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The Effects of Dose, Route, and Repeated Dosing on the Disposition and Kinetics of Tetrabromobisphenol A in Male F-344 Rats

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Studies were conducted to characterize the metabolic and dispositional fate of ^{14}C–tetrabromobisphenol A (TBBPA)—a commonly used brominated flame retardant, in male Fischer-344 rats. The percent of dose eliminated as total radioactivity in feces at 72 h following three different single oral doses (2, 20, or 200 mg/kg) of ^{14}C-TBBPA was 90% or greater for all doses. Most of the dose was eliminated in the first 24 h. At 72 h after administration of the highest dose, the amounts of ^{14}C found in the tissues were minimal (0.2–0.9%). With repeated daily oral doses (20 mg/kg) for 5 or 10 days, the cumulative percent dose eliminated in the feces was 85.1 ± 2.8 and 97.9 ± 1.1, respectively. In all studies radioactivity recovered in urine was minimal, <2%. Repeated dosing did not lead to retention in tissues. Following iv administration, feces was also the major route of elimination. Following iv administration of TBBPA, the radiolabel found in the blood decreased rapidly and could be described by a biexponential equation, consistent with a two-compartment model. The key calculated kinetic parameters are terminal elimination half-life ($t_{½}\beta$) = 82 min; area under the blood concentration-time curve from time 0 to infinity (AUC) = 1440 μg × min/ml; and apparent clearance (CL) = 2.44 ml/min. Although readily absorbed from the gut, systemic bioavailability of TBBPA is low (<2%). It is extensively extracted and metabolized by the liver and the metabolites (glucuronides) exported into the bile. About 50% of an oral dose (20 mg/kg) was found in the bile within 2 h. This extensive extraction and metabolism by the liver greatly limits exposure of internal tissues to TBBPA following oral exposures.

Key Words: **tetrabromobisphenol A; pharmacokinetics; disposition; flame retardant.**

Tetrabromobisphenol A (2,2-bis(4-hydroxy-3,5-dibromophenyl)propane; TBBPA) is a commonly used brominated flame retardant and is primarily used as a reactive flame retardant in epoxy resin circuit boards. More recently used in electronic enclosures made of polycarbonate-acrylonitrile-butadiene-styrene. Other applications of TBBPA include its use as a flame retardant for plastics, paper, and textiles; as a plasticizer; in adhesives and coatings; and as a chemical intermediate for the synthesis of other flame retardants (e.g., TBBPA allyl ether) (de Wit, 2002). Because of these widespread uses humans have been exposed to TBBPA (Hagmar *et al.*, 2000; Jakobsson *et al.*, 2002; Sjödin *et al.*, 2003).

The acute toxicity of TBBPA in rats is low when administered orally (LD50 > 5 g/kg) (IPCS/WHO, 1995). However, repeated oral administration has been reported to disturb the metabolism of heme in rats (Szymańska *et al.*, 2000). The urinary excretion of copro- and uroporphyrins became elevated after two weeks of exposure to TBBPA (250 mg/kg). Recently, nephrotoxicity has been reported to occur in newborn rats given TBBPA orally at 200 or 600 mg/kg/day for 18 days. The effects included polycystic lesions and hyperplasia of the renal tubular epithelium (Fukuda *et al.*, 2004).

A major reason for the low acute toxicity of TBBPA is most likely because of its low level of systemic exposure. A recent review by Hakk and Letcher (2003) described studies that defined the toxicokinetics and the fate of brominated flame retardants. Brady *et al.* (1979) reported that TBBPA was poorly absorbed by Sprague-Dawley rats following a single oral dose of ^{14}C-TBBPA (7 mg/kg). By 72 h, more than 95% of the dose was eliminated in the feces. Similar results were found by Hakk *et al.* (2000) who reported that a single oral dose of 2 mg/kg was excreted mainly in the feces by male Sprague-Dawley rats. Fecal excretion was the major route of elimination when TBBPA (250 or 1000 mg/kg) was administered to female Wistar rats by ip injection. After 72 h, 51–65% of the administered dose was eliminated in the feces and 11–14% was retained in the muscles (Szymańska *et al.*, 2001). These authors suggested that TBBPA may accumulate in tissues following repeated exposure. Hakk *et al.* (2000) also administered a single oral dose of 2 mg/kg ^{14}C-TBBPA to bile duct–cannulated male Sprague-Dawley rats and reported that 72% of the dose was eliminated in the bile by 72 h. In each study referred to above, the excretion of ^{14}C equivalents in the urine was very low. The results of these studies demonstrate that the major route of elimination of ^{14}C equivalents is excretion in feces, regardless of the route of administration. The source of the ^{14}C equivalents found in the feces is the biliary excretion of

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TBBPA metabolites as opposed to poor absorption from the gastrointestinal tract.

Little information is available on the systemic bioavailability of TBBPA following oral administration or on how repeated dosing alters the elimination/tissue retention of TBBPA. Thus, this study was designed to characterize the toxicokinetics of TBBPA after iv dosing, to determine the extent of its systemic bioavailability after oral dosing, and to determine if repeated dosing or dose escalation alters its elimination profile.

