JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 8757–8762 8757
DOI:10.1021/jf1012924

Metabolism of 2,2′,4,4′-Tetrabromodiphenyl Ether (BDE-47) in Chickens

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Polybrominated diphenyl ethers (PBDEs) are an important class of persistent, organic pollutant that, based on previous studies in rodents, are poorly metabolized and bioaccumulate in lipophilic stores of the body. Because humans typically consume the fat and skin of chicken, a single ^{14}C-radiolabeled dose (2.7 mg/kg; 5.64 μmol/kg) of the most common PBDE in the environment, that is, 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47), was administered to determine its metabolic disposition in male broiler chickens. Orally dosed BDE-47 was readily absorbed from the gut of chickens and was estimated to be 73% bioavailable. Cumulative tissue retention at 72 h was 60.2% of the dose. BDE-47 was deposited preferentially in lipophilic tissues, and the decreasing rank order of concentration on a wet weight basis was adipose tissue, skin, gastrointestinal tract, lung, carcass, muscle, liver, and kidney. When concentrations were adjusted for lipid content, the levels of BDE-47 in the principal edible tissues in chicken, that is, adipose tissue, skin, liver, and white and dark meat, were very similar to one another. Excretion of unbound metabolites in excreta was <1% of the dose, but bound radioactivity was a major component of excreta at >12% of the dose. Alkaline hydrolysis of bound material yielded a hydroxylated tetrabromo metabolite. The metabolic pathway of BDE-47 in chicken included mono-oxidation, mono-oxidation/debromination, and debromination. The present results suggest that trimming the fat and skin from chicken would substantially reduce human exposure to PBDEs during the consumption of chicken.

KEYWORDS: BFRs; brominated diphenyl ethers; chicken; metabolism; excretion; BDE-47; tissue disposition; skin

INTRODUCTION

2,2′,4,4′-Tetrabromodiphenyl ether (BDE-47) belongs to a class of flame retardants (polybrominated diphenyl ethers or PBDEs) that are increasingly being detected in the environment and which have been found in humans and the food supply. PBDEs are intentionally produced for their legally required role in consumer product protection. PBDEs are one of many flame retardant chemicals that have been used to meet stringent flammability standards and requirements in consumer products (i.e., furniture, electronics, etc.).

The tetrabromo PBDE congener BDE-47 is the major persistent PBDE found in environmental and wildlife samples (*1*). Its high levels in such samples could be due to exposure, selective absorption, or degradation of more highly brominated PBDEs. In rats and mice (*2*), BDE-47 was efficiently absorbed and primarily stored in adipose tissue of both species. In rats, >80% of the dose was still in the body at 120 h, whereas 14% was excreted in feces and <0.5% was excreted in urine. In mice, 20 and 33% of the dose were excreted in the feces and urine, respectively.

Very little research has been conducted on PBDE levels or pharmacokinetics in chickens. Two studies (*3*, *4*) showed that concentrations of PBDEs in U.S. chicken fat samples ranged from 0.2 to 37.1 ng/g of lipid (sum of eight tri- to hepta-BDEs), which was lower than PBDE levels reported in fish obtained from the Baltic Sea (*5*) or the Great Lakes (*6*), but slightly higher than that in terrestrial animals from Sweden (*7*). The PBDE congener pattern for all chicken samples was the same and strongly indicated that general exposure occurred through contact with the commercial penta formulation (*4*).

Previous rat studies have shown that lipophilic tissues are the major reservoir for PBDEs, that is, adipose tissue, skin, and gastrointestinal (GI) tract. A study in laying hens also showed preferential concentration of BDE-47 into adipose tissue compared to liver or eggs; however, other edible tissues were not analyzed (*8*). In 2007, the per capita consumption of chicken had grown to 38.4 kg compared to 29.5 kg for beef and 22.8 kg for pork (*9*). A recent study has estimated that poultry consumption in the United States is a significant determinant of PBDE body burden (*10*), although quantitative human exposure to PBDEs through poultry consumption is unknown. Because lipophilic tissues such as skin are known to be a principal reservoir for PBDEs and because humans commonly consume the skin from chickens, it would be of high interest to know the pharmacokinetics of a representative PBDE in this food production animal and to determine what exposure to humans can result from

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Published on Web 07/16/2010

chicken consumption. Thus, the objective of this study was to assess the adsorption, disposition, metabolism, and excretion of BDE-47 in broiler chickens, a major source of protein for U.S. consumers.

