Human and Rat ABC Transporter Efflux of Bisphenol A and Bisphenol A Glucuronide: Interspecies Comparison and Implications for Pharmacokinetic Assessment

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Significant interspecies differences exist between human and rodent with respect to absorption, distribution, and excretion of bisphenol A (BPA) and its primary metabolite, BPA-glucuronide (BPA-G). ATP-Binding Cassette (ABC) transporter enzymes play important roles in these physiological processes, and their enzyme localization (apical vs. basolateral) in the plasma membrane allows for different cellular efflux pathways. In this study, we utilized an ATPase assay to evaluate BPA and BPA-G as potential substrates for the human and rat ABC transporters: P-glycoprotein (MDR1), multidrug resistance–associated proteins (MRPs), and breast cancer–resistant protein (BCRP). Based on high ATPase activity, BPA is likely a substrate for rat mdr1b but not for human MDR1 or rat mdr1a. Results indicate that BPA is a potential substrate for rat mrp2 and human MRP2, BCRP, and MRP3. The metabolite BPA-G demonstrated the highest apparent substrate binding affinity for rat mrp2 and human MRP3 but appeared to be a nonsubstrate or potential inhibitor for human MRP2, MDR1, and BCRP and for rat mdr1a, mdr1b, and bcrp. Analysis of ABC transporter amino acid sequences revealed key differences in putative binding site composition that may explain substrate specificity. Collectively, these results suggest that in both rat and human, apical transporters efflux BPA into the bile and/or intestinal lumen. BPA-G would follow a similar pathway in rat; however, in human, due to the basolateral location of MRP3, BPA-G would likely enter systemic and portal blood supplies. These differences between human and rodent ABC transporters may have significant implications for interspecies extrapolation used in risk assessment.

Key Words: metabolism, xenobiotic transporters, physiologically-based pharmacokinetics.

Bisphenol A [4,4′-(propane-2,2-diyl)diphenol; BPA] is a high-production volume chemical used in the manufacture of polycarbonate plastics and epoxy resins (Teeguarden et al., 2005). Widespread inclusion of BPA in consumer products, including the protective lining of metal food cans, polyvinyl chloride pipes, and shatter-proof plastic bottles, has greatly enhanced the potential for human exposure (Doerge et al., 2010). Although BPA is classified as a suspected endocrine-disrupting chemical, which displays aberrant reproductive and developmental effects in laboratory animals, safety concerns regarding low-dose human exposure remain controversial (Volkel et al., 2002).

Xenobiotic pharmacokinetics involve dynamic processes including uptake, distribution, metabolism, and excretion of the parent chemical and its metabolite(s) (Pang et al., 2009). In humans and rodents, BPA undergoes extensive phase II metabolism, via uridine diphosphate-glucuronosyltransferase conjugation, to form the metabolite, BPA-glucuronide (BPA-G) (Pritchett et al., 2002; Kuester and Sipes, 2007). In general, BPA conjugation first occurs presystemically in the gastrointestinal tract, followed by conjugation in the liver during first-pass metabolism (Fisher et al., 2011; Mazur et al., 2010).

Interspecies metabolic differences exist between primates and rodents regarding the excretion of BPA and BPA-G. In human studies, administered BPA is predominantly recovered as the BPA-G metabolite in urine, whereas in rats, the biliary excretion of BPA predominates, with a small percentage of BPA-G excreted in the urine (Inoue et al., 2003; Teeguarden et al., 2005; Volkel et al., 2002). The physicochemical properties of xenobiotics and their metabolites help dictate their biological fate and kinetics, including entry (influx) and exit (efflux) across cellular membranes (Oswald et al., 2007). Although BPA is an uncharged, relatively small, and moderately hydrophobic molecule, BPA-G is a much larger hydrophilic anion at physiological pH levels. In general, smaller uncharged molecules favor passive diffusion, whereas
larger charged molecules require mediated transport to cross cellular membranes (Hirouchi et al., 2009; Morck et al., 2010; Zamek-Gliszczynski et al., 2011).