MATERIALS AND METHODS

Chemicals

Radioactive (Ring ^{14}C) TBBPA (Lot # 3225-235) was obtained from Perkin Elmer Life and Analytical Sciences (Boston, MA). The radiochemical purity of ^{14}C-TBBPA was determined to be 98.9% with a specific activity of 74 mCi/mmol. The chemical purity of the ^{14}C-TBBPA was calculated from an unlabeled TBBPA reference standard obtained from Aldrich (CAS#: 79-94-7) and determined to be 97%. Soluene-350 tissue solubilizer and Pico-Flour 40 scintillation cocktail solution were received from Perkin Elmer (Torrance, CA). Hydrogen peroxide (30%) was obtained from VWR (West Chester, PA). Cremophor, dimethyl sulfoxide, β-glucuronidase (EC 3.2.1.31, Type B-10 from bovine liver), acetate buffer, D-saccharic acid 1,4-lactone, and sulfatase were obtained from Sigma Chemical, (St Louis, MO). Solid phase extraction (SPE) Oasis MCX 3cc-60 mg cartridges were obtained from Waters (Milford, MA). All other reagents used in these experiments were high performance liquid chromatography (HPLC) or analytical grade.

Animal Studies

Animals. Male Fischer-344 (F-344) rats of 8–9 weeks of age (161–190 g) without surgical alteration, with indwelling jugular vein cannula (JVC) or with cannulated bile ducts (BDC) were obtained from Harlan Sprague Dawley Inc. (Indianapolis, IN). All animals were maintained in an Association for Assessment and Accreditation for Laboratory Animal Care–approved animal care facility for at least 1 week before experimentation. Except for the JVC and BDC animals, acclimation time was 5–7 days. The cannulated animals were acclimated only for 1 day to ensure that the cannula remained unobstructed. Animals were housed in Nalgene metabolism cages 24 h prior to dosing and maintained in a temperature controlled (25°C), 12-h light/12-h dark cycle facility. The rats were allowed food and water *ad libitum* except for a 12-h fasting period before administration of TBBPA. Food was returned 2 h after dosing. The food (Teklad 4% Rat Diet 7001, Harlan Teklad, Madison, WI) was ground into a powder to reduce contamination of fecal matter.

Dose selection. The doses used in these studies were based on the results of published toxicity studies (Fukuda *et al.*, 2004; IPCS/WHO, 1995). The doses of 2 and 20 mg/kg represent nontoxic doses, while 200 mg/kg may represent a minimally toxic dose. This lack of acute toxicity was confirmed following a single iv administration of unlabeled TBBPA (20 mg/kg). TBBPA was administered to male F-344 rats ($N = 4$) by the indwelling JVC. Control JVC animals ($N = 2$) were dosed with vehicle only.

Blood samples (300 µl) were collected from the JVC rats at 2, 4, 8, 12, 24, 48, and 72 h into heparinized syringes and replaced with an equal volume of saline. Urine was collected every 24 h up to 72 h from both groups for determination of urine-specific gravity. Body weights, liver and kidney weights, and markers of hepatic and renal injuries were assessed over 72 h. The indices measured were alkaline phosphatase, glucose, alanine aminotransferase, serum protein, blood urea nitrogen, and creatinine. The analyses were performed by The University's Animal Care Diagnostic laboratory, using standardized procedures.

This single iv dose of TBBPA (20 mg/kg) did not alter serum chemistry. Furthermore, body, liver, and kidney weights were not changed significantly from control (data not shown), and therefore, this dose was chosen for the iv dose toxicokinetics studies.

Tissue distribution and excretion studies. Following iv (20 mg/kg) or oral (2, 20, or 200 mg/kg) dosing, the animals were returned to Nalgene metabolism cages for collection of urine and feces. Except where indicated, all doses contained 50 µCi/kg ^{14}C-TBBPA. Urine was collected at 6, 12, 24, 36, 48, and 72 h; feces were collected at 12, 24, 36, 48, and 72 h. The metabolism cages were rinsed after the collection of urine samples with methanol. The cage wash samples were held and analyzed separately from the urine. At the end of the study, animals were subjected to euthanasia by CO_2 inhalation and necropsy. All collected samples were analyzed immediately or stored at − 80°C until analyzed. Blood, bile, feces, and selected tissues (brain, heart, lung, thyroid, kidneys, liver, spleen, stomach, stomach contents, intestine, intestinal contents, cecum, cecum contents, testes, muscle, fat, and skin) were solubilized with Soluene-350 as described by Thomson and Burns (1996). Body composition estimates of 8% for blood, 11% for adipose tissue, 50% for muscle, and 16% for skin were used to estimate percent dose in these tissues (Birnbaum *et al.*, 1980). Radioactivity associated with cage rinse and urine was determined by scintillation counting of diluted samples. All samples were stored in the dark for 48 h to control for chemoluminescence and were corrected for background.

For repeated dosing studies, male F-344 rats ($N = 4$ for each dose) were dosed daily for 1, 5, or 10 days with ^{14}C-TBBPA (20 mg/kg, 50 µCi/kg) by oral gavage. The animals were not fasted. During the 5- and 10-day experiments, urine, cage rinse, and feces samples were collected just prior to the next dose administration. During the 1-day study, urine and cage rinse samples were collected at 6, 12, and 24 h; feces were collected at 12 and 24 h after dosing.

Blood kinetics following iv administration. ^{14}C-TBBPA (20 mg/kg, 50 µCi/kg) was administered iv to nine animals through a JVC in ethanol:cremophor:saline (10:20:70, 1 ml/kg) over 5 s. To insure that the whole dose was delivered, blood (100 µl) was then drawn into the cannula and returned to circulation, and the cannula was then flushed with normal saline (1 ml/kg). Blood samples (300 µl) were collected via the JVC at 0.08, 0.16, 0.25, 0.33, 0.5, 1, 1.5, 2, 4, 6, 12, 24, and 36 h into heparinized syringes. When an aliquot of blood was removed via the JVC, it was replaced with an equal volume of saline. Collection of blood from the animals ($N = 9$) was staggered in order to obtain sufficient blood volumes and time points for pharmacokinetic analysis and to reduce blood loss from the animals.