MATERIALS AND METHODS

Syntheses of Unlabeled and [^{14}C]-2,2′,4,4′-Tetrabromodiphenyl Ether (BDE-47). Phenol (102 mg) was dissolved in HBr (785 mg), cooled to −5 °C, and reacted with bromine (0.26 mL of a 1:1 w/w solution of HBr/Br_2) to form 2,4-dibromophenol. 2,4-Dibromophenol (237 mg) was dissolved in dry dimethylformamide (1 mL), cooled to 0 °C, and then reacted with sodium hydride (46 mg, 60% in mineral oil) for 5 min. After warming to room temperature, 1-fluoro-2,4-dinitrobenzene (0.12 mL) was added to the mixture to form 2,4-dibromo-2′,4′-dinitrodiphenyl ether in 45−60% yield after 2 h (*11*). Reduction to 2,4-dibromo-2′,4′-diaminodiphenyl ether was accomplished with iron powder (189 mg, 100 mesh) and concentrated HCl (30 μL) in 85% ethanol (3.5 mL) by refluxing in a 95 °C oil bath for 2 h (90% yield). BDE-47 was produced from a double Sandmeyer reaction of 2,4-dibromo-2′,4′-diaminodiphenyl ether (100 mg), $NaNO_2$ (50 mg), and Cu(I)Br (102 mg) in 16% HBr (2.25 mL); the reagents were combined at 0 °C and then heated in a 70 °C oil bath for 5 min to produce BDE-47 in 85% yield (*12*). BDE-47 from the final reaction step was purified by preparative HPLC on a C18 Delta Pak column (Waters, Milford, MA) using a 90% acetonitrile/water isocratic elution (99% purity by GC-MS).

2,2′,4,4′-Tetrabromo-[^{14}C]diphenyl ether ([^{14}C]BDE-47) was synthesized in a similar manner starting with [^{14}C]phenol (250 μCi, 54.5 mCi/mmol; Sigma, St. Louis, MO) diluted with unlabeled phenol (94 mg) with the following modification. The reduction to [^{14}C]-2,4-dibromo-2′,4′-diaminodiphenyl ether was accomplished with a 10-fold excess of both triethylamine and formic acid and catalytic amounts of 5% platinum on charcoal in refluxing benzene (10 mL) (75% yield) (*13*). A chemical purity of 98% was determined by GC-ECD, and a >98% radiochemical purity was verified by TLC using conditions described below.

Animals. Four Jumbo Cornish × Rock cross male chickens were purchased (McMurray Hatchery, Webster City, IA) at 1 day of age and raised in-house to 7 weeks of age (~3 kg). Research protocols were approved by the Institutional Animal Care and Use Committee at the ARS Biosciences Research Laboratory prior to the receipt of study animals. Chickens were fed an unmedicated, commercial starter ration until approximately 14 days of age and were subsequently fed a grower ration for the next 5 weeks and through the 72 h study period. The chickens were housed in individual cages equipped with water bottles, feeders, and pans to collect excreta. Feed and water were provided on an ad libitum basis.

Dose and Study Design. Each chicken (n = 4; 3.088 ± 0.271 kg) received a gelatin capsule containing [^{14}C]-2,2′4,4′-tetrabromodiphenyl ether (BDE-47; 2.7 mg/kg of body weight; equivalent to 5.64 μmol/kg of body weight; 8.4 mg/chicken) dissolved in 0.5 mL of peanut oil that had been applied to 4 g of grower ration. After dosing, urine and feces were collected together at 24 h intervals for 72 h. At 72 h animals were humanely slaughtered, and tissues were harvested as described below.

Sample Collection. Excrement from each chicken was collected at 24, 48, and 72 h after dosing. No attempt was made to perform colostomies, which would allow for the separate collection of feces and urine. The colostomy surgery is stressful on animals, a limited number of chickens were available, and little [^{14}C]BDE-47-derived radiolabel was expected to be eliminated in the urine due to the extremely lipophilic nature of the parent compound and because data from previous rat studies suggested a minor role of renal excretion for BDE-47 and metabolites. Seventy-two hours after dosing, animals were slaughtered by CO_2 asphyxiation followed by exsanguination. Blood was collected, and brain, GI tract (composed of duodenum, small intestine, large intestine, and ceca), gizzard (including proventriculus and crop), heart, kidney, liver, lung, skin, and spleen were removed. The GI tract was flushed of contents with 0.01 M NaCl and treated separately as GI tract contents. Aliquots of adipose tissue, white meat, and dark meat were sampled from each carcass. Feet, feathers, and beaks were not analyzed, and the carcass components remaining after tissue removal comprised the residual carcass. All tissues were frozen at −20 °C until analyses began. A set of three control chickens was filleted into white meat (breast) and dark meat (thigh and drumstick) so that live weights of these tissues could be obtained. Average live body weights of white meat and dark meat were 16.4 ± 2.1 and 13.1 ± 1.9%, respectively (data not shown). Previous studies have found that the average fat content of broiler chickens is 12.4% (*14*), and therefore this figure was used for all calculations. Extraction of selected tissues for fat analysis and high-resolution GC-MS quantitation of BDE-47 was by pressurized liquid extraction, as described in ref *8*, except for fat and brain, which were extracted by classical liquid−liquid extraction following homogenization in a Polytron (Brinkmann Instruments, Westbury, NY). Tissue extracts were purified for TLC and GC-MS analyses by using the methods described in ref *4*. The percent lipid composition was determined gravimetrically for adipose tissue, brain, liver, dark and white meat, and skin and found to be 85.6, 30.2, 8.5, 12.1, 3.0, and 47.1%, respectively.

