METABOLISM OF BISPHENOL A IN PRIMARY CULTURED HEPATOCYTES FROM MICE, RATS, AND HUMANS

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ABSTRACT:

Studies have shown that in the rat, bisphenol A (BPA) is metabolized and eliminated primarily as a monoglucuronide, a metabolite without estrogogenic activity. The purpose of this study was to determine the extent of monoglucuronide formation in monolayers of hepatocytes from rats, mice, and humans. Noncytotoxic concentrations of BPA (10, 20, and 35 μM; 1.0 μCi), as assessed by lactate dehydrogenase leakage, were incubated with isolated hepatocytes for 0–6 h. Media were collected and analyzed for metabolites by radiochemical high performance liquid chromatography and liquid chromatography-tandem mass spectrometry. The metabolites identified include a monoglucuronide (major metabolite), a sulfate conjugate, and a glucuronide/sulfate diconjugate (minor metabolites). In hepatocytes of male Fischer-344 rats, the predominate metabolite was the diconjugate (glucuronide/sulfate). Under these conditions, the extent of metabolism by 3 h was similar in all species tested because all BPA was converted to conjugates by 3 h. Initial rates of metabolism in hepatocytes followed the order of mice > rats > humans. However, when extrapolated to the whole liver (i.e., cells per liver), the hepatic capacity for BPA glucuronidation is predicted to be humans > rats > mice. This research was supported in part by The Society of Plastics Industry Inc., and Southwest Environmental Health Science Center (ES 06694).

Bisphenol A (BPA) is used in the production of polycarbonates, epoxy resins, phenolic resins, and diacrylates. Polycarbonates, one of the most widely used plastics, accounts for 60% of the total production of BPA (Perez et al., 1998). Epoxy-based resins are used in a variety of consumer products that include decorative floor manufacturing, lacquer coatings in cans, dental composites and sealants, and as additives in the production of vinyl and acrylic resins. Trace amounts of BPA monomer have been reported to leach out of polycarbonate and epoxy resins (Brotons et al., 1995). Such leaching may result in systemic exposure to BPA monomer, as assessed by lactate dehydrogenase leakage, were incubated with isolated hepatocytes for 0–6 h. Media were collected and analyzed for metabolites by radiochemical high performance liquid chromatography and liquid chromatography-tandem mass spectrometry. The metabolites identified include a monoglucuronide (major metabolite), a sulfate conjugate, and a glucuronide/sulfate diconjugate (minor metabolites). In hepatocytes of male Fischer-344 rats, the predominate metabolite was the diconjugate (glucuronide/sulfate). Under these conditions, the extent of metabolism by 3 h was similar in all species tested because all BPA was converted to conjugates by 3 h. Initial rates of metabolism in hepatocytes followed the order of mice > rats > humans. However, when extrapolated to the whole liver (i.e., cells per liver), the hepatic capacity for BPA glucuronidation is predicted to be humans > rats > mice. This research was supported in part by The Society of Plastics Industry Inc., and Southwest Environmental Health Science Center (ES 06694).

1 Abbreviations used are: BPA, bisphenol A; HC, hepatocytes; HPLC, high pressure liquid chromatography; F-344, Fischer-344; SD, Sprague Dawley; LDH, lactate dehydrogenase; CID, collision-induced dissociation; LC-MS/MS, liquid chromatography-tandem mass spectrometry; MS, mass spectrometry; MS/MS, tandem mass spectrometry; UGT, glucuronosyl transferase.