Blood kinetics following oral administration. ^{14}C-TBBPA (20 mg/kg, 200 µCi/kg), in ethanol:cremophor:saline (10:20:70, 4 ml/kg), was administered by oral gavage to JVC male F-344 rats ($N = 4$). Blood samples (300 µl) were collected through the JVC into a heparinized syringe at 0.126, 0.25, 0.5, 1, 2, 4, 6, and 8 h as described above.

Biliary excretion study. BDC male F-344 rats were dosed with either unlabeled TBBPA (20 mg/kg, $N = 2$) or labeled ^{14}C-TBBPA (20 mg/kg, 50 µCi/kg, $N = 2$), in ethanol:cremophor:saline (10:20:70) by oral gavage. Immediately following dosing, animals were placed in Nalgene metabolism cages that had been modified to permit continuous bile collection. The bile was collected continually for 2 h.

Pharmacokinetics analysis. The blood concentration-time data following iv and oral administration were analyzed by compartmental methods. A computer modeling program (Pharsight WinNonlin, Rainbow Technologies, Inc., 1998–2005) was utilized to fit the data to a suitable multicompartment model using nonlinear regression analysis and assuming first-order kinetics for all processes. The parameters of the model were used to calculate values for the terminal elimination half-life ($t_{½}\beta$), area under the blood concentration–time curve from time zero to infinity (AUC), mean residence time (MRT), apparent clearance (CL), and volume of distribution under steady-state conditions (Vss).

Analytical Methods

HPLC analysis of TBBPA and its metabolites in blood and bile. Blood samples (2 × 50 µl) were solubilized and measured directly by scintillation counting. Because TBBPA partitioned between red blood cell and plasma

(Szymańska *et al.*, 2001), TBBPA was extracted from whole blood for analysis. Immediately after collection, blood samples (150 µl) were mixed with ethyl acetate (450 µl), vortexed, and centrifuged and the organic extracts were removed. The extraction was performed five times and the extracts were pooled. The samples were evaporated to dryness and reconstituted in 100 µl acetonitrile. Aliquots of bile samples (100 µl) were sonicated for 15 min and subjected to SPE on nonconditioned MCX SPE cartridges. After loading, the cartridges were washed with 2 ml water, dried under vacuum for 5 min, and then eluted 4 times with 1 ml methanol. After the eluent was evaporated, the samples were reconstituted in 1 ml methanol and were shaken for 20 min. Aliquots (100 µl) of the reconstituted samples were subjected to HPLC separation (Agilent Technologies, Palo Alto, CA). The samples (100 µl) were injected onto a reverse phase Luna C18(2), 5µ, 250 × 4.6 mm (Phenomenex, Torrance, CA) column coupled with Phenomenex SecurityGuard C18 guard cartridge (4.0 × 3.0 mm) (AJO-4287). The mobile phases consisted of acidified water and acidified acetonitrile that contained 0.05% of acetic acid at a flow rate of 1 ml/min with a total run time of 35 min. The mobile phase gradient was run from 60% water and 40% acetonitrile for the first 5 min, then up to 60% acetonitrile over 5 min, then to 90% acetonitrile over 2 min, and finally to 100% acetonitrile for the last 13 min. Mobile phase was brought back to initial conditions and the column allowed to reequilibrate for 10 min between runs. The eluent from the HPLC was monitored at the UV max (210 nm) for TBBPA with a diode array detector, a flow through beta ram detector (INUS, Tampa, FL), and then fractions were collected at 1 min intervals via an Agilent 220 microplate sampler. Data were acquired with ChemStation for LC 3D, Rev. A 09.03 (1417) data acquisition software by Agilent Technologies. The amount of TBBPA in the dosing solutions and blood was determined based on a 5-point calibration curve. Calibration standards and quality control samples were prepared from concentrated stock solution. The limit of detection (LOD) using DAD-UV detection at 210 nm was 0.26 µg/ml, and the limit of quantification (LOQ) was 0.8 µg/ml. The LOD for ^{14}C by liquid scintillation counting was 26 DPM as determined by the equation described by Zhu *et al.* (2005). To increase the sensitivity for radiometric detection of ^{14}C equivalents in blood following oral administration of ^{14}C-TBBPA, the amount of administered radioactivity was increased from 50 µCi/kg to 200 µCi/kg. This resulted in a LOD for ^{14}C of 0.021 µg/ml.

Metabolite Identification

Tentative identification of phase 2 metabolites. To determine the presence of glucuronide and/or sulfate conjugates of TBBPA, bile was incubated with 5000 U/ml β-glucuronidase or 11 U/ml sulfatase as described by Peter and Caldwell (1994). Untreated bile and buffer without enzyme were incubated under identical conditions to serve as controls. The samples were subjected to SPE procedure as described previously and analyzed by reversed phase HPLC to identify ^{14}C-TBBPA metabolites in bile.

LC–Mass Spectrometry/Mass Spectrometry analysis. HPLC separation of bile sample post–SPE treatment was performed as described for analysis of blood. The LC system was coupled to MSD-Trap SL ion trap mass spectrometer (Agilent Technologies). Analytes were ionized using an electrospray ionization (ESI) source in the negative ion mode over a scan range of m/z 50–1500. Samples were dehydrated with nitrogen at a flow rate of 10 ml/min, drying temperature of 350°C, nebulizer pressure of 60 psi, and capillary voltage of 4000 V. Three masses were selected for analysis: m/z 543 (TBBPA), m/z 718 (TBBPA monoglucuronide), and m/z 895 (TBBPA-diglucuronide). A full mass spectrometry (MS) scan was followed by programmed automatic selection of the two most abundant ions for isolation and fragmentation.

