026	(Brown *et al.*, 1997)
Uterus	VUC	0.002	0.0018	R: (Bruce, 1976) H: (ICRP, 1975; Langlois, 1970)
Body	VDC	1 – remaining tissues – bone		
Hematocrit	HCT	0.45	0.4	R: (Waynford and Flecknell, 1992) H: (Guyton, 1991)
Volumes of distribution (fraction of body weight)				
BPAG	VDC2	0.17 [H]	0.17	Fitted to human data
Molecular weights (g/mole)				
BPA	MW1	228.8		
BPAG	MW2	404.4		
Partition coefficients				
Body:blood	PD1	1.35 [H]	1.35	Muscle and kidney value from (Csanady *et al.*, 2002)
Liver:blood	PL1	1.46 [H]	1.46	(Csanady *et al.*, 2002)
Uterus:blood[a]	PU	1.35	1.35	Muscle value from (Csanady *et al.*, 2002)
Metabolism parameters for liver				
V_{Max} (mg/h/kg$^{0.75}$)	$V_{Max}C1$	1000 ♀ 5000 ♂	850	Estimated
KM (mg/l)	KM1	10	10 [R]	(Kuester and Sipes, 2003)
Receptor or plasma protein content				
Uterine ER content (nmole)	$B_{max}U$	0.006	1.7	(Plowchalk and Teeguarden, 2002)
Plasma binding protein content (μM)	$B_{max}A$	3100	2000.0	R: Estimated using (Mayersohn, 2003) H: (Csanady *et al.*, 2002)
Binding affinities				
BPA:estrogen K_d (nM)	KD1	400 [H]	NA	(Gaido *et al.*, 1997; Kuiper *et al.*, 1998; Kuiper *et al.*, 1997)
BPA:plasma protein K_d (μM)	KDA	195 [R]	100.0	R: Estimated using (Mayersohn, 2003) H: (Csanady *et al.*, 2002)
Uptake rate (kg$^{0.25}$/h)				
From stomach to liver via portal vein	kAC1	10	10 [R]	Estimated
Rates of BPAG elimination (kg$^{0.25}$/h)				
Feces	kEFC	0.008 ♀ 0.013 ♂	0.0	Estimated
Urine	kEUrC	0.75	1.65	Estimated
Cleavage & EH recirculation	kEHRC	0.28	0.5	Estimated
Percentage of BPAG eliminated via the bile (%)	Bile	99	10	Estimated

Note. [R]: rat value used as approximation of the human value; [H]: human value used as an approximation of the rat value.

[a]The uterine tissue partition coefficient is not applied directly in the model. The partition coefficient is adjusted dynamically by the free fraction of BPA in the blood (see *PBPK Model Structure*).

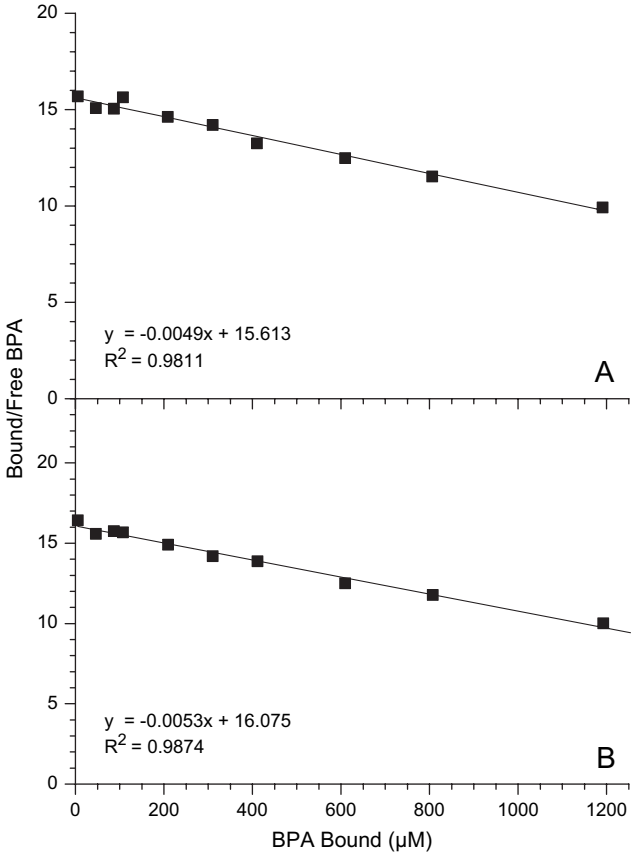

FIG. 1. Scatchard analysis of BPA:plasma protein binding in male (A) and female (B) F344 rats. A linear regression line was fitted to a plot of bound/free BPA as a function of bound BPA and the binding terms B_{max} and K_d were estimated as described in the *Materials and Methods*.

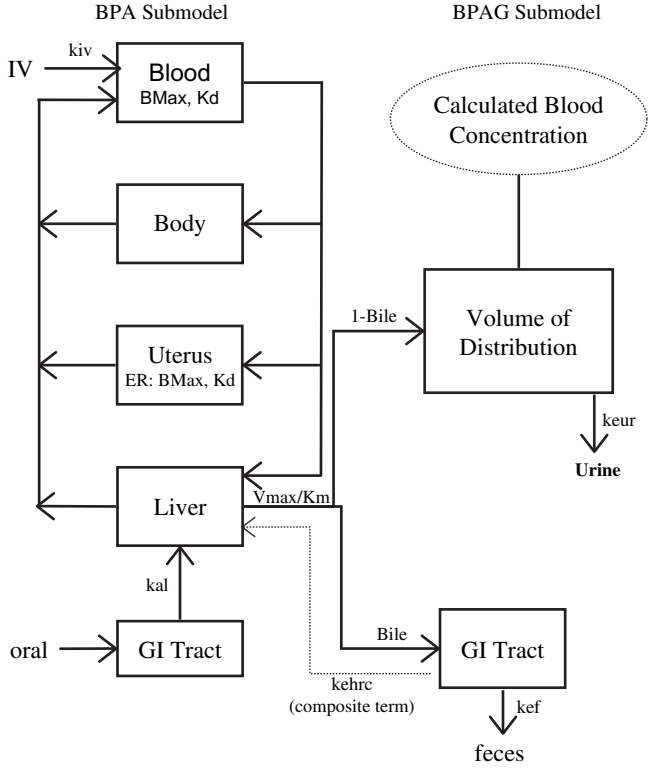

FIG. 2. Diagram depicting the structure of the BPA/BPAG PBPK model.

in the body compartment, vd is the volume of the body compartment, and pd1 is the body:blood partition coefficient. The liver compartment (denoted with an l in equations 5 and 6) contains additional terms for absorption of BPA from the GI tract lumen, metabolism by glucuronosyl transferase, and enterohepatic recirculation of BPA derived from the hydrolysis of BPA-glucuronide:

$$\frac{dA(BPA)_l}{dt} = al1 = Q1 \times (cb1 - cvl1) + rao1 - ram1 + rar1, \quad (5)$$

$$cvl1 = al1/v1/PL1, \quad (6)$$

$$ram1 = \frac{VMAX1 \times cvl1}{(KM1 + cvl1)}, \quad (7)$$

where Ql is the total liver blood flow (l/h) and cvl1 is the exiting venous concentration of BPA (mg/l), ram1 is the rate of hepatic glucuronidation (mg/h), VMAX1 (VMAXC1*$BW^{0.75}$) is the maximum rate of metabolism by the liver, and KM1 is the Michaelis constant (a BPA concentration, mg/l) for glucuronidation.

Oral dosing and absorption of BPA from the GI tract lumen is described by a first-order rate constant (Ka1), which when multiplied by the amount in the lumen gives the rate of BPA absorption (roa1). Absorbed BPA enters the portal circulation and goes directly to the liver. The fate of BPAG produced in the liver and enterohepatic recirculation (rar1, mg/h) are described later, in the section on the BPAG submodel (equations 19–21).