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Materials and Methods

Chemicals. Radiolabeled BPA [propyl-2-14C] (1.0 Ci/ml; 4.0 mg/ml in ethanol) with specific activity of 56 mCi/ml) was provided by Wizard Laboratories (Davis, CA). Radiochemical purity was 99.3%. Unlabeled BPA was supplied by RTI International Laboratory (Research Triangle Park, NC; Log 9176-10-02). The purity of both 14CBPA and unlabeled BPA was confirmed by HPLC and found to be greater than 99%. Purity was reassessed routinely
throughout the course of experiments. BPA-glucuronide standard was provided by the Dow Chemical Company (Midland, MI); Bovine liver β-glucuronidase type B-10, Helix pomatia sulfatase type H-1, and s- saccharic acid 1,4-lactone were obtained from Sigma-Aldrich (St. Louis, MO); Flo-Scent III (Packard BioScience Co., Meriden, CT); Universol Cocktail (ICN Radiochemicals, Irvine, CA); and HPLC grade acetonitrile (Burdick and Jackson, INC., Muskegon, MI) were obtained from the indicated vendors.

**Animals.** Male Fischer-344 (F-344) (250–300g, 77 days old), female F-344 (200–250g, 77 days old), male Sprague-Dawley (SD) (250–300g, 77 days old), female SD (200–250g, 77 days old) rats and male CFI (30–40g, 77 days old) and female CFI (25–30g, 77 days old) mice were purchased from Harlan Sprague-Dawley Inc. (Indianapolis, IN). The animals were housed in polyethylene cages with free access to food (Harlan Teklan 4% mouse/rat diet; Harlan Teklan, Indianapolis, IN) and water. All animals were maintained in an Association for Assessment and Accreditation of Laboratory Animal Care approved animal care facility for at least 1 week before experimentation.

**Hepatocyte Isolation and Treatment.** Mouse and rat hepatocytes. Animals were anesthetized with sodium pentobarbital, the portal veins cannulated and livers perfused for 4 min with Hanks balanced salt solution containing EGTA and HEPES. The livers were then perfused with collagenase solution (6.5 min for the rat, 5.5 min for the mouse). Flow rates for perfusion of rat livers were 40 and 20 ml/min for Hanks balanced salt solution and the collagenase solution, respectively. Flow rates for the perfusion of mouse livers were 5 ml/min for each solution. Following these perfusions the livers were removed and dissociated by massaging through sterile gauze. HC were isolated by slow speed centrifugation (2 × 400 rpm, 3 min). Only those cell preparations with >90% viability (as determined by trypan blue exclusion) were used for primary culture.

**Human hepatocytes.** Human HC were supplied by Clonetics Normal Human Cell Systems (San Diego, CA). They were obtained by the Department of Surgery/Transplantation Institute, University of Pittsburgh. Human HC were obtained in large numbers (up to 18.5 × 10⁶) from split and whole livers using a procedure similar to the collagenase perfusion technique used in rodent and human biopsies (Dorko et al., 1994). Upon arrival of human HC, cell viability was determined by trypan blue exclusion. Only those cell preparations with >85% viability or greater were used for cell culture experiments.

**Cell Incubations.** Approximately 0.5 × 10⁶ cells were plated into six-well tissue culture plates (BD Biosciences, Falcon, Franklin Lakes, NJ) containing Williams medium E (Invitrogen, Carlsbad, CA) supplemented with fetal bovine serum (10% for rats and humans, 1% for mice) (HyClone Laboratories Inc., Logan, UT). After 2 h the monolayers were rinsed once, and medium was replaced with serum-free Williams medium E (Invitrogen, Carlsbad, CA). The cells were then incubated with BPA at 37°C in an atmosphere of 5% CO₂, 95% air. For all hepatocyte experiments, a stock solution of BPA was made in dimethyl sulfoxide 100 times more concentrated than the incubation concentration. For time-dependent metabolism studies, a concentration range of (5–20 μM BPA at 5 μCi/incubation) was used. For concentration-dependent metabolism, a range of BPA concentrations (2.5–30 μM BPA at 0.5 μCi/incubation) were used. Rates of BPA metabolism were determined at an incubation time of 10 min. At appropriate times, plates were placed in liquid nitrogen (to stop BPA metabolism) and stored at −80°C until analysis. Data from isolated HC were used to calculate the in vivo hepatic clearance capacity based on cell number (Kedderis and Held, 1996).

