Toxicokinetics of BDE 47 in Female Mice: Effects of Dose, Route of Exposure, and Mdr1 Transporter

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Introduction

2,2',4,4'-Tetrabromodiphenyl ether (BDE 47) is a polybrominated diphenyl ether (PBDE) congener used in a class of brominated flame retardants (BFRs) commonly used in a variety of highly flammable consumer goods. Concern for the effects of PBDEs has increased significantly in recent years as their presence has been detected in environmental samples and in human tissues at steadily increasing concentrations. Despite its small contribution to the PBDE global production and usage, BDE 47 is the major congener found in environmental samples and human tissue. Limited toxicology studies suggest that BDE 47 is a developmental neurotoxicant and an endocrine disruptor[1]; however, several data gaps exist and must be investigated in order to evaluate the human health risk of BDE 47.

This study investigates the basic toxicokinetic properties of BDE 47 in female C57BL/6J mice. Orn and Wehler[2] have previously reported limited toxicokinetic properties of BDE 47 in male C57 mice following a single oral dose (30 umol/kg): here we report the effects of dose and route of exposure on the absorption, distribution, and excretion following a single, acute dose of ^{14}C-labeled BDE 47. Data from these basic ADME studies will be applied to studies investigating the toxicokinetics of BDE 47 in a developmental model as well as in the development of a physiologically-based pharmacokinetic (PBPK) model.

Materials and Methods

Animals: 10-week old female C57BL/6J mice from Charles River and Jackson Laboratories were pre-adapted in Nalge metabolism cages (3/cage) one week prior to dosing. All animals were individually housed following exposure to BDE 47 and given approximately 1µCi of labeled chemical. Excreta (collected daily), liver, blood, adipose, brain, muscle (abdominal), skin (ears), lung, and kidney samples were analyzed for residual radioactivity. Further analysis by HPLC was conducted for determination of parent compound. [^{14}C]BDE 47 (>97% pure) was generously provided by Great Lakes Chemical Corporation.

Effect of Dose: Mice were administered a single dose of [^{14}C]BDE 47 (0.0, 0.1, 1.0, 10.0, 100 mg/kg) in corn oil by oral gavage (10 ml/kg). Tissues were collected five days following exposure.

Effect of Route: A single dose (1.0 mg/kg) of [^{14}C]BDE 47 was administered intratracheally (i.t. through involuntary aspiration), intravenously (i.v.), intraperitoneally (i.p), and dermally. For i.t. exposure, BDE 47 was dissolved in physiological saline

solution and administered directly into the oropharynx of anesthetized animal. Ethanol:Emulphor®:water (1:1:8) solution was used for i.v. (tail vein, 4ml/kg) and i.p. (4ml/kg) dosing. For dermal exposure, BDE 47 was dissolved in hexane and painted on a shaven area of back skin to which a metal perforated patch was adhered for the duration of the study following exposure. Tissues were collected five days following exposure.
Effect of Mdr1 Transporter: 10-week old female FVB Mdr1a/b$^{+/+}$ and Mdr1a/b$^{-/-}$ mice (Taconic Laboratories) were administered a single, oral dose of [^{14}C]BDE 47 in corn oil by gavage (1.0 or 100 mg/kg). Excretion and tissue distribution was monitored as described above for the effect of dose study.

Results and Discussion

Absorption and elimination were key factors in determining body burden of BDE 47 in female C57BL/6J mice. Oral and intratracheal administration result in approximately 80% absorption while dermal absorption was lower (~40%). Five days after a single, oral dose, a significant amount (>60%) of BDE 47 had been eliminated via the excreta: at the low dose (0.1 mg/kg), 39% and 34% had been excreted in the urine and feces, and at the high dose (100 mg/kg), 13% and 51%, respectively (Fig. 1&2, Table 1). Similar trends were seen for all routes of administration when based on absorbed dose. Tissue distribution was not dependent on dose or route of administration and was primarily dictated by its lipophilicity as it tends to distribute into lipid-rich tissues; adipose, skin, liver, and muscle had the highest concentrations (Table 2).

	Effect of Dose (mg/kg)				Effect of Route (1mg/kg)				
	0.1	1	10	100	i.v.	oral	i.t.	i.p.	dermal
% Urine	39±13	38±8	33±2	13±5	42±5	38±8	32±7	33±8	21±3
% Feces	34±9	41±12	38±7	51±11	17±2	41±12	29±8	29±13	16±2

Table 1. Cumulative percent of dose excreted in urine and feces with respect to dose and route of administration five days after exposure.

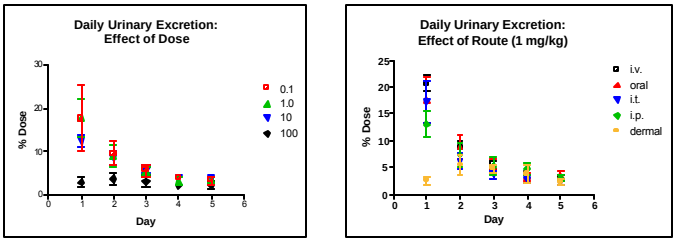

Figure 1. Effect of dose and route of administration on urinary excretion of BDE 47.

