POLYBROMINATED DIPHENYL ETHERS IN CHICKENS

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Introduction

Polybrominated diphenyl ethers (PBDEs) are flame retardants added to items such as electronic, computer, and appliance components. PBDEs are structurally similar to dioxins and PCBs and appear to be persistent and, for certain congeners, bioaccumulative[1]. PBDEs have been found in most environments sampled including Swedish and Canadian biota[2,3], fish from the North Sea[4], sediments and marine life from Japan[5], and fish-eating species in the Arctic[6]. PBDEs are also present in the human population and appear to be on the rise[7]. Although most data has been collected on aquatic species, PBDEs have been detected in terrestrial animals[2]. Air-born transport of PBDEs has been observed[8] and could make PBDEs a global concern, similar to dioxins and PCBs . The presence of PBDEs in livestock animals has not been assessed or reported in the scientific literature.

In a preliminary study at Stockholm University, two chicken fat samples were found to contain relatively high levels of total PBDEs (15-20 ppb). These chickens had originated from a dioxin contamination incident in the Southern US involving a ball clay feed additive. The samples had previously been analyzed for dioxins and furans at our laboratory. We now report the results of further analyses of PBDEs in these and other chickens.

Materials and Methods

Chicken fat samples were collected in June, 1997, and stored at -60 C until analyzed. Samples (1.0 g) were dissolved in methylene chloride, spiked with a recovery standard (1 ng $^{13}C_{12}$-3,3',4,4'-tetrabromodiphenyl ether, ^{13}C-BDE-77[9], CIL, Andover, MA), and homogenized. The PBDEs were isolated by a modified version of EPA Method 1613. Fat samples were treated with 10 g of 40% acid silica, then chromatographed sequentially on a triphasic silica column with hexane and on an alumina column with a 50/50 mixture of methylene chloride and hexane. Chromatography was performed using an automated system from Fluid Management Systems (Waltham, MA). Samples were dissolved in 20 ul of dodecane containing an injection standard (1 ng 4-bromo-2',3',4',5'-tetrachlorobiphenyl, Wellington Laboratories, Guelph, Ontario) for analysis by GC-MS. Ball clay (0.1 g) was suspended in toluene, spiked with the recovery standard, and sonicated for 90 min. The dispersion was filtered through a high density polyethylene filter to remove particulate and the filtrate was concentrated to dryness. The sample was reconstituted in 20 ul of dodecane containing the injection standard (IS) for GC-MS.

GC-MS analyses were performed on a VG Autospec instrument operating in the electron impact selective ion monitoring mode at 2000 mass units resolution. Time windows were established to detect molecular ions for the IS, tetra-, and hexa-congeners, and to detect molecular ions minus Br_2 for the penta- through deca-congeners. Two ions of each cluster were monitored; ratios were found to be within 15% of the theoretical value. Gas chromatography was performed on a 30 meter DB-5MS column (J&W Scientific, Folsom CA) using splitless injection. A standard curve was generated for a mixture of 23 PBDE standards (CIL, Andover MA) from which response

factors relative to the IS were calculated for each PBDE. PBDEs for which no relative response factors (Rrf) were available were assigned the Rrf of the most similar PBDE in the mixture. PBDEs not included in the CIL standard mixture were identified by comparison of retention times to technical formulations of penta-, octa-, and decabromodiphenyl ethers (Great Lakes Chemical, West Lafayette, IN) which had previously been characterized by comparison to synthetic standards. Analysis of the decaBDE formulation showed that our methods did not adequately detect this congener. The estimated limit of detection was over 50 ng and the Rrf approximately 0.00001. Matrix blanks were run using a composite blend of chicken fat. A standard solution of the octaBDE formulation which contained tetra- through deca-BDEs was spiked into 1.0 g of fat matrix or into solvent for cleanup to assess performance and recovery of the analytical method (matrix and method PARs). A PAR and blank were run with each set of three samples.

Results and Discussion

Seven chickens from three different sites which had used dioxin-contaminated ball clay as a feed additive were analyzed for PBDEs. In addition two chickens from sites which did not use the contaminated clay and a composite blend of ten chickens purchased at a Fargo, ND, grocery (matrix blank) were analyzed. Because preliminary analyses of two chicken samples had shown no detectable levels of 3,3',4,4'-tetrabromodiphenyl ether (BDE-77), ^{13}C-BDE-77 was chosen as the recovery standard in this study. Recoveries of ^{13}C-BDE-77 from the cleanup procedure ranged from 73% to 110%. Table 1 shows that matrix and laboratory blanks contained no significant levels of PBDEs. Comparison of a PAR standard analyzed at various times during the study, showed a discrimination for higher brominated congeners in the analytical method (Table 1). This comparison showed that recoveries of tetra- through hepta-BDEs were 60% or better except for BDE-153 and BDE-183 which had average recoveries of 30% and 17%, respectively. The higher brominated congeners were often detected at levels less than 50% of the PAR standard. Using ^{13}C-BDE-77 as the only recovery standard may overestimate recoveries for certain congeners during analysis and lead to incorrect low values. An improved method would include more than one recovery standard and ideally one ^{13}C-labeled standard for each congener measured.