**Determination of Cytotoxicity in Hepatocytes.** HC were incubated for 18 h with BPA over a wide range of concentrations (5–100 μM). Following incubation, the medium was removed and the monolayer rinsed with saline. The cells were then harvested into phosphate buffer containing Triton X-100. Both medium and cells were analyzed for lactate dehydrogenase (LDH) activity (LDH-20 enzymatic kit; Sigma-Aldrich Diagnostics). The LDH activity was expressed as the percentage of LDH released into the medium (LDH in the medium divided by total LDH in cells and medium multiplied by 100).

**Determination of BPA Metabolites in Hepatocytes.** After storage at −80°C, samples were thawed and the medium was removed and placed in 2-ml centrifuge tubes. Plates were then rinsed 2 times with 1 ml of ethanol to lyse remaining cells and obtain intracellular metabolites and parent compound. Initially, ethanol washes were counted on a Beckman scintillation counter (Beckman Coulter, Inc., Fullerton, CA) to determine remaining total cellular radioactivity. However, subsequent ethanol washes have been run on the HPLC to identify the source of cellular radioactivity. Samples from HC medium and ethanol washes were vortexed for 30 s and then centrifuged for 4 min at 12,000 rpm. Aliquots of medium or ethanol wash were injected (100 μl) onto a 300 mm × 3.9 mm Alphabond-C₁₈ 125A 10 μm analytical column (Alltech Associates Inc., Deerfield, IL), eluted with a mobile phase of water/ acetonitrile both containing 0.1% acetic acid, at a flow rate of 1 ml/min. Total run time was 50 min. The mobile phase gradient was run with 95% water and 5% acetonitrile for 5 min then to 5% water and 95% acetonitrile over 25 min. These conditions were maintained for 5 min, until the column was brought back to initial conditions over the next 15 min. The HPLC system used throughout this work was composed of a SP8800 ternary HPLC pump and SP8775 autosampler (Thermo Separation Products, San Jose, CA). The column effluent was monitored in tandem with a UV-visible detector (Spectra-Physics SP8450) at a wavelength of 280 nm and with a β-Ram Flow-Through radiochemical monitor system (IN/US Systems, Inc., Tampa, FL). Bisphenol A eluted at 26 min under these conditions. Bisphenol A-glucuronide generated from HC experiments coeluted with the synthetic BPA-glucuronide standard at 21 min.

**β-Glucuronidase and Sulfatase Assay.** Samples were subjected to enzymatic hydrolysis to identify possible glucuronide or sulfate conjugates of bisphenol A. Samples were incubated at 37°C for at least 24 h with β-glucuronidase (2000 U/ml) or sulfatase (100 U/ml) according to the method of Peters and Caldwell (1994) and analyzed by HPLC with the same method as described above.

**LC-MS/MS.** Metabolites were mass analyzed on a Finnigan TSQ 7000 triple quadrupole mass spectrometer (Thermo Finnigan MAT, San Jose, CA) set to the electrospray negative-ionization mode. For mass analysis in the negative ion mode, the HPLC conditions were similar to those described above except no acetic acid was added to the mobile phase. After on-column separation of BPA and metabolites, the column out flow was introduced into the mass spectrometer ion source in its entirety at 0.3 ml/min. The ions with m/z values corresponding to BPA and putative metabolites were subjected to collision-induced dissociation (CID) using argon gas within the collision cell of the mass spectrometer and the subsequent product ions were mass filtered to produce the corresponding product ion mass spectrum. Metabolite fragmentation patterns observed in the resulting CID-MS/MS spectra were compared with standards (BPA, BPA-glucuronide) to provide further evidence as to metabolite identity.