Quantiation of Radioactive Residues in Excreta and Tissues. Tissues and excreta were analyzed for total radioactive residues so that a full mass balance could be determined. Three to five aliquots of lyophilized excreta (0.1 g) or lyophilized liver, GI tract, gizzard, kidney, lung, heart, and spleen (0.5 g each) were combusted in a tissue oxidizer (Packard, model 307, Meridan, CT), and radiolabeled CO_2 was trapped in CARBO-SORB E (Perkin-Elmer, Waltham, MA) and measured for ^{14}C in a Permafluor scintillator (Packard) using a calibrated liquid scintillation counter (LSC, Beckman LS 6000IC, Fullerton, CA). Skin, adipose tissue, muscle, and brain samples (0.5 g each) were combusted wet using a tissue oxidizer, and the released [^{14}C]CO_2 was assayed by LSC.

Extraction and Analysis of Radioactive Metabolites in Tissues and Excreta. BDE-47 and its metabolites were qualitatively assessed following extraction of adipose tissue, brain, liver, dark and white muscle, and skin. Individual tissues from the four chickens were pooled and homogenized with a mechanical mixer. Five grams of tissue was diluted with about 10 g of Celite filter aid until powdery and then transferred to an accelerated solvent extractor (ASE) cell (Dionex, Sunnyvale, CA) and extracted by pressurized fluid extraction (ASE) using 35:30:35 isopropanol/hexane/methylene chloride. Lipid content was determined gravimetrically and was subsequently removed by passing the extract through a hexane-equilibrated, H_2SO_4−silica gel column (20 g). Delipidated extract was then purified with a triphasic silica column packed from bottom to top with silica (1 g), basic silica (4 g), silica (1 g), acid silica (8 g), silica (2 g), and sodium sulfate (1 g). The extracts were blown to dryness and reconstituted with hexane (1 mL). One microliter was removed and transferred to a GC-MS vial, where 20 μL of nonane containing known amounts of 13 [^{13}C]PBDE internal standards was added. HR-GC-MS methods in selected ion recording (SIR) mode were used to quantitate the amount of parent compound in each of the tissue extracts (see below). The remainder of the extract was applied onto silica gel TLC plates (5 × 20 cm, Uniplate, 250 μm, Analtech, Inc., Newark, DE) and developed with 1:1 hexane/methylene chloride with [^{14}C]BDE-47 used as a standard. Radiolabeled bands on the TLC plates were analyzed using a System 2000 Imaging Scanner (Bioscan, Inc., Washington, DC). Any metabolites detected were scraped, eluted, derivatized with diazomethane, and analyzed by GC-MS (see below).

Lyophilized excreta were pooled each day and extracted sequentially with hexane, ethyl acetate, and methanol (750−1000 mL each extraction) by stirring for 6 h. Aliquots of concentrated extracts were analyzed with silica gel TLC plates (see above). The total amounts of nonextractable radioactivity in excreta were determined by subtracting the radioactivity obtained by solvent extraction from the total radioactivity obtained from combustion analyses. Extractable fecal radioactivity was analyzed for metabolites by gas chromatography−mass spectrometry (*15*) following purification. Briefly, fecal extracts were evaporated to dryness under N_2, reconstituted in 2.0 mL of hexane, and back extracted with 1.0 mL of 0.5 M KOH in 50% EtOH; aqueous and organic layers were then assayed for radioactivity. The aqueous layer was acidified with 1.5 mL of 0.5 N H_2SO_4 and subsequently extracted three times with 1.0 mL of 1:1 hexane/ether. The pooled organic extract was treated with diazomethane (3 h) and evaporated to dryness, the lipids were destroyed by acidification with 200 μL of concentrated H_2SO_4, and the acidic residue extracted three times with 0.5 mL of hexane. The pooled hexane layers were applied to a C-18 SPE cartridge (500 mg; YMC Corp., Ltd., Kyoto, Japan), and six fractions were eluted, that is, 50, 60, 70, 80, 90, and 100% methanol in water. Methoxylated metabolites eluted in the 80% methanol fraction, and parent compound eluted with 100% methanol.