It is well established that active transport can be a major pharmacokinetic determinant in the disposition and metabolism of xenobiotics (Kim et al., 2006). The ATP-Binding Cassette (ABC) superfamily is composed of ATP-hydrolyzing enzymes that actively transport a broad range of substrates (Glavinas et al., 2004). Cytoplasmic ATP-binding domains harness energy from ATP hydrolysis for substrate transport across membranes, whereas membrane-spanning domains, composed of multiple transmembrane (TM) α-helices, form the substrate binding pocket (Aller et al., 2009). Specific amino acids within or near transporter binding pockets have been shown to play important roles in transporter-specific substrate specificity based on hydrophobic, aromatic, and noncovalent interactions (Haimeur et al., 2004a).

ABC efflux transporters are expressed at high levels in organs of excretion, such as the intestine, liver, kidney, and placenta (Prouillac and Lecoeur, 2010). It is widely believed that their predominant role is to prevent the cellular accumulation of potentially toxic substances (Oswald et al., 2007). However, localization of these transporters on the apical or basolateral side of plasma membranes is an important determinant of xenobiotic transport. In the liver, efflux transporters expressed on the apical side of hepatocyte membranes will efflux xenobiotics out of the cell and into the bile, whereas those expressed on the basolateral side will efflux xenobiotics to the blood. In the placenta, membrane localization can be a critical determinant of fetal exposure through the efflux of xenobiotics into the fetal circulation. Additionally, interspecies differences in transporter-based efflux in the small intestine and liver may alter the kinetics of first-pass metabolism or systemic clearance of xenobiotics. Cell-based assays, such as Caco-2 cells, are often used to address permeability across the intestinal barrier; however, characterizing the roles of specific transporters may be difficult as the available chemical inhibitors often cross-react with multiple transporters (Giacomini et al., 2010).

BPA metabolism is often studied in vitro using subcellular microsomal fractions or isolated cryopreserved primary hepatocytes (Kuester and Sipes, 2007; Mazur et al., 2010). Although such techniques are useful for high-throughput pharmacokinetic analyses, the cellular machinery that allows efflux transport in vivo is rapidly disrupted when cells or subcellular fractions are isolated from the intact organ (Li et al., 2009). ATPase assays utilize membranes with individual functioning transporters and allow for the rapid screening and identification of potential substrates and inhibitors.

Given that ABC transporters play a key role in determining the exposure of various organs and tissues to a variety of environmental toxins, drugs, and other xenobiotics, a better understanding of their substrate specificity, cellular localization, and interspecies differences is necessary. This study investigates the interaction of BPA and its primary metabolite, BPA-G, in vitro with specific members of three major subfamilies of human and rat ABC efflux transporters: P-glycoprotein (MDR1), various multidrug resistance–associated proteins (MRPs), and breast cancer–resistant protein (BCRP). To mechanistically evaluate species- and isoform-related differences in substrate specificity, ABC transporter amino acid sequences were analyzed at known substrate binding and recognition sites to identify differences that may impact BPA and BPA-G transport.

### MATERIALS AND METHODS

**Reagents.** BPA (> 99% purity) and acetaminophen (> 99% purity) were purchased from Sigma-Aldrich (St. Louis, MO); BPA-G (> 98% purity) was a gift from the National Institute of Environmental Health Sciences (Research Triangle Park, NC). All chemical reagents, selective ATPase activity inhibitors (orthovanadate), and transporter substrates (verapamil, sulfasalazine, probenecid, and benz bromarone) were included in the ATPase Assay Kits purchased from BD Biosciences Discovery (Woburn, MA).

