Inhibition of Genistein Glucuronidation by Bisphenol A in Human and Rat Liver Microsomes

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Nonstandard abbreviations used: HPLC, high-performance liquid chromatography; MS, mass spectrometry; MS/MS, tandem MS; ESI, electrospray ionization; RSD, relative standard deviation; BPA, bisphenol A; BPA gluc, bisphenol A β-D-glucuronide; genistein gluc, genistein 4’-β-D-glucuronide; SPE, solid phase extraction; m/z, mass to charge ratio; UDPGA, uridine 5’diphosphoglucuronic acid; UGT, UDP-glucuronosyltransferase;
ABSTRACT

Genistein is a natural phytoestrogen of the soybean, and bisphenol A (BPA) is a synthetic chemical used in the production of polycarbonate plastics. Both genistein and BPA disrupt the endocrine system in vivo and in vitro. Growing concerns of altered xenobiotic metabolism due to concomitant exposures from soy milk in BPA-laden baby bottles has warranted the investigation of the glucuronidation rate of genistein in the absence and presence (25 µM) of BPA by human liver microsomes (HLMs) and rat liver microsomes (RLMs). HLMs yield $V_{max}$ values of 0.93 ± 0.10 nmol/min/mg and 0.62 ± 0.05 nmol/min/mg in the absence and presence of BPA, respectively. $K_m$ values for genistein glucuronidation by HLMs in the absence and presence of BPA are 15.1 ± 7.9 µM and 21.5 ± 7.7 µM, respectively, resulting in a $K_i$ of 58.7 µM for BPA. A significantly reduced $V_{max}$ and unchanged $K_m$ in the presence of BPA in HLMs is suggestive of non-competitive inhibition. In RLMs, the presence of BPA resulted in a $K_i$ of 35.7 µM, an insignificant change in $V_{max}$ (2.91 ± 0.26 nmol/min/mg and 3.05 ± 0.41 nmol/min/mg in the absence and presence of BPA, respectively) and an increase in apparent $K_m$ (49.4 ± 14 µM with no BPA and 84.0 ± 28 µM with BPA), indicative of competitive inhibition. These findings are significant because they suggest that BPA is capable of inhibiting the glucuronidation of genistein in vitro, and that the type of inhibition is different between HLMs and RLMs.
INTRODUCTION

Glucuronidation is a major form of phase II xenobiotic metabolism, which is catalyzed by isoforms of the UDP-glucuronosyltransferase (UGT) family. UGTs are promiscuous enzymes capable of conjugating structurally diverse substrates. UGTs are constitutively expressed in a tissue-specific manner, with their expression and activity altered by genetic and environmental factors (Tukey and Strassburg, 2000). While some UGTs exist only in extra-hepatic tissues, most UGTs are expressed abundantly in the liver (Tukey and Strassburg, 2000; Miners et al., 2006). Although UGT isoforms vary between species, several inter-species homologues have been identified. Humans and rats have several orthologous UGTs, including 1A1, 1A3, 1A6, and 1A10. In general, rats have an overall greater capacity for glucuronidation than humans (Elsby et al., 2001; Völkel et al., 2002). Along with the required cofactor uridine 5’-diphosphoglucuronic acid (UDPGA), UGTs catalyze the synthesis of a β-D-glucuronide conjugate. Glucuronide conjugates are generally less biologically active and are more readily excreted than their parent substrates, making UGTs key players in the regulation of xenobiotic metabolism and toxicity.

Genistein [4’,5,7-trihydroxyisoflavone] is a natural phytoestrogen abundantly present in soybeans. Since nearly sixty percent of processed foods contain soy, humans consume a nearly continuous supply of genistein in their everyday diet (Patisaul and Jefferson, 2010). The pleiotropic effects of genistein remain controversial. While some laboratories report therapeutic effects of genistein such as chemoprevention, improved bone health, and amelioration of menopausal symptoms (Goldwyn et al., 2000; Evans et al., 2010), other groups emphasize the phytoestrogen’s adverse health effects, namely endocrine disruption (Jefferson et al., 2005; Casanova et al., 1999). In both humans and rats, genistein causes precocious puberty and altered
menstrual cycles (Strom et al., 2001; Casanova et al., 1999). Additional adverse effects of genistein in rats include inhibited embryonic development and impaired spatial learning (Xing et al., 2010; Ball et al., 2010).

