#28

NRDC
THE EARTH'S BEST DEFENSE

NATURAL RESOURCES DEFENSE COUNCIL

June 11, 2004

VIA FEDERAL EXPRESS

Craig Wilson, Chief
TMDL Listing Unit
Division of Water Quality
State Water Resources Control Board
1001 "I" Street
Sacramento CA 94814

RE: Notice of Public Solicitation of Water Quality Data and Information

2004 Clean Water Act Section 303(d) List

Dear Mr. Wilson:

Enclosed are attachments to be included with the comment letter being sent under separate cover by our NRDC Los Angeles office. Thank you for considering this information.

Sincerely,

Gail de Rita

Gail de Rita

Encls.

www.nrdc.org

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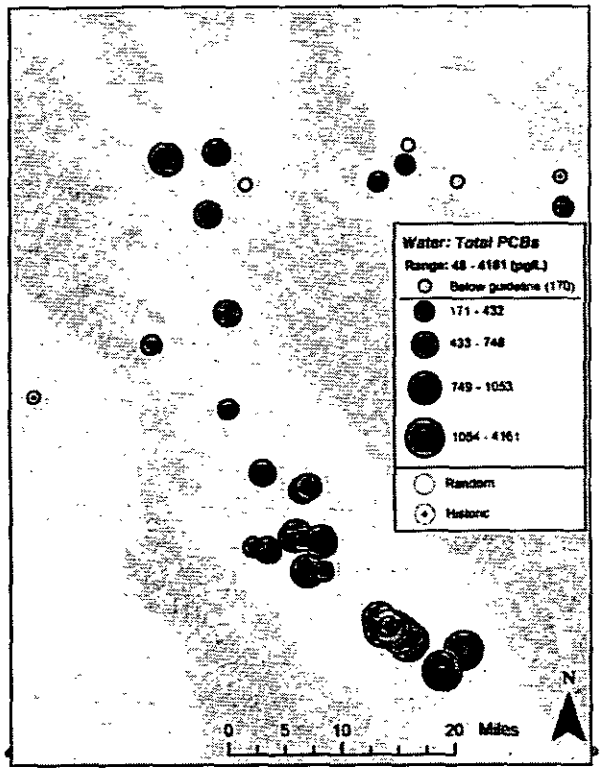


Total PCBs in Water

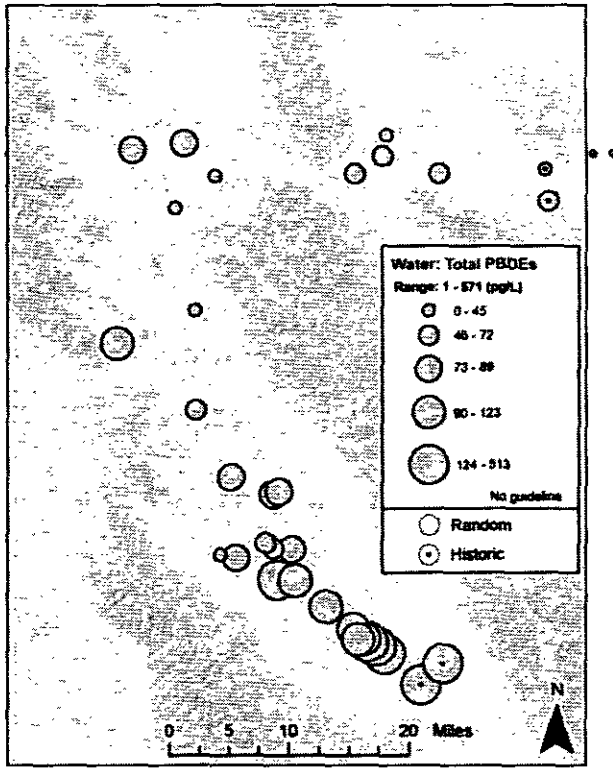
PCB contamination remains one of the greatest water quality concerns in the Estuary, and PCB clean-up is a primary focus of the Regional Water Quality Control Board. Like mercury, PCBs are a problem because they accumulate to high concentrations in some Estuary fish and pose health risks to consumers of those fish. The water quality objective for PCBs in water is designed to prevent unacceptable accumulation of PCBs in humans who consume Estuary fish. **In 2002, the PCB water quality objective was exceeded in 27 of 31 samples (87%) collected from the Estuary.** PCB contamination is greatest in the South Bay; all samples from the South Bay exceeded the objective, with maximum concentrations measured at the southern end of the South Bay. The few samples that did not exceed the objective were from the northern Estuary.

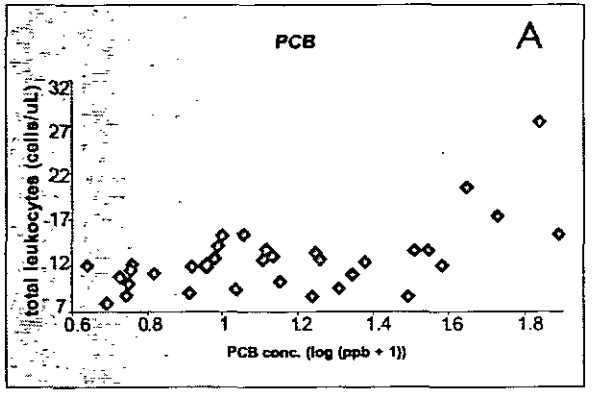
2002

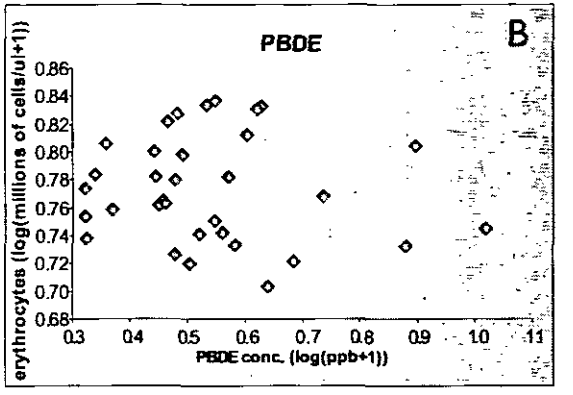
Total PBDEs in Water

PBDEs, a class of flame retardants that were practically unheard of ten years ago, are now found in waters throughout the Estuary. PBDEs are currently on the 303(d) watch list due to increasing concentrations in the Estuary (see page 13) and concerns about their possible effects at the top of the food web. A 2003 California law banned the use of two types of PBDE technical mixtures by 2008.

Tracking the trends in these chemicals is extremely important to determine what effect, if any, the ban will have and if further management actions are necessary. The highest PBDE concentrations in 2002 were measured in waters in the Lower South Bay. Elsewhere, they were present but uniformly low relative to the lower South Bay.

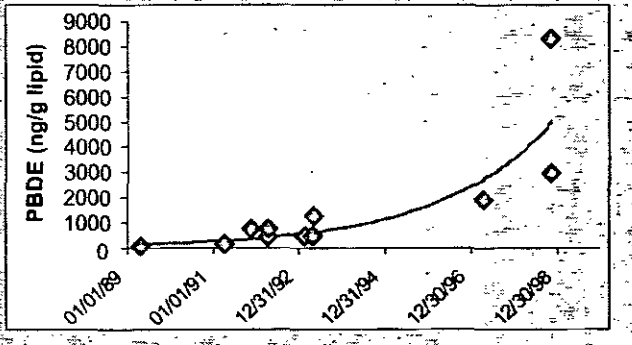






PBDEs in Seal Blubber

PBDE concentrations appear to be rising rapidly in the Bay, raising concern that another legacy contamination problem is developing. Virtually undetectable in samples during the 1980s, over the course of the past 10 years PBDEs have become common in the water, sediments, and food web of the Bay, and concentrations in some samples rival those of other major organic contaminants such as PCBs and DDT.



Perhaps the best record of PBDEs over time is from the analysis of harbor seal blubber by the Hazardous Materials Laboratory of the California Department of Toxic Substances Control. These data illustrate the rapid increase in the Bay food web in the 1990s. In the past few years, significant concentrations of PBDEs have also been measured in terns, cormorants, and fish from the Bay. Furthermore, studies of PBDE concentrations in human blood, fat, and breast milk from the Bay Area have found some of the highest concentrations measured in the world. **Concerns about PBDEs led to legislation in 2003 that will ban two PBDE formulations ("penta" and "octa") in California starting in 2008.** Another major formulation ("deca"), however, has not been banned. The RMP now measures PBDEs in water, sediment, bivalves, fish, and bird eggs, and is establishing a database that can be used to track the success of the PBDE ban and other management efforts.

Reference: She, J., Petreas, M., Winkler, J., Visita, P., McKinney, M., and Kopec, D. PBDEs in the San Francisco Bay Area: measurements in harbor seal blubber and human breast adipose tissue. Chemosphere. 2002 Feb; 46(5):697-707.

Contacts: Seal work - Jianwen She, California Department of Toxic Substances Control, jshe@dtsc.ca.gov
PBDEs in general - Kim Hooper, California Department of Toxic Substances Control, khooper@dtsc.ca.gov
Tom McDonald, California Office of Environmental Health Hazard Assessment, TMCDONAL@oehha.ca.gov

Links to Seal Health

Contaminant concentrations in the blood of Bay harbor seals are high enough to warrant concern for effects on their reproduction and immune systems. PCBs and other priority contaminants reach their highest concentrations at the top of the Bay food web, so fish-eating wildlife such as seals, terns, and cormorants face the highest exposures and greatest health risks. The Bay's harbor seal population has suffered from habitat loss and degradation, including decades of environmental contamination. To explore the possibility of contaminant-induced health alterations in this population, UC Davis researchers measured blood levels of PCBs, DDE, and PBDEs in Bay seals, examined relationships between contaminant exposure and several key natural blood parameters, and compared PCB levels in the present study with levels determined in Bay seals a decade ago. PCBs in harbor seal blood declined slightly during the past decade, but remain high enough that reproductive and immunological effects are possible. A positive association was found between leukocyte counts and PBDEs, PCBs, and DDE in seals (Figure A), and a negative relationship between PBDEs and red blood cell count (Figure B). Although not *necessarily detrimental, these responses serve as* sentinel indications of contaminant-induced alterations in Bay seals, which in individuals with relatively high contaminant burdens could include increased rates of infection and anemia.

Reference: Neale, Jennifer C. C. 2004. Persistent organic contaminants and contaminant-induced immune alterations in the harbor seal, Phoca vitulina. Ph. D. Dissertation, U.C. Davis, Davis, California.

Contact: Jennifer Neale, University of California Davis, jcneale@ucdavis.edu

PBDE (fresh weight corrected) concentrations in CISNet egg samples, 1999-2001.

e = estimated value, NA = not analyzed/not available, ND = not detected.

Station Code	Station	Date	Cruise	Species	Egg Type	# Homogenized	PBDE 047	PBDE 099	PBDE 153
â€¦	â€¦		â€¦	â€¦			ug/kg	ug/kg	ug/kg
RB99A	Richmond Bridge	May-99	1999	Double-Crested Cormorant	RANDOM	10	NA	NA	NA
RB99B	Richmond Bridge	May-99	1999	Double-Crested Cormorant	RANDOM	10	NA	NA	NA
RB99C	Richmond Bridge	May-99	1999	Double-Crested Cormorant	FAIL-TO-HATCH	9	NA	NA	NA
CHINAC	China Camp	March - July 1999	1999	Samuels Song Sparrow	RANDOM	3	NA	NA	NA
BJOHN	Black John's Slough	March - July 1999	1999	Samuels Song Sparrow	RANDOM	7	NA	NA	NA
RBV1	Richmond Bridge	May-00	2000	Double-Crested Cormorant	RANDOM	10	NA	NA	NA
RBV2	Richmond Bridge	May-00	2000	Double-Crested Cormorant	RANDOM	10	NA	NA	NA
RBN1	Richmond Bridge	May-00	2000	Double-Crested Cormorant	FAIL-TO-HATCH	10	NA	NA	NA
CCAV1	China Camp	March - July 2000	2000	Samuels Song Sparrow	RANDOM	6	NA	NA	NA
CCAV2	China Camp	March - July 2000	2000	Samuels Song Sparrow	RANDOM	5	NA	NA	NA
CCAN1	China Camp	March - July 2000	2000	Samuels Song Sparrow	FAIL-TO-HATCH	3	NA	NA	NA
BJAV1	Black John's Slough	March - July 2000	2000	Samuels Song Sparrow	RANDOM	6	NA	NA	NA
BJAV2	Black John's Slough	March - July 2000	2000	Samuels Song Sparrow	RANDOM	6	NA	NA	NA
BJAN1	Black John's Slough	March - July 2000	2000	Samuels Song Sparrow	FAIL-TO-HATCH	6	NA	NA	NA
RMAV1	Petaluma River Mouth	March - July 2000	2000	Samuels Song Sparrow	RANDOM	6	NA	NA	NA
RMAV2	Petaluma River Mouth	March - July 2000	2000	Samuels Song Sparrow	RANDOM	8	NA	NA	NA
RMAN1	Petaluma River Mouth	March - July 2000	2000	Samuels Song Sparrow	FAIL-TO-HATCH	7	NA	NA	NA
RBV1A/B	Richmond Bridge	May-01	2001	Double-Crested Cormorant	RANDOM	10	e 245.23	e 70.62	e 32.49
RBV2A/B	Richmond Bridge	May-01	2001	Double-Crested Cormorant	RANDOM	10	e 111.11	e 53.28	e 22.13
CCAV1	China Camp	March - July 2001	2001	Samuels Song Sparrow	RANDOM	7	e 18.17	e 13.94	e 3.00
CCBV1	China Camp	March - July 2001	2001	Samuels Song Sparrow	RANDOM	6	e 17.59	e 11.51	e 2.43
CCAN1	China Camp	March - July 2001	2001	Samuels Song Sparrow	FAIL-TO-HATCH	4	e 13.88	e 9.65	e 2.29
RMAV1	Petaluma River Mouth	March - July 2001	2001	Samuels Song Sparrow	RANDOM	6	e 7.13	e 5.15	e 1.71
RMAN1	Petaluma River Mouth	March - July 2001	2001	Samuels Song Sparrow	FAIL-TO-HATCH	4	e 4.69	e 2.25	ND

Table 23. BDE concentration in bivalve tissue, 2002. NA = not analyzed, ND = not detected, NS = not sampled, e = estimate.
CFLU - Corbicula fluminea, CGIG - Crassostrea gigas, MCAL - Mytilus californianus.
T-0 = time of bivalve deployment into the Estuary from the source indicated under station name heading.
NA = not available, ND = not detected, e = estimated value

Station Co	Station	Date	CruiseN	Species	BDE 017	BDE 028	BDE 047	BDE 066	BDE 085	BDE 099	BDE 100	BDE 138	BDE 153	BDE 154	BDE 183	BDE 190	Total PBDEs (SFEI)
					ug/kg	ug/kg	ug/kg	ug/kg	ug/kg	ug/kg	ug/kg	ug/kg	ug/kg	ug/kg	ug/kg	ug/kg	ug/kg
BG20	Sacrament	8/27/2002	2002-08	CFLU	ND	ND	47.6	ND	ND	12.7	24.8	ND	ND	ND	ND	ND	85.1
BG30	San Joaqu	8/27/2002	2002-08	CFLU	ND	ND	59.7	ND	ND	17	28.9	ND	ND	ND	ND	ND	105.6
BD50	Napa River	9/6/2002	2002-08	CGIG	ND	ND	29.3	ND	ND	e 12.2	e 6.18	ND	ND	ND	ND	ND	47.68
BD40	Davis Poin	9/6/2002	2002-08	CGIG	ND	ND	43	ND	ND	13.3	e 7.4	ND	ND	ND	ND	ND	63.72
BD30	Pinole Poir	9/4/2002	2002-08	MCAL	ND	ND	10.6	ND	ND	e 4.33	ND	ND	ND	ND	ND	ND	14.93
BD20	San Pablo	9/4/2002	2002-08	CGIG	ND	ND	25.2	ND	ND	e 6.94	e 3.78	ND	ND	ND	ND	ND	35.92
BD15	Petaluma F	9/4/2002	2002-08	CGIG	ND	ND	e 9.05	ND	ND	ND	ND	ND	ND	ND	ND	ND	9.05
BC61	Red Rock	9/5/2002	2002-08	MCAL	ND	ND	12.7	ND	ND	5.58	ND	ND	ND	ND	ND	ND	18.28
BC21	Horseshoe	9/5/2002	2002-08	MCAL	ND	ND	e 9.5	ND	ND	e 3.92	ND	ND	ND	ND	ND	ND	13.42
BC10	Yerba Buer	9/5/2002	2002-08	MCAL	ND	ND	23	ND	ND	e 11.5	e 3.19	ND	ND	ND	ND	ND	37.27
BC10	Yerba Buer	9/5/2002	2002-08	MCAL	ND	ND	22.4	ND	ND	11.3	e 3.15	ND	ND	ND	ND	ND	
BB71	Alameda	9/3/2002	2002-08	MCAL	ND	ND	27.5	ND	ND	e 14.6	e 4.8	ND	ND	ND	ND	ND	46.92
BA40	Redwood (	9/3/2002	2002-08	MCAL	ND	ND	24.2	ND	ND	12.2	e 3.55	ND	ND	ND	ND	ND	39.95
BA30	Dumbarton	9/3/2002	2002-08	MCAL	ND	ND	14.7	ND	ND	e 5.04	ND	ND	ND	ND	ND	ND	19.74
BA10	Coyote Cre	9/3/2002	2002-08	CGIG	ND	ND	18.5	ND	ND	4.73	ND	ND	ND	ND	ND	ND	23.23
T-0	Bodega He	5/31/2002	2002-08	MCAL	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
T-0	Tomales B	5/31/2002	2002-08	CGIG	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Sum of PBDE concentrations in water samples, 2002.

B = blank contamination >30% of measured concentration, b = blank contamination < 30% of measured concentration

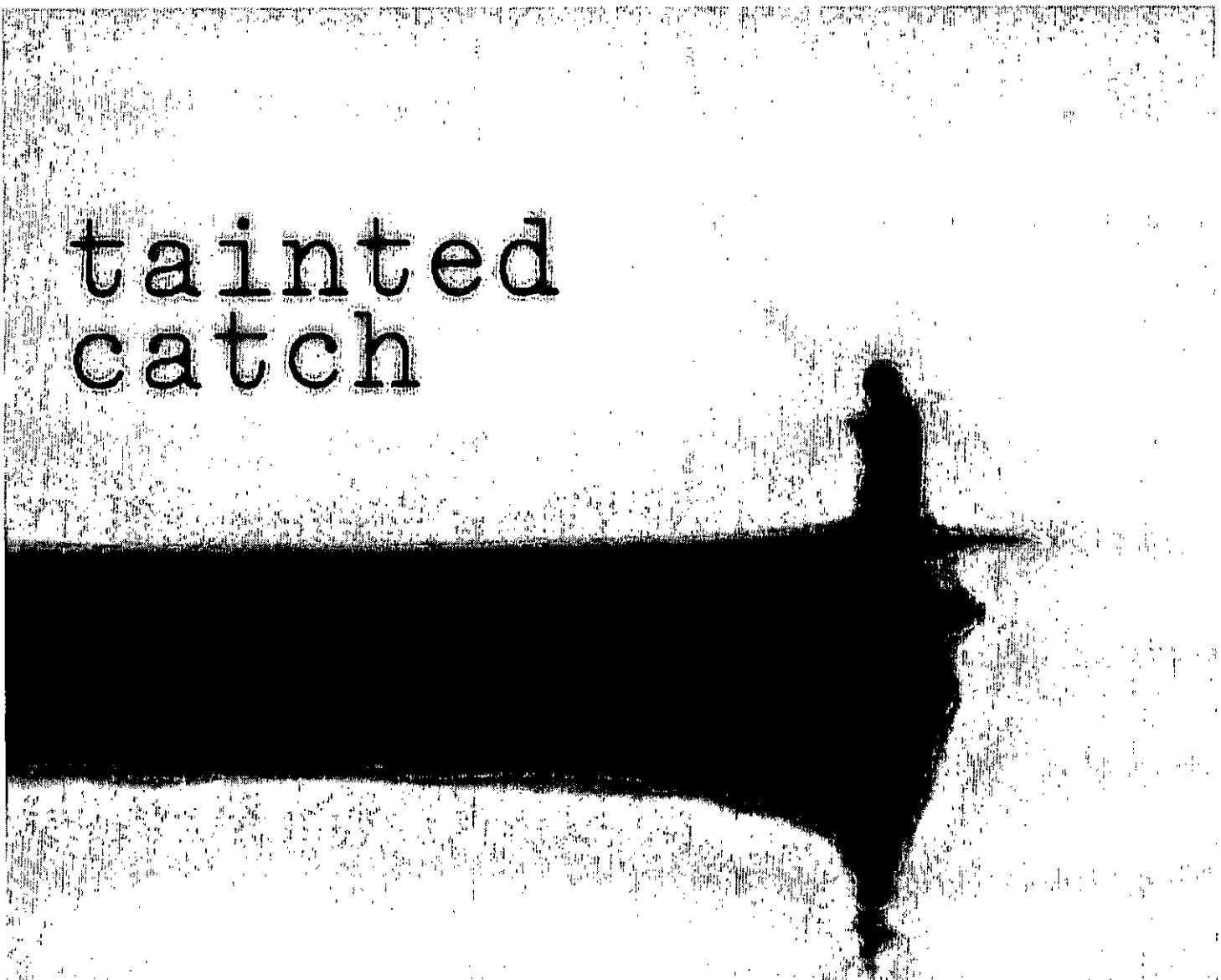
CXXX = coelution, where XXX is the number of the coeluting congener where the value is stored,

ce = coelution (result is for two or more coeluting congeners), e = estimated value, ND = not detected.

SITE_COD	SITE_NAM	COLLECTI	CRUISE_N	BDE 001	BDE 002	BDE 003	BDE 007	BDE 008	BDE 010	BDE 011	BDE 012	BDE 013	BDE 015	BDE 017	BDE 025	BDE 028	BDE 030	BDE 032	BDE 033	BDE 035	BDE 037	BDE 047	BDE 049	BDE 066	BDE 071
				pg/L	pg/L	pg/L	pg/L	pg/L	pg/L	pg/L	pg/L	pg/L	pg/L	pg/L	pg/L	pg/L	pg/L	pg/L	pg/L	pg/L	pg/L	pg/L	pg/L	pg/L	pg/L
BG20	Sacrament	7/30/2002	2002-07	ND	ND	ND	e .241	ND, ce	ND	C008	ND	ND	b 1.145	1 436	e .165	b, ce, e 1.4	ND	ND	C028	ND	e .072	B	b 1.828	B	e .132
BG30	San Joaqu	7/30/2002	2002-07	ND	ND	ND	e .358	ce, e .151	ND	C008	ND	ND	b, e 2.22	e 2.2	e .286	b, ce 2.09	ND	ND	C028	e .255	e .369	b 37.4	b, e 2.49	b 1.41	e 49
BG30	San Joaqu	7/30/2002	2002-07	ND	ND	ND	e .326	ce, e .108	ND	C008	ND	ND	b 1.62	2.13	e .274	b, ce 2.15	ND	ND	C028	0.208	e .226	b 45.9	b 2.58	b 2.66	e .454
SU001W	Suisun Bay	7/29/2002	2002-07	ND	ND	ND	0.464	ce, e .303	ND	C008	ND	ND	b 2.08	2.59	0.399	b, ce 2.25	ND	ND	C028	ND	e .265	b 30.7	b 2.84	b 1.32	e .596
SU002W	Suisun Bay	7/29/2002	2002-07	ND	ND	ND	e .584	ce, e .331	ND	C008	ND	ND	b 3.27	2.96	ND	b, ce 2.67	ND	ND	C028	e .23	e .391	b 45.4	b 3.29	b, e 1.77	e .621
SU003W	Suisun Bay	7/29/2002	2002-07	ND	ND	ND	e .303	ce, e .478	ND	C008	ND	e .165	b 3.94	2.8	e .568	b, ce 3.17	ND	ND	C028	ND	e .326	b 42.9	b, e 3.49	b 2.53	e .748
SU005W	Suisun Bay	7/29/2002	2002-07	ND	ND	ND	e .373	ce, e .277	ND	C008	ND	ND	b 2.74	e 2.7	e .517	b, ce 3.03	ND	ND	C028	0.217	e .329	b 43.1	b 3.18	b 1.4	e .694
SPB001W	San Pablo	7/17/2002	2002-07	ND	ND	ND	0.562	ce .735	ND	C008	ND	0.159	b 3.72	3.74	e .508	b, ce 2.15	ND	ND	C028	e .124	e .306	b 35.9	b, e 3.68	b, e 1.61	0.704
SPB002W	San Pablo	7/17/2002	2002-07	ND	ND	ND	0.555	ce .388	ND	C008	ND	ND	b 1.55	3.31	0.444	b, ce 2.57	ND	ND	C028	e .213	e .339	b 38.2	b 2.57	b 1.31	0.625
SPB003W	San Pablo	7/17/2002	2002-07	ND	ND	ND	0.625	ce, e .925	ND	C008	ND	0.232	b 7	6.44	0.935	b, ce 3.73	ND	ND	C028	ND	e .303	b 62.3	b 8.55	b, e 2.34	1.74
SPB004W	San Pablo	7/17/2002	2002-07	ND	ND	ND	0.531	ce, e .396	ND	C008	ND	e .131	b 1.22	2.4	e .329	b, ce, e 1.5	ND	ND	C028	ND	e .205	b 32.6	b 2.37	b, e 1.23	0.43
BC20	Golden Ga	7/18/2002	2002-07	ND	ND	ND	e .154	ce, e .084	ND	C008	ND	ND	B, e	e .197	ND	B, ce	ND	ND	C028	ND	e .103	B	B, e	B, e	e .078
CB001W	Central Ba	7/16/2002	2002-07	ND	ND	ND	0.582	ce, e .349	ND	C008	ND	ND	b .626	2.52	e .473	b, ce 1.13	ND	ND	C028	ND	ND	B	b 2.31	B, e	0.349
CB002W	Central Ba	7/19/2002	2002-07	ND	ND	ND	2.78	ce 1.17	ND	C008	ND	ND	b 1.12	5.79	0.812	b, ce 2.64	ND	ND	C028	ND	ND	b 56.2	b 5.03	b 1.88	0.771
CB002W	Central Ba	7/19/2002	2002-07	ND	ND	ND	e 2.23	ce, e .876	ND	C008	ND	ND	ND	5.05	0.604	b, ce 2.09	ND	e .105	C028	ND	e .17	b 53.7	b 5.06	b 1.94	0.968
CB003W	Central Ba	7/18/2002	2002-07	ND	ND	ND	0.344	ce, e .28	0.225	C008	0.087	ND	0.573	1.43	e .287	ce .986	ND	ND	C028	ND	ND	b 24.8	1.7	b, e .903	0.273
CB004W	Central Ba	7/19/2002	2002-07	ND	ND	ND	0.714	ce .465	ND	C008	ND	ND	0.452	2.19	0.391	ce .9	ND	ND	C028	ND	ND	b 20.6	1.93	b, e .907	0.366
SB001W	South Bay	7/25/2002	2002-07	ND	ND	ND	0.923	ce, e .373	ND	C008	ND	ND	0.349	2.3	0.442	ce 1.08	ND	ND	C028	ND	ND	b 16.1	2.1	b, e .821	0.495
SB002W	South Bay	7/24/2002	2002-07	ND	ND	ND	2	b, ce 1.38	ND	C008	ND	e .167	1.04	b 5.81	1.34	b, ce 2	ND	ND	C028	ND	ND	b 24.4	b 5.13	B, e	1.45
SB003W	South Bay	7/26/2002	2002-07	ND	ND	ND	1.49	b, ce .614	ND	C008	ND	ND	0.697	b 2.63	0.441	B, ce, e	ND	ND	C028	ND	ND	b 19.3	b, e 2.03	B	0.557
SB004W	South Bay	7/25/2002	2002-07	ND	ND	ND	1.23	b, ce .542	ND	C008	ND	ND	0.658	b 3.32	0 374	b, ce 1.24	ND	ND	C028	ND	ND	b 17.7	b 3.12	B, e	e .529
SB005W	South Bay	7/26/2002	2002-07	ND	ND	ND	1.43	b, ce .644	ND	C008	ND	ND	0 491	b 3.09	e .536	B, ce, e	ND	ND	C028	ND	ND	b 17.7	b, e 2.55	B, e	ND
SB006W	South Bay	7/23/2002	2002-07	ND	ND	ND	2.38	b, ce 1.3	ND	C008	ND	0.164	0.819	b 5.72	e .737	b, ce 1.77	ND	ND	C028	ND	ND	b 18.9	b 4.08	B	e .681
SB007W	South Bay	7/26/2002	2002-07	ND	ND	ND	1.42	b, ce .913	ND	C008	ND	ND	1.52	b 3.83	0.631	b, ce 1.64	ND	ND	C028	ND	ND	b 30.8	b, e 4.36	B	e .692
SB008W	South Bay	7/25/2002	2002-07	ND	ND	ND	2.52	b, ce .98	ND	C008	ND	ND	0.895	b 3.96	e .617	b, ce 1.44	ND	ND	C028	ND	ND	b 24 4	b, e 3.39	B	0.359
SB009W	South Bay	7/26/2002	2002-07	ND	ND	ND	0.956	b, ce .499	ND	C008	ND	ND	0.754	b 3.36	0 408	b, ce 1.27	ND	ND	C028	ND	ND	b 25.4	b 3 48	B, e	0.317
SB010W	South Bay	7/25/2002	2002-07	ND	ND	ND	2.45	b, ce 1.11	ND	C008	ND	0.176	1	b 5.23	0.556	b, ce 2	ND	ND	C028	e 469	ND	b 30.5	b, e 4 45	B, e	0.695
LSB001W	Lower Sou	7/24/2002	2002-07	ND	ND	ND	1.46	ce 1.07	ND	C008	ND	0.172	0.958	5.65	0.756	ce 1.83	ND	0.22	C028	ND	ND	b 28	5 21	b, e 1.32	e 1.26
LSB002W	Lower Sou	7/22/2002	2002-07	ND	ND	ND	2.27	ce 2.27	ND	C008	ND	0.414	2.29	12.7	1.98	ce 4.17	ND	ND	C028	ND	ND	b 51.8	11.7	b, e 2.85	e 1.89
LSB003W	Lower Sou	7/24/2002	2002-07	ND	ND	ND	1.86	ce 1.72	ND	C008	ND	0.352	1.63	9.23	1.45	ce 3.02	ND	ND	C028	ND	ND	b 34.3	8.41	b, e 1.39	1.34
LSB004W	Lower Sou	7/24/2002	2002-07	ND	ND	ND	1.77	ce 1.39	ND	C008	ND	0.219	1.5	8.56	1.12	ce 2.95	ND	ND	C028	ND	ND	b 36.6	8.23	b, e 1.91	e 1.97
LSB005W	Lower Sou	7/23/2002	2002-07	ND	ND	ND	1.18	ce .836	ND	C008	ND	e .138	0.67	5.99	1	ce 2.22	ND	ND	C028	ND	ND	b 30.4	5.77	b 1.24	0.949
LSB006W	Lower Sou	7/22/2002	2002-07	ND	ND	ND	3.96	ce 5.01	ND	C008	e .074	1.23	6.96	26.7	4.5	ce 10.3	ND	e .522	C028	ND, e	0.509	b 123	27.9	b 5.83	e 3.69
C-3-0	San Jose	7/23/2002	2002-07	ND	ND	ND	7.6	ce 5.86	ND	C008	0.09	2.186	b 9.85	32.1	5.67	b, ce 17.37	ND	e .813	C028	e .198	e .834	b 179.5	b 24.33	b 7.9	5.04
C-1-3	Sunnyvale	7/23/2002	2002-07	ND	ND	e 1.9	14.9	ce 6.96	ND	C008	ND	3 61	b 16	36.5	5.67	b, ce 13.4	ND	0.916	C028	e .362	e 1.22	b 103	b 29	b 4.05	3.27

BDE 075 pg/L	BDE 077 pg/L	BDE 085 pg/L	BDE 099 pg/L	BDE 100 pg/L	BDE 105 pg/L	BDE 116 pg/L	BDE 119 pg/L	BDE 126 pg/L	BDE 138 pg/L	BDE 140 pg/L	BDE 153 pg/L	BDE 154 pg/L	BDE 155 pg/L	BDE 166 pg/L	BDE 181 pg/L	BDE 183 pg/L	BDE 190 pg/L	BDE 206 pg/L	BDE 207 pg/L	BDE 208 pg/L	BDE 209 pg/L	Sum of PBDEs (SFEI) pg/L
0.055	ND	B	B	B	ND	ND	ND	ND	B, ce, e	ND	B	B, e	0.225	C138	ND	B	ND	B, e	B	B, e	B	2.931
e .12	ND	B, e	B	B	ND	ND	e .141	ND	ND, ce	ND	B, e	B	B, e	C138	ND	B	ND	B, e	B, e	B	B	57.65
0.134	ND	B, e	B	b 9.68	ND	ND	ND	ND	ND, ce	ND	B, e	B	B, e	C138	ND	B	ND	B	B, e	B, e	B	
ND	ND	B	B	B	ND	ND	ND	ND	ce, e .318	ND	B	B	B, e	C138	ND	B, e	ND	B	B, e	B, e	B	37.178
e .149	ND	B	B	b 9.36	ND	ND	e .255	ND	ce, e .391	ND	B	B, e	ND	C138	ND	B	ND	B, e	B, e	B, e	B	62.551
ND	ND	B	B	b 8.95	ND	ND	ND	ND	ND, ce	ND	B, e	B	B, e	C138	ND	B	ND	B, e	B	B, e	B	60.35
ND	ND	B	B	b 9.17	ND	e .451	ND	ND	ce, e .401	e .175	B	B	B, e	C138	ND	B	ND	B, e	B, e	B, e	B	59.801
0.113	ND	b, e 1.94	B	b 9.92	ND	ND	ND	ND	ce .52	ND	B	B	ND	C138	ND	B	e .308	b 9.31	b, e 13.5	b, e 7.11	B	86.008
ND	ND	B	B	B	ND	e .353	ND	ND	ND, ce	ND	B	B	B	C138	ND	B	ND	B, e	B	B	B	45.39
e .205	ND	B, e	B	b 13.1	ND	ND	e .282	ND	ce, e .527	e .313	B	B	B, e	C138	ND	B	ND	B	B, e	B	B	88.437
ND	ND	B, e	B	B	ND	ND	ND	ND	ce, e .242	ND	B	B	B, e	C138	ND	B	e .317	B	B	B	B	38.319
ND	ND	B, e	B	B	ND	ND	ND	ND	ND, ce	ND	B	B, e	ND	C138	ND	B	ND	B, e	B, e	B, e	B, e	0.197
ND	ND	B, e	B	B	ND	ND	ND	ND	ce, e .222	ND	B	B, e	ND	C138	ND	B, e	ND	B	B	B	B	3.872
0.099	ND	B	B	b 10.5	ND	ND	ND	ND	ce, e .757	ND	B	B	B, e	C138	ND	B, e	ND	B, e	B, e	B	B	75.33
0.093	ND	B, e	B	b 9.88	ND	e .372	0.162	ND	ce .254	e .304	B	B	B, e	C138	ND	B	ND	B, e	B	B, e	B	
ND	ND	1.24	b 35.6	b 6.65	ND	ND	ND	ND	ce .414	ND	b 4.26	b 3.39	e .326	C138	ND	b 2.07	ND	ND	e .826	ND	26.6	109.169
e .073	ND	e .824	b 24	b 4.78	ND	ND	ND	ND	ce, e .201	e .163	b 2.89	b 2.29	0.306	C138	ND	B	ND	ND	ND	ND	12.2	71.782
e .229	ND	e .434	b 11.7	b 2.71	ND	ND	ND	ND	ce, e .45	ND	B	b 1.31	e .309	C138	ND	b .761	ND	ND	e .864	ND	36.8	75.3405
B, e	ND	B	b 17.5	b 4.43	ND	ND	ND	ND	ND, ce	ND	b, e 2.32	b 1.95	e .549	C138	ND	B, e	ND	ND	ND	ND	40.9	99.31
B, e	ND	B	b 15.3	b 3.63	ND	ND	ND	ND	B, e	ND	B	b 1.58	e .253	C138	ND	B	ND	ND	ND	ND	ND	42.44
B, e	ND	B	b 12.5	b 3.08	ND	ND	ND	ND	ND, ce	ND	B	b, e 1.36	e .306	C138	ND	B, e	ND	ND	ND	ND	23.6	62.8
B	ND	B, e	b 14.5	b 3.19	ND	ND	ND	ND	ND, ce	ND	B	b, e 1.52	e .321	C138	ND	B	ND	ND	1.62	ND	e 16.7	58.32
B, e	ND	B, e	b 13.1	b 3.17	ND	ND	ND	ND	B, e	ND	B, e	b 1.24	0.373	C138	ND	B, e	ND	1.72	2.42	1.3	74.1	123.44
B	ND	B	b 25.1	b 5.92	ND	ND	ND	ND	ND, ce	ND	b 2.18	b 2.02	e 411	C138	ND	B	ND	e .716	e 1.14	ND	15.7	89.046
ND	0.164	B	b 23.3	b 5.19	ND	ND	ND	ND	ND, ce	ND	b 2.61	b 2.09	e .417	C138	ND	B	ND	e 1.02	e 1.11	e .682	e 19.3	85.102
B, e	ND	B, e	b 21.2	b 4.93	ND	ND	ND	ND	ND, ce	ND	b 2.16	b 1.84	0.275	C138	ND	B, e	ND	e .815	e 1.05	e .75	21.1	83.875
B	ND	B	b 25.1	b 6.08	ND	ND	ND	ND	ND, ce	ND	b 2.63	b 2.19	0.542	C138	ND	B	ND	2.08	e 1.71	1.28	e 45.3	124.1
ND	ND	0.676	b 23.5	b 5.26	ND	ND	0.13	ND	ce, e .515	ND	b 3.23	b 2.74	e .754	C138	ND	b 1.01	ND	0.802	e 1.92	1.13	e 25.1	102.683
ND	e .224	0.898	b 35.6	b 8.09	ND	ND	0.201	ND	ce .545	ND	b 4.5	b 3.86	0.947	C138	ND	b 1.46	ND	2.08	2.99	1.65	87.8	220.993
0.255	ND	e .594	b 23.8	b 5.65	ND	ND	e .204	ND	ND, ce	ND	b 2.26	b 2.94	e .557	C138	ND	b 1.6	ND	0.555	1.72	1.18	30.7	118.939
e .277	ND	0.687	b 27.3	b 6.32	ND	ND	0.152	ND	ce, e .343	e .217	b 3.4	b 3.25	e .988	C138	ND	b 1.25	e .632	2.12	4.09	e 2.23	57.9	159.542
e .18	ND	e .673	b 22.3	b 4.99	ND	ND	ND	ND	ce .515	ND	b 2.81	b 2.7	e .508	C138	ND	b 1.11	e .372	e 1.1	ND	e .962	e 45.2	122.582
e .288	ND	2.3	b 90.7	b 20.7	ND	ND	0.392	ND	ce, e 1.4	e 483	b 10.3	b 9.25	2.46	C138	ND	b 3.48	0.528	5.05	7.46	4.93	191	512.9
0.543	ND	B, e	B	B	ND	ND	0.497	ND	b, ce, e 1.0	0.38	B	B	B	C138	ND	B, e	0.427	B, e	B, e	B	B	238.327
0.259	ND	b 2.02	b 62.7	b 15.1	ND	ND	0.447	ND	ce, e 1.18	ND	b 9.01	b 7.13	b, e 1.69	C138	ND	b 26.9	e 1.12	B, e	b 10.8	B	B	292.91

tainted catch



Toxic fire retardants are building up rapidly in San Francisco Bay fish — and people

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ENVIRONMENTAL WORKING GROUP

THE POWER OF INFORMATION
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EWG is a nonprofit research organization with offices in Washington, DC and Oakland, CA. EWG uses the power of information to educate the public and decision-makers about a wide range of environmental issues, especially those affecting public health.

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Executive Summary

Levels of a little-known class of neurotoxic chemicals found in computers, TV sets, cars and furniture are building up rapidly in key indicator species of San Francisco Bay fish, according to tests by the Environmental Working Group (EWG.)

Analysis of six species of Bay fish, conducted for EWG by a California state toxics lab, detected polybrominated diphenyl ethers (PBDEs) in every fish sampled. The tests compared fish caught by local anglers with archived samples caught in 1997, and found that PBDE levels more than doubled in halibut and more than tripled in striped bass. Striped bass and halibut are the two most commonly eaten species of Bay fish, and as large, mobile, carnivorous species, are good indicators of overall toxic contamination in the Bay.

These are the first findings for PBDEs in Bay fish. They add to the evidence that the Bay Area is a hotspot for exposure to bromine-based chemicals, widely used in commercial flame retardants, that many scientists warn are "the next PCBs" — a notorious class of chemicals banned in 1977 after evidence that they cause cancer and build up in people and the environment. The European Union has banned two of the most commonly used PBDEs, effective next year, but in the United States they remain virtually unregulated by either state or federal authorities.

PBDEs and other brominated fire retardants (BFRs) are similar in chemical structure to PCBs, which are still found in the bodies of people and animals more than 20 years after they were removed from commercial products in the United States. Recent research on animals has shown that exposure to low levels of PBDEs can cause permanent neurological and developmental damage including deficits in learning, memory and hearing, changes in behavior, and delays in sensory-motor development. Most at risk are pregnant women, developing fetuses, infants and young children, and to a lesser extent, the 10 million Americans with hypothyroidism.

Every day, a typical American comes in contact with dozens, if not hundreds, of consumer goods that contain PBDEs, including electronics, electrical cables, carpets, furniture, and textiles.

Although the pathway by which PBDEs and other brominated fire retardants get into the environment is largely still a mystery, the chemicals are now found worldwide in house dust, indoor and outdoor air and the water and sediments of rivers, estuaries and oceans. PBDEs have been found in the tissues of whales, seals, birds and bird eggs, moose, reindeer, mussels, eels, and dozens of species of freshwater and marine fish.

Rapid Increases in Humans

PBDEs are also building up rapidly in the bodies of people. Levels in Swedish breast milk samples were 55 times higher in 1997 than in 1972. The few breast milk samples collected from U.S. women indicate even higher levels of PBDEs in the bodies of first-time mothers than found in Europe and Canada. Already, scientists say, most Americans may carry in their bodies levels of PBDEs that have been found to cause serious, permanent neurological damage in laboratory animals.

Though still limited, the data on elevated levels of PBDEs in the Bay Area are disturbing. The levels of PBDEs found in San Francisco Bay fish are much higher than those found in commonly eaten fish species from Europe, Japan, the Pacific Northwest and the Great Lakes. Consumption of contaminated fish is believed to be a major route of PBDE exposure for adults. Earlier studies of PBDEs in the blood and breast tissue of Bay Area women, and of harbor seals from San Francisco Bay, have found levels from three to 60 times higher than levels measured in people and animals in Europe. Ninety-five percent of the type of PBDEs that bioaccumulate most readily is used in North America, and much of that amount goes into polyurethane foam sold in California, but it is unknown exactly why contamination is so high in the Bay Area.

In the fall of 2002, EWG researchers collected 22 fish from six of the most commonly eaten species at 10 locations around San Francisco Bay. Analysis conducted under contract by the state Department of Toxics Substances Control's Hazardous Materials Laboratory in Berkeley found that every sample contained seven different PBDEs, in concentrations ranging from trace amounts to more than 60 parts per billion (ppb) in fish tissue. We also tested for PBDEs in fish samples archived from 1997, and found that in five years, levels of the chemicals had increased in four of six species tested.

The California Legislature is considering a ban on some types of PBDEs in consumer products by 2008. AB 302 by Assemblywoman Wilma Chan of Alameda, which passed the Assembly in May 2003 and is pending a vote in the state Senate, would make California the first state in the nation to regulate PBDEs. The bill is an

important first step, but additional action will be necessary to fully protect public health. Some industries, notably many computer makers, are already moving toward safer alternatives, but the rapid buildup of PBDEs in people, animals and the environment makes it imperative that all brominated flame retardants must be phased out quickly.

The Next PCBs?

As highly flammable synthetic materials have replaced less-combustible natural materials in consumer products, chemical fire retardants have become ubiquitous in consumer products. Of the many different kinds of fire retardants, one of the most common is a class of bromine-based chemicals known as polybrominated diphenyl ethers, or PBDEs. Today PBDEs are in thousands of products, in which they typically comprise 5 to 30 percent of product weight. [1] During manufacturing, PBDEs are simply mixed in to the plastic or foam product, rather than chemically binding to the material as some other retardants do, making PBDEs more likely to leach out.

There are 209 structural variants, or congeners, of PBDEs, classified by the number of bromine atoms in a molecule of the chemical: Penta-BDEs have five bromine atoms, octa-BDEs have eight, deca-BDEs have 10, and so on. The commercial PBDE flame retardants are actually mixtures of several different congeners, with the three major products called Deca, Penta, and Octa. (The common name of the commercial product can be somewhat misleading; the Penta product, for example, is actually a mixture of 40 percent tetra-BDE, 45 percent penta-BDE and 6 percent hexa-BDE congeners.) Worldwide, Deca is the most widely used of the PBDEs with 83 percent of the global market by weight, followed by Penta with 11 percent and Octa with 6 percent. [2]

PBDEs are the chemical cousins of PCBs, another family of persistent and bioaccumulative toxins that came to the attention of regulators only after millions of pounds had been released into the environment. In the 26 years since PCBs were banned, numerous studies have documented permanent, neurological impairment to the developing child from low level PCB exposure. [3-7] Recent evidence suggests PBDEs and PCBs may work together to cause adverse health effects. Not only do PBDEs appear to be acting through the same pathways as PCBs and dioxins, but a 2003 study found that early exposure of lab animals to a combination of PCBs and PBDEs affected motor skills ten times more strongly than exposure to the individual chemicals. [8, 9]

PBDE use has skyrocketed in the last three decades, with Penta production almost doubling between 1992 and 2001. [2, 10] The market took off after the ban of a previously popular class of fire retardants, polybrominated biphenyls or PBBs, following

the catastrophic contamination of cattle feed in Michigan during 1973 and 1974 that exposed nine million people to tainted meat and dairy products. [11] Today, half of the PBDEs used worldwide are used in North America, with 73 million pounds being used in 2001. [2] An unknown amount of PBDEs, probably millions of pounds, is also imported into the country each year in manufactured goods. Chemical industry analysts say the North American market for brominated flame retardants is $1 billion a year and growing by about 3.7 percent annually; the European market is a little more than half that size. [12]

The Bromine Oligopoly

Worldwide, eight companies manufacture PBDEs, with the two largest in the U.S.: Great Lakes Chemical Corp. of West Lafayette, Ind., and Albemarle Corp. of Richmond, Va. In 2002, Great Lakes reported total sales for all products of $1.4 billion, up 4 percent from the previous year. Albermarle reported sales of $980 million, up 7 percent. [13, 14] To Americans familiar with toxics issues, the corporations are notorious as the manufacturers of methyl

Products Often Containing PBDEs

Materials used in	Types of PBDEs used	Examples of consumer products
Plastics	Deca, Octa, Penta	Computers, televisions, hair dryers, curling irons, copy machines, fax machines, printers, coffee makers, plastic automotive parts, lighting panels, PVC wire and cables, electrical connectors, fuses, housings, boxes and switches, lamp sockets, waste-water pipes, underground junction boxes, circuit boards, smoke detectors
Textiles	Deca, Penta	Back coatings and impregnation of home and office furniture, carpets, automotive seating, aircraft and train seating
Polyurethane foam	Penta	Home and office furniture (couches and chairs, carpet padding, mattresses and mattress pads) automobile, bus, plane and train seating, sound insulation panels, imitation wood, packaging materials
Rubber	Deca, Penta	Conveyor belts, foamed pipes for insulation, rubber cables
Paints and laquers	Deca, Penta	Marine and industry protective laquers and paints

Source: WHO 1994 [1], Danish EPA 1999 [103]

bromide, a volatile, acutely toxic, ozone-depleting pesticide gas used to fumigate strawberries, tomatoes and other crops. (Albemarle also has the dubious distinction of being a spin-off of Ethyl Corp., whose leaded gasoline additive was banned in the U.S. in 1972.) The main areas of bromine production in the world are southeastern Arkansas, where Great Lakes and Albemarle pump it from underground pools of brine, and Israel, where a company named Dead Sea Bromine extracts it from the briny inland sea. A chemical industry journal describes the global trade in brominated chemicals as "an oligopoly controlled by Albemarle, Great Lakes and the Dead Sea Bromine Group." [15]

Despite their heavy use, until recently data were scarce on the toxicity or environmental fate of PBDEs. But in the last few years, it has become clear that PBDEs and other brominated flame retardants have joined PCBs, DDT and dioxin on the list of persistent, bioaccumulative chemicals contaminating people, animals and the environment everywhere on Earth. PBDEs are now found in house dust, sewage sludge and the water and sediments of rivers, estuaries and oceans. They've been found in the tissues of whales, seals, birds and bird eggs, moose, reindeer, mussels, eels, and dozens of species of freshwater and marine fish. [16-21] Like scores of other industrial chemicals, they have also been found in human breast milk, fat and blood.

The reach of PBDE pollution is global, found essentially everywhere scientists have looked: Belgium, Canada, Czech Republic, Denmark, England, Finland, Germany, Greenland, Ireland, Israel, Japan, Korea, the Netherlands, Norway, Portugal, Russia, Sweden, Switzerland, Taiwan, and numerous U.S. locations. [16, 17, 19, 22-25] PBDEs can travel great distances. They've been found in birds and marine mammals in remote locations including the North Sea, the Baltic Sea and the Arctic Ocean. [26]

Of greatest concern is the exponential rate of PBDE increase in the environment. PBDEs in California harbor seals increased by a factor of 100 between 1989 and 1998, and in Lake Ontario trout by a factor of more than 300 between 1978 and 1998. [27, 28] Similar dramatic increases have been seen in human blood samples from Norway, ringed seals from the Canadian arctic, and gull eggs from the Great Lakes region. [18, 29, 30] In each of these studies, the time it took for PBDEs to double in concentration was remarkably short — from less than two years to five years.

U.S. Dominates Global Use of PBDEs

The problem is global, but the U.S. is clearly a hotspot. The average PBDE concentration found in the breast tissue of California women was among the highest yet reported — three times higher than Swedish tissue samples, 10 times higher than German blood samples and Canadian milk samples, and 25 times higher than Spanish tissue samples. [27]

It is still unknown why U.S. levels are so much higher than in other industrialized nations, but part of the explanation is the kind of PBDEs favored by American manufacturers. North America uses the lion's share of all the various PBDE products — 44 percent of global Deca production by weight and 40 percent of Octa — but uses an estimated 95 percent of global Penta production. [2] The commercial Penta product is almost exclusively used in flexible polyurethane foam for home and office furniture, carpet padding, and mattresses. But only about 7.5 percent of the more than 2.1 billion pounds of foam produced in the United States each year contains penta-DBE. The majority of the Penta-laden foam is sold in California, where components of upholstered furniture are required to meet stringent fire retardancy standards. [31] Research shows that Penta is by far the most likely of the PBDEs to be absorbed by and build up in living organisms.

A separate but related concern is that PBDEs can form polybrominated dioxins and furans (PBDD/Fs) when heated or burned — in a municipal solid waste incinerator, for example. [32] Low levels of the very similar polychlorinated dioxins and furans are known to cause cancer, birth defects and chloro-acne. PBDD/Fs have recently been measured in human tissue samples and the environment in Japan. [32, 33]

Global Use of PBDEs in 2001
(in thousands of pounds)

Commerical PBDE Product	Americas	Europe	Asia	Other	Total	Percent used in the Americas
Deca	54,010	16,760	50,710	2,315	123,700	44%
Penta	15,650	331	331	221	16,530	95%
Octa	3,307	1,345	3,307	397	8,356	40%

Source: BSEF 2002 [93]

PBDEs in Bay Area Fish and People

Results of EWG fish sampling

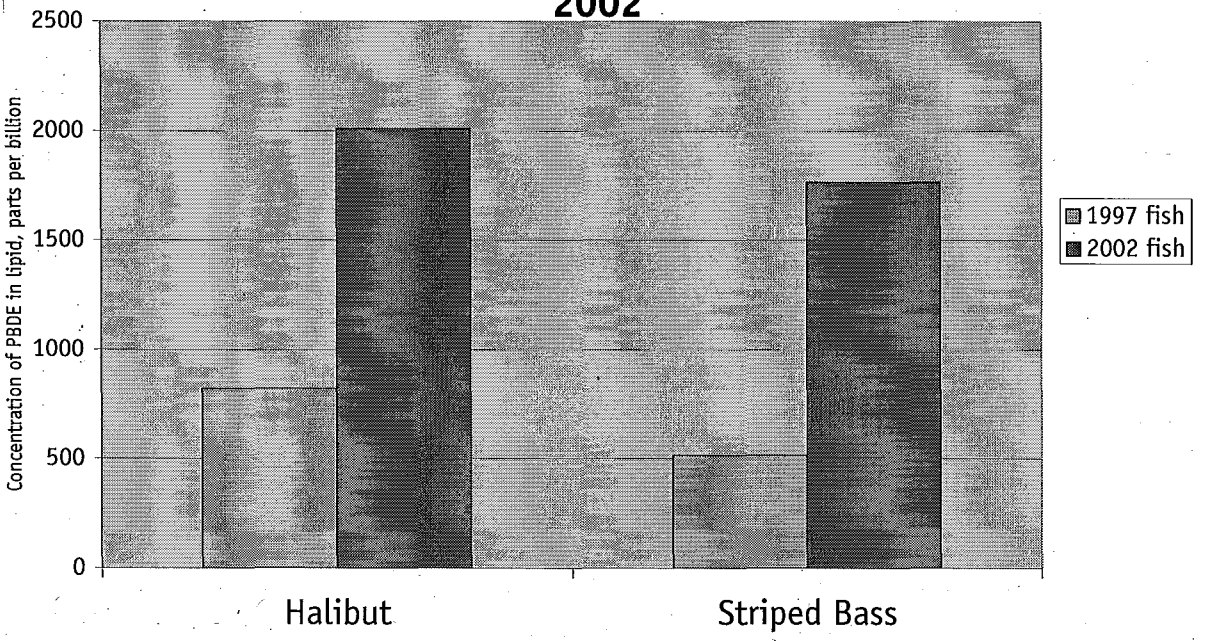
From September to November 2002, EWG researchers visited public piers and other fishing locations around greater San Francisco Bay. We asked anglers to donate fish caught that day they planned to eat. We collected 22 fish from six of the 10 most commonly caught and eaten species in the Bay: halibut, striped bass, white croaker (also called kingfish), walleye surfperch, jacksmelt and leopard shark. Halibut, bass and shark samples were collected from anglers on private or charter boats around South San Francisco or in San Pablo Bay. All other samples were donated by fishermen at public piers in San Francisco, South San Francisco, Alameda, Berkeley, Richmond and Point Pinole. EWG researchers prepared the samples as the species are typically eaten — skinning the shark, halibut and bass but leaving the skin on the croaker, surfperch and smelt. [34]

Samples of the same species caught in 1997 were obtained from the fish tissue archives of the Regional Monitoring Program For Trace Substances (RMP), part of the San Francisco Estuary Institute. The RMP collects and tests Bay fish every three years for PCBs, mercury, pesticides and other contaminants, and in future will include testing for PBDEs. The RMP samples were selected to include fish from the same general areas of the Bay as the fish EWG collected in 2002. Because the RMP did not collect walleye surfperch, we compared shiner surfperch from 1997 to the walleye surfperch we collected in 2002.

Both sets of samples were analyzed under contract by the Public Health Institute in collaboration with California Department of Toxic Substance Control's (DTSC's) Hazardous Materials Lab in Berkeley, where they were tested for 11 different classes of PBDEs by scientists recognized worldwide as pioneers in research on brominated fire retardants. Every sample analyzed by the lab was found to contain the seven most common PBDEs, and four other PBDE congeners were found in some fish. The samples contained levels of PBDEs ranging from 1 to 62 parts per billion (ppb) wet weight.

PBDE levels varied widely between fish species and between individuals of the same species. This variation may stem from difference in the fat content of the particular fish, their diet,

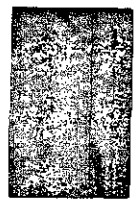
PBDE levels in striped bass and halibut 1997 and 2002



age, metabolism, location in the Bay and/or differences in the way they each species was prepared. While the fish samples compared fish collected from similar geographic areas, there are some differences in the locations represented in the 1997 and 2002 fish. RMP samples were typically collected from boats in the deeper reaches of the Bay, whereas our samples were donated by anglers, in most instances fishing in the shallower, and possibly more polluted, waters from public piers.

In general, white croaker and surfperch had the highest PBDE levels in fish fillets; leopard shark and jacksmelt had the lowest levels. Both the wide range of contaminant levels and the higher concentrations of chemicals in croaker and surfperch have been reported in previous studies of PCBs and persistent pesticides in the same species and locations of the Bay. [34] The single most contaminated fish was a white croaker collected at the Richmond Inner Harbor, a location known for high levels of pollution. This specimen had 62 ppb of PBDEs wet weight.

Comparing EWG's 2002 samples to the RMP's 1997 samples suggests that the concentrations of PBDEs in the fat of San Francisco Bay fish increased rapidly in striped bass and halibut over the past five years. PBDE levels in striped bass fat were



PBDE levels in 2002 San Francisco Bay Fish, ppb wet weight

Fish species	Average Level of PBDEs in fish tissue *	Lowest value	Highest value	Average level of PBDEs in fish fat*	Number of fish tested
Leopard Shark	1	1	NA	474	1
Jacksmelt	6	2	10	282	5
Halibut	13	2	28	2009	4
Striped Bass	17	11	21	1756	4
Walleye Surfperch	22	9	39	672	4
White Croaker	40	17	62	651	4
Overall average	16	1	62	974	22

Sum of the seven most common congeners (PBDE 28, 33, 47, 99, 100, 153, 154)

* NOTE The overall concentration of PBDEs in fish fillets is very different than the PBDE concentration in fish fat. We report the concentrations in fish fillets here because they are more relevant when considering potential human exposure. Concentrations in fish fat are a better way to track changes in contaminant levels over time, because the concentrations of fat in individual fish can vary widely.

3.4 times higher and in halibut fat were 2.4 times higher than samples collected in 1997. According to the California Department of Health Services, bass and halibut are the most commonly eaten Bay fish, and as larger, mobile, carnivorous fish at the top of the food chain, are key indicators of overall Bay contamination. Leopard shark fill a similar ecological niche on the top of the food chain, but our analysis included only one sample from 2002, which makes it less likely to detect a time trend if it were to exist. Less of an increase was observed in white croaker, a smaller, fattier fish that eats lower on the food chain. Surfperch and jacksmelt did not increase.

Based on our analysis, we calculate a doubling rate for PBDE concentrations of 2.8 years for bass and 3.9 years for halibut. The bass and halibut species were roughly the same length and had similar levels of fat when analyzed in 1997, which indicates that the differences we observed are not likely due to different age or fat contents of the fish. The detailed results of our analysis are presented in the Appendix.

PBDEs in Seals, Birds and Water from the Bay

The high level of PBDE contamination and rapid increase in PBDE levels in bass and halibut samples was no surprise. PBDEs have been documented in San Francisco Bay harbor seals, bird eggs, water and sediments. [27, 35, 36] Over the past 20 years, a rising tide of PBDEs has been detected by almost every study that looked at trends over time. In 2002, researchers at DTSC found that PBDE levels in San Francisco Bay Harbor Seals doubled every 1.8 years between 1989 and 1998. [27] Recently published data show a doubling time of 1.6 years in fish collected in the headwaters of the Columbia River in Washington state between 1992 and 2000. [37] PBDE levels in the fat of trout in Lake Ontario increased steadily between 1978 and 1998, by a factor of more than 300. [38] Similar increases in PBDEs over a 15-to-20 year period have been found in Arctic ringed seals and beluga whales. [18, 39]

The levels of PBDEs measured in Bay fish are much higher than those reported for a variety of commonly eaten fish in a variety of locations. These include wild and farmed salmon [40-42], commonly eaten fish in Japan, Sweden, Finland [43-45], similar species in the Great Lakes, Pacific Northwest and Bering Sea, and Washington state trout. [46-49] However, the levels detected in Bay fish are still lower than levels reported in studies of bottom feeding fish like carp collected in Virginia, and fish living downstream from a plastics and textile manufacturer in Sweden. Consumption of fish is thought to be a major route of PBDE exposure for adults. [31]

Comparison of PBDE levels in fish fat 1997 and 2002

Fish Species	Average levels in 1997 fish	Average levels in 2002 fish	Percent increase
Leopard Shark	438	474	8%
Jacksmelt	312	282	no increase
Halibut	821	2009	145%
Striped Bass	516	1764	240%
Surfperch	903	672	no increase
White Croaker	564	652	15%

Concentration of PBDE in lipid, parts per billion

The rapid buildup of PBDEs in the human body was first documented in 1999 by Swedish researchers, who examined archived breast milk samples collected over a 25-year span. They found a 60-fold increase in the concentrations of PBDEs in breast milk between 1972 and 1997 — equivalent to a doubling every 5 years. They noted that the increase was startling, given that levels of many persistent chemicals declined sharply in the same period. [50, 51] Later, Canadian researchers reported a 15-fold increase in PBDE levels in the breast milk of women in Vancouver, B.C. between 1992 and 2002 — a doubling every 2.6 years. [52]

The Swedish findings led to additional studies and the eventual ban of most PBDEs in the European Union, beginning in 2004. However, Europeans' exposure to PBDEs are likely much lower than Americans'. The few breast milk samples collected from U.S. women indicate even higher levels of PBDEs in the bodies of first-time mothers than found in Europe and Canada. [53] There are no historical archives of breast milk samples from the U.S., so it is not possible to track the trend of PBDE buildup in American women as meticulously as it has been documented in Sweden. But the available data are disturbing.

Levels in U.S./Bay Area Women Highest Worldwide

The average PBDE concentrations found in breast tissue, blood, and breast milk samples from studies of U.S. women are the highest yet reported in the world. Two recently published studies

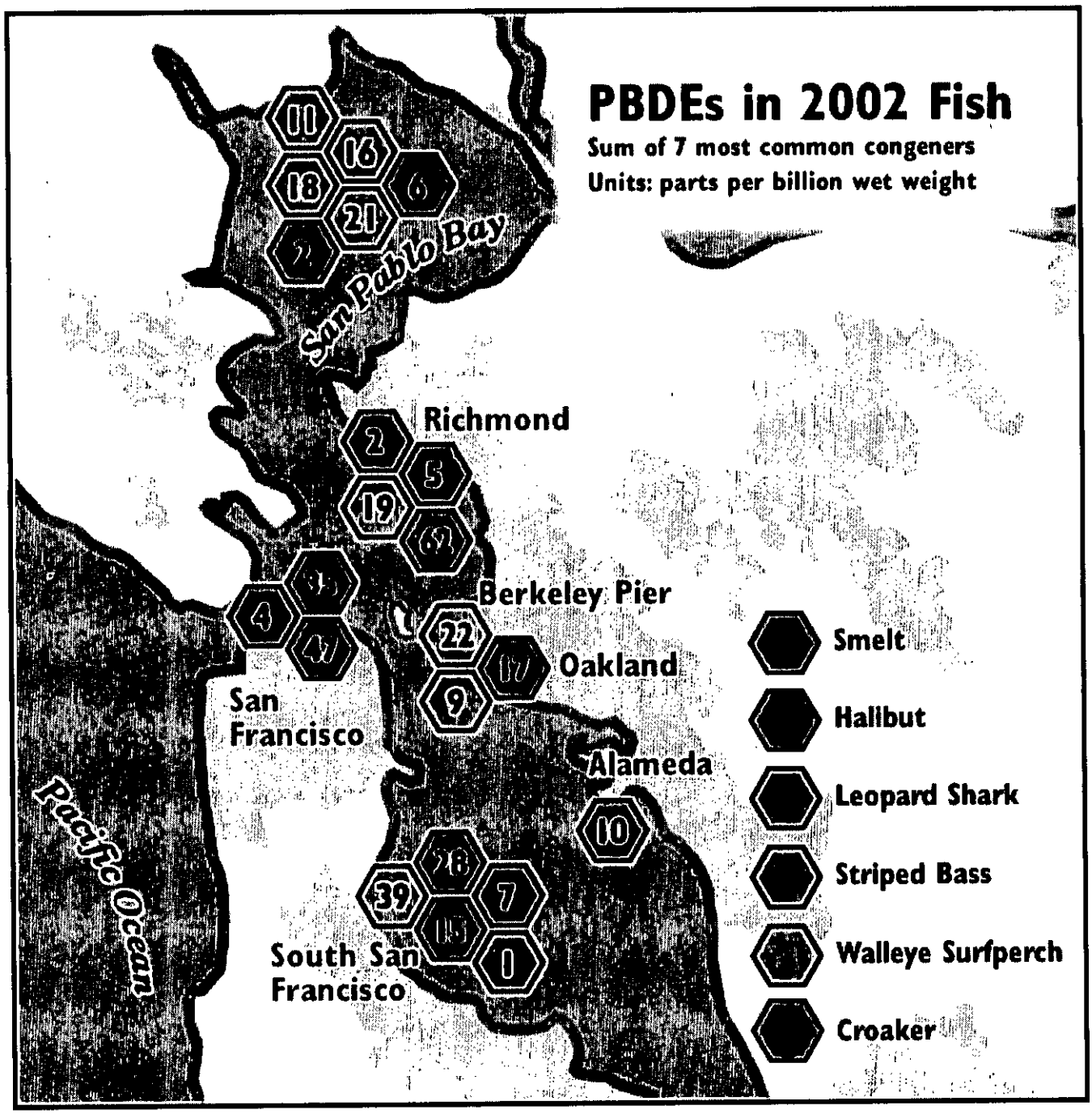
indicate that PBDE levels in Bay Area women have risen by at least a factor of 3 to 5 since PBDEs were first introduced in commercial products about 30 years ago. They also found levels of PBDEs three to five times higher than a study of German blood samples, and 12 to 30 times higher than women's breast milk in Japan, Sweden and Finland. [20, 43, 50, 54] About one in 12 Bay Area women in the two studies had more than 100 ppb of the most common PBDE in her body fat — more than 100 times higher than average levels found in Swedish women during this time period. [55]

A growing body of evidence shows a very low threshold for PBDE to cause permanent impacts to the development of the nervous system. It is hard to say whether PBDE concentrations have reached that point, but scientists are concerned that the margin between known contamination levels and levels that cause health effects in laboratory animals is low — and shrinking rapidly.

Levels of PBDE-47 in California women
parts per billion in lipid

Study	Year of sample collection	Average level of PBDEs	Range	Number of participants
Bay Area women's blood	1960s	Non-detectable	All below 10	42
Bay Area women's breast tissue	late 1990s	29	5 to 196	32
Bay Area women's blood	late 1990s	50	4 to 511	50

Source: Petreas 2003 [55]


PBDEs in 2002 Fish
Sum of 7 most common congeners
Units: parts per billion wet weight
San Pablo Bay
11
16
18
6
21
2
Richmond
2
5
19
62
Berkeley Pier
4
22
Oakland
17
9
San Francisco
Alameda
10
Pacific Ocean
28
39
7
15
0
South San Francisco
Smelt
Halibut
Leopard Shark
Striped Bass
Walleye Surfperch
Croaker

Sidebar

San Francisco Bay Fish: Who's At Risk?