**ATPase activity assays.** Baculovirus-infected insect cells expressing human MDR1, BCRP, MRP2, MRP3, and rat mdr1a, mdr1b and bcrp and mrp2 (rat mpr3 unavailable) transporter isoforms were purchased from BD Biosciences Discovery Labware. Membrane preparations and ATPase assay techniques were used according to Sarkadi et al. (1992) with slight modifications. Insect cell membranes were stored at −80°C until use. ATPase assay buffer comprised 50mM Tris-Mes buffer (pH 6.8), 50mM KCl, 2mM DTT, 2mM EGTA, and 5mM sodium azide and stored at −20°C until use. BPA and BPA-G stock solutions (60mM) were prepared in acetonitrile, and subsequent dilutions (1.95–62.5μM) were prepared using ATPase assay buffer containing 2.5% acetone; final acetone concentration in ATPase assay reaction wells was 0.83%. To perform the ATPase assay, cell membranes were first thawed in a 37°C shaking water bath and diluted to 1 mg/ml using assay buffer. Cell membranes were split into two samples with 400μM (final concentration) of the inhibitor, orthovanadate, added to one sample, whereas an equal volume of ultrapure water was added to the other. Transporter activity was measured as the orthovanadate-sensitive portion of the total ATPase activity. Cell membrane samples (20 μl) were loaded in triplicate onto 96-well flat bottom plates (Microtest, Franklin Lakes, NJ) with and without orthovanadate. Serial dilutions of test chemicals and positive control substrates (20 μl) were added to the wells sequentially and incubated for 5 min at 37°C while shaking (BioTek Synergy HT plate reader; Winooski, VT). The ATPase activity reaction was initiated by adding 20 μl (4mM final concentration) MgATP to each well, followed by 10–60 min of shaking incubation at 37°C (based on each transporter protocol). Reactions were stopped by adding 10% SDS (30 μl) to each well. Liberation of inorganic phosphate was determined by adding 200 μl of detection reagent (2.5 ml of 70mM ammonium molybdate, pH 5.0, and 2.5 ml of 30mM zinc acetate, pH 5.0, mixed with 20 ml of 10% ascorbic acid, pH 5.0) to each cell, followed by a 20-min shaking incubation at 37°C. The inorganic phosphate complex was detected by its absorbance at 800 nm.

**ATPase activity data analysis.** Baseline optical density was determined by subtracting the mean absorbance from triplicate wells with no substrate and inhibited with orthovanadate from wells containing no substrate or inhibitor. Triplicate control values of samples containing the transporter substrate, and inhibited with orthovanadate, were subtracted from control samples containing substrate and no inhibitor. The difference in ATPase activity in the presence or absence of the inhibitor (orthovanadate) represents the transporter-mediated activity. Baseline optical density was subtracted from the net value, determined with the positive control substrate, to give maximum optical density. Maximal ATPase activity was determined for each transporter using strong, specific activators as positive controls. ATPase activity for positive controls was set to 100%, and both BPA and BPA-G at varying concentrations were measured as a percent ATPase stimulation of the positive
controls. According to protocol, verapamil, sulfasalazine, probenecid, and benzbromarone (10–1000 μM) served as positive controls for rat and human MDR1, BCRP, MRP2, and MRP3, respectively. All test samples with BPA and BPA-G at varying concentrations were run in triplicate, with and without orthovanadate, and the net difference in absorbance was divided by the maximum optical density to give percent stimulation of ATPase activity. Net positive stimulatory activity was indicative of an apparent substrate, whereas values within standard error of the baseline threshold were considered non-substrates. ATPase activities with standard error below the baseline threshold were classified as potential substrates.

**Protein sequence analyses.** Rodent and human MDR1 P-glycoprotein (P-gp) and MRP protein amino acid sequences were obtained from GenBank (NCBI). Sequence homology and alignment were performed using the T-Coffee program for only those transporters that displayed significant interspecies differences (Notredame et al., 2000). Protein sequences were analyzed at distinct amino acids that have been reported to impact transporter substrate selectivity. A number of specific amino acid differences were identified among the transporters studied that may be modulating transporter substrate affinity for BPA and BPA-G.