The blood binding and uterine binding submodels are written in molar rather than milligram units (the remainder of the model) for consistency with the way binding constants are typically measured and reported. Unit conversions are placed directly in the equations shown below, although they are handled separately in the code. Binding to the uterine estrogen receptor is described as simple equilibrium binding of a single ligand to a single site on the receptor (equation 8):

$$\text{Receptor Bound} = \frac{BMAXU \times \frac{BPAfree \times 10^6}{MW1}}{\left(KD1 + \frac{BPAfree \times 10^6}{MW1}\right)}, \quad (8)$$

where Receptor Bound is the amount (nmoles) of uterine estrogen receptor bound by BPA, BPAfree is the free concentration (nmoles/l) of BPA in the uterus, BMAXU is the amount (nmoles) of estrogen receptor in the uterus, and KD1 is the disassociation constant (Kd) for BPA binding to ER. Estrogen receptor occupancy is defined as the ratio of BPA occupied receptors to total receptors. Only free BPA is assumed to be available for partitioning from blood to the uterus. The total concentration of BPA in the uterus and BPAfree is calculated as described in equations 9–11:

$$\frac{dA(BPA)_u}{dt} = au1 = Qu \times (cb1free - BPAfree), \quad (9)$$

$$cu1 = au1/vu, \quad (10)$$

$$BPAfree = \frac{MW1}{10^6} \times \left[\frac{au1 \times 10^6}{MW1} \Big/ \left[vu \times PU/FF + BMAXU/\left(KD1 + \frac{BPAfree \times 10^6}{MW1}\right)\right]\right], \quad (11)$$

where Qu is the blood flow to the uterus (l/h), cb1free is the concentration of free BPA in the blood (mg/l), BPAfree is the concentration of free BPA in the exiting venous blood (mg/l), cu1 is the concentration of total BPA in the uterus (mg/l), aul is the amount (mg) of BPA in the uterus, vu is the volume of the

uterus (l), PU is the tissue:blood partition coefficient, and FF is the free fraction of BPA in the blood (calculated dynamically in the model). Equation 11 is an implicit equation and is solved by an iterative procedure in the model.

The approach to partitioning (PU/FF) used for the uterine compartment is different from that used for other tissues in this model. The difference arises both from the method used to measure the partition coefficients (Csanady *et al.*, 2002) and the need to model uterine tissue receptor binding as a function of free rather than total BPA. Csanady and co-workers measured blood:tissue partition coefficients for several tissues using blood directly rather than the standard blood:saline, saline:tissue protocol. The resulting partition coefficients account for the net affect of processes controlling apparent partitioning (*e.g.*, binding to plasma proteins). These partition coefficients can be applied directly in the model for the tissues Csanady and co-workers measured if they are used in a fashion consistent with the way they were measured; that is, if they are applied to total blood BPA rather than free blood BPA.

The situation is different for the uterus for two reasons. First, in the absence of better data, we chose to use the partition coefficient reported by Csanady *et al.* for muscle tissue. As measured, this partition coefficient would reflect partitioning to a muscle-like tissue and the effect of binding to blood proteins, but not receptor binding in a tissue like the uterus. To properly apply the muscle partition coefficient to the uterus, we must separate the receptor binding and partitioning processes in the model. Second, ER binding of BPA in the tissue is expected to be a function of the free material in the blood, not the total. To accommodate these needs, we calculate free concentration of BPA in the blood and allow the tissue and receptor to equilibrate to the free concentration rather than total blood BPA. Because these equations are written based on free BPA concentrations, the muscle partition coefficient reported by Csanady *et al.* was adjusted by dividing by the free fraction of BPA. The adjustment is made in the model because the free fraction of BPA is dynamic, changing if plasma binding is saturated or additional compounds that compete for binding are added.

Free and total BPA are calculated in the plasma compartment according to equations 12 (total amount of BPA in blood), 13 (total concentration in blood), 14 (concentration in plasma), 15 (amount in plasma), 16 (free concentration in plasma), and 17 (free concentration of the plasma binding protein in μM) and 18 (free concentration in blood):

$$\frac{\mathrm{dA(BPA)_b}}{\mathrm{dt}} = \mathrm{ab1} = (\mathrm{Qd}\times\mathrm{cvd1} + \mathrm{Q1}\times\mathrm{cvl1} + \mathrm{Qu} \times \mathrm{BPAfree} - (\mathrm{Qc}-\mathrm{Qu}) \times \mathrm{cb1} - \mathrm{Qu}\times\mathrm{cb1free} + \mathrm{Kiv}), \quad (12)$$

$$\mathrm{cb1} = \mathrm{ab1}/\mathrm{vb}, \quad (13)$$

$$\mathrm{cp1} = \mathrm{cb1}/\mathrm{PBP}, \quad (14)$$

$$\mathrm{ap1} = \mathrm{cp1}\times\mathrm{vp}, \quad (15)$$

$$\mathrm{cp1free} = \frac{\mathrm{MW1}}{10^3}\times\left(\frac{\mathrm{ap1}\times\frac{10^3}{\mathrm{MW1}}}{(\mathrm{vp}\times(1+\mathrm{cafree}/\mathrm{KDA}))}\right), \quad (16)$$

$$\mathrm{cafree} = \frac{\mathrm{BMAXA}\times\mathrm{vp}}{\left(\mathrm{vp}\times\left(1+\mathrm{cp1free}\times\frac{10^3}{\mathrm{MW1}}/\mathrm{KDA}\right)\right)}, \quad (17)$$

$$\mathrm{cb1free} = \mathrm{cp1free}\times\mathrm{PBP}, \quad (18)$$

where Qc is the cardiac output (l/h), cb1 is the total BPA concentration in the blood (mg/l), ab1 is the total amount of BPA in the blood, cvu1 is the BPA concentration (mg/l) in the venous blood exiting the uterus, cb1free is the free BPA concentration in the blood, Kiv is the i.v. dose rate (mg/h), PBP is the blood:plasma partition coefficient, vb and vp are the volumes of the blood (l) and plasma (l), cp1free is the free concentration of BPA in the plasma, cafree is the free concentration of binding proteins in the blood (μM), BMAXA (μM) is the total measured binding protein concentration in the plasma, KDA is the BPA:Binding protein disassociation constant (μM) (see *Plasma Protein and Estrogen Receptor Binding*, below). The equation (16) for cp1free is solved as an implicit equation because (by substitution with equation 17) it contains cp1free. We verified that implantation of these equations led to accurate simulations of the free fraction (Mayersohn, 2003), and therefore concentrations, of BPA.

Glucuronidation is the major pathway for metabolic elimination of BPA and the chemical species formed, BPAG, is inactive for receptor binding and estrogenic activity (Matthews *et al.*, 2001; Snyder *et al.*, 2000). Therefore, the presence of BPAG in the body was described with a volume of distribution, and tissues were not explicitly described. The BPAG produced in the liver is passed to the GI tract lumen via the bile or directly to the volume of distribution in the BPAG sub-model. The fraction of BPAG passed to these two compartments is controlled by the term BILE, which is adjusted for species differences in the elimination of BPAG. In humans, BPAG is eliminated almost entirely by renal excretion, although some biliary elimination followed by complete reuptake cannot be ruled out. We represent human elimination of BPAG as occurring exclusively from the blood via the kidneys, whereas in the rat, BPAG elimination is principally via the bile.

The rate of change of BPAG in the body is determined by its rate of formation and elimination in urine (equation 19). The concentration of BPAG in blood was estimated with the assumption that the concentration in the volume of distribution was equivalent to the plasma concentration and no BPAG entered the red blood cells (equation 20). Therefore, the blood concentration was the product of the concentration in the volume of distribution and the fraction of the blood comprised of plasma (*i.e.*, 1-hematocrit [HCT]):

$$\frac{\mathrm{dA(BPAG)_{VD}}}{\mathrm{dt}} = \mathrm{ad2} = \mathrm{MW2}/\mathrm{MW1}\times\mathrm{ram1}\times(1-\mathrm{BILE}) - \mathrm{keur}\times\mathrm{ad2}, \quad (19)$$

$$\mathrm{cb2} = (\mathrm{ad2}/\mathrm{VD2})\times(1-\mathrm{HCT}), \quad (20)$$

where the ratio of molecular weights converts the rate of metabolism of BPA into the rate of production of BPAG and 1-BILE is the fraction of BPAG produced in the liver passing directly to the volume of distribution. The remaining BPAG is eliminated via the bile. A first-order rate constant describes urinary elimination (keur) of BPAG. The amount of BPAG (ad2) in the compartment is obtained by integrating equation 19. VD2 is the volume of distribution (Table 1: VDC2*BW). Integration of the urinary elimination rate (keur*ad2) is used to determine the total amount in urine, from which the percentage of the administered dose in urine was calculated for comparison with data.