The PBDEs found in EWG and DTSC's tests join a list of known toxins in San Francisco Bay Fish, including PCBs, mercury and dioxins. As a result, state health officials advise that most adults should eat no more than two meals a month of fish from the Bay. The exceptions, however, are crucial.

The Office of Environmental Health Hazard Assessment (OEHHA) says women who are pregnant, breastfeed or considering pregnancy, and children 5 and under, should eat no more than one meal of Bay fish a month. Additional warnings have been issued against eating certain fatty fish, older fish and fish from more severely polluted parts of the Bay.

To further reduce toxic exposure, the Department of Health Services (DHS) says people who eat Bay fish should:

- Eat younger, smaller and less fatty fish.
- Eat several species of fish from a variety of locations.

Fish Consumption Advisories for the San Francisco Bay

Fish Species/Location	All adults	Women who are pregnant, breastfeeding or considering pregnancy (within one year), and children ages 5 and younger
All species in the San Francisco Bay	No more than 2 meals per month	No more than 1 meal per month
Striped bass	No fish over 35 inches long	No fish over 27 inches long
Leopard shark	No advisory	No fish over 24 inches long
Croaker, surfperch, bullhead, gobies or shellfish	None of these species caught in the Richmond Harbor Channel	

OEHHA http://www.oehha.ca.gov/fish/general/sfbaydelta.html and http://www.oehha.ca.gov/fish/preg/index.html

- Skin and trim the fish to remove fat.
- Eat only the fillets, not organs or eggs.
- Cook the fish thoroughly to kill parasites.
- Bake, broil, grill or steam the fish to drain juices, which can reduce toxic levels by a third or more.

Despite official advisories, there is ample evidence that anglers are still risking their health or others' health by eating Bay fish.

In 1998-99, DHS interviewed 1,300 people fishing at more than 150 sites around the greater Bay. Eighty-five percent of the anglers said they eat their catch; the rest give the fish away away or release them). More than half of those surveyed said they share their catch with a woman of child-bearing age or a young child, who are most at risk from exposure. DHS estimated that the average person fishing in San Francisco Bay eats seven ounces of fish in a meal. Other studies by two nonprofit groups, the Asian Pacific Environmental Network and Save the Bay, say some people eat considerably more than that.

About six in 10 of the anglers surveyed by DHS said they had a general awareness that the state had issued fish advisories for the Bay, but only a third could name even one of the precautions to limit consumption of contaminated fish. Disturbingly, DHS found that the anglers' knowledge of the advisories correlated with their race and income: Poorer anglers and people of color were less likely to know about the government's advice to limit fish consumption.

The survey found a number of other indicators that people of color are more at risk of eating unhealthy levels of contaminated Bay fish. African-Americans and Asian-Americans were more likely to exceed the advisory level of two meals a month. African-Americans and Asian-Americans, plus Latinos, also were the most common consumers of croaker, the species that had the highest level of PBDEs, and which other studies have found to contain the highest levels of PCBs and pesticides.Since people of color in the Bay Area are more likely to live in neighborhoods near toxic pollution, anglers in those communities are also more likely to fish in the more highly contaminated parts of the Bay.

Section Reference:

California Department of Health and Human Services (DHS), Environmental Health Investigations Branch and San Francisco Estuary Institute. 2001. San Francisco Bay Seafood Consumption Study. March, 2001. http://www.sfei.org/rmp/sfcindex.htm

Health Risks of PBDEs

A growing body of research in laboratory animals has linked PBDE exposure to an array of adverse health effects including thyroid hormone disruption, permanent learning and memory impairment, behavioral changes, hearing deficits, delayed puberty onset, fetal malformations and possibly cancer. Research also shows that exposure to brominated flame retardants in utero or infancy leads to much more significant harm than adult exposure, and at much lower levels. Many questions still remain, but almost every month brings new evidence that PBDEs pose a significant health risk to developing animals and are very likely to pose a significant health risk to fetuses, infants and children.

These findings echo what researchers have learned about the structurally similar, but much better known, PCBs. Used primarily as electrical insulators, PCBs were found to be rapidly building up in people and animals before they were banned in 1977. Although levels are now declining, PCBs persist in the environment and cause a number of well-documented health problems. Recent studies have shown that PBDEs can act in concert with PCBs and other chemicals through similar mechanisms to increase their effects. [8, 9, 56]

Most unsettling, comparison of PBDE concentrations in the bodies of American women with the levels shown to harm the health of laboratory animals, the margin of safety is slim or may already be eclipsed. If PBDE levels in people continue to rise at anywhere near current rates, any remaining gap will likely be closed within a few years.

Many of the known health effects of PBDEs are thought to stem from their ability to disrupt the body's thyroid hormone balance, by depressing levels of the T3 and T4 hormones important to metabolism. In adults, hypothyroidism can cause fatigue, depression, anxiety, unexplained weight gain, hair loss and low libido. This can lead to more serious problems if left untreated, but the consequences of depressed thyroid hormone levels on developing fetuses and infants can be devastating. [57] One study, for instance, found that women whose levels of T4 measured in the lowest 10 percent of the population during the first trimester of pregnancy were more than 2.5 times as likely to have a child with an IQ of less than 85 (in the lowest 20

percent of the range of IQs) and five times as likely to have a child with an IQ of less than 70, meeting the diagnosis of "mild retardation." [58]

Even short-term exposures to commercial PBDE mixes or individual congeners can alter thyroid hormone levels in animals, and the effects are more profound in fetuses and offspring than in adults. [59-64] These results aren't surprising, but are ominous as data in humans indicate that pregnancy itself stresses the thyroid, and developing fetuses and infants don't have the thyroid hormone reserves adults do to help buffer insults to the system. [65]

Most studies on thyroid hormone disruption by PBDEs have been very short — with exposures of 14 days or less. The real question is how low doses over the long term affect the body's thyroid hormone balance. The answer is important, because the entire U.S. population is exposed daily to low levels of PBDEs, and studies of other thyroid hormone disrupters have found that long-term exposures can cause more serious harm at lower levels of exposure. [66] Although no direct link could be made, one study found higher rates of hypothyroidism among workers exposed to brominated flame retardants on the job. [67]

Because the developing brain is known to be extremely sensitive to exposure to toxins, researchers have begun to examine whether short-term exposures to PBDEs at critical times could have long-term effects. The results are troubling: Doses administered to fetal or newborn mice and rats caused deficits in learning, memory and hearing, changes in behavior, and delays in sensory-motor development. Many of these effects were found to worsen with age and the effects were seen with the higher-weight PBDEs (the usually less harmful deca-BDE) as well as the more readily absorbed lower-weight congeners.

Just One Dose May Be Harmful

Experiments have shown that just one dose of PBDEs at a critical point in brain development can cause lasting harm. [68-70] In two different studies a small dose — as little as 0.8 milligrams per kilogram of bodyweight per day (mg/kg-day) — given to 10-day-old mice caused "deranged spontaneous behavior," significant deficits in learning and memory and reduced ability to adapt to new environments, with these problems often becoming more pronounced with age. [69, 70] This research also demonstrated the heightened sensitivity of the brain at certain critical phases of development and the importance of timing: While earlier exposures caused "significantly impaired spontaneous motor behavior" and "persistent neurotoxic effects," no effects were seen in mice that were exposed later on during development,

despite having similar levels of PBDEs (or their metabolites) in the brain. [69]

Other animal studies have shown that early exposure to PBDEs, often at relatively low levels, can lead to delays in sensory-motor development, hearing deficits, as well as changes in activity levels and fear responses. [68, 71, 72] At this point, scientists do not understand exactly how PBDEs affect neurological development. But there is evidence that PBDEs and/or their metabolites are in fact acting through several different mechanisms, including mimicking thyroid hormones, increasing their rate of clearance in the body, and interfering with intracellular communication. [73]

In addition to their effects on thyroid hormones and neurological development, PBDEs have been linked to a gamut of other health impacts, from subtle to dramatic. For example, two new studies found that early exposure to PBDEs delayed the onset of puberty in male and female rats and decreased the weight of male reproductive organs. [74, 75] In studies of pregnant animals, PBDE exposure was associated with retarded weight gain, enlarged livers and raised serum cholesterol. [76, 77] In utero exposures have also been associated with serious harm to the fetus, including limb and ureter malformation, enlarged hearts, bent ribs, fused stemebrae, delayed bone hardening, and lower weight gain. [76-79] The malformations of the fetus were consistently seen at levels much lower than doses harmful to the mothers — the lowest being 2 and 5 mg/kg-day, respectively.

The few studies that have looked at changes in organ structure have found that semi-chronic PBDE exposure can cause thyroid hyperplasia and enlarged livers at relatively low doses (10 mg/kg-day) and other adverse effects such as hyaline degeneration, focal necrosis and deformation in the kidney, hyperplastic nodules in the liver, decreased hemoglobin and red blood cell counts at higher doses. [76, 78, 80]

Only one PBDE congener has been tested for causing cancer, in a single study more than 15 years ago. High doses of deca-BDE given to rats and mice caused liver, thyroid and pancreas tumors. [80] Deca-BDE is the least easily absorbed and the most rapidly eliminated of the PBDEs, and recent research indicates that other congeners can cause genetic recombination in cells, a sign of likely carcinogenicity. [81] As a result, scientists believe that the congeners with fewer bromines are likely to be more carcinogenic than deca-BDE and have urged that such tests be conducted. [73]

Unanswered Questions

There are many unanswered questions about the health effects of PBDEs. For example, it is unclear to what extent PBDEs are metabolized by the body and what health effects these breakdown products might have. Because the composition of many commercial mixes hasn't been well characterized, there may be harmful congeners or other chemical contaminants that scientists aren't even looking at. [19] And there remains the question of the health effects of polybrominated dioxins and furans, formed when PBDEs are heated or burned.

One of the major debates centers on the health effects of the various PBDE congeners. Scientists have found that PBDEs with fewer bromines (including penta-BDE) are almost totally absorbed by the body, slowly eliminated, highly bioaccumulative, and cause health effects at relatively low levels. In contrast, PBDEs with more bromines (including octa- and deca-BDE) are less readily absorbed, less bioaccumulative, more quickly eliminated by the body, and generally cause health effects at higher doses. [73] But new research suggests that deca-BDE may be more toxic than previously thought. [82] And maybe even more importantly other recent research shows that when exposed to sunlight, the higher-weight, less harmful congeners can be chemically converted to the more bioaccumulative, better absorbed and more toxic lower-weight congeners. [83-86]

Although there are significant differences between how the environment and organisms deal with the various PBDE congeners, the bottom line is that all have the potential to cause serious environmental and health problems — some alone, some through their breakdown products, others by interacting with other toxic chemicals. The chemical industry, trying to save a highly profitable product, is pushing the notion that certain PBDEs are harmless. The evidence already available argues the opposite: to prevent a bad situation from getting worse, all PBDEs should be banned now.

Scientists have really only begun to examine the potential health effects of PBDEs, but as more studies are conducted, the threshold for health effects continues to be set lower and lower, similar to the regulatory trend with lead, mercury and PCBs. One of the lowest doses of PBDEs found so far to harm lab animals was a 2002 study of newborn mice showing neurodevelopmental damage at concentrations of 4 ppb in brain tissue. [69] Many women in the two recent California studies had PBDE levels above this level in her body fat. Scientists at the California Environmental Protection Agency's Office of Environmental Health Hazard Assessment used the state data, plus studies from Indiana and

Texas, to project PBDE levels in the entire U.S. population. If the data used in the model are representative, as many as 15 million Americans have a PBDE body burden of more than 400 ppb — a hundred times the concentration known to cause permanent effects in laboratory animals. [9]

The highest concentrations of PBDEs measured to date in U.S. breast milk are only slightly lower than level of the PBDE cousin, PCBs, shown to cause significant irreparable harm (lower IQ and memory and attention deficits) to a developing baby. [87] Researchers estimate that if the increase in PBDE levels in our bodies continues at this rate, U.S. women will exceed these levels within 6 to 12 years. The costs of this damage will be an increased need for special education, decreased lifetime earnings for affected children, and treatment for the learning, developmental and behavioral disabilities that already affect nearly 12 million U.S. children. [88]

Regulatory Failure

The evidence against PBDEs was strong enough that bans were proposed in Germany, Sweden and the Netherlands in the mid-1980s and early 1990s. Industrial users of the chemicals agreed to voluntarily phase them out in Germany in 1986, with the manufacturers and users in the other two countries later following suit. In 1993 Germany placed official restrictions on PBDE use because of their tendency to release dioxins when burned under its Dioxin Ordinance. [85] As concern spread to other countries, the European Union launched a scientific review of the safety of PBDEs, originally with respect to electronics waste. In February 2003, the EU announced a ban on two common PBDEs (Penta and Octa) in all products as of August, 2004. [89] The EU is also considering a ban on Deca for use in electronic products by July 2006. Pending the completion of further studies, the EU Chemicals Inspectorate will decide whether to ban on Deca in other non-electronic products as well as of 2006. [90]

Even before the ban takes effect, the early efforts to reduce PBDE use in Europe are paying off. Researchers have found that PBDE levels in Swedish breast milk rose exponentially from 1972 to 1997, but since that year have begun to decline: PBDE levels in Swedish women dropped about 30 percent between 1997 and 2001. [91] These results are encouraging. This shows that if regulations are enacted and PBDE use ceases or declines, the human body burden of PBDEs will also decrease after a lag-time of several years or more.

Despite that fact that PBDE concentrations in Americans and their environment are at least ten times higher than those found in Europe, the U.S. government has so far done nothing to counter this rapidly escalating problem. PBDEs are virtually unregulated for use in commercial products. In 1994, EPA determined that the waste stream from the production of Octa and Deca "should not be listed as hazardous." [92] The only other regulation governing PBDEs is the requirement that companies manufacture or use large amounts of Deca report their chemical emissions under the Toxics Release Inventory.

California Bill a First Step

State legislation was introduced in California this year to ban the use of several types of PBDEs by 2008. While this bill, AB 302 by

Assemblywoman Wilma Chan of Oakland, is a welcome first step, it lacks some key provisions to assure that these chemicals are removed from our homes and our bodies as quickly as possible:

- AB 302 exempts the most widely used PBDE product (Deca), which is common in electronics produced in the United States, but the European Union has been considering banning Deca in electronic products by 2006. This is troublesome, as numerous studies have shown that the types of PBDEs in this commercial product can break down into other congeners that are much more bioaccummulative and bioreactive (and are included in the proposed California legislation).

- As passed by the Assembly, the bill gives PBDE producers and users until 2008 to stop using the chemicals. But if PBDE use continues at its current rate for five years, another 365 million pounds of PBDEs will be put into American couches, easy chairs, cars, planes, buses and other consumer products. [93]

- The bill also doesn't require manufacturers to label PBDE-containing products. As it is now, it would remain impossible to know whether the couch or computer you buy contains PBDEs. Labeling would have allowed consumers to make more informed decisions, providing extra incentive to manufacturers and users to speed their conversion to new fire retardants, materials, or design.

Not surprisingly, the European and California drives to regulate PBDEs have met fierce opposition from manufacturers and users. In 1997 Great Lakes, Albemarle, Dead Sea Bromine and other companies formed the Bromine Science and Environmental Forum (BSEF). Ostensibly dedicated to providing "extensive scientific information on bromine and bromine products" and facilitating "open communication about bromine products across the globe," the Forum is in fact a lobbying front dedicated to casting doubt on the mounting evidence against brominated chemicals. [94]

For example, BSEF denies that the burning of bromine-contaminated waste increases the formation of dioxins and furans, though numerous studies show otherwise. The group also tries to downplay the environmental and public health threats of PBDEs, claiming the chemicals are only used "in controlled applications where emissions to the environment are highly unlikely." [94] Yet

volumes of evidence show that PBDEs are not only escaping into the environment, but that they have become a ubiquitous global pollutant.

Chemical manufacturers been vocal opponents against the California legislation. Great Lakes Chemical, which says it spends $2 million a year to lobby against BFR regulation, pushed for a longer phase-out date and the exemption for Deca. [95]

Affordable Replacements

For most uses of PBDEs there are already chemical replacements on the marketplace at equivalent or slightly higher cost. Aluminum trihydroxide and various phosphorous-based compounds are some of the most common alternatives. But rather than replacing one chemical with one that may turn out to be even more toxic, the answer is to redesign products so that chemical flame retardants are not needed to meet fire safety regulations. The U.S. Consumer Product Safety Commission recently reported: "CPSC laboratory tests have demonstrated that the properties of actual filling materials have little or no effect on the small open flame ignition resistance of full-scale chairs" — in other words, the use of flame retardants in foam does little to improve upon the fire safety of foam furniture. [96] For other products, simply increasing the density of polyurethane foam can eliminate the need for chemical flame retardants. This can also be achieved using manufacturing materials that are naturally less flammable. [97]

Some U.S. companies have begun to phase out PBDEs, even without a regulatory mandate. Computer and electronics companies such as Apple, Ericsson, IBM, Intel, Motorola, Panasonic, Phillips and Sony are already producing some PBDE-free products, and some have committed to completely phasing out PBDEs and other brominated flame retardants. [97] The furniture giant IKEA has phased out BFRs in all its products by changing product design, using naturally less-flammable materials, and employing alternative flame retardants if needed. Hickory Springs of Conover, N.C., a major polyurethane foam producer, is working with Akzo Nobel, a chemical manufacturer, to test a non-halogenated phosphorous-based flame retardant. Hickory Springs says it was motivated by requests from companies such as IKEA, Crate & Barrel and Eddie Bauer to stop using PBDEs. [98]

Unfortunately, data on the toxicity of the alternative fire retardants already in use or under development is scarce. This is largely because of well-documented shortcomings of the nation's toxics laws. The chief regulatory statute for commercial chemicals,

the Toxic Substances Control Act (TSCA), is infamous for the lack of authority it provides the Environmental Protection Agency. [99] The looming PBDE crisis is another disturbing illustration of the failures of a regulatory system that allows persistent, bioaccumulative toxins on the marketplace before they have been adequately tested for safety.

Under the current system, the EPA reviews new chemicals through a process that does not require health and safety test data and that discourages voluntary testing. Companies submit only basic toxicity data for fewer than half of all applications for new chemicals, and the government approves 80 percent of these with no restrictions and no requests for tests. Eight of 10 new chemicals win approval in less than three weeks, at an average rate of seven a day. [99]

No Safety Studies on Many Toxic Chemicals

Worse, when TSCA was enacted in 1976, more than 63,000 chemicals already in use were "grandfathered" — granted blanket approval for continued use in consumer and industrial products. In 1998, the EPA and the nonprofit Environmental Defense Fund reviewed all of the toxicity and environmental fate studies publicly available and found no information — not a single test — for 43 percent of the 2,600 chemicals produced in the highest volumes in the U.S. [99, 100]

The chemical industry has since agreed to do more tests to assess potential toxicity to children for a select number of the most widely use chemicals under the Voluntary Children's Chemical Exposure Program (VCCEP). The three most widely used PBDEs were included in the first group of 23 chemicals to be assessed as part of this program, but the usefulness of the VCCEP program is highly limited. Its purpose is to make "health effects, exposure, and risk information" of these chemicals available and provide "the means to understand the potential health risks to children." [101] But because the program is voluntary, chemical manufacturers are unlikely to hand over any information that might be damning for their chemical products, nor do they have much incentive to fill any significant scientific data gaps that are identified in the process.

There is no question that fire safety is important and that making products fire-resistant can save lives. Chemical flame retardants have become ubiquitous over the last few decades, but a wide variety of fire safety strategies exist. Using less-flammable materials or changing the product design so that it is inherently

more fire resistant are chemical-free solutions. Using less toxic chemicals as flame retardants is another option. We do not have to expose ourselves to toxins to protect ourselves from fire.

EWG recommends:

- The EPA must ban all PBDEs as quickly as possible — no later than 2006.

- In the interim, all products containing PBDEs must be labeled so that consumers have the option of choosing products without them.

- All potential replacement fire retardants must be adequately tested to ensure that they are neither persistent, nor bioaccumulative, nor toxic. Changes in product design that decrease the need for chemical fire retardants should be encouraged over simply switching to a different chemical.

- A nationwide biomonitoring program is needed to identify chemicals that are accumulating in our bodies and in the environment, and determine whether levels are increasing or decreasing.

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Appendix

Appendix: Summary data for 1997 and 2002 fish

Fish samples Fish species	Year	Number in sample	Fish length (cm)	Reported moisture	Percent lipid, wet weight
walleye surfperch	2002	4	19 (16-21)	72 (71-85)	3.2 (2.4-4.0)
shiner surfperch	1997	40	12 (11-15)	76 (71-78)	2.5 (1.7-3.9)
halibut	2002	4	72 (69-76)	76 (75-86)	0.5 (0.2-0.9)
	1997	3	76 (59-92)	75 (73-77)	0.3 (0.2-0.4)
jacksmelt	2002	5	35 (33-37)	72 (67-75)	3.2 (0.5-7.4)
	1997	15	26 (21-29)	74 (71-76)	2.1 (1.4-3.2)
white croaker	2002	4	30 (28-31)	72 (70-74)	6.3 (5.2-7.4)
	1997	10	26 (22-29)	71 (70-73)	6.7 (5.2-7.4)
striped bass	2002	4	59 (55-69)	77 (75-78)	1.1 (0.6-2.1)
	1997	6	58 (50-66)	76 (75-77)	0.9 (0.8-1.0)
leopard shark	2002	1	114	79	0.3
	1997	12	96 (92-102)	76 (75-78)	0.25 (0.18-0.34)

Wet weight values, parts per billion

Fish species	Year	tri-BDE (PBDE 28+33)	tetra-BDE (PBDE-47)	penta-BDE (PBDE-99+100)	hexa-BDE (PBDE-153+154)	sum of 7 congeners
walleye surfperch	2002	0.5	17.2	4.3	0.2	22.2
halibut	2002	0.2	8.4	3.2	0.8	12.6
jacksmelt	2002	0.2	2.3	2.5	0.5	5.5
white croaker	2002	0.9	27.4	10.1	1.7	40.1
striped bass	2002	0.3	10.3	4.5	1.4	16.6
leopard shark	2002	0.1	0.9	0.3	0.1	1.4

Lipid adjusted values, parts per billion

Fish species	Year	tri-BDE (PBDE 28+33)	tetra-BDE (PBDE-47)	penta-BDE (PBDE-99+100)	hexa-BDE (PBDE-153+154)	sum of 7 congeners
walleye surfperch	2002	15	508	133	16	672
shiner surfperch	1997	27	669	185	23	903
halibut	2002	24	1286	548	151	2009
	1997	24	641	154	2	821
jacksmelt	2002	11	122	121	29	282
	1997	15	158	124	15	312
white croaker	2002	13	444	166	28	652
	1997	14	395	131	25	564
striped bass	2002	30	1021	526	179	1756
	1997	24	349	118	26	516
leopard shark	2002	26	310	110	28	474
	1997	70	300	60	8	438

Levels of Polybrominated Diphenyl Ether (PBDE) Flame Retardants in Animals Representing Different Trophic Levels of the North Sea Food Web Environmental Science and Technology 1oct02

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The levels of individual PBDE congeners were investigated in the invertebrate species whelk *(Buccinum undatum)*, seastar *(Asterias rubens)*, and hermit crab *(Pagurus bernhardus)*, the gadoid fish species whiting *(Merlangius merlangus)* and cod *(Gadus morhua)*, and the marine mammal species harbor seal *(Phoca vitulina)* and harbor porpoise *(Phocoena phocoena)*. These species are all important representatives of different trophic levels of the North Sea food web. All six major PBDE congeners detected (BDEs 28, 47, 99, 100, 153, and 154) are most prevalent in the commercial Penta-BDE formulation. There is no evidence for the occurrence of the Octa-BDE formulation in the North Sea food web, since its dominant congener, BDE183, was never detected. BDE209, the main congener (>97%) in the Deca-BDE formulation, was detected only in a minority of the samples and always in concentrations around the limit of detection. Since BDE209 is often the major BDE congener in sediments from the

area, the main reason for its low concentrations in biota from the North Sea seems to be a relatively low bioaccumulation potential. This can either be due to a low uptake rate of the very large molecule or a relatively rapid excretion after biotransformation. Since all invertebrates investigated are sentinel species, they are highly representative for the area of capture. The highest lipid-normalized concentrations of PBDEs in the invertebrates occurred near the mouth of the river Tees at the East coast of the UK. The geographical distribution of the PBDEs can be explained by the residual currents in the area. The direction of these currents differs between the summer and the winter season as a result of the presence or absence of vertical summer stratification of the deeper waters north of the Dogger Bank. Summer stratification results in the development of a density-driven bottom water current formed after the onset of vertical stratification of the water column in May leaving the UK coast near Flamborough Head toward the Dogger Bank. In winter, the residual currents run in a more southerly direction and follow the UK coastline. The distribution pattern of the PCBs and p,p'-DDE in the invertebrates was entirely different from that of the PBDEs, which could be expected, since the use of these organochlorines in western Europe peaked in the 1960s and 1970s but has been forbidden more than two decades ago, whereas the production and use of the penta-BDE formulation is of a more recent origin. The higher trophic levels of the North Sea food web were represented by the predatory gadoid fish species whiting and cod and the marine mammal species harbor seal and harbor porpoise. The lipid-normalized levels of the six major PBDE congeners in fish were similar to the levels in the invertebrates, but a biomagnification step in concentrations of generally more than an order of magnitude occurred from gadoid fish to marine mammals. Based on the limited number of samples, no differences could be observed between harbor seal and harbor porpoise. In summary, the results in three species of sentinel invertebrates from a network of stations covering a major part of the North Sea basin showed that the estuary of the river Tees at the UK East coast is a major source for tri- to hexa-PBDEs. Throughout the food-chain, the most marked increase in (lipid-normalized) levels of all six PBDE congeners occurred from predatory (gadoid) fish to marine mammals, agreeing with the transition from gill-breathing to lung-breathing animals. This has serious consequences for the route of elimination of POPs, since their elimination from the blood into the ambient seawater via the gill-membrane is no longer possible.

Introduction

Brominated flame retardants are added to many plastics and printed circuit boards of electronic household equipment and textile and polyurethane foam in furniture and cars for obvious safety reasons. Among the brominated flame retardants (BFRs), there are three commercial formulations that contain the diphenyl ether skeleton. In order of increasing overall bromination, these formulations are named Penta-BDE, Octa-BDE, and Deca-BDE. The global market demands in 1999 were 54 800 metric tons for the Deca-BDE, 8500 tons for the Penta-BDE, and 3825 tons for Octa-BDE (ref: www.bsef.com).

PBDEs are very hydrophobic (log K_{ow} values 4-10) and resistant to degradation. The water solubility and vapor pressure of PBDEs decrease with increasing degree of bromination, whereas hydrophobicity increases (1-3). Two major congeners of Penta-BDE, i.e., BDEs 47 and 99 (numbering is according to the IUPAC nomenclature for PCBs), showed even higher bioaccumulation factors than PCBs of similar hydrophobicity, despite a larger molecular size of the brominated compounds (4). The presence of these congeners and BDE100 in tissues of the deep-sea foraging sperm whales indicates that these PBDEs have become globally dispersed chemicals (5). The temporal trends of the major congeners of the penta-BDE formulation still showed a sharp increase between 1978 and 1998 in lake trout *(Salvelinus namaycush)* from the Great Lakes (6). The levels in Beluga whale *(Delphinapterus leucas)* from the St. Lawrence estuary collected between 1997 and 1999 were on average 20 times higher than the levels in animals collected a decade earlier (7), irrespective of sex. Mixed temporal trends were observed in sediments of several UK rivers entering the North Sea or the Irish Sea (8). In the Rhine-Meuse delta, the levels in yellow eel *(Anguilla anguilla)* from the "Haringvliet" decreased since 1984, and those in eel from the German/ Dutch river Roer, a tributary of the Meuse, since 1993 (8). In Japan, the levels in sea bass and grey mullet from Osaka bay have been rapidly decreasing since 1990 (9). Less is known about the situation for the other two technical PBDE formulations, i.e. the Octa- and the Deca-BDE formulation. Octa-BDE was reported to be absent in freshwater fish from Virginia (10). BDE209 constitutes > 95% of the commercial deca-BDE formulation. The only occasion where this congener and BDE 183 have been reported in relatively high concentrations in wildlife concerns eggs of pererine falcons breeding in Sweden (11). The peregrine falcon *(Falco peregrinus)* belongs to the top-predators of the terrestrial food-web, since this bird of prey feeds mainly on other birds. The uptake of BDEs 183 and 209 in blood of humans working in a recycling factory of electronic household equipment has also been demonstrated (12). Thus, the assumption that BDE209 cannot bioaccumulate due to its large molecular size is not always correct. This is an important observation, since the concentrations of BDE209 are

often much higher than those of the other PBDE congeners in suspended particulate matter and sediments of several continental European, UK, and Irish rivers (8).

Although data on PBDE levels in the estuaries of several rivers entering the North Sea have been published (8), a survey of a number of PBDE congeners representative for the different commercial formulations in a number of animal species representing different trophic levels of the North Sea food web has not yet been carried out. For this purpose, the invertebrate species seastar *(Asterias rubens),* common whelk *(Buccinum undatum),* and hermit crab *(Pagurus bernhardus)* and the predatory gadoid fish species whiting *(Meralangius merlangus)* and cod *((Gadus morhua)* were sampled between 52 and 58° N and 1°W-10°E, covering a large part of the North Sea basin and the Skagerrak, the connection between the North Sea and the Baltic Sea. The highest trophic levels were represented by harbor seals *(Phoca vitulina)* from the German Wadden Sea and harbor porpoises *(Phocoena phocoena)* stranded or by-caught along the Dutch coast. Since the three invertebrate species are sentinel, the geographical distribution of their PBDE levels can give an impression of the location of important input sources into the North Sea basin. For comparison with the PBDEs, the levels of the major PCB congeners CB101, 138/158, and 153 and p,p'-DDE were also analyzed.

Experimental Section

Sampling of Invertebrates and Fish. The majority of the samples were taken with a 5 m beam trawl with a mesh size of 3.5 cm n during cruise 64PE144 with the RV *Pelagia* in August-September 1999. The basic data on the position, sampling date, water depth, and temperature, and salinity at the surface and just above the sea-bottom, are given as Supporting Information in Table SI-1. Large differences in temperature and/or salinity between surface and bottom water are indicative for the occurrence of vertical stratification. The presence of a thermocline or a halocline severely inhibits the exchange rates between the sea surface and the sea bottom. The herring samples were caught with a pelagic net at 51°34' N and 2°47' E in the Southern Bight by the commercial fishing vessel TX 37 in October 2000.

Because it is very hard to obtain a good homogenate from entire animals for analysis of POPs, a number of tissues were selected. The following tissues were excised on board immediately after capture and frozen at -20 °C until further analysis: for the sea star the *pyloric caeca* (part of the intestinal system), for the hermit crab the soft abdomen; for the whelk the whole (soft) body; and for fish the liver and filet from one side of the backbone. No differentiation was made between the sexes except for the whelk. In this case only the

males were taken because the females were used for research on levels and effects of organotin compounds. Samples of invertebrates and fish each contained material from five individual animals. From each station, two such samples were analyzed.

Samples of Marine Mammals. All samples of marine mammals came from beach-stranded animals or animals drowned in fishing nets. The samples of harbor porpoise originated from the southern North Sea and were obtained from Dr. Chris Smeenk and Drs. Marjan Addink of the Museum of Natural History "Naturalis" in Leiden, The Netherlands. The samples of harbor seals were obtained from Dr. Ursula Siebert of the Centre for Research and Technology in Büsum, Germany, and originated from Wadden Sea of Schleswig-Holstein (Germany). For cetaceans and harbor seals, samples of liver and blubber of individual animals were stored for analysis.

Extraction Method for the Determination of POPs. Tissue amounts corresponding to approximately 50 mg of lipid were extracted, using an ultra-Turrax method. After extraction with pentane and acetone, the sample was treated with sulfuric acid, and a silica cleanup was performed. The method has been described in more detail elsewhere as the "NIOZ method" (13).

Analysis of PBDEs. The levels of 15 individual PBDEs (Cambridge Isotope Laboratories, Inc. Andover, MA) were determined by GC/MS. The GC was a Hewlett-Packard 6890; the mass-selective detector a Hewlett-Packard 5973. GC specifications: split-splitless injection, split valve closed for 1.5 min. $T_{injector}$ 270 °C. Column: stationary phase CP Sil-8, 25 m * 0.25 mm * 0.25 μm (Chrompack, NL). Carrier gas He; linear gas velocity 74 cm n s^{-1}, constant flow programmed. Oven temperature program: 90 °C (1.5')/20 °C min^{-1}/190 °C (0')/4.5 °C min^{-1}/270 °C ((5')/10 °C min^{-1}/320 °C ((10'). MSD specifications: negative chemical ionization (NCI) in the SIM mode at the *m/z* ratios of both bromine isotopes (79 and 81) and *m/z)* 487 (for BDE 209 only). Ionization gas CH_4. $T_{ion\ source}$ 210 °C; $T_{transferline}$ 320 °C; $T_{quadrupole}$ 160 °C.

The limit of detection (LOD), defined as a signal of three times the noise level, was established at 0.6 ng g-1 lipid for all BDE congeners except BDE209, when an amount of wet tissue containing 50 mg of lipid was extracted. Other amounts of lipid affect this LOD value proportionally. The LOD for BDE209 was 5 ng g-1 lipid.

Chromatographic Conditions for the Analysis of PCBs and p,p'-DDE. The levels of individual CBs and p,p'-DDE were determined by gas chromatography on a Carlo Erba 5300 (Italy) with electron capture detection (ECD). The specifications of the GC were as follows: 1

μL split-splitless injection, split valve closed for 4 min, $_{Tinjector}$) 300 °C. Column: stationary phase CP Sil-8, 50 m * 0.25 mm * 0.25 μm (Chrompack, NL). Carrier gas H2; constant pressure. Oven temperature program: :90 °C ((2')/10 °C min^{-1}/215 °C ((10')/8 °C min^{-1}/275 ° C ((17'). . The ECD was 300 °C and 90 mL N2 min^{-1} was used as make up gas.

Quality Assurance. Before sample treatment, the internal standards CB112 and decabromobiphenyl (BB209) were added. Although BB209 has been produced in France until recently, no indication of its occurrence has been found in the present set of samples, which were run simultaneously without the addition of this internal standard. Moreover, PBBs were also not detected in any sample from a large set from Dutch coastal waters either (14). Our participation in the first interlaboratory study, which has been organized for PBDEs, showed a good performance for the samples of biota and sediment. Until now, no certified reference materials are available for the determination of PBDEs.

Extraction Method for the Determination of Tissue Lipid Contents. The levels of all POPs have been expressed on the basis of extractable lipids extracted from the same tissue as used for the POP analyses, since the POPs of interests are all very hydrophobic and thus they prefer to accumulate in fatty tissues **(15, 16).** However, the extraction method used for the determination of the different POPs, extracts especially the polar phospholipids incompletely. Therefore a separate extraction of the tissues was performed for the determination its total lipid content, using a dichloromethane/methanol/water solvent system (17). Especially in lean tissues, this method produced substantially higher values than the acetone/pentane/water solvent system used for the extraction of the POPs. For comparison, a table with data of the lipid *contents* as obtained with both methods is given as Supporting Information in Table SI-2.

Multivariate Statistical Analysis. The data of the six major PBDEs, three major PCBs, and p,p'-DDE were subjected to Principal Component Analysis (PCA) to find the underlying pattern and correlation structure of the data set. PCA was performed on the correlation matrix of the lipid-based concentrations. The data were 10log-transformed prior to the calculations to obtain a greater homogeneity of variance. The results have been graphically displayed in the form of a principal components bi-plot. From such figures, several things can be read.

A: The Position of Each Sample in the Plane. Each sample can be conceived as a point in a k-dimensional space, where k is the number of compounds taken into account. The first two principal components (PCs 1 and 2) span the plane on which the projected points show the highest variance. The position of each projected point (= individual sample) on this plane can

be visualized in the form of a so-called covariance biplot (18). The x and y coordinates of each data point represent the ***scores*** for the first and second PC, respectively. The coordinates of each data point can be read in the bi-plot from the lower x-axis for the value of the first PC and the left y-axis for the value of the second PC.

B: The Vectors. The biplot also shows the values of the **correlation (r)** between each original-in this case the logarithm concentration of each compound and the first and second principal components by means of a vector. The x and y coordinates of the endpoint of each vector represent the ***loading.*** The squared length of each vector shows the goodness of fit and is equivalent to the fraction of the total variance that is explained by the sum of the first and second PC (In formula: $(l_{vector})^2 = R^2$). Thus, if a vector of a compound reaches the drawn circle of unit variance (circle $R^2=1$), then all variance in the concentration is explained by this sum. The values for the individual correlations with the PCs plotted can be read by orthogonal projection of the endpoint of each vector on the upper *X*-axis in the plot for the first PC and on the right y-axis for the second second PC. The correlation r between the concentrations of two different compounds is equal to the product of the length of their vectors and the cosine of the angle *R* between them. Thus, the concentrations of two compounds of which the vectors touch the unit-circle (i.e. all variance can be explained by the sum of the first two *PCs),* and point in the almost the same direction (cosine α approaches 1), show a very high positive correlation. In contrast, orthogonal vectors indicate a zero correlation (cosine 90°= 0); a negative correlation is indicated by angles between 90 and 180°.

C: The Relation between the Location of the Datapoints and the Vectors. The orthogonal projection of each datapoint (= individual sample) on each vector--or its extension on the other side of the center of the plot--shows the relative concentration of the accompanying compound in the sample with respect to the mean value, which is represented by the center of the plot.

Results and Discussion

PBDE Congeners Detected. Of the 16 congeners analyzed, six were present as major compounds. The basic statistical data (mean, median, standard error, minimum-maximum values) of the levels of these congeners in the different species and tissues are given in Table 1. The general order of decreasing concentrations is BDE47 > BDE99, BDE100 > BDE153, BDE154 > BDE28. An exception to this rule is the sea star, where BDE100 > BDE47, BDE99. In contrast to the situation in the sea star, BDE100 is remarkably low in the harbor

seal tissues, which might indicate the occurrence of a certain biotransformation capacity in this species. In the invertebrates, the amount of BDE47 is generally < 50% of ΣPBDE except for the shrimps, whereas in fish it is always > 50%. In harbor seal blubber the contribution of BDE47 *to* ΣPBDEs was somewhat higher than in harbor porpoise with values for respectively the mean (SEM/median/min-max range of 63 ± 3.6%/66%/42-80% for harbor seal blubber and 51 ± 3.9%/49%/32-68% for harbor porpoise blubber (n) = 9 for both species). However, far fetched conclusions should not be drawn from these differences in relatively small data *sets,* since for another larger data set of harbor porpoise blubber, the values 60 ± 1.4%/59%/39-88% have been reported (19), which is very similar to our results for the harbor seal.

The congeners BDE66, BDE75, BDE77, BDE119, and BDE138 were present at concentrations around the limit of detection (LOD; 0.6 ng g^{-1} lipid in a tissue containing 50 mg of extractable lipid). The congeners BDE71, BDE85, BDE183, and BDE190 were never detected.

BDE209 was occasionally present in concentrations just above its LOD of 5 ng g^{-1} lipid. However, when the samples contained parts of the digestive system, a presence of BDE209 just above the detection limit cannot be interpreted as unambiguous proof for are al uptake by the organism. Instead, the levels may represent remainders of food present in the digestive system. Since BDE209 is a major compound in suspended particles and sediments from the North Sea and related environments (14), it does apparently not bioaccumulate to a high degree (20). Thus, either its large molecular size decreases the uptake rates, or a relatively rapid biotransformation increases *its* degradation *rate.* The literature presents evidence for both sides. A number of field studies showed that the bioaccumulative properties of BDE209 were much lower than those of the tetra- and pentabrominated congeners that dominate in the Penta-BDE formulation (21, 22). The only occasion where this congener has been reported (together with BDE183) in relatively high concentrations in wildlife concerns eggs of pererine falcons breeding in Sweden (11). The peregrine falcon *(Falco peregrinus)* belongs to the top-predators in the terrestrial food-web, since this bird of prey feeds mainly on other birds. The uptake of BDEs 183 and 209 in blood of humans working in a recycling factory of electronic household equipment has also been demonstrated (12). However, as a result of biotransformation processes, BDE209 had a half-life of less than 10 days in these humans. A relatively rapid metabolism of this compound has also been shown in *rats (23).* A slow but measurable uptake of BDE209 has also been demonstrated in laboratory studies with rainbow trout (24) and juvenile salmon (25). Laboratory studies with ^{14}C-labeled BDE47 in marine invertebrates demonstrated the formation of polar metabolites, and thus a certain metabolic

capacity toward PBDEs does exist even in marine invertebrates, although the fraction metabolized differed from barely detectable to about 50% between the species investigated (26). No clear relations between metabolic capacity and taxonomic group could be observed. Thus, it remains inconclusive whether the main reason for the absence of BDE209 is on the accumulation or on the elimination side of the bioaccumulation process.

TABLE 1: Lipid-Normalized Concentrations in ng g-1 of the Six Major PBDE Congeners Encountered in Animals of Different Trophic Levels of the North Sea Food Web[a]

	BDE28		BDE47		BDE100		E
Br subst.:	(2,2',4-)		(2,2',4,4'-)		(2,2',4,4',6-)		(2,2'
species	mean/ median	(min-max)	mean/ median	(min- max)	mean/ median	(min- max)	mean/ median
					Invertebrates		
sea star (pc)	0.6/0.5	(0.4-1.1)	22/19	(3.4-56)	23/9.1	(2.2-82)	9.3/2.9
hermit crab(abd)	1.4/1.6	(0.8-1.7)	38/29	(8.6-118)	15/11	(2.8-40)	16/9.2
whelk (sp)	1.2/1.0	(1.0-1.6)	10/5.5	(2.6-30)	4.2/2.8	(1.5-9.7)	6.1/4.(
shrimp (wb)	2.6/2.6	(2.4-2.7)	37/37	(35-39)	6.9/6.9	(5.1-8.7)	5.1/5.1
					Fish		
herring							
liver	2.1/2.1	(1.6-2.5)	30/25	(19-52)	9.1/6.9	(5.6-17)	13/12
filet	1.9/1.9	(1.2-2.4)	37/38	(23-47)	9.2/9.3	(6.3-12)	12/11
cod							
liver	6.7/6.2	(2.0-12)	133/99	(63-307)	40/33	(18-93)	15/8.8
filet	2.7/2.1	(1.5-4.5)	43/34	(26-74)	13/12	(5.9-21)	6.3/4.1
whiting							
liver	3.6/3.6	(0.7-6.3)	70/74	(7.6-132)	16/15	(1.7-31)	15/14
filet	1.8/1.8	(1.3-2.4)	26/28	(7.1-40)	8.6/10	(4.2-12)	9.0/9.(
					Marine Mammals		
harbor porpoise							
liver	26/17	(5.0-86)	1331/720	(1.2-4877)	562/285	(0.3-2142)	715/402
blubber	22/21	(7.6-36)	864/796	(245-1312)	242/228	(47-479)	406/350
harbor seal							
liver	16/16	(4.1-28)	1328/368	(95-5065)	83/22	(8.7-271)	454/38
blubber	9.7/2.9	(1.1-49)	1236/210	(57-9248)	82/25	(6.2-543)	396/57

a pc) pyloric caeca; abd) abdomen; sp) soft parts; wb) whole body. < LOD

The absence of BDE183 from any environmental compartment indicates that the environmental occurrence of the Octa-BDE mixture is presently still negligible in the North Sea and the Skagerrak.

The analyses were performed on different tissues of the same fish (liver and filet) and marine

mammals (liver and blubber). The lipid physiology of herring and both gadoid species is quite different; herring stores its depot lipids mainly in muscle ("fatty fish", with a relatively lean liver), whereas the gadoid species whiting and cod use the liver for this purpose ("lean fish", with a very fatty liver and a lean muscle). In both gadoid species, the lipid-normalized PBDE concentrations were higher in the liver than in the filet. This is 11h illustrated for the ratio of PBDE concentrations in liver divided by the sum of the concentrations in liver+filet in Table 2. Here, equal concentrations in both tissue types result in a ratio of 0.5, but the actual mean and median values were 0.67/0.63 for whiting and even 0.79/0.79 for cod. Such a tissue preference for the liver was not found in herring and both marine mammal species. In the case of planar *nonortho* PCBs, PCDDs, and PCDFs, a preference for the liver after lipid normalization is attributed *to* the binding of such planar compounds with a high affinity *to* the phase-I biotransformation enzyme cytochrome P450 1A (27). However, since the levels of CYP1A expression in cod were at the low end of the range observed in different fish species from the North Sea (28), and certainly lower than those in marine mammals, we do not believe that this can explain the observed liver preference of PBDEs in gadoid fish species.

TABLE 2: Tissue Preference of PBDEs, Expressed as Ratios of the Lipid-Normalized Concentrations of BDE47 in Liver to (Liver + Filet) for Fish and Liver to (Liver + Blubber) for Marine Mammals[a]

species	n	mean	SEM	median	min	max
			Fish			
herring	4	0.445	0.057	0.408	0.362	0.604
whiting	6	0.674	0.048	0.729	0.518	0.780
cod	4	0.790	0.022	0.793	0.736	0.841
			Marine Mammals			
harbor porpoise	3	0.524	0.089	0.597	0.347	0.627
harbor seal	3	0.427	0.087	0.354	0.327	0.600

a A ratio of 0.5 indicates equal concentrations of BDE47 in both tissues of the

Biomagnification through the Food Chain. The major biomagnification step in the food chain occurs from fish to marine mammals; the lipid-normalized PBDE levels in blubber and liver were similar and generally more than an order of magnitude higher than in the invertebrates and fish. Surprisingly, there was not a clear difference between the three invertebrate species, the planktivorous herring, or the predatory gadoid species whiting and cod in the present study. In an earlier study, biomagnification related to trophic level was found for tetra- to hexa-PBDEs in the imaginary food chain copepods (mainly *Calanus finmarchius)*-- planktivorous fish (sprat *(Sprattus sprattus),* or small-and large herring

(Clupea harengus))-predatory fish (salmon *(Salmo salar))* from the Baltic and the North Atlantic off Iceland (29). The lipid-normalized levels of the major congeners (BDEs 47, 99, and 100) were up to two times higher in large herring than in zooplankton, whereas the levels in salmon were again 2-3 times higher than those in large herring. However, there may also be a size-dependent component in this field observation, since apart from trophic level, the bioaccumulation process also incorporates a size-dependency component (30). This is due to the fact that the ratio of the total surface area of an animal to its size gets smaller when size increases. The surface area of the gill-membrane to total animal volume also plays a role. Both factors result in lower elimination rates in larger animals.

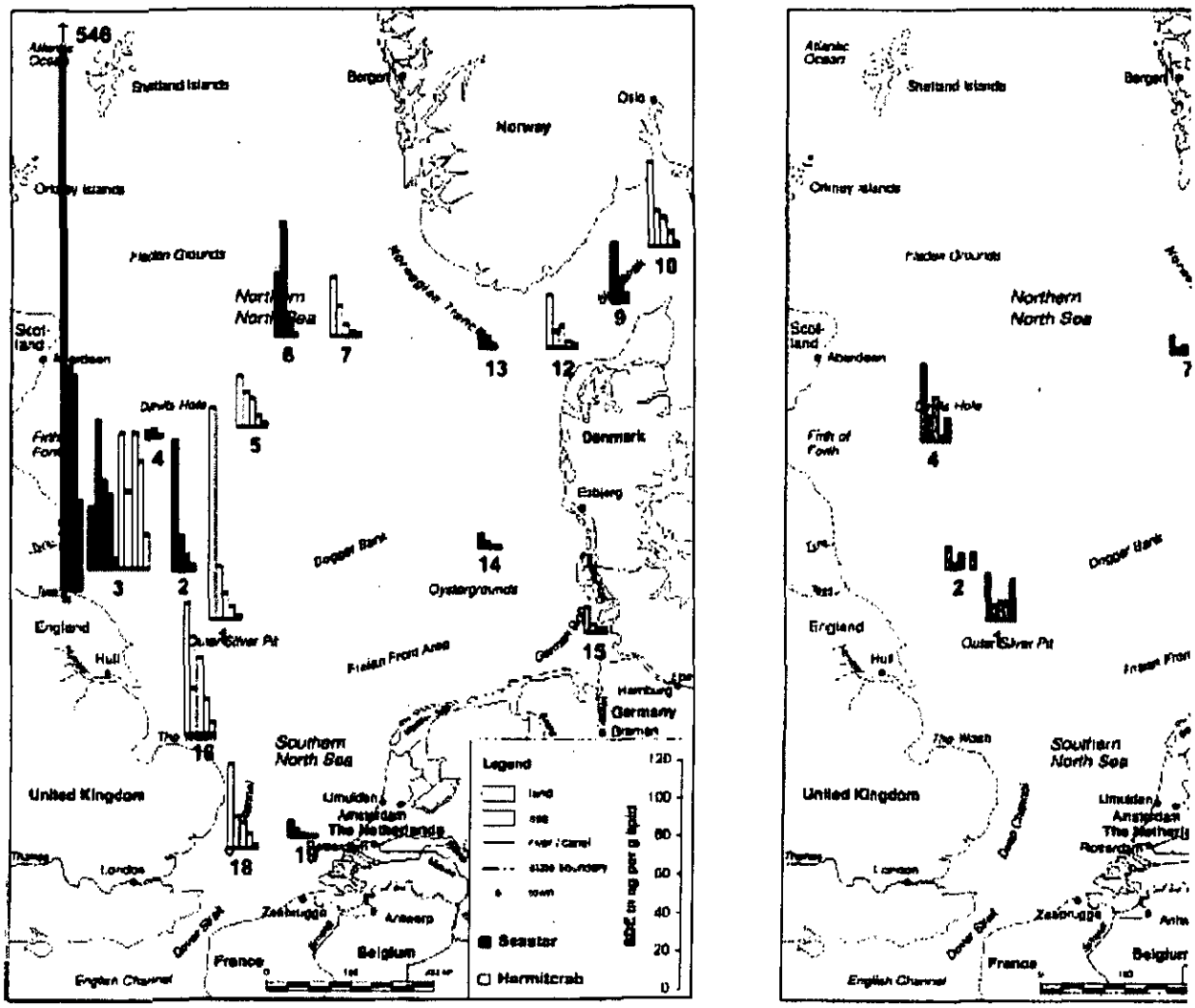
Perhaps the mean and median values of the lipid-normalized PBDE concentrations are biased to higher values for the invertebrates of the present data set, because a number of stations between the UK coast and the Dogger Bank with relatively high exposure levels were present. This may have increased the levels in the sentinel invertebrates more than those in the rapidly migrating gadoids caught in the same area.

To illustrate the observed concentration ranges, the basic statistical descriptors for the data of all six major BDE congeners in the different species have also been given in Table 1. A large difference between median and mean indicates that the levels in the different samples were far from normally distributed. This was especially the case for the harbor seal data, where the mean concentrations are always much higher than the median values, due to the contribution of two animals with very high concentrations, as illustrated with a maximum value of 9248 ng g-1 lipid for BDE47 in blubber.

Geographical Distribution in Invertebrates. The results for the sea star *Asterias rubens* and the hermit crab *Pagurus bernhardus are shown* in Figure 1, and those for the common whelk in Figure 2. In general it can be said that the geographical trend is highly similar in the three species. The concentrations of BDEs in the abdomens of hermit crabs being slightly above those in the pyloric caeca of sea stars, which are higher than the concentrations in the soft parts of whelks.

FIGURE 1. The lipid-normalized concentrations (in ng g^{-1}) of the major PBDE congeners in seastars and hermit crabs from the North Sea. The bars represent, from left to right, BDE47, BDE100, BDE99, BDE154, and BDE153 (order of elution from the GC-column).

FIGURE 2. The lipid-normaliz ng g^{-1}) of the major PBDE cong the North Sea. The bars repres BDE47, BDE100, BDE99, BDE



Since these invertebrates do not migrate over large distances, they are more representative for the site of capture than fish and marine mammals. The highest levels of PBDEs in these invertebrates occurred near the English coast, especially at the latitude of the estuaries of the rivers Tyne and Tees, but also further south. These levels were at least an order of magnitude higher than those found along the coastline of continental Europe. Thus the PBDE levels in the major rivers there (Scheldt, Rhine/Meuse, Ems, Weser, and Elbe) do not appear to affect the levels of BDEs from the penta-BDE formulation in invertebrates living in the receiving waters of the North Sea to a marked degree. We were able to complement our data set from the Pelagia-cruise with the data of a sample of A. rubens taken by CEFAS directly in the main dredged channel at the mouth of the river Tees. The very high levels in this sample confirm that the Tees is a major source for the PBDE congeners of the penta-BDE formulation in the North Sea. The long-term residual currents of the North Sea run in a

counterclockwise direction (3134). As a result, a residual current along the English coast runs south from Scotland to the Wash, at least in the winter season. In the late spring and summer (May-October) however, a density driven bottom water jet-like current develops along the 40 m depth contour (35, 36), which is close to the coastline in the area of the Tees estuary. This is caused by the development of vertical stratification in the water column caused by increasing air temperatures that warm the surface of the North Sea in the late spring and the summer season. Just like the main circulation, this bottom current runs south but it turns east to the Western-flank of the Dogger Bank at Flamborough Head. Thus water and suspended (dredged) particles from the Tees estuary can be transported either to the south (in winter) and to the Dogger Bank in the east (in summer). The data on the invertebrates illustrate that although atmospheric movements are considered as the main transport pathway for POPs on a global scale (37, 38), water-associated transport can be more important on the scale of regional seas. The identification of the river Tees as a significant source of penta-BDE for the North Sea implicates that the background levels due to the PBDEs released into the environment by the evaporation from flame retarded products in individual households can be overruled by the quantities released in large industrial processes. However, it is yet unknown whether the PBDE production facility at Newton Aycliffe or the application of Penta-BDE by the user industries along the river represents the major source (39). However, the Tees is probably not the only major source at the UK East coast, since in an earlier study, surface sediments from the Humber estuary also contained relatively high levels of BDEs associated with both the Penta-and Deca-BDE formulations in a survey of different European rivers (40). High levels of deca-BDE only were found in sediments of the Belgian/Dutch river Scheldt (40). Intermediate levels of deca-BDE were found in sediments from the UK river Thames, the French river Seine, and the Swiss/French/German/Belgian/Dutch Rhine-Meuse estuary. Low levels of all PBDE formulations were present in the German rivers Ems, Weser and Elbe and the Scandinavian rivers Göta, Glomma, Skaien, and Otra (40).

The levels in fish were measured in liver and filet of two gadoid species, whiting and cod. The geographical trends in concentrations of these rapidly migrating species did not correspond with the trends in the invertebrates any more, although a single sample of whiting filet taken directly at the mouth of the river Tees by CEFAS showed also very high levels (Stetra-hexa BDEs > 2000 ng g^{-1} lipid; C. R. Allchin, unpublished data). The Tees has been identified as a major source for PBDEs in an earlier study too (39). The river is also dredged at regular intervals, and the sediment is released at a dispersive site in the outer estuary.

In the Skagerrak, the levels seem to increase from west to east; this might be due to an increasing proportion of Baltic influence.

Comparison of the PBDE Levels in Fish with Other Areas. The comparison of the levels obtained for the North Sea with other areas can be done on the basis of ΣPBDEs or a single congener. We have selected the congener that is always present in the highest concentrations, BDE47, for this purpose. The present levels ranged between 26 and 133 ng g-1 lipid in the different tissues of herring, whiting, and cod. Due to the limited number of data, we have neglected any effects of biomagnification in predatory fish compared to planktivorous fish in this paragraph (29). Sprat *(Sprattus sprattus),* herring, and salmon ***(Salmo salar)*** caught in the Baltic Sea in 1998, showed BDE47 values in the range of 6.8-45.8 ng g^{-1} lipid (29), which is similar to the present range for the North Sea. The levels of BDE47 in yellow eel from the Rhine-Meuse estuary in 1999 were also similar to those in fish from the present study (8). The average levels in lake trout caught in the U.S./Canadian Great Lakes in 1997 were similar to those in fish from the North Sea in Lake Erie and Lake Huron, but the values for Lake Superior (225 ng g^{-1}) and Lake Ontario (380 ng g^{-1}) were about an order of magnitude higher (6). An even higher average concentration of 1590 ng g-1 lipid was found for coho and chinook salmon from Lake Michigan (41). The levels in 1999 in grey mullet and sea bass from Osaka Bay in Japan were similar to those in fish from the North Sea, but the historical levels in this coastal area have been more than an order of magnitude higher in the second half of the 1980s (9). Data from the North Atlantic Ocean office land that can be regarded as background levels due to long-distance transport, concern herring and salmon; these samples showed indeed lower levels than all coastal areas with a concentration range of BDE47 between 2.0 and 7.6 ng g^{-1} lipid (29).

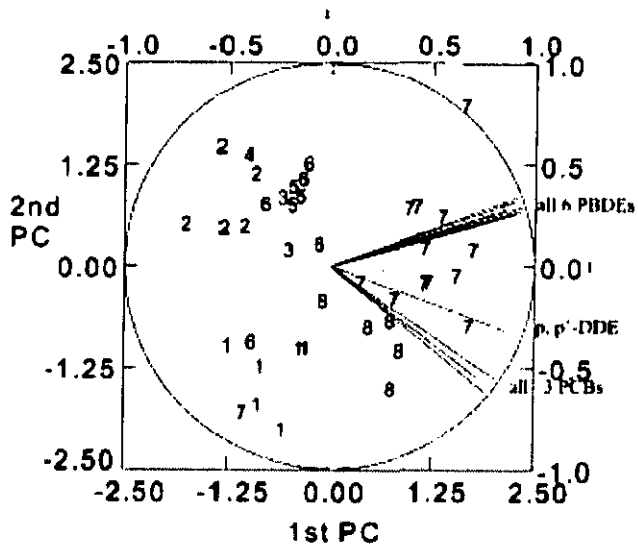


FIGURE 3. Covariance bi-plots of the (10log-transformed) lipid based concentrations (in ng g^{-1}) of the six major PBDEs (BDEs 28, 47, 99, 100, 153, and 154), three major PCBs (CBs 101, 138, and 153), and p,p'-DDE. The lower x-axis and the left y-axis give the scores for the first and second PC, respectively. The upper x-axis and the right y-axis show the values of the correlation (r) between each vector and both PC's. The values for the first and second PC can be obtained by orthogonal projection on the upper X-axis for the first PC and the right y-axis for the second PC. The numbers indicate the different species: 1) *Asterias rubens;* 2) *Pagurus bernhardus;*

3) *Buccinum undatum;* 4) *Clupea harengus;* 5) *Gadus morhua;* 6) *Merlangius merlangus;* 7) *Phocoena phocoena;* 8) *Phoca vitulina.*

Multivariate Statistical Analysis. For a comparison with the PBDEs, the levels of three major PCB congeners (CBs 101, 138, and 153) and p,p'-DDE as the dominant compound of the DDT family were also quantified in the samples. The bi-plot of the (^{10}log transformed) lipid-based concentrations of the six major PBDEs, three major PCBs, and p,p'-DDE in the tissues of all species investigated is given in Figure 3. The vectors of the PBDE and the PCB congeners are clearly clustered, indicating a high covariance of the concentrations of the different congeners within the same class of chemicals. The vector of p,p'-DDE is closer to the PCB cluster, indicating closer resemblance in its environmental behavior with the PCBs than with the PBDEs. Thus, the environmental distribution of the PBDEs in the North Sea food web clearly differs from the older organochlorines. This is in contrast with the situation in the Great Lakes, where a high degree of covariation was found between the levels of PBDEs and PCBs in atmospheric samples (42) and in samples of salmonids (41). In the air samples, both groups of compounds were clearly associated with large industrial and urban centers of this area.

The datapoints of the samples of the different invertebrates, fish species, and marine mammals are also clearly clustered in the bi-plot. A plot of the value of the first PC against the concentration of BDE47 showed a high correlation between the two variables. This means that the lipid-normalized PBDE concentrations increase from left to right in the bi-plot.

Acknowledgments

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Supporting Information Available

Tables of locations and basic characteristics of the sampling stations of the cruise with the RV *Pelagia* in the North Sea and the Skagerrak for all invertebrates, whiting, and cod (Table SI-1) and lipid contents of the samples as percentage of wet weight (Table SI- 2). This material is available free of charge via the Internet at http://pubs.acs.org.

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Identification of 19 Polybrominated Diphenyl Ethers (PBDEs) in Long-Finned Pilot Whale (*Globicephala melas*) from the Atlantic

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Abstract. Nineteen tetra- to hexabrominated diphenyl ethers were identified at ppb concentration in the blubber of pilot whale caught off the coast of the Faroe Islands in 1994 and 1996. Higher total concentrations were found in the pooled samples of young males (3,160 ng/g lipid) and females (3,038 ng/g lipid) compared to adult females (843 ng/g and 1,048 ng/g lipid) and males (1,610 ng/g lipids). The predominant isomers in all samples were 2,2′,4,4′-TeBDE (PBDE #47) and 2,2′,4,4′,5-PeBDE (PBDE #99) accounting for some 70% of the sum of the 19 isomers.

Polybrominated diphenyl ethers (PBDEs) have been used as flame retardants in a variety of consumer products. This includes their accelerated use in electronic boards in computers, radios, and television sets. In 1992 the world annual production was 40,000 t (WHO 1994). The consumption of flame retardants in the United States is expected to rise to $926 million in 2000. About 30% of this market is expected to consist of bromine-based flame retardants (Reish 1997). The PBDEs are mainly used as two formulations, deca-BDE and Bromokal 70-5DE, a mixture of tetra-, penta-, and hexa-BDE. The use of Bromkal 70-5DE has recently been reduced in several countries because of its bioaccumulation potential and toxicity (WHO 1994).

The PBDEs were first discovered in the environment in Sweden in pike, eel, and sea trout samples from Viskan-Klosterfjorden south of Gothenborg in 1981 (Andersson and Blomkvist 1981). These first findings have been confirmed by several others (Jansson *et al.* 1987; Wantanabe *et al.* 1987; de Boer 1989, 1990; Kuehl *et al.* 1991; Loganathan *et al.* 1995). Reported concentrations vary from 8–110,000 ng/g depending on the species or sampling site. Mainly the tetra- and pentabrominated isomers were recently found (Haglund *et al.* 1997; Asplund *et al.* 1997). PBDEs have also been detected in sediment, sewage sludge (Sellström *et al.* 1996; Hartonen *et al.* 1997), birds, and bird eggs (Sellström *et al.* 1993a, 1993b; Peterman *et al.* 1997). Recently, methoxylated bromodiphenyl ethers have been reported in herring and seal samples from the Baltic (Haglund *et al.* 1997). In humans, higher brominated PBDEs were first reported in the early 1990s (Remmers *et al.* 1990; Stanley *et al.* 1991). This is in contrast to the lower brominated congeners found in biota where tetra, penta, and hexa congeners were most abundant. Also recent publications of human concentrations show higher concentrations of the lower brominated congeners (Lindström *et al.* 1997; Haglund *et al.* 1997; Klasson Wehler *et al.* 1997). Although food is considered the main exposure route for humans, the influence from contaminated indoor air and dermal exposure are additional sources (Bergman *et al.* 1997).

The PBDE concentrations in marine mammals are only known for a number of different seal species from the Baltic, the Arctic, and the North Sea. Total concentrations of 90 ng/g, 10–40 ng/g were reported for harbor and ringed seal (Jansson *et al.* 1987), respectively, from the Kattegat and the Baltic. Higher concentrations were measured in juvenile harbor seals (250–650 ng/g), adult male harbor, ringed, and grey seals (230–320 ng/g) and adult female grey seals (280–1,500 ng/g) collected in 1988 (Andersson and Wartanien 1992). The first congener-specific concentrations in a composite ringed seal sample from Svalbard in 1981 and a composite grey seal sample (1979–85) from the Baltic were reported by Jansson *et al.* (1993). Of the three congeners measured, 2,2′,4,4′ TeBDE was clearly the most dominant compound with concentrations of 47 to 650 ng/g reported in ringed seal and grey seal, respectively. The other congeners found were 2,2′,4,4′,5-PeBDE and an unidentified PeBDE congener. The concentrations of the two pentabrominated congeners were comparable in both ringed seal (1.7–2.3 ng/g) and grey seal, (38–40 ng/g). Recently, Haglund *et al.* (1997), in addition to these penta congeners, detected three hexabrominated diphenyl ethers, of which one was identified as 2,2′,4,4′,5,5′ HxBDE (3–27 ng/g).

Consistent with the previously reported high concentrations of chlorinated organic contaminants in marine mammals, brominated organic pollutants were expected to be found in pilot whale blubber. High concentrations of organochlorine contaminants have already been reported in Atlantic pilot whale (Simmonds *et al.* 1994; Abraham *et al.* 1995; Mössner and Ballschmitter 1997). There have been no determinations of

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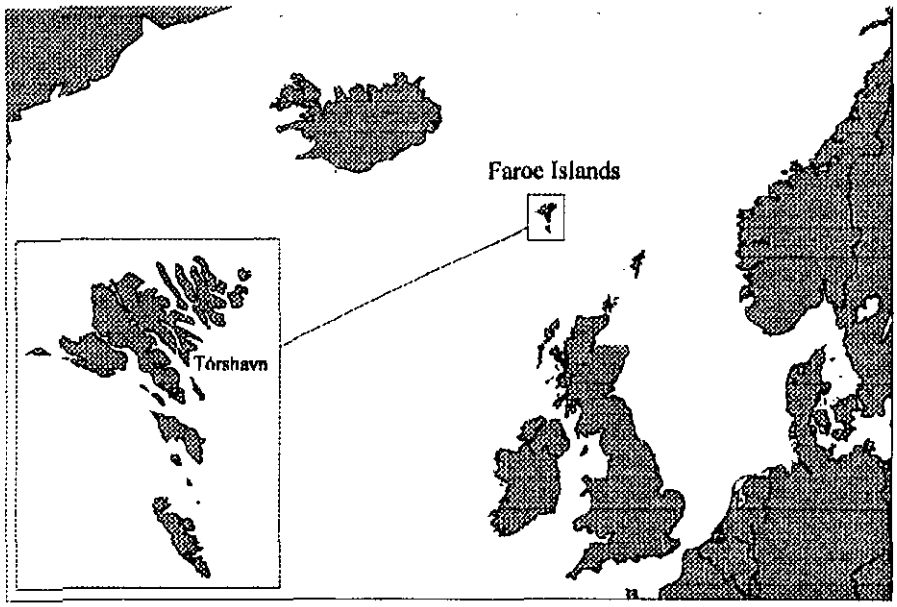

Fig. 1. The sampling sites in Vestmanna and Hvannasund, Faroe Islands, in 1994 and 1996

PBDEs reported for pilot whale in these studies. In the present study the occurrence of PBDE in long-finned pilot whale was investigated both by full scan (SCAN) and selective ion recording (SIR) mass spectrometry to identify and quantify PBDEs.

Materials and Methods

Sampling

Samples of blubber from long-finned pilot whales were collected from two separate schools taken in the traditional drive fishery (Bloch *et al.* 1990). Samples were taken after the whales were opened for cooling. Blubber samples were taken from the ventral side, in the posterial end of the incision already made by the skilled assistants on the quay. The first sampling took place on June 30, 1994, in Hvannasund (Figure 1). Samples were taken from 20 animals, and sex and lengths were determined by local authorities. With length and sex of the individuals known, the approximate age, or rather the degree of sexual maturity, could be inferred (Bloch *et al.* 1993a). The sampling was random from the pod containing a total of 119 whales. From this catch only the adult females were analyzed; they were nine in number and their mean body length was 4.30 m (range 3.95–5.12 m).

