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Disposition and Metabolic Profiling of Bisphenol F in Pregnant and Nonpregnant Rats

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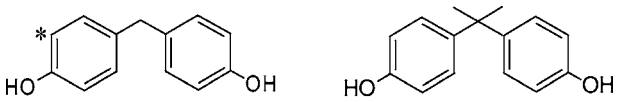
The distribution of bisphenol F (4,4′-dihydroxydiphenyl-methane, BPF) was studied in female Sprague−Dawley rats. Pregnant and nonpregnant animals were gavaged with a single dose of 7 or 100 mg/kg [^{3}H]BPF and were kept for 96 h in metabolic cages. The excretion of BPF residues occurred mainly in urine (43−54% of the administered dose), which was found to contain at least six different metabolites, and to a lesser extent in feces (15−20% of the administered dose). Sulfatase treatment and subsequent high-performance liquid chromatography analyses suggest that the major urinary metabolite (more than 50% of the radioactivity present in urine) is a sulfate conjugate of BPF. At 96 h, BPF residues were detectable in all tissues examined with the largest amounts in the liver (0.5% of the dose). In pregnant rats dosed at day 17 of gestation, BPF residues were detected in the uterus, placenta, amniotic fluid, and fetuses (0.9−1.3% of the administered dose). Large amounts of radioactivity (8−10% of the dose) were still located in the digestive tract lumen at the end of the study. After administration of a single oral dose of [^{3}H]BPF, 46% of the distributed radioactivity was excreted in bile over a 6 h period. In rats, BPF and/or its metabolites very likely undergo enterohepatic cycling, which could be responsible for the relatively high amounts of residues still excreted 4 days after BPF administration. This bisphenol is efficiently absorbed and distributed to the reproductive tract in female rats, and its residues pass the placental barrier at a late stage of gestation in rats.

KEYWORDS: Bisphenol F; endocrine disruptor; residues; metabolism; distribution; rat; biotransformation; placental barrier; fetus

INTRODUCTION

Bisphenol F (4,4′-dihydroxydiphenyl-methane, BPF) is a member of the bisphenol family and is structurally very close to bisphenol A (BPA), a monomer widely used for the manufacture of plastics and epoxy resins. In BPF, the two aromatic rings are joined through a methylene bridge (**Figure 1**). Like BPA, this compound has a broad range of industrial applications. The BPF monomer is polymerized to prepare the epoxy resins and polycarbonates used to manufacture lacquers and varnishes, coatings, adhesives plastics, and other products (*1*). Among the coatings, varnishes, or lacquers used in cans for food-packaging purposes, the most popular are those based on vinilic organosols (novolacs), which include in their composition epoxy resins such as BADGE (bisphenol A diglycidyl ether) and BFDGE (bisphenol F diglycidyl ether) (*2*). BPF is also found in dental materials (*3*) such as restorative materials, liners, adhesives, oral prosthetic devices, and tissue substitutes (*4*). It is well-established that BPA can migrate from packaging materials into food (*5*). Moreover, BPA was detected in human serum, in amniotic fluid, and in 95% of the urine samples obtained from a reference population in the United States, at concentrations of more than 0.1 μg/L (*6*). Likewise, BPF residues may migrate into food from epoxy coatings such as novolac glycidyl ethers (NOGE) (*7*). BPF monomers have been found in canned foods (*8*). BPF has also been detected in environmental media but at lower levels than BPA (*9*). Stachel et al. found low BPF concentrations in freshly deposited sediments (up to 7 μg/kg dry matter) sampled from German rivers and in surface water (0.4 ng/L) taken from the River Elbe and at the mouth of its tributaries (*10*).



[^{3}H]-BPF BPA

Figure 1. Chemical structures of BPF and BPA and the position of ^{3}H (*) labeling.

Since the early 1990s, it has been argued that man-made chemicals used for agricultural, industrial, or domestic purposes

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can be released in the environment, enter the food chain, and produce a number of disorders in animals and possibly in man (*11*, *12*). Moreover, in utero exposure during critical development stages and, more generally, disturbance of the early life environment may have an impact on child and adult health (*13*, *14*). These toxic substances, which are referred to as "endocrine disruptors", are widespread in our surrounding environment. They interfere with the endocrine system and can affect development and/or reproduction in many animal species (*12*) even at low doses (*15*, *16*). Many endocrine disruptor chemicals are estrogenic. Other compounds exhibit distinct biological activities including antiestrogenic, androgenic, or antiandrogenic effects (*17*). The biological activity of endocrine disruptors can be due either to direct binding to steroid hormone receptors or to other mechanisms such as disruption of biosynthesis or metabolism of steroids (*18*, *19*). BPA is a known endocrine disruptor that possesses estrogenic activity (*20*). Likewise, BPF was shown to be a xenoestrogen. When a yeast two-hybrid system was used, BPF was found to be the most estrogenic compound among the molecules present in food-packaging material or used in dentistry (*4*, *21*). The proliferative response of MCF-7 cells (E-screen assay) increased when cells were exposed to BPA but also to BPF (*22*) in a concentration-dependent manner (*23*). The latter authors found that, according to EC_{50} values, MCF-7 cell proliferation was more pronounced with BPF than with BPA, although the two molecules exhibited the same affinity for the estrogen receptor (ER). However, when the mammary cell line MDA-MB453-kb2 (AR^+, Luc^+) was used, BPF was found to exhibit the same antiandrogenic activity as BPA, suggesting that in vitro, BPF had a weakly estrogenic effect on MCF-7 cells and an antiandrogenic effect on MDA-MB453-kb2 cells (*20*). Yamasaki et al. reported that BPF was also estrogenic in vivo based on a uterotrophic assay carried out on immature Sprague−Dawley rats dosed with 200 mg/kg bw for 3 days by subcutaneous route (*24*). In contrast, using the Hershberger assay, these authors found no androgenic or antiandrogenic effects of BPF even at a higher dosage of 1000 mg/kg bw for 10 days (*25*).

