Tissue Disposition, Excretion, and Metabolism of 2,2',4,4',5-Pentabromodiphenyl ether (BDE-99) in Male Sprague-Dawley Rats

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INTRODUCTION

Polybrominated diphenyl ethers (PBDEs), biphenyls (PBBs), and bisphenol-A (i.e.,TBBP-A) are synthesized in large quantities and serve as flame retardants in textiles, and electronic equipment, such as computer circuit boards. Due to their structural similarity to polychlorinated dibenzo-*p*-dioxins and biphenyls, and the fact that their commercial synthesis has increased dramatically over the past 20 years (currently at 150,000 tons per year globally [6)]), there is a growing concern that environmental levels may be increasing and leading to a future toxicological problem. The occurrence of PBDEs in the environment was first reported 20 years ago in sediments from the USA[1)], and shortly thereafter in fish from Sweden [2)]. They have now been identified in human plasma [3)] and adipose tissue [4)]. A continuous increase has been reported for all measured congeners within human milk in Sweden banked from 1972 through 1997 [5)]. Both PBDEs and PBBs have recently been found to be present in relatively high levels in sperm whales, an organism that spends most of its time in deep ocean waters [7)].

Due to their high lipophilicity, bioaccumulation of PBDEs in the environment is expected. However, relatively little is known about their metabolism and tissue disposition in mammalian systems. Therefore, the purpose of the present study was to administer a radiolabelled oral dose of an environmentally abundant polybrominated diphenyl ether congener, i.e. 2,2',4,4',5-pentabromodiphenyl ether (BDE-99), to male Sprague-Dawley rats. Tissue disposition, excretion, and metabolite formation was studied after 72h in conventional and bile-duct cannulated animals.

METHODS

2,2',4,4',5-Pentabromo-[^{14}C]diphenyl ether (BDE-99) was synthesized in-house by accepted methods [8)]. The radiolabel was administered orally (2.2 mg/rat in peanut oil; 1.0 μCi) to six conventional male rats and six bile-duct cannulated rats (Sprague-Dawley). The rats were housed in steel metabolism cages. Urine, feces, and bile were collected at 24h intervals for 72h. The rats were anesthetized with CO_2 and sacrificed. Liver, kidneys, G.I., heart, testes, fat, adrenals, lungs, blood, and thymus were removed. Urine, bile, and blood were assayed for

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radioactivity by counting aliquots in a liquid scintillation counter (LSC). Air-dried feces and lyophilized tissues were combusted in a tissue oxidizer and the ^{14}C counted by LSC.

The pooled, air-dried feces was extracted 3X with hexane, ethyl acetate, and methanol. Silica gel TLC plates were developed with 50:50 hexane:methylene chloride, using BDE-99 as a standard. HPLC analysis was performed with a 20-100% linear gradient of either water to

methanol, or water to acetonitrile on C-18 columns. HPLC fractions were evaporated to dryness on a rotary evaporator, derivatized with diazomethane and submitted for GC/MS analysis.

RESULTS AND DISCUSSION

BDE-99 metabolism to water soluble metabolites or conjugates was low, as evidenced by the low daily excretion levels observed in the urine and bile. Cumulative urinary excretion was less than 1% in conventional rats at 72h, and approximately 0.3% in bile-duct cannulated rats (Table 1). Biliary elimination was only 3.7% over the same period of time. The urine data are similar to the elimination pattern observed in male rats following a single dose of the most common PBDE environmental contaminant, i.e. 2,2',4,4'-tetrabromodiphenyl ether (BDE-47), in which <0.5% of the dose was excreted in urine 5 days after exposure [9]. These excretion results are in agreement with standard *in vitro* biotransformation results conducted on a series of PBDEs, where no significant metabolism was observed for any PBDE tested [7]. It was concluded from the data that a great environmental persistence is likely for the PBDE family.

Feces was the major route of elimination of BDE-99. Forty three percent of the dose was excreted in conventional rat feces in 72h (Table 1). No evidence for enterohepatic circulation existed in the fecal and biliary elimination results. Feces was also the major route of elimination in bile-duct cannulated rats (86% in 72h; Table 1). A comparison with the conventional feces data allows one to conclude that bile salts are necessary for intestinal uptake (0-24h elimination of 22% versus 53%, respectively). Evidence for reactive metabolic intermediates was obtained in the fecal extracts. Only 58% of the ^{14}C from combustion analysis could be extracted from 0-24h conventional feces; less than 18% could be extracted from 48-72h feces. The inextractable ^{14}C was presumably covalently bound to either lipid or protein. In addition, decomposition of chromatographed feces metabolites back to parent BDE-99 was observed on several occasions. Örn *et.al.*[9] also observed covalent binding of metabolites to fecal macromolecules after BDE-47 exposure, and found labile metabolites in the urine. No labile metabolites were observed in urine of the present BDE-99 experiment.