The second sampling was on June 26, 1996, in Vestmanna from a pod of 192 whales. Samples were taken from 50 individuals randomly chosen. In the case of the Hvannasund samples, the whales were subdivided according to sex and maturity. The four subsamples represented were immature females (n = 4, mean body length = 2.97 m, range 2.55–3.5 m) and males (n = 13, mean body length = 3.59 m, range 2.73–4.75 m), mature females (n = 19, mean body length = 4.39 m, range 4.00–4.65 m), and males (n = 8, mean body length = 5.41 m, range 5.11–5.63 m). The average number of mature and immature females and males in an average pilot-whale school was 40% mature females, 20% immature females, 13% mature males, and 26% immature males (Bloch *et al.* 1993b). The sampling from Vestmanna June 26, 1994, was thus biased toward adult males, so fewer immature females than average were represented.

The samples were frozen individually in polyethylene bags, and kept at approximately −20°C until preparation of pooled samples prior to analysis. When subsampling from the individual blubber samples, care was taken to cut away and thus exclude from the subsamples the parts of the blubber that had been in contact with the polyethylene bags. Thus the core of the blubber layer, which was in all approximately 5 cm thick, was used. Subsamples of similar weight were taken from each individual to make up the pooled samples to approximately 40 g. Five pooled fat samples of pilot whale, caught in Vestmanna and Hvannasund, Faroe Islands, in 1994 and 1996, were analyzed (Table 1).

Sample Extraction and Cleanup

The pooled fat samples were homogenized in a mortar with sodium sulfate (1:4). An internal standard consisting of 13 ^{13}C-labeled polychlorinated biphenyls (PCBs), di- through deca-substituted (#15, #28, #47, #52, #101, #105, #118, #138, #153, #156, #180, #194, and #209) was added before the lipid extraction. The homogenates were applied on columns (4.7 cm ID) and the lipids were quantitatively extracted with methylene chloride and hexane (1:1) and gravimetrically determined. Further cleanup was performed on a multilayer silica column, consisting of sulfuric acid on silica, neutral activated silica, and potassium hydroxide on silica, using hexane as eluting solvent. A method blank was cleaned up simultaneously using the same procedure and amounts of solvents as for the samples. After concentration in a rotary evaporator a recovery spike, three ^{13}C-labeled PCBs, tetra through hepta (#80, #128, and #178) dissolved in tetradecane, was added.

Instrumentation, HRGC/MS Analyses

Full-scan HRGC/MS spectra were recorded using a Fisons GC 8000 gas chromatograph coupled to a MD800 mass spectrometer (Micromass, Manchester, UK) scanning from m/z 100 to m/z 700 in 1 s and operating in the electron impact mode (EI). Chromatographic separation was achieved by splitless injection of 2 µl on a nonpolar DB-5 column (J&W, Folsom, CA) using helium as the carrier gas. The GC oven was programmed as follows: 180°C initial hold for 2 min, increase at a rate of 15°C/min to 205°C, followed by an increase of 3.7°C/min to 300°C, final hold at 300°C for 20 min. The same chromatographic conditions were used for selected ion recording SIR MS. When using SIR the two most intense ions of the molecular ion cluster were monitored for TeBDE (m/z 483.7, 485.7), PeBDE (m/z 563.6, 565.6), and HxBDE (m/z 641.5, 643.5) in addition to two

Table 1. Concentrations of the 19 PBDEs in ng/g lipid in five pools of adult male, adult female, juvenile male, and juvenile female pilot whale samples from the Faroe Islands

	Vestmanna, June 1996 19 Females 79% Lipids (ng/g)	Vestmanna, June 1996 8 Males 66% Lipids (ng/g)	Vestmanna, June 1996 13 Young Males 76% Lipids (ng/g)	Vestmanna, June 1996 4 Young Females 72% Lipids (ng/g)	Hvannasund June 1994 9 Females 82% Lipids (ng/g)
Te-BDE (a)	2.9	3.9	5.8	8.5	2.3
Te-BDE (b)	0.8	0.8	1.0	1.4	0.7
Te-BDE (c)	2.4	3.5	7.8	7.9	1.5
Te-BDE (d)	7.5	8.7	6.1	11.2	4.7
Te-BDE #47	529.4	862.4	1782.1	1727.4	411.9
Te-BDE (e)	2.9	3.9	8.1	8.5	2.2
Te-BDE (f)	21.9	28.2	40.2	61.5	13.4
Pe-BDE (a)	4.6	6.8	13.1	12.1	3.5
Pe-BDE (b)	ND	0.2	0.5	0.4	ND
Pe-BDE (c)	1.9	2.8	6.4	6.3	1.5
Pe-BDE (d)	104.4	153.6	280.5	281.1	87.1
Pe-BDE (e)	2.4	3.4	6.4	6.3	2.0
Pe-BDE (f)	ND	1.7	3.6	3.3	0.8
Pe-BDE #99	209.0	292.0	603.6	562.2	164.1
Pe-BDE (g)	4.8	12.4	25.2	19.1	6.3
Hx-BDE (a)	29.2	43.9	67.2	54.6	27.8
Hx-BDE (b)	85.1	123.3	203.7	178.6	77.9
Hx-BDE (c)	3.4	5.8	9.0	10.4	3.5
Hx-BDE #153	35.2	53.2	90.0	77.4	32.0
Sum BDE	1,047.9	1,610.3	3,160.3	3,038.2	843.2

ND = not detected

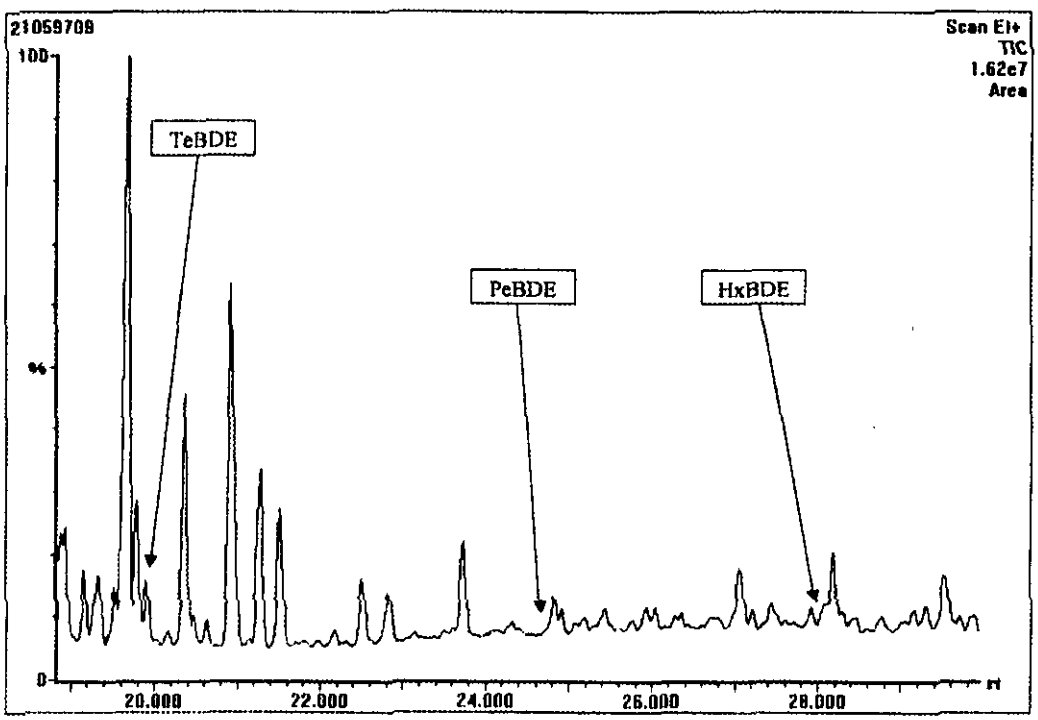

Fig. 2. The total ion current chromatogram of the male whale sample from Vestmanna during a full scan (m/z 100–m/z 700) between 19 and 30 min; indicated on the chromatogram are the retention times and location for TeBDE (19.8 min), PeBDE (24.8 min), and HeBDE (28.1 min)

masses for the ^{13}C-labeled internal standard Deca-PCB (m/z 509.7, 511.7) at a dwell time of 40 ms and an interchannel delay of 1 ms. Quantification was achieved by comparing the relative responses of the target compounds against the internal standard (^{13}C PCB #209) in both the samples and a standard solution. The standard solution contained known amounts of 2,2′,4,4′-TeBDE (BDE #47), 2,2′,4,4′,5-PeBDE (BDE #99), 2,2′,3,4,4′-PeBDE (BDE #85), 2,2′,4,4′,5,5′HxBDE (BDE #153), 2,2′,3,4,4′,5-HxBDE (BDE #138), and IS ^{13}C PCB #209.

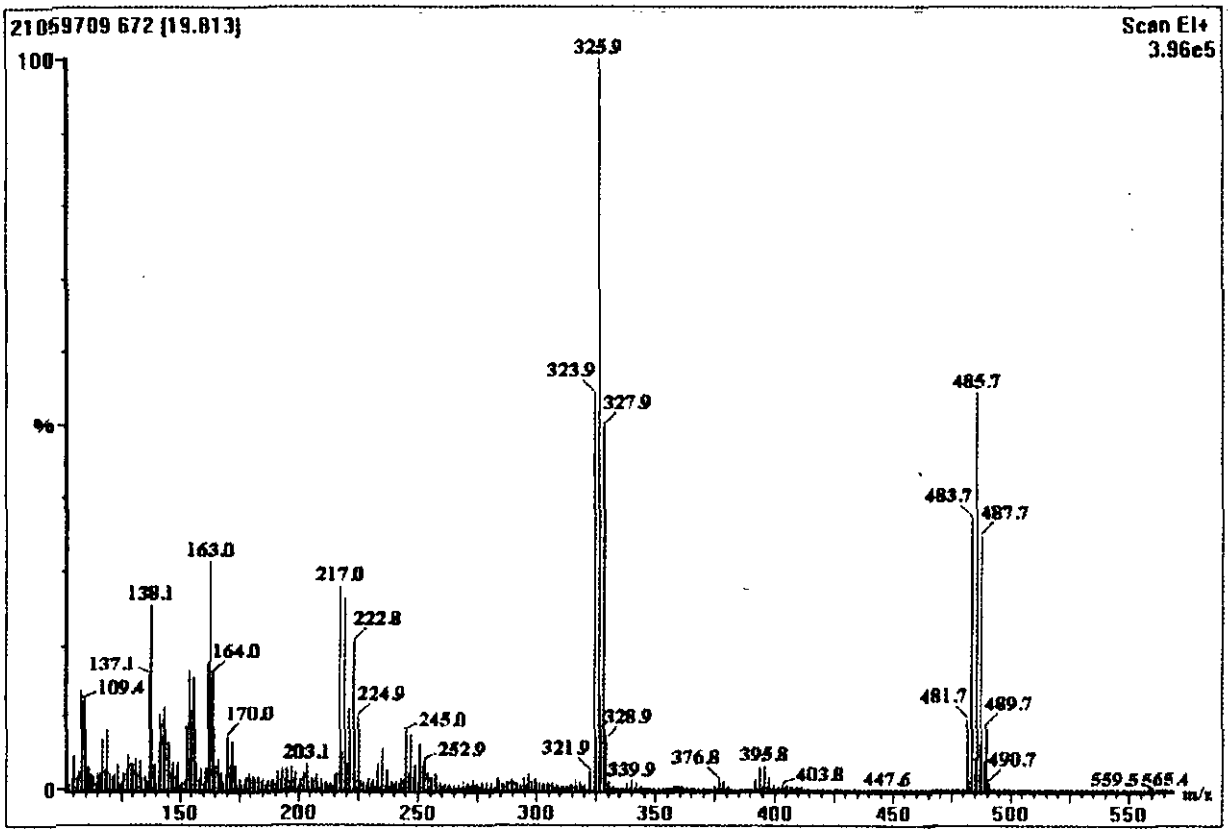

Fig. 3. The mass spectrum of TeDBE eluting at 19.8 min. The molecular ion bromine cluster at mass 481.7 (M^+) indicates the presence of four bromine atoms. The most abundant fragment showing a bromine cluster can be seen at mass 321.9, indicating the loss of two bromine atoms

Quality Assurance and Quality Control

Quality assurance and quality control concerning the analysis of PDBE is extensively discussed elsewhere (Haglund *et al.* 1997; Lindström 1997). In short, positive quantification of PBDE was reported when (1) S/N > 3 for the quantification ion in the SIR GC/MS mode; (2) the ratio of the two most abundant ions in the bromine cluster was within 10% of the theoretical ratio; and (3) concentration of the target compound in a laboratory blank sample, treated the same way as a real sample, was below 10% of the reported concentration in the samples. In this study the laboratory blank samples did not contain PBDE concentration above the detection limit (0.1–0.5 ng/g). The detection limit for the whale samples was 0.1–1.0 ng/g lipids, depending on the sample size (1.9–2.2 g wet weight) and the different responses of the PBDE congeners. All bromine isotope ratios were well within 10% of the theoretical ratio for all PBDE congeners reported in Table 1. Because no ^{13}C-labeled PBDEs are available as internal standard, ^{13}C-labeled PCB was used as an internal standard and added before the sample cleanup. PCBs and PBDE are structurally similar and have been shown to have a similar chemical behavior during extraction and cleanup of biological samples (Haglund *et al.* 1997). Therefore, in this study ^{13}C-labeled PCB was used as an internal standard. The recovery of all internal standards was within acceptable limits, 50–90%. Quantification was performed using a PBDEs mixture containing five PBDE isomers at a concentration of 10 ng/µl (99% purity, Wallenberg Laboratory, Stockholm) and the internal ^{13}C-labeled PCB congeners. For the PBDE isomers not present in the quantification mixture, a relative response factor, similar to the closest eluting PBDE, was assumed.

Results and Discussion

Identification

A total ion chromatogram of the male whale sample from Vestmanna is given in Figure 2. Indicated on this chromatogram are the retention times for the brominated diphenyl ethers. The TeBDE is eluting around 19.8 min, PeBDE at around 24.8 min, and HxBDE at 28.1 min. Due to several other co-eluting compounds the PBDEs do not result in significant peaks in the full-scan chromatogram and are located on the "shoulder" of other more dominating peaks. This is especially the case for both the PeBDEs and HxBDEs, as can be clearly seen in Figure 2. The corresponding mass spectra's generated at 19.8, 24.8, and 28.1 min are given in Figures 3, 4, and 5. Figure 3 shows the mass spectrum of TeDBE eluting at 19.8 min, the molecular ion bromine cluster at mass 481.7 (M^+) indicating four bromine atoms and a very intense bromine cluster at mass 321.9 $(M\text{-}Br_2)^+$, indicating the loss of two bromine atoms. This spectrum of TeBDE is identical to the spectra of TeBDE published in the literature (Andersson and Blomkvist 1981; Buser 1986; Wantanabe *et al.* 1987). The mass spectrum of the compound eluting at 24.8 min is shown in Figure 4. The bromine cluster at mass 559.6 (M^+) indicates the presence of five bromine atoms. Again the typical loss of two bromine atoms creates a base peak fragment at mass 401.8 $(M\text{-}Br_2)^+$. This spectrum is identical to EI spectra of PeBDE in the

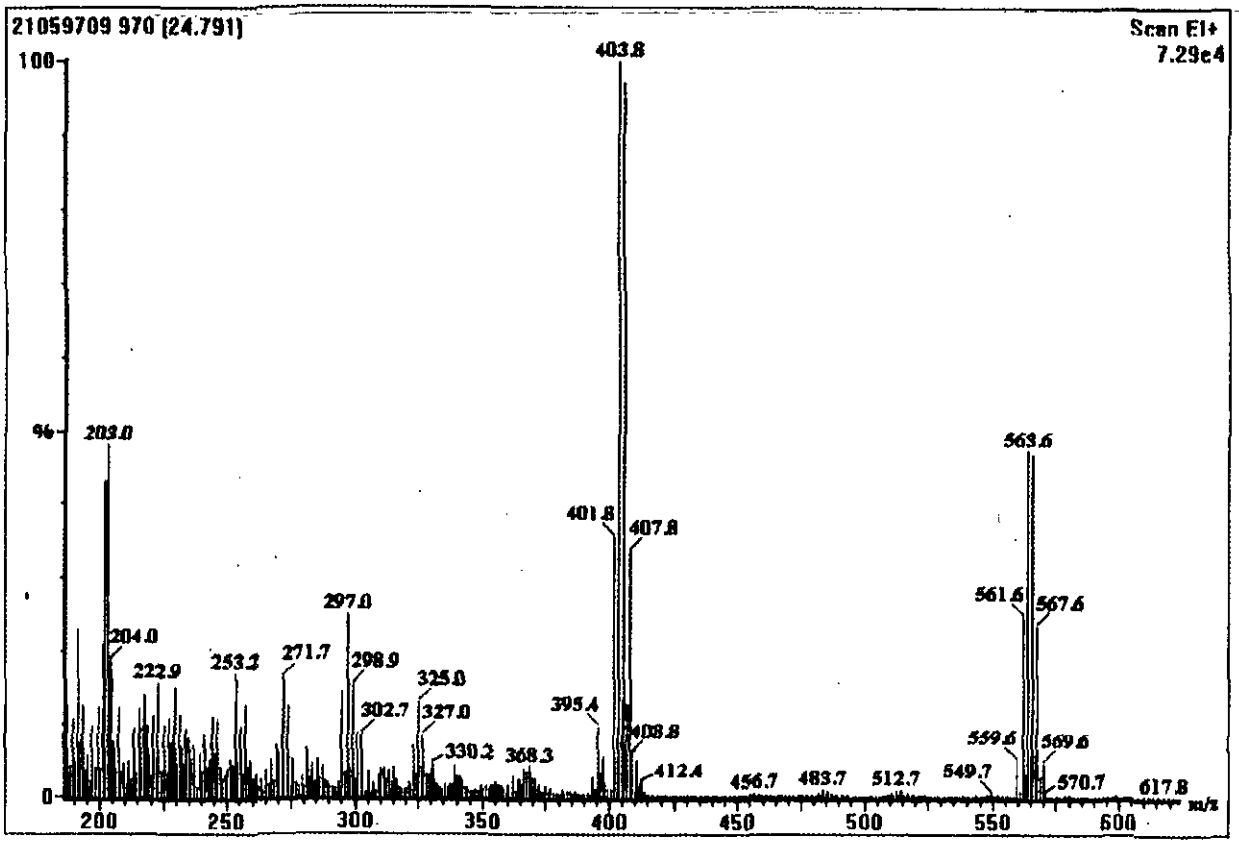

Fig. 4. The mass spectrum of the compound eluting at 24.8 min. The bromine cluster at mass 559.6 (M^+) indicates the presence of five bromine atoms. The most abundant fragment showing a bromine cluster can be seen at mass 401.8, indicating the loss of two bromine atoms

literature by Buser (1986) and Watanabe *et al.* (1987). In Figure 5 the spectra of the shoulder peak at 28.1 min is presented. The molecular ion cluster at mass 641.6 (M^+) and the typical base peak fragment of two bromine losses at 481.7 $(M\text{-}Br_2)^+$ are present, indicating that this compound is HxBDE.

Quantification

After identification of the TeBDE, PeBDE, and HxBDE in the whale tissue, the samples were rerun in the SIR mode. Running a sample in the SIR mode strongly reduces noise and thus enhances the detection limit of the target compounds. In Figure 6 the chromatogram of the most abundant mass 485.7 in the bromine cluster is shown. In total, seven TeBDE congeners were found to comply with the theoretical bromine ratio between $(M\text{-}2)^+/(M\text{-}4)^+$. The largest peak in the chromatogram was identified as 2,2′,4,4′-BDE (BDE #47), showing the same retention time as for BDE #47 in the standard solution. BDE #47 was also identified as a major component in Bromkal 70-5 DE (Sundström and Hutzinger 1976; Sjödin *et al.* 1997). The structures of the other TeBDEs denoted as a, b, c, d, e, and f could not be verified, but it can be assumed that congener f is 2,3′,4,4′-BDE (BDE #66).

In Figure 7 the chromatogram of the most abundant mass of the molecular cluster of PeBDE (563.6) is shown, and eight isomers were positively confirmed as PeBDEs by the theoretical bromine ratio. From these eight isomers the largest peak was verified as 2,2′,4,4′,5-PeBDE (BDE #99), having the same relative retention time as BDE #99 in the standard solution. Tentatively, isomer d in Figure 7 was identified as 2,2′,4,4′,6-BDE (BDE #100). Both BDE #99 and BDE #100 are major peaks in Bromkal 70-5 DE. Four HxBDEs were found in pilot whale.

In Figure 8 the ions corresponding to mass 643.5 are displayed. Again one isomer was found also in the standard solution, the last eluting HxBDE was positively identified as 2,2′,4,4′,5,5′-HxBDE (BDE #153). The structure of other HxBDEs could not with certainty be determined. Although BDE #153 is the major peak in a Bromkal 70-5 DE mixture, in whale this is for all five samples the peak denoted with b and peak a is almost of the same intensity as BDE #153. This indicates that the PBDE pattern in whale is significantly different from Bromkal 70-5 DE, implying that Bromkal is not the only source of exposure. Metabolism of PBDEs by whales can also be a reason for the difference in congener pattern. In Table 1 the concentrations of the 19 PBDEs are given in ng/g lipid for the five pools of adult male and female and juvenile male and female samples. In all sample pools TeBDE #47 was found to be present in the highest concentrations, followed by two PeBDEs, one of which was identified as BDE #99 and one HxBDE.

Considerations

Total concentrations of PBDEs in the five pooled pilot whale samples were significantly higher than the concentrations

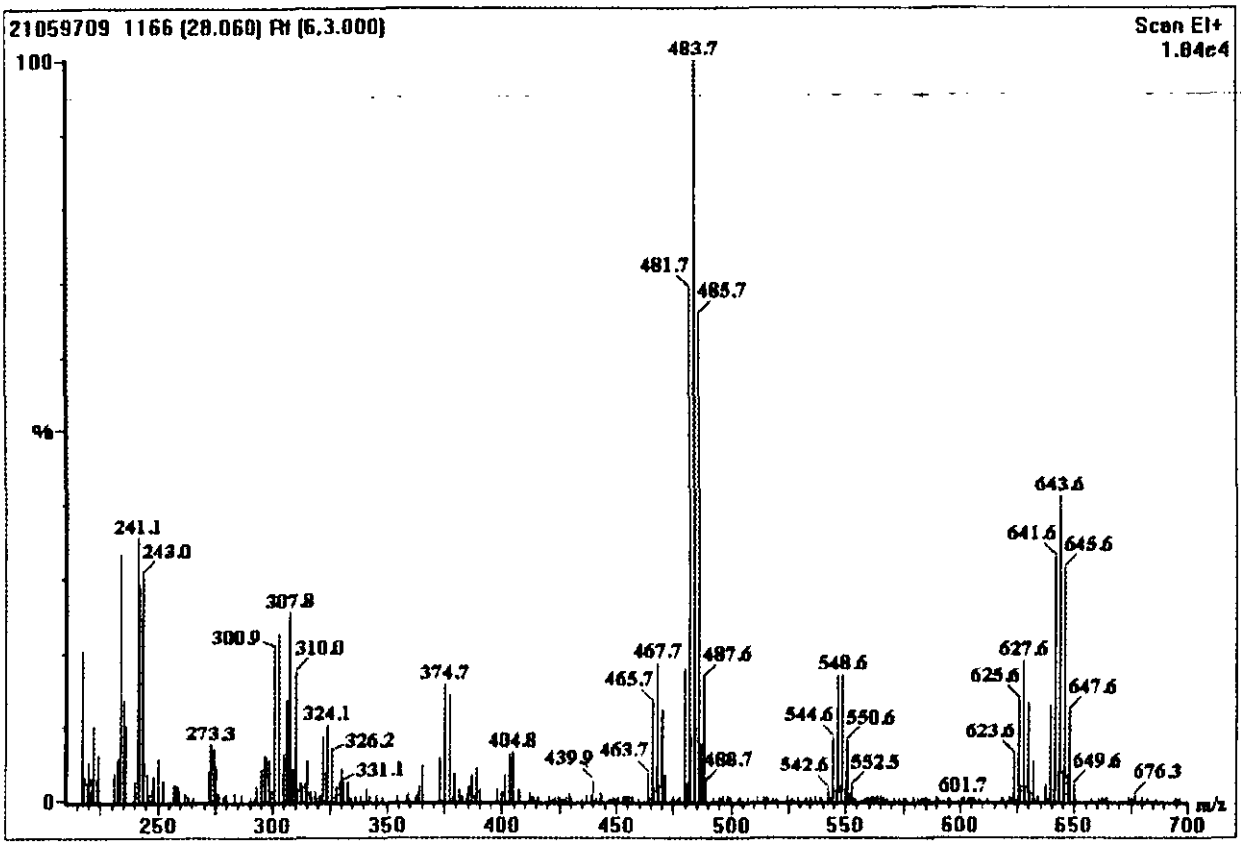

Fig. 5. The spectra of the shoulder peak at 28.1 min. The molecular ion cluster at mass 641.6 (M^+) indicates six bromine atoms present and the typical base peak fragment of two bromine losses at 481.7 $(M\text{-}Br_2)^+$

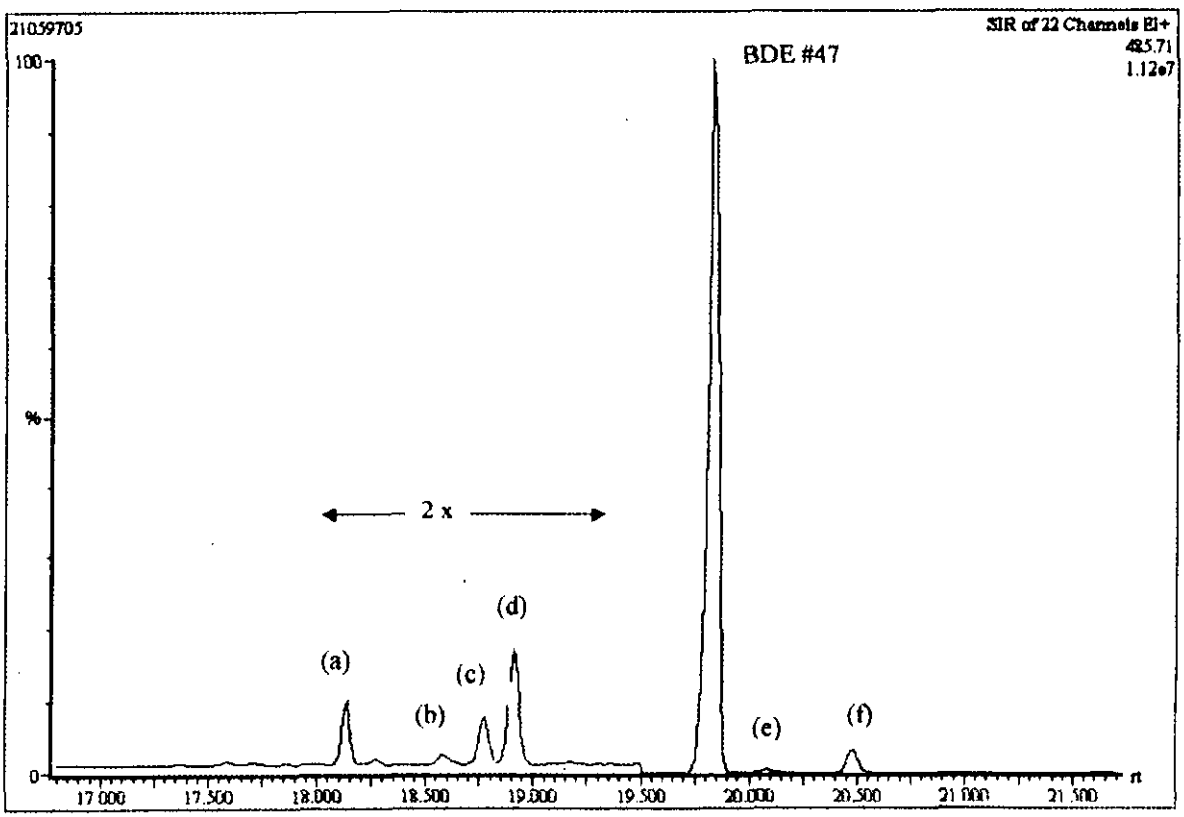

Fig. 6. The chromatogram of the SIR GC/MS run showing the most abundant mass 485.7 in the bromine cluster; a total of seven TeBDE congeners comply with the theoretical bromine ratio of $(M\text{-}2)^+/(M\text{-}4)^+$ and a S/N larger than 3. Between 17.5 and 19.5 min this trace is magnified by a factor of two

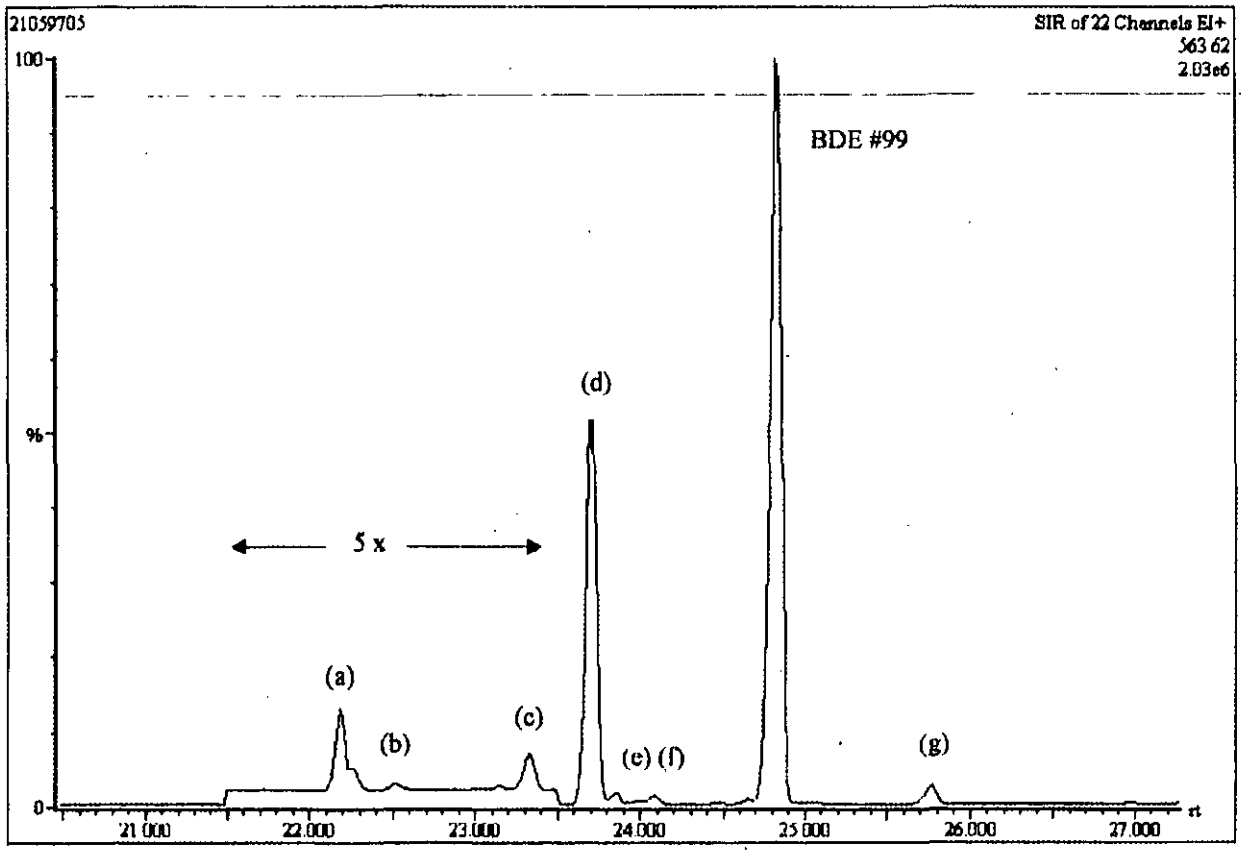

Fig. 7. The chromatogram of the SIR GC/MS run showing the most abundant mass of the molecular cluster of PeBDE (563.6); eight isomers were confirmed as PeBDEs. Between 22.5 and 23.5 and 19.5 min this trace is magnified by a factor of five

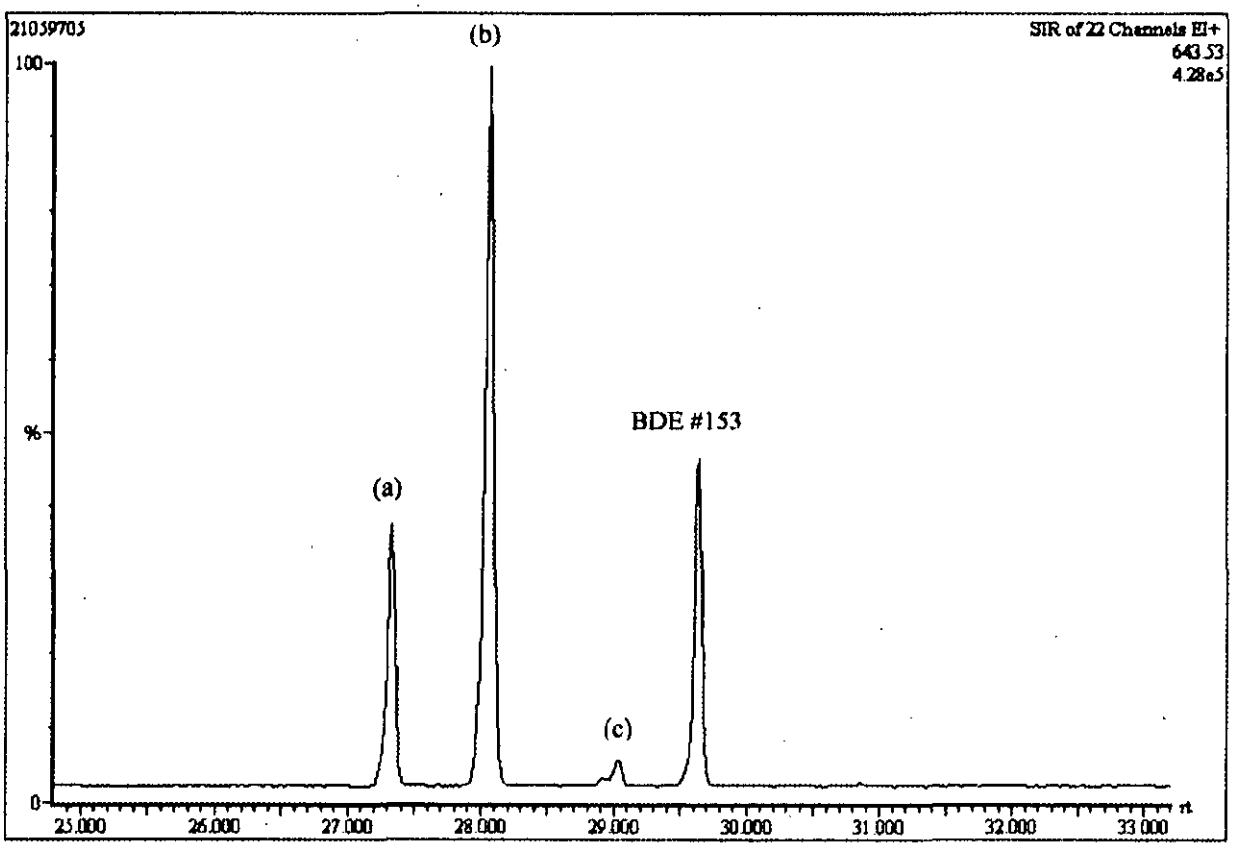

Fig. 8. The ions corresponding to mass 643.5 of the SIR GC/MS run, the last eluting HxBDE was identified as 2,2′,4,4′,5,5′-HxBDE (BDE #153)

previously reported in seals. The PBDE concentrations presented here are among the highest measured in biological samples so far. Also of interest was the fact that for the pooled samples of female pilot whales the concentrations in the samples from 1996 were somewhat higher than the concentrations found in samples from 1994. This is in agreement with other studies, where an increase in PBDE concentrations in the environment are reported and contradicting the observed decrease in concentrations of banned organochlorine compounds such as DDT/DDE and PCB (Sellström *et al.* 1993b; de Boer 1989). But these findings have to be confirmed because there are usually large between-school variations. High concentrations of PBDEs in young animals can be explained by a considerable lactational transfer of these compounds from the females to the offspring. In a recent study on PBDEs in human tissue we have found several of the 19 congeners reported in pilot whale to be present also in the general Swedish population (Lindström *et al.* 1998). On the basis of these findings we strongly recommend further monitoring of PBDEs in human tissue and in biota.

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Rapidly Increasing Polybrominated Diphenyl Ether Concentrations in the Columbia River System from 1992 to 2000

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Concentrations and congener patterns of 32 individual PBDE congeners from mono- through hexa-brominated were investigated in two fish species occupying similar habitats—but having different diets and trophic levels—and surficial sediments from several locations on the major river system of western North America, the Columbia River, in southeastern British Columbia, Canada. Total PBDE concentrations have increased by up to 12-fold over the period from 1992 to 2000 in mountain whitefish from the Columbia River, with a doubling period of 1.6 years. The rate at which PBDE concentrations are increasing in whitefish is greater than has been previously reported worldwide. At the current rate of increase, ΣPBDE will surpass those of ΣPCB by 2003 to become the most prevalent organohalogen contaminant in this region. ΣPBDE in whitefish from the mainstem of the Columbia River range up to 72 ng/g wet weight, concentrations that are 20−50-fold higher than in a nearby pristine watershed affected only by atmospheric contaminant transport. Conversely, ΣPBDE in largescale suckers were approximately an order of magnitude lower than in whitefish, demonstrating the influence of biomagnification and feeding habits. Congener patterns in whitefish from the Columbia River directly correlated with the two major commercial penta-BDE mixtures in use and represent the first time free-swimming aquatic biota such as fish have been found to contain PBDE congener patterns so similar to commercial mixtures. PBDE concentrations in sediments were not linked to a variety of investigated point sources but were instead inversely correlated with the ratio of organic carbon:organic nitrogen in surficial sediments with a pattern suggesting the dominant influence of septic field inputs from the primarily rural population.

Introduction

The Columbia River is the fifth largest river system in North America ranked by flow (6650 m^3/s), draining a total basin area of 673 000 km^2 in the northwestern United States and Canada before discharging into the northeast Pacific Ocean at the Washington State/Oregon border, some 1900 km from its source in two mountain lakes of the Selkirk Mountain range in British Columbia, Canada. Human settlement along the river has been present for over 10 000 years, but the 20th century saw large scale engineering projects (mostly hydroelectric developments) rapidly change the river's hydrology. Anthropogenic activities resulting from this development have had numerous effects on the physical, chemical, and biological characteristics of the region, especially declining salmonid fish populations over the past century (*1*). At present, more than 400 dams exist on the Columbia River system, making it the most hydroelectrically developed river system in the world (*2*). However, while much is known regarding the effects of human development on the physical and biological state of the Columbia River, relatively little is known regarding organic contaminants in this system, especially in the Canadian portion.

Among the organic contaminants of current interest and concern, recent evidence has confirmed that the brominated flame retardants, polybrominated diphenyl ethers (PBDEs), are ubiquitous contaminants present in all environmental compartments at concentrations up to the parts-per-million range (*3*, *4*). In industrialized and pristine regions of North America, PBDE concentrations are increasing rapidly (*3*, *5*) at rates which may be more rapid than was ever observed for PCBs (*6*) and in contrast to declining concentrations of PCBs and PCDD/Fs in the present study area (*7*). Indeed, the 1999 worldwide production volume of PBDEs (sum of the commercial penta-, octa-, and deca-BDE mixtures) at 67 000 tonnes is near the PCB production maximum of 100 000 tonnes in 1970 (*8*). At this rate of PBDE production, which does not appear to be either stabilizing or declining (*3*), only 14 years would be required to surpass the estimated worldwide PCB production of ~1 000 000 tonnes which took place over the five decades from the 1930s until the late 1970s (*9*).

Unfortunately, there are few temporal studies by which to rigorously examine how PBDE concentrations have changed in different regions during the latter 20th and early 21st centuries. Furthermore, little is known of PBDE concentrations and congener patterns in the major North American rivers (i.e. Mississippi-Missouri-Red Rock, St. Lawrence, Mackenzie-Peace, Yukon, Columbia). In the Columbia River, the large number of hydroelectric facilities have, in effect, disconnected various units of the river from each other, thereby preventing unrestricted movement of fish throughout the ecosystem. This disconnection is thought to have greatly constrained the access of anadromous fish to upstream spawning sites, possibly helping to explain large declines in regional fisheries stocks since the early 1900s (*10*−*12*). In addition, dams largely prevent the downstream movement of suspended and bedload sediments, thereby encouraging scouring of the river bed downstream. These sediments are known to be important vectors for hydrophobic contaminants such as PBDEs, which would generally prefer to reside on organic substrates than be dissolved in the water column (*13*−*16*). This compartmentalization of fish populations and sediments makes the Columbia River system a particularly intriguing one in which to examine PBDE

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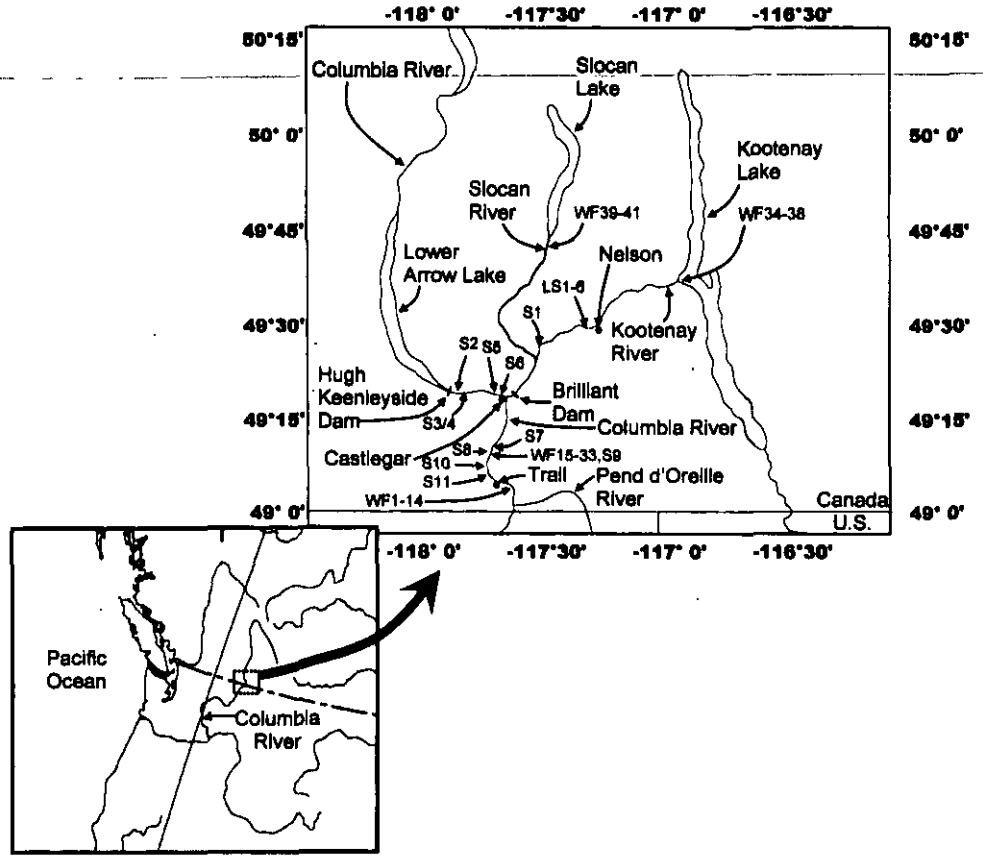

FIGURE 1. Map showing topographic features of the study region discussed in the text and sampling sites for mountain whitefish and surficial sediments in the Columbia and Kootenay River systems in southeastern British Columbia, Canada. Sample numbers correspond to data provided for individual fish and sediments in the Supporting Information.

concentrations and patterns. In addition, increasing concentrations of potentially toxic organic contaminants such as PBDEs may be an additional stressor (*17*–*19*), that when combined with other human influences such as hydroelectric development, may further hinder recovery of some fish populations in the Columbia River.

In the study region of the Columbia River and a major tributary, the Kootenay River, in southeastern British Columbia, Canada (Figure 1), thalwegs are generally shallow (2–12 m) during normal discharge events and river velocities are moderate to high (75 to >150 cm/s), favoring a riverbed composed of medium to large cobbles and small boulders and/or parent bedrock material with a limited littoral area (*20, 21*). Smaller numbers of depositional environments are found where gravels, cobbles, sand, and silt materials collect (*20, 22*). The Kootenay River joins the Columbia River ~10 km downstream from Hugh Keenleyside Dam and 46 km upstream from the Canada–United States border, after which the Columbia River continues its course 1100 km through the United States before discharging into the Pacific Ocean. The average flow of the Columbia River at the Canada–United States border is ~2630 m^3/s, of which 1150 m^3/s is from the Arrow Lakes reservoir system controlled by the Hugh Keenleyside Dam, 790 m^3/s from the Kootenay River system, and 690 m^3/s from the Pend d'Oreille River which enters the Columbia River 3 km before the international border (*22*). In the study region, 25 species of fish have been recorded (*22*), of which mountain whitefish and largescale suckers are considered resident (*21*) and thus good indicators of local contaminant inputs and sources. Of these two species, mountain whitefish are the most abundant sportfish in the Columbia and lower Kootenay Rivers with a population that has remained relatively stable at 35 000–40 000 individuals since the early 1980s (*20, 22*). Whitefish tend to occupy high velocity habitats with cobble or boulder substrates and riffle and run areas adjacent to cobble and boulder deposition areas. Their diet is mainly bottom feeding of aquatic insect larvae (e.g. caddisflies), small mollusks, and occasionally fish (*23*). Most adult whitefish in this area occupy deepwater habitats in the day and relocate to shallow-water habitats for feeding at night (*24*). Juveniles typically reside in shallow nearshore habitats for rearing, as these areas provide the greatest quantity of food and cover (*20, 21*). Largescale suckers, in comparison, use a wider variety of habitats than whitefish with adults residing in both deepwater and nearshore habitats and juveniles occupying shallow stream margins. The diet of immature suckers is mostly planktonic, consisting mainly of water fleas and copepods, and shifts to bottom feeding of invertebrates for adult individuals (*23*). Largescale suckers generally spawn in May and June, while whitefish spawn during the period from November through February (*21*). The population of largescale suckers is estimated to range between 21 000–57 000 and is also thought to be relatively stable (*22*). Together with surficial sediment samples, the resident nature of these two fish species and their different feeding habits offer a means of investigating the concentrations and patterns of PBDEs in this section of the Columbia River system.

To facilitate an understanding of the temporal and spatial trends of PBDEs in a major North American river, we determined the concentrations of 32 individual PBDE congeners from mono- through hexabrominated in mountain whitefish, largescale suckers, and surficial sediments from several locations on the Columbia and Kootenay River systems in southeastern British Columbia, Canada. Whitefish samples obtained over the period from 1992 to 2000 shed insights into how PBDE concentrations have changed in this aquatic system over the past decade. Largescale sucker

samples were taken to help determine how PBDE concentrations and congener patterns differ for fish occupying the same region but with different feeding habits. Surficial sediments were also collected to assist in identifying potential PBDE sources and to examine whether congener patterns and concentrations differed between sediments and fish. Together, these samples form one of the first comprehensive data sets on PBDE concentrations and congener patterns in a major North American river.

Experimental Section

Sample Collection and Preparation. Sampling for mountain whitefish (*Prosopium williamsoni*) in the Columbia and Kootenay River systems took place during the first 2 weeks of July in 1992, 1994/1995, and 2000. Whitefish samples from the Slocan River reference site (WF39-41) were obtained by angling methods in an attempt to avoid tissue damage induced by electroshocking. Angling methods were unsuccessful at the Genelle (WF15-33) and Beaver Creek (WF1-14) sites on the Columbia River, and whitefish were collected using electroshocking. Whitefish samples collected in Kootenay Lake (WF34-38) in early July of 1998 near the outflow to the Kootenay River as well as largescale suckers (*Catostomus macrocheilus*; LS1-6) collected in early July of 2000 downstream of the city of Nelson on the Kootenay River were also obtained by electroshocking. Details on the methods of electroshocking and age determination are provided elsewhere (*25, 26*). Sampling sites are shown in Figure 1; details regarding each location are discussed throughout the manuscript.

Surficial sediments were collected from 11 depositional locations (S1-11) on the Columbia and Kootenay River systems on August 18 and 19, 2001. Samples were collected from the top 2−3 cm of fine-grained silt, clay, and organic materials within 2 m of the shoreline. All materials for collecting sediments were stainless steel or amber glass and were washed with successive rinses of tap water, distilled water, 95% ethanol, and acetone in the field prior to collection. Sediments collected at each site were placed in solvent rinsed amber glass jars and stored on dry ice during sampling and transport and at −20 °C in the laboratory prior to processing and analysis (<30 d to processing from sampling date).

Sample Extraction, Cleanup, and Analysis. Fish muscle tissue was thawed, dissected, and homogenized prior to extraction. Skin and bones were removed during dissection, and the tissue was homogenized with a commercial meat grinder. Following homogenization, samples were subsampled for contaminant analysis and moisture and lipid contents.

Sediment samples were thawed to room temperature, and approximately 10 g (wet) was weighed out for extraction. Additional samples (~2 g) were also taken for moisture content, organic carbon, and organic nitrogen analysis. Moisture content was determined by drying the sample in a 105 °C vented oven for 48 h and weighing the sample before and after drying. Organic carbon and organic nitrogen content were determined using a Leeman CE440 elemental analyzer.

Approximately 10 g of sample (tissue or sediment) was spiked with a suite of ^{13}C-labeled PBDE, PCDD, PCDF, and PCB procedural internal standards (Cambridge Isotope Laboratories; Andover, MA) and processed using procedures described in detail previously (*3, 4*). Tissue samples were ground with sodium sulfate, transferred quantitatively to an extraction column with, and extracted with, CH_2Cl_2/hexane (1:1 v/v). Sediment samples were Soxhlet extracted with toluene/acetone (80:20 v/v), and the extract was acid−base washed prior to other cleanup. Sample cleanup took place in three stages. In the first step, aliquots were passed through a multilayer silica column packed with successive layers of silica gel (basic, neutral, acidic, neutral) and eluted with CH_2Cl_2/hexane (1:1 v/v). The second cleanup step was with a neutral activated-alumina column capped with anhydrous sodium sulfate. The column was washed with hexane followed by CH_2Cl_2/hexane (1:1 v/v) elution to recover the analytes of interest. Eluants from the alumina column were concentrated to less than 10 μL and spiked with 1 ng of a ^{13}C-3,3',4,4'-tetrabromodiphenyl ether instrumental internal standard (10 μL of a 100 pg/μL stock solution) prior to congener-specific PBDE analyses by high-resolution gas chromatography/high-resolution mass spectrometry (HRGC−HRMS).

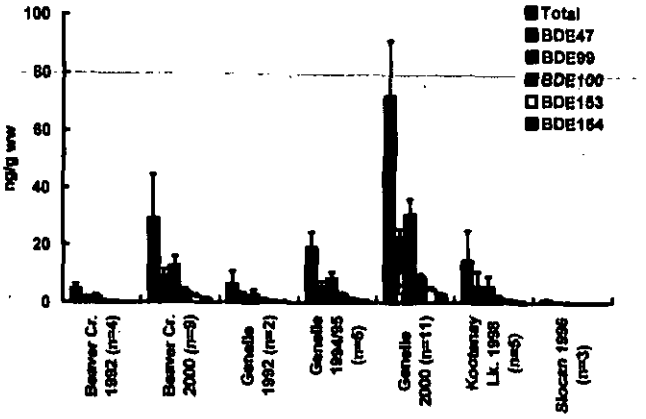

FIGURE 2. Concentrations of total PBDEs and the five major congeners in mountain whitefish muscle from the Columbia River, British Columbia, Canada. Labels (e.g. Genelle 2000 (n = 11)) indicate sampling location, year, and sample size, respectively. Values are in ng/g wet weight and are age normalized using ANCOVA.

Where PCB and PCDD/F analyses were also required from the same sample, the eluant concentrate collected from the alumina column was fractionated with an automated high-performance liquid chromatography (HPLC) system utilizing a carbon fiber column. The four fractions (fraction #1 = di- through tetra-ortho PCBs; #2 = mono-ortho PCBs; #3−4 = nonortho PCBs; #4 = PCDD/Fs; #1−4 = PBDEs) collected from this system were spiked with the corresponding instrumental internal standard(s) and analyzed for the desired target analytes (i.e. PCBs and PCDD/Fs) by HRGC−HRMS. All HRGC−HRMS analyses were performed with the HRMS operating in the positive EI ionization mode at 10 000 resolving power and acquiring data under SIM conditions. The sample workup and the instrumental analyses conditions (for all analyses; PBDEs, PCBs, and PCDD/Fs) and the criteria used for identification and quantitation are described in detail in our previous works (*3, 4*).

Data Analysis. Data compilation and graphing were performed using Microsoft Excel XP (Redmond, WA). Differences between sampling groups were investigated using single factor ANOVA in SPSS v. 10.0 (Chicago, IL). As has been observed elsewhere (*27−29*), PBDE concentrations were positively correlated with fish age and were thus age-normalized using ANCOVA for those sample groups having different mean ages and age distributions. Cluster analysis (with the standardized Euclidean measure and Ward clustering method) was performed using KyPlot v.2.0 b.9 (Tokyo, JPN). Because of the difficulty in representing multivariate data sets, such as multiple congener contributions to total PBDEs, on conventional two- and three-dimensional graphs (e.g. scatter and bar graphs), cluster analysis (CA) was developed as a means of showing similarities and differences of multivariate data sets in a two-dimensional form. A brief description of interpreting the CA plot shown in Figure 4 is presented below.

Results and Discussion

Increasing PBDE Concentrations in Mountain Whitefish. Concentrations and congener patterns of the brominated

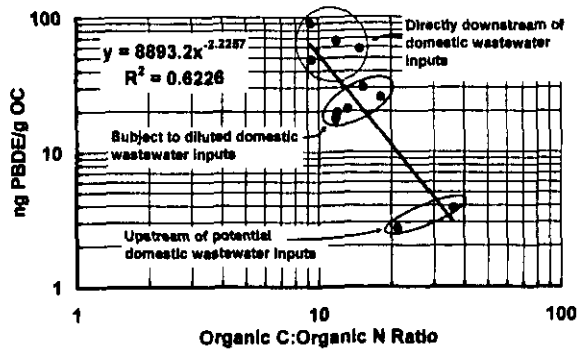

FIGURE 3. Relationship between total PBDE concentrations in Columbia River surficial sediments and the ratio of organic carbon to organic nitrogen content.

flame retardants, polybrominated diphenyl ethers (PBDEs), were determined in 41 mountain whitefish, 6 largescale suckers, and 11 surficial sediment samples from the Columbia and Kootenay River systems in southeastern British Columbia, Canada (Figure 1). Total PBDE concentrations (ΣPBDE; sum of the 13 major congeners shown in Figure 4) in mountain whitefish obtained near Genelle and Beaver Creek on the mainstem of the Columbia River have increased by factors of 11.8 and 6.5, respectively, over the period from 1992 to 2000 (Figure 2). At Genelle (population 800), located approximately 13 km downstream from the city of Castlegar (population 7400) and the Celgar bleached softwood kraft pulp mill, mean ΣPBDE concentrations increased 3.1-fold from 6.1±4.6 ng/g wet weight (ww) to 19.1 ± 5.3 ng/g ww between 1992 and 1994/1995 ($p < 0.011$). The period from 1994/95 to 2000 saw a further increase by a factor of 3.8 to a mean ΣPBDE concentration of 71.8 ± 19.0 ng/g ($p < 3.1 \times 10^{-5}$). The total increase over the period from 1992 to 2000 at Genelle is over an order of magnitude (11.8-fold; $p < 1.2 \times 10^{-5}$). At the confluence of Beaver Creek and the Columbia River, approximately 25 km downstream from the community of Genelle and 9 km downstream from the city of Trail (population 35 300) and the Teck Cominco zinc and lead smelting operation, ΣPBDE concentrations increased by a factor of 6.5 from 4.5 ± 1.8 ng/g ww in 1992 to 29.2 ± 15.4 ng/g ww in 2000 ($p < 0.005$). In the region where the west arm of Kootenay Lake (regional pop. ~34 000 along lakeshore) discharges into the Kootenay River near the city of Nelson (population 9700) and 30 km upstream from the confluence of the Columbia and Kootenay Rivers, ΣPBDE concentrations in mountain whitefish from 1998 were

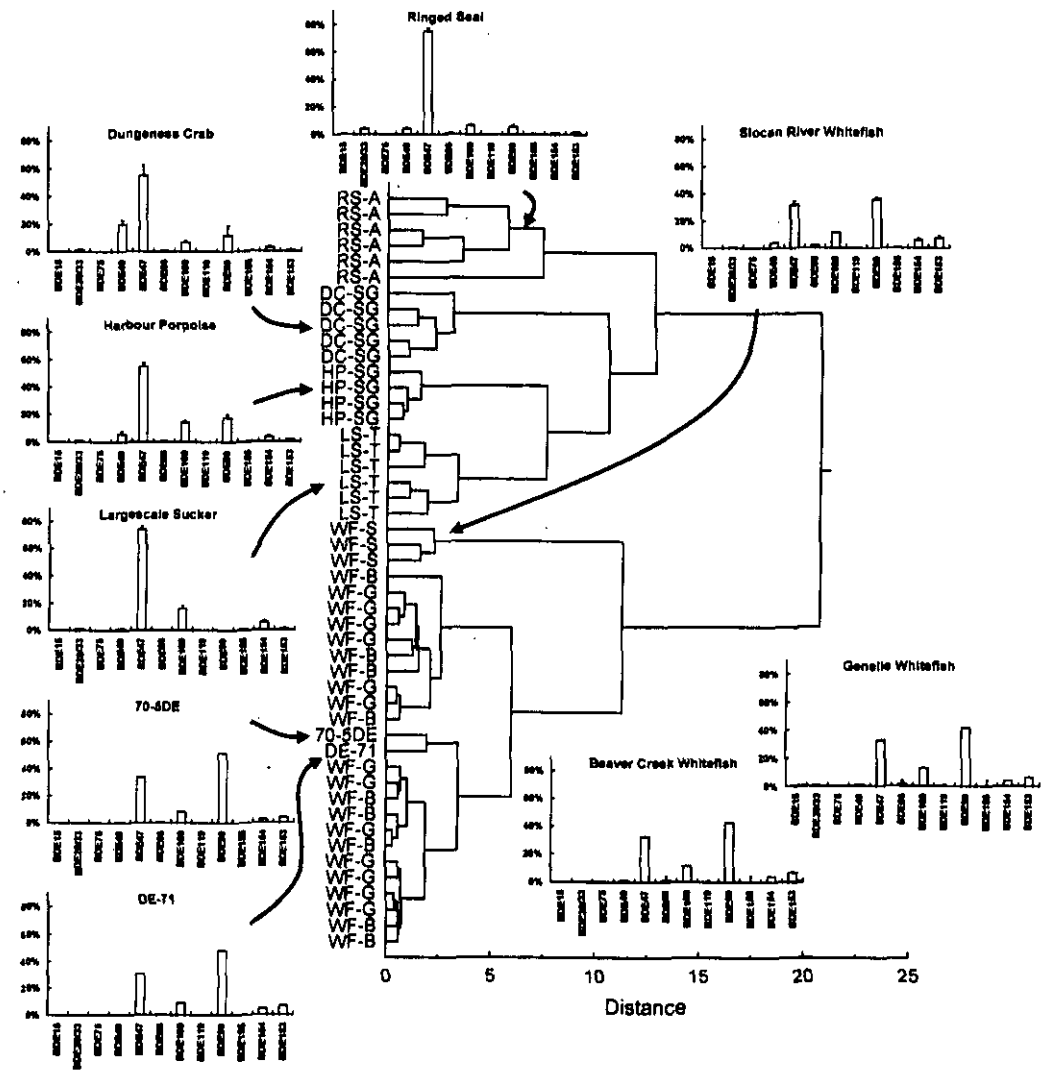

FIGURE 4. Cluster analysis plot of PBDE congener patterns in Columbia River mountain whitefish muscle from Genelle (WF-G) and Beaver Creek (WF-B) collected in 2000, male ringed seal blubber in individuals aged 0−15 years from the Canadian Arctic collected in 2000 (RS-A), Dungeness crab hepatopancreas from the Strait of Georgia off the southwestern coast of British Columbia collected in 1996 (DC-SG), harbor porpoise blubber from the Strait of Georgia collected in 1996 (HP-SG), largescale sucker muscle from near Taghum in the Kootenay River collected in 2000 (LS-T), and two major penta-BDE mixtures (Bromkal 70-5DE and Great Lakes Chemicals DE-71).

significantly lower (14.3 ± 10.4 ng/g) than observed at Beaver Creek or Genelle in 2000 ($p < 0.012$ and $p < 1.3 \times 10^{-5}$). The increase in ΣPBDE concentrations at Genelle is unprecedented in the published literature, with a doubling period of 1.6 years between 1995 and 2000.

On the Slocan River reference site, in an unpopulated pristine area not directly impacted by anthropogenic activities, ΣPBDE concentrations in 1996 were 0.9±0.2 ng/g ww. These concentrations at Slocan are ~20−50 times lower than those at Genelle in 1994/1995 and Beaver Creek in 1996, respectively (single data point for 1996 at Beaver Creek provided in the Supporting Information). The significantly lower ΣPBDE concentrations at Slocan compared to Genelle and Beaver Creek suggests long-range atmospheric deposition is not likely the major source of PBDEs to this region, otherwise we would not expect to see such distinct differences depending on locale. However, the Columbia River at Genelle and Beaver drains a much larger area (~90 000 km²) (*21*) than the Slocan River (~3300 km²) (*30*), which may allow in-stream concentration of high levels of atmospherically deposited PBDEs. On the other hand, the ratio of drainage areas between the Columbia River and the Slocan River (~27) is similar to the ratio of mean flows between these two rivers (2010 and 88 m³/s, respectively; ratio ≈ 23) (*21*, *30*), suggesting that a differential concentration of atmospherically deposited PBDEs between the two watersheds is likely minimal and insufficient to explain the large ΣPBDE concentration differences. Thus, there appears to be a regional source of PBDEs discharging directly into this part of the Columbia River system. Furthermore, the lower ΣPBDE concentrations in whitefish from Kootenay Lake (volume ≈ 36.7 km³) (*31*) compared to the Columbia River are consistent with dilution of influent PBDE sources to the lake, such that there is a reduced fish exposure to PBDEs via the water column in Kootenay Lake versus the Columbia River between Castlegar and the United States border. Knowledge regarding the population distribution, and the suspected domestic wastewater sources of PBDEs in this area (discussed below), appears to support this hypothesis and help understand the spatial distribution of PBDE burdens in whitefish from this region.

PBDE concentrations were also determined in six large-scale suckers sampled in 2000 downstream of the city of Nelson on the Kootenay River at Taghum. These fish had lower PBDE burdens (5.0 ± 1.9 ng/g ww) than whitefish from either the upstream Kootenay Lake ($p < 0.08$) site or the Genelle ($p < 1.3 \times 10^{-5}$) and Beaver Creek ($p < 0.013$) sites on the Columbia River. The lower concentrations found in suckers versus whitefish, especially compared to Kootenay Lake whitefish (likely exposed to more dilute PBDE concentrations), suggests that whitefish may be able to accumulate PBDEs to a greater extent than suckers. Similar findings have been reported between suckers and mountain whitefish and rainbow trout from pristine and populated watersheds in nearby Washington State (*32*). The differing PBDE concentrations between whitefish and suckers occupying similar habitats may result from their relative trophic status. Whitefish are insectivores and piscivores versus the benthic omnivorous behavior of suckers (*32*), and thus the higher PBDE burdens in whitefish may result from an additional bioaccumulatory step up the local food chain (*32*, *33*). Differential metabolic capacities of the two fish species may also help explain the differences, although only qualitative data are available to support this hypothesis (*32*).

Domestic Wastewater as the Major Sources of PBDEs in this Region of the Columbia River. The rapidly increasing PBDE concentrations in whitefish from the Columbia River in southeastern British Columbia, Canada, warranted further investigation into potential sources of these compounds to aquatic systems. We have recently shown that a congener-specific reconstruction procedure using semipermeable membrane devices (SPMDs) deployed in the heavily urbanized and industrialized lower Fraser River (population 2 000 000) suggested that relatively unaltered present-day commercial penta- and octa-BDE formulations are the likely source of PBDEs to this aquatic system (*34*). However, our analysis did not investigate where these PBDEs might be coming from. To better understand potential sources of PBDEs in this region of the Columbia River system, 11 surficial sediment samples (top 1−2 cm of fine organic sediment) were taken from a range of locations throughout the study region (see Figure 1 for locations and Supporting Information Table 1 for concentrations). PBDE concentrations in these surficial sediments ranged from 3.8 to 90.9 ng ΣPBDE normalized to organic carbon content (ng ΣPBDE/g OC), with no prima facie spatial pattern. Other than the same three major congeners (BDEs 47, 99, and 100), congener patterns differed between sediments and whitefish from the same region. BDE47 was the major congener in surficial sediments (46−63% of ΣPBDE), with lesser quantities of BDEs 99 (23−39%) and 100 (6−8%). Interestingly, concentrations of the two major hexa-BDE congeners usually found in the environment and technical mixtures, BDEs 153 and 154, were below detection limits (0.1−0.5 pg/g) in all sediment samples. That BDE99 contributions were greater in whitefish than sediments is unique because previous studies have suggested that BDE99 is significantly less amenable to biological uptake than BDE47. To our knowledge, no previous studies have shown free-swimming aquatic biota (from plankton to fish to marine mammals) with BDE99 as the major congener and a percent contribution similar to major penta-BDE commercial mixtures (*27*, *28*, *32*, *35*−*41*). Thus, sediments are usually enriched in BDE99 contribution compared to aquatic biota, in contrast to our findings here.

The difference in congener patterns between sediments and whitefish in the Columbia River system, in a manner contrary to what is expected and has been reported elsewhere, suggests one or more of the following processes may be operating. Whitefish could have a unique uptake, metabolism, storage, and excretion (UMSE) mechanism distinct from other fish species studied to date (e.g. trout, pike (*42*−*44*)), resulting in preferential accumulation of BDE99 over BDE47. Such a mechanism may be reasonably postulated provided there are no size-limited diffusion restrictions on BDE99 accumulation in whitefish, as the log K_{ow} for BDE99 (~6.5) is slightly greater than that for BDE47 (~6.1) (*13*), thereby favoring bioaccumulation of BDE99. In addition, the PBDE sources that whitefish receive much of their burdens from could be different from the sources to the surficial sediments. However, previous work has shown the whitefish to spend much of their spawning, rearing, and feeding time in the same regions from where sediment samples were collected (*22*, *45*). Indeed, immediately downstream (~1−2 km) of Genelle is a known spawning area and high use rearing habitat for whitefish (*22*). Additional spawning areas and high to moderate use rearing areas are also located adjacent to the remaining sediment samples. Thus, it seems unlikely that sediments and whitefish would be exposed to different PBDE sources when residing in the same area.

