

An ADME Study with 2,2',4,4',-Tetrabromodiphenyl ether (BDE-47) in Chickens

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Introduction.

Polybrominated diphenyl ethers (PBDEs) are synthesized in large quantities and are used as flame retardants in textiles and electronic equipment, especially in computer circuit boards. Due to their structural similarity to polychlorinated dibenzo-*p*-dioxins and biphenyls, their environmental persistence, and a growing database of toxicological effects, this class of compounds has become a new environmental contaminant. A particular group of PBDE congeners is persistent in the environment, e.g. BDE-47, 99, 100, 153 and 154 (Law et al. 2003). BDE-47 (Örn and Klasson-Wehler 1998), BDE-99 (Hakk et al. 2002), BDE-100 (Hakk et al. 2006), BDE-153 (Sanders et al. 2006), BDE-154 (Hakk et al. 2005), and BDE-209 (Mörck et al. 2003), and have already been studied in rats. These studies have shown that lipophilic tissues such as adipose and skin are major depots for persistent PBDE's. Since humans commonly consume the skin of a chicken, it was of interest to conduct an adsorption, tissue disposition, metabolism and excretion study in this production species with the most persistent PBDE found in biota, i.e. 2,2',4,4'-tetrabromodiphenyl ether (BDE-47).

Materials and Methods.

2,2',4,4'-Tetrabromo-[¹⁴C]diphenyl ether ([¹⁴C]BDE-47) was synthesized by starting with [¹⁴C]phenol (250 µCi, 54.5 mCi/mmol; Sigma, St. Louis, MO) diluted with unlabeled phenol (94 mg). 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/Br₂) to form 2,4-dibromophenol. 2,4-Dibromophenol (237 mg) was dissolved in dry dimethyl formamide (1 mL), cooled to 0° C, 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 (Vogel 1989). The reduction to [¹⁴C] 2,4-dibromo-2',4'-diaminodiphenyl ether was accomplished with an excess (10x) of both triethyl amine and formic acid and catalytic amounts of 5% platinum on charcoal in refluxing benzene (10 mL) (75% yield; Cortese and Heck 1977). BDE-47 was produced from a double Sandmeyer reaction of 2,4-dibromo-2',4'-diaminodiphenyl ether (100 mg), NaNO₂ (50 mg), and Cu(I)Br (102 mg) in 16% HBr (2.25 mL); the reagents were combined at 0° C then heated in a 70° C oil bath for 5 min to produce BDE-47 in 85% yield (Rinehart et al. 1987). BDE-47 from the final reaction step was purified by preparative HPLC on a C₁₈ Delta Pak column using a 90% acetonitrile/water isocratic elution (99% purity by GC/MS).

[¹⁴C]BDE-47 was administered orally (2.7 mg/rat in peanut oil; 4.69 µCi) to four chickens (Jumbo Cornish x Rock; 2.711-3.267 kg). The chickens were housed in steel metabolism cages. Excreta (urine/feces) were pooled, and collected at 24 h intervals for 72 h. The chickens were anesthetized with CO₂ and exsanguinated. Abdominal fat, brain, G.I. tract and contents, gizzard, heart, kidneys, liver, lungs, muscle (white=breast and dark=thigh), skin, and spleen were removed. Air-dried excreta and lyophilized tissues were combusted in a tissue oxidizer, and the [¹⁴C]CO₂ counted by LSC. The pooled, air-dried excreta was extracted 3X each with hexane, ethyl acetate, and methanol. Excreta extracts were purified by HPLC (C₁₈; 1 ml/min; mobile

phases, A=H₂O, B=CH₃CN; gradient elution, 60%B to 100%B in 15 min).

Results and Discussion.