RESULTS

Intravenous Administration

When ^{14}C-TBBPA was administered iv, a single radioactive peak that coeluted with TBBPA (Rt = 16.9 min) was detected in blood (Fig. 1). This peak decreased with time, and new peaks were not observed. The concentration-time profile for TBBPA in blood is shown in Figure 2 and can be described by a biexponential equation that is consistent with a two-compartment model. The half-life for distribution ($t_{½}\alpha$) was 5 min and $t_{½}\beta$ was 82 min. The AUC, apparent Vss, and systemic blood clearance (CL) values, were, respectively, 40 µg × min/ml, 126 ml, and 2.44 ml/min. The MRT was 51.6 min (Table 1). Blood concentrations at times greater than 4 h could not be determined accurately because they were at or below the LOQ.

Following iv administration, the predominant route of elimination of TBBPA ^{14}C equivalents was fecal. Within the first 24 h, 73 ± 8% of the administered radioactivity was eliminated in the feces. An additional 7% was eliminated by this route in the next 12 h. Less than 0.5% of the dose was excreted in the urine over the 36 h time course (Fig. 3).

Oral Administration

Following oral administration of ^{14}C-TBBPA (2, 20, or 200 mg/kg) as a single oral bolus dose, 90–95% of the total administered radioactivity was eliminated in the feces over the 72-h collection period (Fig. 4). The peak fecal excretion occurred between 12 and 24 h (>80%). The elimination of ^{14}C equivalents in urine was minimal, less than 1% regardless of the dose administered. Figure 5 shows the percent of dose recovered from tissues at 72 h following oral administration of the highest dose (200 mg/kg). Total percent of dose retained in tissues at 72 h was 0.4%. Tissue disposition was not determined at the two lower doses.

After the rate and route of elimination of ^{14}C-TBBPA following a single oral bolus dose was defined, the effects of repeated daily oral dosing of TBBPA on excretion and tissue distribution of ^{14}C-TBBPA equivalents were determined. After 1, 5, or 10 days of repeated daily dosing of 20 mg/kg of ^{14}C-TBBPA, the average cumulative amounts of ^{14}C equivalents found in the feces were 82, 85, and 98% of dose, respectively (Table 2). The radioactivity found in the urine was negligible, less than 0.5%. The data presented in Table 2 show the recovery of ^{14}C-TBBPA equivalents in excreta and tissues at 24 h after administration of the last dose. That which had not been excreted was in the cecum and intestinal contents. The percent of dose remaining in tissues for the three dosing regimens was less than 1%.

The effect of time on the blood concentration of ^{14}C-TBBPA of rats dosed orally by gavage with ^{14}C-TBBPA at (20 mg/kg, 200 µCi/kg) is shown in Figure 2. The concentration of ^{14}C-TBBPA represented less than 0.1% of the dose with a Cmax of 0.2 µg/ml and a AUC of 24 µg*min/ml. The initial rate of absorption was rapid and reached a maximum at 30 min (Table 3). However, after a decline for 2 h there was small, but consistent increase in blood levels of TBBPA at 4 h. As this small increase in TBBPA was also observed in rats treated iv, it may represent enteric-hepatic recycling, as has been described by Schauer *et al.* (2006). Based on AUC (adjusted for dose) following oral