TABLE 1. Concentrations of Total PBDEs, PCBs, and PCDD/Fs in Columbia River Whitefish from 1992 to 2000[a]

	ΣPBDE (ng/g ww)	ΣPCB (ng/g ww)	ΣPCDD/F (pg/g ww)
Beaver Creek 1992	4.5 ± 1.8	70.3 ± 41.4	73.6 ± 32.3
Genelle 1992	6.1 ± 4.6	89.9 ± 45.6	43.3 ± 27.9
Genelle 1994/1995	19.1 ± 5.3	86.2 ± 62.6	25.6 ± 20.1

[a] Error bars are 95% confidence limits about the mean.

Another hypothesis is that congener-specific partitioning occurs among sediment grains of different sizes and that the smaller grains are enriched in the contribution of higher brominated congeners such as BDEs 99 and 100. This postulate is consistent with the results of previous studies looking into the congener specific partitioning of halogenated contaminants onto sediments of differing sizes (*14, 16, 46–52*). Because of the generally greater organic content and surface area of smaller sediment fractions (i.e. colloidal and particulate organic carbon, organic detritus, silts and clays), heavier congeners have a greater affinity for these sediments than smaller congeners based on equilibrium partitioning theory. Wide flow variations occur in this region of the Columbia River because of the large spring/early summer freshet in May–late June (*22*) followed by the dry season from July–April (range of flows from 172 to 9340 m^3/s at the hydrographic station at Birchbank (sample site S8) (*53*). In combination with seasonal, weekly, and daily discharge variations from two dams (Hugh Keenleyside Dam and Brilliant Dam) immediately upstream of the study region on the Columbia and Kootenay Rivers, respectively (Figure 1), large flows remove much of the bedload smaller than sand and gravels each year at freshet time and continuously throughout the seasons (*20, 22*). In addition, the Columbia River downstream of Castlegar is generally characterized as having moderate to high water velocities with few depositional regions (*20, 22*) and increased bed scour because of the upstream dams which remove much of the sediment load arriving from the headwaters (*54*), thereby favoring little year-to-year accumulation of fine sediments. Overall, because the UMSE processes for PBDEs in whitefish are at present unknown, and because it is unlikely fish and sediments residing in the same region in the shallow (< 12 m as provided in refs *20* and *22*) waters of the Columbia and Kootenay rivers would be exposed to different PBDE sources, the differing congener patterns between whitefish and sediments in the these aquatic systems likely results from the preferential transport of higher brominated congeners out of the local system due to the continual erosion of fine sediments. In addition, whitefish are known to feed heavily on caddisflies in streams. Caddisflies, in both the larval and adult stages, tend to produce webs to capture fine suspended material as a food source. These feeding habits by both whitefish and their caddis fly prey may help explain both the higher PBDE concentrations and predominance of higher brominated congeners we observe in whitefish, as fine suspended sediments tend to concentrate contaminants, especially the more hydrophobic congeners (i.e. BDE99 over BDE47) as discussed above. The intake of fine, suspended particulates by whitefish in this aquatic system may be a major pathway of exposure to higher brominated congeners not generally reported to date in other aquatic systems (e.g. lakes and marine environments) where PBDE concentrations and patterns have less direct dietary influence from sediment associated contaminants than in the present study.

At a first approximation, no obvious point sources of PBDEs (e.g. furniture and textiles production (*28*) or PBDE manufacturing plants) were evident along the Columbia River downstream from Castlegar to the Canada–United States border. Sampling sites were chosen to surround the following potential sources identified from topographic map and "on-the-ground" surveys: automobile "wrecking" operations (potential leaching from polyurethane foams) located immediately adjacent to watercourses, landfills situated within 1 km of the Columbia River or along tributaries, major industries (e.g. pulp and paper mills, hydroelectric dams, smelters) which may utilize or produce PBDEs, below forest fire sites on erodible slopes, adjacent to agricultural land where biosolids may have been applied, and the Castlegar and city of Nelson municipal sewage outfalls. No clear trends were observed in PBDE concentrations compared to any of these potential sources. However, when ΣPBDE concentrations were plotted against the ratio of organic carbon to organic nitrogen (OC:ON) content in each sample, a moderate negative log–log correlation ($R^2 = 0.62$) was observed (Figure 3). Both municipal wastewater and landfill leachate have been previously shown to have decreased OC:ON ratios compared to natural aqueous samples (*55–60*). While no studies have examined OC:ON ratios in leachate from automobile wrecking operations, OC:ON ratios in such leachate are expected to be higher than that of wastewater and landfill leachate because there is not an appreciable source of nitrogen in the source material (e.g. rubber, polyurethanes, and mostly other hydrocarbon polymers) (*61, 62*). Upon further examination of the sediment samples with > 10 ng ΣPBDE/g OC, these locations are near potential point and nonpoint (e.g. septic fields) sources of domestic wastewater, and higher concentrations do not correlate well with known landfill sites. In addition, landfills in the region tend to be located more distant from waterways than populations residing directly adjacent to aquatic systems. Thus, sorption of PBDEs onto saturated subsurface soils will likely hinder their movement in landfill leachate and the resulting groundwater, thereby reducing the effects of landfill leachate relative to the more direct wastewater inputs.

Similar results suggesting municipal wastewater sources of PBDEs to river sediments and biota have been previously discussed (along with the likely exclusion of landfill leachate as a significant source (*40*)) or is evident from the work of other researchers (*28, 40*). PBDE congener patterns in some municipal wastewater biosolids are also similar to the commercial penta-BDE mixtures (*63*), suggesting that municipal wastewater contains similar congener patterns to these technical mixtures and that the land application of the resulting sludge may also be a potential source of PBDEs to aquatic systems through leaching and subsequent transport processes. Higher PBDE concentrations were not observed in sediments immediately adjacent to the limited number of agricultural areas in this region. This suggests the application of PBDE-contaminated biosolids as an agricultural nutrient amendment, and subsequent leaching processes, is not a major PBDE input to this aquatic system. Furthermore, as noted elsewhere for riverine environments enclosed by hydroelectric facilities (*28*), a marine source of PBDEs (either from anthropogenic or natural (*64*) sources) to the whitefish from this portion of the Columbia River is not likely because the number of dams present in the United States region of the river prevents upstream movement of aquatic biota (*20–22*). Overall, the ΣPBDE concentrations in sediments reported here are in the lower end of the range observed elsewhere (*28, 35, 36, 40, 41*), possibly because of the general lack of good depositional areas for fine particulate organic matter, silts, and clays in the mainstem of the Columbia River. In various sediments worldwide, BDE99 has been generally found at a higher contribution to ΣPBDE than BDE47 (*35, 36, 40*), although one instance where BDE47 dominated the congener pattern in sediments has also been observed (*28*). The absence of BDEs 153 and 154 in these surficial sediments, and dominance of BDE47 over the more hydrophobic BDE99, has been discussed above.

A number of small (population <500) communities are prevalent along waterways through the study area, and, in sum, the population of these "rural" residents outweigh those living in the two regional cities (population of 57 000 in the Regional District of Central Kootenay vs 16 300 in Castlegar and Nelson). Thus, since most of these residents live along the shores of the Columbia and Kootenay Rivers and Kootenay Lake, rural septic field inputs to regional waterways would be expected to dominate those from Castlegar (secondary activated sludge treatment) and Nelson (primary

settling treatment). In essence, this "spreads out" the source of PBDEs throughout the region, assuming no large industrial point sources, which were not observed according to our sampling pattern and topographic and field reconnaissance. Furthermore, septic leachate is largely untreated compared to the municipal discharges, and this may favor the release of congener patterns more similar to commercial mixtures. This may help to explain why, if PBDEs were released from municipal wastewaters in the Kootenay and Columbia River systems, our sediment sampling grid did not detect higher PBDE levels near the major communities of Castlegar and Nelson.

Comparisons with PCBs and PCDD/Fs. PCB and PCDD/F concentrations were also determined in whitefish from Genelle in 1992 and 1994/1995 and Beaver Creek in 1992 (Table 1) (*25*, *26*). While total PCB (ΣPCB) and PCDD/F (ΣPCDD/F) concentrations declined slightly at Genelle from 1992 to 1994/1995 following installation of an air-activated sludge secondary treatment system and 100% ClO_2 substitution for Cl_2 in the bleaching process at the pulp mill upstream of Castlegar (*26*), ΣPBDE concentrations increased by a factor of 3.1 over this period of time. If PBDE concentrations continue to rise at Genelle in the same manner as they have since 1992 while PCB levels remain constant or decline, ΣPBDE concentrations in these whitefish will surpass those of ΣPCB (86 ng/g ww) by 2003. Likewise at Beaver Creek, the increasing ΣPBDE concentrations will exceed ΣPCB (70 ng/g) by 2013. Thus, in a relative short period of time (1−2 decades), compared to PCBs when they were commercially produced in North America for five decades from the 1930s to the late 1970s, PBDEs are overtaking PCB burdens in biota from some regions. Such findings further attest to the ready transport and bioaccumulation/biomagnification of PBDEs, a perhaps ironic result if the manufacturing companies had, in fact, chosen these compounds as commercial flame retardants not only because of their inherent fire suppression properties but also because the large molecular size, mass, and hydrophobicity of these compounds was thought to hinder environmental transport and biotic uptake.

PBDE Congener Patterns in Whitefish Compared to Other Aquatic Biota. PBDE congener patterns in whitefish from the Columbia River are remarkably similar to that of the commercial penta-BDE mixtures from Great Lakes Chemical Corporation (DE-71) and Bromkal (70-5DE) (Figure 4). Comparisons among these samples were made on the basis of the relative contributions of the 13 major congeners with >30% of values above MDLs. Concentrations of individual congeners in the two technical mixtures are provided elsewhere (*34*). The key to interpreting cluster analysis plots as shown here is to compare the *x*-axis values where the lines from different samples (and/or sample groups or clusters) meet. The larger the *x*-axis value (or "distance"), the lower the similarity in multivariate patterns between the two samples or groups. In sharp contrast, congener patterns in whitefish from the unpopulated reference area on the Slocan River were distinct from those found in whitefish in the Columbia River, as measured by distance on the cluster analysis plot. Congener patterns are similar in whitefish from the two Columbia River sites (Genelle and Beaver Creek), suggesting a related source of PBDEs for these locations. This difference in PBDE congener patterns between populated and reference sites further suggests that atmospheric deposition (which is likely the only PBDE source for the Slocan River whitefish) is not likely the major PBDE source for whitefish in the Columbia River. Preferential removal of the higher brominated BDEs 99, 153, and 154 from the Columbia River system due to hydrological regimes has been discussed above and may help explain congener pattern differences between the Columbia River whitefish and those from the Slocan River reference site. While a previous report suggested a similarity in congener patterns between DE-71 (the major North American penta-BDE product) and sediments and sewage sludge, these comparisons were made using only the five major congeners (BDEs 47, 99, 100, 153, and 154) and with the assumption that DE-71 contained the same relative proportions of these congeners as 70-5DE (*35*) for which congener distributions had been published (*65*). Previous work has shown DE-71 to contain slightly higher proportions of BDEs 153 and 154, and lower proportions of BDEs 47 and 99, compared to 70-5DE (*34*), although there may be wide variation in the congener patterns between different batches of each commercial product. Although such qualitative insights using the five major congeners are valuable, full congener data and some type of statistical evaluation are needed to provide some measure of confidence on congener pattern comparisons.

Large variations in congener patterns for species occupying similar locales are also evident in the cluster analysis. Both whitefish from Genelle and Beaver Creek have similar congener patterns to whitefish from Kootenay Lake (data not included in cluster plot for clarity but are provided in the Supporting Information; BDE47: 32.7 ± 2.4%, 32.3 ± 2.4%, and 36.3 ± 6.5%; BDE99: 41.6 ± 3.9%, 42.7 ± 3.9%, and 39.0 ± 4.7%; BDE100: 12.7 ± 1.3%, 11.4 ± 1.2%, and 11.0 ± 1.5%; respectively, for Genelle, Beaver Creek, and Kootenay Lake); these patterns are quite distinct from largescale suckers sampled near Taghum on the Kootenay River (BDEs 47, 99, and 100: 73.7 ± 2.5%, 0.2 ± 0.1%, and 16.2 ± 1.9%, respectively). Taghum is located approximately 11 km west of the outlet from Kootenay Lake, and both sites are expected to have the same ambient PBDE patterns. Thus, there appear to be significant species-specific UMSE processes between whitefish and largescale suckers. This is particularly evident in the low contribution of BDE99 in the largescale suckers compared to whitefish (0.2% vs 39.0%), which cannot otherwise be explained in this system where BDE99 seems prevalent as the first or second most abundant congener. Congener profiles in ringed seals from the Canadian Arctic and harbor porpoise and Dungeness crab are also provided for comparison. These samples have distinct congener patterns from the Columbia River fish, further demonstrating the combined effects of species type and location on PBDE patterns.

Acknowledgments

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Supporting Information Available

PBDE concentrations for all individual fish and sediment samples. This material is available free of charge via the Internet at http://pubs.acs.org.

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A comparison of the properties of the major commercial PBDPO/PBDE product to those of major PBB and PCB products

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Abstract

Decabromodiphenyl oxide (DBDPO), a highly effective polybrominated diphenyl oxide (PBDPO) flame retardant (FR) used primarily in electrical and electronic equipment, is the second highest volume brominated flame retardant (BFR) and accounts for 82% of the PBDPO usage globally. The apparent similarities in chemical structure between the DBDPO, polychlorinated and polybrominated biphenyl (PCB, PBB) molecules have led to the presumption that these substances also share similar toxicological and environmental properties. However, DBDPO's physical/chemical properties, applications, environmental release, and toxicology differ substantially from the former PCB/PBB products. DBDPO is a heavier and larger molecule than components of the predominant PCB/PBB products used in the past, and the commercial DBDPO product has a lower water solubility and vapor pressure than the former PCB and PBB products. DBDPO's detection in the environment is generally in sediments near known point sources, and its primary use in thermoplastics limits its environmental release from end products. PBB environmental release has been primarily associated with one accident occurring in the US in 1973. The PCBs, used in applications with a high potential for environmental release, were detected in diverse locations around the world as early as in the 1970s. Current releases of PCB are considered related to an environmental cycling process of congeners previously released into the environment; however, DBDPO's physical/chemical properties do not indicate a similar potential. Extensive testing of the DBDPO commercial product has demonstrated that it is toxicologically and pharmacokinetically different from the predominant PCB and PBB products used in the past. Thus, although the chemical structures of DBDPO, PBB, and PCB appear similar, the properties of DBDPO are distinctly different.

Keywords: PBDPO; PBDE; Polybrominated diphenyl ether; Brominated flame retardant

1. Introduction

Brominated flame retardants (BFRs) comprise about 25% of the total global flame retardant (FR) volume and are used in applications requiring high FR performance or in resins needing an FR active in the gas phase (Hardy, 2000). The bromine portion of the compound is responsible for the molecule's FR activity and is unique in its ability to provide flame retardancy in the gas phase. Societal benefits derived from these FRs include a reduction in deaths, injuries and property losses due to fires (Clarke, 1997; Stevens and Mann, 1999). Environmental benefits include a reduction in fire-derived pollution (Simonson et al., 2000).

BFRs as a class are structurally diverse and include aromatic diphenyl oxides (a.k.a. ethers), cyclic aliphatics, phenolic derivatives, aliphatics, phthalic anhydride derivatives and others (2). Three commercial products, decabromodiphenyl oxide (DBDPO), octabromodiphenyl oxide (OBDPO) and pentabromodiphenyl oxide

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(PeBDPO), compose the BFR group known as polybrominated diphenyl oxides (PBDPOs) or polybrominated diphenyl ethers (PBDEs). The composition, production volumes, uses and toxicology of the three commercial PBDPO FRs DBDPO, OBDPO and PeBDPO are distinctly different (Hardy, 2002).

DBDPO (CAS #1163-19-5) is a highly effective FR used primarily in electrical and electronic equipment. A secondary, but nonetheless important, use of DBDPO is in upholstery textiles where it is applied in a polymer back coat to the fabric. Global market demand in 1999 for DBDPO was estimated at 54,800 ton, which makes DBDPO the second largest volume BFR in use today after tetrabromobisphenol A (TBBPA) (BSEF, 2001). Together, TBBPA and DBDPO account for approximately 50% of all the BFR usage globally. DBDPO's global market volume exceeds those of the other two commercial PBDPO products in 1999: OBDPO – 3825 ton and PeBDPO – 8500 tons. Market demand, 1999, for DBDPO in the regions of the Americas, Europe and Asia was 24,300, 7500 and 23,000 tons, respectively. These regional differences reflect differences in location of end product manufacture.

Some literature references include statements, apparently based on their similar chemical structures (Fig. 1) that the PBDPO FRs have characteristics similar to the former polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs) commercial products (Pijnenburg et al., 1995; de Boer et al., 1998; Eriksson et al., 1998; Van Overmeire et al., 2001). Inherent in these statements is the assumption that all PBDPOs, including DBDPO, pose the same risks as the PCB. However, there are substantial differences between the commercial PCB, PBB and DBDPO products in composition, applications, physical/chemical properties, and toxicology and pharmacokinetics. These differences affect their potential for environmental release and biological uptake. The objective of this paper is to bring these differences to light.

2. Composition and uses of the PCB, PBB and DBDPO commercial products

Commercial production of the PCB was initiated in the 1930s, and a cumulative volume of 501,600,000 kg was sold in the US by the end of their manufacture in the 1970s (Table 1) (Pomerantz et al., 1978; Polychlorinated biphenyls, 2000). A total of 12 different commercial PCB products, marketed by their chlorine content, were sold in the US. Each was a complex mixture of isomers, which included 4–5 congeners, and had chlorine content ranging from 21% to 68% (Tables 1 and 2). Polychlorinated dibenzofurans (PCDFs) and chlorinated naphthalenes were present as trace impurities in some products (Polychlorinated biphenyls, 2000). PCBs were not used primarily as FRs, but some of their applications, such as in electrical transformers and capacitors, took advantage of their high thermal stability. Common applications of the PCB products included use as dielectric fluids in large volume electrical transformers and capacitors, hydraulic fluids, paperless copies, adhesives, and inks. Transformers contained up to 690 gallons of PCB (O'Keefe et al., 1985). These uses all had a relatively high potential for environmental release, such that by 1970, PCBs were recognized as pervasive worldwide environmental contaminants (Pomerantz et al., 1978). Due to the complex composition of the commercial PCB products, approximately 100 different PCB congeners have reached the environment (Pomerantz et al., 1978).

Commercial production of the PBB began in approximately 1970 (NTP, 1983), 40 years after the PCB (Table 1). During their peak use period, PBB products represented <1% of the total sales of fire retardant chemicals, and, according to Di Carlo et al. (1978), would likely have escaped intensive study if they had not been accidentally mixed with animal feed preparations. PBB manufacture was discontinued in the US in 1976 at a cumulative volume of 6,065,454 kg (Di Carlo et al., 1978) and as a result PBB production, distribution and use was not as widespread as the PCB (Pomerantz et al., 1978). Only three commercial PBB products were manufactured, hexabromobiphenyl (HxBB), octabromobiphenyl (OBB) and decabromobiphenyl (DBB), and these three products were based on a limited number of congeners (Norris et al., 1975; Pomerantz et al., 1978; NTP, 1983). The HxBB product, accounting for ~87% of PBB products manufactured, was the primary PBB product in use with ~5,363,636 kg produced between 1970 and 1974 when its manufacture was discontinued

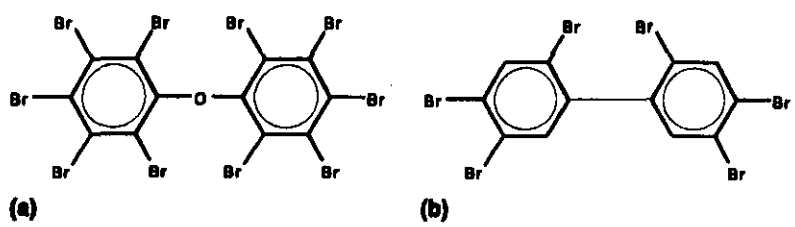

Fig. 1. Chemical structure of decabromodiphenyl oxide (a) and 2,4,5,2',4',5'-hexabromobiphenyl (b).

Table 1
Comparison of PCB, PBB and DBDPO commercial products and their applications

Parameter	PCB	PBB	DBDPO
Commercial production initiated	1930s	1970	1970s
Cumulative volume sold in US by end of production (kg)	501,600,000	5,800,00	In commercial production 1999 volume ~50,000,000
Commercial products sold in US	12 Products of 21–68% Cl content Each product a complex mixture of 40–50 isomers and 4–5 congeners	Three products based on HxBB, OBB or DBB HxBB Product: ~83% of PBB products sold HxBB Product: composition ~60% 2,2',4,4',5,5'-HxBB	1 Product composed of ⩾97% DBDPO
Use and associated potential for release	Large volume electrical transformers capacitors – high Hydraulic fluids – high Paperless copies – high Adhesives high Inks high	Thermoplastic (ABS) used in office equipment – low	Thermoplastics (Styrenics) used in electrical and electronic equipment – low Upholstery textile back coat during textile manufacture: higher
Actual environmental release	Recognized as pervasive worldwide contaminants by 1970	Environmental detection primarily associated with 1973 accidental inclusion in livestock feed in Michigan	Environmental detection generally limited to sediments near point sources
Isomers reaching the environment	~100	Few	1

Table 2
Composition of major PCB, PBB and PBDPO commercial products expressed as % of congeners present in each product

#Halogens/biphenyl or diphenyl oxide	Commercial product			
	A1242 (%)	A1254 (%)	HxBB (%)	DBDPO (%)
Mono	3	–	–	–
Di	13	<0.1	–	–
Tri	38	2	–	–
Tetra	30	17	–	–
Penta	22	49	4	–
Hexa	4	28	63	–
Hepta	–	4	33	–
Octa	–	<0.05	–	[a]
Nona	–	<0.05	–	[a]
Deca	–	–	–	⩾97

– Not known to be present.
[a] Sum of Nona- and OctaBDPO content ⩽3%. NonaBDPO predominates.

(NTP, 1983). The HxBB commercial product has been described as containing penta- through heptabromobiphenyl congeners or as tetra- through octabromobiphenyl congeners (Table 2) (Pomerantz et al., 1978; Di Carlo et al., 1978). The predominant component (~60%) of the HxBB product was 2,2',4,4',5,5'-HxBB (Di Carlo et al., 1978). The HxBB commercial product also contained ~200 ppm bromonaphthalenes, and may have contained trace levels of other compounds. Commercial OBB contained at least four compounds; a heptabromobiphenyl, isomeric octabromobiphenyls, and a nonabromobiphenyl (Di Carlo et al., 1978). The major component of commercial OBB was nonabromobiphenyl, and not octabromobiphenyl. Commercial DBB was composed of 96.8% DBB, 2.9% nonabromobiphenyl, and 0.3% octabromobiphenyl (Di Carlo et al., 1978). The sum of OBB's and DBB's production volumes amounted to ~13% of the total PBB production in the US (Di Carlo et al., 1978).

PBB applications were almost exclusively limited to a particular thermoplastic (acrylonitrile-butadiene-styrene, ABS) used in housings for office equipment. According to Pomerantz et al. (1978), the potential for environmental release associated with this use was low because PBB had little tendency to migrate from the thermoplastic in which they were incorporated. Further, only a few PBB congeners were released to the environment due to the limited composition of the commercial products. PBB environmental release/dispersion was confined mainly to sites of former manufacture and

an area of Michigan following the 1973 accidental inclusion of 500–1000 pounds of the HxBB product in livestock feed (Di Carlo et al., 1978). The HxBB-contaminated feed was unknowingly fed to dairy cattle, pigs, chicken and other farm animals over a period of approximately 9 months, and during that time contaminated dairy products and eggs were widely consumed by farmers and their families, and later, by the general population of Michigan (Di Carlo et al., 1978). This incident resulted in the destruction of at least 29,800 cattle, 5920 hogs, 1470 sheep, and 1.5 million chickens. Also removed from the commercial market were at least 865 ton of animal feed, 17,790 pounds of cheese, 2630 pounds of butter, 34,000 pounds of dry milk products and nearly 5 million eggs. Shortly after this accident, all PBB production in the US was voluntarily discontinued (Di Carlo et al., 1978). Re-initiation of manufacture requires prior approval of the US Environmental Protection Agency (1980, 1987). Once US PBB production was discontinued in 1976, the only PBB remaining in commercial production was DBB. Until 2000 when its production was discontinued, a single company manufactured DBB at one location in France (BFRIP, 1999). Although its toxicology was generally favorable, DBB production was discontinued because of its negative perception as a PBB. At present, there are no known manufacturers of commercial PBBs for use as FRs; some synthesis for use as analytical standards may occur. The major global BFR manufacturers had voluntarily committed to OECD in 1995 not to initiate PBB manufacture (BFRIP, 1995).

The commercial production of DBDPO, in commercial production and use, began in a similar time frame to the PBB, e.g., during the 1970s (Table 1). The composition of the commercial product is $\geqslant 97\%$ DBDPO with remainder nonabromodiphenyl oxide; trace amounts of octabromodiphenyl oxide may be present (Ranken et al., 1994; BFRIP, 1995). Trace analysis of the commercial product for 15 2,3,7,8-substituted brominated dibenzo-*p*-dioxins and dibenzofurans revealed no detectable amounts at the limits of quantitation established by the US Environmental Protection Agency (Ranken et al., 1994). The DBDPO molecule exists as only one structural isomer. The commercial product's primarily application is in thermoplastics (typically styrenics) used in electrical and electronic equipment (Existing Substances Regulation 793/93/EEC, 2000). The potential for environmental release associated with this use is low (WHO, 1994). A secondary application is in upholstery textiles where the DBDPO commercial product is encapsulated in a polymer back coat on the fabric (Existing Substances Regulation 793/93/EEC, 2000). The potential for environmental release associated with this textile use is higher than that in thermoplastics because water is used in the back coating process (Existing Substances Regulation 793/93/EEC, 2000). The 1999 US Toxic Inventory Release (TRI) figures for DBDPO demonstrate this difference in environmental release between the two applications (BFRIP, 1999). DBDPO is not a ubiquitous environmental contaminant and its detection in the environment is generally associated with sediments near known point sources (Hardy, 2000).

3. Physical/chemical properties

The PCB congeners composing the commercial products ranged in molecular weight from 291.98 to 360.86 (Tables 2 and 3) (Polychlorinated biphenyls, 2000). Although numerous trade names were used, the "Arochlor" name is widely recognized as associated with PCB. Arochlor (A) products were designed by a set of four numbers, the first two of which indicated that the molecule was a biphenyl (12 carbons) and the last two indicating the product's average chlorine content, e.g., A1221 contained an average of 21% chlorine (Polychlorinated biphenyls, 2000). The commercial products existed as mobile oils (A1221–1248), viscous liquids or sticky resins (A1250–1262) or solids ($A \geqslant 1268$) (Polychlorinated biphenyls, 2000). Their vapor pressures were such that volatilization was possible (Arochlor 1242, 2000; Arochlor 1254, 2000). Similarly, their water solubilities, although low, were sufficient to allow movement into water (Arochlor 1242, 2000; Arochlor 1254, 2000). All PCB products had good solubility in organic solvents (Polychlorinated biphenyls, 2000).

The composition of the commercial PBB products was more restricted, and generally composed of congeners of higher molecular weight than the PCB. For example, the molecular weight of 2,2′,4,4′,5,5′-HxBB, comprising ~60% of the major PBB commercial product (HxBB), was ~2–2.5 times that of the average molecular weight of A1242 and 1254 (Tables 2 and 3) (2,4,5,2′,4′,5′-HEXABROMOBIPHENYL, 2000). The higher molecular weights of the PBB products were due to bromine's high atomic weight and the greater number of halogen atoms/molecule in the PBB products than in the PCB products.

The three commercial PBB products were all solids. 2,2′,4,4′,5,5′-HxBB's vapor pressure and water solubility were lower than those reported for A1254 and 1242, and its solubility in organic solvents variable (Arochlor 1254, 2000). The HxBB product had little tendency to translocate in soil; a laboratory study indicated that HxBB was retained in the topsoil and did not leach with rainwater (Damstra et al., 1982).

Greater than 97% of the commercial DBDPO product is composed of a single isomer: 2,2′,3,3′,4,4′,5,5′,6,6′-DBDPO which is a large molecule with a molecular weight of nearly 1000 (Tables 2 and 3). The commercial product, a solid, has negligible vapor pressure and water

Table 3
Comparison of the physical/chemical properties of the commercial PCB, PBB, and DBDPO products or of their major components

Property	PCB commercial products	PBB commercial products	DBDPO commercial product
Molecular weight	Range: 291.48–360.86 Average: A1242: 261 A1254: 327	Range: 466–943 HxBB Isomer: 627.40	Range: 880–959.22 DBDPO Isomer: 959.22
Form	A1221-28: Mobile oils A1250-62: Viscous liquids or sticky resins *A* > 1268: Solids	Solid	Solid
Vapor pressure	A1242: 4.0×10^{-4} mm Hg at 21 °C A1254: 7.7×10^{-5} mm Hg at 21 °C	7.6×10^{-5} mm Hg at 90 °C[a]	4.3×10^{-6} Pa at 21 °C
Water solubility (μg/l)	A1242: ~200 A1254: 70	11[a]	<0.1
Solvent solubility (wt%)	Soluble in common organic solvents	Acetone: 6[a] Benzene: 75[a]	Acetone: 0.05 Benzene: 0.48
HLC (atm m^3/mole)	A1242: 3.43×10^{-4} A1254: 2.83×10^{-4}	5.7×10^{-3}[a]	1.93×10^{-8}
Log K_{ow}	A1242: 4.11 A1254: 6.30 (estimated)	9.10 (estimated)[a]	12.61 (estimated) 6.265 (measured)
Log K_{oc}	A1242: 3.35-5.17 A1254: 5.0–6.1	5.088[a]	6.254 (estimated)

[a] 2,2′,4,4′,5,5′-HxBB.

solubility (Stenzel and Nixon, 1997; Stenzel and Markley, 1997). DBDPO also has very limited solubility in organic solvents (Norris et al., 1974, 1975; BFRIP, 1999).

The commonly used PCB products had greater volatility than the DBDPO or HxBB commercial products. For example, the commercial DBDPO product's vapor pressure is 4.63×10^{-6} Pa at 20 °C and remains a solid exerting a vapor pressure of only 5 mm Hg at 306 °C (Norris et al., 1973, 1975; Stenzel and Markley, 1997). A1242, with an average of only three chlorine atoms/molecule, boils at 325–366 °C (Arochlor 1242, 2000). DBDPO should not volatilize significantly from plastics even on exposure to temperatures >250 °C, because of its low mobility and vapor pressure (Norris et al., 1973, 1974, 1975). In contrast, A1248 (with an average of four Cl atoms/molecule) volatilized from polyvinyl chloride at a rate of 19% in 24 h when heated to 87 °C. The water solubilities of the PCB and DBDPO products are also different: DBDPO <0.1 μg/l, A1260 (average of six Cl atoms/molecule) ~25 μg/l, and A1242 ~200 μg/l, and DBDPO is less soluble in organic solvents than common PCB products.

The basic properties of three PCB isomers, a HxBB isomer and the DBDPO isomer were estimated (Meylan, 1999) and compared (Table 4). No data other than the chemical structure were entered in the estimation program. Measured values, where known, were not entered in order to remove any variability introduced by differences in analytical methods. The 2,2′,4,4′-TeCB and 2,2′,4,4′,5-PeCB isomers were chosen for estimation as they were important constituents of major PCB commercial products. Likewise, the 2,2′,4,4′,5,5′-HxBB isomer was chosen because it was the major component of the HxBB commercial product, and DBDPO was chosen because it is the major commercial PBDPO produced and used.

Large differences were found in the estimated physical/chemical properties of the DBDPO and PCB isomers (Table 4). DBDPO's molecular weight, log octanol/water partition coefficient (K_{ow}), Henry's law constant (HLC), organic carbon/water partition coefficient (K_{oc}), and water volatilization half-lives were greater than those of the PCB and HxBB isomers. Conversely, DBDPO's water solubility, vapor pressure, and bioconcentration factor were less than those of the PCB and HxBB isomers. Volatilization may be an important mechanism for the loss of chemicals from the soil and transfer to the air, but DBDPO's estimated vapor pressure, water solubility, HLC and volatilization half-life

Table 4
A comparison of the estimated values (EPIwin v3.04) of some basic properties for specific PCB and HxBB isomers and DBDPO

Estimated parameter	Chemical				
	2,2',4,4'-TeCB	2,2',4,4',5-PeCB	2,2',4,4',5,5'-HxCB	2,2',4,4',5,5'-HxBB	2,2',3,3',4,4',5,5',6,6'-DBDPO
Molecular weight	291.99	326.44	360.88	627.59	959.17
Log K_{ow}	6.335	6.9795	7.6240	9.10	12.6147
Water solubility (mg/l)	0.04859	0.00597	0.001281	0.0001598	0.00000000001052
Vapor pressure (mm Hg)	8.45×10^{-6}	2.22×10^{-6}	5.81×10^{-7}	2.49×10^{-9}	2.19×10^{-13}
HLC (atm m^3/mole)[a]	1.25×10^{-4}	9.24×10^{-5}	6.85×10^{-5}	1.65×10^{-6}	1.93×10^{-8}
HLC (unitless)[a]	5.10×10^{-3}	3.78×10^{-3}	2.80×10^{-3}	6.76×10^{-5}	7.88×10^{-7}
K_{oc}	$4.482 \times 10^{+4}$	$7.41 \times 10^{+4}$	$1.225 \times 10^{+5}$	$1.225 \times 10^{+5}$	$1.796 \times 10^{+6}$
BCF	57,980	138,000	25,200	360.7	3.1
River volatilization Half-life (days)	0.2921	0.6419	2.096	27.51	3915
Lake volatilization Half-life (days)	9.156	13.31	29.5	308.9	42,700

[a] Bond method used for estimating the HLC.

indicate volatilization from water into the atmosphere will not occur. DBDPO's K_{oc} and vapor pressure indicate a high potential for adsorption to organic carbon without subsequent volatilization from sediment/soil. DBDPO's molecular weight, log K_{ow}, water solubility and bioconcentration factor indicate bioaccumulation is not expected. This expectation is corroborated with environmental monitoring that indicates no significant bioconcentration of DBDPO (Existing Substances Regulation 793/93/EEC, 2000).

Based on the comparison of their estimated parameters, DBDPO's environmental behavior is not expected to be similar to those of the PCB and PBB isomers. DBDPO can be expected to achieve only trace quantities in water, to adsorb to particulates in water, not to volatilize from water to the atmosphere, to bind to organic carbon in sediment/soil and not to volatilize from these matrixes to the atmosphere. DBDPO's predicted environmental behavior is distinctly different from that predicted for the PCBs. PCBs are expected to volatilize from water and moist soils and to achieve higher concentrations in water. The estimated HLC's and river/lake volatilization half-lives for TeCB, PeCB, and HxCB predict these compounds will volatilize from water surfaces (Arochlor 1242, 2000). Adsorption to sediments and organic matter is expected to be a major fate process in water. The most water soluble PCBs will be enriched in water relative to the sediment, and the sediment will be enriched with the higher chlorinated PCBs (lowest solubilities in water). Their estimated K_{oc}s suggest they will be immobile in soil and should not leach significantly in most aqueous soil systems although the most water soluble PCBs will be leached preferentially. In the presence of organic solvents, which may be possible at waste sites, PCBs may have a tendency to leach through soil. Volatilization from moist soil surfaces is expected to be an important fate process based on their HLCs. Although the volatilization rate may not be rapid from soil surfaces due to the strong adsorption, the total loss by volatilization over time may be significant. Lower chlorinated congeners are expected to sorb less strongly. The estimated vapor pressures indicate the congeners will exist in both the vapor and particulate phases in the ambient atmosphere, with enrichment of PCBs with the highest vapor pressure (e.g., low Cl-content). Physical removal from the atmosphere is expected via wet and dry deposition with dry deposition important only for congeners associated with particulates. Although adsorption can immobilize PCBs for relatively long periods of time in the aquatic compartment, resolution into the water column has been shown to occur suggesting that the substantial quantities of PCBs contained in aquatic sediments can act as an environmental sink for environmental redistribution of PCBs. Current evidence suggests that the major source of PCB release to the environment may be an environmental cycling process of congeners previously released into the environment (Polychlorinated biphenyls, 2000; Arochlor 1242, 2000). The estimated values reported here support that concept.

4. Molecular configuration and size

The structures of representative PCB, PBB and DBDPO molecules appear similar when drawn in one dimension (Figs. 1 and 2). However, there are important three-dimensional differences in their structures due to the ether linkage and the location/number of halogen atoms. These differences in molecular geometries are expected to influence the molecules' toxicological prop-

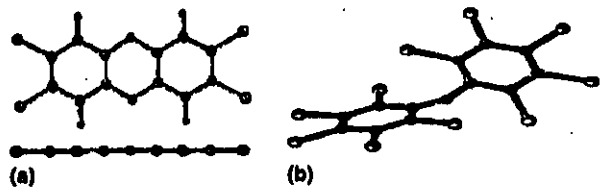

Fig. 2. A 2,3,7,8-substituted chlorodibenzo-*p*-dioxin (a) illustrates the coplanarity of its aromatic rings; whereas DBDPO (b) exists as a nonplanar molecule.

erties by influencing absorption and receptor interactions.

The toxicology of certain PCB isomers, like that of 2,3,7,8-substituted chlorinated dioxins and furans, is related to their ability to exist in a planar or near planar configuration. Unlike those molecules, DBDPO cannot adopt a coplanar conformation (Fig. 2). The ether linkage in the diphenyl oxide molecule introduces a high barrier to rotation and prevents the two aromatic rings from assuming a planar configuration. In addition, the ortho positions of the aromatic rings must be nonhalogen-substituted for a biphenyl or diphenyl oxide molecule to assume a planar or near planar configuration. Halogen substitution of the diphenyl oxide molecule in the ortho positions (2,2′,6,6′) will force the aromatic rings orthogonal to one another; e.g., the phenyl rings will be positioned in space 90° to one another. DBDPO, fully substituted at all ring positions, exists with its two aromatic rings orthogonal to one another (Fig. 2). In addition, the ether bridge in the DBDPO molecule introduces a 120° bend in the alignment of the biphenyl rings. PCB and PBB, having no oxygen bridge, lack this bend (Fig. 3). Given that the toxicology of the dioxin-like PCB and 2,3,7,8-substituted halogenated dioxins and furans is generally accepted to be receptor-mediated, DBDPO's lack of planarity makes it an unlikely candidate for dioxin-like toxicity.

Another structural difference between the chlorinated and brominated molecules relates to molecular geometries. Bromine atoms occupy a considerably larger volume than chlorine atoms, and as a consequence, brominated molecules have a larger molecular volume than do molecules containing a similar number of chlorine atoms. The number of bromine atoms per molecule is also a determinant of molecular size; DBDPO's molecular volume is 25.9% larger than that of 2,2′,4,4′,5,5′-HxBB.

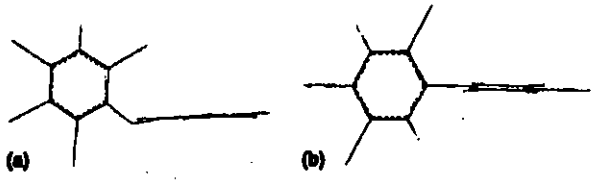

Fig. 3. (a) The ether linkage of the DPO molecule introduces a 120 bend in the alignment of DBDPO's aromatic rings. (b) The HxBB molecule lacks both the ether linkage and the bend.

5. Toxicology

Numerous studies have evaluated the mammalian toxicology of various PCB and PBB congeners, and a complete review of the literature will not be attempted. The present comparison will focus on the repeated dose toxicity, carcinogenicity, blood:liver:adipose tissue ratios, and pharmacokinetics of a representative PCB A1254, the HxBB commercial product, and the commercial DBDPO product in the rat. A1254 was selected for comparison to the DBDPO product because the major PCB products marketed contained ~42% chlorine (i.e., A1242), but patterns in environmental samples more closely resemble those of A1254 (Arochlor 1242, 2000). The HxBB commercial product was selected because it was the primary PBB produced and used. Specific data on the 2,2′,4,4′,5,5′-HxBB isomer, which accounted for ~60% of the HxBB product, were included where available and appropriate.

A no-effect-level (NOEL) of 5 ppm A1254 in the diet was determined in male rats in a repeated dose study of 3 or 5 weeks duration (Matthews et al., 1978). Using a conversion factor of 25 g of feed consumed/day/250 g rat, this equates to a dose of ~0.5 mg/kg body wt. A no-adverse-effect-level (NOAEL) of $\leqslant 3$ mg/kg administered by gavage for 30 days to rats 5 days/week was found for the HxBB commercial product (Gupta et al., 1981). In contrast, the dietary NOEL of the DBDPO product in rats and mice was $\geqslant 100{,}000$ ppm in a 14 day study and $\geqslant 50{,}000$ ppm in a 90 day study (NTP, 1986). Using the same conversion factor as for the A1254 study, these doses are estimated to be ~10,000 and ~5000 mg/kg, respectively, in the DBDPO 14 and 90 day studies. Thus, the NOEL of DBDPO in a 90 day repeated dose study was substantially higher than that determined for either A1254 or the HxBB commercial product in studies only a third as long.

Several PCB products, A1260, 1254 and Kanechlor 500, and the commercial HxBB product are listed by the US National Toxicology Program (NTP) as "reasonably anticipated to be a human carcinogen" (NTP 9th Report on Carcinogens 2000, 2001). A two-year carcinogenicity study of A1260 in rats performed at 0, 25, 50 and 100 ppm in the diet resulted in clear evidence of carcinogenicity in female rats in the form of hepatocellular carcinomas and liver adenocarcinomas, and an increase in thyroid gland follicular cell adenomas or carcinomas in males (Brunner et al., 1996; NTP 9th Report on Carcinogens 2000, 2001). The HxBB commercial product also produced a clear evidence of carcinogenicity in male

and female rats and mice when tested at doses of 0, 3, or 10 mg/kg administered for 5 days/week for 6 months and then held without treatment until 24 months (NTP, 1983). Neoplastic nodules, hepatocellular carcinomas and cholangiocarcinomas were induced in rats and hepatocellular carcinomas in mice. Treatment with the HxBB product also decreased survival and induced frank toxicity in the bioassay.

In contrast, the DBDPO product administered at doses of 2.5% and 5% of the diet produced no (female mice), equivocal (male mice) or some evidence (male and female rats) of carcinogenicity (NTP, 1986). Even at these extraordinarily high doses, the DBDPO product had no effect on survival or body weight, and produced no clinical signs of toxicity in either rats or mice. The US National Toxicology Program (NTP) estimated the average amount of DBDPO consumed per day for two years to be 1120 and 2240 mg/kg for low and high dose male rats, respectively, and 1200 and 2550 mg/kg for low and high dose female rats, respectively. Likewise, NTP estimated the average DPDPO consumed per day by mice to be 3200 and 6650 mg/kg for low and high dose male mice, respectively, and 3760 and 7780 mg/kg for low and high dose female mice, respectively. No evidence of carcinogenicity was observed in female mice receiving 2.5% or 5% DBDPO in the diet (~3760 or 7780 mg/kg/day). Equivocal evidence of carcinogenicity was observed in male mice due to an increase in the combined incidence of hepatocellular adenomas or carcinomas in both dose groups (~3200 or 6650 mg/kg/day); however, this finding may have been influenced by the larger number of early deaths in control male mice compared to the treated male mice. The combined incidence of hepatocellular adenomas and carcinomas in male mice treated with DBDPO was well within the historical range. Some evidence of carcinogenicity in male and female rats was observed by increased incidences of neoplastic nodules of the liver in low dose (2.5%, ~1120 mg/kg/day) males and high (5%, ~2240 mg/kg/day males, ~2550 mg/kg/day females) dose groups of each sex. (The term "neoplastic nodule" is no longer used by NTP to describe hepatoproliferative lesions in rats. This change in nomenclature was made subsequent to a peer review of representative hepatoproliferative lesions from two-year carcinogenicity studies. The peer review found the use of this poorly defined and understood term had permitted some potentially useful drugs and chemicals to be unfairly categorized as carcinogens (Maronpot et al., 1986).) DBDPO is not listed as a carcinogen by NTP (NTP 9th Report on Carcinogens 2000, 2001), the International Agency for Research on Cancer (IARC) (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, 1990) or the US Occupational Safety and Health Administration (OSHA) (OSHA Standard 1990, 2001).

With respect to the pharmacokinetics of PCB in general, Matthews and Dedrick (1984) said "The pharmacokinetics of PCBs are complicated by numerous factors, not the least of which is the existence of up to 209 different chlorinated biphenyls. Whereas all PCB congeners are highly lipophillic and most are readily absorbed and rapidly distributed to all tissues, PCBs are cleared from tissues at very different rates, and the same congeners may be cleared at different rates by different species. With the exception of special situations in which PCBs may be passively eliminated in lipid sinks, e.g., milk or eggs, clearance is minimal prior to metabolism to polar compounds. Rates of PCB metabolism vary greatly with species and with the degree and positions of chlorination. Mammals metabolize these compounds most rapidly, but even among mammalian species rates of metabolism vary greatly. In all species studied, the more readily metabolized chlorinated biphenyls have adjacent unsubstituted carbon atoms in the 3–4 positions. Congeners that do not have adjacent unsubstituted carbon atoms may be metabolized very slowly and are therefore cleared very slowly. These PCBs not readily cleared concentrate in adipose tissue". The pharmacokinetics of the HxBB commercial product share certain characteristics with the above (Damstra et al., 1982).

Both A1254 and 2,2′,4,4′,5,5′-HxBB were well absorbed from the gastrointestinal tract at 45–99% and ~90% of an oral dose, respectively (Damstra et al., 1982; Arochlor 1254, 2000). Both were initially distributed to the liver and skeletal muscle. A1254's subsequent disposition was largely dependent upon the rate at which each of the isomers composing the mixture was metabolized (Arochlor 1254, 2000). Each isomer's rate of metabolism was dependent on the degree and position of chlorine substitution. Those PCB isomers readily metabolized were readily excreted in the feces whereas those not easily metabolized were redistributed to the skin and adipose tissue, and stored. 2,2′,4,4′,5,5′-HxCB was not metabolized to any significant extent and thus only 2% of an oral dose was excreted in 21 days (Birnbaum, 1983). 2,2′,3,3′,6,6′-HxCB was readily metabolized and cleared and ~93% of this isomer was excreted (Birnbaum, 1983). Similar to 2,2′,4,4′,5,5′-HxCB, 2,2′,4,4′,5,5′-HxBB showed no evidence of metabolism, was slowly eliminated in the feces (~7% in 6 weeks), and was redistributed over time to skin and adipose tissue (Damstra et al., 1982). Pomerantz et al. (1978) estimated that < 10% of an oral HxBB dose would be excreted by rats.

The pharmacokinetics of the DBDPO isomer were distinct from those of A1254 and 2,2,4,4′,5,5′-HxBB. DBDPO was poorly absorbed from the gastrointestinal tract (<0.3–2% of an oral dose), >99% of an oral dose was excreted in the feces in 72 h, and some evidence of metabolism was found (Norris et al., 1973, 1975, 1974;

NTP, 1986; El Dareer et al., 1987). Further, DBDPO showed no preferential tissue distribution (NTP, 1986). DBDPO's poor absorption from the gastrointestinal tract was expected for a molecule of this size, weight and poor solubility. Following oral administration of ^{14}C-DBDPO, only trace levels of radioactivity (a total of <1% of the dose) were found in organs/tissues at any time point. The whole body half-life was <24 h. The parent molecule (and all metabolites) was rapidly eliminated; >99% of the dose was recovered in the feces and gut contents within 72 h of oral dosing. The overwhelming route and form of elimination was by fecal excretion as the parent molecule. Less than 0.01% of the oral dose was excreted in the urine. DBDPO was capable of being metabolized; the parent molecule and three metabolites were detected in feces following oral or intravenous (IV) dosing of rats. IV administration of DBDPO shifted elimination of the parent molecule to

Table 5
Comparison of the mammalian repeated dose toxicity, carcinogenicity, blood–liver–adipose tissue ratios, and pharmacokinetics of Arochlor 1254, the HxBB Commercial Product, and the DBDPO commercial product

End point	Arochlor 1254	HxBB commercial product	DBDPO commercial product
Repeated dose	3 or 5 week male rat diet	30 day rat and mice oral (5 days/week)	14 day rat and mice diet
	NOEL = 5 ppm (~0.5 mg/kg)	NOAEL ⩽ 3 mg/kg	NOEL ⩾ 100,000 ppm (~10,000 mg/kg) 90 day rat and mice diet NOEL ⩾ 50,000 ppm (~5,000 mg/kg)
Carcinogenicity	2 year rat diet 0, 25, 50, 100 ppm	2 year rat and 6 mice gavage: 0, 3, 10 mg/kg 5 days/week for 6 months held for 24 months	2 year rat and mice diet 0, 25,000, 50,0000 ppm
	Clear evidence in f rats: Hepatocellular carcinomas and liver adenocarcinomas	Clear evidence in m and f rats mice: Hepatocellular carcinomas	No evidence in f mice; Equivocal evidence in m mice (thyroid tumors); Some evidence in m and f rats (hepatic neoplastic nodules)
	Sufficient evidence of carcinogenicity in animals by NTP Reasonably anticipated to be a human carcinogen by NTP	Sufficient evidence of carcinogenicity in animals by NTP Reasonably anticipated to be a human carcinogen by NTP	Insufficient evidence of carcinogenicity in animals by NTP Neither known to be nor reasonably anticipated to be a human carcinogen by NTP
Blood:liver:adipose tissue ratio	1: 22: 359 (Rats)	1: ??: 340 (Rat)[a]	1:7:2 (Rat)
Pharmacokinetics	Well absorbed: ~45–95% oral dose	Well absorbed: ~90% oral dose[a]	Poorly absorbed: <0.3–2% oral dose
	Initial distribution to liver and muscle; subsequent disposition largely dependent upon rate at which each isomer metabolized: those readily metabolized – readily excreted, those not readily metabolized – redistribution to skin and adipose tissue where stored	Initial distribution to muscle, liver; redistribution with time to skin and adipose tissue[a]	
	Rate of metabolism dependent on degree and position chlorination	No evidence of metabolism[a]	Some evidence of metabolism
	Eliminated in feces: 2,2′,4,4′,5,5′-HxCB persistent, not metabolized to any significant extent, 2% of dose excreted in 21 days 2,2′,3,3′,6,6′-HxCB-readily metabolized and cleared (~93% dose)	Slow elimination in feces: ~7% of dose in 6 weeks[a]	Rapid elimination in feces: > 99% of dose in 72 h

[a] 2,2′,4,4′,5,5′-HxBB.

metabolites. After IV administration, 60% of the dose was excreted as metabolites and only 40% was excreted as parent molecule.

The blood:adipose tissue ratios of A1254 and 2,2′,4,4′,5,5′-HxBB in the rat are similar, 1:359 and 1:340, respectively, and indicate preferential distribution from blood to adipose tissue (Kodavanti et al., 1998). DBDPO, however, does not exhibit a similar preferential distribution. DBDPO's blood to adipose tissue ratio, calculated from the results of NTP's pharmacokinetic studies (NTP, 1986), is 1:2 (see Table 5).

6. Conclusion

DBDPO, which represents ~82% of the PBDPO/PBDE FRs used commercially, differs from the major PCB and PBB commercial products formerly in use in terms of its physical/chemical and environmental properties, applications, and toxicology. The DBDPO commercial product's water solubility and vapor pressure are considerably lower and its components are larger and heavier than those of the major PCB and PBB commercial products. The DBDPO commercial product is not used in PCB-like applications that had a high potential for environmental release. Further, the likelihood that DBDPO, a solid used primarily in electrical and electronic equipment, could undergo a rapid large scale loss to the environment is much less than that of liquid PCBs used in large quantities as functional fluids subject to leaks and spills.

The DBDPO isomer's behavior in the environment is expected to be different from those of the isomers found in the major PCB and PBB products. DBDPO is expected to achieve only trace quantities in water, to adsorb to particulates in water, not to volatilize from water to the atmosphere, to bind to organic carbon in sediment/soil and not to volatilize from these matrixes to the atmosphere. In contrast, PCB isomers are expected to volatilize from water and moist soils and to achieve higher concentrations in water, and current evidence suggests the major source of PCB release to the environment is an environmental cycling process of congeners previously released into the environment. DBDPO's physical/chemical properties do not indicate a potential for environmental cycling analogous to that of the PCB.

As a result of differences in properties and uses, the environmental release of PCB, PBB and DBDPO has been very different in magnitude and location. PCBs were steadily released into the environment, in many countries, presumably over decades, and were recognized as pervasive, world-wide contaminants as early as in 1978. PBB environmental release is predominantly associated with a 1973 accident occurring in the US, and detection of the DBDPO isomer in the environment is primarily associated with sediments near known point sources. DBDPO's primary use in dense thermoplastics limits the likelihood and magnitude of its environmental release.

The structures of representative PCB, PBB and DBDPO appear similar. However, DBDPO cannot assume the coplanar configuration required for the dioxin-like toxicity exhibited by some PCB molecules, and its molecular size and volume are considerably larger than the components of the primary PBB product, HxBB. These structural characteristics affect DBDPO's biological uptake, accumulation, and toxicology. Both A1254 and 2,2′,4,4′,5,5′-HxBB were moderately to well absorbed whereas DBDPO was very poorly absorbed. 2,2′,4,4′,5,5′-HxB and components of A1254 were metabolized and cleared very slowly, and subsequently stored in adipose tissue. DBDPO was rapidly excreted with a short whole body half-life and showed no preferential distribution to adipose tissue. The NOEL/NOAEL in repeated dose studies (~30 days) for A1254 and the HxBB product were 1000 and 166 orders of magnitude lower than that of DBDPO in a 90-day study. Both A1254 and the HxBB product produced clear evidence of carcinogencity in rats, and the HxBB product induced frank toxicity and mortality at the doses tested. DBDPO, at doses 255 times higher and administered for a period four times longer than HxBB, produced no overt toxicity, minimal organ effects, no mortality, and only some evidence of carcinogencity in rats. Thus, the toxicological effects and pharmacokinetic behavior of the DBDPO product are not analogous to those of A1254 or the HxBB product.

This comparison of chemical structures, physical properties, applications, environmental behavior, repeated dose toxicology and pharmacokinetics demonstrates the substantial differences between the DBDPO commercial product and the major PCB and PBB products in former use. DBDPO's properties do not mimic those of the major PCB and PBB products formerly used.

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Polybrominated diphenyl ether flame retardants in the North American environment

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Abstract

North America consumes over half of the world's production of polybrominated diphenyl ether (PBDE) flame retardants. About 98% of global demand for the Penta-BDE mixture, the constituents of which are the most bioaccumulative and environmentally widespread, resides here. However, research on the environmental distribution of PBDEs in North America has lagged behind that in Northern Europe. Examination of available governmentally maintained release data suggests that Deca-BDE use in the US substantially exceeds that in Canada. Penta-BDE use probably follows a similar pattern. PBDE demand in Mexico is uncertain, but is assumed to be comparatively modest. Recent research examining air, water, sediment, sewage sludge and aquatic biota suggests that Penta-BDE constituents are present in geographically disparate locations in the US and Canada. The less brominated congeners have been observed in areas distant from their known use or production, e.g. the Arctic. PBDEs have been detected in low concentrations in North American air, water and sediment, but much higher levels in aquatic biota. Increased burdens as a function of position in the food web have been noted. PBDE concentrations in US and Canadian sewage sludges appear to be at least 10-fold greater than European levels and may be a useful barometer of release. In general, PBDE concentrations in environmental media reported in North America are comparable or exceed those observed elsewhere in the world. In contrast to Europe, environmental burdens are increasing over time here, consistent with the greater consumption of the commercial mixtures. However, data remain relatively scarce. Deca-BDE in the North American environment appears largely restricted to points of release, e.g. urban areas and those where PBDE-containing sewage sludges have been applied. This lack of redistribution is likely due to its extremely low volatility and water solubility. Penta-BDE and Deca-BDE products are used in different applications and this may also be a factor controlling their environmental release.

Keywords: Polybrominated diphenyl ether; Retardant; North America

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are a group of flame retardant additives used in thermoplastics, polyurethane foam and textiles. PBDEs are compositionally similar to polybrominated biphenyls (PBBs), although their molecular configurations differ due to the presence of an ether linkage between the phenyl rings (Hardy, 2002). The linkage also makes the PBDEs more structurally similar to the thyroid hormone thyroxine and, accordingly, these compounds may interfere with endocrine system function (Meerts et al., 2000). PBB use was largely discontinued after their accidental introduction into cattle feed in the state of Michigan (US) in 1973 (Di Carlo et al., 1978). Three major PBDE commercial mixtures are commonly used: Deca-BDE, Penta-BDE and Octa-BDE. According to 1999 data (Table 1), these constitute 71.5%, 24.4% and 4.1% of the total North American PBDE market, respectively (personal communication, Lawrie McLaren, Bromine Science and Environmental Forum). PBDE distribution in the European environment has been studied in earnest for a number of years. However, until recently, comparatively little

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Table 1
Major PBDE commercial products in use in North America in 1999

Commercial PBDE mixture	1999 North American demand (tonnes)	Percent of 1999 global demand (%)	Major component PBDE congeners
Penta-	8290	97.5	BDE-47, 99, 100, 153, 154, 85
Octa-	1375	35.9	BDE-183, 153, unknown octa- and nona-BDEs
Deca-	24300	44.3	BDE-209, unknown nona-BDEs
Total	33965	50.6	

research has been done on their occurrence in North America, despite their disproportionate consumption here. The 1999 data indicate that 50.6% of total global demand for all PBDEs and 97.5% of that for the Penta-BDE product(s), believed to be the most environmentally problematic, reside in North America. Consumption here represents 36% of global Octa-BDE demand; the remainder residing in Asia (53%) and Europe 12%.

The Penta-BDE product is now used predominantly to flame retard polyurethane foam. However, some use of this mixture in circuit boards apparently occurred until the mid-1990s (Sakai et al., 2001). Many of these electronics remain in service and their future disposal may be an avenue for the release of Penta-BDEs to the environment. The constituents of Penta-BDE (Table 1; primarily BDE-47, -99 and -100, with smaller contributions from BDE-153, -154 and -85; note that PBDE congeners are named by number and position of bromines analogous to the PCBs) are volatile and persistent enough to permit their long-range transport. Their high lipophilicities enable significant bioaccumulation in animals and humans. For these reasons, and their detection in increasing concentrations in human breast milk (Betts, 2002), a ban on the use of Penta-BDE has been proposed in the European Union, effective in 2003. Consumption of Penta-BDE has already dropped in northern Europe (Renner, 2000) and Japan (Watanabe and Sakai, 2001). However, continued North American usage provides a source for global redistribution via export of products and large-scale environmental circulation patterns.

To date, the PBDEs have not been designated as persistent, bioaccumulative and toxic (PBT) chemicals by the US EPA or Environment Canada, although the properties of the Penta-BDE products certainly fulfill the physical criteria. The Deca-BDE products, consisting mostly of BDE-209 (Table 1), are used primarily in thermoplastics and back-coatings of textiles. BDE-209 is much less environmentally mobile and bioavailable due to its exceeding high K_{ow} and low water solubility and volatility (Hardy, 2002). However, concern has been expressed that it may be degraded in the environment to less brominated compounds (Renner, 2000). Assessment of risks associated with Deca-BDE use, versus its benefits, continues in the European Union. Asia was reported to use about 42% of the world's Deca-production, likely due to their substantial manufacture of electronics, but no Penta-BDE in 1999 (Brominated Science and Environmental Forum; http://www.bsef.com).

2. Sources and mechanisms of PBDE release

Environmental release of PBDEs may occur during their initial synthesis, incorporation into polymers or related finished products, during use of said products or as a result of their ultimate disposal or recycling (Danish Environmental Protection Agency, 1999). North American PBDE production is currently dominated by two US companies, both with major manufacturing facilities in Arkansas. Thus, releases from PBDE synthesis are unlikely to be responsible for their widespread detection in the environment. In contrast, facilities incorporating PBDEs into polymers and the subsequent use of these in electronics, automobile padding,

Table 2
Highest PBDE concentrations reported in various North American environmental matrices

Matrix	Sum of Congeners	Concentration	Units	Comments, year of collection
Sewage sludge	BDE-47, 49, 99, 100, 153, 154	2290	ng/g (dry)	US biosolids, 2000
	BDE-209	4890	ng/g (dry)	US biosolids, 2000
Surface water	BDE-47, 99, 100, 153, 154, 183	0.158	ng/l	Lake Michigan, 1999
Outdoor air	BDE-47, 99, 100, 153, 154	2100	pg/m^3	Yukon, Canada, possible incineration source, 2001
	BDE-209	0.35	pg/m^3	Chicago, USA, particle phase, 1997
Sediment	BDE-47, 99, 100	132	ng/g (dry)	Stream near a US polyurethane foam plant, 2000
Finfish	BDE-47, 99, 100, 153, 154	47,900	ng/g (lipid)	Fillet from Virginia river carp, 1999
Crustaceans	BDE-15, 17, 28/33, 47, 49, 66, 75, 99, 100, 119, 153, 154, 155	480	ng/g (lipid)	Dungeness hepatopancreas from Prince Rupert, Canada, 1995
Pinipeds	Not specified	3100	ng/g (lipid)	Male bottlenose dolphin blubber; Gulf of Mexico, 1990
	BDE-15, 17, 28/33, 47, 49, 66, 75, 99, 100, 119, 153, 154, 155	2300	ng/g (lipid)	Male harbor porpoise blubber, Tsawwassen, Canada, 1993

Specific PBDE congeners detected in the samples varied. However, major congeners detected were BDE-47, 99, and 100, except in sludge where BDE-209 was occasionally dominant. See text for references.

furniture and textiles are much more widespread. The percentage of PBDE in these polymer-based products may be as high as 30% by weight. Although initially considered nondispersive, releases from these operations and the disposition of PBDE-containing products during and following their useful lifetime merit additional scrutiny.

Separate PBDE production statistics for the US and Canada have not been released. However, some insights regarding relative PBDE demand in these countries may be gleaned from the chemical release inventories maintained by their respective environmental agencies. Unfortunately, Penta-BDE is not listed in either inventory, but Deca-BDE is tracked due to its designation as a high production volume chemical. It should be noted that data in the inventories are ultimately self-reported by industry and only encompass large-volume users. The 2001 US EPA Toxic Reduction Inventory or TRI (http://www.epa.gov/tri/) indicates that 148 facilities released about 912 tonnes of Deca-BDE. In contrast, the Canadian National Pollutant Release Inventory (http://www.ec.gc.ca/pdb/npri/npri_home_e.cfm) only lists two Ontario facilities that handle large amounts of Deca-BDE (>10 tonnes). The sum of releases from these Canadian facilities is reported to be less than 1% of the US total, i.e. about 5 tonnes. Neither Canadian facility manufactures PBDEs. The combined release figure represents less than 4% of the 24,300 tonnes of Deca-BDE reported in use in North America in 1999 (Brominated Science and Environmental Forum; http://www.bsef.com). It should also be recognized that the total human population, and its density, are about 10-fold greater in the US than Canada. Thus, handling and consumption of PBDEs would be expected to be more intense in the US. No data on use or environmental burdens of PBDEs in Mexico were found. However, production of electronics, automobiles and other presumably PBDE-containing products in Mexico for domestic use and export is increasing.

The relatively low volatilities and aqueous solubilities of the PBDEs suggest that the bulk of the environmental

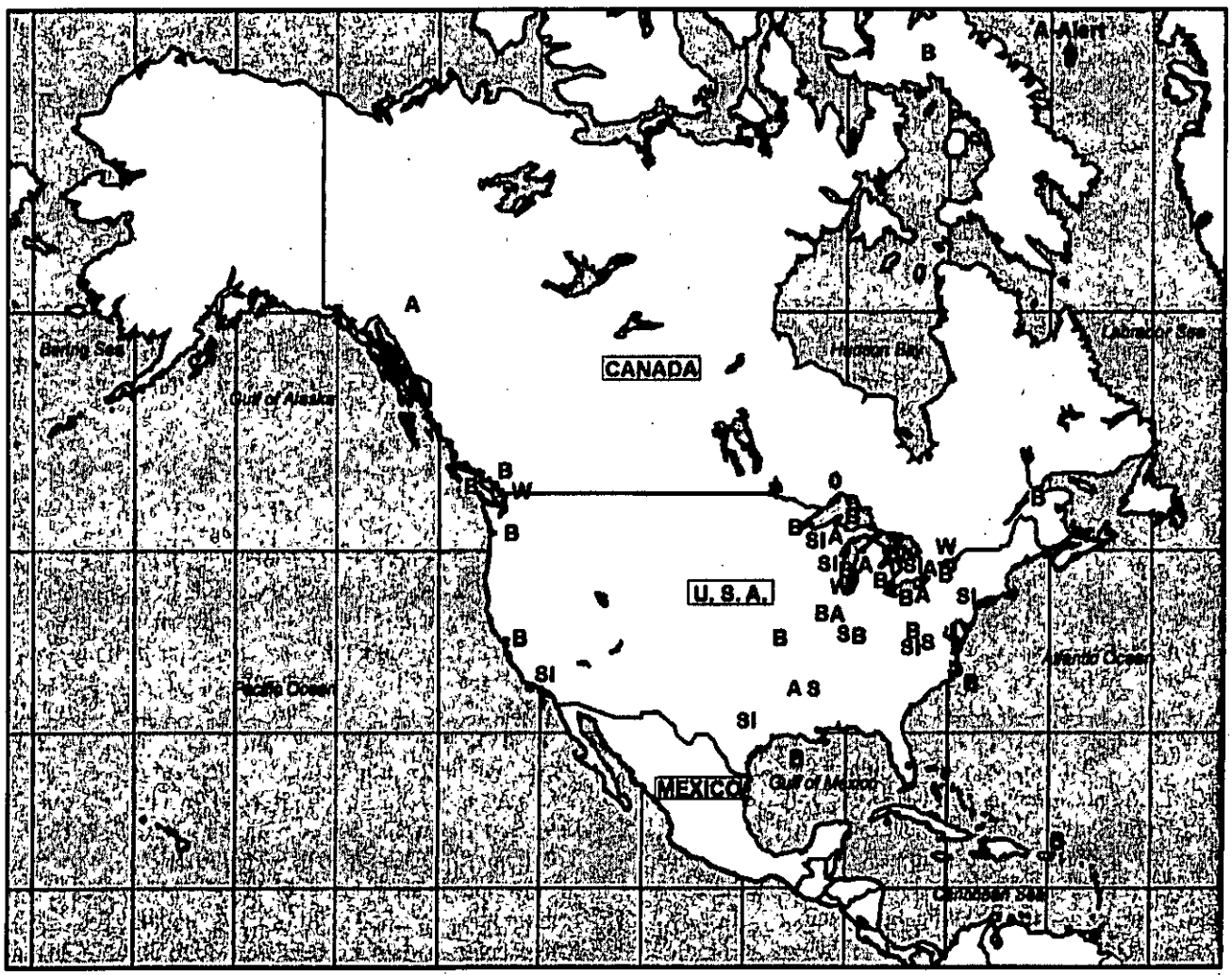

Fig. 1. Locations of studies examining PBDE burdens in North American environmental samples. Note that data are not available for large portions of the continent and scarce for all media. W = surface water; A = air; S = sediment or soil; SI = sewage sludge; B = aquatic biota.

burden of these chemicals will eventually reside in either sediments or soils (Palm et al., 2002). Air and water are likely transport media. The lipophilicities of PBDEs indicate that the less brominated components constituting Penta-BDE will accumulate in aquatic organisms. The available data on the distribution of PBDEs in these media in North America are summarized below and in Table 2. The paucity of published information for North America is evident in Fig. 1. Studies to date have been clustered around the Great Lakes, Mid-Atlantic and Pacific Coast (Fig. 1). An attempt has been made below to put these data in the context of known global levels. While some data on marine pinipeds are presented here, burdens in marine mammals and terrestrial organisms are not the focus of the current paper.