Bile is secreted into the lumen of the small intestine, where glucuronides are often subject to hydrolysis of the conjugate by the intestinal microflora; BPA released by hydrolysis may then be reabsorbed (equation 21):

$$\frac{\mathrm{dA(BPAG)_{gut}}}{\mathrm{dt}} = \mathrm{ag2} = \mathrm{BILE}\times\frac{\mathrm{MW2}}{\mathrm{MW1}}\times\mathrm{ram1} - \mathrm{kehr}\times\mathrm{ag2} - \mathrm{kef}\times\mathrm{ag2}. \quad (21)$$

A single first-order rate constant, kehr, is used to describe the rate of BPAG hydrolysis and re-absorption. The rate of BPA reabsorbed into the liver (rar1 in equation 5) is the product of kehr, the amount of BPAG in the gut lumen (ag2), and the ratio of the molecular weights. A first-order rate constant is also used to describe fecal elimination (kef). Integration of the fecal elimination rate (kef*ag2) determined the total amount in feces, from which the percentage of the administered dose was calculated for comparison.

Model Parameterization

Physiological parameters. The physiological parameters used in the rat and human models are summarized in Table 1. Body weights of rats were study specific (Pottenger *et al.*, 2000). Tissue volumes and blood flows were obtained from Brown *et al.* (1997), except for the uterine compartment. Uterine tissue

volumes were reported elsewhere for the rat (Bruce, 1976) and human (Langlois, 1970).

Tissue distribution parameters. Csanady and co-workers measured the human tissue:blood partition coefficients of bisphenol A and the rat and human tissue:blood partition coefficients for the phytoestrogen daidzein using the vial equilibration technique (Csanady *et al.*, 2002). The reported BPA partition coefficients in humans were 1.35 for muscle and kidney, 1.46 for liver, and 3.31 for fat. A value of 1.35 was assumed for the bulk tissue compartments for the human, and the liver value was used directly. Based on the finding that average tissue:blood partition coefficients for daidzein were not statistically significantly different between rats and humans, the human BPA partition coefficients were assumed to be a good proxy for rodent values, and were thus used in the rat model (Table 1). The blood:plasma partition coefficient has been measured in adult and 21-day-old male and female, as well as pregnant F344 rats and adult male and female Sprague-Dawley rats. The blood:plasma partition coefficient had a range of 0.72–0.94, with a mean value of 0.83 and a standard error of the mean (S.E.M.) of 0.015 (Mayersohn, 2003). This value was used directly in the model.

A volume of distribution for BPAG has not been reported for rats. Völkel estimated a volume of distribution at steady state (VD_{ss}) for this glucuronide of 0.43 l/kg (Völkel *et al.*, 2002) using a noncompartmental approach corresponding to a three-compartment model. The VD_{ss} is an overestimation of the VD_0 (volume of distribution at Tzero) for models of greater than one compartment (O'Flaherty, 1981). This was verified here through simulation of the Völkel data, and a much smaller VD of 0.17 l/kg was assumed for humans (Table 1). In the absence of other data, this value was adopted for rats as well (Table 1). The value compares well to the measured VD for morphine-6-glucuronide, which was reported to be between 0.28 and 0.43 l/kg in adult humans (Barrett *et al.*, 1996).

Metabolism, excretion, and enterohepatic recirculation. The values of parameters for metabolism, excretion, and enterohepatic recirculation in rats were estimated by fitting the oral gavage data of Pottenger *et al.* (2000). The K_m for hepatic glucuronidation was assumed to be 10 μM, the middle of the range of values reported for rats and humans after measurement in microsomal preparations and isolated hepatocytes (Kuester and Sipes, 2003). The reported sex differences in pharmacokinetics were accommodated by estimating parameter values separately for male and female rats (Table 1). The close interrelationship between absorption, metabolism, and enterohepatic recirculation prevented determination of unique values of the corresponding model parameters by either visual or formal optimization (ACSL Optimize, AEGIS technologies). However, different aspects of the blood BPA or BPAG time course varied in their sensitivity to these model parameters. For example, rates of enterohepatic recirculation do not affect the BPA T_{max} or C_{max}, but do influence later blood concentrations. The rates of metabolism and uptake both influence BPA T_{max} and C_{max}, but the rate of uptake has a stronger influence on BPA T_{max}. These differences in sensitivity allow simultaneous estimates of these parameters to be made with the given data sets.

Simulating i.v. route pharmacokinetics in rats required the use of different rates of hepatic glucuronidation, enterohepatic recirculation, and fecal elimination. Table 2 summarizes parameters that are different between the oral route and i.v. route simulations. This discrepancy is addressed further in the *Discussion*.

Plasma protein and estrogen receptor binding. In adult rats and humans, the principal BPA binding protein in the plasma is believed to be albumin, to which it binds with relatively low affinity (Mayersohn, 2003). Bisphenol A also binds to sex hormone binding globulin (SHBG) in humans (Bendridi *et al.*, 2002; Dechaud *et al.* 1999), but does not appear to bind significantly to α-fetoprotein in rats (Milligan *et al.*, 1998). Minor binding to other plasma proteins such as transthyretin is possible (Ishihara *et al.* 2003), but rat and human binding data are not available. The binding characteristics of BPA have been characterized *in vitro* using plasma obtained from rats (see *Plasma protein binding,* above) and human blood (Csanady *et al.*, 2002; Mayersohn, 2003). In both cases, composite values of B_{max} and K_d (KDA) were estimated reflecting the contribution of all plasma proteins (including SHBG in human plasma) to binding. The B_{max} and KDA reported by Csanady *et al.* (2002) for humans are 2000 μM and 100 μM, respectively, and derived here for female F344 rats are 3100 μM and 195 μM, respectively (Fig. 1, Table 1).

TABLE 2
IV Route Parameters for the Rat PBPK Model

Parameter	Symbol	Value	Reference
Metabolism parameters for liver			
V_{max} (mg/h/kg$^{0.75}$)	$V_{max}C1$	250	Estimated
Rates of BPAG elimination (kg$^{0.25}$/h)			
Cleavage & EH recirculation	kEHRC	0.038	Estimated
Feces	kEFC	0.0068	Estimated

Limited data are available for estimating the affinity constant (KD1) of BPA for the rat estrogen receptor. Bisphenol A appears to have a greater affinity for ERβ than for ERα (Kuiper *et al.*, 1997, 1998; Matthews *et al.*, 2001). Estimates of its relative affinity for ERα compared to E2 range from 0.006% to 0.05% (Kuiper *et al.*, 1998; Matthews *et al.*, 2001; Nagel *et al.*, 1998). A K_D of 400 nM (representing a relative affinity of 0.05% compared to the K_D of 0.2 nM for E2) was used here (Teeguarden and Barton, 2004). The K_D used for E2:ER binding was 0.2 nM (Nagel *et al.*, 1998; Zava and Duwe, 1997). This value is plausible based on a review of published values, which are highly variable (Plowchalk and Teeguarden, 2002).