**Results**

**Cell Cytotoxicity.** After 18 h of incubation of rat HC with concentrations of BPA ranging from 5 to 50 μM, the LDH released at these concentrations was the same as that of control HC incubated for 18 h. At BPA concentrations of 75 and 100 μM, BPA-induced cytotoxicity was apparent as assessed by the increased LDH activity in the media (Fig. 1). Based on these LDH values, noncytotoxic
concentrations of BPA (less than 40 μM) were used for metabolism studies with incubations time of 3 h or less.

In preliminary experiments, human and rat HC were incubated for 12 h in the presence of 14C-labeled BPA (35 μM). The resulting radiochromatogram shows four peaks (Fig. 2). Two of the four peaks were putatively identified by coelution with BPA and the authentic BPA monoglucuronide. BPA eluted at 26 min (peak D) and the BPA-glucuronide eluted at 21 min (peak C). Additional metabolites eluted at 4 and 19 min (peaks A and B).

**β-Glucuronidase and Sulfatase Assay.** To further characterize the metabolites of BPA produced by HC, samples from male human HC were incubated with β-glucuronidase or sulfatase to determine whether these treatments altered the HPLC profile. Treatment with these enzymes resulted in a change in the HPLC profile. Incubation of the samples with β-glucuronidase resulted in loss of peak C (BPA-glucuronide) with a subsequent and equivalent increase in peak D (BPA). Additionally, peak A decreased with a corresponding increase in peak B. Incubation of HC samples with sulfatase caused a decrease in peaks A and B with a corresponding increase in peaks C and D. Since β-glucuronidase and sulfatase both affected peak A, this metabolite is most likely a diconjugate of BPA. Peak B decreased only when incubated with sulfatase, suggesting a BPA-sulfate conjugate.

**LC-MS/MS.** Bisphenol A metabolites generated from hepatocytes were separated via HPLC and subjected to mass spectral analysis. The ions with m/z values corresponding to BPA metabolites were subjected to CID.

Peak D (Rf, 26 min) was determined to be BPA. This peak had the identical HPLC profile and electrospray mass spectrum as the BPA standard ([M – H]– ion of m/z 227). The CID product ion spectrum from [M – H]– m/z 227 produced fragment ions at m/z 210 and 198 (Table 1). Plausible chemical formulas for these fragment ions are C14H10O2 (m/z 210) and C13H10O2 (m/z 198).

The major metabolite (Peak C; Rf, 21 min) was identified as the BPA monoglucuronide. This peak, which was sensitive to enzymatic hydrolysis with β-glucuronidase, had the identical HPLC profile as the BPA monoglucuronide standard. The deprotonated molecule ([M – H]−, m/z 403) of this metabolite, when subjected to CID yielded fragment ions of m/z 227, 192, and 176. The fragment ion observed at m/z 176 corresponds to the radical anion, C19H16O6. Another fragment ion signal at m/z 192 is characteristic of the radical anion, C19H16O7. The major fragment ion signal at [M – H]− 227 represents the m/z of BPA, which resulted from the loss of glucuronide to give the aglycone.

Metabolite A (Rf, 4 min) was determined to be a glucuronide/sulfate diconjugate metabolite (Table 1). The CID-MS/MS spectra (Fig. 3) of the [M – H]− ion at m/z 483 produced product ion signals at m/z 403, 307, and 227. The major product ion signal observed at [M – H]− 307 resulted from a loss of a glucuronide. The product ion observed at m/z 403 resulted from the loss of SO3. Another product ion signal at m/z 227 represents the [M – H]− of BPA, which resulted from the loss of SO3 and glucuronide. This confirmed the presence of a diconjugate metabolite of BPA containing both glucuronide and sulfate moieties.

Metabolite B (Rf, 19 min) was identified as BPA-sulfate. The resulting CID-MS/MS product ion spectrum of the [M – H]− ion at m/z 307 contained fragment ions at m/z 227 and 212. The major product ion signal was observed at m/z 212, presumably results from the loss of SO3 and methyl radical to give a radical anion, m/z 212. The signal observed at m/z 227 ([M – H]− of BPA) resulted from the loss of SO3.