3. PBDEs in outdoor air

Data on PBDE burdens in North American air are scarce. One of the earliest published reports did, however, concern Deca-BDE associated with atmospheric particulates, obtained near an Arkansas PBDE manufacturing plant (De Carlo, 1979). Due to its low volatility and strong sorption to particulates, BDE-209 is unlikely to be transported via air significant distances from points of initial release. Deca-BDE's primary use in environmentally stable and relatively dense thermoplastics and textile backcoatings may also impede its release after production and subsequent redistribution.

Strandberg et al. (2001) detected PBDEs in every atmospheric sample collected between 1997 and 1999 from urban and remote sites in the Great Lakes region. Concentrations were similar to those of organochlorine pesticides. Total levels (summed particulate and gas phases) ranged from 4.4 to 77 pg/m^3. To minimize the variability of the data, only samples taken at 20 ± 3 °C were described. BDE-47 was the dominant congener reported, followed by -99 and -100. Mono- to tribrominated congeners were not examined. Thus, totals present may have actually been higher. In general, they reported that PBDE and PCB concentrations in air were correlated. In most samples PCBs were more abundant, but in urban Chicago samples BDE-47 concentrations rivaled those of the common PCB congeners. BDE-209 was only detected in atmospheric particulates from the Chicago area.

In southern Ontario, Canada, Gouin et al. (2002) reported that PBDE concentrations in air samples collected in 2000 from a rural site, prior to the spring bud burst, ranged between 88 and 1250 pg/m^3. Highest levels were believed to be related to release of PBDEs from snowpack during the spring melt. Following the bud burst, atmospheric levels dropped to between 10 and 230 pg/m^3. These concentrations were comparable to those of coincident PCBs. Major congeners reported were the more volatile BDE-17, -28 and -47. These values are similar to those reported for European, Japanese and Taiwanese air samples summarized by de Wit (2002).

Concentrations of ΣPBDEs in air samples from more northern latitudes, i.e. Alert (Ellesmere Island, Nunavut, Canada), Dunai (Siberia, Russia) and Tagish (Yukon, Canada), ranged from 10 to 2100 pg/m^3 (Bidleman et al., 2001). The levels of PBDEs in Dunai air were the lowest and those from Tagish the greatest among the three sites. Relatively high levels of mono- to hepta-BDEs were detected at Tagish, with maximum concentrations occurring during the summer months. The higher air temperatures at Tagish compared to Alert and Dunai, combined with closer proximity to populated areas in the southern Yukon, may explain the more elevated levels. However, local sources such as incineration of PBDE-containing products cannot be ruled out. Field blanks showed only very low PBDEs levels.

4. PBDE occurrence in sewage sludge

As PBDEs are hydrophobic, resistant to degradation and widely used in North American products, it is logical to assume that some enter sewage treatment plants (STPs) and will subsequently be concentrated in high organic carbon-containing sewage sludges. Therefore, examination of sludge PBDE burdens may be a useful monitoring strategy, as this material will integrate releases from multiple sources and thus may be indicative of relative environmental discharges. In the US over half of the sludge generated is now disposed of by application onto agricultural and other lands. This may ultimately serve as a conduit for wider dispersal. Nonetheless, few studies have examined PBDE burdens in sludge. In all 11 sludge samples collected from four different regions of the US, Hale et al. (2001a) detected the constituents of Penta-BDE. The sludge had been stabilized in preparation for eventual land application. Concentrations (total of BDE-47, -99, -100, -153 and -154) were similar between samples, ranging from 1100 to 2290 μg/kg on a dry weight basis (dw), despite differences in facility location, industrial base and sludge stabilization process. Concentrations of BDE-47, -99 and -100 exceeded those of the major PCB congeners and other halogenated contaminants present. In contrast, levels of BDE-209 varied substantially between samples, ranging from 84.8 to 4890 μg/kg.

PBDEs have also recently been determined in stabilized biosolids from several Wisconsin communities by the Wisconsin Public Health Laboratory (Sonzogni, unpublished data). Average concentrations (dw) in sludge from three Lake Superior watershed communities were 767, 1327 and 510 μg/kg for BDE-47, BDE-99 and BDE-209, respectively. Mean values in sludge from eight Lake Michigan watershed communities were 507, 706 and 466 μg/kg for the same congeners. PBDEs congeners were 30 to 50 times higher in concentration than the most abundant PCB congeners in these samples. In a single biosolid sample from Ontario Canada, the Penta-BDE concentration (sum of BDE-47, -99,

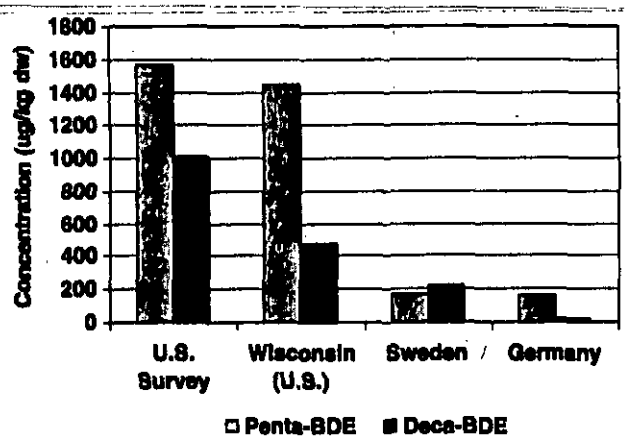

Fig. 2. Mean PBDE concentrations in sewage sludges determined in a multi-state US investigation (11 STPs; Hale et al., 2001a,b) and studies conducted in Wisconsin (11 STPs; Sonzogni, unpublished data), Sweden (3 STPs; de Wit, 2002) and Germany (8 STPs; Kuch et al., 2001). See text for PBDE congeners included in these Penta-BDE totals. Deca-BDE consisted of BDE-209. Note: Large variations in Deca-BDE concentrations levels between STPs were seen in the multi-state US and German studies. Median Deca-BDE concentrations therein were 389 and below quantitation, respectively.

-100, -153 and -154) was lower than in the abovementioned US samples, but BDE-209 was higher, i.e. 637 and 6930 μg/kg (dw), respectively (Hale, unpublished data).

These North American values are substantially higher than those reported in European sludges (Fig. 2). Available data on PBDE in European sludges have recently been summarized (de Wit, 2002). Samples examined from Sweden and Germany in the 1980s were low, between 0.4 and 30 μg/kg dw. However, sludges taken more recently exhibit higher levels. For example, Kuch et al. (2001) reported an average of 162 μg/kg total PBDEs in sludge from eight German plants. Interestingly, Deca-BDE was only detected in one of the facilities, at a concentration of 135 μg/kg. They noted no relationship between PBDE burden and facility size. Sellstrom et al., as summarized by de Witt (2002), noted that the sum of BDE-47, -99 and -100 ranged from 105 to 205 μg/kg in three Stockholm Sweden treatment plants. BDE-209 levels were higher, between 170 and 270 μg/kg.

5. Concentrations in surface waters and STP effluents

Limited data are available regarding PBDE concentrations in water. In the Fraser River (British Columbia, Canada), Ikonomou et al. (2002a) examined congener profiles using semi-permeable membrane devices (SPMDs). In these, BDE-47 dominated, followed by -99 and -100, the major constituents of the Penta-BDE product. BDE-209 was not determined. However, due to its low water solubility and extreme tendency to partition to particles, it likely would not have been detectable with this approach. BDE-49 was detected in the SPMDs, but has not been reported in commercial PBDE products. Consequently, it may represent a breakdown product. Lake Michigan water column concentrations of total PBDEs were reported to have increased from an average of 0.031 to 0.158 ng/l from 1997 to 1999, the latter rivaling coincident PCB levels (Stapleton and Baker, 2001). Lower concentrations have been reported for Lake Ontario, i.e. 0.006 ng/l (Lucky et al., 2001). de Boer et al. (2003) recently reported extremely high BDE-209 concentrations, up to 4600 μg/kg (dw), associated with suspended particulate matter from the western Scheldt in the Netherlands. The Deca- source was hypothesized to be the textile industry in Antwerp.

As PBDEs have been reported in STP sludges in significant concentrations, it is logical to assume that treatment plant effluents may be a source for the release of these hydrophobic chemicals. Kuch et al. (2001) observed concentrations of BDE-47 and -99 up to 1.1 ng/l in German STP effluents. They noted that 97% of the PBDEs in the influent were particle-associated. BDE-47 was the only PBDE congener they detected in surface waters, ranging from <0.2 to 0.71 ng/l. de Boer et al. (2003) also observed BDE-47 and -209 on particulates associated with treatment plant influents and effluents in the Netherlands. Concentrations on the effluent particles ranged from 11 to 35 and 310 to 920 μg/kg (dw), respectively. Interestingly, median effluent values exceeded those in the influent, attributed to smaller particle size in the latter. No published data on PBDE concentrations in North American STP effluents were found. PBDE burdens therein are likely a function of the STP's efficiency at removing suspended particulates from its effluent.

6. Concentrations in sediments and soil

Aquatic sediments and terrestrial soils are probable deposition sites for PBDEs (Palm et al., 2002). Unfortunately, few measurements of PBDE burdens in these matrices in North America have been made. Burdens in sediment appear to be a function of distance from the source and their organic carbon content. Dodder et al. (2002) reported results of the analysis of four surficial sediment samples from Hadley Lake in Indiana (US). The lake is near a research and development facility of a PBDE producer. BDE-209 was the major congener detected, followed by BDE-99, -153, -154, -47 and -100. BDE-209 concentrations ranged from 19 to 36 μg/kg (dw). All other PBDE congeners were less than 5 μg/kg. This distribution suggests the presence of elevated concentrations of the more brominated commercial mixtures.

PBDEs were detected (above 0.5 μg/kg dw) in 22% of surficial sediments taken from 133 sites in freshwater tributaries of Virginia (Hale et al., 2001b). BDE-47 was the dominant congener, followed by -99 and -100. The maximum sediment concentration detected was 52.3 μg/kg (1210 μg/kg on a total organic carbon basis). Stream sedi-

ment adjacent to a recently closed polyurethane foam manufacturing facility in North Carolina contained up to 132 μg/kg Penta-BDE (Hale et al., 2002). Soil outside a building where the foam was extruded contained 76 μg/kg. While low relative to sludge and fish tissues, these values exceed those in most European sediments reported to date. An exception involved sediments from the River Skerne and the Tees estuary in the UK. (Allchin et al., 1999). There, up to 368 μg/kg BDE-47 and 898 μg/kg BDE-99 were detected. The source was believed to be a PBDE manufacturing facility. PBDE production in the European Union ended in 1997. Deca-BDE levels up to 3190 μg/kg were observed in sediments from the River Calder, downstream of a UK STP. A review of sediment core data from the Baltic, Norway, Germany and the Wadden Sea suggested that PBDE burdens have increased over time, escalating significantly in recent years (deWit, 2002). However, recent reductions in Penta-BDE use in Europe may reverse this trend.

7. PBDE concentrations in aquatic organisms

While the Deca-BDE mixture constitutes 72% of the total PBDE demand in North America, and 82% globally, BDE-209 has rarely been reported in wildlife. This congener has been less frequently measured in part because of analytical difficulties related to its low volatility in gas chromatographic systems and poor thermal stability. The paucity of reports is also related to its limited bioavailability, due in turn to its large molecular volume and extreme hydrophobicity. In a laboratory feeding study using rainbow trout, Kierkegaard et al. (1999a) noted only limited BDE-209 uptake. However, its accumulation was surpassed by that of lower brominated congeners, present either as impurities in the original test material or subsequently produced via metabolism or degradation of BDE-209. Others have reported significant bioconcentration from water and bioaccumulation from food of the lower brominated PBDEs, e.g. BDE-47, rivaling the most bioaccumulative PCBs (Burreau et al., 1997; Gustafsson et al., 1999). Thus, it is not surprising that an increasing number of reports of PBDE burdens in North American aquatic organisms are emerging. However, data remain limited geographically (Fig. 1). The observation that consumption of contaminated fish may be a major uptake route for PBDEs in humans further reinforces the importance of these observations (Sjodin et al., 2000).

One of the first reports of PBDEs in North American aquatic biota was in bottlenose dolphins collected in 1987 and 1988 during a mass mortality event along the US mid-Atlantic and Gulf coasts (Kuehl et al., 1991). Blubber samples from three females were screened and contained a mean of 200 μg/kg of total tetra- to hexa-PBDEs. All values presented for biota are on a lipid basis for ease of comparison, unless noted. In examining these organisms, the authors noted that there were nearly as many brominated as chlorinated compounds present. Some of these, however, may have been natural marine products. A second mass mortality event involving dolphins occurred in the Gulf of Mexico in 1990. Adult females from the Gulf contained similar concentrations, mean 190 μg/kg (Kuehl and Haebler, 1995). However, adult males exhibited much higher levels, mean of 3100 μg/kg. The differential may be related to lactational or other reproduction-related losses. Immature dolphins contained intermediate PBDE burdens. Ikonomou et al. (2002a) reported a PBDE concentration of 2300 μg/kg in blubber from a male harbor porpoise collected at Tsawwassen in British Columbia in 1993. Levels in four additional males from more rural areas were lower, between 350 and 740 μg/kg. In all cases the major congener detected was BDE-47, followed by -99 and -100.

Data for teleosts are more widely available. Livers from nine blue marlins, obtained off the coast of Puerto Rico in 1991, were observed to contain a range of tri- to hexa-PBDEs (BDE-17, -28/33, -47, -66, -85, -99, -100, -138, -153 and -154 (Greaves and Harvey, 2000). Total PBDE concentrations were modest, less than 100 μg/kg on a lipid basis. PCBs and DDT metabolites were present at 10-fold higher concentrations. Interestingly, two methoxy- derivatives of tetrabromodiphenyl ethers were detected at concentrations exceeding the PBDEs. These chemicals have been reported in biota collected elsewhere, but it is uncertain whether these represent PBDE metabolites or were derivatives of natural products originating from marine sponges or algae (Asplund et al., 2001).

PBDEs were reported in carp obtained in 1991 from the Buffalo River in New York (Loganathan et al., 1995). Tetrabrominated congeners constituted 94–96% of the total observed, with penta- and hexa-congeners contributing the remainder. BDE-209 was not observed above the detection limit (0.1 μg/kg wet). Samples collected from freshwater rivers around the state of Washington in 1997 showed a range of PBDE concentrations (Johnson and Olson, 2001). Highest levels were observed in samples from urbanized watersheds. Tetra- and penta-congeners constituted 95% or more of the PBDEs detected. The concentration of the total pentabrominated homologues in suckers was lower than in other species. The highest ΣPBDE concentration (wet weight: whole mountain whitefish from the Spokane River) was 1250 μg/kg. However, levels in other species (e.g. rainbow trout fillet) from this location exceeded this value when calculated on a lipid basis, i.e. 19,300 versus 8390 μg/kg, respectively. These concentrations are comparable to maximum values reported in fishes from Virginian rivers and lakes obtained between 1998 and 1999 (Hale et al., 2001b). Levels in Virginia fishes varied significantly between sites. Interestingly, a fillet from carp obtained from a rural location exhibited the greatest burden, i.e. 47,900 μg/kg (lipid). These levels are similar to the highest noted in Europe, i.e. in a perch fillet from the River Viskan in Sweden, 36,900 μg/kg (Sellstrom et al., 1993). In general, PCB and PBDE tissue burdens were correlated, but excep-

tions occurred, suggesting that distinct sources of PCBs and PBDEs may exist. BDE-47 was reported to be the dominant PBDE present and species variations in congener patterns were noted in the Virginia fish.

PBDEs have also been reported in biota from the St. Lawrence estuary collected from 1997 to 2000 (Lebeuf et al., 2001). A variety of organisms were considered, from benthic worms to beluga whales. In general, concentrations increased with trophic level and wet weight concentration was correlated with tissue lipid content. Except in marine mammals, PBDE concentrations rivaled those of PCBs and common organochlorine pesticides. BDE-47, -99 and -100 were reported to constitute 78% to 100% of the PBDE congeners present. BDE-209 was not assessed.

Several studies examining PBDE burdens in aquatic organisms have been performed in the Great Lakes region. Lipid-normalized concentrations in sunfish from Hadley Lake, near a PBDE research and development facility in Indiana, contained a mean of 2400 μg/kg (Dodder et al., 2002). A carp exhibited a similar burden. Levels exceeded those of coincident chlorinated pesticides and PCBs. BDE-99 was relatively low in the carp compared to BDE-47, as has been previously described. This is likely due to PBDE metabolism. In laboratory exposures, carp exposed to both BDE-47 and -99 displayed the same accumulation pattern as seen in field-collected specimens, i.e. diminished -99 relative to -47 levels (Stapleton et al., 2002). This would seem to indicate that carp have heightened capacity to metabolize certain PBDEs relative to other fish species. In the Indiana fish the congener composition was enriched in the more brominated congeners, relative to other reports. PBDEs in sediments, discussed above, also suggested contamination by mixtures more brominated than Penta-BDE. Total PBDE concentrations in fish from Lake Superior (smelt), Ontario (smelt) and a relatively remote Missouri lake (sunfish) were about an order of magnitude lower and BDE-47 was the dominant congener present. In Chinook and coho salmon collected from Wisconsin tributaries of Lake Michigan in 1996, PBDE concentrations varied from 773 to 8120 μg/kg (Manchester-Neesvig et al., 2001). Values were less than 10% of coincident PCBs. BDE-47 was the dominant congener present. BDE-99 and -100 levels were comparable to each other. Biomagnification of the less brominated congeners in a Lake Michigan food chain has recently been reported (Stapleton and Baker, submitted for publication). Concentrations in the highest predator, lake trout, were comparable to the abovementioned Lake Michigan associated salmon. In contrast, total PBDEs were two orders of magnitude lower in mysid shrimp. Luross et al. (2002) also examined lake trout, obtained in 1997. Average burdens in whole fish from Lake Ontario, Superior, Huron and Erie were 434, 392, 251 and 117 μg/kg (lipid), respectively.

Whole carp and large mouth bass from the Detroit River, obtained in 1999, contained 40.7 and 163 μg/kg (lipid) of PBDEs, respectively (Rice et al., 2002). Carp obtained from the Des Planes River (Illinois) contained 281 and 78.3 μg/kg at sites near Joliet and Riverside, respectively. Interestingly, these fish contained appreciable burdens of the heptabrominated congeners (e.g. BDE-181 and -183), on occasion surpassing BDE-47. BDE-190 was also detected. This pattern is apparently related to the presence of more brominated PBDE mixtures and possibly biotransformation, as BDE-181 is not present in the Octa- or Deca- mixtures at appreciable concentrations. Unfortunately, BDE-209 was not analyzed for in the fish and sediment results were not reported.

Aquatic organisms have also been examined from a variety of pristine, harbor and paper mill locations in British Columbia (Ikonomou et al., 2002a). Total PBDE concentrations were 4.2 to 480 μg/kg (lipid) in Dungeness crab (hepatopancreas) and 12 to 340 μg/kg in English sole (liver). Ikonomou et al. (2002b) also recently found up to 1000 μg/kg total PBDE in whitefish within a section of the Columbia River, receiving municipal and other wastes, over an order of magnitude higher than 1992 levels.

8. Temporal trends of PBDEs in the North American environment

Temporal trends from Europe (Sellstrom et al., 1993; Kierkegaard et al., 1999b) and Japan (Ohta et al., 2001) indicated a sharp increase in the concentration of PBDEs up to the mid-1980s, followed by a significant drop or leveling off in these compounds in biota. This may reflect decreased usage of Penta-BDE there. In contrast, recent data from North America indicate that concentrations of these compounds have increased significantly during the past two decades. Luross et al. (2000) reported that PBDEs increased by 300-fold over the past 20 years in Lake Ontario lake trout. Similar trends were observed in walleye from Lake St. Clair and lake trout from Lake Michigan (Hickey et al., 2002). Moisey et al. (2001) observed a 60-fold increase in PBDEs in herring gull eggs from the Great Lakes. Similar trends have been observed in marine mammals in the Arctic, St. Lawrence Estuary (Lebeuf et al., 2002) and California. Stern and Ikonomou (2000) reported a 75-fold increase in PBDEs in belugas from Baffin Island between 1982 and 1997. Ikonomou et al. (2002b) observed a 10-fold increase in PBDEs between 1981 and 2000; and She et al. (2002) reported a 65-fold increase PBDEs in harbor seals from California between 1988 and 2000.

9. Summary

Despite the paucity of studies, PBDE releases to the North American environment have been occurring since at least the late 1970s. Environmental concentrations appear to be increasing in all environmental compartments here. In some areas, levels of the total tetra- to hexabrominated PBDEs now rival those of PCBs and the organochlorine

pesticides. This is particularly evident in some of the rapidly responding media of transport, i.e. air, water and sewage sludge. North America is now the major consumer of PBDEs in the world, particularly the more environmentally problematic Penta-BDE products. As such, it probably is also the major source of PBDEs to the global environment. In addition to transboundary contamination via atmospheric processes, importation of PBDE-containing products from North America by other countries will remain an avenue for global PBDE redistribution. The less brominated PBDEs are becoming widely dispersed from urban to remote areas, following the same pattern as the now largely banned persistent organic pollutants (POPs), i.e. the PCBs, DDTs and chlordanes. These same PBDEs are bioaccumulating in lipid reservoirs of aquatic organisms and being transferred up the food chain, ultimately to humans. Recently, PBDE burdens in breast milk from North American women were reported to exceed those previously reported in Swedish women and to be increasing dramatically over time (Betts, 2002). In contrast to the less brominated congeners, BDE-209 appears to exhibit a more limited geographical distribution, associated with points of release. Biological uptake of Deca-BDE also appears lower. However, due to its greater use, further study of its environmental fate is merited.

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The toxicology of the three commercial polybrominated diphenyl oxide (ether) flame retardants

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Abstract

Three commercial polybrominated diphenyl oxide flame retardants (PBDPO, PBDE) are manufactured: decabromodiphenyl oxide (DBDPO), octabromodiphenyl oxide (OBDPO) and pentabromodiphenyl oxide (PeBDPO). The composition, production volumes, uses and toxicology of the three products differ. In 1999, DBDPO accounted for ~82% of the global PBDPO usage. DBDPO has been extensively tested. DBDPO was not acutely toxic, was not irritating to the skin or eye, and did not induce skin sensitization. No evidence of genotoxic effects was detected in the Ames Salmonella, chromosome aberration, mouse lymphoma, or sister chromatid exchange tests. No cytogenic changes were observed in the bone marrow of rats (parents and offspring) undergoing a one-generation reproduction test. DBDPO did not adversely affect development or reproduction in rats. DBDPO's no-adverse-effect-level (NOAEL) in repeated dose studies was $\geqslant$1000 mg/kg body weight. No, equivocal, or some evidence of carcinogenicity, dependent on genus and sex, was found in mice and rats at 2.5% and 5% of the diet administered for 2 years. DBPDO was poorly absorbed from the gastrointestinal tract (< 0.3–2% oral dose), had a short half-life (< 24 h) compared to PCB 153 (only 2% of an oral dose eliminated by rats in 21 days), and was rapidly eliminated via the feces (> 99% in 72 h). In contrast, components of the PeBDPO product were well absorbed and slowly eliminated, OBDPO's effect level in a 90-day study was ~100 mg/kg, PeBDPO's no-effect-level (NOEL) in a 30-day study was 1 mg/kg, and OBDPO induced developmental toxicity in the rat. In aquatic species, neither DBDPO nor OBDPO were toxic to aquatic organisms or bioconcentrating. Components of the PeBDPO product bioconcentrated in fish but produced little evidence of adverse effects. © 2002 Published by Elsevier Science Ltd.

Keywords: PBDPO; PBDE; Polybrominated diphenyl ether; Brominated flame retardant; Toxicology; Toxicity

1. Introduction

Brominated flame retardants (BFRs) comprise approximately 25% of the volume of flame retardants (FR) used on a global scale, and are used in applications requiring high FR performance or in resins needing a FR active in the gas phase (Hardy, 2000a). BFRs as a class are structurally diverse and include aromatic diphenyl oxides (a.k.a. ethers), cyclic aliphatics, phenolic derivatives, aliphatics, phthalic anhydride derivatives and others. The bromine portion of the compound is responsible for the molecule's flame retardant activity and is unique in its ability to provide flame retardancy in the gas phase. BFR toxicology reviewed in this paper is that of the commercial polybrominated diphenyl oxide products (PBDPO) which are commonly known as decabromodiphenyl oxide (DBDPO; CAS#1163-19-5), octabromodiphenyl oxide (OBDPO; CAS#32536-52-0) and pentabromodiphenyl oxide (PeBDPO; CAS# 32534-81-9). Throughout this paper, the abbreviations "DBDPO", "OBDPO" and "PeBDPO" will refer to the commercial product, unless otherwise stated. The PBDPOs are commonly referred to in Europe as the polybrominated diphenyl ethers or PBDEs.

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2. The commercial PBDPO products

Only three commercial PBDPO products are manufactured. The composition, production volumes, uses and toxicology of the three commercial PBDPO flame retardants are distinctly different from one another. DBDPO together with Tetrabromobisphenol A (TBBPA) make up approximately 50% of all BFR usage globally (Hardy, 2000a). Global market demand in 1999 for DBDPO was estimated at 54,800 metric tons (Bromine Science and Environmental Forum, 2001). Market demand, 1999, for DBDPO in the regions of the America's, Europe and Asia was 24,300, 7500 and 23,000 metric tons, respectively (Bromine Science and Environmental Forum, 2001). These regional differences reflect differences in the location of end product manufacture.

The remaining 50% of the global BFR volume is composed of a number of different BFR structural types and includes the two other commercial PBDPO flame retardants, OBDPO and PeBDPO. OBDPO and PeBDPO are produced and used in substantially smaller quantities than DBPDO. OBDPO and PeBDPO 1999 market demand were estimated at 3825 and 8500 metric tons, respectively (Bromine Science and Environmental Forum, 2001).

DBDPO's main application is in high impact polystyrene (HIPS) for electronic enclosures (Hardy, 2000b). A comparatively minor, but important, use of DBDPO is to flame retard upholstery fabric where it is applied as a fabric back coat encapsulated in latex (Hardy, 2000b). The composition of the commercial DBPDO product is $\geqslant$ 97% in purity (Existing Substances Regulation 793/93/EEC, 2000a). OBDPO, a mixture of brominated diphenyl oxide congeners ranging from nona- to hexa-, is used to flame retard business equipment constructed of acrylonitrile–butadiene–styrene (ABS) plastic (Existing Substances Regulation 793/93/EEC, 2000b). PeBDPO, a highly viscous liquid composed of tetra-, penta- and hexa-BDPO congeners, is used to flame retard polyurethane foam that is used as cushioning in upholstery (Existing Substances Regulation 793/93/EEC, 2000c).

The potential toxicological effects of the three commercial PBDPO products vary with their degree of bromination such that the toxicity of the tetra- through deca-BDPO molecules declines with increasing levels of bromination. For example, only minimal effects were observed following lifetime administration of DBDPO at doses $\geqslant$ 1000 mg/kg/d whereas the no-effect-level (NOEL) for PeBDPO in a 90-day study was < 2 mg/kg/d. This paper will summarize and compare the toxicology of the three commercial PBDPO products.

3. Toxicology of the DBDPO-commercial product

All studies summarized (Table 1) were performed using a commercial DBDPO product unless otherwise stated. The DBDPO molecule has a high molecular weight (959.17). Trace analysis of the commercial product for 15 2,3,7,8-substituted brominated dibenzo-*p*-dioxins and dibenzofurans revealed no detectable amounts at the limits of quantitation established by the US Environmental Protection Agency (Ranken et al., 1994). DBDPO's measured water solubility (< 0.1 μg/l) (Stenzel and Markley, 1997a) and vapor pressure (4.63×10^{-6} Pa) (Stenzel and Nixon, 1997a) were negligible. DBDPO's solubility in organic solvents is also extremely low: acetone 0.05%, benzene 0.48%, methylene bromide 0.42%, xylene 0.87%, and 0.2% in toluene (Norris et al., 1973; WHO, 1994). DBDPO is often assumed to be lipophilic due its presumed similarity to PCBs. However, no formal fat solubility study has been performed, and pharmacokinetic studies show no appreciable affinity of DBDPO for adipose tissue (NTP, 1986). DBDPO's blood:liver:adipose ratio in the rat was 1:7:2 compared to Arochlor 1254's ratio of 1:22:359 (Kodavanti et al., 1998). DBDPO's measured octanol/water partition coefficient ($\log K_{ow}$) was 6.265 (MacGregor and Nixon, 1997a), but its $\log K_{ow}$ estimated by EPIwin, v3.4, was 12.61 (Meyland and Howard, 1999). It is apparent that DBDPO has a very low solubility coefficient in water and most organic solvents.

3.1. DBDPO mammalian toxicology

DBDPO has undergone extensive testing in mammalian species (Table 1). DBDPO was not acutely toxic, was not irritating to the skin or eye, and did not induce skin sensitization in a human patch test (Norris et al., 1973, 1974, 1975; NTP, 1986). Repeated dermal application to rabbits' ears did not induce a chloracne-like response (WHO, 1994). The soot and char combustion products from a high impact polystyrene/DBDPO/antimony trioxide matrix also were not acutely toxic and did not induce a chloracne-like response (Pinkerton et al., 1989). No evidence of a genotoxic effect was detected in the Ames Salmonella, chromosome aberration, mouse lymphoma, or sister chromatid exchange tests (NTP, 1986; WHO, 1994; Existing Substances Regulation 793/93/EEC, 2000a). No cytogenic changes were observed in the bone marrow of rats (parents and offspring) undergoing a one-generation reproduction test using a former DBDPO-commercial mixture of 77% purity (Dow FR-BA-300) (Norris et al., 1974). Gavage administration of DBDPO (0.1 nmol/kg/d) to rats over 14 days did not induce hepatic cytochrome P450, cytochrome P450 reductase, UDP-glucuronyl-transferase, benzo[a]pyrene hydroxylase, *p*-nitroanisole demethylase, or EPN detoxification (Carlson, 1980a).

No adverse effects in either parent or F1 animals were noted in a dietary one-generation reproduction test utilizing doses up to and including 100 mg of a 77% DBDPO mixture/kg body weight (Norris et al., 1974).

Table 1
DBDPO toxicology summary

Test	Results
Water solubility[a]	< 0.1 μg/l
Vapor pressure[a]	4.63×10^{-6} Pa[a]
Octanol/water partition coefficient[a]	6.265
Rat oral LD50	> 2000 mg/kg
Rabbit dermal LD50	> 2000 mg/kg
Rat inhalation LC50	> 48.2 mg/l
Rabbit eye irritation	Not an irritant
Rabbit skin irritation	Not an irritant
Human skin sensitization	Not a skin sensitizer
Ames[b]	Not mutagenic
Mouse Lymphoma[b]	Not mutagenic
Sister chromatid exchange[b]	Did not induce
Chromosome aberration[b]	Did not induce aberrations
14-day rat and mice oral (diet)[b]	NOEL ⩾ 100,000 ppm (10% of diet or ~10,000 mg/kg/d)
90-day rat and mice oral (diet)[b]	NOEL ⩾ 50,000 ppm (5% of diet or ~5000 mg/kg/d)
30-day rat (diet)[c]	NOEL = 0.01% (8 mg/kg/d)
Rat 1 generation reproduction[c]	NOEL ⩾ 100 mg/kg/d (highest dose tested)
Rat developmental, days 0–19 gestation[a]	NOEL ⩾ 1000 mg/kg/d (maternal and fetal)
Rat developmental, days 6–15 gestation[c]	NOEL ⩾ 1000 mg/kg/d (maternal) NOEL = 100 mg/kg/d (fetal)
Rat and mouse carcinogenicity (diet)[b]	25,000 (2.5%) or 50,000 (5%) ppm for 2 years (~3200–7780 mg/kg/d mice, ~1120–2550 mg/kg/d rats) Negative, equivocal or some evidence of carcinogenicity No effect body weight or mortality Minimal evidence of chronic toxicity
Rat carcinogenicity (diet)[c]	*NOEL ⩾ 1 mg/kg/d for 2 years (highest dose tested)*
Rat hepatic enzyme induction	Did not induce hepatic enzymes: cytochrome P450, cytochrome P450 reductase, UDP-glucuronyl-transferase, benzo[a]pyrene hydroxylase, *p*-nitroanisole demethylase, or EPN detoxification
Rabbit skin acnegenicity	Not acnegenic, soot and char not acnegenic
Rat pharmacokinetics (oral and IV)[b]	Poorly absorbed (< 0.3–2%) from GI tract Rapidly eliminated (> 99% in 72 h) Half-life < 24 h
Ready biodegradation[a]	Not readily biodegradable
Anaerobic sediment degradation	Not degraded after 32 weeks[a] or 2 years
Aqueous photodegradation[c]	Half-life ≫ 90 days, products not lower BDPOs
Organic solvent photodegradation	Half-life < 15 min, sequential reductive debromination, PBDFs formed from degradants
Solid surface photodegradation (sand, soil, sediment)	Half-life in sand ~37 h, some evidence of sequential reductive debromination, not as pronounced as in organic solvents, no evidence of 2,2′,4,4′-TeBDPO formation
Fish LC50, 48 h	> 500 mg/l
Algae EC50, marine, 96 h	> 1 mg/l
Fish bioconcentration	Not bioconcentrating: BCF < 5 (60 μg/l) and < 50 (6 μg/l) 6 wk
Sediment organism chronic, 28 d (2% and 5% organic carbon)[a]	Lumbriculus variegates: 28-day EC50 > 5000 mg/kg dry wt of sediment, NOEL ⩾ 5000 mg/kg dry wt of sediment

[a] Studies performed under Good Laboratory Practices and using today's commercial DBDPO product as test article.
[b] Test article 94–99% DBDPO.
[c] Test article only 77% DBDPO.

No evidence of maternal or fetal toxicity or developmental effects was detected in a developmental test in the rat ($n \approx 25$ pregnant females/dose) at 1000 mg/kg body weight utilizing a composite of today's commercial DBDPO product produced by three manufacturers and administered from days 0–19 of gestation (Schroeder,

2000). The test article composition was 97.34% DBDPO, 2.66% nona- and octa-bromodiphenyl oxide. An earlier study, using the former commercial product of only 77% DBDPO purity (Dow FR-BA-300) and administered on gestation days 6–15, also was negative for maternal toxicity and developmental effects (Norris et al., 1973, 1974, 1975). Some fetal variations were observed in this earlier study, which prompted performance of the second study using today's $\geqslant 97\%$ purity commercial DBDPO product. A brief account of a non-standard study reported a disruption in habituation in adult mice which were exposed on post-natal day 3 to a single oral dose of 20.1 mg lab-synthesized DBDPO/kg (Viberg et al., 2001a). The test article was laboratory synthesized, and the purity not specified. Animals exposed on day 3 to 2.3 mg/kg were not similarly affected nor were animals treated with either dose on day 19 or on day 10 with 1.34, 13.4 or 20.1 mg/kg.

DBDPO administered at 10% and 5% of the diet for 14 and 90 days, respectively, produced no adverse effects in rats and mice (NTP, 1986). In the 14-day study, DBDPO doses up to 10% of the diet in rats ($n = 5$ rats/sex/dose) and mice ($n = 5$ mice/sex/dose) produced no mortality, no effect on body weight, and no compound-related clinical signs or gross pathologic effects (histopathology was not performed). In the 13 week study, DBDPO doses up to 5% of the diet in rats ($n = 10$ rats/sex/dose) and mice ($n = 10$ mice/sex/dose) produced no mortality, no effect on body weight, and no compound-related gross or microscopic pathologic effects. Doses up to 5% of the diet for two years were also well tolerated by rats ($n = 50$ rats/sex/dose) and mice ($n = 50$ mice/sex/dose) with no effect on body weight or mortality and only minimal evidence of organ effects (NTP, 1986). The US National Toxicology Program (NTP) estimated the average amount of DBDPO consumed per day in the two year study to be 1120 and 2240 mg/kg for low and high dose male rats, respectively, and 1200 and 2550 mg/kg for low and high dose female rats, respectively. Likewise, NTP estimated the average DPDPO consumed per day by mice in the two year study was 3200 and 6650 mg/kg for low and high dose male mice, respectively, and 3760 and 7780 mg/kg for low and high dose female mice, respectively. Organ effects reported in high dose male rats (~2240 mg/kg/d) at the conclusion of the two year study consisted of thrombosis and degeneration of the liver, fibrosis of the spleen, and lymphoid hyperplasia. Degeneration of the eye was observed in low dose female rats (~1200 mg/kg/d). This later effect has been correlated with exposure to artificial light due to cage placement, and as a result, long term studies presently incorporate cage rotation into the study design. The DBDPO two year study was conducted prior to NTP instituting cage rotation as a part of their experimental protocols. In mice, granulomas in the liver of low dose males and hypertrophy in the liver of low (~3200 mg/kg/d) and high (~6650 mg/kg/d) dose males were observed. Follicular cell hyperplasia was observed in thyroid glands of dosed male mice. The US NTP concluded "... effects observed in these studies must be attributed to the approximately 95% pure preparation used rather than to pure DBDPO" (NTP, 1986).

An earlier repeated dose study using a DBDPO material of lower (77%) purity, Dow FR-BA-300 (Norris et al., 1973, 1974, 1975), produced somewhat different results from those of NTP which used a test article of $\geqslant 95\%$ DBDPO (NTP, 1986). In a 30-day-feeding study 5 male rats/group were administered the DBDPO mixture in the diet at 0%, 0.01%, 0.1% and 1.0% which corresponded approximately 0, 8, 80 and 800 mg/kg body weight (Norris et al., 1973, 1974, 1975). No overt signs of toxicity were detected in any dose group. Liver weights were statistically increased in the 1.0% and 0.1% dose groups compared to the control group. Gross pathologic changes were limited to hepatomegaly in 2 of 5 rats at the 1.0% dose level. Centrilobular cytoplasmic enlargement with minimal vacuolation was observed in 2 of 5 rats at the 1.0% dose level. Thyroid hyperplasia was detected in a non-dose-related manner: in 1 of 5 rats at the 1.0% dose level and in 3 of 5 rats at the 0.1% dose level. Hyaline droplet tubular cytoplasmic changes were detected in the kidneys of 4 of 5 rats at the 1.0% dose level. A dose of 8 mg/kg/d was established as a NOEL and 80 mg/kg/d as a marginal-effect level. The 77% DBDPO-commercial product is no longer manufactured and the results of the 1974 30-day study are not applicable to the $\geqslant 97\%$ DBPDO-commercial product in use today.

Two two-year carcinogenicity bioassays have been conducted (Kociba et al., 1975; NTP, 1986). The first, a single species study performed at a top dose level of 1 mg/kg using a DBDPO material of only 77% purity, produced no evidence of carcinogenicity or toxicity in rats (Kociba et al., 1975). The second, conducted at 2.5% and 5% of the diet in rats and mice using a DBDPO material more closely resembling today's commercial product, produced no, equivocal and some evidence of carcinogenicity depending on genus and sex (NTP, 1986). No evidence of carcinogenicity was observed in female mice receiving 2.5% or 5% DBDPO in the diet (~3760 or 7780 mg/kg/d). Equivocal evidence of carcinogenicity was observed in male mice by an increase in the combined incidence of hepatocellular adenomas or carcinomas in both dose groups (~3200 or 6650 mg/kg/d); however, this finding may have been influenced by the larger number of early deaths in control male mice compared to the treated male mice. The large number of early deaths in the control males may have decreased expression of hepatocellular adenomas or carcinomas in this group. The combined incidence of hepatocellular adenomas and carcinomas in male mice treated with DBDPO was well within the historical range. Some evidence of carcinogenicity in

male and female rats was observed by increased incidences of neoplastic nodules of the liver in low dose (2.5%, ~1120 mg/kg/d) males and high (5%, ~2240 mg/kg/d – males, ~2550 mg/kg/d – females) dose groups of each sex. (The term "neoplastic nodule" is no longer used by NTP to describe hepatoproliferative lesions in rats. This change in nomenclature was made subsequent to a peer review of representative hepatoproliferative lesions from two-year carcinogenicity studies. The peer review found the use of this poorly defined and understood term had permitted some potentially useful drugs and chemicals to be unfairly categorized as carcinogens (Maronpot et al., 1986).) DBDPO is not listed as a carcinogen by NTP (2001), the International Agency for Research on Cancer (IARC, 1990) or the US Occupational Safety and Health Administration (OSHA, 2001).

Taken as a whole, the no-adverse-effect-level (NOAEL) for DBDPO in repeated dose studies is at least 1000 mg/kg body weight. DBDPO's low toxicity is likely related to its poor absorption and rapid elimination (NTP, 1986). Pharmacokinetic studies have shown that DBPDO is poorly absorbed (0.3–2% oral dose), has a short half-life (24 h) compared to PCB 153 (< 2% of an oral dose was eliminated by rats in 21 days), can be metabolized, and is rapidly eliminated in the feces (> 99% in 72 h) (Norris et al., 1974, 1975, 1986; El Dareer et al., 1987; Morck and Klasson Wehler, 2001).

3.2. DBDPO absorption, distribution, elimination

The uptake, distribution and elimination of DBPDO after oral or intravenous (IV) dosing in the rat have been evaluated in several studies (Norris et al., 1974, 1975; NTP, 1986; El Dareer et al., 1987; Morck and Klasson Wehler, 2001). These processes were monitored by following total ^{14}C-radioactivity after administration of labeled-DBDPO or by following total bromine content via neutron activation after administration of DBDPO. NTP evaluated the uptake and disposition of DBDPO in the rat as part of the two year bioassay. Four studies were performed and the results were reported in the 1986 NTP report (NTP, 1986) and in the publication of El Dareer et al. (1987). Earlier studies are reported in Norris et al. (1974, 1975). Recently, similar work was performed by Morck and Klasson Wehler (2001).

In the dietary NTP-sponsored studies conducted by El Dareer et al. (cf. NTP, 1986; El Dareer et al., 1987), DBDPO treatment for 7 days at varying dose levels preceded treatment with the radiolabeled compound. Pretreatment dose levels were 51,000, 25,400, 4730, 2510, 496 and 238 ppm in the diet. Test articles used for pretreatment in the ^{14}C-DBDPO studies (NTP, 1986; El Dareer et al., 1987) closely resembled today's commercial product which is ⩾ 97% DBDPO [4]. In the studies conducted by Norris et al. (1974, 1975), a single dose of ^{14}C-DBDPO was administered orally or bromine tissue levels were monitored by neutron activation after repeated administration of DBDPO for 3, 6 or 12 months. The test article for the neutron activation experiments was the former low purity product "Dow FR-300-BA" composed of 77.4% DBDPO, 21.8% nonabromodiphenyl oxide and 0.8% OBDPO. In the Morck and Klasson Wehler (2001) study, ^{14}C-DBDPO was synthesized in the laboratory.

All studies showed similar results. The NTP studies by El Dareer et al. (cf. NTP, 1986; El Dareer et al., 1987) showed that DBDPO was poorly absorbed (2–0.28% of the oral dose) from the gastrointestinal tract at all pretreatment doses (277–50,000 ppm in the diet, respectively) and rapidly eliminated. The whole body half-life was < 24 h. Excretion in the urine accounted for ⩽ ~ 0.01% of the dose. Feces was the major route of elimination and > 99% of the dose was recovered in the feces by 72 h post-dosing. At all oral doses tested (277–50,000 ppm in the diet), the majority of the test article (~98–70%, respectively) was eliminated as the parent molecule. Three metabolites were detected in the feces and ranged from ~2% to 30%, respectively, of the total recovered ^{14}C-label. The highest percentage of metabolites (~30% of the dose) was present in the feces of animals pretreated with higher doses of DBDPO (25,000 and 50,000 ppm) in the diet. The identity of the metabolites was not determined.

Only trace levels of the ^{14}C-label were detected in any organ or tissue at any time point (24, 48 or 72 h post-dosing with the radiolabel) (NTP, 1986; El Dareer et al., 1987). The maximum total ^{14}C-activity detected in the body at any time was only ~1% of the oral dose. The maximum ^{14}C-activity, calculated as the sum of the radioactivity in liver, kidneys, lungs, spleen, brain, muscle, skin, fat, and blood, was detected in the 277 ppm treatment group 24 h post-dosing. Studies utilizing IV administration of 1 mg ^{14}C-DBPDO/kg and bile duct canulation showed that the ^{14}C-label was excreted in the bile as the parent molecule and three metabolites. Approximately 60% of the dose was eliminated as metabolites after IV administration. The bile contained 7.17% of the IV dose within 4 h post-doing, and 2.2% of the dose was excreted in the bile per hour.

The above results (NTP, 1986; El Dareer et al., 1987) are consistent with earlier reports by Norris et al. (1974, 1975). Norris et al. (1975) administered 1 mg/kg ^{14}C-DBDPO orally to 3 male and 3 female rats. The level of radioactivity found in the expired air and urine, measured at 24 h intervals over a 16-day period, was < 1%. The principal route of excretion was the feces. The rate of excretion was the same for both sexes. Within the first 24 h post-dosing, 90.6% of the administered dose was detected in the feces, and 99% of the ^{14}C-activity was accounted for by day 2. Tissues (adipose, heart, skin, adrenals, spleen, liver, pancreas) taken on day 16 post-dosing showed no ^{14}C-label with the exception of the

adrenal (0.01% of the dose) and spleen (0.06% of the dose). The ^{14}C-activity in these two tissues was at the limit of detection. The half-life of the disappearance of ^{14}C-activity from the body of DBPDO-treated rats was < 24 h.

Norris et al. (1974, 1975) also measured bromine concentrations (via neutron activation analysis) in the kidney, skeletal muscle, serum testes, liver and adipose tissue in male and female rats maintained on diets providing 1, 0.1, 0.01 and 0 mg DBPDO mixture/kg/d for 6 or 12 months. The composition of the DBPDO mixture (Dow FR-300-BA) was 77.4% DBPDO, 21.8% nonabromodiphenyl oxide and 0.8% OBDPO. After 180 days of treatment, mean bromine levels in the control and treatment groups in liver, kidney, skeletal muscle, serum and testes were statistically comparable. The mean bromine level in adipose tissue from the 0.1 mg/kg/d dose group (~3.3 μg/g) was statistically greater than the control mean (~1.7 μg/g). After 12 months on treatment, bromine concentrations in both the liver and adipose tissue were statistically comparable to controls.

Norris et al. (1975) evaluated the elimination of bromine from liver and adipose tissue. Male rats were maintained for 90 days on diets providing a dose of 1 mg DBDPO mixture (Dow FR-300-BA)/kg/d and then placed on control diet. Kidney, serum, adipose tissue, and liver were analyzed for bromine by neutron activation analysis. On recovery day 0 there was no difference in bromine content in kidney or serum between the control and treated rats. After 10 days on the control diet, bromine concentrations in the liver of treated rats were comparable to controls. Adipose bromine levels in the treated group (~2.5–4 μg/g) were higher than the controls (~0–2 μg/g) during the recovery period.

Morck and Klasson Wehler (2001) reported similar results. Male rats were gavaged with a single dose of ^{14}C-DBDPO (3 μmol/kg; 0.00288 μg/kg). Feces were the predominant excretory route and contained ~90% of the dose within 3 days. Only trace amounts were eliminated in the urine (< 0.5% of the dose). Approximately 9.5% of the dose was recovered in the bile within 3 days. Approximately 3% of the dose remained in tissues at 72 h post-dosing. The majority of the ^{14}C-activity was detected in the liver followed in declining amount in the muscle, skin, adipose tissue and colon wall plus contents. Eight phenolic metabolites were reported in the feces, and included di-substituted penta- to octa-BDPOs. Trace amounts of three nona-BDDPOs were also reported.

Based on the findings of NTP (1986), Norris et al. (1974, 1975) and El Dareer et al. (1987), DBDPO is poorly absorbed from the gastrointestinal tract as would be expected for a molecule of this size, weight and poor solubility. Following oral administration of ^{14}C-DBDPO, only trace levels of radioactivity were found in organs/tissues at any time point. The parent molecule (and all metabolites) was rapidly eliminated – > 99% of the dose was recovered in the feces and gut contents within 72 h of oral dosing. The overwhelming route and form of elimination was by fecal excretion as the parent molecule. Less than 0.01% of the oral dose was excreted in the urine. DBDPO was capable of being metabolized; the parent molecule and three metabolites were detected in feces following oral or IV dosing of rats. IV administration of DBDPO shifted elimination of the parent molecule to metabolites. After IV administration, 60% of the dose was excreted as metabolites and only 40% was excreted as parent molecule. Recent studies by Morck and Klasson Wehler (2001) performed at a substantially lower dose reported similar findings.

3.3. DBDPO aquatic toxicology and environmental degradation

DBDPO was not acutely toxic to fish (Biodegradation and bioaccumulation data of existing chemicals based on the CSCL Japan, 1992) or marine algae (Walsh et al., 1987), and was not expected to be chronically toxic in aquatic species due to its large molecular weight, negligible water solubility, and the lack of toxicity exhibited by OBDPO (Existing Substances Regulation 793/93/EEC, 2000a). DBDPO was not toxic (NOEC ⩾ 5000 mg/kg dry wt sediment) to the sediment oligochaete, *Lumbriculus variegatus*, when tested over a 28-day period in sediments with either 2% or 5% organic carbon (Krueger et al., 2001). A 6-week bioconcentration study in Japanese carp reported a BCF of < 5 (60 μg/l) and < 50 (6 μg/l) (Biodegradation and bioaccumulation data of existing chemicals based on the CSCL Japan, 1992). No measurable accumulation of DBDPO in rainbow trout was observed after a 48 h exposure to 20 μg ^{14}C-DBDPO/L whereas 2,2′,4,4′-tetrachlorobiphenyl accumulated over 50 times its original concentration in water during the same time period (Norris et al., 1973, 1974, 1975).

Recently, Kierkegaard et al. (1997, 1999) investigated the uptake by trout of a DBDPO product no longer manufactured, Dow FR-300-BA, and which contained only 77.4% of the DBDPO isomer. The remainder of the test article was composed of nona- (21.8%) and octa-BDPO isomers (0.8%). Rainbow trout were force-fed homogenized cod in which the test article was suspended over a period of 16, 49 and 120 d. Doses ranged between 7.5 and 10 mg/kg/d. The results indicated that only a very small proportion of the test material was taken up during the 120-day exposure phase. Uptake was estimated to be 0.02–0.13% based on the muscle concentrations of the total hexa- to DBDPO isomers present, or ~0.005% of the DBDPO component only. 2,2′,4,4′-TeBDPO, 2,2′,4,4′,5-PeBDPO and 2,2′,4,4′,6-PeBDPO were present in similar concentrations in the liver and muscle of control and treated

fish, and so were not related to treatment with the test article (i.e., were not metabolic products). The concentrations of some hexa-, hepta-, octa- and nona-BDPO congeners were also found to increase with exposure in liver and muscle. Some of these congeners were not detectable in the test article and it was thought that their presence was the result of either a metabolic process or an efficient absorption process of trace amounts initially present in the food/test article use. The study was not able to distinguish between these two possibilities (Kierkegaard et al., 1999). The results of this study are consistent with previous work showing insignificant bioconcentration of DBDPO in fish, and do not provide definitive evidence that DBDPO is debrominated metabolically (Existing Substances Regulation 793/93/EEC, 2000a).

DBDPO was not readily biodegradable (Biodegradation and bioaccumulation data of existing chemicals based on the CSCL Japan, 1992) nor was DBDPO degraded by anaerobic sediment over a 32 week (Scheffer, 2001) or 2 year time frame (de Wit, 2000). DBDPO leaching from polymers was insignificant (Norris et al., 1973, 1974; Moller et al., 2000), as expected for a molecule of negligible water solubility and vapor pressure.

Norris et al. (1973, 1975) reported investigating the photolysis of DBDPO by sunlight in organic solvent or water (Norris et al., 1974, 1975), and predicted different routes of photodegradation for the DBDPO molecule in water or organic solvents based on the behavior of other halogenated aromatic compounds. Halogenated aromatics photodegraded by reductive dehalogenation when dissolved in solvents capable of proton transfer. However, in water, photodegradation proceeded via an oxidative process of hydroxylation leading to the formation of phenolic compounds. Once photohydroxylation was initiated, it was expected to accelerate as electron-withdrawing halogens were replaced by electron releasing hydroxyl groups. The resulting hydroxylated species were expected to adsorb light more strongly and this ultimately could result in rupture of the aromatic ring. Laboratory findings correlated with the predictions. Minimal evidence of DBDPO (98% purity) aqueous photodegradation was found over a period of 3 months exposure to natural sunlight; degradants were not lower brominated diphenyl oxides. Evidence for degradation of only 0.57% of the amount initially present (10 g/8 l water) was detected after 98 days of exposure to sunlight. The minimal degradation was likely related to DBDPO's extremely poor water solubility (< 0.1 μg/l) and stability. However, in octanol at 7 ppm, DBDPO decomposed with a half-life of 4 h. In xylene (a strong absorber of UV light) DBDPO photodegraded by reductive debromination with a half-life of 15 h on exposure to a 125 watt Hg lamp. In comparison, neither Arochlor 1242 nor 1260 showed any evidence of degradation after 350 h.

DBDPO degradation via reductive debromination in organic solvents (xylene, hexane, toluene, methanol/water) to lower brominated diphenyl oxides has been demonstrated (Norris et al., 1974, 1975; Watanabe and Tatsukawa, 1987; Eriksson et al., 2001). A further stepwise formation of polybrominated dibenzofurans was also observed (Watanabe and Tatsukawa, 1987; Eriksson et al., 2001). However, organic solvent photodegradation of DBDPO was not anticipated to be an environmentally relevant degradation mechanism (WHO, 1994; Existing Substances Regulation 793/93/EEC, 2000a).

The potential photodegradation of DBDPO adsorbed to sand, soil or sediment was also investigated (test article composition unknown but contained nonaBDPO and trace levels of octaBDPO) (Sellstrom et al., 1998). The DBDPO half-life in sand was ~37 h in natural sunlight. Evidence of reductive debromination was seen, although the amounts of nona-, octa- and heptaBDPO detected was not nearly so pronounced as in the toluene experiments carried out by this group. This indicates that either a stepwise reductive debromination pathway was less significant in environmental media or that in these media the lower brominated products formed themselves degrade at a faster rate than in toluene. Although small amounts of nonaBDPO formed, the subsequent formation of octaBDPO was a small fraction of this and the subsequent formation of heptaBDPO a small fraction of this. No 2,2′,4,4′-TeBDPO was detected. Thus, although it appears possible for reductive debromination of DBDPO to occur, the amounts of lower brominated diphenyl oxides formed would be very small and would also undergo similar degradation. Further, 2,2′,4,4′-TeBDPO, the primary PBDPO detected in the environment, does not appear to be produced.

4. Toxicology of the OBDPO-commercial product

All studies summarized were performed using the commercial OBDPO product as test article (Table 2). The molecular weight of the OBDPO molecule is 801 g/m. The commercial OBDPO product is a mixture of PBDPO congeners ranging from hexa- to nona-BDPO (Existing Substances Regulation 793/93/EEC, 2000b). Like DBDPO, OBDPO's measured water solubility (1 μg/l) (Stenzel and Markley, 1997b) and vapor pressure (6.59×10^{-6} Pa) (Stenzel and Nixon, 1997b) were negligible. OBDPO's measured octanol/water partition coefficient was 6.29 (MacGregor and Nixon, 1997b).

Like DBDPO, OBDPO was not acutely toxic (WHO, 1994; Existing Substances Regulation 793/93/EEC, 2000b), was not irritating (WHO, 1994; Existing Substances Regulation 793/93/EEC, 2000b), was not mutagenic (WHO, 1994; Existing Substances Regulation

Table 2
OBDPO toxicology summary

Test	Results
Water solubility[a]	< 1 μg/l
Vapor pressure[a]	6.59 × 10-6 Pa
Octanol/water partition coefficient[a]	6.29
Oral LD50	> 28,000 mg/kg
Dermal LD50	> 2000 mg/kg
Inhalation LC50	> 50 mg/l
Eye irritation	Not an irritant
Skin irritation	Not an irritant
Ames[a]	Not mutagenic
Sister chromatid exchange	Did not induce
Unscheduled DNA synthesis	Did not induce
Chromosome aberration	Did not induce
28-day rat oral (diet)	Hepatic centrilobular hypertrophy at 100, 1000, 10,000 ppm diet: scattered necrosis at 10,000 ppm (1000 mg/kg/d), liver wt increased
90-day rat oral (diet)	Effects consistent with above at 100, 1000, 10,000 ppm diet/d
14-day rat inhalation, 8 h/d	Hepatocytomegally and degeneration at $\geqslant$ 12 mg/m^3, NOEL = 1.2 mg/m^3
90-day rat inhalation, 6 h/d 5 d/week[a]	Hepatic centrilobular hypertrophy at 15 and 200 mg/m^3, liver wt increased at 200 mg/m^3, NOEL = 1.0 mg/m^3, NOAEL = 15 mg/m^3
Rat developmental (day 6–15 of gestation)	Developmental toxicant NOEL = 25 mg/kg/d (maternal), 2.5 mg/kg (fetal)
Rabbit developmental (day 7–19 gestation)	Not a developmental toxicant NOEL = 5 mg/kg/d (slight fetotoxicity at maternally toxic dose)
Rat hepatic enzyme induction	Induced hepatic enzymes: cytochrome P450, cytochrome P450 reductase, UDP-glucuronyl-transferase, *p*-nitroanisole demethylase, EPN detoxification, NADPH cytochrome *c* reductase.
Biodegradation[a]	Not readily biogradable
Fish LC50, 48 h	> 500 mg/l
Daphnid EC50[a]	EC50 > water solubility
Daphnid chronic, 21 D[a]	NOEC > water solubility
Fish bioconcentration	Not bioconcentrating: BCF < 4 at 8 wk

[a] Studies performed under Good Laboratory Practices using the current commercial product.

793/93/EEC, 2000b), was not acutely toxic to fish (WHO, 1994) or daphnia (WHO, 1994), was not chronically toxic to daphnia (Graves et al., 1997), and did not bioconcentrate in fish (CITI, 1982; WHO, 1994; Existing Substances Regulation 793/93/EEC, 2000b). However, OBDPO's mammalian toxicology data demonstrated its properties on repeated exposure are different from that of DBDPO. OBDPO induced liver effects in 14-, 28- and 90-day studies (12 mg/kg for 14 days or ~100 mg/kg for 28 days) whereas DBDPO did not at significantly higher levels (10% diet for 14 days) (WHO, 1994; Existing Substances Regulation 793/93/EEC, 2000b). OBDPO was also effective in inducing hepatic enzymes whereas DBDPO was not (Carlson, 1980a,b). OBDPO produced developmental effects in the rat, but not the rabbit (WHO, 1994).

4.1. OBDPO mammalian toxicology

In a 14-day-inhalation study in rats (WHO, 1994), doses up to 1200 mg/m^3 for 8 h/d had no adverse effect on food consumption, body weight, gain, hematology, serum chemistry or urinalysis. Liver weights were increased in dose groups $\geqslant$ 12 mg/m^3. Doses $\geqslant$ 12 mg/m^3 induced hepatocytomegaly and evidence of hepatocellular degeneration.

In a 28-day study in rats (International Research and Development Corporation, 1976a), OBDPO doses of 100 or 1000 ppm in the diet did not affect appearance, behavior, mortality, feed consumption or bodyweight gain. Liver weights were increased. Compound-related histopathological liver changes included enlarged centrilobular and mid zonal hepatocytes, and were consistent with hepatic enzyme induction. Slight to moderate thyroid hyperplasia was observed at 1000 ppm. Dose-related increase in total bromine levels in the liver were detected. A NOEL was not determined.

In a second 28 study in rats (WHO, 1994), dietary doses of 0, 100, 1000 and 10,000 ppm produced no effect on appearance, behavior or mortality. Centrilobular and mid zonal hepatocytomegaly was observed at all doses. At the high dose, hepatocellular vacuolization and

scattered necrosis was observed. Serum enzyme levels were not affected. Recovery was evident 4-weeks post-dosing. Bromine levels in the liver were increased in a dose-dependent manner, and a rapid decline was observed during the recovery period. A NOEL was not established.

In a 90-day study in the rat (International Research and Development Corporation, 1977), dietary doses of 0, 100, 1000 or 10,000 ppm produced results similar to the 28-day study. Evidence of recovery was observed post-dosing. Dose-related increase in liver bromine levels were detected. A NOEL was not established.

In a 90-day-inhalation study in the rat (WIL Research Laboratories, Inc., 2001), OBDPO doses of 0, 1.0, 15 or 200 mg/m^3 produced a NOEL and NOAEL for systemic toxicity of 1.0 and 15 mg/m^3, respectively. Groups of 10 male and 10 female rats were exposed to the current commercial product for 6 h/d 5 d/week for 13 consecutive weeks. The commercial product was micronized in order to generate particulates capable of being aerosolized. No effect body weight, food consumption, or mortality was observed at any dose. No clinical signs of toxicity were observed. Ophthalmologic exams were normal. No toxicologically significant effects on hematology or serum chemistry parameters were detected. Test article-related decrease in mean T4 levels were detected in both sexes of the 15 and 200 mg/m^3 dose groups. Mean TSH levels were increased in the 15 and 200 mg/m^3 males and in the 200 mg/m^3 females. Mean thyroid weights were comparable in the control and treated groups, and no histopathological effects were detected in the thyroid gland. No thyroid hormone-related clinical signs of disease or effects on body weight were detected. Absolute and relative-to-body-weight mean liver weights were increased in both sexes in the 200 mg/m^3 group. Centrilobular hepatocellular hypertrophy, consistent with hepatic enzyme induction, was observed in all males and in 6 of 10 females in the 200 mg/m^3 group and in 3 of 10 males and females each of the 15 mg/m^3 group. Ovaries of 3 of 10 females in the 200 mg/m^3 group had no visible corpora lutea. A high incidence of lung inflammation was noted in both sexes at 200 mg/m^3. The NOAEL was based on the high incidence of lung inflammation and on the > 60% decrease in T4 levels in the 200 mg/m^3 group.

In a dose range finding study, a dose of 50 mg/kg/d on days 6–15 of gestation to female rats (Hazleton Laboratories, 1986), OBDPO produced effects in the conceptus including reduced average fetal body weight, increased embryo/fetal death (resorption) and retarded ossification. The NOAEL for the conceptus was 25 mg/kg and for the dams 50 mg/kg.

In a developmental study in the rat (WHO, 1994), OBDPO administered on days 6–15 of gestation ($n = 25$ pregnant females/dose) induced reduced average fetal body weights (10 and 25 mg/kg), increased embryo/fetal death (resorption), fetal malformation/variations and delayed skeletal ossification (25 mg/kg). The embryo/fetal NOAEL was 10 mg/kg, and the maternal NOAEL $\geqslant$ 25 mg/kg.

A second developmental study in the rat (Argus Research Laboratories, Inc., 1985a), also with test article administration from day 6–15 of gestation ($n = 25$ pregnant females/dose), produced a maternal NOEL of 25 mg/kg and a fetal NOEL of 2.5 mg/kg based on post-implantation loss.

OBDPO did not induce developmental effect in the rabbit (Breslin et al., 1989). OBDPO was administered to female rabbits ($n = 26$ females/dose) at doses up to 15 mg/kg body weight on days 7–19 of gestation. No effect of treatment was found in maternal mortality, number of pregnancies, numbers of litters with viable pups, corpora lutea/dam, implantations/dam, live fetuses/litter, percentage of resorptions, and fetal body weight. No evidence of teratogenic activity was detected. Slight fetotoxicity (delayed ossification) was observed at 15 mg/kg.

OBDPO did not induce skin sensitization in the guinea pig (Wenk, 1996). OBDPO was effective in inducing rat hepatic enzymes. OBDPO administered orally by gavage to male rats at 0.1 mmol/kg/d over 14 days was effective in inducing hepatic cytochrome P450, cytochrome P450 reductase, UDP-glucuronyl-transferase, *p*-nitroanisole demethylase, and EPN detoxification (Carlson, 1980a). Increase in activity of these enzymes ranged from 66–182% above the control animals. Administration of 0.78 μmol/kg/d orally for 90 days also resulted in increased EPN detoxification, *p*-nitroanisole demethylation, cytochrome P450, and NADPH cytochrome *c* reductase (Carlson, 1980b). Return to control levels was a slow process, as indicated by measurements made 30 and 60 days after cessation of treatment.

OBDPO was not mutagenic in the Ames Salmonella test, did not induce unscheduled DNA synthesis in human fibroblast cells in vitro, did not induce sister chromatid exchange in Chinese hamster ovary cells in vitro, and did not induce chromosome aberrations in an in vitro cytogenic assay using human peripheral blood lymphocytes (WHO, 1994; Existing Substances Regulation 793/93/EEC, 2000b).

4.2. OBDPO aquatic toxicology and environmental degradation

OBDPO was not acutely toxic to fish or daphnia at the limit of its water solubility (WHO, 1994; Existing Substances Regulation 793/93/EEC, 2000b). OBDPO was not chronically toxic to *Daphnia magna* in a 21-day life-cycle study at the limit of its water solubility Graves et al., 1997. OBDPO's bioconcentration factor determined in an 8 week study in Japanese carp was < 4

(CITI, 1982; WHO, 1994). OBDPO was not readily biodegradable (Schaefer and Haberlien, 1996).

5. Toxicology of the PeBDPO-commercial product

All studies summarized were performed using a commercial product unless otherwise indicated (Table 3). The molecular weight of the PeBDPO molecule is 564. The current commercial PeBDPO product is a mixture of tetra- (~34%), penta- (~55%) and hexa-BDPO (~12%) congeners (Existing Substances Regulation 793/93/EEC, 2000c). The two major isomers in the current commercial product are 2,2′,4,4′,5-PeBDPO followed by 2,2′,4,4′-TeBDPO. The next most prominent isomer in the commercial product is 2,2′,3,4,4′-PeBDPO followed by lesser amounts of 2,2′,4,4′6-PeBDPO, 2,2′,4,4′,5,6-HxBDPO and 2,2′,4,4′,5,5′-HxBDPO.

The measured water solubility of PeBDPO, given as the sum for the commercial product, was 13.3 μg/l (Stenzel and Markley, 1997c). The water solubility determined in this study for the 2,2′,4,4′,5-PeBDPO and 2,2′,4,4′-TeBDPO were 2.4 and 10.9 μg/l, respectively (Stenzel and Markley, 1997c). PeBDPO's measured vapor pressure was 4.69×10^{-5} Pa (Stenzel and Markley, 1997d) and its measured octanol/water partition coefficient was 6.58 (MacGregor and Nixon, 1997c).

5.1. PeBDPO mammalian toxicology

Like DBDPO and OBDPO, PeBDPO was not acutely toxic, was not irritating, was not mutagenic, and did not induce skin sensitization in guinea pigs. Like OBDPO on repeated exposure, PeBDPO's results were different from that of DBDPO. The liver was the primary organ affected in repeated dose studies. Liver effects were induced by PeBDPO at ~100 mg/kg in a 28-day study, the NOEL in a 90-day study in rats was < 2 mg/kg, and a NOEL in a subsequent 30-day study was identified as 1 mg/kg. Also unlike DBDPO, components of the PeBDPO product were well absorbed and only slowly eliminated. All studies summarized were performed using a commercial PeBDPO product, unless stated otherwise.