Modeling Approach

The rat PBPK model for BPA and BPAG was developed to simulate blood and uterine concentrations after exposure to BPA by routes relevant to human exposure (oral) and experimental characterization of BPA pharmacokinetics (i.v). Bisphenol A metabolism was attributed solely to the liver, although the ability of intestinal tissues to glucuronidate BPA has recently been demonstrated (Inoue *et al.* 2003). Because BPA administered by the i.v. route should be subject to predominantly hepatic rather than intestinal metabolism, i.v. BPA kinetic data (Upmeier *et al.*, 2000) were used to estimate the hepatic metabolism of BPA, as well as the BPA volume of distribution. The oral route kinetic data were more complete, containing blood BPA and BPAG concentration time course data and cumulative elimination of total radioactivity in the feces and urine of male and female rats; these data were used for fitting the remaining parameters. Rates of uptake, fecal excretion and enterohepatic recirculation as well as urinary clearance of the glucuronide were fitted to the available oral (Pottenger *et al.*, 2000) rat pharmacokinetic data. Simulation of these data required the estimation of oral-route–specific rates of glucuronidation, which were still attributed to the liver.

The model was extended to humans by means of physiological parameters obtained from the literature and fitting of the remaining parameters to oral-route pharmacokinetic data obtained from male and female volunteers (Völkel *et al.*, 2002).

Plasma protein binding of BPA was incorporated into both the rat model and the human model, and simulations of free and protein bound BPA were compared to published data (Csanady *et al.*, 2002; Mayersohn, 2003). The rat model was used to simulate ER binding in the uterus after oral exposure to BPA, and various dose metrics were tested for correlations with uterine weight gain observed in separate oral-route experiments (Twomey, 1998).

Sensitivity Analysis

The numerical values of measured and estimated model parameters are not known with absolute certainty. An evaluation of the impact of uncertainty in the parameters on model estimates of blood BPA and BPAG concentrations was performed by conducting a sensitivity analysis. The analysis was carried out by measuring the change in model output for a 1% change in a particular model

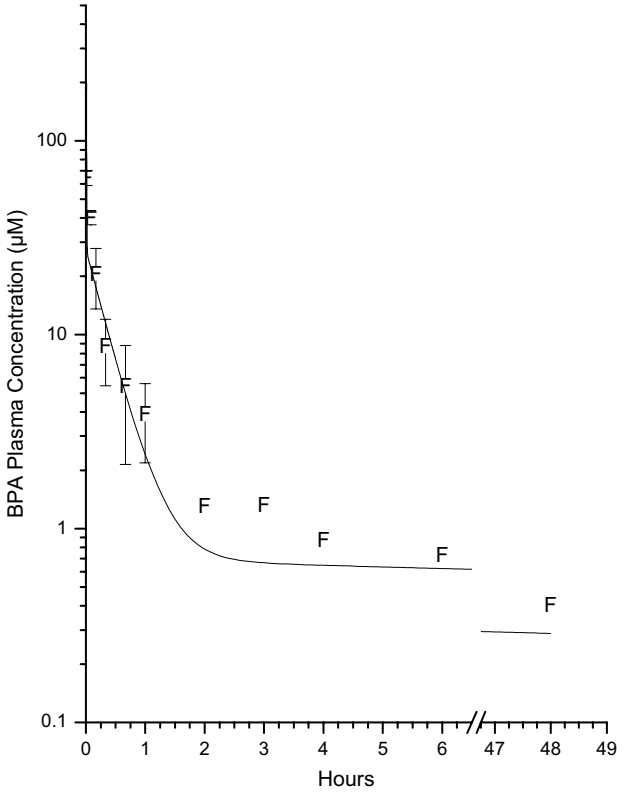

FIG. 3. Simulated (lines) and observed (symbols, F designates females) plasma BPA concentrations in female DA/HAN rats after i.v. dosing of 10 mg/kg BPA. Data are from Upmeier *et al.* (2000).

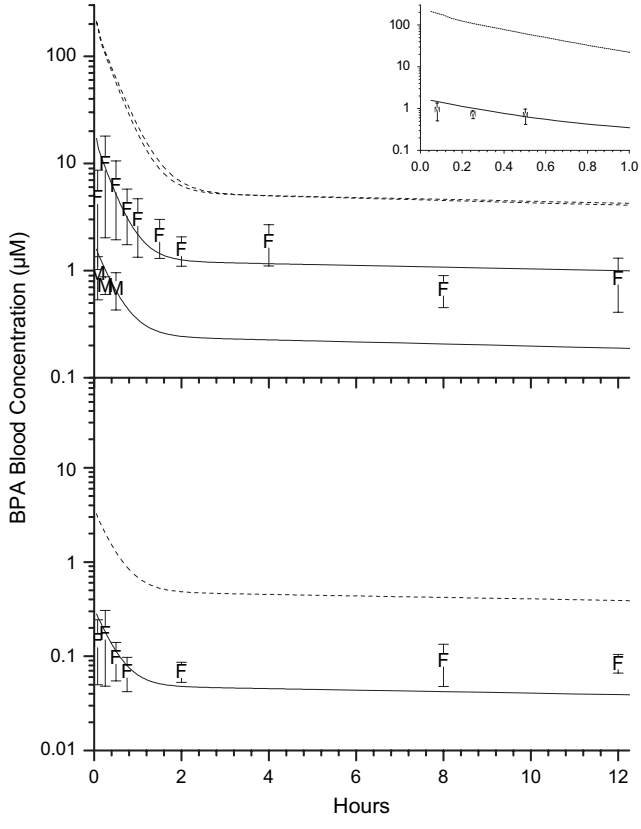

FIG. 4. Simulated (lines) and observed (M, male; F, female) blood BPA concentrations in F344 rats following oral gavage dosing of 10 or 100 mg/kg BPA. Solid lines indicate use of V_{max}'s fitted to oral data while dotted lines indicate use of V_{max} obtained from i.v. data. Inset depicts the male rat time course over a shorter interval. Data are from Pottenger *et al.* (2000).

parameter when all the other parameters were held fixed. A normalized sensitivity coefficient of 1 indicates that there is a one-to-one relationship between the fractional change in the parameter and model output. A positive value for the normalized sensitivity coefficient indicates that the output and the corresponding model parameter are directly related and a negative value indicates they are inversely related. Time series sensitivity coefficients are reported to convey the time dependence of the various model parameters on BPA and BPAG kinetics.

The rat sensitivity analysis was conducted under the conditions of the female oral 100 mg/kg oral dose study, and the human sensitivity analysis was conducted under conditions of the human oral study (5 mg/person, females). These doses were selected to allow evaluation of model behavior in the range of the experimental range. Normalized sensitivity coefficients were reported as a function of time and presented here for all parameters with sensitivity coefficients greater than 0.1.

RESULTS

Rat Intravenous Dose Pharmacokinetics

Following administration of 10 mg/kg BPA, simulated and observed BPA plasma concentrations at the T_{max}, as well as during distribution and elimination/enterohepatic recirculation dominated phases of the time course, were in excellent agreement (Fig. 3). The fitted V_{max} for hepatic glucuronidation was 250 mg/hr/$BW^{0.75}$ (average body weight, 0.140 kg). Partitioning into the lumped body tissues with a partition coefficient of 1.35 (Csanady *et al.*, 2002) described the volume of distribution of BPA well.

Rat Oral Route Pharmacokinetics

Simulated blood BPA concentrations after oral exposure to 100 mg/kg of BPA agreed well with observed values at all time points in females, and in males when rates of hepatic glucuronidation were significantly increased over the value estimated from the i.v. data (Fig. 4). The fitted V_{max} for the male (average BW 0.209 kg) and female (average BW 0.148 kg) data were 5000 and 1000 mg/h/$BW^{0.75}$, reflecting a significant sex-dependent difference in pharmacokinetics reported by Pottenger *et al.* (2000).