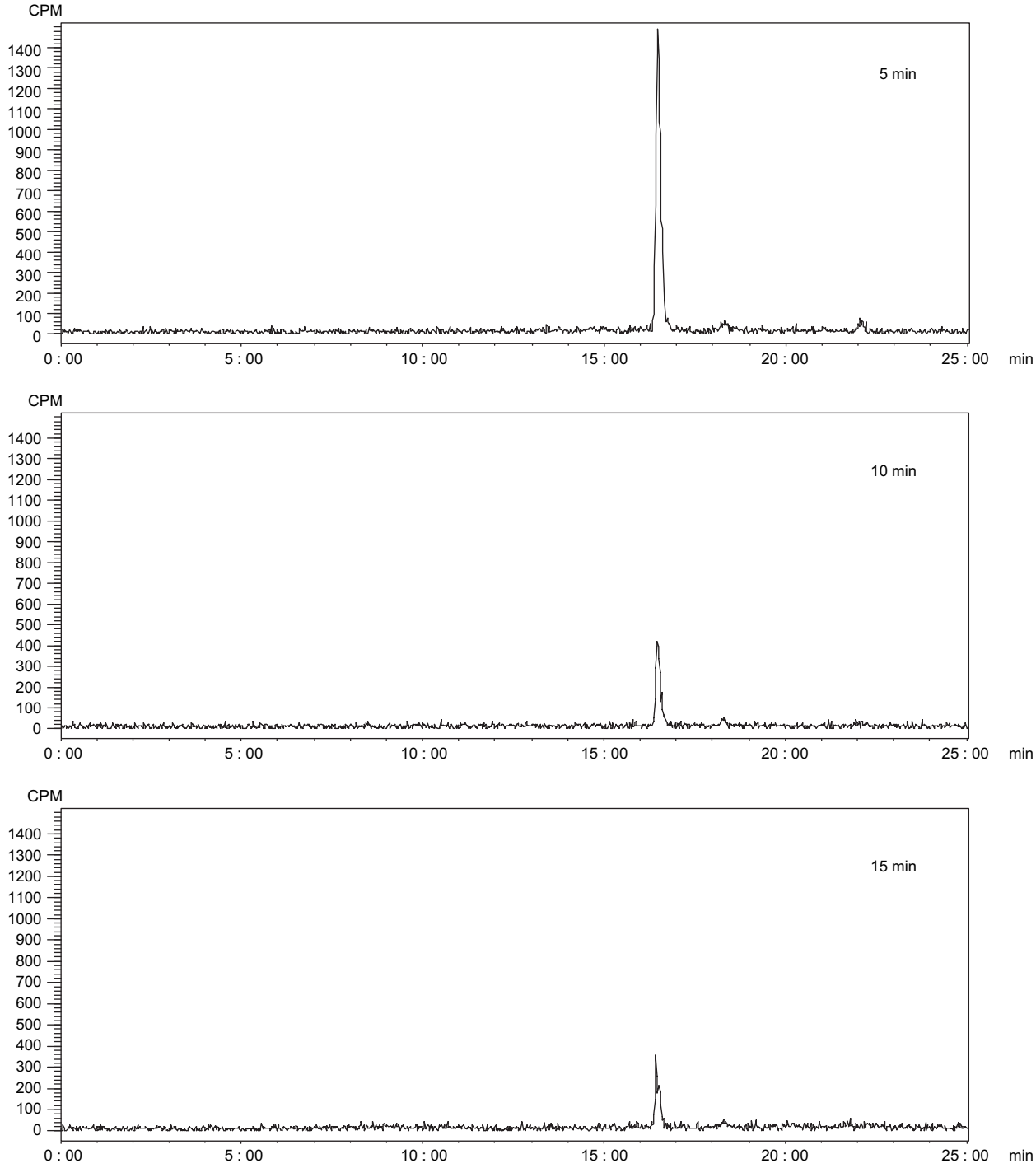

FIG. 1. Representative radiochromatograms of extracted whole-blood samples obtained from male F-344 rats at 5, 10, and15 min following iv administration of ^{14}C-TBBPA (20 mg/kg, 50 μCi/kg).

administration (24 μg*min/ml) and the AUC following iv administration (1440 μg*min/ml), the systemic bioavailability was estimated to be 1.6% after oral dosing. HPLC analysis of blood also revealed the presence of 3–4 small peaks that did not coelute with TBBPA. The chemical identity of these metabolites was not determined.

Oral Administration to Bile Duct–Cannulated Rats

Following oral administration of ^{14}C-TBBPA (20 mg/kg, 50 μCi/kg) to two BDC rats, 47% and 51% of the dose was excreted in the bile within 2 h. HPLC analysis of bile revealed two major radioactive peaks at retention times of 11.2 and 15.5

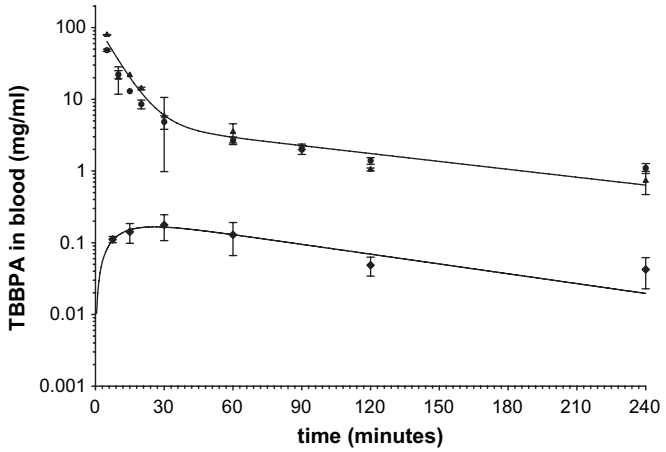

FIG. 2. Whole-blood concentration of TBBPA (▲) and ^{14}C equivalents (●) expressed as μg/ml following iv administration of ^{14}C-TBBPA (20 mg/kg, 50 μCi/kg) and oral administration of ^{14}C-TBBPA (20 mg/kg, 4 ml/kg, 200 μCi/kg) (♦) to male F-344 rats. The TBBPA concentration following iv administration was calculated based on the UV chromatogram of extracted blood at 210 nm, and the TBBPA concentration following oral administration was calculated based on the ^{14}C content of fractions that eluted between 16 and 19 min. Data are expressed as mean ± SD ($N = 3 \pm$ SD to $4 \pm$ SD).

TABLE 1
Kinetic Parameters for TBBPA following Administration of ^{14}C-TBBPA (20 mg/kg, 50 μCi/kg, iv) to Male F-344 Rats

	$t_{½}$ (min)	AUC (μg·min/ml)	CL (ml/min)	Vss (ml)	MRT (min)
Value	82.1	1440	2.44	126	51.6

Note. $t_{½}\beta$, terminal half-life; CL, systemic clearance.

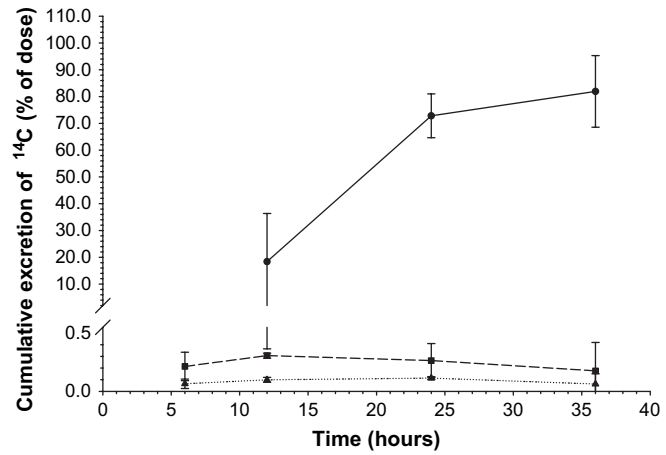

FIG. 3. Cumulative excretion of total radioactivity in urine (■), feces (●), and cage rinse (▲) expressed as percent of dose following iv administration of ^{14}C-TBBPA (20 mg/kg, 50 μCi/kg) to male Fischer-344 rats. Data are expressed as mean ± SD ($N = 4 \pm$ SD at 24 h and $N = 2$ at 36 h).