Table 1. Tissue Distribution of [^{14}C]-2,2′,4,4′-Tetrabromodiphenyl Ether (BDE-47) Derived Total Residues in Jumbo Cornish × Rock Cross Male Chickens (n = 4) after a Single Oral Dose in Peanut Oil (2.7 mg/kg; 5.64 μmol/kg)

excreta	% dose	tissues	% dose
0−24 h	14.00 ± 4.86	adipose[a]	3.25 ± 1.04
24−48 h	4.28 ± 0.83	brain	0.002 ± 0.0005
48−72 h	3.69 ± 0.44	residual carcass	36.83 ± 2.83
GI contents	0.69 ± 0.37	GI tract	3.10 ± 1.35
		gizzard	0.04 ± 0.01
total excreted	22.6	heart	0.04 ± 0.01
		kidney	0.11 ± 0.02
		liver	0.47 ± 0.05
		lung	0.07 ± 0.02
		muscle (dark)[b]	1.30 ± 0.11
		muscle (white)[c]	0.91 ± 0.36
		plasma	0.06 ± 0.02
		skin	14.15 ± 2.83
		spleen	0.003 ± 0.001
		total retained	60.33

[a] A 16.5 g aliquot (average) of abdominal adipose tissue. [b] A 105.5 g aliquot (average) of dark (thigh) muscle. [c] A 260.5 g aliquot (average) of white (breast) muscle.

Methoxylated metabolites were analyzed by gas chromatography−electron impact mass spectrometry (GC-MS) on a VG Auto Spec (Micromass, Beverly, MA) mass spectrometer operating at 70 eV, using a model 5890 GC (Hewlett-Packard, Palo Alto, CA) with a 15 m DB-5 ms column (J&W Scientific, Folsom, CA) run from 70 to 310 at 10 °C/min. Full-scan mode was utilized, and scan range was from 300 to 750 mass units.

Nonextractable radioactive residues in excreta were hydrolyzed under alkaline conditions, as follows. Lyophilized excreta (0−24 h; 11.56 g) in a 250 mL Ehrlenmeyer flask were diluted with 10 mL of 10% NaOH in H_2O (w/v), and the contents were heated at 45−50 °C for 48 h. Concentrated HCl was added to neutralize the solution, which was then extracted twice with ethyl acetate.

Plasma was prepared by adding 5 mL of 2 mg/mL heparin solution to each of the blood collection vials and then centrifuging the contents to cause cells to separate from plasma.

Plasma was assayed for radioactivity by counting aliquots with a Packard model 1900 CA liquid scintillation counter (LSC; Meridan, CT).

RESULTS

Dosing with BDE-47 had no apparent acute adverse effect on the health of the broilers because each chicken continued to consume feed and gained about 4.9% of the starting body weight during the 72 h experimental period (data not shown). BDE-47 was readily absorbed from the GI tracts of chickens because > 60% of the administered dose remained in the carcass fractions at 72 h. Lipophilic tissues contained the greatest amounts of the dosed radioactivity (**Table 1**), that is, adipose tissue (3.25 ± 1.04%; based on an average 19.0 g aliquot), skin (14.15 ± 2.83%), and GI tract (3.10 ± 1.35%). In addition, the residual carcass contained a significant portion of the dose, that is, nearly 37% due presumably to adipose tissue and white/dark muscle that remained because only aliquots of these tissues had been removed initially. Whole body estimates of total dose in the dark and white meat were 5.54 ± 0.31 and 1.65 ± 39%, respectively, and the estimate of BDE-47 in all adipose tissue compartments was 79.43 ± 10.91% (data not shown). Only 0.002 ± 0.0005% of the dose, representing 0.19 ppm of PBDE-47 equivalents, was detected in the brain of chickens, an organ thought to be a principal target for PBDE toxicity. The amount of total radioactive residues in all other internal organs was < 1% of the administered dose.