Understanding the distribution and metabolic routes followed by BPF in mammals is a necessary step to assess the risk associated with the use of this chemical for the manufacture of food contact plastics. Both the pharmacological and the toxicological properties of BPF may be modified when the parental compound is metabolized, and no data are currently available regarding the bioavailability and distribution of BPF and its residues. Key information is thus required on these issues using in vivo animal models. From a histological point of view, rodent placentation at late stages of gestation is relatively close to human placentation, as both are of the chorioallantoidian type with histiotrophic nutrition of the embryo. In CD1 mice, it was demonstrated that BPA crosses the placental barrier, and both the parent compound and the conjugated metabolites were present in fetuses (*26*). Given the structural similarity of the two molecules, this could also be the case for BPF.

The disposition of tritium-labeled BPF ([^{3}H]BPF) was investigated following a single oral dose (7 and 100 mg/kg bw) administered to pregnant and nonpregnant Sprague−Dawley rats. The main goals of this study were (i) to investigate for the first time the distribution and disposition of BPF in an animal model in vivo, (ii) to evaluate the potential of BPF residues to cross the placental barrier in rat at a late stage of gestation, (iii) to determine the influence of the gestational status on BPF disposition, and (iv) to obtain a metabolic profile of BPF in vivo.

MATERIALS AND METHODS

Chemicals. ^{3}H-labeled BPF ([^{3}H]BPF), with a specific activity of 300.36 MBq/mmol, was purchased from Izotop (Budapest, Hungary). It was stored in ethanol at −20 °C. Before the experiments, the solution was evaporated to dryness under a nitrogen stream and resuspended in propylene glycol (Sigma Aldrich, l'Isle d'Abeau Chesnes, France). Its purity was greater than 99.3% based on radiochromatographic analysis. Unlabeled BPF (>98% pure, CAS #620-92-8) was purchased from Sigma Aldrich (L'Isle d'Abeau Chesnes, France).

Other chemicals, solvents (analytic grade), and scintillation cocktails were purchased from the following sources: ammonium acetate, Sigma Aldrich; methanol and acetonitrile, Scharlau Chemie S.A. (Barcelona, Spain); ethanol and acetic acid, Merck (Briare-Le-Canal, France).

Animals. *Metabolic Balance and Tissue Distribution.* Eight adult nonpregnant Sprague−Dawley female rats and eight adult pregnant Sprague−Dawley female rats (8−10 weeks old) were obtained from Charles River Laboratories (Saint-Germain sur l'Arbresle, France). The animals were individually housed in stainless steel metabolic cages with free access to water and to a semisynthetic diet (18% casein, 39% wheat starch, 24% sucrose, 8% peanut oil, 3% cellulose, 8% minerals and vitamins), under a 12 h light/dark cycle. After a 5 day acclimatation period, nonpregnant rats were weighed and randomly divided into two groups of four animals: nonpregnant, low dose (NP-LD) and nonpregnant, high dose (NP-HD). Likewise, pregnant animals were divided into two groups: pregnant, low dose (P-LD) and pregnant, high dose (P-HD). There was no significant difference in calculated mean weights between NP-LD and NP-HD groups (211.4 ± 4.9 g) or between P-LD and P-HD groups (285.2 ± 12.2 g). Groups NP-LD and P-LD were individually gavaged with a single nominal oral dose of 7 mg/kg bw [^{3}H]BPF (2046 and 2035 MBq, respectively, adjusted with unlabeled BPF for the pregnant group) dissolved in propylene glycol (1 mL per rat). NP-HD and P-HD groups were individually dosed with 100 mg/kg bw of a [^{3}H]BPF solution (2146 and 2072 MBq, respectively), adjusted with unlabeled BPF in propylene glycol (1 mL per rat). For P-LD and P-HD groups, oral administration was carried out at day 17 of gestation. Urine and feces were collected once every 24 h over a 96 h period. Animals were euthanized by exsanguination after cervical dislocation 96 h after BPF administration. Blood, liver, ovaries, uterus, vagina, muscle (back leg), peritoneal fat, the digestive tract including its contents (duodenum to rectum), brain, kidney, and the remaining carcass were collected for quantification of radioactivity. For pregnant rats, uteri were individually processed to separate fetuses, placenta, amniotic fluid, and the uterus itself. The metabolic cages were washed with ethanol (30−50 mL), and aliquots were removed for the determination of residual radioactivity.