Bisphenol A [(BPA) 4,4’-isopropylidenediphenol] is a synthetic monomer used in the synthesis of polycarbonate plastics and epoxy resins. BPA is a fairly ubiquitous compound, found as a common component in plastic baby bottles, as well as food and beverage containers. BPA can leach from plastic containers into contents, especially when containers are exposed to heat and/or excessive wear, placing humans at a direct risk of exposure (Brede et al., 2003). BPA has estrogenic activity both in vitro and in vivo across numerous species (Kuiper et al., 1998; Perez et al., 1998; vom Saal et al., 1998). Endocrine disrupting properties of BPA are routinely reported, and include inhibited embryonic development, altered postnatal growth rate, and induction of precocious puberty (Xing et al., 2010; Howdeshell et al., 1999).

The UGT isoforms that are primarily responsible for the glucuronidation of genistein and BPA have been identified. The primary human UGTs implicated in the glucuronidation of genistein are hepatic UGTs 1A1, 1A6, and 1A9, as well as extra-hepatic UGTs 1A10 and 2B17 (Doerge et al., 1999; Pritchett et al., 1998; Tang et al., 2009; Liu et al., 2007). Although the predominant UGTs responsible for the glucuronidation of genistein in rats have not been definitively identified, they are suspected to be UGTs 1A1, 1A6, and/or 1A10, since these isoforms are orthologous to the human UGTs (Tukey and Strassburg, 2000). The predominant UGT responsible for the glucuronidation of bisphenol A in rats is UGT2B1, which is homologous to human UGTs 2B7 and 2B17, neither of which is the primary UGT involved in human BPA glucuronidation (Elsby et al., 2001). Instead, human UGT2B15 is the primary UGT responsible for BPA’s glucuronidation in humans (Hanioka et al., 2008a).
Genistein and BPA share common metabolic pathways, with glucuronides being the predominant conjugate of each substrate formed in both human and rat liver microsomes. In addition to similar metabolism, genistein and BPA also exhibit comparable adverse effects of endocrine disruption when individuals are exposed during gestation and/or neonatal development. Humans and rats are routinely exposed to both endocrine disrupting compounds simultaneously in their everyday lives, and interactions between bisphenol A and genistein have been observed both in vitro and in vivo, warranting the analysis of genistein and BPA in a co-exposure scenario (Xing et al., 2010; Dolinoy et al., 2007). The goal of the present study was to investigate the inhibitory effect of BPA on the glucuronidation of genistein in vitro during simultaneous exposure to both endocrine disrupting compounds. Hepatic UGT inhibition was investigated in HLMs and RLMs using binary incubations of BPA and genistein.
METHODS

Chemicals and reagents. Chemicals were purchased from the sources indicated: Bisphenol A, genistein, ammonium acetate, UDPGA, alamethicin, and magnesium chloride (Sigma-Aldrich, St. Louis, MO); bisphenol A β-D-glucuronide (BPA gluc) and genistein 4’-β-D-glucuronide (genistein gluc; TRC Canada, North York, Ontario); ethyl acetate (pesticide grade), methanol (LC/MS grade), and formic acid (Fisher Scientific, Fair Lawn, NJ); and high purity solvents acetonitrile and water, both of HPLC grade (Honeywell Burdick and Jackson, Muskegon, MI). Bond Elut Plexa cartridges (60 mg, 1 mL) were purchased from Varian Inc. (Palo Alto, CA). Human liver microsomes (HLMs; pooled from 50 donors, mixed gender) and rat liver microsomes (RLMs; pooled from 100 female and 100 male Wistar rats) were purchased from XenoTech (Lenexa, Kansas).