**Metabolic Profile.** Hepatocytes from all species incubated with 20 μM [14C]BPA exhibited a time-dependent loss of radiolabeled BPA from the medium. This decrease represents an uptake of BPA into HC, as seen with an increase in cellular radioactivity at early time points (Fig. 4). The cellular radioactivity at these early time points (10 min, 30 min, and 1 h) represents predominately [14C]BPA, while at later time points (2, 3, 5, and 6 h) it represents [14C]BPA metabolites that have yet to be secreted back into the medium. Three hours after addition of [14C]BPA, more than 95% of parent BPA had been eliminated from the medium.

Once [14C]BPA was taken up into HC, it underwent conjugation. This can be seen with the time-dependent formation of BPA metabolites (Fig. 4) and their excretion into the medium. The percentages of metabolites found in the culture media are shown in Tables 2 and 3. The appearance of [14C]BPA-metabolites in the medium followed the loss of [14C]BPA from medium. The metabolic profile for male and female F-344 rat HC revealed glucuronide (30 and 86%), sulfate (1 and 0%), and glucuronide/sulfate (70 and 10%) conjugates of BPA, respectively. HC of male and female SD rats produced glucuronide (58 and 100%), sulfate (2 and 0%), and glucuronide/sulfate diconjugate (30 and 0%). Male and female CF1 mouse HC formed the monoglucuronide of BPA (100 and 86%). Time-dependent formation

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**TABLE 1**

<table>
<thead>
<tr>
<th>Peak Identification</th>
<th>HPLC Rf</th>
<th>m/z of [M – H]−</th>
<th>Fragmentation Losses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucuronide/Sulfate</td>
<td>4</td>
<td>483 (94)</td>
<td>403 (7), 307 (20), 227 (3)</td>
</tr>
<tr>
<td>Sulfate</td>
<td>19</td>
<td>307 (78)</td>
<td>227 (13), 212 (44)</td>
</tr>
<tr>
<td>Glucuronide</td>
<td>21</td>
<td>403 (50)</td>
<td>227 (32), 192 (10), 176 (15)</td>
</tr>
<tr>
<td>Bisphenol A</td>
<td>26</td>
<td>227 (98)</td>
<td>210 (65), 198 (25)</td>
</tr>
</tbody>
</table>

a See Fig. 2.
b HPLC retention time.
c Numbers in parentheses, relative abundance (% of highest peak).
of the BPA-glucuronide by human hepatocytes is shown in Fig. 5. Male human HC (n = 2) primarily produced glucuronide (85 and 75%). Minor metabolites, sulfate (0 and 7.5%), and glucuronide/sulfate conjugates (1 and 0%) were also observed. Female human HC (n = 3) produced glucuronide (93, 84 and 55%) and sulfate (0, 0 and 2%), and glucuronide/sulfate conjugates (4, 2 and 43%; Table 3).

Concentration-Dependent Metabolite Formation. When various concentrations of BPA (2.5–30 μM) were incubated for 10 min with hepatocytes, two maximal rates of glucuronidation were apparent depending on the concentration range of BPA (Fig. 6). For these initial rate studies the incubations were short (10 min) to maintain high concentrations of BPA (greater than 85%) in the cell. From these experiments the data are graphed as initial velocity (nmol/min/0.5 × 10^6 cells) versus substrate concentration. Kinetic data obtained from HC exhibited a biphasic curve in all species tested (Fig. 6). Because of this biphasic nature the data could not be evaluated by classic Michaelis-Menten analysis, hence V_{max} values were obtained from a visual examination of the curve taking the average rate obtained at the highest plateau (Table 5). The velocity at the lower plateau was approximately one-half of that observed at the higher plateau.