Biliary Excretion of [^{3}H]BPF Radioactivity after a Single Oral Administration. Four 16 week old Sprague−Dawley female rats (284.0 ± 20.9 g) were gavaged with a single dose of [^{3}H]BPF (1.5 mg/kg bw) dissolved in 1 mL of propylene glycol. The rats were anesthetized with ethyl carbamate (1.2 g/kg, intraperitoneal route), and the bile duct was cannulated with a Folioplast catheter (0.3 mm × 0.7 mm). Bile collection began 2 h after administration of BPF. Bile was collected at 30 min intervals for 6.5 h, and then, the animals were euthanized by exsanguination.

Sample Processing. Radioactivity was measured in aliquots of urine and bile on a Packard scintillation analyzer (model Tricarb 2200CA; Packard Instruments, Meriden, CT) using Packard Ultima Gold as scintillation cocktail (Packard Instruments, Downer Grove, IL). For all vials, sample quenching was compensated by the use of quench curves and external standardization.

Radioactivity in rat carcasses, homogenized tissues and feces, blood, and amniotic fluid samples was determined by complete combustion using a Packard Oxidizer 306 (Packard Instruments), followed by ^3H_2O quantification on a Packard scintillation analyzer (scintillation cocktail: Packard Monophase S and Packard Permafluor E+, 14:5, v/v). Three replicates were analyzed for each sample.

For each rat, the contents of the digestive tract were emptied into a glass flask, washed with 20−30 mL of 0.9% NaCl, and homogenized using a Polytron homogenizer (Kinematica, Lucerne, Switzerland). The

Table 1. Ninety-Six Hour Metabolic Balance of BPF in P and NP Sprague–Dawley Rats Dosed with 7 mg BPF/kg bw (LD) or 100 mg/kg bw (HD)[a]

	%			
	NP-LD	P-LD	NP-HD	P-HD
urine	42.85 ± 4.62	44.39 ± 4.11	43.80 ± 3.55	53.72 ± 2.97
feces	14.31 ± 4.01	18.99 ± 1.68	18.29 ± 1.71	18.96 ± 2.63
digestive tract content	8.51 ± 2.69	8.14 ± 2.01	9.95 ± 3.53	10.49 ± 3.18
tissues	0.53 ± 0.04	0.85 ± 0.16	0.46 ± 0.07	0.75 ± 0.07
liver	*0.48 ± 0.03*	*0.75 ± 0.20*	*0.43 ± 0.08*	*0.54 ± 0.07*
uterus	*<0.01*	*0.07 ± 0.02*	*<0.01*	*0.18 ± 0.05*
fetuses + placenta		1.32 ± 0.33		0.91 ± 0.21
carcasses	8.05 ± 1.43	8.38 ± 1.43	5.97 ± 0.56	6.69 ± 0.57
cages	1.18 ± 0.55	1.34 ± 0.25	1.65 ± 0.36	2.25 ± 0.48
total	**75.5 ± 13.3**	**83.4 ± 10.1**	**80.1 ± 9.8**	**93.8 ± 10.2**

[a] Values are means of four animals per group ± SEM.

radioactivity in three aliquots taken from each of these samples was then measured using the oxidizer.

For each pregnant rat, three fetuses and the corresponding placenta were randomly selected and radioactivity in the samples was measured after combustion. The liver, head, and the rest of the body of each fetus were processed separately.

Radio-high-performance Liquid Chromatography (HPLC) Profiling. All HPLC analyses were performed on a HP1050 apparatus (Hewlett-Packard, Waldbronn, Germany) equipped with a Rheodyne model 7125 injector (Rheodyne, Cotati, CA) connected to a Radiomatic Flo-One/β 610 TR instrument (Radiomatic, La-Queue-Lez-Yvelines, France) to measure radioactivity using Flow-Scint II as the scintillation cocktail (Packard Instruments) or to an HP 1050 UV detector set at 270 nm to establish metabolic profiles. The reverse phase HPLC system consisted of a C18 guard precolumn (18 mm × 4.6 mm, 5 μm) (Interchim, Montluçon, France) coupled to a two-column system: (i) a Kromasil C18 column (250 mm × 4.6 mm, 5 μm) (Interchim) and (ii) a Nucleodur C18 column (250 mm × 4 mm, 5 μm) (Macherey-Nagel, Hoerdt, France). Mobile phases were ammonium acetate buffer (20 mM, adjusted to pH 3.5) and acetonitrile: A, 95:5 v/v, and B, 10:90 v/v, respectively. The flow rate was 1 mL/min at 35 °C. The two-step gradient was used as follows: 0−6 min 100% A; 6−18 min linear gradient from 100% A to A:B 60:40 v/v; 18−34 min A:B 60:40 v/v; 34−36 min linear gradient from 40 to 100% B; 36−56 min 100% B. Urine samples (ca. 333 Bq) were injected in mobile phase A with a final volume of 500 μL.