Formation of glucuronide metabolites. Reagent pool was prepared by mixing together HLMs (0.0625 mg/mL) or RLMs (0.025 mg/mL) with alamethicin (25 µg/mg of protein in incubation), magnesium chloride (4 mM), and Tris-HCl buffer (pH 7.4 at 25°C, 50 mM). Microsomal protein concentrations were optimized through range-finding experiments with varying concentrations of HLMs and RLMs ranging between 0.5 to 0.025 mg/mL (data not shown). For enzyme kinetics studies, varying concentrations of either BPA or genistein were added (10, 25, 50, 100, 175, and 250 µM). Equal aliquots of reagent pool were placed in incubation vials (1.5 mL center drain glass vials, Sigma) and pre-incubated in a shaking water bath (80 strokes/min) at 37°C for 15 minutes. Reactions were initiated by the addition of UDPGA (5 mM), bringing each incubation volume to a total of 200 µL. After a 30 minute incubation period, reactions were terminated via addition of 400 µL of ice-cold acetonitrile. Duration of incubation was optimized by testing and analyzing total metabolite formation after 0,
15, 30, 45, and 60 minute incubations (data not shown). Samples were then centrifuged at 2,800 rpm for 20 minutes and supernatant was collected for eventual solid phase extraction (SPE) cleanup and high performance liquid chromatography-tandem-mass spectrometry (HPLC-MS/MS) analysis.

**Inhibition studies.** Reagent pools were prepared as previously described. To determine $K_i$, samples contained varying concentrations of genistein (10, 25, 50, 100, 175, 250 μM) in the absence (0 μM) and presence (25 μM) of BPA. The concentration range, 10 to 250 μM for genistein was chosen because it represented the high end of the environmentally relevant dose while allowing for good quantitative measurement with the available instrumentation. For IC$_{50}$ determination in HLMs, samples contained varying concentrations of BPA (0, 5, 10, 25, 50, 100, 250 μM) in the presence of 100 μM genistein.

**Sample Preparation:** Analytes were isolated from their matrix using SPE with Bond Elut Plexa cartridges and sequential additions of elution solvents ethyl acetate, methanol, and acetonitrile, as previously described by Coughlin *et al.*, 2011. SPE extracts were evaporated to dryness under vacuum and a stream of nitrogen before being reconstituted in 100 μL of 50% acetonitrile in water.

**Standard Solutions:** Initial genistein stock solutions were prepared by dissolving genistein in methanol; BPA, BPA gluc, and genistein gluc were initially dissolved in acetonitrile. All subsequent standards, ranging from 1 μg/mL to 1000 μg/mL, were prepared via serial dilution in acetonitrile and were stored at -20°C.

**Blank Controls:** Two sets of blank controls were used: incubation blanks and SPE blanks. Incubation blank controls contained all of the same reagents and substrates as the samples, however, the blanks were terminated via addition of ice-cold acetonitrile prior to
initiation of the reaction with UDPGA. SPE blank controls contained high purity water in place of standard solutions, and were processed alongside samples during SPE using all of the same supplies and reagents. The use of blank controls is especially important when analyzing ubiquitous compounds such as BPA, which is known to be prevalent in numerous laboratory supplies due to its use as a plasticizer (Stiles et al., 2007). The use of plastics was minimized wherever possible, such as using glass incubation vials instead of plastic, in an effort to reduce contamination with BPA.

**Analytical Conditions:** Analytical conditions were similar to those optimized by Coughlin et al., 2011, with minor modifications. Chromatographic separation was carried out with a Discovery C8 column (Supelco, St. Louis, MO; 50 x 4.6 mm I.D., 5 μm) and a Krud Katcher Ultra In-Line Filter guard column (Phenomenex; 0.5 μm). A gradient of solution A (10% acetonitrile in 2 mM ammonium acetate, pH 9), and solution B (acetonitrile) was used as follows: 0 to 3 min 45% B, 3 min to 14 min 45% to 89% B, 14 min to 18 min 89% B, 18 min to 18.2 min 89% to 45% B, 18.2 min to 20 min 45% B.