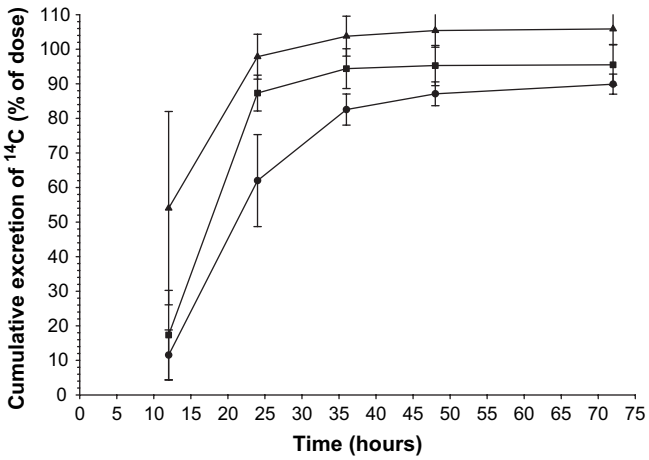

FIG. 4. Comparison of the cumulative excretion of total radioactivity in feces following administration of different oral doses of ^{14}C-TBBPA (2 mg/kg [■], 20 mg/kg [▲], and 200 mg/kg [●]) to male Fischer-344 rats. Data are expressed as the mean percent of dose $N = 4 \pm$ SD

min, (33% and 63% of bile derived ^{14}C equivalents, respectively). Incubation of bile with β-glucuronidase resulted in the disappearance of both peaks and an increase in a peak that coeluted with the parent TBBPA (Rt = 16.9 min). Incubation with sulfatase did not alter these peaks (data not shown). These data suggest the presence of two metabolites that contain a glucuronide moiety in the bile of rats orally dosed with TBBPA.

Metabolite Identification

The two suspected glucuronide conjugates of TBBPA had UV spectral characteristics similar to TBBPA, and MS/MS

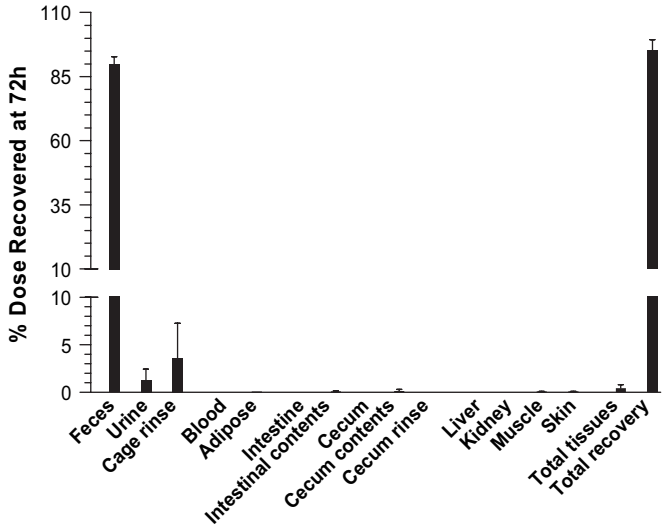

FIG. 5. Excretion and tissue distribution of TBBPA (200 mg/kg, 50 μCi/kg) following single po administration. Feces, urine, cage rinse, and tissues were collected, solubilized, and analyzed by LSC. Data expressed as mean percent of dose ± SD ($N = 4$)

TABLE 2
Percent of Dose Recovered One Day following Administration of ^{14}C-TBBPA (20 mg/kg, 50 μCi/kg, po daily dose) for 1, 5, or 10 Days to Male F-344 Rats ($N = 4 \pm$ SD)

	1 day	5 days	10 days
Feces	81.91 ± 3.59	85.05 ± 0.5	97.92 ± 1.12
Urine	0.4 ± 0.19	0.2 ± 0.01	0.35 ± 0.11
Cage rinse	0.1 ± 0.07	0.06 ± 0.01	0.12 ± 0.03
Brain	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Cecum	0.17 ± 0.06	0.06 ± 0.04	0.01 ± 0.00
Cecum contents	8.43 ± 3.65	1.12 ± 0.23	0.69 ± 0.21
Cecum rinse	0.10 ± 0.07	0.06 ± 0.04	0.02 ± 0.01
Adipose tissues			
Kidney fat	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mesentary fat	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.02
Testes fat	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Thoracic fat	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Total fat	0.01 ± 0.00	0.00 ± 0.00	0.01 ± 0.01
Heart	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Intestine	0.09 ± 0.03	0.04 ± 0.04	0.01 ± 0.01
Intestine contents	1.92 ± 0.52	0.38 ± 0.17	0.26 ± 0.17
Kidneys	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Liver	0.07 ± 0.03	0.01 ± 0.00	0.01 ± 0.00
Lung	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Muscle	0.05 ± 0.05	0.01 ± 0.01	0.01 ± 0.00
Skin	0.02 ± 0.02	0.00 ± 0.00	0.00 ± 0.00
Spleen	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Stomach	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Stomach contents	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Testes	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Thyroid	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Bladder urine	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Total recovery	93.35 ± 5.89	86.99 ± 2.80	99.36 ± 0.93

analysis of these metabolites revealed ions of m/z 718 (major peak) and m/z 895. The isotope pattern obtained for these ions was indicative of molecules containing four bromine atoms (Figs. 6B and 6D). After fragmentation, the major metabolite (Rt = 15.5 min) with a m/z 718 yielded a simple spectrum with a daughter ion of m/z 543, which resulted from a loss of 176 (Fig. 6A). Fragmentation of the minor metabolite (Rt = 11.2 min, m/z 895) yielded daughter ions at m/z 718 (loss of 176 amu) and m/z 543 (loss of 2 × 176) (Fig. 6C). Based on these results the metabolites were identified as TBBPA-glucuronide and TBBPA-diglucuronide. A noteworthy observation was that storage of samples in methanol resulted in a reduction of the TBBPA-glucuronide peak, with a new peak appearing with a longer Rt and the addition of 15 amu observed in the mass spectrum (m/z 733). These results suggest methylation of TBBPA-glucuronide upon storage in methanol.