Discussion

Isolated HC from humans, rats, and mice were used to study the metabolism of BPA in a cellular system that closely simulates in vivo conditions. Freshly isolated hepatocytes represent a qualitative in vitro model for xenobiotic metabolism with the same phase I and phase II metabolites being formed as in vivo (Billings et al., 1977). These HC systems complement microsomal and purified enzyme systems in elucidation of the various steps involved in the biotransformation of chemicals and in the determination of the relative importance of each in an integrated system.

In this study, biotransformation experiments were conducted at concentrations of BPA that did not cause cytotoxicity over 18 h of incubation. Results showed clear evidence of cytotoxicity of BPA at concentrations of 50 μM or greater as evidenced by significant LDH leakage into hepatocyte medium. Other investigators have also shown BPA at higher concentrations to be cytotoxic to rat HC. For example Nakagawa and Tayama (2000) reported that BPA caused an impairment of mitochondrial function and consequent decrease in cellular levels of ATP. However, BPA was relatively nontoxic at concentrations below 250 μM and toxic above 500 μM. The differences observed in cytotoxic concentrations of BPA between these two studies may be attributed to different experimental design. In the studies reported here, monolayers of HC were incubated for 18 h with BPA. In the Nakagawa and Tayama study, suspensions of HC were incubated for only 3 h with BPA. Monolayers incubated for 18 h can be more sensitive because molecular/chemical changes may occur early but not be expressed as cytotoxicity until later. Indeed, Nakagawa and Tayama reported that BPA at 250 μM, which was noncytotoxic at 3 h, was slightly cytotoxic at later time points.

Cytotoxicity and metabolism of BPA appeared to be directly related. At noncytotoxic concentrations, BPA is extensively metabolized to the BPA monoglucuronide and other minor conjugates. At cytotoxic concentrations, the loss of BPA from media and the formation of the BPA metabolites were reduced significantly (data not presented). This suggests that BPA and/or nonconjugated metabolites contribute to the cytotoxicity. These metabolites might include products of cytochrome P450 catalyzed reactions such as 5-hydroxybisphenol A or its quinone derivative (Atkins and Roy, 1995). However, none of these nonconjugated metabolites were detected in our studies that used noncytotoxic concentrations of BPA. Nakagawa and Tayama (2000) provided further evidence for the importance of conjugation in reducing cytotoxicity. They showed that blocking BPA glucuronidation with salicylamide induced BPA cytotoxicity. As stated in the introduction, the BPA-glucuronide has also been shown not to bind to the estrogen receptor (Matthews et al., 2001). Thus, conjugation of the parent molecule was found to significantly reduce its cytotoxic and estrogenic potential.

BPA-glucuronide was the major metabolite in HC from all species except those of male F-344 rats. In this case, a BPA-glucuronide/sulfate diconjugate was the major metabolite formed. Diconjugates have been identified for other compounds. For example, an acyl glucuronide/sulfate conjugate of naproxen has been identified as a major biliary metabolite in male SD rats (Jaggi et al., 2002). Why HC from F-344 male rats form the diconjugate is not known. It may relate to different isoforms of glucuronosyl transferase (UGT) and/or sulfotransferase enzymes responsible for BPA metabolism. The biological relevance of this diconjugate also is not known. It has not been identified as a urinary metabolite of BPA in F-344 male rats in vivo (Pottenger et al., 2000). The major metabolite in urine from F-344 rats was the BPA monoglucuronide. Although several minor unidentified metabolites of BPA appeared in the urine of male F-344 rats, none correlated with the retention time of the diconjugate found in this study, despite very similar analytical conditions. Another explanation for this difference between the in vitro and in vivo results may relate to biliary excretion of BPA conjugates. If the BPA-glucuronide/sulfate diconjugate is preferentially excreted in bile, intestinal microflora containing considerable hydrolytic activity toward glucuronides and sulfates could release the aglycone (i.e., BPA), which can then be...
reabsorbed and enter enterohepatic circulation (Parkinson, 1998). It is interesting that hepatic microsomes from male F-344 rats showed a reduced capacity to form the monoglucuronide of BPA (Yokota et al., 1999). Similar results were obtained in the present study when comparing the glucuronidation of BPA among male and female SD and F-344 rats. It would be expected that the sulfate conjugate might form to a greater degree in HC of F-344 male rats. However, the glucuronide/sulfate diconjugate was the predominate metabolite at prolonged times of incubation. These results suggest that the monoglucuronide serve as precursor of the glucuronide/sulfate diconjugate.