**Data Analyses:** All incubations for enzyme activity and inhibition were performed in triplicate for each substrate concentration, and each reconstituted extract was injected into the HPLC-MS/MS in triplicate. Replicate injections were averaged together for each sample. All incubations were performed three times, and reported values represent the overall average of these nine determinations ± relative standard deviation (RSD). Reported values have been blank-subtracted from raw data prior to calculation of analyte quantity. Michaelis-Menten values of apparent $K_m$ and $V_{max}$ were obtained by analyzing Lineweaver-Burk plots using GraphPad Prism 5.04 (La Jolla, CA), and confirmed via manual calculation using the Michaelis-Menten equation:
where $V_{\text{max}}$ represents the maximal reaction rate, $[S]$ represents the substrate concentration, and $K_m$ is the Michaelis constant, which is defined as the substrate concentration at half of the maximal reaction rate. $K_i$ values were found graphically using Lineweaver-Burk plots, and verified via manual calculation using the following equation:

$$K_m^{\text{apparent}} = \frac{(1 + \frac{[I]}{K_i}) \cdot K_m}{K_m}$$

where $K_m^{\text{apparent}}$ is the Michaelis constant in the presence of inhibitor, $K_m$ is the Michaelis constant in the absence of inhibitor, $[I]$ represents the inhibitor concentration, and $K_i$ represents the dissociation constant. IC50 values were found graphically based on plots created using GraphPad Prism 5.04. Unpaired student t-tests were performed using GraphPad Prism 5.04 to determine statistical significance between curves of 0 and 25 μM BPA. Data acquisition was performed using Xcalibur 2.0.7 and analyzed using Qual Browser 2.0.7 (Thermo Fisher Scientific). Quantitation was calculated in Excel 2003, using manually integrated peak areas using genesis peak integration and 15-point smoothing with Xcalibur 2.0.7.
**RESULTS**

**BPA glucuronidation kinetics.** BPA glucuronidation kinetics were best modeled using the Michaelis-Menten equation (Figure 1). When incubated with pooled male and female HLMs, the $V_{\text{max}}$ for BPA glucuronidation was determined to be $4.71 \pm 0.30 \text{ nmol/min/mg protein}$, and the $K_{\text{m}}$ was $45.8 \pm 8.9 \mu\text{M}$.

**Genistein glucuronidation kinetics.** The enzyme kinetics of genistein glucuronidation were best fit using the Michaelis-Menten model. With pooled male and female HLMs the mean $V_{\text{max}}$ for genistein glucuronidation was $0.93 \pm 0.10 \text{ nmol/min/mg protein}$. When co-incubated with 25 $\mu\text{M}$ BPA, the mean $V_{\text{max}}$ for genistein glucuronidation significantly decreased to $0.62 \pm 0.05 \text{ nmol/min/mg protein}$. $K_{\text{m}}$ values remained relatively unchanged between samples incubated with HLMs in the absence $(15.1 \pm 7.9 \mu\text{M})$ and presence $(21.5 \pm 7.7 \mu\text{M})$ of 25 $\mu\text{M}$ BPA (Figure 2). When genistein glucuronidation was performed with pooled male and female RLMs, mean $V_{\text{max}}$ values of $2.91 \pm 0.26$ and $3.05 \pm 0.41 \text{ nmol/min/mg protein}$ were achieved in the absence and presence of 25 $\mu\text{M}$ BPA, respectively. $K_{\text{m}}$ values for genistein glucuronidation by RLMs were $49.4 \pm 14 \mu\text{M}$ in the absence of BPA and $84.0 \pm 28 \mu\text{M}$ in the presence of 25 $\mu\text{M}$ BPA (Figure 3).