It was also useful to express the distribution of total radioactive residues on a concentration basis (nmol of BDE-47 equiv/g of tissue fresh weight). Tissues with the highest concentrations of

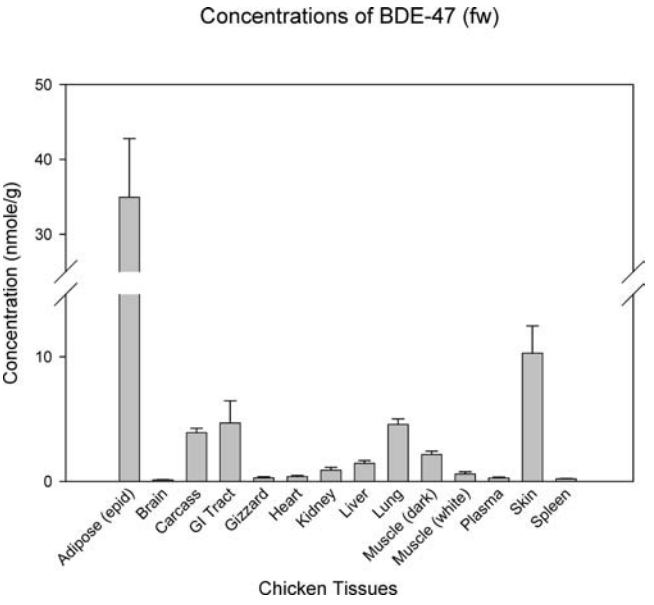

Figure 1. Concentration (nmol/g of tissue) of [^{14}C]BDE-47-derived tissue residues at 72 h in tissues on a fresh weight basis (fw) of Jumbo Cornish × Rock cross male chickens (n = 4) that were administered a single oral dose of radiolabeled BDE-47.

Table 2. Excretion (Percent of Dose) of BDE-47, Unbound Metabolites, and Bound Metabolites from Chickens Following a Single Oral Dose of [^{14}C]BDE-47 (2.7 mg/kg; 5.64 μmol/kg)

excreta	% parent	% free metabolite	% bound[a]	% total
0−24 h	6.6	0.25	7.2	14.01 ± 4.88
24−48 h	1.6	0.20	2.5	4.28 ± 0.83
48−72 h	1.1	0.10	2.5	3.69 ± 0.44

[a] Bound material could be partially hydrolyzed by base, and a monohydroxy-tetraBDE was identified.

radioactive residues were generally lipophilic, and the trend for tissues containing > 4 nmol/g was adipose tissue > skin > GI tract > lung (**Figure 1**). Dark and white muscle had a lower concentration of BDE-47 at 72 h, but concentrations in dark meat were nearly 4-fold higher than in white meat (2.2 vs 0.6 nmol/g; **Figure 1**). Plasma levels and concentrations (0.06% of dose; 0.25 nmol/g, respectively) of BDE-47 at 72 h were low relative to other tissues.

Brain, fat, dark and white muscle, and skin pooled extracts contained exclusively parent compound as assessed by TLC analyses (data not shown). In liver approximately 35% of the radioactivity was present as metabolites. The mean, lipid-adjusted concentrations of BDE-47 derived radioactivity as determined by GC-MS isotope dilution were 360 (brain), 5640 (white meat), 5560 (liver), 7470 (dark meat), 2240 (fat), and 7430 (skin) ng/g.

Nearly 22% of the administered dose was eliminated in urine and feces by 72 h. Elimination of radioactive residues was highest from 0 to 24 h (14%, **Table 1**) and declined to < 4% of the dose by 48−72 h. Less than 1% of the dose remained in the lumen of the GI tract at the time of sacrifice (**Table 1**).

Solvent-extractable radioactivity comprised approximately 49% of the total excreta radioactivity at 24 h; extractable radioactivity was composed of parent compound and free metabolites (**Table 2**). The remainder of the radioactivity was nonextractable and was presumably bound to biological macromolecules. The proportion of nonextractable radiocarbon increased with time, reaching 67% of the excreted radioactivity at 48−72 h (**Table 2**). Radioactivity that was extractable from excreta was partitioned

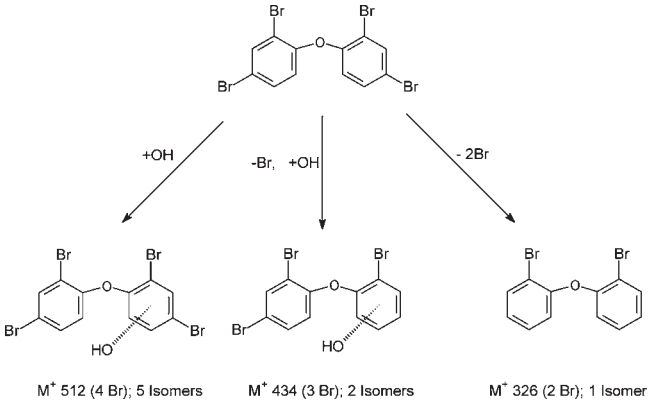

Figure 2. Major oxidation/debromination and debromination metabolites of [^{14}C]BDE-47 detected in the excreta of Jumbo Cornish × Rock cross male chickens (n = 4) following a single oral dose. Metabolites and their isomers were characterized by GC-MS after treatment with diazomethane.

on reversed phase HPLC, and free metabolites with a greater polarity than the parent compound were detected (data not shown), but cumulatively represented < 1% of the administered dose (**Table 2**).