Recent reports (Yokoto et al., 1999) indicate that the UGT isoform UGT2B1 is involved in BPA glucuronidation in male Wistar rats. In that study 65% of the male rat liver microsomal UGT activities toward BPA were absorbed by the anti-UGT2B1 antibody. These results suggest that 35% of the metabolism of BPA might be mediated by other UGT isoforms. In female Wistar rats 65% of the activity remained unabsorbed to this antibody, which suggests that other isoforms are responsible for the glucuronidation of BPA in female Wistar rats (Yokoto, 1999). The identification of the isozymes responsible for the phase II metabolism of BPA in various strains and species may provide further insight into possible species differences in the in vivo metabolism of BPA.

Contributions to the glucuronidation of BPA by different isoforms of UGT may also explain the biphasic response observed when different concentrations of BPA were incubated with hepatocytes. A low capacity, high specificity UGT could catalyze glucuronidation at low concentrations, whereas a high capacity, low specificity (promiscuous) UGT may be predominant at higher concentrations. Alternative explanations for the observed effect may be substrate activation of UGT. A biphasic curve was not observed when detergent-activated microsomes were incubated with various concentrations of BPA (unpublished data). In hepatocytes higher concentrations of BPA may serve to activate UGT and thus result in an increased rate of glucuronidation at higher substrate concentrations. Activation of CYP3A in hepatocytes by high substrate concentrations has recently been reported by Witherow and Houston (1999). Another explanation is that at 10 min of incubation, at low substrate concentrations, insufficient BPA has diffused from the medium to the microsomal UGT.

**TABLE 2**

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>% of Total Dose in Hepatocyte</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F-344&lt;sup&gt;a&lt;/sup&gt;</td>
<td>S-D</td>
</tr>
<tr>
<td>Glucuronide/Sulfate</td>
<td>70</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>Sulfate</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Glucuronide</td>
<td>30</td>
<td>58</td>
<td>100</td>
</tr>
<tr>
<td>Bisphenol A</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Peak identified from Fig. 2.

<sup>b</sup> See Fig. 4 for representative time course for formation of these metabolites.

**TABLE 3**

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>% of Total Dose in Hepatocyte Medium</th>
<th>Fem-1</th>
<th>Fem-2</th>
<th>Fem-3</th>
<th>Male-1</th>
<th>Male-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucuronide/Sulfate</td>
<td>4</td>
<td>2</td>
<td>43</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sulfate</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>7.5</td>
<td>75</td>
</tr>
<tr>
<td>Glucuronide</td>
<td>93</td>
<td>84</td>
<td>55</td>
<td>85</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>Bisphenol A</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Fig. 5.** Time-dependent glucuronide formation in isolated hepatocytes from human male and female hepatocytes.

Isolated hepatocytes (0.5 × 10<sup>6</sup>) were incubated with 20 μM BPA in a modified Williams E solution at 37°C for the indicated times.