**RESULTS**

**Characterization of ATPase Activities**

BPA and its metabolite BPA-G were assessed for efflux transport specificities among rat mdr1a, mdr1b, bcrp, mrp2 and human MDR1, BCRP, MRP2, and MRP3 transporters. It is important to note that even in the absence of a substrate, baseline ATPase activity exists in transporter membranes. Thus, it is possible to achieve negative ATPase activity percent values below baseline in the presence of a slowly transported substrate or potential inhibitor.

With respect to the MDR1 subfamily, our results demonstrated markedly different ATPase activation profiles for BPA among human MDR1, rat mdr1a, and rat mdr1b (Fig. 1). Human MDR1 displayed little BPA-stimulated ATPase activity (0–62.5 μM), with the highest stimulation (approximately 20% of positive control) occurring at 30 μM. BPA (0–62.5 μM) demonstrated a less stimulatory effect on rat mdr1a than human MDR1 as it displayed little to no ATPase activity. However, BPA with rat mdr1b demonstrated a significant increase in ATPase activity to about 60% of positive control. BPA-stimulated rat mdr1b ATPase activity followed the bell-shaped curve commonly observed among MDR1 substrates (Buxbaum, 1999), with maximum activity at approximately 15 μM BPA.

Significant differences in ATPase activity were also observed among the MRP subfamily in both rats and humans toward the metabolite BPA-G. Human MRP3 displayed high ATPase activity toward BPA-G to above 80% of positive control, with a continuous increase in stimulation that correlated with increasing BPA-G concentration (0–62.5 μM; Fig. 2). Rat mrp2 displayed low to moderate stimulation (approximately 30% of positive control) in response to BPA-G at concentrations greater than 30 μM, whereas human MRP2 showed little to no stimulation, with a slight inhibitory profile, in response to BPA-G. A similar trend occurred in these MRP transporters with respect to BPA. Although human MRP3 and rat mrp2 showed moderate ATPase stimulation (upwards of 30% of positive control) with the parent compound BPA, human MRP2 showed little BPA-induced ATPase stimulation (< 10% of positive control). Based on varying concentration profiles, ATPase activities determined at 30 μM best represented all transport families assayed with both BPA and BPA-G (Figs. 3A and B).

The BCRP subfamily of ABC transporters displayed slight differences in response to BPA, with human BCRP showing higher ATPase activity (approximately 20% of positive control at 30 μM BPA) than rat bcrp, which displayed little to no stimulation (≤ 10% of positive control at 30 μM BPA; Fig. 3A). In response to the metabolite BPA-G, both rat and human BCRP demonstrated little stimulation (< 5% of positive control; Fig. 3B).
indicating that BPA-G is likely not a substrate for the rat and human BCRP isoforms. These results are similar to that observed for the rat and human MDR1 isoforms in which BPA-G also did not appear to be a substrate, rather appearing to inhibit the ATPase activity of human MDR 1 and rat mdr1a and mdr1b (Fig. 3B).

FIG. 3. Interspecies comparison of human and rat ATPase activities of ABC efflux transporters with BPA (30μM; A) and BPA-G (30μM; B). ATPase activity for positive controls was set to 100%.
Transporter Amino Acid Sequence Analyses
In this study, initial comparisons of the full-length protein amino acid sequence revealed that the full-length human MDR1 sequence is 87 and 80% identical to the rat mdr1a and rat mdr1b isoforms, respectively, whereas the full-length rat mdr1a and rat mdr1b sequences were found to be 84% identical. Human MRP2 was found to have 78 and 46% amino acid sequence identity with the rat mrp2 and human MRP3 isoforms, respectively, whereas rat mrp2 and human MRP3 were found to be 45% sequence identical. Among these isoforms, protein amino acid sequence analyses confirmed the presence of known highly conserved amino acids required for general ABC transporter function including those present in the ABC signature and Walkers A and B motifs (Glavinas et al., 2004; Kim et al., 2006).