Simulations of blood BPAG concentrations in females following 100 mg/kg oral dosing under-predicted peak concentrations, but accurately reflected the time of the peak concentration (T_{max}) (Fig. 5). In males, which required higher rates of glucuronidation to fit observed blood BPA concentrations, modeled peak BPAG concentrations agreed well with those observed experimentally, but at later times during the

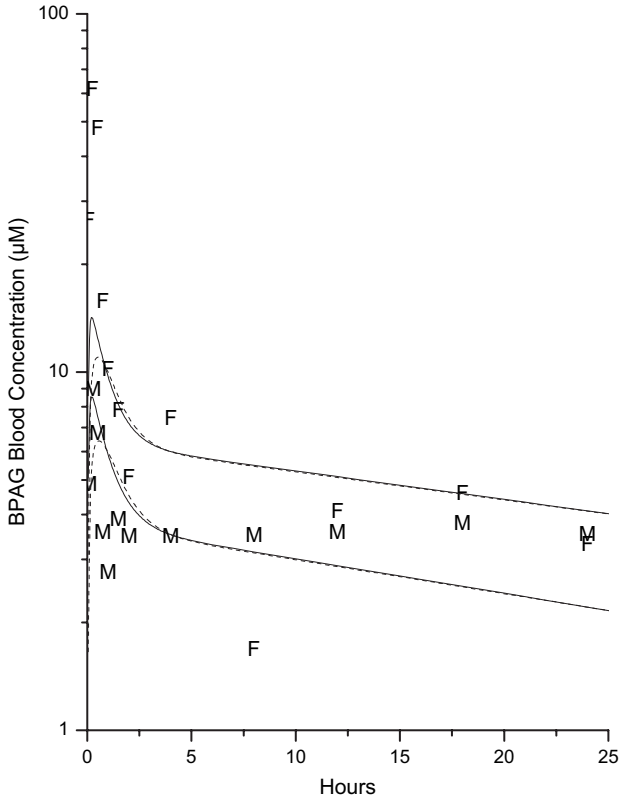

FIG. 5. Simulated (lines) and observed (M, male; F, female) blood BPAG concentrations in F344 rats following oral gavage dosing of 100 mg/kg BPA. Solid lines indicate use of V_{max}'s fitted to oral data, and dotted lines indicate use of V_{max} fitted to i.v. data. Data are from Pottenger *et al.* (2000).

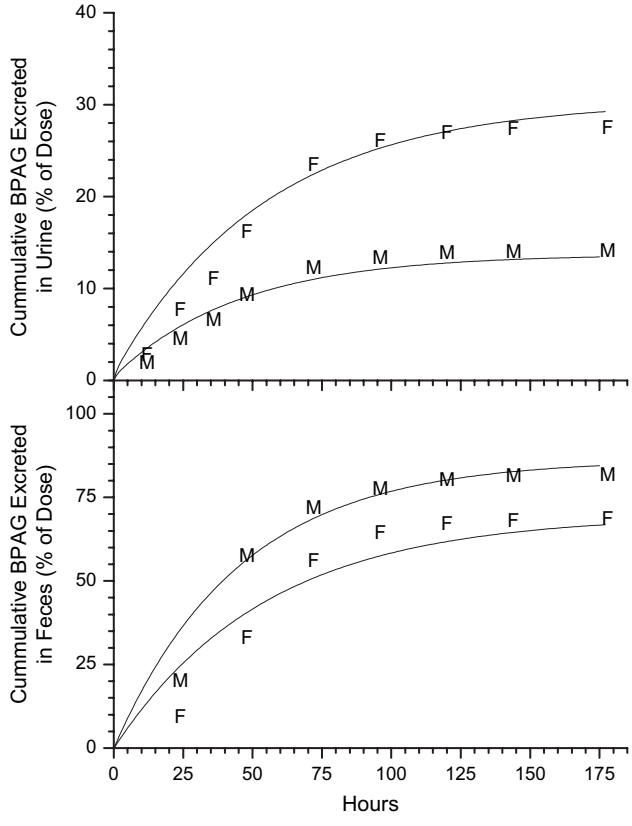

FIG. 6. Simulated (lines) and observed (M, male; F, female) cumulative % of dose eliminated by F344 rats in urine (panel A) or feces (panel B) after oral gavage dosing of 100 mg/kg BPA. Data are from Pottenger *et al.* (2000).

clearance/enterohepatic recirculation dominated phase, the model either under-predicted or over-predicted blood BPAG concentrations.

Urine and fecal elimination data provide a basis for verification of the rates of urinary and biliary elimination used in the model, ensuring that attributions of elimination to these two routes are consistent with the available data. For both males and females, there was good agreement between the predicted and observed cumulative percentages of dose eliminated in urine (Fig. 6, panel A) and feces (Fig. 6, panel B), although the early time points are over-predicted (consistent with the under-prediction in blood in Figure 5 or the absence of appropriate time delays in the model to reflect movement through the bowels or excretion through the bladder and urination).

Uterine Weight and Simulated Uterine Estrogen Receptor Binding

Estrogen receptor occupancy dose metrics—percent receptor occupancy at 6 h after dosing (POCCU1) and the 24 h AUC for occupied uterine receptor (AUCUB1)—following oral gavage administration of BPA were compared to increases in uterine wet weights reported by Twomey (1998) (Table 3). Two comparisons were made, one that included the affect of plasma protein binding restrictions on free BPA, and one that did not.

In the absence of plasma protein binding, 100% of plasma BPA is capable of equilibrating with uterine estrogen receptors. Using this assumption in the model results in poor correlations between receptor occupancy (POCCU1) and increases in uterine wet weight (Table 3), as evidenced by the absence of a response at high levels (70%) of receptor occupancy and a doubling of uterine weight with a modest change (85% to 96%) in occupancy. The trends are similar when AUCUB1 is considered as the dose metric. In contrast, the correlation between receptor occupancy and uterine response is stronger when the free, ''available'' concentration of BPA is restricted by plasma protein binding to ~6%. No observable uterine weight increase is reported when receptor occupancy is low (~1–15%), a response is observed when occupancy reaches ~25%, and there is approximately a doubling of uterine wet weight when receptor occupancy approximately doubles to 63% (Table 3).

Human Pharmacokinetics

After oral administration of 5 mg BPA, the human model accurately simulated plasma BPAG concentrations for most of

TABLE 3
Correlating Uterine Receptor Dose Metrics and Uterine Response

Oral dose (mg/kg)	Percent occupancy	Percent occupancy	Uterine weight (% control)[a]
	Without binding	With binding	
0	0	0	100
10	9.6	0.63	100
100	73.1	14.0	100
200	84.8	25.2	124
800	96.4	62	200
	ER-BPA complex AUC Without binding	ER-BPA complex AUC With binding	
0	0	0	100
10	0.025	0.002	100
100	0.19	0.04	100
200	0.23	0.07	124
800	0.26	0.17	200

[a]Twomey, 1998.

the time course (<20 h) in both males and females, In general, however, it under-predicted BPAG at the 24–48 h post-exposure interval (Fig. 7). Adjusting the rate of urinary elimination affected simulated concentrations at all but the earliest time points and did not improve overall fits to these data. The model accurately predicted the cumulative urinary elimination of BPAG in both males and females (Fig. 8).

Plasma BPA was below the limit of detection (9 nM) at all time points (Völkel *et al.*, 2002). Modeled BPA concentrations (Fig. 7) were sensitive to the rate of hepatic glucuronidation, but the BPAG concentrations were not. This allowed the estimation of a lower bound of the rate of hepatic glucuronidation: the smallest rate required to keep peak plasma BPA concentrations below the limit of detection. The lower bound estimate on the rate of hepatic glucuronidation was 850 mg/h/$BW^{0.75}$.