Enzymatic Hydrolyzes. To determine if a major peak corresponded to conjugates in the radiochromatograms, samples were treated with β-glucuronidase from *Escherichia coli* (type VII, Sigma Chimie, St. Quentin Fallavier, France) and sulfatase from *Aerobacter aerogenes* (type VI, Sigma Chimie). Hydrolysis conditions were as indicated by the supplier, except that 20 μL of 10% D-saccharic acid-1,4-lactone was added with sulfatase during incubation to inhibit possible β-glucuronidase activity.

Statistical Analyses. Mean values were compared using Student's *t*-test.

RESULTS

Excretion of Radioactivity. The total radioactivity recovered over the 4 day study, expressed as a percentage of the administered dose, was equal to 83.2%: 79.4 ± 11.7% for nonpregnant rats (NP-LD and NP-HD groups) and 86.7 ± 9.9% for pregnant rats (P-LD and P-HD groups) (**Table 1**). Most of the excreted radioactivity was recovered in urine. Ninety-six hours of cumulated urinary excretion of radioactivity accounted for ca. 44% of the administered dose, except for the P-HD group in which the amount of radioactivity recovered in urine was 53.7%. No significant difference was observed between groups.

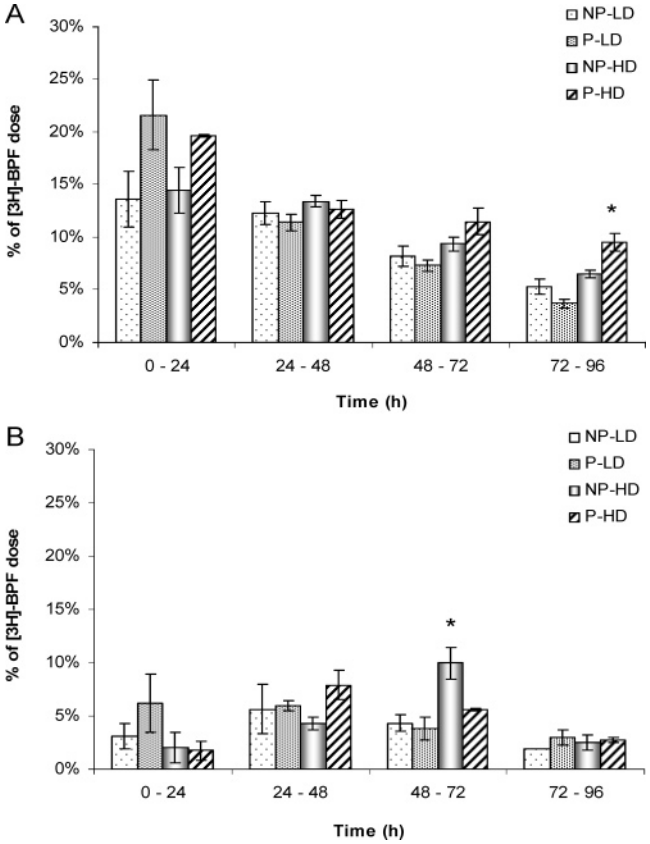

Figure 2. Residual urinary (**A**) and fecal (**B**) levels of radioactivity measured every 24 h in pregnant (P) and nonpregnant (NP) rats following a single oral dosage of 7 mg/kg (LD) or 100 mg/kg (HD) [^{3}H]BPF. Results (mean ± SEM, $n = 4$ for each group) are expressed as a percentage of the administered radioactivity. *Significantly different ($p < 0.05$) from the other groups.

Excretion of urinary radioactivity decreased over the 4 day study (**Figure 2A**) but remained relatively high (>3%) up to 96 h. At that time point (urine collected between 72 and 96 h), a higher proportion of radioactivity (9.6%) was recovered in the P-HD group than in the other groups. Lower amounts of residues were excreted by fecal route (14−20%), with no significant difference between groups. Fecal excretion reached a maximum around 48 h (**Figure 2B**), but 2.5−3% of the administered radioactivity was still excreted in 72−96 h feces. Additional radioactivity (8−10% of the administered [^{3}H]BPF dose) was recovered in the contents of the digestive tract at the end of the study (**Table 1**).

Distribution in Tissues. The amount of [^{3}H]BPF residues measured in tissues ranged from ca. 0.5−0.8% in nonpregnant rats and pregnant rats, respectively, with no significant difference between the four groups. Most of the tissular radioactivity was located in the liver and, in pregnant animals, in the uterus. Higher amounts of residues were found in the uteri of pregnant rats dosed with 7 mg/kg than in pregnant rats dosed with 100 mg/kg although mean weights of uteri (4.6 and 5.2 g for animals from groups P-LD and P-HD, respectively) were not significantly different (data not shown). All other tissues contained no more than 0.05% of the administered radioactive dose. Relatively high amounts of radioactivity (6−8.4%) were still located in the carcass at the end of the study (**Table 1**).