Lineweaver-Burk plots were constructed to determine $K_{\text{i}}$ values as well as the type of enzyme inhibition on genistein glucuronidation by BPA. According to the Lineweaver-Burk plots, BPA exerted non-competitive inhibition for genistein glucuronidation in HLMs with a $K_{\text{i}}$ value of $58.7 \mu\text{M}$, and competitive inhibition for genistein glucuronidation in RLMs, with a $K_{\text{i}}$ value of $35.7 \mu\text{M}$ (Figure 2B and Figure 3B, respectively). Variation in $V_{\text{max}}$, $K_{\text{m}}$, and $K_{\text{i}}$ values among replicates was minimal for both HLMs and RLMs (Supplemental Figures 1 through 6).
The effects of varying concentrations of BPA on glucuronidation activity in HLMs are shown in Figure 4. The \( \text{IC}_{50} \) value of BPA for genistein glucuronidation in HLMs was 37.0 \( \mu \text{M} \).
The work presented here shows that BPA inhibits the glucuronidation activity of genistein in both human and rat liver microsomes. The glucuronide is the predominant metabolite of genistein formed in both humans and rats. Inhibition of genistein’s glucuronidation therefore increases the bioavailability of genistein in the presence of BPA, thus allowing genistein to have a prolonged pharmacological effect. Extended biological action of genistein could exacerbate the compound’s adverse effects, especially among populations that are particularly vulnerable to the endocrine disrupting effects of the natural phytoestrogen, such as neonates. Alternatively, metabolism of genistein and BPA could be transferred to susceptible extra-hepatic organs, further contributing to endocrine disruption. Such an alteration in metabolic capacity is noteworthy, considering that both humans and laboratory rodents are routinely exposed to BPA and genistein simultaneously in their everyday lives (Thigpen et al., 2004; Patisaul and Jefferson, 2010).

It is interesting that the type of UGT enzyme inhibition elicited by BPA is different in HLMs and RLMs. In HLMs, the presence of BPA induced a significant decrease in $V_{\text{max}}$, while $K_m$ remained unaffected. These characteristics, along with the Lineweaver Burk plot for co-exposures to genistein and BPA in HLMs are suggestive of non-competitive enzyme inhibition (Figure 2B). On the other hand, increased $K_m$ values and unchanged $V_{\text{max}}$ values in the presence of inhibitor are hallmarks of competitive inhibition. These trends, coupled with the Lineweaver Burk plots obtained from incubations of genistein and BPA with RLMs suggest competitive inhibition (Figure 3B).

The disparity in UGT inhibition type caused by BPA between species may be due to a variety of reasons. One possible explanation for the difference in inhibition type of genistein
glucuronidation observed between human and rat liver microsomes is that different UGT isoforms may be responsible for genistein’s metabolism in the different species. It is reasonable to consider that UGT isoforms are present at different levels in HLMs as they are in RLMs, thus contributing to different glucuronidation capacities between the two species. Additionally, it has been previously reported that BPA may inhibit the activity of human UGT 1A6, which is one of the key isoforms responsible for the metabolism of genistein (Hanioka et al., 2008b). This interaction could be implicated in the altered metabolism of genistein observed during binary exposures in HLMs, and may help to explain why the glucuronidation rate of BPA appears to be independent of the presence of genistein (previous experiments, data not shown).

Interactions between BPA and genistein and their subsequent biological effects have been reported both in vitro and in vivo. Xing et al demonstrated a significant synergistic interaction between BPA and genistein in embryonic development of cultured post-implantation rat embryos when these two compounds are present simultaneously during organogenesis (Xing et al., 2010); supporting the hypothesis that BPA and genistein act in a synergistic manner when present simultaneously. Conversely, Dolinoy et al. report a non-synergistic effect between the two by demonstrating that while BPA exposure during early stem cell development alters epigenetic patterning in mice, concomitant exposure to genistein a dietary component that provides methyl donors, negates the DNA hypomethylation effect (Dolinoy et al., 2007). The study described here showed a competitive inhibition in rat and a noncompetitive inhibition of glucuronidation in human microsomal culture. Such contributions have added to our understanding of the effects of simultaneous exposures to BPA and genistein, but more work needs to be done in order to fully elucidate the true nature and extent of the interactions. While effects of chemical mixtures are often assumed to be additive, there are likely multiple levels of regulatory interactions between
genistein and BPA; that may be synergistic or antagonistic depending upon the level and species selection. The study described in this paper contributes to this growing body of research, and highlights the complexity of outcomes that occur with co-exposures of these endocrine disrupting compounds.