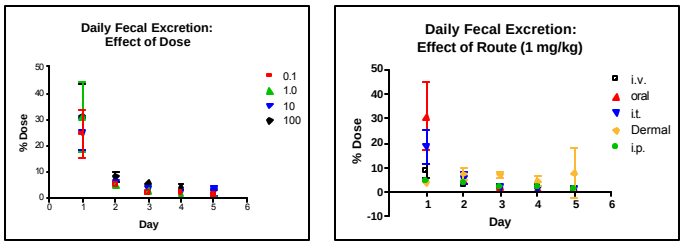

Figure 2. Effect of dose and route of administration on fecal excretion of BDE 47.

Up to 40% of the dose was excreted in the urine as unmetabolized parent compound (data not shown); a surprising result for this high molecular weight compound. Because the chemical is not bound and not metabolized[2], we hypothesize that BDE 47 may be a substrate for active transport. Furthermore, there was a dose-dependent effect on urinary excretion, suggesting a saturation of the process by which BDE 47 is eliminated into the urine. The mdr1 transporter is a relatively well-studied transporter known to be involved with a large number of xenobiotic substrates; specifically large, non-polar chemicals[3]. Mice deficient in this transporter did not have altered urinary excretion patterns, demonstrating that P-glycoprotein is not a key transporter of BDE 47 (Fig 3). The potential role of active transport is of particular interest because of stark species differences in excretion patterns of BDE 47; rats do not excrete BDE 47 in the urine whereas mice readily excrete BDE 47 via urine and feces.

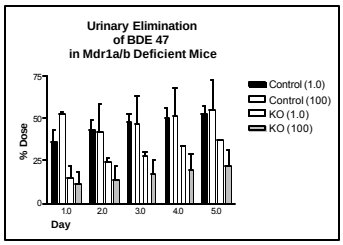

Figure 3. Cumulative urinary excretion of BDE 47 in FVB Mdr1a/b $^{+/+}$ and Mdr1a/b$^{-/-}$ female mice.

Conclusion

Limited toxicology studies suggest that BDE 47 is a developmental neurotoxicant and an endocrine disruptor, which underlies the need to understand the kinetics of this flame retardant in both adults and developing offspring. The results of this study demonstrate that BDE 47 has ADME properties similar to other lipophilic PAHs with the exception of an unexpected rapid excretion pattern of unmetabolized parent compound in the urine and feces in mice. This research has further characterized a major pharmacokinetic species difference in the elimination patterns of BDE 47 as rats do not excrete BDE 47 in the urine. Identifying the underlying mechanism for these alterations will have implications when developing a PBPK model and may ultimately contribute to an explanation of the diversity in measured human BDE 47 blood concentrations.

References

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2. Orn and Klasson-Wehler 1998. Metabolism of 2,2'4,4'-tetrabromodiphenyl ether in rat and mouse. Xenobiotica 28, 199-211.
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Acknowledgements

Partial funding provided by the NHEERL-DESE Training in Environmental Sciences Research, EPA CT 826513. This abstract does not necessarily reflect EPA policy.

Tissue	0.1 mg/kg	1.0 mg/kg	10.0 mg/kg	100 mg/kg
Liver	19.94 ± 1.37	135.81 ± 30.74	1688.29 ± 113.87	16596.11 ± 2790.88
Blood	2.28 ± 0.42	13.04 ± 3.06	178.67 ± 22.02	2223.75 ± 565.94
Fat	110.35 ± 27.34	1204.00 ± 390.19	17712.53 ± 879.81	176142.40 ± 34063.23
Brain	1.92 ± 0.12	7.17 ± 3.08	109.05 ± 10.53	1210.48 ± 182.30
Muscle	11.76 ± 6.43	39.16 ± 20.84	644.13 ± 196.05	20706.41 ± 26727.68
Skin	22.34 ± 1.98	130.27 ± 63.59	2229.30 ± 195.76	23712.89 ± 4147.87
Lung	8.15 ± 1.49	55.78 ± 13.87	1045.00 ± 362.15	8038.20 ± 2607.81
Kidney	3.91 ± 0.70	20.83 ± 7.24	369.37 ± 66.51	4058.72 ± 872.23

Tissue	i.v.	oral	i.t.	dermal
Liver	221.72 ± 12.02	135.81 ± 30.74	179.44 ± 31.78	14.38 ± 6.91
Blood	26.39 ± 13.34	13.04 ± 3.06	20.06 ± 5.32	1.41 ± 0.75
Fat	1502.13 ± 193.65	1204.42 ± 390.19	1206.60 ± 377.61	93.27 ± 28.00
Brain	11.01 ± 2.34	7.17 ± 3.08	11.04 ± 1.40	1.07 ± 0.54
Muscle	78.81 ± 23.94	39.16 ± 20.84	59.10 ± 33.80	6.11 ± 2.94
Skin	204.12 ± 59.32	130.27 ± 63.59	182.32 ± 36.17	6.03 ± 3.51
Lung	115.15 ± 83.31	55.78 ± 13.87	554.20 ± 152.67	6.42 ± 2.16
Kidney	40.39 ± 4.13	20.83 ± 7.24	34.67 ± 3.55	3.89 ± 1.39

Table 2. Effect of dose and route of administration on the tissue concentrations (ppb) of BDE 47 five days following exposure.