The concentration of [^{3}H]BPF residues in tissues was calculated and was expressed in ng/g BPF equivalent (ppb) (**Table 2**). Residual levels ranged from 100 to 800 ppb in the LD groups and from 500 to 13000 ppb in the HD groups; the

Table 2. Levels of [^{3}H]BPF Residues Measured 96 h after the Administration of a Single Oral Dose of 7 mg BPF/kg bw (LD) or 100 mg/kg bw (HD) to P and NP Sprague–Dawley Rats[a]

	NP-LD	P-LD	NP-HD	P-HD
liver	832.5 ± 51.5	679.0 ± 174.8	13072.6 ± 2007.8	7558.0 ± 990.5
kidney	319.3 ± 25.0	268.3 ± 22.5	4571.2 ± 890.8	3535.3 ± 490.9
brain	159.9 ± 15.5	88.0 ± 9.2	1411.7 ± 399.8	499.4 ± 76.5
muscle	160.6 ± 17.8	121.3 ± 20.8	1506.4 ± 430.6	778.8 ± 187.3
fat	105.1 ± 12.2	85.8 ± 15.4	1743.7[b] ± 282.4	500.4 ± 68.7
ovary	196.9 ± 18.4	133.7 ± 12.9	2257.6 ± 649.9	1179.8 ± 288.9
uterus	217.8 ± 7.1	197.4 ± 30.6	3025.5 ± 1141.3	6486.8 ± 1103.7
vagina	449.1 ± 180.0	209.5 ± 54.0	4145.3 ± 798.3	3838.2 ± 960.3
blood	91.1 ± 38.5	137.1 ± 9.3	2540.1 ± 697.6	1343.9 ± 253.1
placenta		241.8[c] ± 24.4		2141.8 ± 161.6
amniotic fluid		195.1[c] ± 16.4		2691.0[d] ± 111.6
fetuses		193.9[d] ± 9.4		2892.6[d] ± 109.5

[a] Results are expressed in ng/g (ppb) of BPF equivalent (values are means of four animals per group ± SEM). [b] Significantly different ($p < 0.05$) from corresponding values in P-HD animals. [c] Significantly different ($p < 0.05$) from corresponding blood values. [d] Significantly different ($p < 0.01$) from corresponding blood values.

highest concentrations were found in the liver. For each dose, pregnant and nonpregnant animals were compared. No significant difference was found, except for the concentrations measured in fat, which were lower in pregnant rats dosed with 100 mg/kg BPF than in pregnant rats dosed with 7 mg/kg.

Intrauterine Compartment. In rats dosed with 7 mg/kg [^{3}H]-BPF, 1.23 ± 0.38% of the radioactivity was located in the fetuses (whole litter) and 0.09 ± 0.03% in the placenta. Lower but not significantly different values were found in animals dosed with 100 mg/kg (fetuses, 0.88 ± 0.21%; placenta, 0.05 ± 0.01%). Residual concentrations of [^{3}H]BPF residues were similar in fetuses, placentas, and amniotic fluids in the P-LD and P-HD groups, respectively. These values were all significantly higher than those calculated for blood, with the exception of placentas in the HD group.

The determination of radioactivity levels in different parts of the fetuses (head, liver, and the rest of the body) enabled homogeneous distribution of [^{3}H]BPF to be demonstrated in both the LD and the HD groups (**Figure 3**). Residual concentrations of BPF were identical in the liver and in the rest of the body but also in the head of the fetuses, which contained ca. 25% of fetal radioactivity. This distribution was the same for both BPF doses.

Biliary Excretion in Cannulated Rats. Bile samples from rats dosed with 1.5 mg/kg bw [^{3}H]BPF were collected every 30 min starting 2 h after oral BPF administration (**Figure 4**). The highest amounts of radioactivity were excreted in the earliest fractions. More than 46% of the administered [^{3}H]BPF dose was recovered 6 h after bile collection started, indicating extensive biliary excretion of BPF residues under our experimental conditions.

Radiochromatographic Profiling of Urine. Figure 5 shows a radiochromatographic profile obtained for a 0−96 h pool of urine from a pregnant rat dosed with 100 mg/kg bw [^{3}H]BPF. No tritium exchange was observed. Unchanged BPF, coeluting with the authentic standard, was detected with a retention time (R_T) of 32.5 min. The major metabolite of BPF (>50% of urine radioactivity) had a R_T of 22.9 min (peak 5). At least five other radioactive peaks were detected, suggesting the existence of multiple metabolic pathways for BPF. After collection and treatment with appropriate enzymes, peak 5 (R_T 22.9 min) was successfully hydrolyzed by sulfatase, whereas β-glucuronidase did not affect its retention time (data not shown). In addition, on the HPLC analysis obtained after sulfatase treatment, peak 5 disappeared almost completely, yielding a peak eluted at 32.5 min (data not shown), suggesting that the major metabolite present in urine was very likely a sulfate conjugate of BPF.