Sensitivity Analysis

With few exceptions, the rat and human models showed significant sensitivity (>0.1) to the same parameters. Sensitivity coefficients for the rat are presented here, and important differences with the human model are noted. The modeled BPA concentrations showed sensitivity to parameters that can be grouped into three categories: those that control uptake, distribution, and elimination. Sensitivities varied significantly with time (Fig. 9). Parameters controlling uptake and metabolism—the rate of uptake, metabolic constants, liver blood flow, cardiac output, and the body:blood partition coefficient—had a strong impact on BPA concentrations at early time points (1 h or less). Bisphenol A concentrations at later time points were sensitive to parameters controlling elimination, including V_{max} and enterohepatic recirculation (Fig. 9). The human model was not sensitive to the rate of fecal elimination because

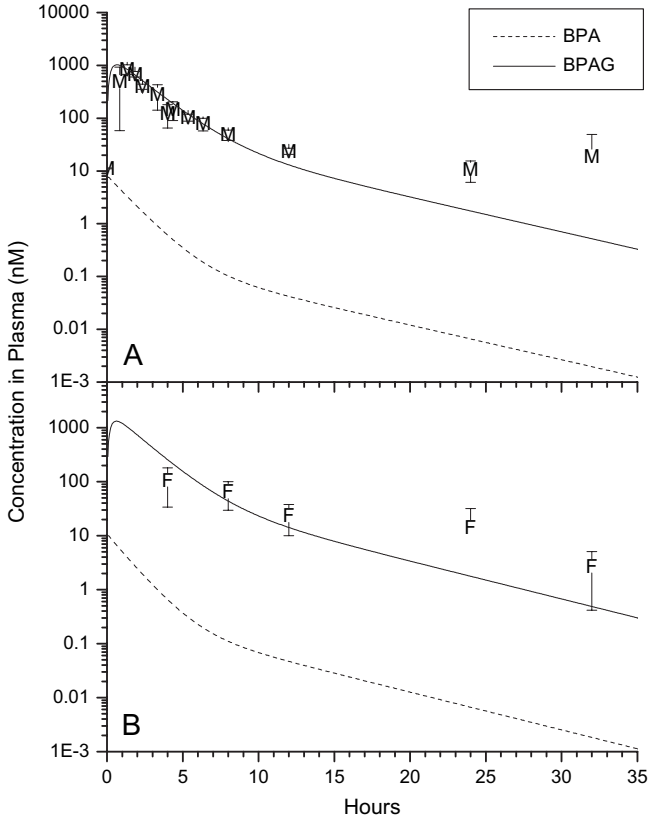

FIG. 7. Simulated (solid line) and observed (M, males; F, Females) plasma BPAG concentrations in six male (panel A) and three female (panel B) human volunteers following administration of 5 mg BPA/person. Blood BPA was not observed experimentally but simulations (dashed line) are presented to demonstrate that the parameterization of the model keeps peak blood BPA concentrations below the limit of detection (9 nM). Data are from Völkel (2002).

biliary elimination of BPAG and enterohepatic recirculation are not as quantitatively important as they are in the rat.

Modeled BPAG concentrations showed high sensitivity to the rate of uptake of BPA only in the first few minutes after oral exposure (Fig. 9). BPAG concentrations were sensitive at all time points to parameters controlling its distribution, namely the volume of distribution, as well as to the fraction of BPAG excreted via the bile (Fig. 9). Modeled BPAG concentrations also showed sensitivity to rates of renal elimination and enterohepatic recirculation that increased with time post-exposure (Fig. 9). The human model showed sensitivity to the same parameters with similar general trends, but sensitivity coefficients could be somewhat larger or smaller than those for the rat.

DISCUSSION

Non-compartmental analyses have been the standard practice for evaluating BPA pharmacokinetics (Sun *et al.*, 2002;

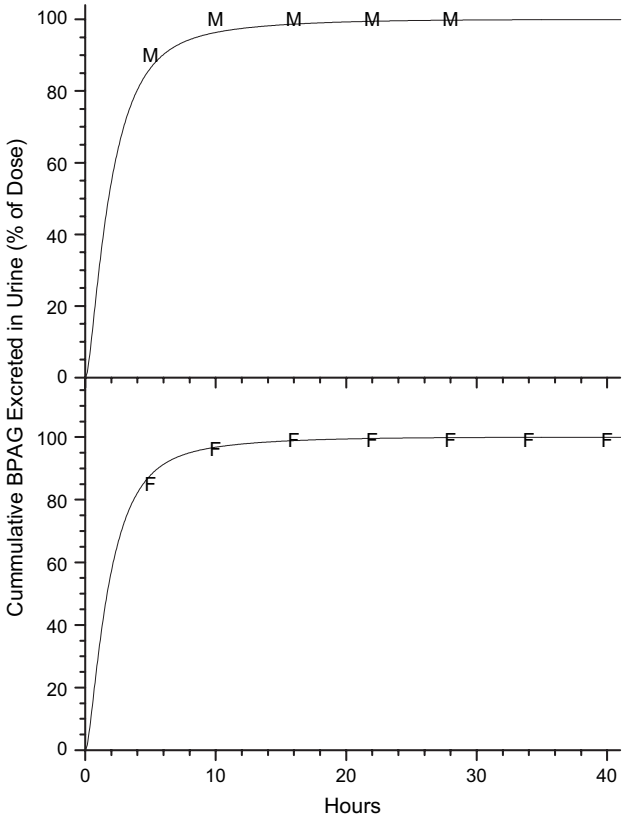

FIG. 8. Simulated (lines) and observed cumulative % of dose eliminated in urine in three male (M) and three female (F) human volunteers administered 5 mg of BPA. Data are from Völkel (2002).

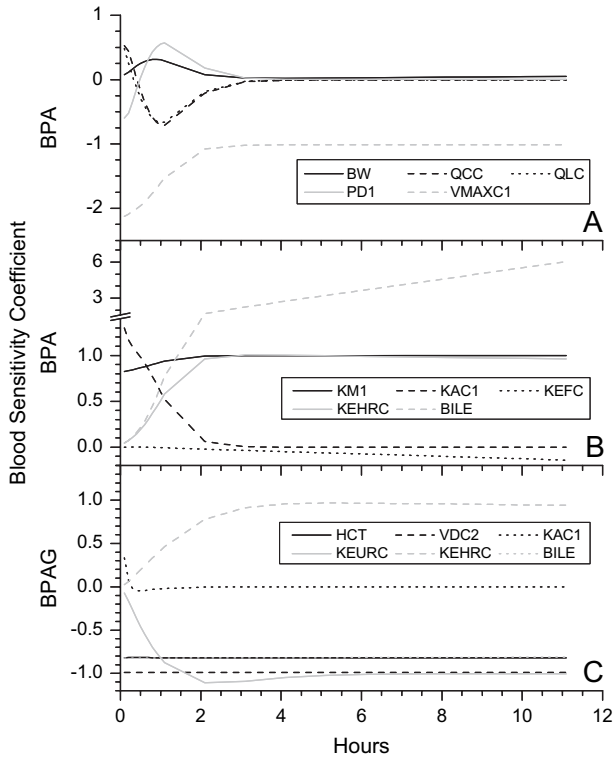

FIG. 9. Sensitivity coefficients for BPA (positives, panel A, negatives, panel B) and BPAG (panel C) for the rat model. Sensitivity coefficients followed the same pattern in humans where differences are not (see *Results*) noted.

Upmeier *et al.*, 2000; Völkel *et al.*, 2002; Yoo *et al.* 2000). Such approaches are simple and provide useful estimates of volumes of distribution and apparent elimination rates that can be used to make rough estimates of steady-state concentrations expected after long-term exposure. These ''models'' operate outside physiological constraints (*e.g.*, partitioning, hepatic blood flow limitations on metabolic clearance) and without direct representation of aspects of physiology/biochemistry (*e.g.*, tissue and receptor binding, enterohepatic recirculation, renal/biliary elimination), which differ across species or may be important for characterization of BPA pharmacokinetics in the context of safety assessment. A PBPK model of BPA pharmacokinetics was developed to provide a physiological context in which the physiological and biochemical processes controlling BPA pharmacokinetics, such as plasma protein binding, estrogen receptor binding, urinary and biliary elimination/enterohepatic recirculation, could be incorporated, and with which BPA and BPAG pharmacokinetics could be evaluated more completely.