Protein sequences were further analyzed to identify nonconserved amino acids in putative ABC transporter substrate–binding regions that may have key roles in substrate specificity. These amino acid differences among the rat and human MDR1 isoforms were found exclusively in the TM helical binding pocket regions and included Gly64 (amino acid numbering based on the human MDR1 protein sequence), Ser196, Ile306, Val338, Ile340, Ala342, Leu762, Ser943, Leu975, Val981, and Gln990 (Fig. 4A). Among the rat and human MRP isoforms, differences at amino acids that may be a factor in substrate specificity were also identified and included Cys208 (amino acid numbering based on the human MRP1 protein sequence), Glu210, Trp261, Lys267, Lys319, Lys347, Pro448, Trp553, and Phe594 (Fig. 4B).

**DISCUSSION**
It is well established that ABC transporters play a fundamental role in the absorption, distribution, metabolism, and excretion of endogenous and exogenous chemicals, and transporter membrane localization can directly influence these processes (Glavinas et al., 2004). The apical localization of efflux transporters in the liver and intestine facilitates biliary excretion and substrate transport back into the intestinal lumen, respectively, whereas basolateral localization in both liver and intestine facilitates efflux of the substrate into blood circulation (Fig. 5) (Leslie et al., 2005). Insight into the physiological role of ABC transporters is critical for the pharmacokinetic assessment of BPA. Moreover, delineating inter- and intraspecies differences of BPA and BPA-G efflux is necessary to reduce uncertainties associated with allometric scaling of rodent kinetic parameters for human applications.

ABC efflux transporters use ATP as an energy source to transport substrates across cell membranes, and it is generally accepted that substrate-stimulated ATPase activity correlates well with substrate transport (Glavinas et al., 2004). Important interspecies differences in the efflux transporter specificities for BPA and BPA-G between rat and human ABC transporters.
were demonstrated in this study. ATP assays conducted with human P-gp (MDR1) and rat P-gp (mdr1a and mdr1b), which are apically localized, showed significant differences in BPA-stimulated ATPase activity. Among these P-gp isoforms, rat mdr1b demonstrated the highest apparent affinity for BPA as a substrate compared with human MDR1 or its rat ortholog, mdr1a. These results suggest a physiological preference for BPA efflux into the intestinal lumen and/or hepatobiliary excretion in rat.

Conflicting evidence exists regarding the efflux of BPA by P-gp. Using Caco-2 cells, BPA was previously reported to be a P-gp substrate (Yoshikawa et al., 2002), whereas human P-gp ATPase assays with limited BPA concentrations showed no stimulation of activity (Jin and Audus, 2005). To our knowledge, this is the first report assessing the potential role of the different rat P-gp isoforms (mdr1a and mdr1b) to efflux BPA. To date, the influence of efflux transporters has yet to be evaluated within established PBPK models, which use BPA rat liver metabolism data scaled for human applications (Teeguarden et al., 2005). Information about the transporter-mediated efflux kinetics of BPA can be used to parameterize the mechanistic components of such PBPK models more accurately.