DISCUSSION

Regardless of the BPF dose and the gestational status of rats, urine was found to be the major excretion route of BPF residues. Urinary excretion accounted for almost half the administered radioactivity, while fecal excretion accounted for less than 20%. BPA residues are predominantly excreted by fecal route in rodents (*27*, *28*). For instance, in female rats, Pottenger et al. reported that 70% of a single administered oral dose (10 or 100 mg/kg bw) was recovered in feces after 7 days (*29*). Our results suggest that despite the structural similarity between BPA and BPF, the favored excretion route for the latter molecule is the urinary route. This may be due to different metabolic pathways for these two compounds. Nevertheless, the extent to which a

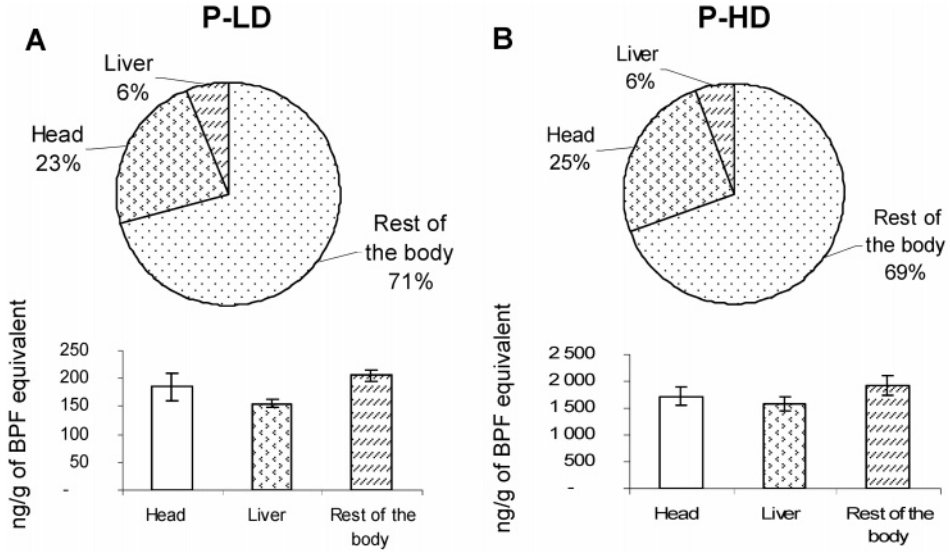

Figure 3. Residual levels of radioactivity recovered in the head, liver, and rest of the body of fetuses from pregnant Sprague–Dawley rats dosed with 7 mg/kg (P-LD) (**A**) or 100 mg/kg (P-HD) (**B**). Results are expressed as a percentage of the radioactivity recovered in fetuses and in ng/g of BPF equivalent (mean ± SEM).

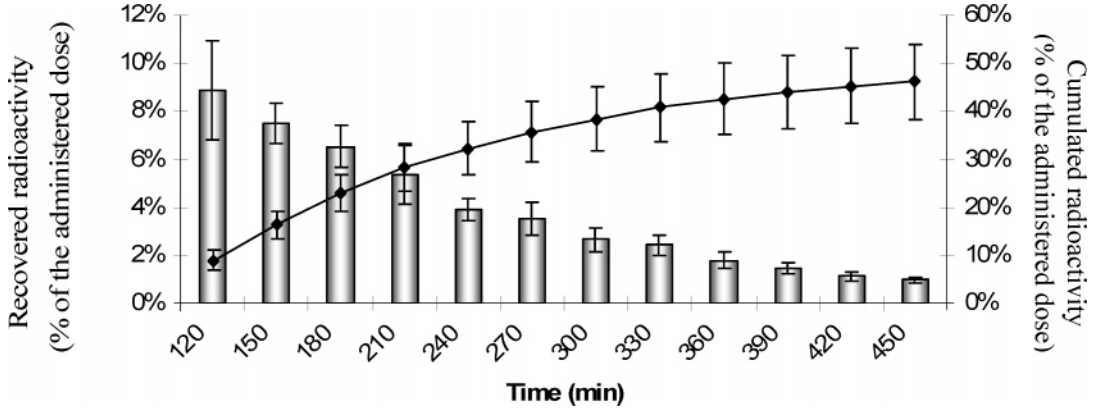

Figure 4. [^{3}H]BPF residues (bar chart) and cumulative biliary excretion (plot) in samples collected at 30 min intervals starting 2 h after administration of 1.5 mg/kg of [^{3}H]BPF (% of the administered dose; mean values ± SEM, $n = 4$ animals).