Free metabolites were derivatized to methyl ethers with diazomethane, analyzed by GC-MS, and characterized as five monohydroxy tetrabromodiphenyl ether isomers [M^+ 512 (4 Br); M − 15 (497, 4 Br); M − BrO (418, 3 Br); M − 2Br (354, 2 Br); M − Br_2CH_3 (339, 2 Br); M − Br_2CO (311, 2 Br)], two monohydroxy tribromodiphenyl ether isomers [M^+ 434 (3 Br); M − CH_2Br (342, 2 Br)], and one dibromodiphenyl ether isomer [M^+ 326 (2 Br)] (**Figure 2**). Parent BDE-47 had EI-GC-MS fragmentations as follows: M^+ 482 (4 Br); M − HBr (402, 3 Br); M − 2Br (324, 2 Br). Base-catalyzed hydrolysis of excreted nonextractable radioactivity also released a monohydroxy tetrabromodiphenyl ether metabolite [M^+ 512 (4 Br); M − 15 (497, 4 Br)].

DISCUSSION

Absorption. Data showing that > 60% of the dose was accounted for in the tissues at 72 h (**Table 1**) implied that BDE-47 was well absorbed from the gut of chickens. In addition, nearly 73% of the dose could be considered bioavailable when free and bound fecal metabolites were also considered (**Table 2**). The intestinal absorption of BDE-47 in chickens is comparable to absorption of PBDEs in mammals and fish. In studies conducted with BDE-47, BDE-99, and BDE-153 (*16*−*18*) utilizing 1.0 μmol/kg of bw single oral doses in male rats, the series of PBDE congeners were well absorbed (75, 85, and 70%, respectively). High bioavailability of lower brominated PBDEs was also measured in fish (*19*), mice (*20*), blue mussels (*21*), and rats (*22*), where 40−70% retention of BDE-47, -99, -100, and -153 was observed.

Tissue Distribution. The major sites for the deposition of [^{14}C]BDE-47 total residues in chickens were the lipophilic tissues, that is, adipose tissue, skin, and GI tract (**Table 1**). If the residual carcass (largely made up of the lipophilic tissues fat and skin) were included, these compartments would comprise > 57% of total residues. When expressed on a concentration basis (fresh weight), the principal edible tissues, that is, dark and white meat, skin, and adipose tissue, would appear to have a very different pattern of BDE-47 disposition. BDE-47-derived ^{14}C was 5−17-fold more concentrated in the skin than in the muscle, whereas it was 16−58-fold more concentrated in the adipose tissue than in muscle (**Figure 1**). The concentration of radioactive residues in chicken fat was 35 nmol/g (16.8 μg/g) on a fresh weight (fw) basis, which converts to total body fat content of 13.0 μmol of BDE-47 (3 kg average/broiler; 12.4% total body fat) (*14*). Because the dose received by each chicken was 16.9 μmol of BDE-47, the total fat burden was estimated to be ∼77% of the dose, suggesting distribution was largely to the lipid stores within each tissue. For instance, the 3-fold higher concentration of BDE-47 in dark meat versus white meat (**Figure 1**) could be adequately explained by the greater fat content of dark meat (12.1%) when compared to that of white meat (3.0%). On the basis of calculations derived from control chickens that dark and white meat comprised 13.1 and 16.4% of the live body weight, respectively (see Material and Methods), 5.2 and 1.8% of the [^{14}C]BDE-47 dose was contained in these edible tissues. In addition, skin contained 14.1% of the administered dose (**Table 1**). When BDE-47 content within six selected tissue extracts (determined by GC-MS) was expressed on a lipid-adjusted basis (lw), the disposition differences among the various tissues disappeared, except for brain. The lipid-adjusted concentration of BDE-47 in dark and white meat, skin, and liver were all between 5500 and 7400 ng/g, whereas the concentration in brain was only 350 ng/g. The concentration (lw) of BDE-47 in the adipose tissue was intermediate, that is, 2200 ng/g, which was probably due to non-steady-state conditions resulting from a single oral dose and only a 3 day withdrawal.