In vitro analyses offer distinct advantages, but they are also limited in their utility. Xenobiotic incubations with liver microsomes provide a great model for quick screenings of drug-to-drug interactions. Since the majority of predominant UGTs involved in the metabolism of BPA (rat UGT 2B1, human UGT 2B15) and genistein (UGTs 1A1, 1A6, and 1A9) are hepatically expressed, the glucuronidation of genistein in the absence and presence of BPA can be easily assessed using liver microsomes. Overall conjugation pathways, however, cannot be investigated using liver microsomes, due to their inherent limited capacity for sulfation. Finally, studies using liver microsomes exclude the analysis of enzymes that are expressed extra-hepatically, whereas in vivo studies allow for the analysis of the contribution of extra-hepatic as well as hepatic enzymes in the metabolism of xenobiotics.

Potential human risk from exposures to xenobiotics is often assessed by data generated from animal models. Such a method is often sufficient, since the animal data frequently mirrors human data. In some circumstances, however, data generated from laboratory animals does not directly parallel the effects observed in humans, and alternate models are then required to predict risk to human health. The data presented here offer such an example of an incidence where animal data and human data do not match at the levels of genistein and BPA tested, and therefore human risk assessment for co-exposures to genistein and BPA should not necessarily be based solely upon data generated from rat models.
Countless xenobiotics share the metabolic pathway of glucuronidation, however not all xenobiotics exhibit similar biological endpoints and increased susceptibility to adverse effects when exposed during particular windows of exposure. Additionally, practical binary exposure scenarios do not abound for all compounds. Genistein and BPA make for a unique mixture of xenobiotics to be investigated simultaneously due to their common metabolic pathways, similar biological effects, and prevalent everyday co-exposures.

In conclusion, BPA induced non-competitive inhibition for genistein glucuronidation in HLMs, and competitive enzyme inhibition for genistein glucuronidation in RLMs. Altered metabolism of genistein in the presence of BPA may impact the phytoestrogen’s toxicity and demonstrated the need for analysis of the effect on glucuronidation of the two endocrine disrupting compounds in a binary exposure.
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AUTHORSHIP CONTRIBUTIONS

Participated in research design: Coughlin, Thomas, and Buckley

Conducted experiments: Coughlin

Contributed new reagents or analytic tools: Thomas

Performed data analysis: Coughlin

Wrote or contributed to the writing of the manuscript: Coughlin and Buckley
REFERENCES


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FOOTNOTES

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**Figure 1:** Enzyme kinetic plots of BPA glucuronidation using pooled human liver microsomes. (A) Michaelis-Menten plot. (B) Lineweaver-Burk plot. $V_{\text{max}} = 4.71 \pm 0.30$ nmoles/min/mg and $K_m = 45.8 \pm 8.9 \mu M$.

**Figure 2:** Enzyme kinetic plots of genistein glucuronidation (pooled data) using pooled human liver microsomes in the absence (0 µM; open circles) and presence (25 µM; closed circles) of BPA. (A) Michaelis-Menten plot. (B) Lineweaver-Burk plot. In absence of BPA, $V_{\text{max}} = 0.93 \pm 0.10$ nmoles/min/mg and $K_m = 15.1 \pm 7.9 \mu M$. In presence of BPA, $V_{\text{max}} = 0.62 \pm 0.05$ nmoles/min/mg and $K_m = 21.5 \pm 7.7 \mu M$.

**Figure 3:** Enzyme kinetic plots of genistein glucuronidation (pooled data) using pooled rat liver microsomes in the absence (0 µM; open circles) and presence (25 µM; closed circles) of BPA. (A) Michaelis-Menten plot. (B) Lineweaver-Burk plot. In absence of BPA, $V_{\text{max}} = 2.91 \pm 0.26$ nmoles/min/mg and $K_m = 49.4 \pm 14 \mu M$. In presence of BPA, $V_{\text{max}} = 3.05 \pm 0.41$ nmoles/min/mg and $K_m = 84.0 \pm 28 \mu M$.