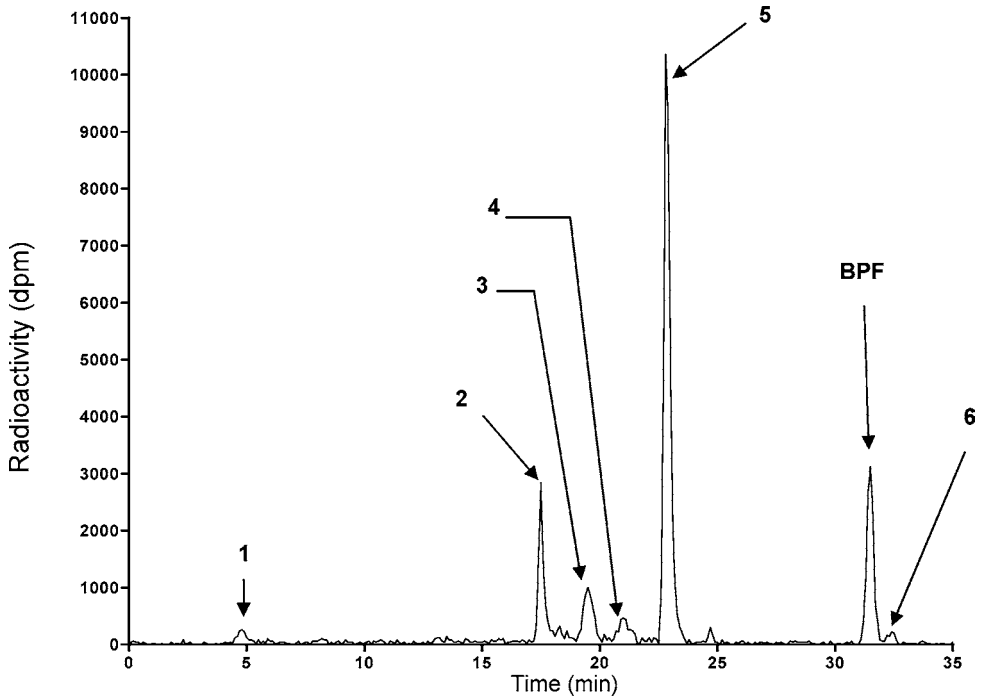

Figure 5. Typical radiochromatographic profile for 0–96 h pooled urine collected from a rat dosed with 100 mg/kg bw [^{3}H]BPF.

xenobiotic (and corresponding metabolites) is eliminated into the bile rather than excreted in the urine may be influenced by a number of physicochemical factors including polarity and molecular weight. The fact that BPF bears no methyl substituents on the central carbon of the molecule makes it slightly more water soluble than BPA thus facilitating urinary excretion. Furthermore, the substantial difference between the molecular weights of BPA and BPF conjugates and the fact that the threshold molecular weight for appreciable biliary excretion in the rat is in the range 275−375 (*30*) could also partly explain the quantitative differences observed between BPF and BPA. In our case, excretion of BPF residues in urine gradually decreased over the 4 day study period but remained quantitatively significant up to 96 h. Approximately 5% of the administered dose was recovered from 72 to 96 h urinary samples. Moreover, in these samples, significantly higher amounts of radioactivity (10%) were excreted by pregnant rats dosed with 100 mg/kg BPF. In a comparable low-dose study carried out on BPA, a rapid decrease in urinary radioactivity levels was demonstrated in rats gavaged with a single oral dose of labeled BPA, with very small amounts of radioactivity being excreted later than 48 h after BPA administration (*28*). Our results showed that, in rats, urinary excretion of BPF may last longer than that of BPA. According to radio-HPLC analyses of pooled urine samples, BPF appeared to be efficiently metabolized in Sprague−Dawley rats. At least six different metabolites were detected, and the parent compound accounted for less than 25% of the radioactivity present in urine. Although no study of BPF biotransformation has been published so far, these results are in good agreement with the data available for BPA in rodents. The latter molecule is extensively metabolized by conjugation (*31*−*34*), as well as by oxidative pathways, with very low amounts of the parent molecule being excreted in urine (*26*). However, whereas the major urinary metabolite in female rats was identified as BPA glucuronide (*35*), we found by incubation with sulfatase that BPF was mainly conjugated with a sulfate group. This difference could be linked to the differences in chemical structure between BPF and BPA. BPF glucuronide may also be more easily deconjugated in the gut and subsequently reabsorbed in large quantities.

Fecal excretion reached a maximum around 48 h and still accounted for 2.5−3% of the administered radioactivity in 72−96 h samples, with no significant difference between groups. By the end of the study, 8−10% of the administered radioactiv-

ity was still present in the contents of the digestive tract, again, with no difference with respect to the gestational status or the administered dose. These data strongly suggest enterohepatic cycling of BPF residues in rats, since the radioactivity excreted over the last 24 h of the study was far below the amount of radioactivity remaining in the contents of the digestive tract. Additional experiments carried out in bile duct-cannulated rats confirmed massive biliary excretion of BPF residues. Nearly half of a 1.5 mg/kg bw [^{3}H]BPF dose was excreted in bile samples collected 2−8 h after administration of BPF. The highest radioactivity levels were found in bile collected immediately after cannulation (e.g., 2−2.5 h after administration of BPF), demonstrating rapid uptake and excretion of the molecule. The amount of residues excreted in bile further strengthens the hypothesis of enterohepatic cycling of BPF or its metabolites. Such a cycle has already been suggested for BPA in rats (*29*, *36*). It was also postulated that the biliary excretion of BPA residues could be greater in female rats than in males and lower in pregnant rats (*37*).

