An ADME Study with 2,2',4,4',5,6'-Hexabromodiphenyl ether (BDE-154) in Male Sprague-Dawley Rats

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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, such as 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 is emerging as a new environmental contaminant. A particular group of PBDE congeners is persistent in the environment, e.g. BDE-47, 99, 100, 153 and 154.[1] BDE-47 [2], 99 [3], 100 [4] and 209 [5] have already been studied in rats. Therefore, to help complete the picture of mammalian metabolism of persistent PBDEs, we conducted an adsorption, tissue disposition, metabolism and excretion study in conventional and bile-duct cannulated male Sprague-Dawley rats of 2,2',4,4',5,6'-hexabromodiphenyl ether (BDE-154).

Materials and Methods:

2,2',4,4',5,6'-Hexabromo-[^{14}C]diphenyl ether (BDE-154) was synthesized in-house. [^{14}C] Phenol (54.5 mCi/mmol; Sigma, St. Louis, MO) was diluted with cold phenol to a specific activity of 7070 dpm/ug, and then treated with liquid bromine to form [^{14}C] 2,4,6-tribromophenol. 2,5-Dibromo-4-fluoro-nitrobenzene was synthesized from 2,5-dibromofluorobenzene by the method of Chen et al.[6] Equimolar amounts of 2,5-dibromo-4-fluoronitrobenzene and [^{14}C]2,4,6-tribromophenol were coupled in dry DMF and sodium hydride at 100^{B}C (18 h) to yield [^{14}C] 2,2',4',5,6'-pentabromo-4-nitrodiphenyl ether (60% yield).[7] Reduction to [^{14}C] 2,2',4',5,6'-pentabromo-4-aminodiphenyl ether was accomplished with 5% platinum on charcoal, triethyl amine, and formic acid (80% yield).[8] [^{14}C]BDE-154 was produced through a Sandmeyer reaction with $NaNO_2$/Cu(I)Br in 33% HBr (50% yield) [9] and purified by RP- HPLC (>98% radiochemical purity by TLC/HPLC; >95% chemical purity by GC/MS).

[^{14}C]BDE-154 was administered orally (1.9 mg/rat in peanut oil; 0.97 mCi) to four conventional male rats and four bile-duct cannulated rats (Sprague-Dawley; 271-278g and 203-232g, respectively). The conventional rats were housed in steel metabolism cages, and the bile-duct cannulated rats maintained in restraining cages.[10] Urine, feces, and bile were collected at 24 h intervals for 72 h. The rats were anesthetized with CO_2 and sacrificed. Adrenals, blood, epididymal fat, G.I. tract, heart, kidneys, liver, lungs, muscle (longisimus dorsii), skin, spleen, testes, and thymus were removed. Quantities of [^{14}C] in urine, bile, and plasma were assayed in a liquid scintillation counter (LSC). Air-dried feces and lyophilized tissues were combusted in a tissue oxidizer, and the [^{14}C]CO_2 counted by LSC. The pooled, air-dried feces was extracted 3X each with hexane, ethyl acetate, and methanol. Silica gel TLC plates (Analtech, Inc. Newark, DE) were developed with 1:1 hexane:methylene chloride, using [^{14}C]BDE-154 as a standard. Radiolabelled bands on silica TLC plates were quantitated using a System 2000 Imaging Scanner (Bioscan, Inc., Washington, D. C.).

Results and Discussion:

More than 31% of the dose remained in the body of conventional rats at 72 h (table 1). The greatest amounts of BDE-154 at 72 h were found in the residual carcass, GI tract, adipose tissue and liver (table 1). No other tissues in conventional or bile-duct cannulated rats contained more than 1% of the ^{14}C at 72h. Since the residual carcass contained 24% of the dosed ^{14}C it was further fractionated into skin and muscle. Approximately one-half of the carcass radioactivity deposited in the skin (data not shown). When the tissue disposition data was expressed on a concentration basis, the lipophilic tissues, i.e. adipose tissue, adrenals, GI tract, skin and liver, contained the highest concentrations of ^{14}C (>5 nmol BDE-154/g tissue fresh weight; table 1, in parentheses).

Of the tissues that were analyzed previously in BDE-47, 99, 100 and 209 treated rats, the lipophilic tissues, e. g. adipose, adrenals, GI tract, skin, also contained the highest concentration (fig. 1).[2, 3, 4, 5] The liver to fat ratio of BDE-154, a hexabromo PBDE, in male rats was 0.25. A single oral TCDD dose at 24h had a liver to fat ratio of 10.5.[11] A specific dioxin binding protein has been identified in mammalian liver [12], cytochrome1A2 (CYP1A2). The L/F ratios of PBDEs appear to be directly related to the level of bromination for these similar doses, i.e. BDE-47 = 0.007 (4 Br); BDE-99 = 0.12 (5 Br); BDE-100 = 0.18 (5 Br); BDE-209 = 5.0 (10 Br).