TABLE 3
Pharmacokinetics of TBBPA following Administration of ^{14}C-TBBPA (20 mg/kg, 200 μCi/kg, po) to Male Fischer-344 Rats

AUC μg × min/ml	$t_{½}$ (min)	Cmax μg/ml	Tmax hours	Bioavailability
24 ± 10	95 ± 20	0.19 ± 0.08	0.53 ± 0.31	1.6%

Note. $t_{1/2}$, terminal half-life; Cmax, maximum blood concentration; Tmax, time to reach Cmax.

DISCUSSION

The results of the studies reported here and elsewhere demonstrate that following oral administration, TBBPA undergoes extensive absorption from gastrointestinal tract, but has a very low systemic bioavailability. The major reasons for the low systemic bioavailability relate to the ability of the liver to absorb TBBPA from the portal vein and to conjugate it with glucuronic acid for elimination in the bile. Indeed, 50% of the dose of TBBPA was recovered in the bile, primarily as glucuronide conjugates within 2 h of oral administration. Additional excretion of TBBPA equivalents into the bile would be expected over the next several hours (Hakk *et al.*, 2000).

Fecal excretion was the major route of elimination of TBBPA regardless of dose or route of administration. At a comparable dose (20 mg/kg), the excretion rate appeared somewhat slower following iv as compared to oral administration. This is most likely explained by more extensive tissue distribution following iv dosing, although that could not be ascertained by the study design (tissue collected only at late time points). Szymańska *et al.* (2001) reported retention of TBBPA in adipose tissue (3–6%) and muscle (11–14%) following ip administration of large doses of TBBPA to female Wistar rats. Also, the elimination in the feces, when expressed as percent of dose, was slower following oral administration of the highest dose of TBBPA. This most likely represents saturation of metabolic and/or transport processes in the liver. However, by 72 h the percent dose excreted was nearly the same as that observed after the two lower doses (Fig. 4). In addition, repeated daily dosing did not appear to alter the rate or route of TBBPA excretion. Rats excreted nearly the entire daily dose in the feces before administration of the next dose.

Because of the rapid excretion of TBBPA, extensive tissue distribution studies were not performed. However, tissues were collected at 72 h after administration of the highest dose (200 mg/kg) and at 24 h after the last dose in the repeated dose studies. These tissues included the thyroid gland and the kidney, two tissues that have been reported to be altered by TBBPA (Fukuda *et al.*, 2004; Meerts *et al.*, 1999). Similar concentrations of ^{14}C in these tissues were obtained at 24 h after 1, 5, or 10 daily doses of TBBPA. Internal tissues, including the thyroid and kidneys, had minimal levels of ^{14}C-TBBPA equivalents. Radioactivity that had not been excreted within 24 h was associated with cecum and intestinal contents. The results of these studies demonstrate that TBBPA will not accumulate in tissues of rats following oral exposure to low levels of TBBPA. These results obtained from rats can be