Bisphenol A is glucuronidated in both the liver and intestinal tissues (Inoue *et al.*, 2003). First pass metabolism of BPA therefore involves both intestinal and hepatic tissues, and oral route pharmacokinetic (PK) data cannot be used to determine the individual contribution of each of these tissues to metabolic clearance. However, i.v. route PK data can be used to determine the contribution of the liver to metabolic clearance of BPA. Several i.v. route PK data sets were available, each from a different laboratory (Sun *et al.*, 2002; Upmeier *et al.*, 2000; Yoo *et al.*, 2000), and in different strains of rats. These PK data are not consistent with one another, and some systematic review was required to determine which data were most appropriate for use in developing the PBPK model. The discrepancies in these data take the form of large differences in apparent volumes of distribution (VD). Yoo and co-workers administered BPA at four dose levels to male Sprague-Dawley rats, 0.2, 0.5, 1, and 2 mg/kg, and reported serum BPA concentrations over approximately 2 h (Yoo *et al.*, 2000). The non-compartmental analyses of these data give an average VD_{ss} of 5.2 l/kg, greater than 5 times body weight. The VD_0 calculated as Dose/C_0 is ~2.5 l/kg. Sun *et al.* administered BPA to male Wistar rats in doses of 10 or 20 mg/kg and reported BPA concentrations in plasma (Sun *et al.*, 2002). They did not report PK characteristics for BPA, but, we determined that these data have the same characteristics as those reported by Yoo *et al.*, a VD_0 of ~2.5 l/kg. In contrast, Upmeier *et al.* reported plasma concentrations ~4 times higher than those reported by Sun and colleagues for the same 10 mg/kg dose, with a correspondingly lower VD_0 (0.60 l/kg). The difference in VD, almost fourfold, is unexpected and difficult to attribute

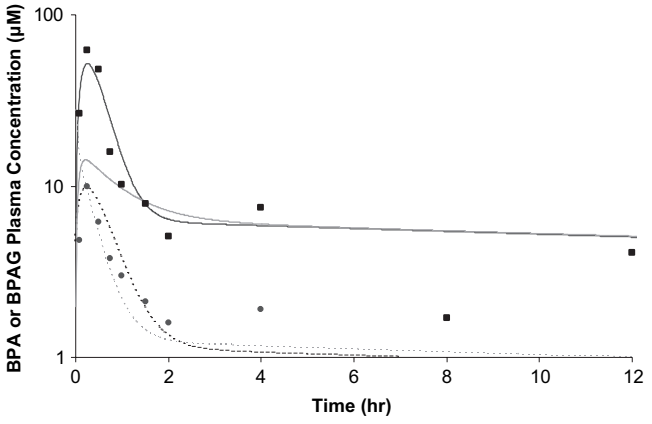

FIG. 10. Simulations of plasma BPA (dotted lines) and BPAG (solid lines) after oral administration of 100 mg/kg BPA in female F344 rats by two BPA models: Model 1 (the subject of this paper, gray lines) and Model 2 (black lines), a revision of Model 1 that reflects an elaboration of gastrointestinal tract handling of BPAG. Points represent data from Pottenger *et al.* (2000).

to physiological differences in the various strains of rats. Csanady *et al.* (2002) found essentially no differences in the volumes of distribution estimated for rats and humans from measured tissue partition coefficients and tissue volumes: the rat value was 1.4 l/kg and the human value was 1.5 l/kg. This approach would approximate a VD_{ss}; the VD_0 would be expected to be lower. The ratio of VD_0 to VD_{ss} reported by Yoo *et al.* is ~0.5. This suggests that the VD_0 based on the Csanady *et al.* (2002) approach would be near 0.7 (1.4 l/kg*0.5), very close to the 0.6 l/kg observed by Upmeier and coworkers (2000). Finally, we found it impossible to simulate the Yoo *et al.* and Sun *et al.* i.v. data sets with the PBPK model, even using the much higher partition coefficients and clearances reported by these authors (data not shown), whereas the Upmeier *et al.* data were simulated well using the characteristics of partitioning reported by Csanady *et al.* (2002), which are also successfully used here to model the oral-route data sets. It is not clear to what the differences in these data sets should be attributed, only that they exist and are most likely the result of experimental differences. None of the authors report recovery of BPA from whole blood, nor do they evaluate the stability of BPAG in their samples during processing. The consistency between the tissue distribution of BPA implied by the Upmeier *et al.* i.v. data, the Pottenger *et al.* oral-route data, and those measured by Csanady *et al.* (2002) led us to rely on the Upmeier *et al.* data for parameterization of the PBPK model and estimation of the hepatic contribution to total metabolic clearance.

Rates of BPA glucuronidation estimated from fitting the i.v. data were 4–20 times lower than the corresponding rates fitted to the oral data. Because strain and sex differences in oral-route and i.v. route studies confound direct comparison of these rates, it is difficult to reach conclusions as to the exact magnitude of the differences. However, the intrinsic metabolic clearance rates reported by Yoo *et al.*, ~100 ml/min/kg, are the same as those used to simulate the Upmeier *et al.* data (95 ml/min/kg), which are in female rather than male rats of a different strain. This consistency supports our estimation of these rates and suggests that strain differences alone do not account for the differences in BPA glucuronidation rates observed in the oral and i.v. studies. Instead, the difference can probably be attributed to glucuronidation of BPA in the GI tract tissues. The rate constant for enterohepatic recirculation used to fit the i.v. data was much lower than for the oral route data. Although experimental variability cannot be ruled out, it seems unlikely that route differences would be as high as a factor of 7. Instead, it is likely that, like the larger oral route V_{max} for glucuronidation, the difference is attributable to oversimplification of hepatointestinal handling of BPA and BPAG.

Although successfully describing BPA pharmacokinetics after oral administration only requires a representation of total (GI tract plus hepatic) metabolic clearance, the less exact simulation of oral-route BPAG pharmacokinetics by the model may be the result of oversimplification of the GI tract compartment in the model. The under-representation of peak BPAG concentrations in female rats, for instance, may be the consequence of attributing BPA glucuronidation solely to the liver, where it is eliminated in the bile before returning to the blood. Specifying metabolism in the GI tract would allow production of BPAG in these tissues and passage directly to the blood, increasing peak concentrations after oral exposure. We confirmed this hypothesis by simulation, but we found blood concentrations of BPAG were then significantly over-predicted. This observation is consistent with the findings of Inoue *et al.*, who, using isolated perfused intestinal segments, showed that rat intestinal tissues not only glucuronidate BPA but also secrete a portion of the BPAG produced back into the intestinal lumen (Inoue *et al.*, 2003), reducing the amount delivered to the blood. However, these more complex descriptions of GI tract handling of BPA and BPAG proved difficult to implement. Even the simplest of the alternative model structures we considered (see Model 2 in the Supplementary Material online), added three to four parameters to the model, expanding the parameter space considerably. Model 2 was a revision of the initial model that included first-order glucuronidation of BPA in the GI tract, movement of the glucuronide from the GI tract to both blood and the GI tract lumen, hepatic clearance of blood BPAG, and hydrolysis of BPAG in the GI tract lumen (see Figure 1 in the Supplementary Material online). Using the hepatic V_{max} derived from the i.v. data (Upmeier *et al.*, 2000), and adding GI tract metabolism of BPAG, simulations of blood BPA and BPAG (female rat 100 mg/kg dose group [Pottenger *et al.*, 2000]) were improved relative to the initial model (Fig. 10) while maintaining fits to the urine and feces data (see Figure 2 in the Supplementary Material online). This model structure also allowed a significant reduction in the first order rate constant for oral uptake (from 10 to ~1). Simulations of the male 100 mg/kg and female 10 mg/kg dose oral route

pharmacokinetic data (Pottenger *et al.*, 2000) were less satisfying (data not shown); attempts to fit these data required variation of several model parameters including the rate of BPAG hydrolysis in the GI tract, hepatic and GI tract glucuronidation of BPA, and/or the fraction of BPAG produced in the GI tract passing to the blood and GI tract lumen. Unique values of the added parameters were not obtainable, and the parameter values appeared to vary in unexpected ways, such as the requirement for a higher hepatic V_{max} for the female 10 mg/kg dose group than the 100 mg/kg dose group. Although Model 2 shows greater fidelity to the underlying physiology and biochemistry relevant to BPA pharmacokinetics and improved ability to fit one of the oral-route rat data sets, implementing this version of the model with complete success for both oral doses would require additional experimental data necessary to obtain better estimates for the additional parameters. It also appears that the initial model, by lumping some processes such as GI tract and hepatic glucuronidation, masks some variability in the underlying processes, allowing a more consistent parameter set to be obtained. The simpler model was retained to keep the level of model detail consistent with the experimental data and to ensure that the need for additional research on GI tract handling of BPA and BPAG be clear. Attractive targets for additional research include *in vitro* characterization of BPA glucuronidation in hepatocytes and enterocytes in both humans and rats, hepatic clearance of blood BPAG, and further characterization of enterocyte processing of BPAG.