TLC and HPLC analysis of the conventional fecal extracts indicated that only minor amounts (<10%) of metabolites were present. GC/MS analysis following methylation indicated the presence of two monomethoxy pentabromodiphenyl ether metabolites [M^+ 590 (5 Br); M-15 (575); M-80 (512, 4 Br); M-94 (496)], and two de-brominated monomethoxy tetrabromodiphenyl ether metabolites [M^+ 512 (4Br); M-15 (497); M-80 (434, 3 Br); M-94 (418)]. Sufficient amounts of purified material were not available to perform ^1H-NMR spectroscopy on these metabolites, therefore, absolute stereochemical assignments could not be made. The majority of the ^{14}C in the fecal extracts (>90%) was present as parent BDE-99. This was confirmed by GC/MS [M^+ 560 (5 Br); M-80 (480, 4Br); M-158 (402); M-265 (295)] and ^1H-NMR [8.10 (s), 7.95 (s), 7.62 (d), 7.33(s), 7.11 (d)].

Bile was analyzed for metabolites. The metabolites were characterized after methylation by GC/MS. Two monohydroxy pentabromodiphenyl ether metabolites were identified [M^+ 590 (5 Br); M-15 (575); M-31 (559); M-80 (512)]. Two dihydroxy pentabromodiphenyl ethers were characterized by mass spectrometry [M^+ 620 (5 Br); M-15 (605)]. No glucuronide or sulfate conjugates were identified in the bile. Evidence was also obtained to suggest the presence of two thio-substituted pentabromodiphenyl ethers [M^+ 606 (5Br); M-15 (591); M-79 (527)]; perhaps formed through the mercapturic acid pathway. Metabolism studies with BDE-47 also suggested the presence of a thiol metabolite in addition to five monohydroxy metabolites[9].

Disposition data revealed that BDE-99 was preferentially deposited in adipose tissue, blood, carcass, and GI tract (Table 1). No other tissues in conventional or bile-duct cannulated rats contained more than 1% of the ^{14}C at 72h. The conventional carcass contained nearly 39% of the dosed ^{14}C, and was further fractionated into skin, bone, brain, eyes, and muscle. The majority of the carcass ^{14}C deposited in the skin. When the tissue disposition data is expressed on a concentration basis, the lipophilic tissues, i.e. adipose tissue, skin, and adrenals, contained the highest concentrations of ^{14}C (data not shown). Of the tissues that were analyzed in BDE-47 treated rats, the adipose tissue also contained the highest concentration [9]. The disposition data contrasted with those reported for toxic dioxins[10]. Up to 55% of a 2378-TCDD dose was deposited in the liver after 24h, where a specific dioxin binding protein has been identified[11], i.e. CYP1A2. Apparently, PBDEs do not induce, or serve as suitable ligands for, CYP1A2.

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Table 1. Recoveries of ^{14}C from male rats dosed orally with 2,2',4,4',5-pentabromo-[^{14}C]diphenyl ether (BDE-99) in a conventional and bile-duct cannulated study.

Percent of Dose

Tissue/Excreta	Conventional (n=6)	Cannulated (n=6)
Urine		
0-24h	0.4 ± 0.06	0.1 ± 0.08
24-48h	0.3 ± 0.1	0.2 ± 0.2
48-72h	0.2 ± 0.01	0.05 ± 0.03
Bile		
0-24h	----	0.6 ± 0.8
24-48h	----	1.7 ± 1.1
48-72h	----	1.4 ± 1.0
Feces		
0-24h	22.3 ± 15.8	52.5 ± 26.9
24-48h	14.8 ± 5.4	30.4 ± 19.9
48-72h	6.0 ± 1.6	3.6 ± 5.9
Adrenals	0.1 ± 0.02	0.01 ± 0.006
Adipose (epidydimal)	3.8 ± 1.1	0.8 ± 0.6
Blood	1.4	0.9
Carcass	38.8 ± 5.2	2.0 ± 1.2
G.I. tract	6.1 ± 0.7	1.5 ± 1.4
Heart	0.03 ± 0.005	0.01 ± 0.009
Kidney	0.1 ± 0.005	0.03 ± 0.02
Liver	0.9 ± 0.20	0.3 ± 0.1
Lungs	0.1 ± 0.02	0.04 ± 0.04
Testes	0.06 ± 0.02	0.08 ± 0.06
Thymus	0.06 ± 0.03	0.01 ± 0.006
Total Recovery	95.5	96.2