PeBDPO was not genotoxic in the Ames Salmonella, *Saccharomyces cerevisiae*, or in vitro cytogenics test using human lymphocytes (Existing Substances Regulation 793/93/EEC, 2000c).

No treatment-related effects were observed in the number of resorptions, litter size, fetal mortality, fetal body weight, incidence of fetal gross external or internal variations when PeBDPO was administered at doses of 0, 10, 100 or 200 mg/kg to female rats ($n = 25$ pregnant females) on days 6–15 of gestation (Argus Research Laboratories, Inc., 1985b; Existing Substances Regulation 793/93/EEC, 2000c). No evidence of developmental effects was detected. The maternal NOEL was 10 mg/kg/d based on a decrease in weight gain. The fetal NOEL was 100 mg/kg based on a slight non-statistically significant decrease in fetal body weight.

Male and female rats ($n = 10$/sex/dose) were fed PeBDPO in the diet at 0, 100 or 1000 ppm for 28 days (International Research and Development Corporation, 1976b). Liver weights were increased in high dose animals. Hypertrophy of centrilobular and mid zonal hepatocytes was observed in 3/5 males at the mid dose, and in all animals at the high dose. Slight to moderate thyroid hyperplasia was observed in 1/5 and 3/5 male rats at the mid and high doses, respectively. Bromine content in the liver was increased by treatment.

Male and female rats ($n = 30$/sex/dose) were fed PeBDPO in the diet at 0, 2, 10 or 100 mg/kg body wt/d for up to 90 days (WIL Research Laboratories, 1984). Serum thyroxin (T4) levels were reduced by > 20% in both sexes of the mid and high dose groups at day 28, but were reduced in mid-dose males only at the end of the dosing period. No treatment-related effect on serum triiodothyronine (T3) levels was observed. Liver weights were increased in animals in the 100 mg/kg dose group. Heptocytomegaly was detected after 28 or 90 days of treatment in all dose groups except low dose females. Recovery from this effect was evident but incomplete at 24 weeks post-dosing. Very slight to slight thyroid hyperplasia was detected at 28 and 90 days in ca. half of the animals in the 100 mg/kg dose group. Recovery was underway within 6 weeks post-dosing, and complete by 24 weeks post-dosing. Tissue bromine levels increased during compound administration, and declined during the recovery period but did not reach control levels at 24 weeks post-dosing. The NOEL was < 2 mg/kg. The European Union risk assessment of PeBDPO concluded the slight thyroid hyperplasia and reductions in plasma T4 levels were indirect consequences of hepatic enzyme induction, and due to species differences in thyroid metabolism, were not likely relevant to human health (Existing Substances Regulation 793/93/EEC, 2000c).

In a subsequent 30-day study (WIL Research Laboratories, 1985), PeBDPO at doses up to 1 mg/kg body wt in the diet produced no treatment-related changes in survival, body weight, food consumption, behavioral or clinical signs, hematology, clinical chemistry, macroscopic or histopathologic changes were observed. A NOEL of 1.0 mg/kg/d was defined.

A fourth subchronic study was recently conducted utilizing a PeBDPO-commercial product no longer manufactured (Bromkal DE-75) (Fattore et al., 2001). Doses of 0, 2.5, 25 and 250 mg/kg/d were administered by gavage to male and female rats ($n = 5$/sex/dose) for 28 days. No clinical signs of toxicity or effects on body weight or food consumption were detected. Absolute liver and kidney weights were statistically increased in the high dose groups. A significant increase and de-

Table 3
PeBDPO toxicology summary

Test	Results
Water solubility[a]	13.3 μg/l (sum for commercial product) 2.4 μg/l (2,2′,4,4′,5-PeBDPO) 10.9 μg/l (2,2′,4,4′-TeBDPO)
Vapor pressure[a]	4.69×10^{-5} Pa
Octanol/water partition coefficient[a]	6.58
Oral LD50	7400 or 5800 mg/kg in male or female Wistar rats, respectively
Dermal LD50	> 2000 mg/kg
Inhalation LD50	> 200 mg/l
Eye irritation	Slight irritation
Skin irritation	Not an irritant
Ames	Not mutagenic
Chromosome aberration[a]	Did not induce aberrations
Guinea pig skin sensitization[a]	Did not induce
28-day rat oral (diet)	Hepatic centrilobular hypertrophy at 100 and 1000 ppm, thyroid hyperplasia and increased liver wt at 1000 ppm, NOEL < 100 ppm (<~ 10 mg/kg/d)
30-day rat oral (diet)	NOEL = 1 mg/kg/d (highest dose tested)
28-day rat oral (gavage)	Hepatic hypertrophy, liver wt increased at 250 mg/kg/d, NOEL not stated
90-day rat oral (diet)	Hepatocytomegally at day 28 and 90 at 2, 10 or 100 mg/kg/d (except in 2 mg/kg/d females), thyroid hyperplasia, T4 decreased at 10 and 100 mg/kg/d at day 28 but not at day 90, liver wt increased at 100 mg/kg/d, NOEL < 2 mg/kg/d
Rat developmental	Not developmental toxicant at 200 mg/kg/d (highest dose tested). NOEL = 10 mg/kg/d (maternal). NOEL = 100 mg/kg/d (fetal)
Rat pharmacokinetics	PeBDPO product: 2,2′,4,4′-TeBDPO was isomer detected at highest levels after feeding 2.9 ppb for 21 d, bioaccumulation of components decreased with increasing DPO bromination 2,2′,4,4′-TeBDPO: Readily absorbed from GI tract (~95%) Poorly metabolized Slowly eliminated (14% in 5 days) 2,2′,4,4′,5-PeBDPO: % Absorption not reported Poorly metabolized Slowly (43% in 3 days), but more rapidly eliminated than 2,2′,4,4′-TeBDPO
Rat hepatic enzyme induction	Induced hepatic enzymes: cytochrome P450, cytochrome P450 reductase, UDP-glucuronyl-transferase, benzo[a]pyrene, *p*-nitroanisole demethylase, EPN detoxification, NADPH cytochrome *c* reductase, PROD, EROD
Competition with thyroxin for in vitro binding to human transthyretin (TTR)	2,2′,4,4′-TeBDPO and 2,2′,4,4′,5-PeBDPO did not compete, in vitro metabolite of 2,2′,4,4′-TeBDPO did compete
In vitro binding to AH Receptor as indicated by luciferase induction	Practically non-existent
Immune system	Did not adversely affect proliferation and immunoglobulin synthesis in vitro Natural killer cell activity not affected in vivo (mice) Suppression of ASRBC response in vivo at 1000 mg/kg (mice)
Neonatal mice behavior	2,2′,4,4′-TeBDPO and 2,2′,4,4′,5-PeBDPO: NOEL behavior > 10 mg/kg 2,2′,4,4′ TeBDPO NOEL learning > 10 mg/kg 2,2′,4,4′,5-PeBDPO NOEL learning between 0.8 and 12 mg/kg
Fish LC50, 48 h	⩾ 500 mg/l (*Oryzias latipes*)
Fish LC50, 96 h[a]	⩾ Water solubility (*Oncorhynchus mykiss*)
Fish egg mortality, injection	2,2′,4,4′-TeBDPO and 2,2′,4,4′,5-PeBDPO NOEC ⩾ 12 μg/egg (*Oncorhynchus mykiss*)
Fish fry liver morphology and cP450 activity, injection into embryos	Little to no effects at top dose of 4 μg/egg (50 μg/g fresh weight)
Fish reproduction (diet)	No effect on spawning success (*Gasterpsteis aculeatus*)

(*continued on next page*)

Table 3 (*continued*)

Test	Results
Fish bioconcentration	Bioconcentrating: BCF PeBDPO product = 14,350, BCF 2,2′,4,4′,5-PeBDPO = 72, BCF 2,2′,4,4′-TeBDPO > 10,000
Algae EC50, 96 h, freshwater[a]	NOEC > water solubility
Daphnid EC50, 48 h[a]	NOEC = 4.9 μg/l, EC50 = 14 μg/l
Daphnid chronic, 21 day[a]	NOEC = 5.2 μg/l, LOEC = 9.8 μg/l
Sediment organism chronic, 28 day[a]	EC50 > 50 mg/kg dry wt in *Hyalella*, *Chironmus*, *Lumbriculus* sp
Soil nitrification organisms, 28 day[a]	NOEC > 1 mg/kg soil dry wt
Terrestrial plant, 21 day[a]	NOEC > 1000 mg/kg dry wt in 4/6 spp
Earthworm, 14 day[a]	NOEC > 500 mg/kg soil dry wt
Ready biodegradation[a]	Not readily biodegradable

[a] Studies performed according to Good Laboratory Practices using the current commercial product.

crease, respectively, were detected in hepatic EROD levels and vitamin A levels in the mid- and high-dose groups. Hepatic PROD was statistically increased at the high dose. Histopathologic findings consisted of enlargement of hepatocytes and an increase in "fat cells" in the liver.

A PeBDPO product composed of ~83% Te/PeBDPO congeners administered orally by gavage to male rats at 0.1 mmol/kg/d for 14 days was effective in inducing hepatic cytochrome P450, cytochrome P450 reductase, UDP-glucuronyl-transferase, benzo[a]pyrene hydroxylase, *p*-nitroanisole demethylase, and EPN detoxification (Carlson, 1980a). Administration of doses as low as 0.78 μmol/kg/d orally for 90 days also resulted in increased EPN detoxification, *p*-nitroanisole demethylation, cytochrome P450, and NADPH cytochrome *c* reductase (Carlson, 1980b). Return to control levels was a slow process, as indicated by measurements made 30 and 60 days after cessation of treatment.

5.1.1. PeBDPO absorption, distribution and elimination

Preliminary results of a mass balance study of the current commercial PeBDPO product (Great Lakes Chemical DE 73) in the rat were recently reported (Hakk et al., 2001). PeBDPO was administered in feed at the low level of 2.9 ppb, a rate designed to mimic environmental levels. Male rats (n = 8/group) were fed diets treated with PeBDPO at 32 ng/d/rat for 21 days. The total dose administered was 672 ng. Isomers present in the product, in descending order, were 2,2′,4,4′,5-PeBDPO, 2,2′,4,4′-TeBDPO, 2,2′,4,4′,5,5′-HxBDPO, 2,2′,4,4′,6-PeBDPO, 2,2′,4,4′,5,6′-HxBDPO, and 2,2′,3,4,4′-PeBDPO. 2,2′,4,4′,5-PeBDPO and 2,2′,4,4′-TeBDPO comprised ~47 and ~29, respectively, of the total dose; however, 2,2′,4,4′-TeBDPO was the component found with the highest bioaccumulation in treated rats. 2,2′,4,4′-TeBDPO was present in the liver and carcass at 0.69% and 55.9% of the dose, respectively. 2,2′,4,4′,5-PeBDPO was present in the liver and carcass at 0.46% and 30.6% of the dose, respectively. Bioaccumulation in the liver generally decreased with increasing bromination of the components of the product. The results were generally consistent with previous work using individual isomers.

2,2′,4,4′-TeBDPO was readily absorbed, poorly metabolized and only slowly eliminated by the rat (Om and Klasson-Wehler, 1998). Absorption of a 15 mg/kg body weight oral dose ^{14}C-2,2′,4,4′-TeBDPO was ~95%. Approximately 14% of the oral dose was eliminated in the feces in 5 days. Less than 0.5% of the dose was eliminated in the urine in over that period. Approximately 3% of the ^{14}C-activity in feces was present as metabolites. The parent molecule was the major ^{14}C-labelled compound detected in all tissues analyzed, and the only brominated compound detectable in kidney, brain and adipose tissue. Adipose tissue had the highest levels of ^{14}C-activity. Five hydroxylated metabolites were detected in trace amounts in the liver. The ^{14}C-levels in plasma were low and predominately due to the parent molecule.

In the mouse (Om and Klasson-Wehler, 1998), 2,2′,4,4′-TeBDPO was well absorbed, but metabolized and excreted more readily than in the rat. Absorption of orally administered ^{14}C-2,2′,4,4′-TeBDPO was ~93%. Approximately 20% and 33% of the dose was excreted in the feces and urine, respectively, in 5 days. Thus, the mouse eliminated a total of ~53% of the oral dose in 5 days compared to elimination of only 14% of the dose by the rat in the same time period. Mice eliminated at least 39% of the oral dose in the form of metabolites. Hydroxylated metabolites, considered similar in identity to those in the rat, were detected. Adipose tissue had the highest levels of ^{14}C-activity, and the parent molecule was the major brominated compound detected in adipose tissue, lung, kidney and brain. The liver contained trace amounts of hydroxylated metabolites. ^{14}C-Activity in plasma was too low for quantitation.

2,2′,4,4′,5-PeBDPO was poorly metabolized in the rat, but more readily eliminated than 2,2′,4,4′-TeBDPO in that species (Hakk et al., 1999). Following oral administration of ^{14}C-2,2′,4,4′,5-PeBDPO to male rats, 43% of the dose was excreted within 3 days. Feces was the major route of elimination. Only minor amounts of metabolites were detected and the majority (> 90%) of the ^{14}C-activity in the feces was present as the parent

molecule. At 72 h post-dosing, ^{14}C-activity was detected mainly in the adipose tissue (4%), blood (1%), carcass (39%) and GI tract (6%). No other tissues contained more than 1% of the^{14}C-activity at 72 h. The majority of ^{14}C-actiivty in the carcass was detected in the skin.

The half-life in perirenal fat of two PeBDPO isomers following administration of a single 300 mg/kg oral dose of a commercial PeBDPO product to rats was 25–47 days (von Meyerinck et al., 1990).

5.1.2. PeBDPO and the immune system

No effects on mitogen-induced DNA proliferation or immunoglobulin synthesis were observed after exposure of human lymphocytes in vitro to concentrations up to 10^{-5} M. These functions of human peripheral lymphocytes, i.e., proliferation and immunoglobulin synthesis, were insensitive to the direct action of the test article (Fernlof et al., 1997).

Female mice were orally exposed to PeBDPO acute single doses up to 500 mg/kg, or to subchronic daily doses totaling up to 1000 mg/kg over a 14-day period (Fowles et al., 1994). Significant suppression of the anti-sheep-red-blood-cell response was seen only in mice exposed on a subchronic basis to 1000 mg/kg, an exposure that also resulted in decreased thymus weight. Natural killer cell activity was not altered by treatment.

The immunologic effects of 2,2′,4,4′-TeBDPO (18 mg/kg) and a commercial PeBDPO product (18 or 36 mg/kg) was evaluated in rats and mice (Damerud and Thuvander, 1998). Animals appeared to have been dosed for 14 days. Neither test article adversely affected the immunologic parameters studied in rats. Parameters related to the spleen and evaluated in rats were not affected at any dose of either compound. In mice, 2,2′,4,4′-TeBDPO deceased the numbers of splenocytes and PeBDPO at 36 mg/kg decreased the in vitro production of IgG.

5.1.3. PeBDPO and thyroid hormones

Homeostasis of thyroid hormone synthesis and secretion is controlled by a sensitive feedback mechanism, which involves the hypothalamus, the pituitary, and the thyroid gland (O'Connor et al., 1999). Thyroid Stimulating Hormone (TSH), secreted by the pituitary, is particularly important in this feedback mechanism. TSH induces the thyroid to synthesize T4 that is then 5′-mono-deiodinated to the more biologically active T3 or inner ring deiodinated to rT3 that has no biological function. The rate of TSH release is controlled by the amount of thyrotropin-releasing hormone (TRH) secreted by the hypothalamus, as well as by the circulating concentrations of T3 and T4. Reductions in circulating T3 and T4 concentrations trigger the pituitary to secrete TSH that increases the synthesis of T3 and T4 by the thyroid gland and may be accompanied by thyroid hyperplasia. The thyroid hormones are eliminated from the body primarily by conjugation reactions in the liver. T4 is conjugated with glucuronic acid in a reaction catalyzed by thyroxine-UDPGT. T3 is conjugated with sulfate in a reaction catalyzed by phenol sulfotransferase. The conjugated products are excreted in the bile.

Perturbations of thyroid hormone homeostasis can occur through several mechanisms including direct action on the thyroid gland through inhibition of synthesis (e.g., thioamides, aniline derivatives, substituted phenols) or release of thyroid hormones (e.g., excess iodine, lithium) (O'Connor et al., 1999). However, a wide variety of chemicals and drugs such as phenobarbital (PB), spironolactone, chlorinated hydrocarbons, calcium channel blockers, and polychlorinated biphenyls are known to induce hepatic microsomal enzymes or inhibit 5′-deiodinase (e.g., erythrosine), both of which result in a reduction the circulating concentrations of the thyroid hormones. In general these agents are less potent in altering thyroid economy than agents that directly target the thyroid.

Although both T3 and T4 circulate in the bloodstream, the majority of the T3 utilized by cells is derived from the intracellular conversion of T4 (O'Connor et al., 1999; Schussler, 2000). In circulation, both T3 and T4 are highly protein bound with only a small fraction of their total present as free hormone. This high degree of protein binding serves to maintain equilibrium between the extracellular and intracellular pools of these important regulatory hormones. The primary binding proteins for T4 and T3 are thyroxin binding globulin (TGB), prealbumin or transthyretin (TTR), and albumin (ALB). At normal mean free T4 concentrations, TBG, TTR and ALB are only 18.4%, 0.16% and 0.0016% saturated, respectively (O'Connor et al., 1999). In most mammals including humans, TGB, a globulin, is the principal thyroid hormone binding protein; ~74% of the total bound-T4 is bound to TGB (Schussler, 2000). TTR and ALB bind only 11% and 15%, respectively, of the total (Schussler, 2000). In contrast to most mammals, the rat utilizes TTR as the major T4 plasma binding protein. Approximately 75% of T4 in rat serum is bound to TTR and only ~25% to ALB.

In both rats and man, TTR is the principal T4-binding protein in cerebrospinal fluid (CSF), and TTR is synthesized in the choroid plexus and secreted into the CSF. TTR may be important in maintaining thyroid hormone equilibrium in the CNS, although its exact function is unclear. TTR does not, however, appear to serve a T4 transport function into the CNS from the blood. Transport of T4 into brain cells primarily occurs via the blood brain barrier. Further, at least one group of mammals is known to exist successfully without TTR (Palha et al., 1997, 2000; Schussler, 2000). The TTR-nul mouse has decreased protein bound and total T4, normal free T4, and exhibits apparent good health (Palha et al., 1997, 2000). In the TTR-nul mouse, TTR influences

thyroid hormone levels in the choroid plexus, but not in the brain (Palha et al., 2000). Interference with the blood–choroid–plexus–CSF–TTR-mediated route of T4 into the brain caused by absence of TTR did not produce measurable features of hypothyroidism (Palha et al., 2000). In the rat, T4 is transported into the brain primarily through the blood–brain barrier, and not via the choroid plexus and CSF (Blay et al., 1993).

Several studies have investigated the potential of the commercial PeBDPO product or its major components to interact with the thyroid hormone system. In general, in vivo studies found no effect on plasma TSH levels, mild depression of free or bound plasma T4 levels, and mild hepatic enzyme induction. In vitro studies found that none of 17 PBDPO isomers tested, including 2,2′,4,4′,5-PeBDPO and 2,2′4,4′-TeBDPO, competed with T4 binding to TTR, that ca. half of the 17 isomers could be converted in vitro by induced microsomes to products which could compete with T4 for binding to TTR, and that hydroxylated derivatives of PBDPO isomers most closely resembling T4 did not interact significantly with thyroid hormone cellular receptors. 2,2′,4,4′-TeBDPO is a major component of the PeBDPO product and one of the eight isomers converted to a hydroxylated derivative which competed with T4 in vitro for binding to TTR. This isomer was poorly metabolized in vivo, however.

In a 90-day study in the rat (WIL Research Laboratories, 1984), serum T4 levels were reduced by > 20% in both sexes in the 10 and 100 mg/kg dose groups at day 28, but were reduced only in the 10 mg/kg animals at day 90. No treatment-related effect on serum T3 levels was observed. Slight thyroid hyperplasia was observed in approximately half the animals in the 100 mg/kg group at 28 and 90 days. These minor changes were considered indirect consequences of hepatic enzyme induction, and due to species differences in thyroid hormone metabolism are not likely relevant to humans (Existing Substances Regulation 793/93/EEC, 2000c).

2,2′,4,4′-TeBDPO was administered to rats for 14 days at 1, 6, or 18 mg/kg (Hallgren and Darnerud, 1998). No effect on plasma TSH levels was found at any dose. Plasma levels of free T4 were unaffected at 1 or 6 mg/kg. At 18 mg/kg, free T4 in plasma was decreased, but to a lesser extent than an equimolar dose of a PCB. Minimal induction of hepatic EROD was found at 6 and 18 mg/kg (EROD levels were increased about 3× compared to controls whereas PCB induced EROD ~6000×). MROD and UDPGT induction was less than EROD. PROD induction by TeBDPO at 8 mg/kg was approximately equal to that of PCB, although a very high standard deviation was found in the TeBDPO-treated group.

Female mice were treated orally with PeBDPO in acute single doses of 0, 0.8, 4.0, 20, 100 or 500 mg/kg, or subchronic daily doses totaling 0, 250, 500, or 1000 mg/kg over a 14-day/yr period (Fowles et al., 1994). Hepatic PROD activity was induced 3–5-fold in mice exposed acutely or on a subchronic basis at doses > 250 mg/kg. Hepatic EROD activity and total microsomal cytochrome P450 content were significantly induced only in mice treated on a subchronic basis. Maximum induction of EROD was 3.3-fold. Total serum T4 concentrations were significantly lower in mice treated acutely at all doses except 100 mg/kg. Total and free T4 concentrations were dose-dependently decreased in treated mice following subchronic exposure.

2,2′,4,4′,5-PeBDPO and 2,2′,4,4′-TeBDPO, did not compete with thyroxin in vitro for binding to human TTR (Meerts et al., 1998a, 2000). An in vitro incubation product of 2,2′4,4′-TeBDPO did compete for binding to TTR. 17 PBDPO congeners ranging from di- to hexa-isomers were tested in vitro for competition with T4 in binding to human TTR (Blay et al., 1993; Hallgren and Darnerud, 1998). None of the 17 PBDPO isomers competed with T4 for binding to human TTR. The 17 congeners were then incubated with rat microsomes induced with PB, beta naphthaflavone (NF), or clofibrate (CL), and the incubation products tested for competition with T4. The NF- or CL-induced microsomes did not produce any PBDPO metabolites that competed with T4. Nine of the 17 PBDPO congeners incubated with PB-induced rat microsomes generated products that competed with T4 for binding to human TTR (60% inhibition): 4,4′-; 2,4,4′-; 2,4,6-; 2,2′,4,4′-; 2,2′,4,6′-; 2,4,4′,6-; 3,3′,4,4′-; 2,2′,4,4′,6-; and 2,3′,4,4′,6-PBDPO. Of these nine, only 2,2′,4,4′-TeBDPO and 2,2′,3,4,4′-PeBDPO are components of the commercial PeBDPO product and, in an earlier in vivo study, 2,2′,4,4′-TeBDPO was found to be very poorly metabolized by the rat and excreted primarily as the unchanged parent molecule (Om and Klasson-Wehler, 1998). Lans et al. (1994) reported that hydroxylated PCBs, PCDDs, and PCDFs can inhibit T4 binding to TTR in vitro, but not to TGB, and concluded these chemicals may cause different effects in rodents and man. Therefore, the significance to both man and rat of the in vitro binding of the nine metabolites, and in particular the 2,2′,4,4′-TeBDPO isomer, to TTR is questionable.

Hydroxylated derivatives of three PBDPO were synthesized and tested in vitro for binding to the thyroid hormone cellular receptors (Marsh et al., 1998). The three isomers were 1,3,5-, 1,3,3′,5′- and 1,3,3′,5,5′-PBDPO. All were hydroxylated in the *para*-position. None of these congeners are known to be present in the commercial PeBDPO product, but were selected for study because they were the most similar PBDPO congeners to T4 and T3. Essentially no competition with T3 or T4 for the alpha thyroid hormone receptor was found. The three hydroxyl derivatives tested had 200 to > 2500 times less affinity for the alpha receptor than did

T3 or T4. Similar results were found for competition between the hydroxylated tri- and penta-congeners with the beta receptor. The hydroxylated tetra-congener showed 4 and 14 times less affinity for the beta receptor than did T4 and T3, respectively. Since these three hydroxylated PBDPO congeners are the most likely to have affinity for the thyroid hormone receptor, other hydroxylated PBDPO congeners should have even less potential to bind.

2,2′,4,4′-TeBDPO did not compete in vitro with ^{123}I-T4 for binding sites in rat choroid plexus homogenates (Sinjari et al., 1998). ^{123}I-T4 binding to choroid plexus homogenates derived from female rats treated with 6 or 18 mg 2,2′,4,4′-TeBDPO/kg/d for 14 days was 80% and 63%, respectively, of the controls. 2,2′,4,4′-TeBDPO's binding potency relative to T4 was $\ll 1$.

5.1.4. Other studies

No evidence for activation of the AH receptor was found in vitro for either 2,2′,4,4′-TeBDPO or 2,2′,4,4′,5-PeBDPO (Meerts et al., 1998b). 2,2′,4,4′-TeBDPO was antagonistic to TCDD's induction of luciferase in vitro. 17 PBDPO congeners were evaluated in vitro in rat hepatoma cells for luciferase expression mediated by the AH receptor (Sanderson et al., 1996). Ten of the 17 PBDE congeners did not induce luciferase expression, and four of these 10 were antagonistic to TCDD's induction of luciferase. These four were 4,4-; 2,2′,4,4-; 3,3′,4,4′-; and 2,2′,3,4,4′,5′-PBDPO isomers. Only 2 of 17 PBDPO congeners induced luciferase expression to a measurable extent and these were 2,3,4,4′5,6- and 2,3,3′,4,4′,5,6-substituted. An EC50 could only be determined for the 2,3,4,4′,5,6-isomer. Therefore, only 2 of the 17 congeners tested showed potential in vitro, as determined by the expression of luciferase, for a possible activation of the AH receptor, and 4 of the 17 congeners tested opposed the action of TCDD.

In a subsequent study (Pettersson et al., 2001), 2,2′,4,4′-TeBDPO, 2,2′,4,4′,5-PeBDPO, 2,2′,4,4′,5,5′-HxBDPO, and a PeBDPO-commercial product no longer manufactured (Bromkal 70) were tested in vitro for their EROD induction potency in cultured chick embryo livers. The maximum induction of all four compounds was less than the positive control, 2,3,7,8-TCDD. 2,2′,4,4′-TeBDPO and Bromkal 70 produced only minor EROD induction, whereas 2,2′,4,4′,5-PeBDPO and 2,2′,4,4′,5,5′-HxBDPO produced 63% and $\geqslant 49\%$, respectively, of the 2,3,7,8-TCDD maximum EROD induction at 10^{-9} M. The report concluded that the potency was "low compared to other dioxin-like compounds".

In a non-standard developmental study, 2,2′,4,4′-TeBDPO did not effect behavior or learning in the neonatal mouse; the NOEL on learning was reported to be between 0.8 and 12 mg/kg for 2,2′,4,4′,5-PeBDPO (Eriksson et al., 1998). Neonatal mice (on day 10 of life) were administered a single dose of 2,2′,4,4′-TeBDPO (0.7 or 10.5 mg/kg) or 2,2′,4,4′,5-PeBDE (0.8 or 12 mg/kg). Spontaneous behavior at 2 and 4 months of age and swim maze performance at 5 months were evaluated. Mice at the highest dose of both compounds were reported as hypoactive early in the 60-minute behavioral test and hyperactive toward the end of the test. This change was reportedly more pronounced at 4 months. On the swim maze, treated mice performed equally as well as the controls during the 4-day-acquisition phase. On movement of the platform on day 5, mice treated with 2,2′,4,4′,5-PeBDPO at 12 mg/kg did not improve in finding the new location. In summary, 2,2′,4,4′-TeBDPO and 2,2′,4,4′,5-PeBDPO had no effect on behavior at the low dose. 2,2′,4,4′-TeBDPO was reported to have no effect on learning at either dose. 2,2′,4,4′,5-PeBDPO was reported to affect learning at the high dose only. In a subsequent study, adult mice exposed as neonates to nicotine were reported to have "increased susceptibility" to 2,2′,4,4′,5-PeBDPO in terms of altered habituation behavior (Ankarberg et al., 2001). Similar results were reported for 2,2′,4,4′,5,5′-HxBDPO (Viberg et al., 2001b).

5.2. PeBDPO aquatic and terrestrial toxicology

PeBDPO was not acutely toxic to fish (Palmer et al., 1997a) or algae (Palmer et al., 1997b) at the limit of its water solubility. Effects were seen in *D. magna* below the limits of its water solubility after acute or chronic administration (Existing Substances Regulation 793/93/EEC, 2000c), but may have been due to physical impairment rather than a direct toxic effect. The 48-h daphnia EC50 was 14 μg/l and the NOEC was 4.9 μg/l based on the mean measured concentration. In a 21-day daphnid life-cycle study, the NOEC was 5.3 μg/l based on a slight reduction in mean body length. No effect on daphnia reproduction was found. The no effect concentration in a fish early life stage test was approximately equal to the water solubility of the product (Wildlife International, 2000a). The overall NOEC was 8.9 μg/l (LOEC = 16 μg/l) with statistically significant effects seen on juvenile fish length and weight at day 60 post-hatch at a concentration of 16 μg/l.

In an 8 week bioconcentration study in Japanese carp (Existing Substances Regulation 793/93/EEC, 2000c) the commercial PeBDPO product as a whole was found to bioconcentrate (BCF ~14,350). However, differences in bioconcentration of the various component of the product were observed. The major constituent of the product, 2,2′,4,4′,5-PeBDPO, showed no significant accumulation (BCF = 73), but the BCF of 2,2′,4,4′-TeBDPO was 35,000. This laboratory finding correlates with measured environmental levels where the major component of the commercial PeBDPO

product is frequently the minor component found in biota (Existing Substances Regulation 793/93/EEC, 2000c).

2,2′,4,4′-TeBDPO and 2,2′,4,4′,5-PeBDPO, when injected into newly fertilized rainbow trout eggs at concentrations up to 12 μg/g, did not induce signs of TCDD-like toxicity or sac fry mortality (Hornung et al., 1996). Hornung stated "Studies using commercial mixtures of PBDEs have also produced little or no TCDD-like effects as measured by effects on cytochrome P450 activity, liver morphology and reproduction in adult three-spined stickleback (*Gasterosteus aculeatus*), and liver morphology and cytochrome P450 activity in rainbow trout early life stages." Pike, fed rainbow trout injected with 2,2′,4,4′-TeBDPO, 2,2′,4,4′,5-PeBDPO arid 2,2′,4,4′,5,5′-HxBDPO dissolved in trout-lipid-extract, had uptake efficiencies of ~90%, 60% and 40%, respectively, for the three isomers (Burreau et al., 1997). The dissolution of the compounds in the lipid extract was believed to have assisted their uptake. The decline in uptake with increasing bromination was explained by the slow diffusion through aqueous phases of highly hydrophobic substances and a dependence on molecular weight. Maximum uptake at a molecular weight of 450 was observed over the series of polychlorinated biphenyl, polychlorinated naphthalene and the three PBDPO isomers compared in the study.

The commercial PeBDPO product, injected into newly fertilized rainbow trout embryos (4 μg/embryo), had little or no effects on fish fry liver morphology (Norrgren et al., 1993). Only a 2–3 fold increase in EROD activity, compared to controls, was detected when injected at 0.18 μg/embryo (theoretical concentration on fresh weight basis = 10 μg/g). No clinical signs similar to those of M74-affected fish were detected.

PeBDPO did not affect fish reproduction when incorporated in the diet (Holm et al., 1993). Numbers of eggs laid and spawning success of female sticklebacks fed freeze-dried chironomids with PeBDPO for 3.5 months was comparable to controls. Hepatic cytochrome P450-dependent EROD activity was not induced. PeBDPO uptake efficiency in the treated females was approximately 20%.

The 28-day EC50 after chronic exposure to the sediment organisms *Hyalella azteca* (Wildlife International, 2000b), *Chironmus riparius* (Wildlife International, 2000c), and *L. variegatus* (Wildlife International, 2000d) to the PeBDPO-commercial product was > 50 mg/kg dry sediment. The 28-day NOEC in Hyalella, Chironomus and Lumbriculus was 6.3, 16, 3.1 mg/kg dry sediment, respectively.

PeBDPO was not toxic to earthworms in a 14-day study (NOEC ~500 mg/kg soil dry weight) (Wildlife International, 2000e) or to soil nitrification organisms in a 28-day study (NOEC ~1 mg/kg soil dry weight (Inveresk, 1999). In 4 of 6 of terrestrial higher plant species tested in a 21-day study, no effect was found at 1000 mg/kg dry wt (Wildlife International, 2000f). In 1 plant species, the NOEC was 125 mg/kg and in another the EC25 was 154 mg/kg.

6. Conclusion

BFRs comprise about 25% of the volume of FR used globally in applications requiring high FR performance or in resins needing a FR active in the gas phase. BFRs as a class are structurally diverse and include aromatic diphenyl oxides (a.k.a. ethers), cyclic aliphatics, phenolic derivatives, aliphatics, phthalic anhydride derivatives and others. PBDPO flame retardants, made up of three commercial products, are representative of just one class of BFRs. The composition, production volumes, uses and toxicology of the three commercial PBDPO/PBDE products are distinctly different from one another and are not representative of all BFRs due to the variety of structural types.

In 1999, the global usage of the PBDPO products was ~81.7% DBDPO, ~5.7% OBDPO, and ~12.7% PeBDPO. The DBDPO-commercial product is ⩾ 97% in purity, whereas the OBDPO product is a mixture of hexa- to nona-BDPO congeners and the PeBDPO product is a mixture of tetra- to hexa-BDPO congeners. DBDPO is used to flame retard styrenic resins in electronic equipment and in upholstery fabric. OBPDO is used to flame retard business equipment composed of ABS resins. PeBDPO's sole application is to flame retard flexible polyurethane foam typically used for upholstery cushions.

The DBDPO, OBDPO and PeBDPO molecules are high molecular weight compounds ranging from 564 (PeBDPO) to 959 (DBDPO). The measured water solubility of the commercial products indicates their solubility is related to the degree of bromination on the diphenyl oxide molecule: DBDPO (< 0.1 μg/l) < OBDPO (1 μg/l) < PeBDPO (13 μg/l for the sum of its major components). Their vapor pressures follow the same pattern: DBDPO (4.6×10^{-6} Pa) < OBDPO (6.6×10^{-6} Pa) < PeBDPO (4.7×10^{-5} Pa). Recent measurements of individual isomers have found the same relationship: water solubility and vapor pressure decrease with increasing bromination (Tomy et al., 2001; Wong et al., 2001). Although the water solubility and vapor pressures of all three commercial products are quite low, their measured values are sufficiently different to impact their movement into and in the environment. Environmental monitoring for components of the commercial products indicates ~50–70% of "PBDEs" detected are due to one isomer, 2,2′,4,4′-TeBDPO, a component of only one PBDPO-commercial product. In contrast, DBDPO is infrequently detected in environmental samples and where found is generally confined to

sediments near point sources of release and not in biota (Ranken et al., 1994; Existing Substances Regulation 793/93/EEC, 2000a,b).

Differences also exist between the three products with respect to bioconcentration potential. DBPDO, OBDPO and the major isomer, 2,2′,4,4′,5-PeBDPO, in the PeBDPO-commercial product did not bioconcentrate in laboratory fish studies. The PeBDPO product as a whole and its 2,2′4,4′-TeBDPO isomer did bioconcentrate. Despite the ability to bioconcentrate, there is little evidence of acute or chronic effects in aquatic species due to the commercial PeBDPO product or its major components.

The mammalian repeated dose toxicity of DBDPO, OBDPO, and PeBDPO's are dissimilar. For example, the NOAEL for DBDPO in subchronic and/or chronic studies in the rat or mouse was at least 1000 mg/kg/d whereas the NOEL for PeBDPO in a 30-day study in the rat was 1 mg/kg/d. This difference in effect levels between the two products is likely related to differences in the absorption, metabolism, and elimination of the isomers in each product. DBDPO was minimally absorbed from the gastrointestinal tract (0.3–2%), had a relatively short half-life (< 24 h), and was rapidly eliminated via fecal excretion (> 99% in 72 h). In contrast, oral absorption of 2,2′,4,4′-TeBDPO was estimated as ~95% with less than 14% eliminated in 5 days by the rat. 2,2′,4,4′,5-PeBDPO was also slowly eliminated, only 43% of an oral dose in 72 h, but at a rate faster than 2,2′,4,4′-TeBDPO. These differences in pharmacokinetic behavior correlate with environmental monitoring results indicating bioaccumulation of components of the PeBDPO product but not of DBDPO. Thus, increasing the number of bromine atoms/molecule from 4 bromines in 2,2′,4,4′-TeBDPO to 10 in DBPDO resulted in a significantly reduced oral absorption, a dramatically shortened half-life, a substantial increase in the percent of dose eliminated, and a reversal in bioconcentration potential from a highly bioconcentrating isomer to one with little or no potential to bioconcentrate (Table 4).

"PBDEs" have been described as potential endocrine disruptors, apparently because of minimal effects of components of the commercial PeBDPO product on the thyroid (Sinjari et al., 1998; Meerts et al., 1998a, 2000; Bergman, 2000). The principle effects observed include a reduction in serum T4 levels in vivo and competition by hydroxylated derivatives with T4 for binding to TTR in vitro. In general, in vivo repeated dose studies found no effect on plasma TSH levels, mild depression of free or bound plasma T4 levels, and mild hepatic enzyme induction. The depression of the plasma T4 levels was likely related to the hepatic enzyme induction. In vitro studies found that none of 17 PBDPO isomers tested, including 2,2′,4,4′,5-PeBDPO and 2,2′4,4′-TeBDPO which are the major components of the PeBDPO product, competed with T4 for binding to TTR, and

Table 4
A comparison of the uptake, half-life and elimination of DBDPO and 2,2′,4,4′-TeBDPO

Property	Molecule	
	DBDPO	2,2′,4,4′-TeBDPO
Half-life (rat)	< 24 h	≫5 d
% Oral dose absorbed (rat)	< 0.3–2%	95%
% Oral dose eliminated (rat)	> 99% in 72 h	< 14% in 5 d
Bioconcentration factor (fish)	< 5 or < 50 at 60 and 6 μg/l, respectively	49,000

that PB-induced microsomes converted ca. half of 17 isomers in vitro to products which could compete with T4 for binding to TTR. Of the PBDPO isomers converted in vitro to metabolites that competed for binding to TTR, only a few, including 2,2′,4,4′-TeBDPO, are known to be present in the commercial PeBDPO product. However, 2,2′,4,4′-TeBDPO is poorly metabolized in vivo.

The relevance of the depression of T4 levels and TTR binding studies to humans and most mammals is questionable. The rodent thyroid is known to have greater sensitivity to derangement by drugs, chemicals and physiologic perturbations than humans (Capen, 1996, 1997). This greater sensitivity is related to T4's shorter half-life in rodents than in humans due to the substantial differences between the species in the transport proteins and the easily induced UDP-glucuronyl transferase in rodents (Capen, 1996, 1997). The plasma T4 half-life in rats is considerably shorter in (12–24 h) than in humans (5–9 d). Circulating T4 is bound primarily to TGB in humans and monkeys, but this high affinity protein is not present in rodents, birds, amphibians or fish. Thus, rodents which have short-lived T4 transported by a low affinity binding protein are more susceptible than humans to decrease the circulating thyroid hormone levels brought about by hepatic enzyme inducers. Capen (1997) stated "The activation of the thyroid gland during treatment of rodents with substances that stimulate thyroxine catabolism is a well-known phenomenon and has been extensively investigated with PB and many other compounds. It occurs particularly with rodents, first because UDP-glucuronyl transferase can easily be induced in rodent species, and second because thyroxine metabolism takes place very rapidly in rats in the absence of TGB." Finally, while competition with T4 for TTR binding may be important for rodents, it has little relevance to humans and most mammals in which TTR is only a minor thyroid binding protein.

In summary, the composition, production volumes, applications, toxicology and bioconcentration of the three commercial PBDPO products, DBPDO, OBDPO

and PeBDPO, are distinctly different. A recent publication (Darnerud et al., 2001) suggested that a LOAEL value of 1 mg/kg/d was "reasonable for compounds or mixtures belonging to the PBDE group". This suggestion is inappropriate for the commercial DBDPO product for which a number of repeated dose studies provide a NOAEL of at least 1000 mg/kg/d. Thus, it is recommended to avoid using the terms "PBDPO" or "PBDE", and instead to specify the product or isomers under discussion or study.

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Review

Polybrominated diphenyl ether (PBDE) flame retardants

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Abstract

Polybrominated diphenyl ether, PBDE, flame retardants are now a world-wide pollution problem reaching even remote areas. They have been found to bioaccumulate and there are concerns over the health effects of exposure to PBDEs, they also have potential endocrine disrupting properties. They are lipophilic compounds so are easily removed from the aqueous environment and are predicted to sorb onto sediments and particulate matter or to fatty tissue, aiding their distribution throughout the environment. PBDEs are structurally similar to PCBs and DDT and, therefore, their chemical properties, persistence and distribution in the environment follow similar patterns. Concentrations of PBDEs found in environmental samples are now higher than those of PCBs. Evidence to date demonstrates that PBDEs are a growing problem in the environment and concern over their fate and effects is warranted. The manufacture of reactive and additive flame retardants is briefly discussed and their fate and behaviour in the environment is assessed. PBDE toxicology is reviewed and methods of analysis are evaluated.

Keywords: Polybrominated diphenyl ethers, PBDEs; Flame retardants

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1. Introduction

Flame retardants are chemical substances used in various products such as plastics, textiles and furnishing foam to reduce their fire hazards by interfering with the combustion of the polymeric materials. The use of both inorganic and organic flame-retardants has achieved high levels of fire safety (Kuryla and Papa, 1991). Brominated flame-retardants (BFRs) are the cheapest way of improving fire resistance. Alternatives are available, such as phosphorus and metal based compounds, but these are more costly and can pose manufacturing problems.

Flame retardants can be used as either reactive or additive chemicals. The reactive flame-retardants are incorporated into the polymeric materials by covalent bonding between the polymer and the flame retardant, whereas the additive types are dissolved in the polymer (Bergman, 1989; Teuerstein and Eurobrom, 1998; Renner, 2000a). Additive flame retardants are sometimes volatile and can tend to bleed, so their flame retardancy may be gradually lost and they can enter the environment more easily. High molecular weight products are being developed to enable plastics to be made more permanently fire retarding by the additive mechanism. Leaching of the flame-retardants into the environment is less from the reactive retardants than from additives.

Although BFRs are a highly diverse group of compounds the flame retardancy mechanism is basically the same for all compounds (Troitszch, 1990; Danish EPA, 1999). With the application of heat they decompose before the matrix of the polymer, preventing the formation of flammable gases. Halogen containing flame retardants act primarily by a chemical interfering with the radical chain mechanism taking place in the gas phase during combustion. High energy OH and H radicals formed during combustion are removed by bromine released from the flame retardant. Thermal stability with respect to the polymer is the critical factor in choosing a flame retardant. They decompose at approximately 50°C below the host polymer and hence serve as good flame-retardants. Most organobromine compounds have this thermal characteristic due to weak carbon–bromine bonds. If the retardant decomposes and/or volatilises at a temperature well below or above that at which the polymer decomposes then the retardant will be ineffective (Price, 1998). In general, aliphatic bromines have less thermal stability than aromatic bromine compounds. Hence, aromatic bromine compounds are used extensively as flame retardants all over the world. Consequently, a considerable spread of these compounds has been found world-wide in biological and in sediment samples (Jansson et al., 1987). Their distribution throughout the environment and their toxicity exhibits many parallels with PCBs (polychlorinated biphenyls) and DDT (Sellstrom et al., 1993).

Polybrominated diphenyl ethers (PBDE), tetrabromobisphenol A (TBBPA), tetrabromophthalic anhydride, dibromoneopentylglycol and brominated styrene are the most commonly used reactive flame retardants. In this review, only one group of halogenated aromatic compounds, polybrominated diphenyl ethers, will be considered.

PBDEs are structurally similar to PCBs and PBB (polybrominated biphenyls) (Fig. 1) and so have similar properties, they have a large number of congeners depending on the number and positions of the bromine atoms on the two phenyl rings. The total number of possible congeners is 209, and the number of isomers for mono-, di-, tri-, tetra-, penta-, hexa-, hepta-, octa-, nona- and decabromodiphenyl ethers are 3, 12, 24, 42, 46, 42, 24, 12, 3 and 1, respectively (WHO, 1994).

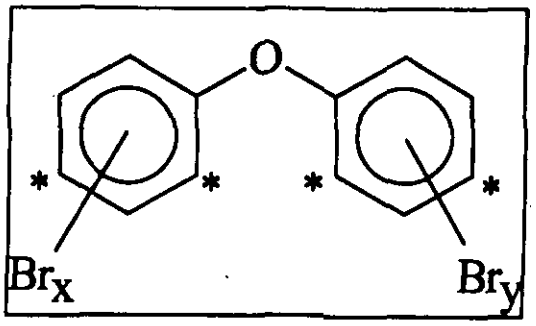

Fig. 1. Chemical structure of polybrominated diphenyl ether, * denotes most active sites of substitution.

The consequences of the spread of these compounds in the environment and in human health are not assessed adequately, because of the shortage of information on quantities produced, where used, and the quantities found in environmental samples. The information on their toxicology is also very meagre, although isolated cases of the occurrence of these compounds have been related to human illnesses including cancer and mass mortality of marine mammals. The object of this review is to report available data on production of these compounds, their use, method of sample-analysis and their toxicology.

1.1. Manufacture of polybrominated diphenyl ethers

Most preparations of PBDEs reported describe the bromination of diphenyl ethers with bromine in presence of catalysts (Om et al., 1996). The authors synthesised PBDEs in the laboratory by coupling phenol and bromobenzene. After coupling the compounds are further brominated by refluxing with aluminium tribromide or Iron III bromide (Friedel Kraft Catalyst) to polybrominated aromatic compounds. The bromination of diphenyl is fairly specific and substitution first occurs in the 4- positions relative to the ether oxygen (Underwood et al., 1930) and then in the 2- positions (Fig. 1) of each phenyl ring with the formation of 2, 2, 4, 4′-tetrabromodiphenyl ether.

Most industrially manufactured PBDEs contain mixtures of brominated diphenyl ethers, their isomers and homologues (Elvers et al., 1992). The commercial PBDEs are predominantly penta- (PeBDE), octa-(OBDE) and decabromodiphenyl ethers (DeBDE). PeBDE is a mixture with tetra- in which tetra-BDE (TeBDE) is a major component (WHO, 1994). The commercial PBDEs are stable compounds with boiling points ranging between 310 and 425°C. They are hydrophobic (especially the higher brominated diphenyl ethers) and lipophilic substances.

According to IPCS (International Programme for Chemical Safety) published by the World Health Organisation (WHO, 1994), there are eight world major manufacturers of PBDEs in Europe the USA and Japan. WHO state the annual global consumption of PBDEs is 40 000 t (30 000 t DeBDE, 6000 t OBDE, 4000 t PeBDE).

As the properties of PBDEs make them efficient flame retardants, their demand is increasing rapidly and, therefore, so is their production. According to the UK Department of Trade and Industry (DTI, 1999), in Europe the market for flame retardants is approximately 200 000 t year^{-1}, in which the demands for organobromines are 64 000 t. No figures for the scale of current production and use of PBDEs in the UK are available.

Table 1
Use of penta-, octa- and deca-bromodiphenyl ethers in resins, polymer and substrates. From: EBFRIP, 1990

Resins and polymers	DeBDE	OBDE	PeBDE
Acrylonitrile-butadiene styrene		X	
Epoxy-resin	X		
Phenolic resins	X		X
Polyacrylonitrile	X		
Polyamide	X	X	
Polybutylene terephthalate	X	X	
Polyethylene/cross-linked polyethylene	X		
Polyethylene terephthalate	X		
Polypropylene	X		
Polystyrene/high impact polystyrene	X	X	
Polyvinylchloride	X		X
Polyurethane			X
Unsaturated polyesters	X		X
Rubber	X		X
Paints/lacquers	X		X
Textiles	X		X

1.2. Use of brominated flame retardants

The use of PBDEs as flame retardants has been able to reduce fire hazards significantly over the past years and they are now extensively used in controlling the risks of fire. However, from the pollution perspective it is important to know which flame-retardants are used, where they are used and how much is disposed of as waste. Some of this information is very sparse or difficult to obtain.

Following an EC risk assessment, PeBDE is likely to be banned (Chemistry in Britain, 2001). Although it only constitutes 10% of the BFR market it is one of the most abundant congeners in the environment, suggesting it may be a degradation product of higher congeners or that the results are from historical discharges.

Table 2
The various applications of resins in which PBDE are used (WHO, 1994)

Resin or polymer	Principal applications	Final product
Acrylonitrile-butadiene-styrene	Molded parts	Television sets, computer casings, hairdryers, automotive parts
Epoxy	Circuit boards, protective coatings	Computers, ship interiors, electronic parts
Paints/laquers	Coatings	Marine and industry laquers for protection of containers.
Phenolics	Printed circuit boards	Paper laminates/glass prepegs for printed circuit boards
Polyacrylonitrile	Panels, electrical components	Lighting panels, housing of electrical appliances
Polyamide	Electrical connectors, automotive interior parts	Computers, connectors, automotive industry, transportation
Polybutylene terephthalate	Electrical components, connectors	Switches, fuses, stereos
Polyethylene/cross-linked polyethylene	Cross-linked wire cable, foam tubing, weather protection, moisture barriers	Power cables, insulation of heating tubes, marine appliances, building control instruments
Polyethylene terephthalate	Electrical components	Boxes, relays, coils, bobbins
Polypropylene	Conduits, electronics devices	Television and electronic devices, electro-mechanical parts, underground junction boxes
Polystyrene/high impact polystyrene	Television cabinets and back covers, electrical housing	Smoke detectors, office machines, housing of electrical appliances
Polyvinyl chloride	Cable sheets	Wire and cables, floor mats
Polyurethane	Cushioning/packaging materials	Furniture, sound insulation, wood imitation
Rubber	Transportation	Conveyor belts, foamed pipes for insulation
Textiles	Coatings	Carpets, automotive seating, furniture, tents, military safety clothing
Unsaturated (thermoset) polymers	Circuit boards, coatings	Electrical equipment, military/marine applications, construction panels

Table 3
The use of PBDE flame retardants in some consumer products in the UK (DTI, 1999)

Consumer products	Tonnes used
Upholstered furniture	1500
Electrical goods, e.g. vacuum cleaners, plugs, sockets	85
DIY products, e.g. sealants, expanding foams	25

The PBDE flame retardants are used in resins and polymers (Table 1). DeBDE and TBBPA are the two most used of the PBDE flame retardants, the detail of which is given in DTI (1999) report. The major uses are in high impact polystyrene, flexible polyurethane foam, textile coatings (not clothing), wire and cable insulation, electrical and electronic connectors and other interior parts (Tables 2 and 3). In the USA, 80–90% of PBDEs used are for these applications.

PVC has been reported to be the largest end-use of PBDE flame retardant material in Europe and polypropylene is the largest growing end-use material. However, commercial confidentiality restricts information on markets serviced by the industry and the tonnages used are not available in the open literature although a rise in global demand for has been reported at a rate of 3–6% per year (DTI, 1999). Table 4 shows the demands world-wide in 1999.

2. Polybrominated diphenyl ethers in the environment

There have been some concerns about the use of PBDEs as they are a potential risk to health and the environment. They are resistant towards acids and bases as well as heat and light and also to reducing or oxidising compounds (Pijnenburg et al., 1995; Allchin et al., 1999), so are, therefore, persistent in the environment. However, they are likely to be more susceptible to environmental degradation than PCBs due to the C–Br bond being weaker than the C–Cl bond (Hooper and McDonald, 2000). In vitro biotransformation tests show PBDEs to be very persistent (more so than PCBs) because there was no biotransformation. There was no genotoxic response observed to the Mutatox test but PBDEs can effect the regulation of hormones (De Boer et al., 1998). Trace levels have been detected in remote areas suggesting they are now a world wide problem (Jansson et al., 1987; De Boer et al., 1998; Allchin et al., 1999).

PBDEs are of environmental concern because of their high lipophilicity (Table 5) and high resistance to degradation processes. They are expected to readily bioaccumulate.

One of the ways to release PBDEs to the environment is through waste disposal. Products containing PBDEs are disposed of in the normal domestic wastes to landfills and incineration. From the landfills they may leach out when they are used as additive chemicals, and during incineration they produce toxic dioxins. This can become a persistent source of emission of these compounds to the environment. However, no

Table 4
Worldwide demand for brominated flame retardants (metric tonnes) in 1999. (Renner, 2000b)

Compound	*Americas*	*Europe*	*Asia*	*Total*
DeBDE	24300	7500	23000	54800
OBDE	1375	450	2000	3825
PeBDE	8290	210	–	8500

Table 5
Lipophilicity and solubility value for PBDE congeners (Alcock et al., 1999), where Log K_{ow} is the octanol-water partition coefficient

PBDE congener	Log K_{ow}	Solubility mg/l
DeBDE	> 5	0.02–0.03
OcBDE	5.5–8.9	< 0.01
HxBDE	6.86–7.92	4.08E–03
PeBDE	6.64–6.97	9.00E–07
TeBDE	5.87–6.16	0.07
TrBDE	5.47–5.58	0.38

studies have been done on the fate of PBDE containing products in landfills.

Some PBDEs are produced naturally (Jansson et al., 1987; Faulkner, 1998; Renner, 2000a). A number of polybrominated phenoxy phenols have been found in sponges, and sponges are known to produce methoxylated PBDEs. A large number of brominated compounds have been found to be naturally occurring although not the specific compounds of concern. Faulkner (1998) describes some marine organisms containing homoperoxidase enzymes that can produce highly brominated metabolites even from the low concentrations of bromide in seawater. How far these compounds could be a contributory factor in the pollution of the marine environment is not clear.

Environmental fate of PBDEs is not well documented. While the fate of lower congeners has been looked at, very limited information is available for DeBDE. Approximately 75% of the

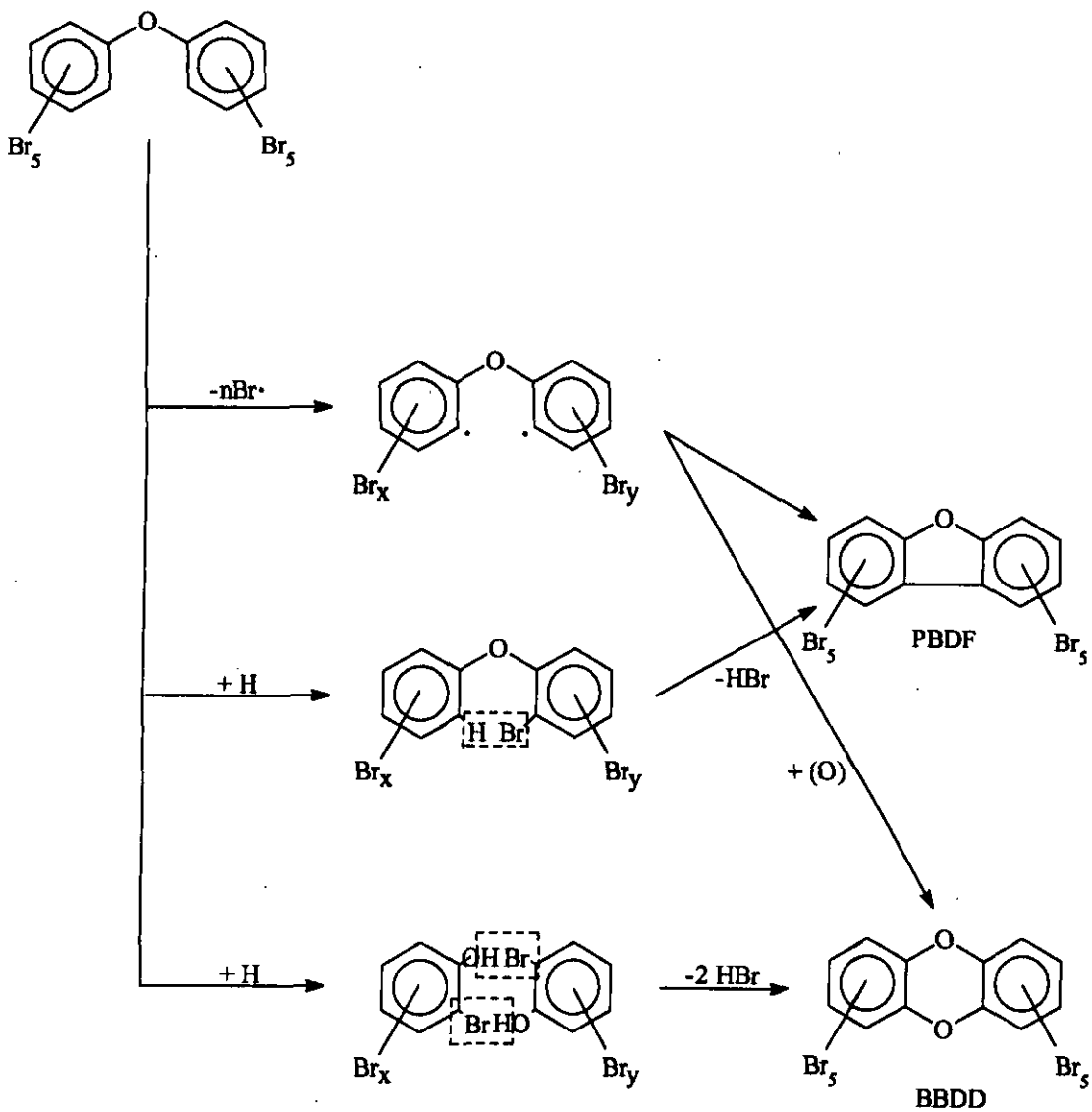

Fig. 2. Possible mechanisms for the formation of PBDF and PBDD from DeBDE (Bieniek et al., 1989).

PBDE congeners used as flame retardants are DeBDE making it important to understand its degradation and debromination in relation to the occurrence of PBDEs in environmental samples. On the basis of chemical structure, DeBDE is fully brominated and there is only one congener. Commercial DeBDE is typically composed of 97–98% DeBDE (WHO, 1994).

Despite DeBDE having the highest consumption, the majority of the PBDEs found in the environment are lower brominated compounds. This discrepancy can be explained if the higher compounds degrade to tetra, penta and hexa congeners. This theory is disputed by one of the manufacturers of PBDE, Albermarle Corporation, who theorise that the congeners found in the environment are from historic emissions (Renner, 2000a). The Great Lakes manufacturers also attribute the rise in levels to historic activities (ENDS, 2000a), such as the offshore oil industry. They believe that the EC Risk Assessment overestimates the amount of PBDEs entering the environment through current normal flame-retardant use. CEFIC claim that the rising levels in human lipids could be linked with smoking. The ECs Scientific Committee for Toxicity, Ecotoxicity and the Environment (CSTEE) are concerned that levels are rising in humans because of increased human exposure (ENDS, 2000a).

Biodegradation is not an important pathway for the PBDEs, but photolysis possibly plays an important role in their transformation (Fig. 2) (Bieniek et al., 1989). The compound is readily photodegraded in presence of xylene. In water, photoxylation has shown no favoured route and the hydroxyl substituted degradation products decomposed rapidly with increased UV-absorption (during a period of 3 months) with the formation of polybrominated dibenzofurans (PBDF) and polybrominated dibenzodioxins (PBDD) which are toxic compounds. Watanabe et al. (1987) identified PBDF from the photolysis of DeBDE in hexane solution by ultraviolet and sunlight. The formation of dechlorinated biphenyl ethers and chlorinated dibenzofurans from the photolysis of PCDE (polychlorinated diphenyl ethers) in an organic solvent was also reported earlier (Choudhry et al., 1977; Mamantov, 1985). Following the same mechanism, the formation of PBDF results from the photolysis of PBDEs, since their chemical structures are essentially the same as those of the PCDEs.

The transformation of PBDEs has also been shown by a pyrolysis experiment (Hurtzinger and Thoma, 1987) with a pyrolysis temperature from 400 to 800°C resulting in the formation of PBDF and PBDD. Therefore, incineration possibly would produce these compounds and release them into the environment. Dumler et al. (1989) suggested that the formation and emission of PBDD/PBDF is possible from a number of different sources. They can form during industrial synthesis of brominated flame-retardants and they may form during conditions of formulation of the polymer. They may also form during use of some consumer products at elevated temperatures (e.g. hair dryer, television sets) and evaporate and contaminate indoor air and they can also form during accidental fire from flame retardant articles in homes or commercial buildings.

The release of PBDFs into the atmosphere from a television set that had been on for several hours was monitored. Detectable levels of lower congeners were released as the television set warmed (Bruckman et al., 1998) as demonstrated in Table 6. There is controversy over PBDEs in television sets, environmental groups want their use phased out but fire safety campaigners support the bromine industry. Recent legislation has allowed PBDEs to be removed without being replaced with another flame retardant, as a result the number of television set fires has increased (ENDS, 2000b).

Pijnenburg et al. (1995) observed that PBBs and PBDE are slow in degradation in the envi-

Table 6
Emissions of PBDFs (pg/m^3) from a television set in use (Bruckman et al., 1998)

	Above TV	Centre of room	Ambient air
Tri-BDF	143	25	< 0.05
Tetra-BDF	11	2.7	0.16
Penta-BDF	0.5	0.5	< 0.5
Hexa-BDF	< 0.1	< 0.1	< 0.05

ronment. The reductive debromination of Firemaster (mixture of PBBs) took place in Pine River sediments (St Louis, Michigan, USA) and demonstrated that bromines were selectively removed from *para*- and *meta*-sites. It has been reported (Morris et al., 1993) that debromination of Firemaster PBB compounds could also take place by microorganisms isolated from Pine River sediments and that organic co-contaminants, petroleum products and heavy metals inhibited insitu debromination in the highly contaminated Pine River sediments.

Watanabe et al. (1987) reported that both PBB and PCB are found extensively distributed in the industrialised urban areas and these compounds are absent in rural Japan. PBBs are found in rivers, whereas PCBs have much wider distribution because of their higher mobility. Higher brominated compounds adsorb to the sediments and have less mobility and so are concentrated in sediments near their points of sources.

Congeners of different PBDEs have been reported from biological and sediment samples even in the remote environment. Concentrations of PBDEs in Sperm whale blubber were 50 times higher (100 ng/g wet weight) than total PBB concentrations found (De Boer et al., 1998). As Sperm whales fish at great depths these results indicate high PBDE levels have reached deep ocean waters. Allchin et al. (1999) found PBDEs in sediments and biota. The samples were collected from locations in UK where brominated flame retardants are known to be manufactured or handled in significant quantities. These were the estuaries of the Rivers Nith, Great Ouse, Ribble and Tees. The tetra-, penta- and deca-BDE were found in most sediment samples but DeBDE was not detected in biological samples. The highest concentration of PBDE congeners, TeBDE and 2,2′,4,4′,5-PeBDE, at 368 and 898 ng/g dry weight, respectively, were recorded from the Tees estuary and 239 and 319 ng/g dry weight, respectively, recorded from the River Tees and its tributary Skerne where the Great Lakes manufacturing site at Newton Aycliff is located. The samples analysed from the River Great Ouse were low in PBDE congeners down river from a landfill site at Elstow Brook which receives waste from the Newton Aycliff flame retardant manufacturing site. The concentration of PBDEs recorded was up to 3.9 ng/g dry weight. The samples from the Rivers Calder and Ribble, where the Sewage Treatment Works (STW) are, showed low concentration of PBDEs upstream of the STW and higher concentration downstream. A maximum of 3190 ng/g dry weight of deca-BDE was recorded from the sediment samples indicating that possibly the STW is the source. DeBDE has also been found in sewage samples (De Boer, 2000a) at relatively high concentrations (up to 920 ng/g dry weight) in STWs in the Netherlands.

Haglund et al. (1997) studied the level of PBDEs in herring, salmon muscle, ringed seal blubber and grey seal blubber, they recorded total concentrations of 36, 298, 380 and 468 ng/g, respectively. These levels are higher than those found in terrestrial species and show biomagnification. Seasonal variations in concentrations have been observed (Sellstrom et al., 1993), lipid based levels in herring were higher in Spring than Autumn probably due to a lower fat content in Spring. The bioconcentration factors found by Sellstrom et al. (1993) for grey seal blubber and Herring muscle were 19 and 4.3 for 2,2′,4,4′-TeBDE and 2,2′,4,4′,5-PeBDE, respectively. The concentrations of TeBDE, PeBDE and HxBDE found by Haglund et al. (1997) in human fatty tissue in Sweden were 9.1, 2.9 and 1.7 ng/g lipid, respectively, which are much higher than those of PCBs reported in studies of human fatty tissue in North America (Stanley et al., 1991).

Sediment samples from the Baltic Sea showed low levels at the bottom of core samples but the concentration of PBDE increased rapidly with the decreasing depth (Nylund et al., 1992). They found that with the decreasing sediment depth the amount of TeBDE increased 4–8 fold and that of PeBDE increased 10–20 fold. This probably indicates the use of increasing amount of these substances with time. PBDEs were reported in sediment from Buffalo River in the Great Lakes, USA. TeBDE accounted for 94–96% of the total and was found to be the most bioaccumulative. PeBDE accounted for 3–5% and only 1% was HxBDE. DeBDE was less than the limit of detection (0.1 ng/g tissue) (Loganathan et al., 1995).

Research in the Netherlands (De Boer, 2000a) showed high levels in Antwerp near to the textile industry. Concentrations were as high as 4600 ng/g dry weight in suspended particulate matter, this reduced the further from the textile industry the samples were taken. Fish samples contained no DeBDE although marine mussels did, this may be due to particulates being present, as the mussels were not depurated before analysis.

PBDEs are also reported from marine and fresh water fish of the North Sea, Japan and Sweden. The presence of PBDEs was reported from the livers of cod from the North Sea (south, central and north). TeBDE dominated the results with occasional PeBDE. The levels of these compounds decreased from south to north (south 170 ng/g, central 54 ng/g and north 26 ng/g) (De Boer, 1989). This trend is perhaps due to the distance from the land where these chemicals were used. In Japan, PBDEs were reported from estuary sediments, marine fish, marine organism and river sediments (Watanabe et al., 1987). The study showed that TeBDE dominated in marine fish and marine organisms, whereas DeBDE was found at the highest level in the Osaka River sediments. Jansson et al. (1987) and Sellstrom et al. (1993) reported the occurrence of PeBDE and TeBDE in the muscle tissue of osprey, in newborn starlings and in guillemot eggs. The results were 14.0, 2.3–4.2 and 24–260 ng/g, respectively. TeBDE was found in greater concentrations in fish eating species such as in Osprey, 2,2′,4,4′-TeBDE reached a concentration of 1800 ng/g in muscle, demonstrating its ability to biomagnify.