**Fig. 6.** Effect of BPA concentrations on rate of formation of BPA-glucuronide by male F-344 rat hepatocytes (n = 3).

Isolated hepatocytes (0.5 × 10<sup>6</sup>) were incubated with (2.5, 5, 10, 15, 20, 30 μM) BPA in a modified Williams E solution at 37°C for 10 min. At 10 min, BPA-glucuronide was the only metabolite present.
Example calculation of in vitro data extrapolated to in vivo

\[ V_{\text{max}} = 0.36 \text{ nmol/min/0.5} \times 10^6 \text{ hepatocytes} \]

1. \( 0.36 \times 2 \times 60 = 43.2 \text{ nmol/h/10}^6 \text{ hepatocytes} \)
2. Avg. wt. of rats = 279 g
3. Liver wt. = 4% of body wt.
4. Avg. liver wt. = 11.19 g
5. Male Fischer factor: 128 \times 10^6 hepatocytes/g rodent liver (Kedderis and Held, 1996)
6. Total hepatocytes in liver = 11.19 g \times 128 \times 10^6 = 1431 \times 10^6 hepatocytes
7. Extrapolate to whole liver = 43.2 nmol/h/10^6 cells \times 1431 \times 10^6 hepatocytes (whole liver) *Total = 61.82 \mu\text{mol/h}

### TABLE 5

**In vitro BPA glucuronidation rates extrapolated to hepatic capacity for BPA glucuronidation**

Hepatic capacity was estimated by multiplying the higher \( V_{\text{max}} \) observed in monolayers containing 0.5 \times 10^6 hepatocytes by the total number of hepatocytes per liver. Hepatocytes per liver were estimated from the values reported by Kedderis et al. (1996) and were 128 and 137 \times 10^6 cells/g for rodent and human liver.

<table>
<thead>
<tr>
<th>Species</th>
<th>( V_{\text{max}} ) ( \mu\text{mol/h} )</th>
<th>Hepatic Capacity ( \text{nmol/min/0.5} \times 10^6 \text{ Hepatocytes} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female Fischer-344</td>
<td>0.46</td>
<td>46.5</td>
</tr>
<tr>
<td>Female Sprague-Dawley</td>
<td>0.39</td>
<td>54.5</td>
</tr>
<tr>
<td>Female CFI mouse</td>
<td>0.50</td>
<td>13.8</td>
</tr>
<tr>
<td>Female human</td>
<td>0.27</td>
<td>8 (mmol/h)</td>
</tr>
<tr>
<td>Male Fischer-344</td>
<td>0.36</td>
<td>61.8</td>
</tr>
<tr>
<td>Male Sprague-Dawley</td>
<td>0.45</td>
<td>79.9</td>
</tr>
<tr>
<td>Male CFI mouse</td>
<td>0.82</td>
<td>23.6</td>
</tr>
</tbody>
</table>

When BPA metabolism was studied in human hepatocytes, the results were similar to those obtained in rodent hepatocytes. BPA-glucuronide was the major metabolite formed by male and female human hepatocytes (Fig. 5). Minor amounts of the BPA sulfate and the diconjugate were formed. The only exception was in the hepatocytes obtained from one female. In this case the amount of diconjugate formed was nearly equivalent to that of BPA-glucuronide.

Human hepatocytes did differ from those obtained from rats and mice with respect to the rate of BPA-glucuronidation. Overall, the rate of formation of BPA-glucuronide was mice > rats > humans, when data are presented as nmol/min per 0.5 \times 10^6 hepatocytes. When the total hepatic capacity to glucuronidate BPA was calculated as shown in Table 4, this rank order was reversed (humans > rats > mice) (Table 5). This change in rank order reflects the total hepatocyte population (cells per liver) available to humans as compared with rodents. Hepatocytes per gram of liver were based on the estimates provided by Kedderis and Held (1996).

In summary, the extensive metabolism of BPA in primary cultured HC indicates that first-pass metabolism and rapid elimination of BPA would be probable following oral exposure. Humans appeared to have the largest capacity for glucuronidation, when the rates were extrapolated to the whole liver, although the confirmation of this finding is currently in progress with additional studies in human hepatocytes. This extensive conjugation of BPA can be considered an important detoxification reaction with respect to its estrogenic effects. Indeed, the BPA-glucuronide has been shown by Matthews et al. (2001) not to serve as a ligand for the estrogen receptor.

### References