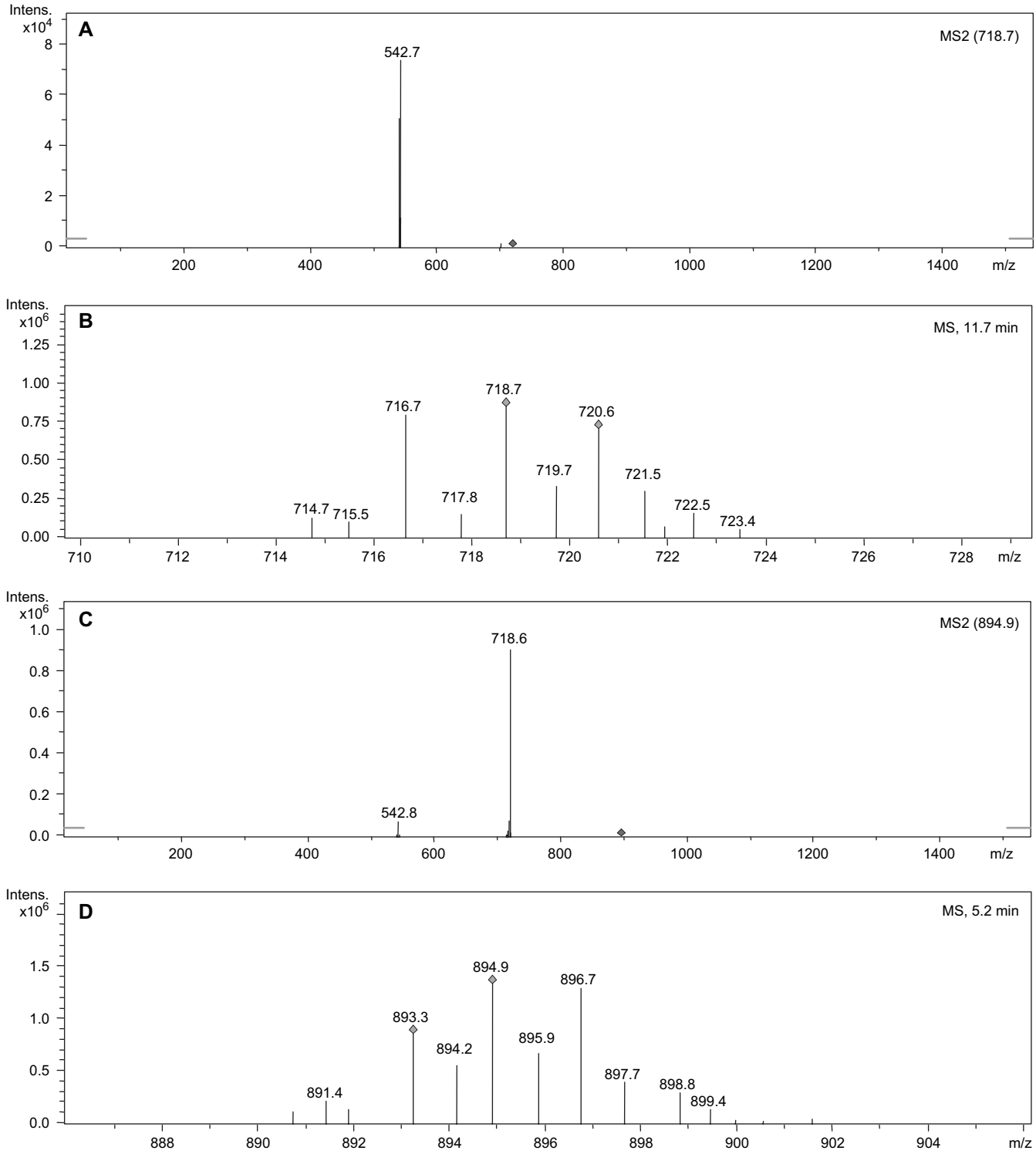

FIG. 6. Electrospray mass spectrum of TBBPA-glucuronide ([M-H] ion): (A) showing fragmentation of m/z 718 gives a loss of 176 amu; (B) isotope pattern of four bromine atoms. Electrospray mass spectrum of TBBPA-diglucuronide ([M-H] ion): (C) showing a fragmentation of m/z 895 gives a loss of 2 × 176 amu, (D) isotope pattern of four bromine atoms.

extrapolated to humans. Schauer *et al.* (2006) demonstrated that following administration of a low oral dose of TBBPA (0.1 mg/kg) to humans, no parent compound could be detected in serum. As in rats, an extensive first-pass effect limits systemic bioavailability of TBBPA and subsequent exposure following oral exposure. Hagmar *et al.* (2000) also concluded that the short half-life of TBBPA would limit its accumulation in the tissues of computer technicians during potential occupational exposure to TBBPA. In fact, Jakobsson *et al.* (2002) reported serum levels of TBBPA in computer technicians ranged from nondetectable to only 3.4 picomol/g lipid.

The major metabolites identified in this study were a mono- and a diglucuronide of TBBPA. They were the key metabolites excreted in the bile. Glucuronides were expected because previous reports in the literature identified them for TBBPA, bisphenol A, and related compounds (Hakk *et al.*, 2000; Pottenger *et al.*, 2000; Schauer *et al.*, 2006; Völkel *et al.*, 2005). Other metabolites of TBBPA may also have been produced at low levels, but they were not observed because of the analytical methodology employed herein. These other metabolites could include tribromobisphenol or a TBBPA-glucuronide-sulfate conjugate (Hakk *et al.*, 2000; Schauer *et al.*, 2006). However, these metabolites would have little influence on the pharmacokinetic behavior of TBBPA. Clearly, glucuronidation appears to be the key physiological process that governs the clearance of TBBPA and determines its oral systemic bioavailability. TBBPA derivatives with substituted hydroxyl groups would be expected to have different absorption, distribution, metabolism and excretion characteristics. They are also the key functional groups that facilitate extensive conjugation. Results of recent studies with TBBPA bis [2,3 dibromopropyl ether] underscore the importance of the hydroxyl groups. This compound, with very limited water solubility, is poorly absorbed from the gastrointestinal tract. The small amount (2–3%) that was absorbed was retained by the liver, where it was slowly metabolized to metabolites (unidentified) that are ultimately excreted into the bile (unpublished results). This extremely slow metabolism is directly related to the lack of free hydroxyl groups. Free hydroxyl groups provide sufficient water solubility to promote absorption from the intestine.

TBBPA is considered to be of low acute toxicity. However, reports in the literature suggest that it causes immunosuppresion (Pullen *et al.*, 2003), nephrotoxicity in newborn mice (Fukuda *et al.*, 2004), liver and kidney toxicity in female rat dams and their offspring (Tada *et al.*, 2006), and neurotoxicity (Mariussen and Fonnum, 2003). It should be stressed that these toxicities were suggested from *in vitro* studies (immunosupression and neurotoxicity) or were produced in newborn animals/dams exposed to excessive doses of TBBPA. *In vitro* studies using lymphocytes or brain synaptosomes expose these tissues directly to excessive amounts of TBBPA. They do not account for the excessive first-pass clearance of TBBPA by the liver, which greatly reduces their exposure *in vivo*. Similarly, newborn rats are deficient in glucuronidation activity (Wishart, 1978), where excessive doses of TBBPA may overwhelm glucuronidation capacity and lead to higher systemic exposure. Thus, it is important to consider such factors in the extrapolation of these toxicological findings to relevant human exposures to TBBPA.

In summary, following oral administration, TBBPA undergoes extensive absorption from the gastrointestinal tract. However, because of the extensive first-pass effect of the liver (and perhaps intestinal mucosa), systemic bioavailability of TBBPA is low. This low bioavailability explains the relative lack of toxicity following oral administration of single or repeated doses of TBBPA.

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