BDE-154 metabolism to water soluble metabolites was low, as evidenced by the low daily excretion levels observed in the urine and bile. Cumulative urinary excretion was approximately 1.0% in conventional rats at 72h, and about 0.3% in bile-duct cannulated rats (table 1). The low urine excretion data are similar to those observed in male rats following a single oral dose of BDE-47, 99, 100 and 209 where <1.0% of the dose was excreted in urine 3 days after exposure, respectively.[2, 3, 4, 5] Cumulative biliary elimination of BDE-154 was 1.3% over the same period of time (table 1). Biliary excretion at 72h with BDE-99, 100 and 209 dosed rats was 3.9, 1.7 and 9.5% in the bile, respectively.[2, 3, 4, 5]

Feces was the major route of elimination of BDE-154. Approximately 62 and 66% of the dose was excreted in conventional and bile duct-cannulated rat feces in 72h, respectively (table 1). Fecal elimination results indicated enterohepatic circulation, because 24-48h fecal elimination was greater than 0-24h (32.4% vs. 22.8%; table 1). Daily fecal excretion was greater for BDE-154 than BDE-47, 99 or 100, but less than BDE-209 (fig. 2).[2, 3, 4, 5] Only 42% of the ^{14}C determined to be in feces by combustion analysis could be extracted into organic solvents from 0-24h conventional feces; compared to 26% from 24-48h feces and about 36% from 48-72h feces. The nonextractable ^{14}C was presumably covalently bound to either lipid or protein. The covalent binding of PBDE metabolites to rat fecal macromolecules has been previously observed with BDE-47 and 99.[2, 3] TLC analyses of the 0-72h conventional fecal extracts indicated that an average of 36% of the extractable fecal ^{14}C was parent compound (Rf = 0.80). Hexane and ethyl acetate extracts contained moderately polar metabolites (Rf = 0.30), while the majority of the methanol extracts contained very polar metabolites (Rf = 0.00). Metabolites were derivatized to their methyl ethers with CH_2N_2, analyzed by GC/MS, and found to be two monohydroxylated, three monohydroxylated debrominated, and a two dihydroxylated di-debrominated isomers. Because 64% of fecal extracts and all the nonextractable fecal ^{14}C are the products of metabolism, metabolites of BDE-154 accounted for at least 24% of the dose. The in vivo metabolism data for BDE-154 agrees substantially with in vivo results obtained for BDE-47, 99 and 100, but contradicts standard in vitro results conducted on the persistent PBDEs, where metabolism was very low.[13]

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Table 1. Recoveries of ^{14}C from male rats dosed orally with 2,2',4,4',5,6'-hexabromo-[^{14}C]diphenyl ether (BDE-154; 4.1 mg/kg) in a conventional and bile-duct cannulated study.

Percent of Dose		
Excreta	Conventional (n=4)	Cannulated (n=4)
Urine 0-24h	0.72 ± 0.19	0.15 ± 0.17
24-48h	0.28 ± 0.06	0.15 ± 0.21
48-72h	0.05 ± 0.02	0.01 ± 0.03
Bile 0-24h	----	0.6 ± 7.5
24-48h	----	0.42 ± 0.31
48-72h	----	0.24 ± 0.22
Feces 0-24h	22.8 ± 9.4	51.5 ± 34.4
24-48h	32.4 ± 5.5	13.8 ± 11.9
48-72h	6.6 ± 2.1	0.94 ± 0.75
Tissues (nmol/g f.w.)		
Adrenals	0.07 ± 0.03 (21.79)	0.008 ± 0.009
Adipose (epid.)	1.82 ± 0.24 (29.62)	0.13 ± 0.11
Plasma	0.04 ± 0.002 (0.04)	0.01 ± 0.01
Carcass	24.3 ± 4.0 (24.30)	2.78 ± 1.67

G.I. tract 3.77 ± 0.73 (17.87) 24.2 ± 46.3

Heart 0.03 ± 0.01 (0.78) 0.010 ± 0.012

Kidney 0.06 ± 0.02 (0.34) 0.034 ± 0.033

Liver 0.68 ± 0.12 (5.96) 0.3 ± 0.39

Lungs 0.09 ± 0.03 (2.19) 0.031 ± 0.032

Muscle (0.47)

Skin (7.52)

Spleen 0.02 ± 0.01 (0.78) 0.002 ± 0.002

Testes 0.1 ± 0.01 (0.94) 0.01 ± 0.01

Thymus 0.1 ± 0.04 (4.55) 0.023 ± 0.040