**Figure 4:** The effects of BPA at concentrations ranging from 0-250 µM on the glucuronidation of genistein in pooled male and female HLMs. The IC$_{50}$ value of BPA for genistein glucuronidation in HLMs is 37.0 µM.
Figure 1
Figure 2

nmol gen gluc/min/mg

[genistein], μM

0 100 200 300

0 0.5 1.0 1.5

0 μM BPA

25 μM BPA

1/[gen], μM

1/(nmol/min/mg)

A

B
Figure 3

A

B

nmol gen gluc/min/mg

[genistein], µM

0

100

200

300

0

1

2

3

4

1/(nmol/min/mg)

1 /[gen], µM

0 µM BPA

25 µM BPA
Figure 4

nmol gen gluc/min/mg

[BPA], µM
Supplemental Data

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Supplemental Figure 1: Enzyme kinetic plots of genistein glucuronidation (rep 1) using pooled human liver microsomes in the absence (0 μM; open circles) and presence (25 μM; closed circles) of BPA. (A) Michaelis-Menten plot. (B) Lineweaver-Burk plot. In absence of BPA, \( V_{\text{max}} = 1.13 \pm 0.05 \) nmoles/min/mg and \( K_m = 11.4 \pm 2.6 \) μM. In presence of BPA, \( V_{\text{max}} = 0.69 \pm 0.03 \) nmoles/min/mg and \( K_m = 13.8 \pm 2.4 \) μM.
Supplemental Figure 2: Enzyme kinetic plots of genistein glucuronidation (replicate 2) using pooled human liver microsomes in the absence (0 μM; open circles) and presence (25 μM; closed circles) of BPA. (A) Michaelis-Menten plot. (B) Lineweaver-Burk plot. In absence of BPA, $V_{\text{max}} = 0.89 \pm 0.06$ nmoles/min/mg and $K_m = 24.0 \pm 6.0$ μM. In presence of BPA, $V_{\text{max}} = 0.57 \pm 0.04$ nmoles/min/mg and $K_m = 30.8 \pm 7.1$ μM.

Supplemental Figure 3: Enzyme kinetic plots of genistein glucuronidation (replicate 3) using pooled human liver microsomes in the absence (0 μM; open circles) and presence (25 μM; closed circles) of BPA. (A) Michaelis-Menten plot. (B) Lineweaver-Burk plot. In absence of BPA, $V_{\text{max}} = 0.91 \pm 0.04$ nmoles/min/mg and $K_m = 21.1 \pm 3.6$ μM. In presence of BPA, $V_{\text{max}} = 0.60 \pm 0.03$ nmoles/min/mg and $K_m = 24.9 \pm 5.5$ μM.
Supplemental Figure 4: Enzyme kinetic plots of genistein glucuronidation (replicate 1) using pooled rat liver microsomes in the absence (0 μM; open circles) and presence (25 μM; closed circles) of BPA. (A) Michaelis-Menten plot. (B) Lineweaver-Burk plot. In absence of BPA, $V_{\text{max}} = 2.83 \pm 0.33$ nmoles/min/mg and $K_m = 63.0 \pm 20$ μM. In presence of BPA, $V_{\text{max}} = 2.68 \pm 0.14$ nmoles/min/mg and $K_m = 82.9 \pm 11$ μM.

Supplemental Figure 5: Enzyme kinetic plots of genistein glucuronidation (replicate 2) using pooled rat liver microsomes in the absence (0 μM; open circles) and presence (25 μM; closed circles) of BPA. (A) Michaelis-Menten plot. (B) Lineweaver-Burk plot. In absence of BPA, $V_{\text{max}} = 3.14 \pm 0.26$ nmoles/min/mg and $K_m = 58.3 \pm 14$ μM. In presence of BPA, $V_{\text{max}} = 3.57 \pm 0.34$ nmoles/min/mg and $K_m = 96.5 \pm 22$ μM.
Supplemental Figure 6: Enzyme kinetic plots of genistein glucuronidation (replicate 3) using pooled rat liver microsomes in the absence (0 μM; open circles) and presence (25 μM; closed circles) of BPA. (A) Michaelis-Menten plot. (B) Lineweaver-Burk plot. In absence of BPA, $V_{\text{max}} = 2.79 \pm 0.13$ nmoles/min/mg and $K_m = 33.28 \pm 5.4 \mu M$. In presence of BPA, $V_{\text{max}} = 3.59 \pm 0.37$ nmoles/min/mg and $K_m = 74.6 \pm 20 \mu M$. 