In a study conducted by Örn and Klasson-Wehler (*2*), BDE-47 as a single oral dose (14.5 mg/kg) was also readily distributed to adipose tissue in both Sprague−Dawley rats and C57B1 mice when concentrations were expressed on a fresh weight basis. The distribution pattern of BDE-47 into murine tissues was generally the same as in the present study with chickens, that is, fat ≫ liver > lung > kidney > brain. In rats, BDE-47 concentrations (fw) were about 60−140 times more concentrated in adipose tissue than in the internal organs, whereas in mice adipose tissue concentrations were about 10−25 times greater than in internal organs. In another single oral dose study with F344 rats and B6C3F1 mice (0.5 mg/kg) (*16*) in which tissues were analyzed quickly after the dose was given (24 h later), BDE-47 deposited to the highest degree in adipose tissue (> 1% dose/g of tissue), and the rank order for the remaining tissues was also liver > lung > kidney > brain. BDE-47 tissue concentrations at 24 h were approximately 5-fold higher in mice than in rats under equimolar doses, which may indicate enhanced uptake and transport in mice when compared to rats, perhaps facilitated by carrier proteins. At these similar dose levels (0.1−1.9 nmol/g) (*2*, *16*), low concentrations of radiochemical were found at 1 or 5 days in the brains of rats and mice, respectively, as the parent BDE-47. Evidently there is a limited capacity for BDE-47 to cross the blood−brain barrier in vertebrates.

Reinforced by the above data, a common assumption in assessing exposure and body burdens of lipophilic, environmental contaminants has been that they distribute equally to the lipid stores within the organism. This is generally true for tri to hexa members of the PBDE family, as has been reported in glaucous gulls (*23*), male seals (*24*), and hedgehogs (*25*). In a carefully controlled, laboratory feeding study in male rats, in which doses were delivered in oil or dust vehicles, adipose tissue to whole carcass ratios of tri- to hexa-PBDEs, including BDE-47, were near unity at steady-state conditions (*22*). However, in the same rats, the ratio was underestimated in liver and overestimated in plasma for the same congeners; therefore, it was concluded that adipose tissue was a suitable matrix for the estimation of body burdens of the lower brominated PBDE congeners. In a survey of chlorinated dioxins and furans (PCDD/Fs) in edible tissues of commercially available U.S. whole chicken fryers, it was observed that marked differences in PCDD/Fs existed when viewed on a wet weight basis but that these differences disappeared when the data were expressed on a lipid-adjusted basis (*26*). These data and

others have validated the approach of using fat as a reliable indicator of the level of these contaminants in any edible tissue for which lipid composition is known.

As stated above, using estimates for the total body fat of whole broilers (12.4%), approximately 77% of the [^{14}C]BDE-47 dose was estimated to be retained in body fat in the present study. Pirard and De Pauw (*8*) provided a daily PBDE mixture to chickens for 14 weeks (3.4 mg/kg of feed) and estimated a total BDE-47 body burden of only 0.15% of the BDE-47 portion of the dose. The discrepancy between the results of these two studies probably was due to the PCDD/F contaminants present in the study of Pirard and DePauw (*8*). The dose, which was contaminated with 0.95 ng of TEQ/kg of feed and delivered daily, would induce a battery of hepatic metabolizing enzymes, particularly cytochromes 1A1 and 1A2, which are involved in the oxidative metabolism of polycyclic aromatic hydrocarbons, for example, PCDD/Fs and PBDEs (*27*, *28*), although not being induced by PBDEs themselves (*29*). Whereas in poultry it is possible to predict PCDD/F contamination of edible tissues if the daily intake of PCDD/Fs can be estimated (*30*), due to differential toxicokinetics and metabolism, this would not appear to be possible with PBDE congeners in poultry, particularly when feed is cocontaminated with CYP inducers.

Transport and Elimination. A dosing study seeking to generate toxicokinetic data on PBDEs was conducted in chickens (*8*). Hens were fed a diet for 14 weeks containing a mixture of 3.4 mg of PBDEs/kg of feed containing all of the persistent tetra- through hepta-PBDEs along with numerous dioxin and furan congeners. The study was unique in that absorption of PBDEs was based on intake levels minus excretion levels, and no tissue levels were directly measured. A high excretion of BDE-47 after 2 weeks (62%) was attributed to debromination of higher brominated PBDE congeners mediated by gastrointestinal bacteria. However, because direct absorption of BDE-47 was not measured in the study, and numerous other PBDEs, dioxins, and furans were present in the dose to confound the results, the reason for the high rate of excretion is speculative. Our study with pure BDE-47 indicated that a much lower rate of elimination occurred (22% in 72 h), and it would be hard to draw firm conclusions on the different results from these two studies. However, PBDE elimination has been shown to be facilitated by carrier proteins in mammals (*20*, *31*). Because colostomies were not performed with the present set of chickens and, therefore, urinary carrier protein mechanisms could not be studied, it is not known if mammalian protein analogues, such as α2u-microglobulin, mouse major urinary protein (mMUP), and serum albumin, also exist in avian species to facilitate the elimination of xenobiotics such as PBDEs. mMUP is constitutively expressed in some tissues and is under developmental and hormonal control in other tissues (*32*). Therefore, it could be hypothesized that inducible carrier proteins may exist in chickens and may be facilitating the high rate of BDE-47 excretion at the 2 week dosing period in the Pirard and De Pauw (*8*) study, particularly in a dose contaminated with PCDD/Fs. A highly polar, labile urinary metabolite of BDE-47 was characterized in mouse urine that rearranged to the parent compound during purification (*2*). Subsequent research has demonstrated that this polar "metabolite" was probably parent BDE-47 bound to mMUP (*20*) because about 40% of the urinary ^{14}C was associated with mMUP as the parent compound. In the Örn and Klasson-Wehler (*2*) study purification of metabolites for identification purposes would undoubtedly have released BDE-47 associated with the carrier protein during excretion. Similarly, HPLC analysis of [^{14}C]BDE-47-dosed mouse urine suggested that a majority of the radioactivity was in the form of metabolites; however, only starting material was detected in the urine following mass spectral analysis of purified radioactivity (*16*).