Nearly 61% of the dose remained in the body of chickens at 72 h (table 1). The greatest amounts of BDE-47 at 72 h were found in the residual carcass, GI tract, adipose tissue and skin (table 1). No other organ in chickens contained more than 1% of the ¹⁴C at 72h. The residual carcass contained almost 37% of the dosed ¹⁴C, and based on the tissue data, was presumed to be associated primarily with the adipose tissue. Greater than 85% and 50%, respectively, of a single, oral BDE-47 dose was retained in rat and mouse tissues in a previous study (Örn and Klasson-Wehler 1998) When the tissue disposition data was expressed on a concentration basis, the lipophilic tissues, i.e. adipose tissue, GI tract, lungs, and skin contained the highest concentrations of ¹⁴C (>3.5 nmol BDE-47/g tissue fresh weight; table 1). Of the tissues that were analyzed previously in BDE-47, 99, 100, 153, 154 and 209 treated rats, the lipophilic tissues, e. g. adipose, adrenals, GI tract, and skin, were also those with the highest concentration of PBDEs. The liver to fat ratio (L/F) of BDE-47 in chickens was very low, i.e. 0.034, and is the same as observed in mice and rats where the L/F of BDE-47 was also low; i.e. 0.088 and 0.007, respectively (Örn and Klasson-Wehler 1998). Hepatic sequestration would have been indicated if L/F were in excess of 0.3 (Diliberto et al. 1999).

Almost 21% of the dose was excreted in excreta over 72 h. It was not possible to distinguish the individual contributions of urine and feces to excrete BDE-47 in chickens since colostomies were not performed. In rats it was observed that feces was the major route of excretion, while in mice, urine played an even more significant role in excretion of BDE-47 following a single oral dose (Örn and Klasson-Wehler 1998). Only 49% of the ¹⁴C in 0-24h excreta (by combustion analysis) could be extracted into organic solvents; non-extractables increased to 57% at 24-48h and about 67% at 48-72h. It was hypothesized that the non-extractable ¹⁴C was covalently bound to either lipid or protein, however, neither basic hydrolysis (10% NaOH, 50° C, 48h) nor acid hydrolysis (6 M HCl, sealed ampoules, 90° C, 42h) could release any radiolabel into organic extracts. The covalent binding of PBDE metabolites to fecal macromolecules has been previously observed in rats (Örn and Klasson-Wehler 1998, Hakk et al. 2002; Hakk et al. 2006; Hakk et al. 2005; Mörck et al. 2003), and was shown to occur with BDE-47 whether the dose was oral or i.v (Örn and Klasson-Wehler 1998; Staskal et al. 2006). Since biological activation would be necessary for covalent binding to occur, the non-extractables must be considered part of the overall metabolism of BDE-47. The excreta extracts contained less than 1% of the administered dose as hydroxylated and hydroxy/debrominated metabolites (by GC/MS). The metabolism data for BDE-47 in chickens agreed substantially with results obtained for other PBDEs, in that adipose tissue and skin were major stores of BDE-47 in chickens. The overall metabolism of BDE-47 is between that observed in the mice (high metabolism) and rats (low metabolism).

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Table 1. Average recoveries of ^{14}C from chickens dosed orally with 2,2',4,4'-tetrabromo- ^{14}C]diphenyl ether (BDE-47; 2.7 mg/kg; 4.69 μCi).

<u>Excreta</u>	<u>Percent of Dose</u>	<u>Concentration (nmol/g fw)</u>
Urine/Feces		
0-24h	14.01	---
24-48h	4.28	---
48-72h	3.68	---
Subtotal	21.97	
<u>Tissues</u>		
Adipose*	3.25	43.46
Brain	0.002	0.13
Carcass (residual)	36.84	4.12
G.I. tract	6.95	13.55
GI contents	0.69	14.53
Gizzard	0.04	0.20
Heart	0.04	0.48
Kidney	0.11	1.20
Liver	0.47	1.51
Lungs	0.07	4.06
Muscle* (white)	0.91	0.78
Muscle* (dark)	1.29	2.23
Plasma	0.061	0.28
Skin	10.28	3.82
Spleen	0.00	0.21
Subtotal	60.94	

* Indicates a representative subsample was removed for analysis. Remainder of tissue belongs to the Residual Carcass.

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