Nineteen congeners of PBDEs were found in Long-Tailed Pilot whales off the coast of the Faroe Islands (Lindstrom et al., 1999) in 1996. The concentrations found ranged from 834 ng/g lipid in young males and 3160 ng/g lipid in adult females. 2,2′,4,4′-TeBDE and 2,2′,4,4′,5-PeBDE account for 70% of the congeners. The levels are higher than those observed in 1994. High levels found in adults are from the contaminated food chain and the levels in young, which subsist on mother's milk, can be explained by lactational transfer from mothers to young.

The metabolism of PBDEs has been investigated by feeding trout DeBDE spiked food (Kierkegaard et al., 1999). The levels in the muscle tissue increased from < 0.6 ng/g fresh weight to $38(\pm 14)$ ng/g after 120 days, the liver concentration also increased. Over a short period of time the levels of hexa and nona congeners increased. After depuration, DeBDE rapidly decreased and the concentrations of the lower congeners were unaffected, demonstrating that DeBDE was metabolised by the trout. Metabolic processes did not readily produce tetra and penta congeners.

Andersson and Blomkist (1981) studied species of Pike, Bream and Eel from the Viskan river system of Sweden. They recorded 27-mg/kg fat of PBDE in Pike muscle. In the liver of the same specimen the figure was 110 mg/kg. However, 2,2,4,4′-TeBDE was by far the most abundant PBDE congener present and was between 70 and 80% of the total PBDEs found in all samples.

Sellstrom and Jansson (1995) analysed TBBPA in a printed circuit board and in sediment samples from upstream and down stream of a plastic industry that used TBBPA. It is used here as a reactive flame-retardant, therefore, it should not enter the environment. However, a small fraction (4 μg free TBBPA/g TBBPA) remained unreacted with the plastic and therefore, leached out of the product. In sediment samples, the TBBPA concentration was found to be 34 ng/g dry weight upstream of the industry and 270 ng/g dry weight down stream. The level of the unreacted TBBPA from the circuit board was 700 ng TBBPA/g filings with 14–20% TBBPA. TBBPA was also found in two sewage samples at levels similar to sediment samples taken upstream from the plastic industry. One sewage treatment works takes landfill leachate from a landfill accepting plastics waste, and the other sample was from a works connected to known TBBPA users. DeBDE has also been found in sewage samples (De Boer, 2000a) at relatively high concentrations (up to 920 ng/g dry weight) in sewage treatment works in the Netherlands.

The data above indicates the use of flame retardants has lead to polybrominated compounds entering the environment world-wide and accumulating in fatty tissues and sediments even when used as reactive compounds. This needs further

investigation of various environmental samples for their implication in hazards.

3. Methods of analysis

Due to the similarities of PBDEs with PCBs the methods of analysis for PCBs are also applicable to PBDEs. Modifications have been made to the widely published PCB methods.

3.1. Sample extraction

The majority of analytical methods have concentrated on the lower PBDE congeners and limited studies have been undertaken on the higher molecular weight compounds (octa and deca congeners). Analysis of DeBDE can pose problems in that it is both light sensitive and unstable at the high temperatures which are required for gas chromatography (De Boer, 2000a,b). These thermally labile properties are what make it a good flame retardant. To avoid degradation problems, the sample extraction may need to be protected from exposure to UV light and the GC column temperature needs to be kept to the minimum possible.

An interlaboratory study involving 20 laboratories world-wide has been done (De Boer, 2000b), looking at seven samples from the aquatic environment. The laboratories looked at TeBDE, PeBDE and DeBDE congeners. The results for 2,4,2′,4′-TeBDE and 2,3,4,2′,4′-PeBDE showed good agreement between the laboratories. The results for most of the other congeners demonstrated that further work is needed. The results for DeBDE showed that significant work is needed, the relative standard deviation for this compound was 48%.

For the analysis of sediment samples, different extraction methods have been used. Air drying and subsequent drying over sodium sulfate followed by soxhlet extraction (Watanabe et al., 1987; Allchin et al., 1999) with hexane/acetone and cleaned through partially deactivated alumina was used for UK and Japanese sediment and biota samples. De Boer (2000a) also used soxhlet extraction with hexane/acetone and followed this with a more extensive cleanup. The cleanup involved HPLC over two PL gel columns and then further purification by elution over silica gel and sulfuric acid treatment. For the analysis of sewage sludge and sediment samples Nylund et al. (1992) used hexane and diethyl ether for solvent extraction using centrifugation followed by liquid/liquid extraction using acetone and hexane, then hexane, diethyl ether and undecane. The cleanup step was done by shaking with 2-propanol and TBA sulfite reagent and achieved recoveries of 60–103%. De Boer (1989) used pentane/dichloromethane for soxhlet extraction followed by fat removal over alumina and fractionation through silica.

Biota samples were analysed (Lindstrom et al., 1999) after initially drying over sodium sulfate and then by applying to columns and extracted using methylene chloride and hexane (1:1). Cleanup was performed on a multilayer silica column, consisting of sulfuric acid on silica, neutral activated silica and potassium hydroxide on silica, hexane was used for elution. Andersson and Blomkist (1981) used hexane/acetone and hexane/diethylether extraction followed by the removal of fat with sulfuric acid for biota samples, they also used silica for the cleanup.

PBDEs were extracted from human milk (Meironyte et al., 1999) with a lipophilic gel Lipidex 5000, and further purified by gel permeation on partly deactivated alumina and silica gel. The average recoveries for the internal standards were 86–102%.

Hartonen et al. (1997) used supercritical fluid extraction with solid phase trapping for the analysis of sediment samples and achieved recoveries of $> 95\%$ with the addition of modifiers. Recoveries varied with different sediments, altering the modifiers depending on sample matrix increased the recovery. Florisil was used as the trapping material. The extraction was at 120°C and 374 bar using 20-min static extraction and a 40-min dynamic extraction, the carbon dioxide flow rate was kept at 1 ml/min. The results were compared to a 20-h soxhlet extraction with acetone/hexane followed by a cleanup over an acid silica column eluted with pentane/heptane.

3.2. Analysis by GC-MS

Most workers analysing PBDEs, have used gas chromatography (GC) with electron capture detection or gas chromatography coupled to mass spectrometry (GC/MS) with negative chemical ionisation detection to identify and quantify PBDE congeners in environmental samples as well as in commercial products (Jansson et al., 1987; Nylund et al., 1992; Sellstrom et al., 1993; Hartonen et al., 1997; Sjodin et al., 1998; Allchin et al., 1999; De Boer, 2000a). Typically, a chromatographic separation of extracted samples are achieved by temperature programming of the oven with a capillary column and helium as carrier gas. Until recently, quantitative analyses were done by using technical PBDE products (e.g. BK 70) due to the lack of availability of pure reference standards for most PBDE congeners.

4. Toxicology

4.1. General

Toxicology is essentially a study of the interacting chemicals or mixtures of them with living organisms, producing harmful effects and the determination of the levels of these effects (Ballantyne et al., 1994). The toxicity is quantitatively-related to degree of exposure and the chemical dose, it is the potential for a material to produce injury in biological systems. The end point of the effect can vary depending on the factors such as physicochemical properties of the substance, its bioconversion, the conditions of exposure and the presence of bioprotective mechanisms.

It is now clear that dioxin (TCDD) exerts most, if not all, of its toxic effects by binding to a specific receptor of the steroid hormone type (Ballantyne et al., 1994). A receptor is a specific protein situated in the cell membrane or at an intracellular site. The binding and interaction of drug molecules mediate the actions of most drugs with the receptor leading to a molecular change in the receptor, which sets off a chain of events leading to a response. Receptors are highly specific and interact with a limited number of structurally-related molecules. It has also been observed (Helleday et al., 1999) that PBDEs have the same effects as PCBs and DDT in terms of inducing genetic recombination, which causes a number of diseases including cancer.

Data on the toxicology of the PBDE flame retardants is limited. The main findings published so far are changes in liver weight accompanied by histological alterations in animals given relatively large doses (Fernlof et al., 1997). The reported results are found either due to accidental exposure or from a few experimental works on laboratory animals. The well-known case of accidental exposure was in Michigan, USA in 1973 where Firemaster (mixture of 12 PBBs) was accidentally mixed into farm animals feed in place of magnesium oxide (Sleight, 1998). As a result, approximately 450 kg of Firemaster was mixed into dairy, livestock and poultry feed and distributed throughout Michigan. A year later, PBB was identified in a laboratory in Maryland and Firemaster was banned. By this time, the contaminants were widespread resulting in illnesses in farm animals and human population. Tolerance levels were immediately established, initially at 1 ppm then to 0.02 ppm (fat basis) for milk and meat. The cows and other livestock that exceeded this limit were disposed of. Concerns about its consequence on human health are still on going.

This accident initiated, probably for the first time, research into the toxicology of the PBDE flame retardants. In Firemaster, the toxicity is caused by four congeners (Sleight, 1998) that comprise only 15–20% of the mixture. These are 2,4,5,3′,4′-pentabromo biphenyl, 2,3,4,2′,4′,5′-hexabromo biphenyl, 2,4,5,3′,4′,5′-hexabromo biphenyl and 2,3,4,5,3′,4′-hexabromo biphenyl. The precise mechanisms of toxicity of the halogenated aromatic hydrocarbons are unknown. However, the polyhalogenated biphenyls are unique, as they are the only class in this group in which there is a single carbon–carbon bond between the aromatic rings. The two rings can rotate freely if the other halogens on the rings do not interfere with the rotation. Thus, 3,4,5,3′,4′,5′-HxBB with no bromines ortho to the bridge carbon is very toxic. Whereas, 2,4,

5,2',4',5'-HxBB with two bromines on the carbon ortho to the bridge carbon cannot rotate freely around the bridge, does not bind to the TCDD receptor and is relatively non-toxic. So the toxicity of the PBB congeners appears to be related to the substitution pattern on the two aromatic rings.

4.2. Human exposures

Swedish research (ENDS, 1998; Meironyte et al., 1999) demonstrated that BFRs pose an increasing risk to human health. The level of BFRs in humans is an upward trend. One TeBDE congener was first detected in human milk in 1972 and since then the level of these compounds have been increasing exponentially as the levels of PCBs and DDT are decreasing (Renner, 2000b). A level of 4 ng/g lipid of TeBDE was detected in 1997 (Meironyte et al., 1999; Hooper and McDonald, 2000) which is a concern for its consequence on health despite it being below levels which are shown to be detrimental to animal brain development. Canadian research (Renner, 2000b) compared levels of PBDE in human breast milk in 1981 and 1992, an increase of nearly 2 orders of magnitude was observed, from 0.21 to 16.24 ng/g milk lipid. It is doubling every 5 years and even an immediate and complete ban will not stop levels from rising for some time. Hooper et al. (1998) found 10–120 pg/g fat of dioxin congeners in rural Kazakstan.

Levels airborne PBDE have been observed in an electronics recycling plant (Sjodin et al., 2001) and in office dust in Sweden (ENDS, 1998). TBBA and two PBDE congeners were found, which probably originate from VDU screens and from office furniture. Workers exposed to DeBDE during manufacturing were examined (Bahn et al., 1980) and were found to have a higher than normal prevalence of primary hypothyroidism and a significant reduction in sensory and fibula motor velocities. It could not be confirmed whether these effects were due to exposure to DeBDE or PBB, which was also produced earlier in the plant. Ott and Zober (1996) reported morbidity during exposures (1975–1988) to PBDD and PBDF. Among potentially exposed men, 2,3,7,8-TeBDF/TeBDD concentration in blood lipid ranged from non-detected to 112 ng/kg and from non-detected to 478 ng/kg fat, respectively. The immunological studies showed that the immune system of exposed workers was not adversely affected by exposure to these substances for 13 years.

These findings are reported at a time when BFRs are reported to be causing problems in human health particularly the consequence of the presence of these compounds in a human body causing endocrine disruption and abnormal development in major organs (Cheek et al., 1999; De Vito et al., 1999). The endocrine system is a ductless network of various bodies (e.g. kidney, pancreas and glands) secreting hormones into the blood system. Any interference or disruption causes health problems (Mullins, 1984). A decrease, absence or increase in the hormonal activity could cause the disruption in the endocrine system (Laycock and Wise, 1984).

Synthetic endocrine disrupting chemicals are persistent in world-wide population and can be passed from generation-to-generation. The problem is magnified because the level of natural hormones required for various body functions is extremely low compared to the endocrine disrupting pollutants present in living tissues which are at concentrations a million times higher than the hormone level. Exposure to these chemicals early in developing life (in the womb) can cause permanent abnormalities in the development of the brain. Dioxins, PCBs and BFRs are among the pollutants that cause endocrine disruption and are present in the environment. These compounds also interfere with the protein, transthyretin, which transports thyroxin around the body exposing it to the developing foetus causing interference in the development of the brain. Structural similarities between PBDEs and know immunotoxic halogenated aromatic compounds suggests that PBDEs might also effect the immune system.

At the 'Dioxin 1998' conference on organohalogen pollutants held in Sweden in 1998, Brouwer (1998) presented a review of the recent work on the endocrine disrupting effects of BFRs on the thyroid system. PBDEs, like many halogenated organics, imbalance the level of the hor-

mone thyroxin when exposed to these compounds. At 'Dioxin (2000)', brominated flame-retardants were still high on the list of priorities.

It has been suggested that TBBA may have endocrine disrupting properties. German scientists have already demonstrated that TBBA has estrogenic properties because it binds to estrogen receptors in breast cancer cells. Bergman (1989) also found that it binds strongly with the transport protein in the blood that normally binds the hormone thyroxin. This suggests that TBBA may interfere with thyroxins role in controlling growth and development. Hoque et al. (1998) concluded from an epidemiological study that exposure to PBB compounds may cause an increased risk of cancer of the digestive and lymph system.

Another study by Fernlof et al. (1997) examined the effect of PBDEs on human lymphocyte functions in exposures to determine the immunotoxic potential of these substances. No effects on mitogen-induced proliferations or immunological synthesis were observed. The study concluded that certain functions of human peripheral lymphocytes, such as proliferation and immunoglobulin synthesis are insensitive to the direct action of certain PBDEs.

Hardell et al. (1998) studied the role of PBDEs in non-Hodgkin lymphoma. The levels of TeBDE in fatty tissue were measured in 42 male and female cancer patients, 19 with non-Hodgkin lymphoma (NHL), 23 with malignant melanoma (MM) and 27 controls without diagnosis of cancer. The level of PBDE in the control group was similar to the MM group but higher than the NHL group. The MM groups occupations included professional car, bus and truck drivers (at possible risk of exposure to PBDE flame-retardants used in vehicle manufacture). However, computer operators (at possible risk of exposure to PBDEs used in VDUs) had lower levels. Due to the small sampling numbers, the results were inconclusive.

4.3. Some experimental results on laboratory animals

Different species of animals vary greatly in their susceptibility to the polyhalogenated aromatic hydrocarbons (WHO, 1994). Guinea pigs, chickens and monkeys are very sensitive, rats, mice, cattle, sheep and swine have intermediate sensitivity and hamsters are highly resistant. PBBs can have non-lethal but toxic effects on farm

Table 7
Toxicological observations (WHO, 1994)

Mammals	Dose	Observations
Single exposure		
Rat	126–2000 mg/g body wt.	14 days, produced no toxicity or pathological changes
Rabbit	200–2000 mg/g body wt.	14 days, no mortality
Short-term exposure		
Rat	0, 100, 1000 mg/g body wt.	No adverse effect on kidney, liver, appearance, food consumption or weight gain. Sight increase in bromine concentration in tissue
Mouse	0, 20, 50, 100 mg/g body wt.	No effect on health or body weight
Long-term exposure		
Mouse	3200–6650 mg/kg body wt.	113 weeks, all killed, carcinomas increased in males
Rat	0, 0.01, 0.1, 1.0 mg/kg body wt.	2 years, no effect, only slight increase in bromine concentration in fatty tissue

animals which lowers food consumption and consequently milk or egg production and weight gains are adversely affected (Sleight, 1998).

WHO (1994) produced some experimental data on the toxicity of PBDEs mainly with the administration of deca-BDE. Laboratory mammals are given doses of commercial DeBDE and observed for different levels of exposures, as shown in Table 7.

4.4. Calculation of toxic equivalent factors (TEF)

PBDEs, DBDs and DBFs, PCBs and PBBs have been found to be toxic. The toxicity of these compounds varies with the substitution position of the chlorine and bromine on the two aromatic rings, and decreases with increasing number of bromine or chlorine atoms. 2,3,7,8-TCDD has been found to be the most toxic halogenated aryl hydrocarbon (Safe, 1990).

Safe (1992) developed a method of relative toxicity's of individual halogenated aromatics relative to TCDD (i.e. toxic equivalents) based on the common receptor-mediated mechanism of action of toxic halogenated aromatics and their structure-activity relationship. The derived toxic equivalents are used for hazard and risk assessment. Provisional TEF values have been recommended for the toxic aromatic congeners which are shown in Table 8.

The TEF have proved to be useful in estimating the toxicity of complex mixtures of chlorinated dibenzodioxins and dibenzofurans (Barnes, 1991). The above values may change with time as more bioassay data becomes available although an international consensus has formed about the TEF values for addressing environmental pollution by toxic aromatic compounds.

5. Discussion

The bioaccumulation of lower brominated congeners is greater than those with a higher bromine content. The higher concentration of TeBDE than other congeners in biological samples world-wide indicates selective bioaccumulation of this congener. One mechanism of this selection is that the TeBDE can pass through the membranes and get into the cell. The PBDEs with higher bromine substitution and, therefore, higher molecular masses cannot pass through the membrane and are 'filtered' out and accumulate in the environment. In the river sediments from Osaka, Japan and marine core sediments from the Baltic Sea, DeBDE constituted a major part and penta and tetra were the minor components of the PBDEs concentration. The results of the concentrations reported so far are not always related to their source. The mechanisms of debromination are not yet fully understood, although it has been suggested that it may either be done by photochemical processes or by metabolic biodegradation.

It has been suggested that the toxicity of the polybrominated biphenyls is related to the nature of the carbon bond to the phenyl rings, with no bromine ortho to the bridge carbon, it makes the designated carbon more toxic. This was observed during a study of carcinogenicity of PBBs on affected farm animals and the human population after the Michigan accident. The results implied that polybrominated compounds, due to the larger atomic size of bromine than chlorine, are less free to rotate than chlorinated ones, and would be less toxic than chlorinated compounds. This is in con-

Table 8
Proposed toxic equivalent factors for the toxic halogenated aromatics (Safe, 1990)

Congener	TEF	Congener	TEF
PCDDs	0.001–1.0	PCBs	0.00002–0.1
PCDFs	0.001–0.5	PBBs	Same values as PCBs
Brominated and bromo/chloro dibenzodioxins and dibenzofurans	Same values as PCDDs and PCDFs	PCDEs complanar and mono-ortho co-planar congeners	0.001

trast to other theories that the lower chemical reactivity of polybrominated compounds will lead to an increase in their toxicity. The resistance of these chemicals to be metabolised will result in bioaccumulation and interference with biochemical processes of cell division, DNA replication and will encourage malignancy.

The DTI (1999) report concluded that the benefits of using PBDEs outweighs the risks to human health and the environment, although the data reported in this review demonstrates that the information on these chemicals is far from adequate at the present time and further studies are required on their use, transformation in nature and their impact on the environment.

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Table of Contents

Polybrominated Biphenyl and Diphenylether Flame Retardants: Analysis, Toxicity, and Environmental Occurrence

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I. Introduction

Polybromobiphenyls (PBBs) and polybromodiphenylethers (PBDEs) are presently being used as flame retardants in electronic equipment, plastics, building materials, and carpets. There are many standards and regulations applicable to flame retardants; those issued by the American Society for Testing of Materials (ASTM) alone account for more than one hundred (Arias 1992). The advantage of these compounds for industry is their high resistance toward acids, bases, heat, light, and reducing and oxidizing compounds. However, this high resistance becomes a great disadvantage when these compounds are discharged into the environment, where they persist for a long time. Furthermore, brominated dibenzofurans and dibenzodioxins may be formed when these flame retardants are heated (Watanabe and Tatsukawa 1990).

The basic formulas of the PBBs and PBDEs are shown in Figure 1. Throughout this chapter, the systematic numbering system developed by Ballschmiter and Zell (1980) for the 209 theoretically possible polychlorinated biphenyl (PCB) congeners has also been adopted for the corresponding PBB and PBDE congeners.

PCBs may now be encountered globally, and they have caused one of the major environmental problems as a result of their chronic and diffuse input into the environment (Tanabe 1988). The similarity in molecular structure of the PBBs and, to a lesser degree, the PBDEs, with the PCBs, give rise to great environmental concern. Much of the quantities of PBB and PBDEs produced will eventually reach the marine environment, where they are likely to accumulate because of their persistence or resistance to degradative processes. Because of their environmental properties, the continued release of PBBs and PBDEs represents an increasing risk to the environment. The purpose of this chapter is to present the existing data in a form that will lead to increased knowledge of this problem, eventually enhancing the proper measures.

II. Analysis

Because of the similarity of the character of PBBs and PBDEs to PCBs, the methods for analysis of PCBs, which are abundantly available in the literature, will also be applicable to analysis of PBBs and PBDEs. The necessary modifications are described herein.

A. Analysis of PBBs

Extraction and cleanup techniques for the analysis of PBBs in fatty tissues and sediments are identical to extraction and cleanup techniques for PCB analysis. Fehringer (1975) describes the extraction of PBBs with dichloromethane from dry animal food. Cleanup is performed with Florisil columns. Soxhlet extraction with dichloromethane/*n*-pentane, followed by

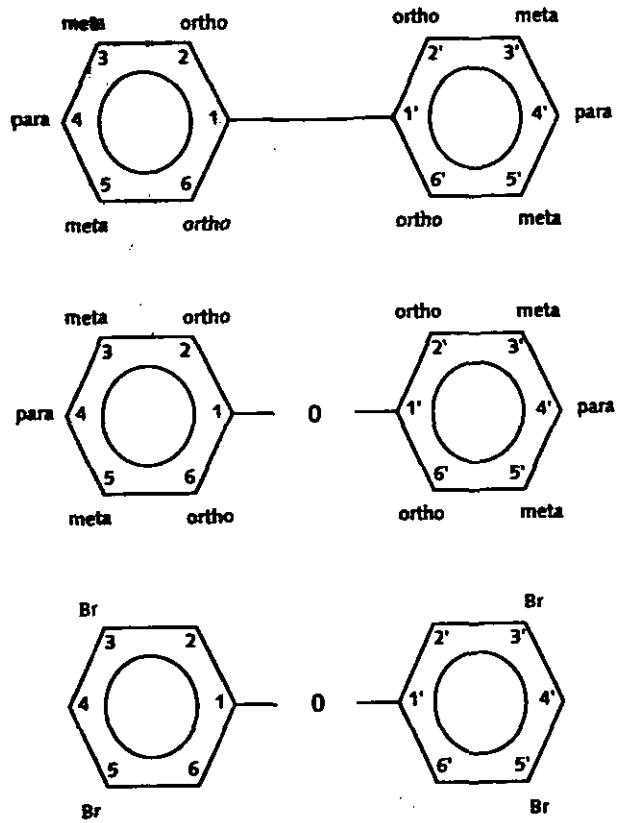

Fig. 1. Basic formulas of brominated fire retardants: (a) Polybromobiphenyls (PBBs); (b) Polybromodiphenylethers (PBDEs); (c) 3,3′,5,5′-tetrabromodiphenylether.

cleanup through alumina columns and fractionation over SiO_2 columns, results in recoveries greater than 95%. Saponification (de Boer 1988a) may be an alternative, but decomposition of some PBB congeners may occur as in the case of PCBs (van der Valk and Dao 1988).

Gas chromatography (GC) is the obvious method for the final analysis of PBBs. Several methods using packed columns are described by Fehringer (1975), de Kok et al. (1977), Sweetman and Boettner (1982), Domino et al. (1980), Mulligan and Caruso (1980), and Erickson et al. (1980). The most frequently used stationary phases are SE-30, OV-1, and OV-17. Oven temperatures vary between 240° and 300°C. The PBBs elute after the PCBs, but higher chlorinated PCBs may interfere with lower brominated PBBs. Polychlorinated terphenyls (PCTs) may also interfere with PBBs

(Wester and de Boer 1993). The separation of PBBs on a capillary 10 m × 0.25 mm i.d. OV-101 column is described by Farrell (1980).

For detection of peaks after separation, either an electron capture detector (GC-ECD) (Fehringer 1975; Domino et al. 1980; Farrell 1980; Sweetman and Boettner 1982) or a mass spectrometer (GC-MS) (de Kok et al. 1977; Erickson et al. 1980) is most frequently used. Mulligan and Caruso (1980) used a plasma-emission detector. Because of the possible interference of PCB and PCT congeners, GC-MS analysis will probably be the *most advantageous technique. The use of negative chemical ionization* (NCI) as ionization technique for the GC-MS analysis is advantageous because of its high sensitivity for compounds with four or more bromine atoms (Wester and de Boer 1993). The sensitivity of NCI for these compounds is approximately 10 times better than that of ECD. The use of narrow bore (0.15 mm i.d.) capillary GC columns is advised to obtain the required resolution.

As for all mixtures of chemical compounds with a similar structure, only the quantification of individual brominated biphenyls (BB) congeners (instead of quantification on the basis of technical mixture equivalents) will allow a comparative study of the environmental occurrence, behavior, and toxicokinetics of PBBs. However, no such results have yet been reported, although some individual BB congeners are available as standards.

B. Analysis of PBDEs

Extraction and cleanup techniques for the analysis of PBDEs in fatty tissues and sediments are similar to extraction and cleanup techniques for PCB and PBB analysis (see Section IIA). Watanabe et al. (1987) describe PBDE analysis in fish and sediments using an acetone/hexane extraction, fat removal by concentrated sulfuric acid, and cleanup on a Florisil column. Andersson and Blomkist (1981) describe PBDE analysis in fish by the subsequent use of a hexane/acetone and a hexane/diethylether extraction, fat removal by concentrated sulfuric acid, and cleanup on a silica column. The analysis of PBDEs described by de Boer (1989) is performed by soxhlet extraction with pentane/dichloromethane, fat removal over alumina columns, and fractionation over silica. In contrast to the PCBs and the PBBs, the more polar PBDEs elute in the second silica fraction together with some organochlorine pesticides.

A multiresidue method including the analysis of PBDEs is described by Jansson et al. (1991). The PBDEs are extracted from biological samples with hexane/acetone and hexane/diethylether, treated with sulfuric acid and cleaned by SX-3 Biobeads gel permeation and silica gel column chromatography. However, the mean recovery of 2,2′,4,4′-tetra-BDE for this method is only 49%.

Both GC-ECD and GC-MS with electron impact (EI) or NCI may be

used for the final analysis of PBDEs. On nonpolar or moderately polar capillary columns, 2,2′,4,4′-tetra-BDE and 2,2′,4,4′,5-penta-BDE elute late in the gas chromatogram of the second silica fraction between *p,p′*-DDT and octachloronaphthalene. The plasticizer di(2-ethylhexyl)phthalate, originating from septa, for example, may coelute with 2,2′,4,4′-tetra-BDE.

The technical mixture Bromkal 70 5DE is used as the external standard for quantification in most studies. For reasons described in section II A, *there is a need for individual bromodiphenylethers as analytical standards*, but they are not commercially available. Wolf and Rimkus (1985) described the synthesis of 2,2′,4,4′-tetra-BDE for the analysis of this congener in fish.

III. Environmental Fate and Occurrence

A. Use

In the Netherlands, the annual consumption of PBBs and PBDEs is 250 and 2500 tonnes, respectively; in Sweden, it is 1400–2000 t/yr (Svensson and Hellsten 1989); and in Japan, between 1975 and 1987, the consumption increased from 2500 to 22,100 t/yr (Watanabe and Tatsukawa 1990).

B. Environmental Fate

Higher brominated compounds have a lower solubility in water than the corresponding chlorinated compounds. The volatility of PBBs is lower than the volatility of the corresponding PCBs. The vapor pressure (P_{vp}) of hexabromobiphenyl and hexachlorobiphenyl is 4.52×10^{-10} Pa and 3.94×10^{-8} Pa, respectively. The P_{vp} of decabromobiphenyl is $< 7.4 \times 10^{-4}$; and for decachlorobiphenyl, it is 5.31×10^{-10} Pa. Higher BBs, as in flame retardants, attach strongly to sediment close to discharge points. Lower BBs have a greater solubility in water and are more easily distributed in the aquatic environment (Watanabe and Tatsukawa 1990).

Both PBBs and PBDEs are slowly degraded in the environment. Since 1970, limited *in situ* reductive debromination of Firemaster mixture seemed to occur in Pine River sediments (St Louis, Michigan, U.S.). In 1988, sediment cores contained 10–12% non-Firemaster PBB compounds. It appeared that bromines were selectively removed from the *meta* and *para* positions.

Microorganisms isolated from Pine River sediment were capable of debrominating Firemaster PBB compounds. Organic cocontaminants, petroleum products, and heavy metals inhibited *in situ* debromination in the most contaminated Pine River sediments (Morris et al. 1993). According to Watanabe et al. (1986), decabromodiphenylether dissolved in hexane can be degraded to nona-, octa-, hepta-, and hexabromodiphenylethers. Ruzo

et al. (1976) studied photodegradation of PBBs dissolved in hexane. PBBs with bromides at the *ortho* positions degrade most rapidly. Mills et al. (1985) carried out a photolysis experiment with hexa-BB components from PBB mixtures dissolved in hexane. It was found that these components could be transformed into tetra- and penta-BB components under the influence of UV light. Buser (1986) has described the formation of bromodibenzodioxins and bromodibenzofurans from PBDE by thermolysis.

Bioaccumulation and metabolism of PBBs and PBDEs in higher organisms are described in section IV.

C. Environmental Occurrence

Residues of PBDE found in samples from the environment are listed in Table 1. In sediments of the Baltic Sea, an increasing trend in the concentration of PBDEs was observed between 1973 and 1990. This increasing trend is consistent with the temporal trend of concentrations in guillemot eggs (Sellström et al. 1990). In recent years, levels of PBDE have been increasing significantly in the Baltic Sea. Levels of PBDE in sediment from 1987 are about sixfold those of 1980 (total of PBDE congeners of Bromkal at 0.44 and 2.9 ng/g IG, respectively). PBDE levels are now of the same magnitude as PCB levels are (Nylund et al. 1992).

In sediments of Osaka Bay in Japan, tetra-, penta-, and hexa-BDE were found, and in 7 of 15 riverine and estuarine samples, deca-BDE was found in higher concentrations (Watanabe and Tatsukawa 1990). In mussels from Osaka Bay, only tetra-BDE was found (Watanabe et al. 1987). PBDEs were detected in the liver of cod from the North Sea. TBDE concentrations decreased from the southern to the northern part of the North Sea, but no temporal trend was observed between 1978 and 1987 (Fig. 2). 2,2′,4,4′-tetra-BDE was identified in fish from a sewage pond in Schleswig Holstein, Germany (Wolf and Rimkus 1985). In herring from the North Sea, 2,2′,4,4′-tetra-BDE was identified (de Boer 1990). In a pike from southwest Sweden sampled in 1981, PBDE was found by Andersson and Blomkist (1981) (Table 1). Sellström et al. (1993) confirmed the extremely high PBDE concentration in the same pike and perch samples (2–35 mg/kg lipid weight) and showed that it predominantly consisted of 2,2′,4,4′-tetra-BDE. Other trout and bream samples from southern Sweden contained high concentrations of 2,2′,4,4′-tetra-BDE as well. PBBs and PBDEs have also been detected in fish-eating birds and seals (Tables 1 and 2). The most frequently occurring PBB compound was hexa-BB. PBDEs were found in eels and livers of cormorants from Dutch freshwater areas (Rhine Delta and River Rur). With the exception of the River Rur, temporal decreases have been identified for PBDE concentrations in organisms from the Netherlands. The concentrations in eels from the River Rur have increased, possibly as a consequence of the use of PBDEs in German mining areas (de Boer 1990).

Table 1. PBDE concentrations in the environment (DW = dry weight; WW = wet weight; lipid = lipid basis).

Matrix	Location	Concentration (μg/kg)	Compound	References
Sediment	Osaka Bay (Japan)	11–30 DW	tetra-, penta-, hexa-BDE	Watanabe and Tatsukawa (1990)
–	Rivers (Japan)	33–375 DW	deca-BDE	Watanabe and Tatsukawa (1990)
Mussels	Osaka Bay (Japan)	15 WW	tetra-BDE	Watanabe et al. (1987)
Cod liver	Northern North Sea	26 lipid	tetra-BDE	de Boer (1989)
–	–	3 –	penta-BDE	de Boer (1989)
–	Central North Sea	54 –	tetra-BDE	de Boer (1989)
–	–	6 –	penta-BDE	de Boer (1989)
–	Southern North Sea	170 lipid	tetra-BDE	de Boer (1989)
–	–	22–26 lipid	penta-BDE	de Boer (1989)
Herring	Southern North Sea	100 lipid	tetra-BDE	de Boer (1990)
Eel	Rur River	1.4×10^3 lipid	tetra-BDE	de Boer (1990)
Pike	Southwest Sweden	27×10^3 lipid	PBDE[1]	Sellström et al. (1990)
Pike liver	Southwest Sweden	110×10^3 lipid	PBDE[1]	Sellström et al. (1990)
Eel	Southwest Sweden	17×10^3 lipid	PBDE[1]	Sellström et al. (1990)
Trout and bream	South Sweden	100–170 lipid	tetra-BDE	Sellström et al. (1993)
Cormorant liver	Rhine Delta	28×10^3 WW	PBDE	de Boer (1990)
Baltic Guillemot egg	Baltic Sea	2×10^3 lipid	PBDE[1]	Sellström et al. (1993)
Osprey	–	$0.16–1.9 \times 10^3$ lipid	PBDE[1]	Sellström et al. (1993)
Ringed Seal	Northern Ice Sea	51 lipid	PBDE[1]	Sellström et al. (1993)
Baltic Grey Seal blubber	–	728 lipid	PBDE[1]	Sellström et al. (1993)
Seal blubber	Baltic Sea	730 lipid	PBDE	Andersson and Blomkist (1981)
–	–	26 lipid	tetra-BDE	Andersson and Blomkist (1981)

[1]Mainly tetra-BDE.

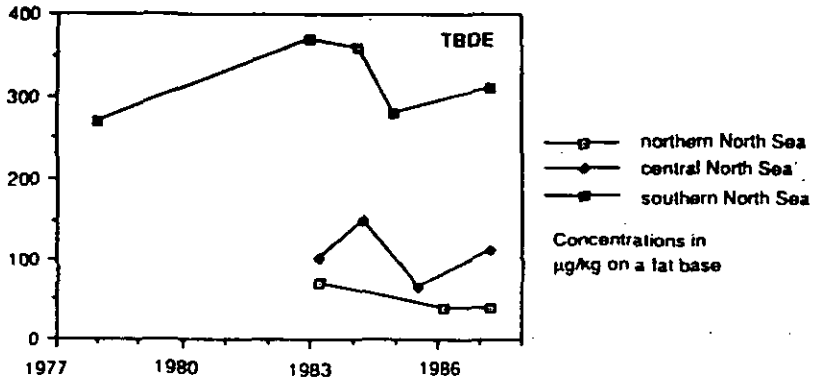

Fig. 2. Concentrations of 2,2',4,4'-TBDE in cod liver from different regions of the North Sea (source: de Boer 1989).

IV. Toxicokinetics

A. Bioaccumulation

The degree of bioaccumulation is an important factor in the overall environmental risk of a compound. The accumulation potential of organic compounds has often been estimated successfully by measuring their partitioning coefficient in a two-phase solvent system of octanol and water (K_{ow}). *n*-Octanol is used as a model compound that should mimic the lipid

Table 2. Total (Σ-) PBB and PBDE concentrations calculated as technical mixture equivalents in herring, seals, and sea birds (µg/kg lipid) (Source: Jansson et al. 1987, 1993).

		Σ-PBB		Σ-PBDE	
Organism	Area	1987	1993	1987	1993
Herring	Baltic Sea		0.16		528
	Bothnian Gulf		0.09		123
	Skagerrak		0.27		735
Seal	Baltic Sea	20/26	90	728	
	Kattegat	3	10		
	Spitsbergen	4	40		
	Northern Ice Sea		0.42		51
Guillemot	Baltic Sea	160	370		
	North Sea		80		
	Northern Ice Sea	50	130		
Sea eagle	Baltic Sea	280		350	

Table 3. Some log K_{ow} values and log BCF (in Guppy, *Poecilia reticulata*) values for PBBs (Gobas 1989).

Compound	log K_{ow}	log BCF
4,4'-di-BB	5.72	5.43
2,4,6-tri-BB	6.03	5.06
2,2',5,5'-tetra-BB	6.50	6.16
2,2',4,4',6,6'-hexa-BB	7.20	5.85

pool in biota. Compounds with a high K_{ow} usually have a high affinity for animal lipids.

Accumulation and Excretion Data of PBBs. The log K_{ow} values and bioconcentration factors (log BCF) of some PBBs are given in Table 3. Gobas et al. (1989) reported BCF and K_{ow} values of polybrominated benzenes and biphenyls and could not detect a correlation between these values when K_{ow} exceeded 6. Possible reasons for an incorrect determination of BCF values are the elimination via feces or lower bioavailability due to absorption of the compounds to other molecules or to dissolved organic matter.

Zitko (1977) and Zitko and Hutzinger (1976) studied the bioaccumulation of PBBs in young Atlantic salmon (*Salmo salar*). The PBB was supplied through the water and food. To establish bioconcentration factors, a mixture of 388 µg PBB consisting of equal amounts of three di-bromo, one tri-bromo, and two tetra-bromobiphenyls was supplied to 3 L water to which the fish were exposed for 96 hr. The biomagnification factors were measured in an experiment where the fish were fed with a PBB-spiked diet. The same mixture of PBB compounds was used. The bromine content of the food was 7.75 µg g^{-1}. The reported bioconcentration and biomagnification factors are given in Table 4.

Table 4. Bioconcentration and biomagnification factors of PBBs on a wet weight basis in Salmon (*Salmo salar*) (Sources: Zitko and Hutzinger 1976; Zitko 1977).

Compound	Bioconcentration	Biomagnification
2,6-di-BB	1.2×10^6	0.179
2,4-di-BB	1.3×10^6	0.318
3,4-di-BB	63×10^3	0
2,5,4'-tri-BB	425×10^3	0.449
2,2',4,5'-tetra-BB	314×10^3	0.589
2,3',4',5-tetra-BB	110×10^3	0.571
C_6H_6	0	0
Firemaster BP-6	n.a.	1.00

For all PBBs, bioconcentration factors are three to four orders of magnitude higher than biomagnification factors. 3,4-di-BB was not accumulated at all from the food and hardly from the water. The corresponding 3,4-di-CB shows the same effect (Zitko 1977). No explanation was given for this phenomenon by the authors.

Bruggeman et al. (1982) showed for PCBs that solubility of the different congeners depends on chlorine substitution at the *ortho* position. Because 3,4-di-CB has no *ortho*-Cl, low solubility of the congeners may be the reason for low accumulation.

In Zitko's study (Zitko, 1977), compounds with a low bromine content bioconcentrated more strongly from water than compounds with a high bromine content. PBBs with more than six bromine atoms were hardly bioconcentrated at all.

However, from food, compounds with one to four bromine atoms were accumulated more when the bromine content was higher. PBBs with six to eight bromine atoms were only accumulated to a slight degree from food.

Zitko (1977) found hexa-BBs in fish tissue after exposure to a diet spiked with only octa-BBs. This dehalogenation is not known for higher chlorinated biphenyls. The accumulation of Firemaster from food appeared to be higher than the accumulation of other PBBs.

The half-life for excretion from fish was determined for two PBB congeners, Firemaster and OB (which is an octa-BB product) (Table 5).

Accumulation of PBDE. In Table 6, the log K_{ow} values of PBDEs are given. The accumulation of PBDEs with a low bromine content is greater than that of higher brominated compounds (Sellström et al. 1990). This is apparent from the often-used PBDE mixture Bromkal 70-5 DE, PBDE concentrations in sediments, and the PBDE pattern in herring and seals. Table 7 shows that the relative concentrations of tetra-BDE in seals is much higher than the tetra-BDE content in Bromkal and in sewage sludge.

Higher brominated diphenylethers are decreased in fish and fish consumers relative to the concentrations in the commercial products. This was not in agreement with what was found with the PCB congeners. The relative concentrations of PBDE congeners in herbivorous mammals in Sweden

Table 5. Biological half-lives of PBBs in fish, following uptake from food or water (Zitko 1977).

Compound	Uptake from water	Uptake from food
2,2',4,5' tetra-BB	21 d	28 d
2,4',5 tri-BB	13 d	26 d
Firemaster	n.a.	93 d
OB	n.a.	93 d

Table 6. Log K_{ow} values of PBDE (Watanabe and Tatsukawa 1990).

PBDE	log K_{ow}
di-BDE	5.03
tri-BDE	5.47–5.58
tetra-BDE	5.87–6.16
penta-BDE	6.64–6.97
hexa-BDE	6.86–7.92
octa-BDE	8.35–8.90
deca-BDE	9.97

were the same as the concentrations in the commercial products (Jansson et al. 1993).

Comparison of accumulation of PBB and PBDE. Norris et al. (1974, 1975) studied the accumulation and excretion of deca-BDE and octa-BB in rats. The laboratory animals were fed for 2 yr with octa-BB or deca-BB 0.1 mg/kg fodder and during this period, at regular intervals, two animals were sacrificed. Deca-BDE caused an initial increase of bromine concentration in the liver, which did not increase further after 30 d. Bromine concentration in fat tissue increased only slightly. Octa-BB caused a rapid increase of bromine in the liver and in fat tissue; this increase was constant during the experiment. In an experiment with ^{14}C-octa-BB and ^{14}C-deca-BDE, the radioactivity of deca-BDE disappeared after 2 d through the feces. The ^{14}C of the octa-BB decreased to 33% during the first day. Later, the radioactivity decreased slowly, and after 16 d, 75% had disappeared. No toxic effects were found in the animals. The author concluded that deca-BDE does not accumulate, in contrast to octa-BB. The disadvantage of this study is that different numbers of bromine atoms were used. It may be that the difference in behavior is a consequence of the unequal bromine substitution and not of the difference between bromobiphenyl and bromodiphenylether.

Table 7. Percentages of PBDE congeners of Bromkal 70-5 DE (Sellström et al. 1990; Jansson et al. 1993).

	2,2',4,4'-tetra-BDE	penta-BDE (not defined)	2,2',4,4',5-penta-BDE
Bromkal 70-5 DE	44	8	48
Sewage sludge	40	9	51
Seal	89–92	3–5	2–6
Herring	62–80	6–11	9–21

No more general information is available about the differences between accumulation factors of PBBs and PBDEs. It is known that PCBs and PCBEs have accumulation factors of approximately the same magnitude (Zitko 1977). The validity of this relation is not confirmed for PBBs and PBDEs.

Comparison of accumulation of PBB, PBDE, and PCB. PBBs and PCBs with one to four bromine or chlorine atoms have accumulation factors of approximately the same magnitude, but PBBs with more than five bromine atoms accumulate less than the corresponding PCBs. Higher chlorinated biphenyls are persistent in fish, while higher brominated biphenyls are partly debrominated (Zitko 1977; Zitko and Hutzinger 1976). On the other hand, 2,2′,4,4′,5,5′-He-BB was found to magnify by a factor of 140 in the food chain herring – grey seal (Jansson et al. 1993).

B. Interactions with Cytochrome P450

In a qualitative sense, the structure-effect relationships for BB congeners show a high similarity with those of chlorinated biphenyls (CBs). Quantitatively, bromine substitution appears to have a stronger effect than chlorine substitution on induction of the P450IA subfamily (3-MC type induction; Andres et al. 1983). This may be due to a higher affinity of BB congeners for the cytosolic Ah-receptor, because the same dose (150 μmol/kg, MW = 486) of BB-77 caused an eight times higher induction of ethoxyresorufin-O-deethylase (EROD) activity than CB-77, while the affinity for the Ah-receptor was also eight times higher.

2,3,7,8-Tetrachlorodibenzodioxin shows the highest affinity for the low capacity/high activity cytosolic receptor protein, which triggers all subsequent events; the activity of PBBs is three to four orders of magnitude lower.

Interactions between PCBs and the cytochrome P450-dependent monooxygenase (MO) systems were reviewed for fish-eating seabirds (Walker 1990) and marine mammals (pinnipeds, cetaceans, and the polar bear) (Boon et al. 1992).

The composition of Firemaster BP-6 and FF-1 is given in Table 8. The amount of Firemaster BP-6 produced is higher than that of Firemaster FF-1 (Safe 1984; Aust et al. 1987). BB-153 contributes more than 50% to both mixtures; it is a PB-type inducer, i.e., it induces the P450IIB subfamily. Mixed-type inducers present in BP-6 are BB-118 (2.9%) and BB-167 (7.95%) (Robertson et al. 1980). The pure 3-MC type inducers BB-77, BB-126, and BB-169 contribute 0.16%, 0.08%, and 0.29% to this mixture, respectively. The contributions of individual congeners of BBs to BP-6 are very different from the contribution of CB congeners to any of the Aroclor or Clophen mixtures (Schulz et al. 1989). It has been shown (Aust et al. 1987) that the BB congeners of BP-6 are entirely responsible for induction

Table 8. Composition of Firemaster BP-6 and Firemaster FF-1 (Source: Silberhorn et al. 1990). Systematic numbering of individual congeners after the system developed for PCBs by Ballschmiter and Zell (1980).

No.	Structure	BP-6 (%)	FF-1 (%)
Di-BB 15	4.4′	<0.020	–
Tri-BB 18	2,2′,5-	0.050	–
26	2,3′,5-	0.024	–
31	2,4′,5-	0.015	–
37	3,4′,4-	0.021	–
Tetra-BB 49	2,2′,4,5′-	0.025	–
52	2,2′,5,5′-	0.052	–
53	2,2′,5,6′-	<0.013	–
66	2,3′,4,4′-	0.028	–
70	2,3′,4′,5-	0.017	–
77	3,3′,4,4′-	0.159	0.03
Penta-BB 95	2,2′,3,5′,6-	–	0.02
99	2,2′,4,4′,5-	–	0.03
101	2,2′,4,5,5′-	2.69	1.54
114	2,3,4,4′,5-	0.08	–
118	2,3′,4,4′,5-	2.94	0.8
126	3,3′,4,4′,5-	0.079	<0.01
Hexa-BB 138	2,2′,3,4,4′,5′-	12.3	5.23
141	2,2′,3,4,5,5′-	0.10	–
149	2,2′,3,4′,5′,6-	2.24	0.78
153	2,2′,4,4′,5,5′-	53.9	55.2
156	2,3,3′,4,4′,5-	0.98	0.37
157	2,3,3′,4,4′,5′-	0.526	0.05
167	2,3′,4,4′,5,5-	7.95	3.37
168	2,3′,4,4′,5′,6-	<0.025	–
169	3,3′,4,4′,5,5′-	0.294	0.15
Hepta-BB 170	2,2′,3,3′,4,4′5-	0.256	1.66
172	2,2′,3,3′,4,5,5′-	–	0.15
174	2,2′,3,3′,4,5,6′-	–	0.24
180	2,2′,3,4,4′,5,5′-	6.97	23.5
187	2,2′,3,4′,5,5′,6-	0.392	–
189	2,3,3′,4,4′,5,5′-	–	0.51
Octa-BB 194	2,2′,3,3′,4,4′,5,′-	–	1.65
196	2,2′,3,3′,4,4′,5′,6-	–	0.31
203	2,2′,3,4.4′,5,5′,6-	–	0.30
Total		92.09	95.89
Approximate molecular weight		628	650

of the MO system in rats; thus, there is no additional effect of impurities, such as brominated dibenzofurans in the mixture.

Another mixture of PBBs is Firemaster FF1. Next to BB-153, BB-180 dominates in this mixture, which is also a PB-type inducer. In general, the 3-MC or mixed-type inducers show a somewhat lower contribution to this mixture compared with BP-6, with the exception of BB-170.

P450 induction occurs at lower body burdens in infant compared to adult female rats: when 1 mg kg^{-1} was injected during a period of 18 d into the mother animals, it caused (a mixed function) MO induction in the sucklings but not in the mothers (Aust et al. 1987).

Carlson (1980a,b) investigated the inducing potential of PBDEs with a mixture of a low overall bromination (24% tetra-BDE congeners, MW = 502; and 50% penta-BDE congeners, MW = 582), a higher overall bromination (45% hepta-BDE congeners, MW = 662; and 30% octa-BDE congeners, MW = 822), and the deca-BBE congener only (BB-209, MW = 982). Both mixtures induced *O*-ethyl *O*-*p*-nitrophenyl phenylphosphothionate and Uridine Diphosphate-glucuronyltransferase, which catalyzes the conjugation of hydroxylated compounds to glucuronic acid (phase II metabolism). *p*-Nitroanisole demethylase and arylhydrocarbon hydroxylase were induced most by the mixture of lowest bromination. The deca-BBE congener BB-209 did not cause any enzyme induction. A long-term study where rats were injected with a daily dose of 0.8–3 μmol/kg of both mixures for 90 d showed that the abovementioned enzymes were induced at a concentration of as low as 1 μmol/kg. Moreover, the enzymes remained induced even 30–60 d after the termination of exposure. These results demonstrate that these inducers are not only potent but that their effects may be long-lasting.

C. Biotransformation of PBBs

In vitro metabolism and structure-effect relationships. Aust et al. (1987) concluded that BBs with H atoms at an *ortho*- and *meta*- (*o,m*) position, and a maximum of four bromine atoms, were metabolized by P450IA in microsomal systems of rats. Mills et al. (1985) drew similar conclusions, with the remark that bromine substitution would be necessary at least at one *meta*- or *para*-position for metabolism to occur because 2,2′-di-BB was not metabolized at all, whereas 2,2′,4,4′-tetra-BB was metabolized. Rates of metabolism decreased in the order 4,4′-di-BB > 3,4,4′-tri-BB > 3,3′,4,4′-tetra-BB > 2,3,3′4′-tetra-BB > 2,3′,4′,5-tetra-BB > 2,2′,4,5′-tetra-BB. Thus, metabolism by microsomes from 3-MC-induced rats decreased with increasing numbers of *ortho*-Br atoms and thus with an increasing energy barrier for a planar configuration. Such quantitative structure-activity relationships for PBBs are not available for marine organisms.

In the case of PCB congeners with vicinal H atoms in the *o,m* positions, they appear to be always capable of being metabolized in the polar bear, and metabolized in seals and cetaceans only when a maximum of one *ortho*-Cl is present, and persistent in seabirds (Walker 1990; Boon et al. 1992).

In contrast to the situation for 3-MC-induced rats, 2,2′-di-BB was metabolized very rapidly by microsomes from PB-induced rats. According to Mills et al. (1985) and Aust et al. (1987), congeners with vicinal H atoms at *m,p* positions are metabolized by P450IIB. The rates decreased in the order: 2,2′-di-BB > 2;2′,4,5′-tetra-BB > 2,3′,4′,5-tetra-BB > 2,2′,4,5,5′-penta-BB.

In the case of PCBs in marine animals, congeners with vicinal H atoms in the *m,p* positions appear capable of being metabolized by seabirds, seals, and the polar bear, but much less so by cetaceans, which lack P450IIB enzymes.

Because different groups of marine animals show such a varying scope for metabolism of PCBs, it is highly unlikely that the structure-effect relationships discussed above for the biotransformation of PBBs in rats also represent the situation in marine animals. Studies of these processes are urgently needed.

In vivo metabolism. Metabolites of congeners from Firemaster were detected in pigs (a hydroxylated penta-BB) and dogs (2,2′,4,4′,5,5′-hexabromobiphenylol) (Safe 1984). Zitko and Hutzinger (1976) reported a dibromodiphenylol in salmon. Of the more extensively studied PCBs, hydroxylated as well as methylsulfone metabolites have been reported (Jansson et al. 1987; Safe 1984). The metabolites are possibly formed via an epoxide, but this is not necessarily the first step (Bush and Trager 1985; Trager 1989) leading either to a hydroxylated compound (phenolic metabolite) or to binding of a reactive intermediate (epoxide) to glutathione (GSH).

Millis et al. (1985) compared the toxicokinetics of the P450IA inducers 3,3′,4,4′-tetra-BB (BB-77, MW = 486) and 3,3′,4,4′,5,5′-hexa-BB (BB-169, MW = 646). A single dose of 21.3 μmol/kg of a congener was administered to one of two groups of rats. The concentration of BB-169 in liver and adipose tissue and the activity of aryl hydrocarbon hydroxylase (AHH) reached a maximum 1 d after injection and remained at this maximum throughout the rest of the experiment. In contrast, the levels of BB-77 and AHH activity declined sharply after 2 d in the other group of rats. The decline of BB-77 was attributed to metabolism; in contrast to BB-169, BB-77 possesses vicinal H atoms. At the end of the experiment, livers of animals injected with BB-169 showed histological changes similar to tetrachlorodibenzo-*p*-dioxin (TCDD) effects, but the livers of BB-77 injected animals did not, even though the affinity for the TCDD receptor protein was 10 times higher for BB-77 than for BB-169. It may be concluded that metabolism of BB-77 decreased its TCDD-type toxicity.

The rate of *in vitro* metabolism of BB-77 is inhibited by the addition of BB-169. At equal concentrations (3 μM), the metabolism of BB-77 is al-

most completely inhibited (Mills et al. 1985) because BB-169 binds to the enzyme receptors and cannot be metabolized and because vicinal H atoms are lacking. BB-169 induces both P450IA1 and IA2, but only binds to IA2 (Voorman and Aust 1987). The BB-169/protein complex appeared to be more stable than P450IA2 by itself. The binding between BB-169 and P450IA2 was noncovalent and could be broken down by extraction with dichloromethane. BB-169 also inhibits the estradiol-2-hydroxylase activity of purified P450IA2 in a noncompetitive way (Voorman and Aust 1988).

V. Toxicity of PBBs

A. Acute Toxicity

The acute toxicities of a number of PBB and PCB mixtures are summarized in Table 9. Firemaster BP-6 appears to have a similar acute toxicity to rats as the PCB mixtures Aroclor 1254 and Kanechlor 500.

Gupta et al. (1983b) administered a dose of 100 mg Firemaster BP-6 kg^{-1} to rats on 22 occasions over a period of 1 mon. After 90 d, all females had died, but 62% of the male rats still survived. In a similar experiment with a daily dose of 30 mg kg^{-1}, all animals of both sexes survived for 90 d.

B. Toxic Effects in Relation to Cytochrome P450 Induction

The toxicity of BB congeners strongly depends on their molecular structure. Induction of the P450IA subfamily of P450 is the precursor of a whole spectrum of possible effects at more integrated levels of biological structure: weight loss, thymus atrophy, and changes in the liver, such as proliferation of the smooth endoplasmatic reticulum (location of the P450 system), increased RNA and protein content, decreased DNA content, cell necrosis, liver enlargement, and hepatic porphyria (Render et al. 1982; Jensen et al. 1983; Koster et al. 1980).

Table 9. Acute toxicities of PBB and PCB mixtures. AHH = aryl hydrocarbon hydroxylase activity (Sources: [1]Gupta et al. 1983a; [2]Andres et al. 1983; [3]Safe 1984).

Mixture	Species/Sex	Details	$LD_{50}/EC_{50}/LC_{50}$
Firemaster BP-6 (PBB)	Rat (F)		LD_{50}: 65 mg/kg/d[1]
Firemaster BP-6 (PBB)	Rat (M)		LD_{50}: 149 mg/kg/d[1]
Firemaster BP-6 (PBB)	Rat	AHH activity	EC_{50}: 50–55 mg/kg[2]
Aroclor 1254 (PCB)	Rat	AHH activity	EC_{50}: 50–55 mg/kg[2]
Kanechlor 500 (PCB)	Rat	AHH activity	EC_{50}: 50–55 mg/kg[2]
Firemaster BP-6 (PBB)	Rat	Oral	LD_{50}: 21.5 g/kg[3]
Hexa-BB	Rabbit	Skin	LD_{50}: 5 g/kg[3]
Firemaster FF 1 (PBB)	Mink	Food	LC_{50}: 3.95 mg/kg[3]

C. PBBs and Cancer

The carcinogenicity of PBBs and PCBs has been reviewed by Silberhorn et al. (1990). The formation of tumors is a multistage process. The first phase is an irreversible mutation of DNA, the initiation phase. The growth of an initiated cell to a tumor is the promotion phase. There are strong indications that PBBs, PCBs, dioxins, dibenzofurans, and related compounds are not mutagenic compounds but do promote the carcinogenicity of mutagenic compounds, such as nitrosamine and certain polyaromatic hydrocarbons (PAHs) (Safe 1984; Jensen et al. 1983; Kavanagh et al. 1985). The latter may well be of importance for the marine environment because halogenated biphenyls often co-occur with PAHs derived from oil or combustion processes. Tumor promotion has been reported for 3-MC type (BB-169; Jensen et al. 1983; Kavanagh et al. 1985) as well as PB-type inducers (BB-153; Kavanagh et al. 1985). The tumor-promoting capacity of Firemaster BP-6 is greater than that of its dominant congener, BB-153 (Jensen et al. 1982).

Safe (1984) and Aust (1987) reported liver cancer in rats from PBBs without the addition of an initiator, but the single doses of 200 mg kg^{-1} and 1 g kg^{-1}, respectively, were high. Initiating compounds may have been present in the diet, because in the second study, for example, neoplastic nodules were observed after 6 mon, which developed into malignant tumors after a period of 2 yr. (Jensen and Sleight 1986).

D. Dermal Toxicity

Like PCBs, PBBs cause chloracne; in monkeys, this occurred at a concentration of 50 mg kg^{-1} in the diet (Safe 1984). After 20 wk, exposure at a dose of 2 mg/animal twice weekly, Firemaster FF-1 also caused skin papillomas in previously initiated mice (Poland et al. 1982).

E. Neurotoxicity

Firemaster BP-6 caused chronic and subchronic neurotic symptoms, such as irritation, changed behavior and decreased muscular control (Safe 1984).

F. Immunotoxicity

PBBs caused thymus atrophy, hypersensitivity, decreased antibody response, and decreased resistance against infections in guinea pigs (Safe 1984). However, the mechanism remained unclear. A decreased immune response in rats and mice occurred after exposure to Firemaster BP-6 at either 30 mg kg^{-1} for 22 d or 10 mg kg^{-1} for 6 mon. Doses of 0.1–100 mg kg^{-1} for 30 d resulted in a reduction of B- and T-helper cells (Aust et al. 1987). In general, immunosuppression by PBBs occurs at levels that also cause a number of the other toxic effects described.

G. Effects on Reproduction and the Regulation of Steroid Hormones

Because the placenta is not an efficient barrier to PBBs and related lipophilic compounds, these enter the developing embryo during pregnancy. A second source after delivery is a mother's milk (Safe 1984). Over a period of 7 mon, a dietary concentration of 0.3 mg kg^{-1} of Firemaster FF-1 caused a longer sexual cycle and decreased progesterone concentrations in monkeys, and weights of neonates decreased. A dose of 40 and 200 mg/kg Firemaster BP-6 administered to rats once during pregnancy caused no teratogenic effects. Malformations of fetuses occurred at single doses of 400 and 800 mg/kg. The 800 mg/kg doses resulted in much higher frequency than at 400 mg/kg (Beaudoin, 1977).

A dietary concentration of 100 mg PBBs kg^{-1} caused decreased egg production and nesting behavior in Japanese quail (Aust et al. 1987). Newton et al. (1982) reported increased hydroxylation rates of testosterone to 7α- and 6β-hydroxytestosterone in rats fed 100 mg kg^{-1} PBB for 4 mon. A reduction of testosterone to dihydrotestosterone and dihydroandrosterone also was found in microsomes from both sexes.

Byrne et al. (1988) fed rats for 5–7 mon with doses of 1–50 mg kg^{-1} food. Lowered concentrations of serum corticoid hormones were already observed at low doses of Firemaster BP-6, together with a decrease in weight of the adrenals.

H. Influence on Vitamin A and Thyroid Hormone Regulation

A single dose of 2 mg kg^{-1} of BB-169 caused an increase by a factor of 2 in vitamin A breakdown products in urine and feces (Cullum and Zile 1985).

Gupta et al. (1983a) found decreased serum TT4 and triiodothyronine concentrations after exposure over 125 d to concentrations of 0.1–10 mg Firemaster BP-6 kg^{-1}.

VI. Toxicity of PBDEs

Most toxicological studies concerning PBDEs are conducted with a commercial BDE composition consisting of 77.4% deca-BDE, 21.8% nona-BDE, and 0.8% octa-BDEs (Norris 1974; Norris 1975; National Toxicology Program (NTP) 1986). Toxicity data of this BDE-mixure do not reflect the toxicity of all BDE congeners. Absorption of this mixture from the gastrointestinal tract is low (approximately 1% in rats) and toxicity will be low compared to that of other BDE congeners (see below).

A. Acute Toxicity

Intragastric intubation of single doses of up to 2000 mg kg^{-1} of commercial BDE to female rats did not affect growth rate, and no gross pathological effects were encountered during a 14-d post-treatment observation period (Norris et al. 1975).

In feeding studies, rats and mice of both sexes were exposed to a range of 5–100 g kg^{-1} of commercial BDE in the diet during 14 d and 13 wk, respectively. There were no effects on survival, body weight, or food consumption, and no gross or microscopic pathological effects (NTP 1986).

In another study with rats (Norris et al. 1975), diet concentrations were 0.1–10 g kg^{-1} commercial BDE during 30 d. Concentrations of 1 and 10 g kg^{-1} caused increased liver weight and a dose-related thyroid hyperplasia. Histopathological investigations revealed liver and kidney lesions at the highest concentration. A concentration of 0.1 mg kg^{-1} commercial BDE in food, corresponding to 8 mg kg^{-1}, body weight, is considered as an unequivocal no-effect level.

B. Toxic Effects in Relation to Cytochrome P450 Induction

As is the case with BB congeners, the toxicity of BDE congeners strongly depends on their molecular structure. BDEs induce the same isoenzymes of cytochrome P450 as PBBs and PCBs. Possible effects are described in section V B.

A dose of 0.1 mMol kg^{-1} d^{-1} BDE (main components: 24.6% tetra-BDEs, 58.1% penta-BDEs, and 13.3% hexa-BDEs) administered to male rats during 14 d increased cytochrome P450 more than an equal molar dose of a BDE mix consisting of 45.1% hepta-BDEs, 30.7% octa-BDEs, and 13.0% nona-BDE; an equal molar dose of high-purity deca-BDE did not significantly increase cytochrome P450. Penta-BDE increased liver/body weight with 64%, octa-BDE with 45%, and deca-BDE with 25% (Carlson 1980a).

C. Carcinogenicity

In feeding studies, female and male rats and mice were exposed to 25 and 50 g kg^{-1} commercial BDE in the diet during 103 wk, resulting in increased incidence of neoplastic nodules in livers of male and female rats. There was equivocal evidence of carcinogenicity for male mice as shown by increased incidences of hepatocellular adenomas and carcinomas. However, these were not increased in comparison with control groups of earlier experiments. Incidences of follicular adenomas or carcinomas of the thyroid gland were only marginally increased. There was no evidence of carcinogenicity for female mice receiving commercial BDE. Effects observed in these studies were attributed to the less brominated BDEs of the commercial mixture (NTP 1986).

D. Mutagenicity

Commercial BDE was not mutagenic in bacteria, *Salmonella typhimurium*, or in a mouse lymphoma assay. It did not induce chromosomal effects like sister-chromatid exchanges or chromosomal aberrations in Chinese hamster ovary cells *in vitro* (NTP 1986).

E. Dermal Toxicity

ommercial BDE did not irritate the skin of rabbits and rats and was only ildly irritating to the eyes of rabbits. External exposure caused no chlor-cne of the ear of rabbits (Norris et al. 1975).

F. Effects on Reproduction, Embryotoxicity, and Teratogenicity

In sticklebacks, *Gasterosteus aculeatus*, Holm et al. (1993) found a decrease in spawning success. Female fish received a dietary dose of approximately 0.5 mg Bromkal (for composition, see Table 7) in 3.5 mon (concentration in the food 346 mg kg^{-1}, with fish consuming 2% of their body weight/d). Uptake efficiency was approximately 20%. Only 2 of 10 fish spawned, compared with 8 of 10 in the controls.

Commercial BDE did not affect the reproductive capacity of rats. Concentrations in the diet of 3, 30, or 100 mg kg^{-1} given 90 d before mating as well as during mating, gestation, and lactation had no effects on the number of pregnancies or on survival and weight of the neonates (Norris et al. 1974).

Oral administration of 2–15 mg kg^{-1} body weight per d^{-1} of a PBDE mixture with a lower overall degree of bromination (0.2% penta-BDEs, 8.6% hexa-BDEs, 45% hepta-BDEs, 33.5% octa-BDEs, 11.2% nona-BDE, and 1.4% deca-BDE) on gestation days 7–19 to pregnant rabbits did not cause teratogenic responses. At 15 mg kg^{-1} slight fetal toxicity was observed by an increase in the incidence of delayed ossification of the breast bone (Breslin et al. 1989).

Commercial BDE caused no teratogenic response in fetuses of rats intubated with 10–1000 mg kg^{-1} d^{-1} on gestation days 6–15. Fetal toxicity only occurred at 1000 mg kg^{-1} as subcutaneous edema and a delayed ossification of normally developed bones of the fetal skull (Norris et al. 1975).

VII. Risk Evaluation and Recommendations

Both classes of brominated fire retardants have properties similar to those of related compounds, such as the PCBs, dibenzodioxins and dibenzofurans, and the PAHs. Thus, their toxicity is likely to interfere with the toxicity of these related compounds because the important mechanisms of toxicity of PBBs and PBDEs are also shown by them.

The scientific basis for a risk evaluation of PBBs and especially the PBDEs in the aquatic environment is very small. To improve this, more knowledge is required, especially in the following areas:

1) their actual concentrations in different compartments of the marine environment, especially in sediments and different classes of biota. As for all commercial mixtures of compounds with the same basic structure, only ...ification of individual congeners will allow comparative environ-... none of the individual BDE congeners and only some BB congeners are yet available as analytical standards, there is an urgent need for more.

2) the toxicity mechanisms of the individual congeners in marine animals, including toxicokinetic aspects, in order to gain insight into the toxicity of congener mixtures of the same class and congeners of the different classes of related compounds.

Because of the scarcity of data, it may be only tentatively concluded that the present concentrations of PBBs and PBDEs in marine food chains of the Baltic Sea, the North Sea, and the North Atlantic Ocean do not by themselves yet appear to represent a great environmental risk.

Despite this fact, their replacement by environmentally less harmful alternatives is strongly recommended because they are often used in open systems. Much of the quantities of PBB and PBDEs produced will thus eventually reach the marine environment, where they are likely to accumulate because of their resistance to degradative processes. Finally, because of their environmental properties, the continued release of PBBs and PBDEs represents an increasing risk to marine organisms.

Summary

Data on two classes of brominated polyaromatic flame retardants are reviewed with emphasis on analytical aspects, occurrence, fate, and toxicity in the environment. Concentrations of brominated fire retardants are quantified as equivalents of commercial mixtures. Because different congeners behave differently in the environment and show large differences in toxicity, future studies would benefit from the availability of analytical standards of individual congeners.

The main environmental properties and mechanisms of toxicity of the PBBs and PBDEs are similar to those of the structurally related PCBs and dibenzodioxins. Although the present concentrations of brominated fire redardants do not yet appear to represent a major environmental risk in marine food chains, their replacement by environmentally less harmful alternatives is recommended.