Less than 1% of the radioactive dose was located in rat tissues 96 h after administration of BPF. The target organ was liver (ca. 0.5% of the radioactivity). The radioactivity present in all other tissues, with the exception of the uterus, accounted for less than 0.05% of the administered dose. No significant difference was found between groups. Calculated residual levels ranged between 100 and 800 ppb in rats dosed with 7 mg/kg. With the 100 mg/kg dose, these levels were higher but roughly corresponded to similar values multiplied by a 100/7 ratio, suggesting no dose effect for BPF residue distribution on a quantitative basis. No significant differences were found between pregnant and nonpregnant rats, with the exception of the residual levels calculated for fat in animals dosed with 100 mg/kg. Although this difference was not observed for the lowest dose, the fact that significantly lower residual levels were found in the fat of pregnant rats dosed with 100 mg/kg is of interest since it may be linked with depot lipid mobilization during late gestational stages. This issue requires further confirmation using a broader range of BPF doses and larger groups of rats.

The amount of BPF residues present in the uteri of nonpregnant rats was below 0.01% of the administered dose. In pregnant rats, 0.07 and 0.18% of the radioactivity was located in the uteri of rats dosed with 7 and 100 mg/kg BPF, respectively, with a significant difference, suggesting a dose−effect relationship. In previous studies, in uterotrophic assays in rats dosed with 200 mg/kg, BPF results were positive (*24*, *38*). However, in our study, uterus weights were not significantly different in the two groups of pregnant rats, and no uterotrophic effects were observed in these adult animals. Therefore, the increase in the weight of the uterus due to an estrogenic effect does not explain the difference observed. In all groups, BPF residues were still detectable in blood 96 h after the rats were gavaged with BPF. As compared to blood, the residual levels detected in the placenta (with the 7 mg/kg dose) and in fetuses and amniotic fluid (with both doses) were significantly higher. Previous studies demonstrated that BPA is efficiently distributed in the reproductive tract and passes the placental barrier in rodents (*26*, *28*, *39*). At a late stage of gestation in mice, residual levels of BPA calculated for placenta, uterus, and amniotic fluid were found to be twice as high as in blood (*26*), which is consistent with the results obtained for BPF in rat. Transplacental passage of BPA residues has also been demonstrated in rats (*28*, *40*). Kurebayashi et al. suggested that in rats, fetal exposure occurs only when mothers are dosed at a late stage of gestation (day 18, as compared to days 12 and 15) (*28*). We found that 1.2% of a 7 mg/kg or 0.9% of a 100 mg/kg dose was located in fetuses 96 h after oral administration of BPF at day 17 of gestation. Moreover, regardless of the dose, fetal radioactivity was homogeneously distributed with equal residual levels measured in the head and liver of the fetus. We thus hypothesize that BPF (or its metabolites) may reach the brain of developing rat fetuses, as already reported for BPA in male and female offspring in mice (*41*) and in female offspring in Wistar rats (*42*). The structural characterization and biological activity of BPF residues will need to be assessed in order to investigate the possible consequences of fetal exposure.

This first study of BPF distribution in an animal model demonstrates that this bisphenol, like its structural analogue BPA, is efficiently absorbed by oral route and is distributed to the whole organism, including the reproductive tract and fetuses. In contrast with BPA, BPF and its metabolites are mainly excreted in urine. Relatively high amounts of residues, 7−9% of the dose, were still present in the carcasses and tissues of rats 96 h after a single oral administration of BPF. At that time point, similar quantities of radioactivity were still present in the digestive tract, and as enterohepatic cycling is highly probable in rat, this could partly explain the apparently slower elimination of this bisphenol as compared to BPA. The metabolic fate of BPF now needs to be investigated in order to better understand the potential toxicity of this endocrine disruptor.

ABBREVIATIONS USED

AR, androgen receptor; BADGE, bisphenol A diglycidyl ether; BFDGE, bisphenol F diglycidyl ether; BPA, bisphenol A; BPF, bisphenol F; Bq, bequerel; bw, body weight; EC_{50}, effective concentration 50; ER, estrogen receptor; HPLC, high-performance liquid chromatography; ip, intraperitoneal; Luc, luciferase; NOGE, novolac glycidyl ether; NP-HD, nonpregnant, high dose; NP-LD, nonpregnant, low dose; P-HD, pregnant, high dose; P-LD, pregnant, low dose; ppb, parts par billion; R_T, retention time; SEM, standard error of the mean.

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