The levels of PBDEs in each chicken sample are shown in Table 2 on a whole weight basis. Three congeners which appeared to be hexa-BDEs were found in most samples but could not be positively identified due to lack of standards. The pattern of PBDEs in chickens was found to be different from patterns found in fish and fish-eating mammals[2,3] with higher levels of penta-BDEs than tetra. This may be due to different sources of exposure or to differences in rates of absorption and retention between species. Total PBDE levels in the chickens were lower than levels reported for Great Lakes fish[3] but higher than levels found in terrestrial animals in Sweden[2].

In addition to PBDEs, these samples had previously been analyzed for dioxins and furans by high resolution GC-MS methods. Those values are also reported in Table 2 for comparison to the PBDE levels. The total PBDEs did not correlate to TCDD/F TEQs in this small set of samples. Ball clay used as a feed additive had been identified as the source of dioxins and furans in these chickens and was also analyzed for PBDEs. No PBDE levels above background were found in the clay (data not included). The source of PBDEs appeared to be localized. The two chickens with the highest levels (19.4 and 30.3 ppb) were from the same production site, while a composite sample of chickens from North Dakota had generally low levels. From this data it appears that animals raised for food, i.e. chickens, can be a dietary source of PBDEs contributing to human exposure.

Table 1. PBDEs (ngs) in the octaBDE formulation standard, recovered from matrix and method blanks spiked with the octaBDE standard (PARs), and in matrix and method blanks.

			PARs			Blanks		
PBDE[1]	Rrf	octa std	Matrix	Method		Matrix		Method
47	0.285	13.4	7.46	6.80	6.57	0.13	0.08	0.058
77	0.245	nd	nd	nd	nd	nd	nd	nd
100[2]	0.250	0.45	0.54	0.46	0.52	0.02	0.01	0.008
119	0.367	0.44	0.46	0.40	0.35	nd	nd	nd
99	0.250	2.65	2.57	2.15	2.40	0.15	0.08	0.056
24:28[2]	0.250	0.29	0.43	0.33	0.35	nd	nd	nd
85	0.107	0.13	0.23	0.17	0.12	nd	nd	nd
154[3]	0.055	108.19	70.34	59.24	53.94	0.01	0.01	0.004
153	0.055	1162.10	409.39	339.81	297.93	0.03	0.03	0.014
27:38[3]	0.055	12.73	13.39	11.52	9.69	nd	nd	nd
28:48[4]	0.017	31.96	30.49	24.95	20.43	nd	nd	nd
29:09[4]	0.017	18.71	24.4	18.51	15.51	nd	nd	nd
183[4]	0.017	17232.1	3942.9	2615.8	2420.7	0.10	0.17	0.090
31:21[4]	0.017	155.13	164.41	113.00	96.39	nd	nd	nd
32:24[4]	0.017	65.80	76.94	52.75	44.34	nd	nd	nd
35:41[4]	0.017	1554.02	979.98	539.72	408.22	nd	nd	nd
203[4]	0.017	110.73	86.71	46.31	32.35	nd	nd	nd
37:28[4]	0.017	228.27	161.54	87.09	59.09	nd	nd	nd
209	0.00001	0.76	0.75	0.33	0.22	nd	nd	nd

[1] PBDEs are identified by the IUPAC numbering system or, when unknown, by their GC retention time (min:sec). [2] Used Rrf of BDE-99. [3] Used Rrf of BDE-153. [4] Used Rrf of BDE-190. nd= not detected.

Table 2. Concentrations of major PBDEs (ng/g) and total TCDD TEQs (pg/g) in chickens on a whole weight basis. Percent lipid in each sample is also given.

	Chicken[2] #									
PBDE[1]	253	254	259	262	266	286	287	288	306	Comp[3]
47	0.80	1.16	1.32	1.06	1.30	3.46	6.99	5.33	1.26	0.11
100	0.39	0.35	0.31	0.29	0.34	1.00	2.02	1.53	0.32	0.02
99	1.34	1.22	2.00	1.77	2.09	5.59	13.36	8.86	2.08	0.12
154	0.27	0.27	0.23	0.23	0.25	0.92	2.02	1.42	0.25	0.01
153	0.31	0.25	0.29	0.26	0.31	0.85	2.73	1.56	0.31	0.03
26:40[4]	0.02	0.01	0.01	0.01	0.01	0.04	0.11	0.08	0.01	nd
26:57[4]	0.002	0.001	nd	0.003	0.003	0.01	0.02	0.01	nd	nd
27:41[4]	0.01	0.01	0.01	0.01	0.01	0.03	0.10	0.06	0.01	nd
183	0.18	0.13	0.12	0.13	0.12	0.11	2.95	0.56	0.10	0.14
EPBDE	3.32	3.38	4.28	3.76	4.45	12.00	30.29	19.42	5.33	0.43
TCDD TEQ	1.19	2.81	22.54	19.35	na	na	20.77	21.24	9.56	0.47
% lipid	87.4	92.9	89.3	88.8	91.3	87.8	86.3	94.1	85.5	86.1

[1]PBDEs are identified by the IUPAC numbering system or by GC retention times (min:sec). [2]Chickens 253 and 254 were not exposed to contaminated ball clay; chickens 259-306 were fed contaminated ball clay. [3]Composite chicken sample, average of two matrix blanks from Table 1. [4]Unknown hexa-BDE. nd= not detected. na = not analyzed.

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