A principal goal of pharmacokinetic modeling of BPA, whether by classical or physiologically based approaches, is determining the propensity of BPA to accumulate during lifetime exposure to small doses in humans. In the absence of human data, it may be tempting to apply rodent models of BPA pharmacokinetics to predict steady-state concentrations in humans from terminal elimination phase half-lives derived from BPA concentration time courses. This approach should be used with appropriate caution, and care should be given to understanding its limitations. Our analyses here demonstrate that the net rate of elimination in the terminal elimination phase is more sensitive to the characteristics of enterohepatic recirculation in the rat than in the human. This is consistent with reports demonstrating that elimination in the human is dominated by urinary elimination rather than biliary elimination (Völkel *et al.*, 2002), which suggests that the quantitative importance of enterohepatic recirculation of BPA/BPAG in humans is negligible. The PBPK model developed here incorporates these species differences through differences in the fraction of BPAG secreted into the bile and different rates of enterohepatic recirculation. Because BPA pharmacokinetics are influenced by the pharmacokinetics of BPAG, which shows significant species differences, it is important that predictive pharmacokinetic models address the pharmacokinetics of both BPA and BPAG.

After submission of this manuscript, a rat PBPK model for BPA was published by Shin and co-workers (2004). The Shin *et al*. model represents a larger number of tissues directly (*e.g.*, spleen, heart, lung, testis), although it does not include a uterine compartment, blood binding of BPA, receptor binding of BPA, or enterohepatic recirculation of BPAG. An unusual approach was taken to represent BPA GI tract tissue concentrations. This tissue was represented as being in equilibrium with blood with a partition coefficient of 46, although the high concentrations of BPA observed in the GI tract are the result of both partitioning and enterohepatic recirculation from a compartment external to the body. Given the much lower partition coefficients for other tissues reported by these authors (~1–5), it is likely that enterohepatic recirculation, rather than equilibrium with blood is responsible for the high measured BPA concentrations in the GI tract. Representing the GI tract as being in equilibrium with blood adds a compartment with an effective BPA volume of distribution of ~524 ml for a 250-g rat and results in an inappropriately high volume of distribution for BPA. We confirmed our expectation that a model structured like the Shin *et al.* model, but without the high partitioning in the gut compartment, does not accurately simulate these or other i.v. route data (data not shown) because the volume of distribution is too low.

Shin *et al.* present simulations of blood and tissue BPA concentrations for a single repeat dose i.v. data set. These simulations agree well with the observed data. No effort appears to have been made to test the model against the larger body of i.v. data, which include steady-state blood concentrations after i.v. infusion, as measured and reported by these authors and several other published i.v. infusion or i.v. bolus studies. Attempts to simulate the Upmeier i.v. data (Upmeier *et al.*, 2000) would have revealed both the need to include enterohepatic recirculation and a BPAG submodel, and the apparent difference between these data and their own. Similarly, efforts to simulate the rat oral-route data (Pottenger *et al.*, 2000) would have also revealed the need to include a submodel for BPAG and enterohepatic recirculation. Without demonstrating an ability to simulate rat (Pottenger *et al.*, 2000) or human (Völkel *et al.*, 2000) oral-route pharmacokinetic data, and without using published human tissue partition coefficients for BPA (Csanady *et al.*, 2002), the model was scaled and used to predict human blood concentrations after oral exposure. The confidence in such predictions is very low, and great care should be exercised in interpreting the results of the human simulations.

Völkel reported blood concentrations of BPA in human volunteers exposed orally to 5 mg BPA as below the analytical detection limit of 9 nM even though this was a dose considerably higher than worst case estimates of daily human exposures from the European Union risk assessment of 0.6 mg/day (European Commission, 2003). This worst-case estimate of human exposure is highly conservative and is not consistent with other estimates of less than 1 μg/person/day based on biomonitoring data (Arakawa *et al.*, 2004). The human PBPK model was fitted to this upper bound (9 nM) on BPA blood

concentration and so can provide only upper bound estimates of BPA concentrations after low oral exposure. The accurate predictions of blood BPAG concentrations and urinary elimination of BPAG by this model, however, raise the possibility of its use as a tool for exposure assessment. Both blood BPAG and urinary BPAG are biomarkers of exposure that have been used to estimate human exposure to BPA (Arakawa *et al.*, 2004; Ikezuki *et al.*, 2002; Ouchi and Watanabe, 2002). Bisphenol A is rapidly eliminated as the glucuronide through the urine after oral ingestion, with ~90% of the dose present in urine 6 h after ingestion. Reports of total BPA in spot samples of human urine are often accompanied by crude estimates of daily BPA exposure made by multiplying urinary BPA concentrations by an estimated urine output of 2l or by multiplying urinary BPA/g creatinine by an assumed daily creatinine elimination of 1.2 g/day (Arakawa *et al.*, 2004; Ouchi and Watanabe, 2002). It is clear, given the rapid urinary elimination of orally ingested BPA, that the amount of BPA found in urine spot sampling studies would be related to short-term exposure rates and patterns, and the time between ingestion of the prior meal, previous voiding of the bladder, and the time of urine collection, rather than longer term, average BPA exposures. These simple (*i.e.*, spot sampling) approaches do not account for the pharmacokinetics of BPA in humans, and they do not address the influence of ingestion and sampling patterns on these predictions. Limitations in these simple approaches to exposure estimation may be overcome through the use of a PBPK model that directly accounts for these important processes. Given urinary concentrations and some information about the exposure pattern and sampling time post-exposure, BPA exposures resulting in the observed urine concentration could be estimated with the model. An alternative experimental approach that would also overcome limitations of the spot sampling method would be to collect urine over a full 24 h period and report total BPA excreted.

The tissue-level response to exposure to weakly estrogenic compounds is determined by the magnitude, timing, and duration of exposure (Plowchalk and Teeguarden, 2002), and it is expected to correlate best with dose metrics closely related mechanistically to the response being studied. Uterine ER binding has been proposed as a relevant dose metric for the effects of weakly estrogenic compounds such as BPA (Plowchalk and Teeguarden, 2002). Recently, Degen *et al.* (2002) showed a poor correlation between blood concentrations of several weakly estrogenic compounds—BPA, genistein, and octylphenol—and uterine wet weight in the standard uterotrophic assay, demonstrating the limitations of blood dose metrics for predicting response to this class of compounds (Degen *et al.*, 2002). In light of these findings, we have adopted the approach proposed by Plowchalk (Plowchalk and Teeguarden, 2002), and correlated simulated estrogen receptor binding with uterine wet weight after oral exposure to BPA in rats. The results demonstrate clear correlations between receptor occupancy and uterine tissue response only when the ''free'' pharmacologically active BPA is allowed to partition into uterine tissue. This finding highlights the importance of incorporating the key processes controlling response to these compounds—absorption, tissue distribution, metabolic and other clearances, receptor binding, and restriction of free concentrations by plasma protein binding—into pharmacokinetic models expected to be used in safety assessments.

SUPPLEMENTARY MATERIAL

Supplementary data include a figure describing an elaboration of the PBPK model presented here (Model 2), simulated urinary and fecal elimination of BPA corresponding to Model 2, and the unpublished plasma protein BPA binding data reported by Mayersohn (2003). The supplemental data can be obtained through the publisher's web site (http://toxsci.oupjournals.org) by searching for the abstract to this manuscript.

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