Although P-gp (MDR1) transports a wide variety of structurally unrelated compounds, substrate specificity has been reported (Tang-Wai et al., 1995). Although highly conserved P-gp amino acids are critical for general ATP hydrolysis and transporter function, differences in nonconserved amino acids positioned within or near the binding pocket appear to play a critical role in substrate specificity via the introduction of hydrogen bonds, charged groups, and differences in size and/or hydrophobicity. These changes can result in large changes in substrate binding affinity because unfavorable contacts may reduce optimal interaction. Through mutational and structure-function studies, a number of amino acids involved in P-gp substrate specificity have been identified (Aller et al., 2009; Hafkemeyer et al., 1998; Loo and Clarke, 1994). Our results revealed that rat mdr1b differs from human MDR1 and rat mdr1a at a number of these amino acids (Fig. 4A). In TM8 and TM12 of human MDR1 and rat mdr1a, two leucine residues appear to play key roles in substrate-protein interaction, whereas rat mdr1b has methionine residues at these positions (M759 and M972) (Aller et al., 2009; Hafkemeyer et al., 1998). Methionine sulfur atoms can form unique interactions with nonprotein constituents and are highly susceptible to oxidation, which can lead to changes in substrate binding pocket conformation. In TM11 of human MDR1 and rat mdr1a, a serine residue is believed to directly modulate substrate binding, and mutation to an alanine (the residue present in rat mdr1b-A940) leads to alterations in substrate specificity. Collectively, we hypothesize that the substrate-binding pocket of rat mdr1b differs from human MDR1 and rat mdr1a both in primary sequence and higher order structure, which may be the molecular basis underlying the specificity of rat mdr1b for BPA.

Upon entering a hepatocyte or enterocyte, BPA may be either effluxed out of the cell or metabolized to BPA-G (Fig. 5). Comparing our results for BPA-induced stimulation of ATPase activity with efflux proteins from the MRP and BCRP family, the rat mpr2 isoform demonstrated higher activity than human MRP2, whereas BCRP was the sole efflux transporter for which human ATPase activity was greater than rat. Although identifying positive stimulation of efflux transporters is critical, knowledge of possible inhibition or slow transport interactions that lower
baseline activity (below 0%) is important to understanding possible chemical-chemical interactions. Out of the eight different rat and human efflux transporter assays evaluated in this study, six assays conducted with the BPA-G metabolite demonstrated ATPase activity below the baseline. With the anionic BPA-G, neither rat nor human MDR1 or BCRP isoform(s) demonstrated detectable efflux transport. The observed decrease below baseline activity may indicate potential inhibitory effects and requires further investigation (Giacomini et al., 2010). Importantly, inhibiting efflux transport processes may also adversely affect target dose concentrations of therapeutic agents or clearance capacity for other xenobiotic exposures.

The MRP subfamily has a general affinity to efflux hydrophilic organic anions including glucuronate and glutathione conjugates (Glavinas et al., 2004). Our results indicate that significant inter- and intraspecies differences toward BPA-G were observed between MRP2 and MRP3. Human MRP3 demonstrated the highest apparent substrate affinity for BPA-G (rat mrp3 was unavailable for purchase), suggesting potential basolateral transport preference of this metabolite into the blood supply. Among MRP2 isoforms, rat isoforms demonstrated higher BPA-G-induced ATPase stimulation than human isoforms. These results indicate preference for hepatobiliary or intestinal lumen excretion within rat versus human. The implication of these findings is highly significant in light of a recent report indicating expression of MRP2 is approximately 10-fold higher in rat liver than in either monkey or human livers (Li et al., 2009). In liver and intestine, the apical localization of MRP2 would facilitate efflux back into the intestinal lumen or excretion into bile within the liver, whereas the basolateral localization of human MRP3 would provide a pathway entrance into systemic blood flow.

Currently, the MRP family of transporters is not well characterized, and the majority of MRP substrate specificity studies involve human MRP1 amino acids (Campbell et al., 2004). In this study, we identified MRP3 isoform-specific differences at specific amino acids that may be a factor in MRP3 substrate specificity for BPA-G (Fig. 4B). For example, Human MRP3 was found to differ from both human MRP2 and rat mrp2 with the presence of two tryptophan amino acids at positions 260 and 539. Due to their bulky aromatic side chains, these amino acids are believed to contribute significantly to MRP substrate specificity by altering the structure of the substrate binding pocket (Ren et al., 2001). These and other differences in the human MRP3 amino acid sequence support our findings that human MRP3 is distinct in its apparent preference for BPA-G.