Chicken BDE-47 metabolites (**Figure 2**) were similar to those identified in previous rat and mouse studies. For example, five monohydroxy tetra-BDE metabolites of BDE-47 were identified in rat and mouse feces (*2*). The formation of monohydroxy metabolites suggested that both direct oxidation and NIH-shift oxidation could occur through an arene oxide intermediate after its formation through cytochrome P450 oxidation of parent BDE-47. Three monohydroxy tetra-BDE metabolites of BDE-47 were also detected in the feces of an intravenously dosed mouse (*20*). Bile collected from rats following intravenous administration of BDE-47 contained of two glutathione conjugates, which for steric and electronic considerations were tentatively assigned as substituents of carbons 5 or 6 (*16*). Urine from these rats contained two polar metabolites identified as a glucuronide and sulfate conjugates of 2,4-dibromophenol. None of the previous studies in mice and rats have reported debromination of BDE-47, as reported in the present study with chickens by identification of dibromo-BDE and two monohydroxy tribromo-BDEs. However, oxidative debromination has been reported to occur in rats with BDE-99 (*31*) and BDE-100 (*33*). Debromination of PBDEs may be catalyzed by deiodinases, although uridine diphosphate glucuronyl transferase and glutathione-*S*-transferase may also play a role (*34*). Toxicologically, some of the putative metabolites formed in this study may have had endocrine-disrupting potential. Direct oxidation between carbons 2 and 4 of either ring would have resulted in a phenolic metabolite with two ortho bromo substituents, a requirement for the thyroid hormone thyroxine (T4)-like structure. Similarly, if a debromination of BDE-47 was accompanied by a bromination adjacent to the phenolic group, this metabolite would have triiodothyronine (T3)-like structure. Hydroxylated PBDEs are known to bind with a higher affinity than the native T4 and T3 for sites on the thyroid hormone transport protein transthyretin (TTR) (*35*). Displacement of native thyroid hormones from TTR with these hydroxylated PBDE metabolites could occur through structural mimicry. Additional interference of the thyroid hormone system with hydroxlyated PBDEs may occur through affinity to the thyroid hormone receptors α1 and β, although PBDE metabolite affinity is lower than the native hormones (*36*).

In conclusion, BDE-47 was well absorbed from the intestines of chickens and deposited preferentially into lipophilic tissues following a single oral dose. Concentrations on a fresh tissue weight basis in adipose tissue and skin were far higher than in dark and white meat, but, when adjusted for tissue lipid content, were very similar. The data suggest that trimming the fat and skin from chicken would substantially reduce human exposures to PBDEs. Excretion of free metabolites in the feces amounted to $<1\%$ of the administered dose after 72 h and yielded products that were oxidized, debrominated, or a combination of both. The presence of reduced (debrominated) metabolites may be of interest especially for higher brominated PBDEs, which could debrominate to more persistent PBDEs in avian systems.

SAFETY

The toxicity of BDE-47 is believed to be minimal; however, all treated animals and samples derived from them were considered to be radioactive and were handled and disposed of by proper laboratory methods.

ACKNOWLEDGMENT

We thank Colleen Pfaff, who performed the animal studies and prepared the tissues samples; Barb Magelky, who conducted the

tissue combustion analyses; Dee Ellig, who helped to rear and dose the chickens; and Margaret Lorentzsen, who performed the mass spectral analyses.

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Received for review April 6, 2010. Revised manuscript received June 24, 2010. Accepted July 6, 2010. The use of trade, firm, or corporation names in this publication is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the U.S. Department of Agriculture or the Agricultural Research Service of any product or service to the exclusion of others that may be suitable.