Remarkable physiological differences exist between primates and rodents in the pharmacokinetic behavior of BPA. In vivo studies indicate that the bioavailability of orally administered BPA is low in rats, monkeys, and humans (Doerge et al., 2011a,b; Volkel et al., 2002). In humans and nonhuman primates (monkeys), ingested BPA is rapidly metabolized in the gastrointestinal tract and liver, and over 95% of the BPA is excreted in urine as BPA-G; only a few percent (2–3%) of an orally administered dose of BPA was accounted for in feces of monkeys (Kurebayashi et al., 2002). In contrast, rodent studies show that most (85%) BPA is eliminated in feces as BPA, whereas the remainder (15%) is excreted in urine as BPA-G (Pottenger et al., 2000).

Understanding the role and impact of transporter proteins on the disposition of xenobiotics such as BPA and BPA-G will help provide a mechanistic framework to describe the influx and efflux of materials in target organs in PBPK models. Characterizing active transport in PBPK models becomes important when concentration gradients are observed between blood and an organ that cannot be described by thermodynamic properties (i.e., tissue:blood partition coefficient) or other interactions, such as nonspecific binding. In the case of BPA-G, transporters may be the primary reason for differences in excretion between rodents and primates. Furthermore, potential BPA-G inhibition of other transport processes could alter the efflux pharmacokinetics of the parent BPA in both the liver and intestine, thus changing its intracellular concentration and possibly driving BPA efflux via alternate uninhibited transport pathways (Fig. 5).

In summary, our results indicate that BPA generally stimulates the ATPase activity of the rat apical transporters mdr1b, mrp2, and bcrp, whereas BPA-G stimulates mrp2, suggesting possible transport preferences of both BPA and BPA-G into the intestinal lumen and hepatobiliary excretion in rats. In humans, BPA induces increased ATPase stimulation for the efflux transporters MDR1, MRP2, and BCRP located apically and MRP3 located basolaterally (Fig. 5). MRP3 showed the highest ATPase activity in response to BPA-G with no stimulation of the apical transporters. This suggests possible transport preferences of BPA-G into the blood supply of the liver or portal blood supply of the small intestine in humans. Our human MRP3 data support assumptions used by Fisher et al. to describe the pharmacokinetics of orally administered BPA in monkeys and humans using a PBPK model (Fisher et al., 2011). For the BPA PBPK model, extensive metabolism of BPA to BPA-G was predicted in the small intestine (enterocytes), and the systemic uptake of BPA-G from the intestine was described using a large first-order rate constant, consistent with active transport of BPA-G from the enterocytes into the blood supply. Further interspecies studies are needed to address MRP differences across rodents themselves because mrp3-deficient mice dosed with BPA demonstrate lower BPA-G levels compared with wild-type (Hirouchi et al., 2009).

Additional in vitro transport studies are needed to assess BPA systemic clearance adequately. Following the identification of key transporter specificities for BPA and BPA-G through ATPase assay measurements, other techniques including membrane vesicles and cell-based assay systems can be carried out to delineate the quantitative aspects of the bidirectional transport of these compounds at environmentally relevant concentrations (Giacomini et al., 2010). In vitro methods including hepatic and Caco-2 cells can be used to gain a mechanistic and quantitative understanding of the transport kinetics of BPA and
BPA-G. This will greatly reduce the uncertainties of PBPK model parameter values currently used to describe the oral uptake of BPA and BPA-G into the portal blood perfusing the liver of both rats and humans and the biliary secretion of BPA-G. Clarifying the histological localization for many transporter proteins is necessary to understand physiological function and may prove critical for assessing BPA fetal-placental transfer (Prouillac and Leceouer, 2010). Furthermore, future PBPK exposure models assimilated with high-throughput in vitro clearance data, derived using subcellular liver microsomes or hepatocyte suspensions that have limited transport activities, must evaluate whether transport kinetics are the rate-determining step in hepatic elimination (Hirouchi et al., 2009).

REFERENCES


BPA AND BPA-G ABC TRANSPORTER EFFLUX