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Swedish Pesticide Policies 1972–93: Risk Reduction and Environmental Charges

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Contents

I. Introduction

Recent decisions by the Swedish parliament and government to reduce the use of agricultural pesticides by 75% over 10 years have received international attention. Steps taken to reduce the risks connected with the use of nonagricultural pesticides, which constitute the majority of pesticides used in Sweden, have not attracted the same attention. The Swedish Ordinance on Pesticides defines the term "pesticide" as "a chemical product that is intended for use to protect against damage to property, sanitary nuisances, or other comparable nuisances caused by plants, animals, or microorganisms." The ordinance covers not only agricultural pesticides but also wood preservatives used by industry. Of 8914 metric tons of pesticide active ingredients (a.i.s) sold in 1993, 4856 tons (54%) consisted of creosote, a wood preservative used by industry. Industrially used chromium compounds

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The significance of brominated flame retardants in the environment: current understanding, issues and challenges

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1. BFRs – yesterday and today

Fire has been a major cause of property damage and death throughout recorded history and to the present day. In fact, in the United States, over 3 million fires are reported annually, which result in 29 000 injuries, 4500 deaths, and direct property losses estimated in excess of US$8 billion (Gann, 1993). During the past several decades, modern technology has responded to this challenge by introducing heat resistant chemicals to reduce the chances of ignition and burning of a wide range of textiles, plastics, building materials, and electronic equipment used in commerce and in residential homes. By some estimates, the use of flame retardant chemicals has saved annually many lives and hundreds of millions of dollars in property damage (Spiegelstein, 2000). By the turn of this century, flame retardant chemicals had become the second largest additive used by the plastics industry, resulting, in part, to a market value for flame retardant chemicals estimated at nearly US$2.2 billion (Tullo, 2000).

The earliest flame retardant formulations date back to about 450 BC, when the ancient Egyptians used alum to reduce the flammability of wood (Hindersinn, 1990). By about 200 BC, the ancient Romans included vinegar with alum to further reduce fire hazards in wooden buildings (Hindersinn, 1990).

Advancements of chemistry in modern times has resulted in the use of more than 175 different flame retardant chemicals, divided into four major groups: inorganic, halogenated organic, organophosphorus and nitrogen-based compounds and mixtures (EHC, 1997). Halogenated organic flame retardants are generally classified as either chlorinated or brominated flame retardants (BFRs). BFRs are further classified as either reactive or additive materials. The reactive BFRs, which include compounds such as the tetrabromobisphenol A (TBBPA) and derivatives, are chemically bonded into plastics (EHC, 1995). The additive BFRs, which include the polybrominated diphenyl ethers (PBDEs) and hexabromocylododecane (HBDD), are used as additives in a wide variety of polymers and resins (EHC, 1997). The additive BFRs generally are believed to be more easily released to the environment than the reactive BFRs (Hutzinger et al., 1976; Hutzinger and Thoma, 1987).

PBDEs were the first group of BFRs to be detected in the environment. In 1979, the presence of BDE-209 (deca-BDE) was measured in soil and sludge samples collected from areas surrounding PBDE manufacturing facilities in the US (de Carlo, 1979). Two years later, Anderson and Blomkist (1981) reported the presence of PBDEs in samples collected along the Visken River in Sweden. Jansson et al. (1987) first suggested that PBDEs were global contaminants by demonstrating their presence in tissue samples of fish-eating birds and marine mammals collected from the Baltic Sea, North Sea and Arctic Ocean. Similar reports confirmed the widespread distribution of PBDE congeners in marine fish, shellfish, and sediments collected in the Pacific region and elsewhere (Watanabe et al., 1987). PBDEs were also reported in cod liver and herring from the North Sea (de Boer, 1989), and in fresh water eels in the Netherlands (de Boer, 1990). Stafford (1983) confirmed the presence of PBDEs in North America, reporting elevated concentrations of several congeners in the eggs and tissues of fish-eating birds from six US states and Ontario, Canada. Watanabe et al. (1992) were among the first to suggest a global long-range transport process for PBDEs

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based, in part, on studies of air particulate samples collected from Japan and Taiwan.

In recent years, scientists and environmental regulatory agencies in North America and Europe have raised new concerns about the presence of PBDEs in the environment. Deep sediment core samples collected from the Bornholm Deep located in the southern Baltic Sea and analyzed by Nylund et al. (1992) indicate, in contrast to other persistent organic pollutants such as PCBs, dioxins, and DDT, that PBDE levels have increased exponentially since the late 1970s. And, nearly every environmental monitoring program conducted during the past decade has shown sharply increasing levels of PBDEs in wildlife, particularly in Nordic countries where this trend sharply contrasts with a general decrease in the occurrence of dioxins, PCBs and some chlorinated pesticides in marine mammals and aquatic wildlife (Bergman, 2000; Hooper and McDonald, 2000). These observations are particularly troubling since PBDEs (BDE-47, -99, -100 and -153, in particular), similar to the dioxins and PCBs, are highly lipophilic compounds and readily bioaccumulate through the food web (Sellstrom et al., 1993).

A second concern among scientists and regulatory authorities, raised most recently, is prompted by evidence suggesting high levels of PBDEs in humans. PBDEs have been detected in human adipose tissue, blood serum, and in human breast milk (Stanley et al., 1991; Klasson Wehler et al., 1997; Norén and Meironyté, 1998). Studies of nursing Swedish women by Meironyté et al. (1999) suggest that the concentrations of several PBDE congeners in breast milk have doubled every 5 years over the past 25 years. The major route of exposure appears to be through the diet, although data on this and other routes of exposure are limited at this time. A related concern is the increasing exposure of infants and young children to PBDEs and the uncertainties associated with the risk of adverse effects during early developmental stages.

At present, much remains uncertain regarding the toxicity of PBDEs in humans (Darnerud et al., 2001). There is limited evidence, both supporting and negating, the carcinogenicity of deca-brominated BDE (Kociba et al., 1975; NTP, 1986). There is general agreement within the scientific community that data are insufficient to fully evaluate deca- and other PBDE congeners as human carcinogens (IARC, 1990). The most sensitive end points of PBDE toxicity observed in animal bioassays appear to be effects on thyroid function, and particularly induction of thyroid hyperplasia and alteration of thyroid hormone production (Fowles et al., 1994). These and related findings are consistent with adverse effects reported for other organohalogens such as PCBs (Brucker-Davis, 1998). Recent studies by Meerts et al. (1998, 2001) raise new concerns regarding the estrogenicity of PBDEs. Several pure di- to hepta-brominated PBDEs have been shown to act via a dioxin-like Ah-receptor mediated pathway in vitro as either agonists or antagonists, prompting speculation that in vivo metabolism of certain PBDEs may produce more potent pseudoestrogens (Meerts et al., 2001). These and other effects and their significance should be the focus of urgently needed future toxicological studies with PBDEs.

2. Overview of this special issue

The manuscripts found in this special issue of *Chemosphere* provide a state-of-the-science understanding of the occurrence and significance to human health and wildlife posed by PBDEs in the environment. This issue is mainly dedicated to the technical session on BFRs held in August 2000 in Monterey, CA, USA as part of the 20th Annual International Symposium on Halogenated Environmental Organic Pollutants and POPs. The work presented herein reflects the evolution of the scientific understanding of BFRs by scientists working in North America, Europe, and Japan during the past decade. The Annual International Dioxin Symposium and Periodic Technical Workshops and symposia hosted by Environment Canada and by Stockholm University have been important venues in this regard.

Manuscripts are organized into five sections: Introduction; Analytical; Environmental levels; Environmental fate and sources; and Toxicology and risk assessment. In the Introduction section, de Wit provides an extensive overview of BFRs in the environment, reflecting the considerable effort undertaken by the Swedish Environmental Protection Authority in 1999–2000 (de Wit, 2000).

The three manuscripts included in the Analytical section describe the challenges posed by efforts to accurately measure PBDEs at trace levels in different environmental compartments. No longer regarded as an undesirable interference in gas chromatography analysis for other organohalogens, considerable effort is underway to establish an analytical protocol for PBDEs.

Seven manuscripts are included in the Environmental levels section, reflecting a broadening of our understanding of the occurrence of PBDEs in various geographical regions and environmental compartments. These studies address the occurrence of BFRs in aquatic birds, fish, marine mammals, and wildlife, as well as in human tissues (blood serum and breast milk) from Japan, Europe and North America. The results, which highlight the similarities and differences in PBDE levels in biota and humans, support the hypothesis that long-range transport and inputs from local and regional sources are both important in the evaluation of PBDEs in the environment.

Three manuscripts are included in the Environmental fate and sources section. On-going work in this field of

study is focused on resolving two important areas of uncertainty, the source(s) of releases to the environment and mechanisms for long-range global transport of PBDEs.

This special issue concludes with three manuscripts in the Toxicology and risk assessment section. Two papers, one by Hardy and the second by McDonald, frame the opposing viewpoints in the current debate concerning the toxicological significance to humans posed by exposure to PBDEs. The third paper by Wenning is one of only a few risk assessment studies conducted thus far; and identifies the uncertainties and limitations in our current understanding of human exposure to the three predominant PBDE products sold commercially in North America and Europe.

3. Closing

As co-editors of this special issue of *Chemosphere*, we are especially grateful to the scientists and peer-reviewers located in North America, Europe, and the Asia-Pacific region who participated in the preparation and review of the manuscripts presented here. We owe a special acknowledgement to Dr. Kim Hooper at California EPA (Berkeley, CA) who initiated this process at the Dioxin2000 meeting held in Monterey, California last August. Dr. Hooper, like many scientists involved in environmental monitoring and research, identified the need for a forum to communicate the complex issues and common themes associated with understanding chemical behavior in the environment and its significance to both human and ecological health. We trust this special issue of *Chemosphere* contributes to that goal.

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A perspective on the potential health risks of PBDEs

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Abstract

The polybrominated diphenylethers (PBDEs) are a class of chemicals widely used as flame retardants. Concentrations of PBDEs in some human and marine mammal populations are increasing. The toxicological endpoints of concern for environmental levels of PBDEs are likely to be thyroid hormone disruption, neurodevelopmental deficits and cancer. Unfortunately, the available toxicological evidence for these endpoints is surprisingly limited, given their widespread use, bioaccumulative potential, and structural similarity to thyroid hormones and polychlorinated biphenyls (PCBs). Available evidence suggests that the PBDE congeners likely to bioaccumulate (i.e., those observed in human tissues and other biota) have the propensity to disrupt thyroid hormones, cause neurobehavioral deficits and possibly cause cancer in laboratory animals. It is unclear whether current concentrations of PBDEs in human tissues would be expected to adversely impact human health. Since nearly all individuals are exposed to low levels of PBDEs, the potential health impacts also should include assessment at the population level. This paper summarizes the available toxicological evidence for PBDE-induced thyroid hormone disruption, neurodevelopmental deficits, and, for some congeners, cancer, and provides a perspective on the potential risks of the PBDEs for human health.

Keywords: PBDE; PCB; Toxicity; Risk; Thyroid hormone; Neurodevelopment; Cancer; Review

1. Background

The polybrominated diphenylethers (PBDEs) are widely used flame retardants whose concentrations are rising in human tissues and biota (Hooper and McDonald, 2000). PBDEs and their metabolites are structurally similar to PCBs and thyroxine (Fig. 1) and DDT (Hooper and McDonald, 2000). PBDE residues are now found in sediments, marine mammals, fish, bird eggs and human milk, serum, and adipose tissue (reviewed in Darnerud et al., 2001). Researchers have reported increasing PBDE concentrations over time in guillemot eggs in Sweden (Sellström et al., 1993), in blubber of beluga whales in Canada (Stern and Ikonomou, 2000) and in adipose tissue of seals near San Francisco (United States) (She et al., 2001). Over the past 20 years, concentrations of PBDEs also have been increasing as contaminants in human breast milk samples from Sweden and Germany (Norén and Meironyté, 2000; Schröter-Kermani et al., 2000). Similar concentrations of PBDEs have been reported in human tissue samples collected in recent years from Spain (Meneses et al., 1999), Israel (de Boer et al., 1998), Finland (Strandman et al., 2000), and Canada (Ryan and Patry, 2000), with somewhat higher concentrations in the United States (She et al., 2001). A high degree of inter-individual variability was observed in the concentrations of PBDEs in human adipose tissue samples, ranging from 17 to 462 ng/g lipid in the United States (She et al., 2001) and from 0.6 to 98 ng/g lipid in Sweden (Hardell et al., 1998). The reason for the wide variability is not known, but may be due to the different

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Measurements of PBDEs reported in the air above Chicago (United States) were 5–10 times higher than PBDE concentrations in the air of rural locations in the Great Lakes region near Chicago (Strandberg et al., 2001). PBDEs have also been measured in indoor air (IPCS, 1994; Sjödin et al., 2001). Airborne PBDEs exist both as a gas and associated with particulate matter, dependent on several factors including the chemical properties of the congeners and the environmental conditions (Strandberg et al., 2001; Sjödin et al., 2001).

2. Low-dose toxicity

Current concentrations of PBDEs in human tissues range from roughly 1 to about 400 ng/g lipid (Hardell et al., 1998; Sjödin et al., 1999; She et al., 2001) and may be rising rapidly in some populations (Ryan and Patry, 2000). Based on available toxicity data and structural and mechanistic similarities with the PCBs, the toxic endpoints likely to be the most sensitive for the PBDEs are thyroid hormone disruption, neurobehavioral toxicity and, for some congeners possibly cancer. Some evidence is available for estrogenic activity of PBDE (Thayer et al., 2000; Meerts et al., 2001), but more studies are needed to determine if low-dose exposures to PBDE have estrogenic activity in humans or other species.

2.1. Thyroid effects

The chemical structure of PBDEs closely resembles that of the thyroid hormones, 3,3′,5-triiodothyronine (T3) and 3,3′,5,5′-tetraiodothyronine (thyroxine, T4). Thyroid hormones, like metabolites of PBDEs, are hydroxylated, halogenated-diphenylethers (Fig. 1). Recent in vitro studies have shown that hydroxylated metabolites of PBDEs bind with high affinity to thyroid hormone transport protein (i.e., transthyretin) (Meerts et al., 2000) and bind to thyroid hormone receptors TR-α1 and TR-β (Marsh et al., 1998), although the latter binding is with low affinity. Metabolism studies of 2,2′,4,4′-tetra-BDE in rodents resulted in the formation of five different hydroxylated tetra-BDE metabolites (Örn and Klasson-Wehler, 1998). Moreover, hydroxy-PBDEs and methoxy-PBDE have been detected in the blood plasma of salmon from the Baltic Sea at concentrations that were similar to those of the major PBDEs (Asplund et al., 1999) (Fig. 1). There are no toxicity data on methoxy-PBDEs.

All of the PBDE products (penta-, octa- and deca-BDE (technical)) disrupt thyroid hormone balance, although the potency of the deca-BDE appears much lower than the rest of the class (reviewed in Hooper and McDonald, 2000; Darnerud et al., 2001; Zhou et al., 2001a). Penta-BDE exerted clear effects on the thyroid system of rodents, including decreased T4 in rats and mice exposed orally for 14 days (18 mg/kg) (Fowles et al., 1994; Darnerud and Sinjari, 1996), hyperplasia among rodent following administration via the diet for 90 days (10 mg/kg) (Dow Chemical Company, 1991), and decreased T4 among mice 8 days after a single oral exposure (0.8 mg/kg) (Fowles et al., 1994). 2,2′,4,4′-Tetra-BDE, the major congener in most human and animal tissue samples, reduced thyroid hormone levels among female rats following oral administration for 14 days (18 mg/kg) (Hallgren and Darnerud, 1998). Zhou et al. (2001a) administered orally to female rats one of three commercial PBDE mixtures (penta-, octa- and deca-BDE (technical)) for 4 days at dosages of 0, 0.3, 1, 3, 10, 30, 100 or 300 mg/kg body weight. Dose-related reductions in serum T4 levels were observed for the penta- and octa-BDE (technical), but not the deca-BDE (technical). Benchmark dose analyses of the data, modeled with a Hill equation, indicated that the 95% lower confidence bound on the effective dose resulting in a 20% reduction of serum T4 (LED_{20}) was about 7 mg/kg for the penta-BDE (technical) and 5 mg/kg for the octa-BDE mixture (Zhou et al., 2001a). In additional experiments initially reported by Zhou et al. (2000), this research group administered penta-BDE (technical) to pregnant rats from gestational day 6 to postnatal day 21 at doses of 0, 1, 10, and 30 mg/kg. Serum concentrations of T4 were depressed in a dose–response trend over all doses in both the fetuses and dams on gestational day 21 and in the offspring at postnatal day 4 and 14, although in pair-wise comparisons T4 concentrations in the lowest dose groups were not statistically different from controls (Zhou et al., 2001b).

To put these observations in perspective, coplanar PCBs (e.g., "dioxin-like" PCBs such as PCB 77, 126, 169) reduce thyroid hormone levels in rodents at levels 100–1000-fold lower (Seo et al., 1995) than those used in the PBDE studies. However, background tissue concentrations of PBDEs in human populations are much higher than the concentrations of dioxins and dioxin-like PCBs. It should also be noted that rodents are generally believed to be more sensitive to thyroid hormone disruption than humans (US EPA, 1998), although wide variability in thyroid hormone status is observed in the general population (see below). Also, preliminary data suggest that PBDEs function additively to reduce T4 levels when co-administered with PCBs (Aroclor 1254) or chlorinated paraffins (Hallgren and Darnerud, 1998).

The mechanism of thyroid hormone disruption by PBDEs has not been fully characterized, but may arise from at least two different mechanisms. First, PBDEs exhibit a moderate potential to induce liver enzymes of different enzyme families, including cytochrome P450 1A1 induction (i.e., Ah-receptor- or dioxin-like induction) and cytochrome P450 2B induction (i.e.,

many of the same neurological effects as non-coplanar PCBs (see below). *ortho*-Substituted, non-coplanar PCBs, which have low affinity for the Ah receptor, exhibit a wide range of toxicities, including neurotoxic effects (Fischer et al., 1998; Brouwer et al., 1998). There is growing recognition that reliance on the use of toxic equivalency factors (TEF) based on relative Ah receptor activity for the PCBs may be insufficient for endpoints such as neurotoxicity (Fischer et al., 1998; US EPA, 2000). Non-coplanar PCBs comprise the greatest concentration of PCBs in most biological samples. It has been suggested that a significant portion of the neurobehavioral deficits associated with PCB exposure in monkeys and humans may be due to non-coplanar congeners (reviewed in Fischer et al., 1998; Brouwer et al., 1998).

There are at least three possible mechanisms by which PBDEs, like non-coplanar PCBs, can adversely affect brain development: (1) thyroid hormone disruption, (2) disruption of second messenger communications, and (3) alteration of neurotransmitter systems. Evidence from animal studies suggests that all three mechanisms may be operative for the PBDEs.

Thyroid hormone imbalance is a well-established mechanism of neurodevelopmental deficits in rodents and humans (Porterfield, 2000; Morreale de Escobar et al., 2000). Thyroid hormones regulate brain development in both the fetal and neonatal periods (Morreale de Escobar et al., 2000). Thyroid hormones control proliferation of neuronal and glial cells, regulate neuronal migration and differentiation, and regulate neuronal connectivity and myelination. They also control normal cytoskeletal assembly and stability, which is essential for proper neuronal migration and outgrowth. Thyroid hormones also regulate the development of cholinergic and dopaminergic systems serving the cerbral cortex and hippocampus (Porterfield, 2000).

Both maternal and fetal thyroid hormone levels are important for proper brain development (Porterfield and Hendrich, 1993; Haddow et al., 1999; Pop et al., 1999; Morreale de Escobar et al., 2000). The mother is the only source of thyroid hormone for the fetus during the first trimester and is the major source in the second trimester, two periods in which maternal thyroid abnormalities in humans are well associated with reduced intelligence in progeny (reviewed in Morreale de Escobar et al., 2000). As noted above, exposure of pregnant rodents to PBDEs reduced thyroid hormone levels in the dams, fetuses and offspring (Zhou et al., 2000, 2001b).

PBDEs may be functioning to perturb intracellular second messenger systems, as has been observed for non-coplanar PCBs. These communication systems, which include the Ca^{2+} homeostasis in neuronal cells, inositol phosphates and protein kinase C, play a vital role in neuronal growth and normal cellular physiology (Fischer et al., 1998). An initial report by Derr-Yellin and Kodavanti (2001) suggests that this mechanism is operative for lower molecular weight PBDEs. Penta-BDE (technical) (but not octa-BDE (technical)) stimulated the release of arachidonic acid through a phospholipase 2 (PLA_2)-dependent mechanism in cerebullar granule cells in vitro, as has been demonstrated with neurotoxic PCBs. PLA_2 activity is associated with learning and memory, and arachodonic acid is a second messenger for synaptic plasticity (Derr-Yellin and Kodavanti, 2001). Additionally, chlorinated analogues of the PBDEs, namely di- to penta-chlorinated diphenylethers, showed equivalent or greater activity to *ortho*-substituted PCBs in perturbing second messenger systems (Kodavanti et al., 1996; Fischer et al., 1998). Also, an initial report by Anderson et al. (2001) indicated that penta-BDE (technical) and PCBs inhibited phosphorylation of mitogen activated protein kinase (MAPK) in tissue slices from the hippocampi of rats. MAPK is a component of a key signaling pathway thought to be related to long-term potentiation of nerve cells, learning and synaptic maturation. Disruption of this pathway during brain development is believed to contribute to cognitive dysfunction in adulthood (Anderson et al., 2001).

The observed neurotoxicity in rodent studies of PBDEs could be a result of alterations in neurotransmitter systems (Eriksson, 1997), which may or may not be mediated through the thyroid system (Porterfield, 2000). Viberg et al. (2000) noted that neurobehavioral responses following postnatal exposure to PBDEs were consistent with alterations to the cholinergic system. Clearly, more research is needed to characterize the developmental neurotoxicity of PBDEs.

2.3. Cancer

Although the more bioaccumulative forms of PBDEs (i.e., tri- to hexa-BDEs) have not been tested for carcinogenicity, there is reason to suspect that they might be carcinogenic in standard two-year rodent bioassays. Only the fully brominated deca-BDE (technical) has been tested for carcinogenic potential and produced some evidence of carcinogenicity in male and female rats and equivocal evidence in male mice (NTP, 1986). In that bioassay, dose-related increases in liver neoplastic nodules (adenomas) were clearly related to deca-BDE treatment in both male and female rats. Acinar cell adenoma of the pancreas also were increased among high-dose male rats. Statistically significant increases in hepatocellular adenomas and carcinomas (combined) were observed in male mice relative to controls, but the increases were within the range of historical controls. Marginal increases in thyroid gland follicular cell adenomas or carcinomas (combined) were observed for male and female mice. Some concerns about the bioassay were raised and considered by the NTP peer

worthy that a dose-related trend in reduced serum thyroid hormone concentrations was observed among the dams, fetuses and newborn rats. Additional thyroid function and neurodevelopmental studies in rats and mice are needed, and these studies should include the use of lower dose levels as well as groups of iodine-deficient animals to better assess the risks to sensitive human populations.

3.2. Potential health risks

Since nearly all individuals are exposed to low-levels of PBDEs as evident by tissue monitoring data, the potential health impacts should include assessment at the population level. Thyroid hormone disruption is associated with many adverse health outcomes, including goiter, benign and neoplastic thyroid diseases, and neurodevelopmental toxicity (Hill et al., 1989; Morreale de Escobar et al., 2000). The developing brain is sensitive to thyroid hormone disruption. For example, recent studies have shown that relatively small decreases in maternal serum T4, free T4 or other indicators of thyroid abnormalities can have a negative impact on the intelligence and psychomotor skills of children (Pop et al., 1999; Haddow et al., 1999; Morreale de Escobar et al., 2000). It is clear that there is a distribution of individuals with varying degrees of thyroid competency. At any given time, a small fraction of the population is clinically hypothyroid, and a larger fraction has a moderate level of (non-clinical) hypothyroidism or thyroxinemia (Pop et al., 1999; Haddow et al., 1999; Morreale de Escobar et al., 2000). Intertwined with the distribution of thyroid competency is the fact that a significant proportion of the population is iodine deficient (Hollowell et al., 1998). Theoretically, body burdens of PBDEs, PCBs, and chlorinated dioxins (which may function additively to disrupt thyroid hormone balance) could shift the population distribution slightly, pushing some fraction of the population towards increased risk.

The health risks posed by PBDEs at low doses cannot be adequately characterized at this time. The most sensitive endpoints (i.e., thyroid hormone disruption, neurobehavior deficits and possibly cancer) associated with the most bioactive PBDE congeners (tri- to octa-BDE) have not been sufficiently tested. Studies that establish a no-observable effect level for thyroid hormone disruption or for neurodevelopmental toxicity are needed, as are animal cancer studies of the lower PBDE congeners (tetra- to octa-BDE). PBDEs have exhibited potential for induction of the cytochrome 1A1 family (Ah receptor induction) and the cytochrome 2B1 family (pentoxyresorufin-*o*-deethylase, PROD induction) and are shown to be Ah receptor agonists or antagonists in vitro, dependent on their bromine substitution (Meerts et al., 1998). Although dioxin-like activity appears to be low ($\sim 10^{-5}$) relative to TCDD, current concentrations of PBDEs in some human populations (She et al., 2001) are roughly 20,000-fold higher than TCDD (Hooper and McDonald, 2000), and are rising rapidly. Importantly, the effects of PBDEs, PCBs and dioxins on thyroid hormone disruption and enzyme induction may be additive (Hallgren and Darnerud, 1998), and may increase the overall risks of health effects associated with dioxin-like compounds. From a risk assessment perspective, considerable empirical and mechanistic evidence suggest that on a population level cancer risks of dioxin-like compounds are consistent with a linear-to-low-dose model for Ah receptor induction, compelling the US EPA to employ such an approach for modeling the risks of TCDD (US EPA, 2000).

Estimates of dietary intake of PBDEs in Sweden were about 1-million-fold lower than the lowest dose which resulted in adverse effects in animal studies (~1 mg/kg day, thyroid hormone reduction) (Darnerud et al., 2001). Although this provides some comfort that current environmental concentrations are not involved in adverse health outcomes, many factors could considerably narrow that gap. For example, tissue concentrations among US populations are about 5–10-fold higher than in Sweden, and in each population tissue concentrations in individuals can vary more than 50-fold. In addition to dietary sources, there are non-dietary exposures that are the primary routes of exposure for some PBDE congeners, as evident by different congener patterns among persons of different occupations (Sjödin et al., 1999). She et al. (2001) reported that when PBDE congener patterns among seals and humans were normalized for 2,2′,4,4′-tetra-BDE (PBDE 47), the contribution of certain penta-BDE congeners (PBDE 100, 153, and 154) was small in seals but prominent in humans, suggesting selective bioaccumulation or that routes other than the diet contributed to human body burdens. Moreover, there is good evidence that humans also have direct dietary exposures to hydroxy-PBDEs at concentrations that are as high as the major PBDE congeners (Asplund et al., 1999). Activity of the hydroxy-PBDEs may be much higher than the PBDEs for thyroid hormone disruption (Meerts et al., 2000) and estrogenic activity (Meerts et al., 2001). Additionally, humans are exposed to methoxy-PBDEs, possibly from environmental conversion of PBDEs, which are at concentrations higher than the major PBDE congeners and whose toxicity has not been studied (Asplund et al., 1999).

Moreover, for TCDD, PCBs and presumably other bioaccumulative agents such as PBDEs, the daily intake for the fetus and infant is much higher than that of adults on a body weight basis. As evidence of fetal exposures, humans at birth have tissue concentrations of TCDD that are up to 25% of maternal levels (Hooper and McDonald, 2000). Furthermore, lactational exposures of highly lipophilic compounds like the PBDEs,

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Brominated Flame Retardants: A Novel Class of Developmental Neurotoxicants in Our Environment?

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Brominated flame retardants are a novel group of global environmental contaminants. Within this group the polybrominated diphenyl ethers (PBDE) constitute one class of many that are found in electrical appliances, building materials, and textiles. PBDEs are persistent compounds that appear to have an environmental dispersion similar to that of polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT). Levels of PBDEs are increasing in mother's milk while other organohalogens have decreased in concentration. We studied for developmental neurotoxic effects two polybrominated diphanylethers, 2,2′,4,4′-tetrabromodiphenyl ether (PBDE 47) and 2,2′,4,4′,5-pentabromodiphenylether (PBDE 99)—congeners that dominate in environmental and human samples—together with another frequently used brominated flame retardant, tetrabromo-bis-phenol-A (TBBPA). The compounds were given to 10-day-old NMRI male mice, as follows: PBDE 47, 0.7 mg (1.4 μmol), 10.5 mg (21.1 μmol)/kg body weight (bw); PBDE 99, 0.8 mg (1.4 μmol), 12.0 mg (21.1 μmol)/kg bw; TBBPA, 0.75 mg (1.4 μmol), 11.5 mg (21.1 μmol)/kg bw. Mice serving as controls received 10 mL/kg bw of the 20% fat emulsion vehicle in the same manner. The present study has shown that neonatal exposure to PBDE 99 and PBDE 47 can cause permanent aberrations in spontaneous behavior, evident in 2- and 4-month-old animals. This effect together with the habituation capability was more pronounced with increasing age, and the changes were dose–response related. Furthermore, neonatal exposure to PBDE 99 also affected learning and memory functions in adult animals. These are developmental defects that have been detected previously in connection with PCBs. *Key words:* adult, brominated flame retardants, developmental neurotoxicology, memory and learning, neonatal, polybrominated diphenyl ethers, spontaneous behavior. *Environ Health Perspect* 109:903–908(2001). [Online 20 August 2001]

http://ehpnet1.niehs.nih.gov/docs/2001/109p903-908eriksson/abstract.html

Brominated flame retardants are a novel group of global environmental contaminants (*1,2*). Within this group the polybrominated diphenyl ethers (PBDEs) are used in large quantities as flame-retardant additives in polymers, especially in the manufacture of a variety of electrical appliances, including television and computer casing, building materials, and textiles (*3,4*). Because PBDEs are mixed, not chemically bound, into the material they are used in, they may migrate from the material. One of the earliest reports of PBDE in our environment came in 1981 (*5*). Since then, PBDEs have been shown to be persistent compounds that appear to have an environmental dispersion similar to that of polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT) (*2,6*). PBDE has been found in various wildlife species and in human tissues (*1,7*). The PBDE congeners that dominate in environmental and human samples are 2,2′,4,4′-tetrabromodiphenyl ether (PBDE 47) and 2,2′,4,4′,5-pentabromodiphenyl ether (PBDE 99). These agents together with tetrabromo-bis-phenol-A (TBBPA) have also been detected in human plasma samples (*6,8*) and in mother's milk (*9,10*). A study on certain organochlorine and organobromine contaminants in milk from native Swedish mothers showed that the concentration of PBDEs has increased continuously from 1972 to 1997, whereas those of organochlorine compounds such as PCB and DDT have decreased (*9,10*). The concentration of PBDEs in 1997 was still lower than those of PCBs and DDT. The concentration for PCBs was 324 ng/g lipid, for DDT 14 ng/g lipid, and for PBDEs 4 ng/g lipid. However, of special concern is that the PBDEs show an exponential increase from 1972 to 1997 with a rate of increase that doubled in 5-year increments (*9,10*).

In mammals, the fetus can be directly exposed during gestation via maternal intake of toxic agents. During the neonatal period, offspring may be contaminated by ingesting mother's milk or by direct exposure to xenobiotics (*11*). In many mammalian species a rapid growth of the brain occurs during perinatal development—the so-called brain growth spurt (*12*). In humans, this period begins during the third trimester of pregnancy and continues throughout the first 2 years of life. In mouse and rat this period is neonatal, spanning the first 3–4 weeks of life, during which the brain undergoes several fundamental phases, such as axonal and dendritic outgrowth and the establishment of neural connections. During this period, animals also acquire many new motor and sensory abilities (*13*) and their spontaneous motor behavior peaks (*14*). The brain growth spurt is associated with numerous biochemical changes that transform the feto-neonatal brain into that of the mature adult (*15,16*). One of the major transmitter systems that undergo rapid development is the cholinergic system, which is involved in many behavioral phenomena and cognitive functions (*17,18*).

In several reports we have shown that low-dose exposure to both persistent and nonpersistent environmental agents during the neonatal period disrupts adult brain function. Among the toxicants that induce such neurotoxic effects are DDT (*19,20*), pyrethroids (*20*), organophosphate (*21*), nicotine (*22*), paraquat and MPTP (*23*), and certain PCBs (*24*). The induction of behavioral and cholinergic disturbances in the adult animal has often been limited to a short period during neonatal development, around postnatal day 10 (*19,21,22*). Those studies also showed that the induction of these disturbances occurs at doses that apparently have no permanent effect when administered to the adult animal. Furthermore, the exposure level for nicotine, PCB, and DDT were also in the same order of magnitude to which humans can be exposed (*20,22,24*). Exposure to PCB, DDT, or nicotine during this phase of development can also lead to an increased susceptibility to toxic agents at adult age, indicating that neonatal exposure to toxic agents can potentiate and/or modify the reaction to adult exposure to xenobiotics (*25–27*).

In view of an increasing amount of PBDEs and TBBPA in the environment and in mother's milk, we undertook the present study to investigate possible behavioral effects of PBDEs and TBBPA when given during the rapid development of the neonatal mouse brain.

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Materials and Methods

Animals and Chemicals

We used male NMRI mice to make this study comparable with our earlier studies, which were performed on male mice. Pregnant NMRI mice were purchased from Charles River, Uppsala, Sweden. Following parturition, each litter, adjusted within 48 hr to eight to ten mice by euthanasia of remaining pups, was kept together with its respective mother in a plastic cage in a room with an ambient temperature of 22°C and a 12 hr light:12 hr dark cycle. At an age of 10 days, pups were exposed to the vehicle or the test compounds. To keep litters and conditions standardized and as close to normal as possible during the neonatal period, we exposed both sexes. At 4 weeks male mice were weaned and were placed and raised in groups of four to seven in a room for male mice only. The animals were supplied with standardized pellet food (Lactamin, Stockholm, Sweden) and tap water *ad libitum*.

The polybrominated diphenyl ethers PBDE 47 and PBDE 99 were synthesized at the Wallenberg Laboratory (*28,29*), University of Stockholm, Sweden. Tetrabromo-bis-phenol-A (TBBPA) was purchased from Aldrich (Steinheim, Germany) and was recrystallized from chloroform. The purity of the compounds exceeded 98%. The substances were dissolved in a mixture of egg lecithin (Merck, Darmstadt, Germany) and peanut oil (*Oleum arachidis*) (1:10) and then sonicated with water to yield a 20% weight:water (w:w) fat emulsion vehicle containing various concentrations of the compounds. The substances were administered orally, at a volume of 10 mL/kg body weight (bw), via a PVC tube (diameter 1.0 mm) as one single dose on postnatal day 10. The amounts of the different compounds given were as follows: 2,2′,4,4′-tetrabromodiphenyl ether (PBDE 47), 0.7 mg (1.4 µmol), 10.5 mg (21.1 µmol)/kg bw; 2,2′,4,4′,5-pentabromodiphenyl ether (PBDE 99), 0.8 mg (1.4 µmol), 12.0 mg (21.1 µmol)/kg bw; tetrabromo-bis-phenol-A (TBBPA), 0.75 mg (1.4 µmol), 11.5 mg (21.1 µmol)/kg bw. Mice serving as controls received 10 mL/kg bw of the 20% fat emulsion vehicle in the same manner. Each treatment group comprised mice from 3–4 different litters.

Behavioral Tests

Spontaneous behavior. We tested spontaneous behavior in the male mice at ages 2 and 4 months, as described previously (*19,24*). We tested eight mice, randomly selected from three to four different litters, once only, and the tests were performed between 0800 and 1200 hr under the same ambient light and temperature conditions. We measured motor activity over 3 × 20 min in an automated device consisting of cages (40 × 25 × 15 cm) placed within two series of infrared beams (low level and high level) (Rat-O-Matic; ADEA Elektronik AB, Uppsala, Sweden) (*30,31*). Locomotion was registered when the mouse moved horizontally through the low-level grid of infrared beams. For rearing, vertical movement was registered at a rate of 4 counts per second, whenever and as long as a single high-level beam was interrupted—the number of counts obtained was proportional to time spent rearing up. To measure total activity, a pick-up (mounted on a lever with a counterweight) with which the test cage was in contact registered all types of vibration within the test cage, such as mouse movements, shaking (tremors), and grooming.

Swim maze. We administered the behavior test to male mice at the age of 5 months. We tested 16–18 mice, randomly selected from three to four different litters, for swim maze performance. The swim maze was of Morris water maze type (*32*): a circular gray tub, 73 cm in diameter, filled with water at 23°C to a depth of 13 cm from the brim. In the center of the northwest quadrant of the pool, a platform was submerged 1 cm beneath the water surface. The platform was formed of metal mesh and had a diameter of 12 cm. We observed the mouse's ability to locate the submerged platform for 5 days, the animals being given five trials each day between 0900 and 1400 hr. Before the first trial each day, the mouse was placed on the submerged platform for 30 sec. It was then released from the south position with its head toward the side of the tub and allowed 30 sec to locate the platform. If the mouse failed to find the platform within 30 sec, it was placed on the platform. After each trial, the mouse remained on the platform for 30 sec. The mice received five trials per day on 4 consecutive days. On the fifth day the platform was moved to the center of the northeast quadrant for reversal trials; otherwise the procedure was the same. Latency to locate the platform constituted the total search time of five trials, maximum 150 sec. Trials 1–20 (days 1–4) measured the mouse's spatial learning ability and trials 21–25 (day 5) its relearning ability. The experimental design of the swim maze test was the same as used earlier in the experiment where mice were exposed to the PCBs (*24*).

Statistical Analysis

Spontaneous behavior. The data were subjected to a split-plot analysis of variance (ANOVA). Pairwise testing between PBDE 47-, PBDE 99-, TBBPA-, and vehicle-treated groups was performed with Tukey's honestly significant difference (HSD) test (*33*).

Habituation capability. From the spontaneous behavior test we calculated a ratio between the performance period 40–60 min and 0–20 min for the three different variables: locomotion, rearing, and total activity. We used the following calculation: 100 (counts locomotion 40–60 min/counts locomotion 0–20 min), 100 (counts rearing 40–60 min/counts rearing 0–20 min), and 100 (counts total activity 40–60 min/counts total activity 0–20 min). This ratio was used to analyze alteration in habituation between 2-month-old and 4-month-old mice. These data were subjected to a split-plot ANOVA.

Swim maze. The data from day 1 to day 4 were subjected to a split-plot ANOVA. Pairwise testing between PBDE 47-, PBDE 99-, TBBPA-, and vehicle-treated groups was performed with Tukey's HSD test. Statistical analysis for the behavioral data of day 5 was submitted to paired *t*-test (difference between trial 1 and trial 5) and one-way ANOVA with pairwise testing between PBDE 47-, PBDE 99-, TBBPA-, and vehicle-treated groups, using Duncan's test.

Results

There were no clinical signs of dysfunction in the treated mice throughout the experimental period, nor were there any significant deviations in body weight gain in the PBDE 47-, PBDE 99-, and TBBPA-treated mice, compared with the vehicle-treated mice.

Spontaneous Behavior

Figures 1 and 2 show the results from the spontaneous behavior variables locomotion, rearing, and total activity in 2- and 4-month-old NMRI male mice exposed to a single oral dose of either 1.4 µmol or 21.1 µmol/kg bw of one of the compounds PBDE 47, PBDE 99, TBBPA, with corresponding controls receiving 10 mL/kg bw of the 20% fat emulsion vehicle. Two months after the exposure there were significant treatment × time interactions [$F(12,126) = 12.30$ $F(12,126 = 13.38$, $F(12,126) = 10.45$], for the locomotion, rearing, and total activity variables, respectively. Pairwise testing among PBDE 47, PBDE 99, TBBPA, and control groups showed a significant dose-related change in all three test variables. In control mice there was a distinct decrease in activity in all behavioral variables over 60 min. Mice receiving the higher dose of PBDE 99 (12 mg) or PBDE 47 (10.5 mg) displayed significantly less activity than controls during the first 20-min period (0–20 min), but during the third 20-min period (40–60 min) they were significantly more active than the controls. Mice receiving the lower dose of PBDE 99 (0.8 mg) showed significantly less rearing and total activity than controls during the first 20-min period

(0–20 min). During the last 20-min period (40–60 min) these mice were significantly more active than the controls in the locomotion variable. In mice receiving TBBPA there were no significant change in the variables locomotion, rearing, and total activity compared with controls.

Four months after neonatal exposure to the different brominated flame retardants there were still significant treatment × time interactions [$F(12,126) = 21.41$ $F(12,126) = 21.09$, $F(12,126) = 27.12$], for the locomotion, rearing, and total activity variables, respectively (Figure 2). Pairwise testing among PBDE 47, PBDE 99, TBBPA and control groups showed a significant dose-related change in locomotion, rearing, and total activity. Pairwise testing between PBDE 99 and control groups showed a significant dose-related change in all three test variables. Mice receiving the lower and the higher dose of PBDE 99 (0.8 mg or 12 mg) were significantly less active than controls during the first 20-min period (0–20 min), but during the last 20-min period (40–60 min) they were significantly more active than the controls. Mice receiving the higher dose of PBDE 47 (10.5 mg) displayed significantly less locomotion, rearing, and total activity than controls during the first 20-min period (0–20 min), but during the last 20-min period (40–60 min) they were significantly more active than the controls. In mice receiving TBBPA there were no significant change in the variables locomotion, rearing, and total activity compared with controls.

Habituation Capability

By analyzing the habituation ratio between performance period 40–60 min and 0–20 min, we obtained information about the ability to habituate to a novel environment which can be used to analyze changes in habituation with age. The results from the habituation ratio, calculated from the spontaneous behavior variables locomotion, rearing, and total activity in 2- and 4-month-old NMRI male mice are given in Table 1. The habituation capability was shown to significantly ($p \leq 0.001$) decrease with age in mice exposed to PBDE 47 [10.5 mg (21.1 μmol)] and PBDE 99 [0.8 mg (1.4 μmol) and 12.0 mg (21.1 μmol)]. In mice neonatally exposed to TBBPA or the vehicle, no significant change in habituation ratio was observed.

Swim Maze Behavior

Mice receiving the higher dose of PBDE 47, PBDE 99, and TBBPA were tested for swim maze performance. Figure 3 shows the performance of 5-month-old mice neonatally exposed to PBDE 47, PBDE 99, TBBPA, or the vehicle. During the acquisition period of spatial learning ability, measured from day 1 to day 4, all mice, regardless of treatment, improved their ability to locate the platform [$F(3,161) = 215.46$]. Split-plot ANOVA revealed no significant treatment × time interactions among PBDE 47, PBDE 99, TBBPA, and controls [$F(8,161) = 0.54$]. On day 5 the platform was relocated for relearning by reversal trials. In the first trial on day 5, control mice displayed longer latency than in the last trial on day 4. This is normal behavior during relearning because, initially, the mouse searches near the previous platform location (*34*). However, controls significantly improved their ability to find the new location (paired t-test trial 1 vs. trial 5, $p \leq 0.01$), indicating normal relearning in

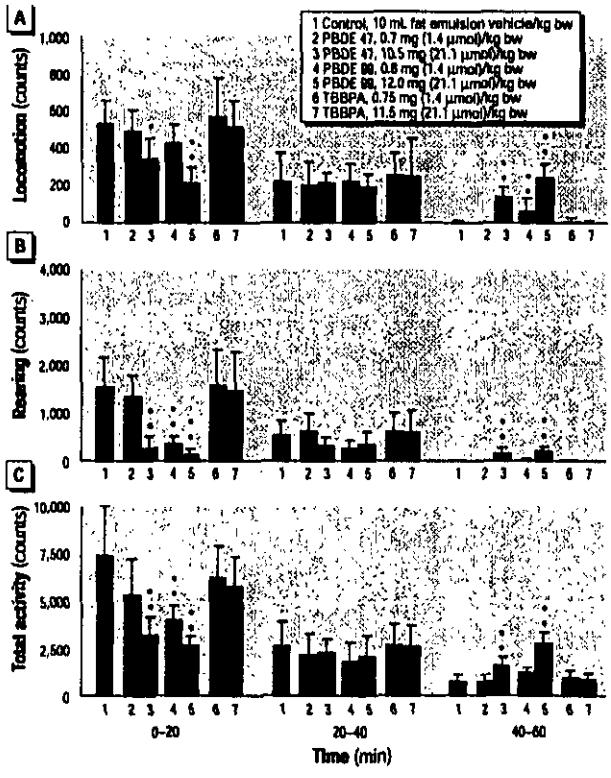

Figure 1. Spontaneous behavior of 2-month-old NMRI male mice exposed at neonatal day 10 to a single oral dose of PBDE 47, PBDE 99, TBBPA, or the 20% fat emulsion vehicle. Statistical analyses of behavioral data were done by ANOVA using a split-plot design (*33*). Pairwise testing between PBDE- and TBBPA-exposed and control groups was performed with the Tukey HSD test. The height of each bar represents the mean ± SD of 8 animals.
*$p < 0.05$ and **$p < 0.01$ compared to control.

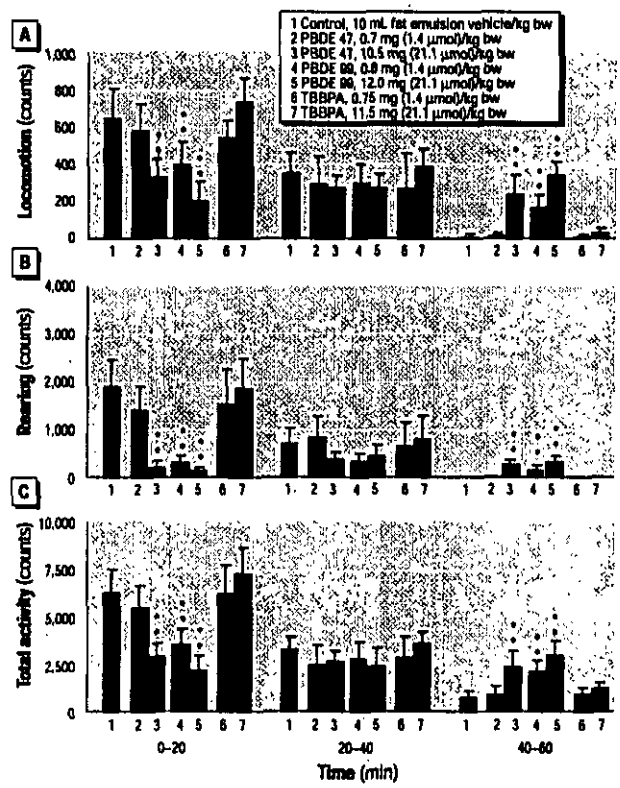

Figure 2. Spontaneous behavior in 4-month-old NMRI male mice exposed to a single oral dose of PBDE 47, PBDE 99, TBBPA, or the 20% fat emulsion vehicle at neonatal day 10. Statistical analyses of behavioral data were done by ANOVA using a split-plot design (*33*). Pairwise testing between PBDE- and TBBPA-exposed and control groups was performed with the Tukey HSD test. The height of each bar represents the mean ± SD of 8 animals.
*$p < 0.05$ and **$p < 0.01$ compared to control.

control animals. In mice receiving the higher dose of PBDE 99 (12.0 mg) the difference in latency between trial 1 and trial 5 differed significantly from controls [$F(3,57) = 3.42$; Duncan, $p \leq 0.01$].

Discussion

Behavior is a major function whereby animals adapt to changes in the environment. Changes in behavior may reveal the influence of chemical pollution on our natural environment. Spontaneous behavior is especially meaningful in environmental toxicology because it reflects function that is important for survival of the individual and for the species in the wild—for example, the mobility needed to search for food, to mate, and to elude predators (*35*).

Our study has shown that neonatal exposure to PBDE 99 and PBDE 47 can cause permanent aberrations in spontaneous behavior. This effect and the habituation capability appear also to worsen with age. Furthermore, neonatal exposure to PBDE 99 also affected learning and memory functions in adult animals. Exposure to TBBPA in the same dose range did not cause any significant change in the investigated behavioral variables. Whether the absence of neurotoxic effects of TBBPA is related to altered uptake or metabolism is not known, but TBBPA is known to have a short half-life (*36,37*).

The spontaneous motor behavior data showed a dose–response-related disruption of habituation in mice treated with both PBDE 99 and PBDE 47. Habituation—a decrease in locomotion, rearing, and total activity variables in response to the diminishing novelty of the test chamber over 60 min—was demonstrated in the control animals, but mice treated with PBDE 99 and PBDE 47 were obviously hypoactive early in the 60-min test period, whereas toward the end they became hyperactive. This nonhabituating behavior profile has also been reported in adult mice neonatally exposed to ortho-substituted PCBs, such as PCB 28, PCB 52, PCB 153 (*24,38,39*), and also in mice neonatally exposed to coplanar PCBs, such as PCB 77, PCB 126, and PCB 169 (*24,40,41*). This indicates that some PBDEs can be potent inducers of behavioral aberrations and that the changes can be similar to those observed earlier for some ortho- and coplanar PCBs. Furthermore, the effects were induced at doses that did not affect the animals' weight gain and at doses comparable to those used in the PCB studies.

The results from the spontaneous behavior tests further indicate that the functional disorder worsens with increasing age: The aberrations were more pronounced in 4-month-old than in 2-month-old mice. In mice receiving the higher dose of PBDE 99 (12.0 mg/kg bw) the difference, compared with controls, in the locomotor variable during the first 20-min period was about 68% in 4-month-old mice compared with about 58% in 2-month-old mice, a difference that was significant (*t*-test, $p \leq 0.01$). This time-dependent effect was evident in mice given the lower dose (0.8 mg/kg bw) where a significant decrease in the locomotor variable was seen in the 4-month-old but not in the 2-month-old mice. The time-dependent effect was also evident in this treatment group during the last 20-min period, where a significant increase in activity in the rearing and total activity variables was seen in the 4-month-old but not in the 2-month-old mice. That this functional disorder can worsen with age is supported by the significantly reduced habituation capability in 4-month-old mice compared to 2-month-old mice. Both the change in spontaneous motor behavior profile, time dependent and dose related, and the reduced habituation capability indicate the advance of brain dysfunction induced at the time of rapid brain development in the neonatal mouse. Significantly, neonatal exposure to certain ortho-substituted and coplanar PCBs can cause this change in spontaneous motor behavior profile, both time dependent and dose related (*24,41*).

In the present study, the ability of adult mice to learn and memorize was observed in a swim maze of the Morris water-maze type. This maze revealed that mice exposed to the higher dose of PBDE 99 (12 mg/kg bw) performed significantly worse than control animals. The swim maze allowed a 4-day acquisition period followed by reversal trials on the 5th day, when the platform was moved. In control mice and in mice given PBDE 99, PBDE 47, and TBBPA, latency to locate the platform decreased during the acquisition training, and all tested animals performed equally well at the end of the acquisition period. In the reversal trials on the fifth day, however, mice exposed to the higher dose of PBDE 99 did not improve in

Table 1. Habituation capability in 2-month-old and 4-month-old NMRI male mice exposed neonatally to PBDE 47, PBDE 99, or TBBPA[a].

Treatment	Habituation ratio (age) 2-Month-old	4-Month-old	*F*-Value	*p*-Value
Locomotion				
Control	1.34 ± 1.45	1.45 ± 1.92	0.02	NS
PBDE 47 l	0.55 ± 0.90	0.91 ± 1.41	0.48	NS
PBDE 47 h	41.6 ± 3.57	69.5 ± 11.2	56.5	0.001
PBDE 99 l	16.6 ± 9.61	39.6 ± 6.08	40.9	0.001
PBDE 99 h	113 ± 11.6	185 ± 48.3	20.7	0.001
TBBPA l	2.15 ± 2.43	1.58 ± 1.86	0.35	NS
TBBPA h	1.43 ± 1.62	3.93 ± 4.87	2.36	NS
Rearing				
Control	0.31 ± 0.52	0.27 ± 0.47	0.02	NS
PBDE 47 l	0.00 ± 0.00	0.10 ± 0.32	1.00	NS
PBDE 47 h	75.2 ± 24.5	152 ± 51.1	18.6	0.001
PBDE 99 l	8.89 ± 4.59	44.7 ± 10.1	104	0.001
PBDE 99 h	157 ± 26.7	222 ± 28.2	21.0	0.001
TBBPA l	0.17 ± 0.36	0.36 ± 0.58	0.82	NS
TBBPA h	0.35 ± 0.57	0.33 ± 0.35	0.01	NS
Total activity				
Control	11.7 ± 2.14	13.1 ± 2.86	1.53	NS
PBDE 47 l	15.9 ± 2.14	16.2 ± 4.25	0.05	NS
PBDE 47 h	50.6 ± 7.35	79.7 ± 11.7	44.8	0.001
PBDE 99 l	31.2 ± 2.03	61.1 ± 1.86	1,181	0.001
PBDE 99 h	101 ± 7.36	138 ± 14.4	51.3	0.001
TBBPA l	15.7 ± 2.66	15.6 ± 1.77	0.01	NS
TBBPA h	15.2 ± 2.58	17.3 ± 2.40	3.41	NS

Abbreviations: h, high; l, low; NS, not significant.
[a]Habituation capability is the ratio between performance in spontaneous motor behavior period 40–60 min and 0–20 min in 2-month-old and 4-month-old NMRI male mice exposed to a single oral dose of either PBDE 47, l = 1.4 μmol and h = 21.1 μmol/kg bw; PBDE 99, l = 1.4 μmol and h = 21.1 μmol/kg bw; TBBPA, l = 1.4 μmol and h = 21.1 μmol/kg bw; or 20% fat emulsion vehicle at neonatal day 10. Statistical analyses of behavioral data were done by ANOVA using a split-plot design (*33*).

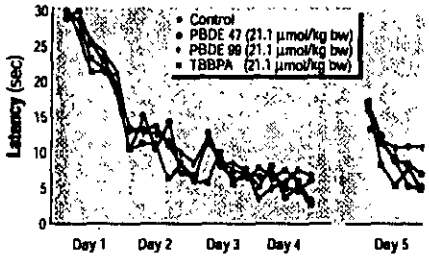

Figure 1. Swim-maze performance of 5-month-old NMRI male mice exposed to a single oral dose of PBDE 47, PBDE 99, TBBPA, or the 20% fat emulsion vehicle at neonatal day 10. Latencies to reach the platform were measured during acquisition (days 1–4) and during reversal trials (day 5). Behavioral data, days 1–4, were analyzed by ANOVA using a split-plot design (*33*). Statistical analyses of behavioral data for day 5 were done by paired *t*-test and ANOVA 1-way combined with Duncan's test. Each point represents the mean of 16–18 animals.

finding the new location of the platform, an improvement that was seen in control mice and mice exposed to PBDE 47 and TBBPA.

The maze learning/memory test and the spontaneous behavior test reveal that PBDE 99 is more potent in causing neurotoxic effects than PBDE 47. This indicates differences in neurotoxicity among different PBDE congeners—changes such as those seen earlier for different PCB congeners. In previous studies we observed altered new/reversal learning in swim-maze performance in adult mice neonatally exposed to PCB 52 (*38*) and PCB 126 (*41*) but not after neonatal exposure to PCB 28 (*38*). Furthermore, in animals showing defects in new/reversal learning, the nicotinic receptors in the brain were affected. The behavioral performance in tasks requiring attention and rapid processing of information in humans and new/reversal learning and working memory in animals may involve cholinergic transmission (*42*), and the cholinergic system is one of the major transmitter systems that correlate closely with cognitive function (*43,44*). Whether neonatal exposure to PBDEs can affect cholinergic receptors in the brain is therefore of special interest and calls for further studies, because in neurodegenerative diseases, such as Alzheimer and Parkinson, there is a change in cholinergic nicotinic receptors in the cerebral cortex and hippocampus (*45,46*).

Do we have a novel class of developmental neurotoxicants in our environment? It is particularly worth noting that neonatal exposure to PBDE 99, and to a lesser degree PBDE 47, affected spontaneous behavior, habituation capability, and learning and memory in adult mice, similar to those observed earlier for PCBs. That different PBDE congeners can have different potency in causing neurotoxic effects is important when comparing the levels of PBDEs in the environment and in mother's milk, but also when comparing levels of PBDEs to the levels of certain PCBs. In our earlier studies we have seen that the amount of ortho-substituted PCB congeners, such as PCB 52 and PCB 153, found in the brain 24 hr after a single oral administration to 10-day-old mice is about 3–5 per mille of the administered dose (*24*). Data on actual tissue levels of PCBs in infants are few. The amounts of these different PCBs given in our studies resulted in a brain tissue concentration (ppb levels) that can be of the same order of magnitude observed in infants less than 1 year of age (*47*).

In human studies it is difficult to distinguish between exposure of offspring by transplacental or by breast milk transfer. However, both human and animal data from a variety of species suggest that accumulation of highly persistent chemicals ingested via milk far exceeds the contribution made by maternal–fetal transfer (*47*). Although the total amount of PCB in mother's milk is higher than that of PBDE, it is important to compare the levels of single congeners. The concentration of PBDEs found in native Swedish mother's milk was 4 ng/g lipid in 1997 (*9,10*). The amount of PBDE 47 was 2.3 ng/g lipid and PBDE 99 0.5 ng/g lipid. The levels of PCB 52 in Swedish mother's milk was 1 ng/g lipid in 1996 and for PCB 153 73 ng/g lipid in 1997 (*9,10*). From the same study (*9*) it is also worth noting that the amount of DDT, a well known neurotoxic compound, was about 6 times higher than PBDE 47—14 ng/g lipid. Human epidemiologic studies suggest that perinatal exposure to PCBs can have developmental neurotoxic effects (*48–51*). Experimental studies in animals have shown that commercial mixtures of PCBs can cause behavioral aberrations and changes in brain neurotransmitter metabolism (*52–54*). Exposure of mice, rats, and monkeys to commercial mixtures of PCBs during development can produce long-term neurobehavioral changes (*55,56*). Recently, Rice and Hayward (*57*) have shown cognitive defects in adult monkeys exposed postnatally, from birth to 20 weeks of age, to a PCB mixture representative of the PCB congeners typically found in human breast milk. In our animal model we have seen that certain PCB congeners, known to be present in the environment and human milk, given during a critical phase of neonatal development, when the maturation of the developing brain and CNS is at a stage of critical vulnerability, induce persistent neurotoxic effects (*24*). In these studies, the lowest dose of the PCB congeners, PCB 52 and PCB 153, shown to induce developmental neurotoxic effects is the same as the one used in the present study of PBDE 99—1.4 μmol/kg bw.

Our present findings that developmental exposure to PBDE can cause similarities in behavioral disturbances as seen earlier for PCBs is of special interest, not only for PBDE as a single agent but for possible interactive effects between these persistent environmental agents and the present background levels of PCBs. Given the increasing concentration of PBDEs in mother's milk, we call for future research into PBDEs as potential neurotoxicants.

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An overview of brominated flame retardants in the environment[*1]

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Abstract

The presence of brominated flame retardant (BFR) chemicals, and particularly polybrominated diphenyl ethers (PBDEs), tetrabromobisphenol A (TBBPA) and hexabromocyclododecane (HBCD), has become of increasing concern to scientists over the past decade. Environmental studies conducted primarily in Europe, Japan and North America indicate that these chemicals are ubiquitous in sediment and biota. The levels of PBDEs seem to be increasing, and several trends, including in humans, indicate that this increase may be rapid. The occurrence of high concentrations of certain PBDE isomers may be sufficient to elicit adverse effects in some wildlife. There is also concern that levels could cause adverse effects in sensitive human populations such as young children, indigenous peoples, and fish consumers. However, our knowledge about these chemicals, their sources, environmental behavior, and toxicity is limited, making risk assessment difficult. In this paper, the current state of knowledge is reviewed and areas for further research recommended to improve future monitoring and risk assessment efforts.

Author Keywords: Brominated flame retardants; Polybrominated diphenyl ethers; Tetrabromobisphenol A; Hexabromocyclododecane

Article Outline

1. Introduction

Flame retardants are substances used in plastics, textiles, electronic circuitry and other materials to prevent fires. Some of the technical flame retardant products contain brominated organic compounds including polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCD), tetrabromobisphenol A (TBBPA) and polybrominated biphenyls (PBBs). The structures for these are shown in Fig. 1. Many of these substances are persistent and lipophilic and have been shown to bioaccumulate.

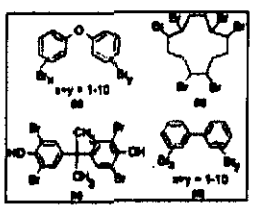
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Fig. 1. The chemical structures of (a) PBDEs, (b) HBCD, (c) TBBPA, and (d) PBBs.

Some brominated flame retardants are additives mixed into polymers and are not chemically bound to the plastic or textiles and therefore may separate or leach from the surface of their product applications into the environment. Additives include PBBs, PBDEs and HBCD. Others, such as TBBPA, are reactive and are bound to the material chemically. However, some of the reactive flame retardant may not have polymerized and may be released to the environment. Knowledge about some of these substances is very limited and hinders environmental authorities from carrying out adequate risk assessments.

The major companies producing brominated flame retardants are Albemarle Corporation (previously Ethyl Corporation) (US), Great Lakes Chemical Corporation (US and UK), Dead Sea Bromine (Israel) and Eurobrom (Netherlands). Other companies include Riedel de Haen (Hoechst Group),

Ceca (ATOCHEM, France), Potasse et Produit Chimiques (Rhone Poulenc Group), Warwick Chemicals (UK) and Albermarle S.A. (Belgium), as well as Nippo, Tosoh and Matsunaga, all from Japan (Arias; KEMI and WHO). The total world production of all brominated flame retardants in 1992 was estimated at approximately 150 000 metric tons/year. Forty percent of the distribution was to North America, 30% to the Far East and 25% to Europe (Arias and KEMI).

The majority of environmental studies conducted to date have focused on PBDEs, and to some extent on TBBPA and HBCD. Other brominated organic substances are also of concern, such as polybrominated biphenyls (PBBs) and polybrominated dibenzo-*p*-dioxins and furans (PBDD/Fs). Concern about PBBs was raised after a major poisoning accident in the US in 1973. PBDD/Fs are formed, for example, when plastics containing brominated flame retardants are heated (welding of mats, melting of polymers). However, coverage of PBBs and PBDD/Fs is outside the scope of this paper. Interested readers are referred to the following reports for PBBs (KEMI; WHO and de) and recent reports on PBDEs and PBDD/Fs by WHO/ICPS for more information (WHO; WHO and WHO).

1.1. Polybrominated diphenyl ethers (PBDEs)

There are theoretically 209 PBDE congeners. Technical PBDE products are produced by brominating diphenyl ether in the presence of a catalyst. The major technical products contain mainly pentaBDEs, octaBDEs or decaBDE, but contain other PBDEs as well. The general compositions of the technical products are given in Table 1. The individual PBDE congeners are numbered according to the IUPAC system used for numbering PCBs based on the position of the halogen atoms on the rings.

Table 1. The general compositions of PBDE-based flame retardant commercial products given in percent of BDE congeners present (WHO/ICPS, 1994b)

(<1K)

Annual worldwide production of penta-, octa- and decaBDE technical products in 1990 was estimated to be 4000, 6000 and 30 000 metric tons, respectively (Arias, 1992). The estimated world market for PBDEs as well as TBBPA and HBCD in 1999 is given in Table 2 (www.bsef.com). Consumption of PBDEs for 1999 in the European Union was estimated to be 150 metric tons penta-, 400 metric tons octa- and 7000 metric tons decaBDE technical products (De Poortere, 2000).

Table 2. Estimated world market demand for PBDEs, TBBPA and HBCD in 1999 given in metric tons (www.bsef.com)

(<1K)

Accumulated amounts in electric products still in use in 1994 in the Nordic countries were estimated to be 250 metric tons of PeBDE and 5500 metric tons of OcBDE and DeBDE combined (Hedelmalm et al., 1995). The estimated amounts supplied to Sweden alone in 1991 were 17 metric tons of PeBDE and 626 metric tons of OcBDE and DeBDE combined (Hedelmalm et al., 1995). The

combined total annual amounts of penta-, octa- and decaBDE technical products recently imported by Sweden are given in Table 3. For 1993, approximately 20 metric tons of PBDE were imported by Sweden and the same amount was imported in plastics (KEMI, 1995). The amount of PBDE imported in ready-made products the same year was estimated by the Swedish National Chemicals Inspectorate to be approximately 400 metric tons (KEMI, 1995).

Table 3. The import of brominated flame retardants by Sweden in metric tons (National Swedish Chemicals Inspectorate, www.kemi.se/kemstat/kortstat/mangditon.htm)a

(<1K)

PBDEs are lipophilic and have some structural similarities to PCB and PCDD/F. Bromkal 70-5DE is one of several PeBDE technical products that contains lower brominated PBDEs such as 2,2',4,4'-TeBDE (BDE-47), 2,2',4,4',5-PeBDE (BDE-99) (Sundström and Hutzinger, 1976) and 2,2',4,4',6-PeBDE (BDE-100), as well as two newly identified triBDEs, one additional tetraBDE, one additional pentaBDE, three hexaBDEs and one heptaBDE (Sjödin et al., 1998). PBDEs were first discovered in Sweden in fish samples taken downstream from several textile industries on River Viskan (Andersson and Blomkvist, 1981). In some countries, industries are voluntarily replacing the lower brominated PBDEs with other flame retardants. PeBDE technical products are currently in the process of being banned within the European Union.

PeBDE technical products are used in epoxy resins, phenol resins, polyesters, polyurethane foam and textiles (WHO/ICPS, 1994b). OcBDE technical products are used in acrylonitrile butadiene styrene, polycarbonate and thermosets. DeBDE products are used in most types of synthetic materials including textiles and polyester used for printed circuit boards (OECD, 1994).

1.2. Tetrabromobisphenol A (TBBPA)

TBBPA is covalently bound to plastic and is used in electronic circuit boards. Annual worldwide production was estimated to be 50 000 metric tons/year in 1992 (KEMI, 1994). Estimated accumulated amounts of TBBPA in products in the Nordic countries in 1994 were 4000 metric tons (Hedelmalm et al., 1995). The estimated amount supplied to Sweden alone in products in 1991 was 334 metric tons. More recent information on market demand is given in Table 2 and on the import of TBBPA by Sweden in Table 3. One moiety of TBBPA's molecular structure is similar to that of the thyroid hormone thyroxine, except that the iodine atoms have been replaced by bromines (see Fig. 1 and Fig. 2). The dimethylated derivative of TBBPA (MeTA) may have some use as a flame retardant, but may also be the result of methylation of TBBPA in sediment (Watanabe et al., 1983).

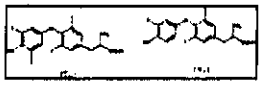
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Fig. 2. The chemical structures of the thyroid hormones 3,3',5,5'-tetraiodo--thyronine (thyroxine or T_4) and its 5'-deiodinated congener 3,3',5-triiodothyronine (T_3).

1.3. Hexabromocyclododecane (HBCD)

HBCD is produced by bromination of cyclododecane in a batch process. HBCD has been used for about 20 years. Approximately 11 000 metric tons are incorporated in articles made of polystyrene and in textile backcoating for the EU market. It is used in foams and expanded polystyrene. End products include upholstered furniture, interior textiles, automobile interior textiles, car cushions and insulation blocks in trucks and caravans as well as in building materials such as house walls, cellars, roofs and parking decks, against frost heaving in roads and railway embankments, packaging material, video cassette recorder housing and electric equipment (Margareta Palmquist, National Swedish Chemicals Inspectorate, personal communication). World demand for HBCD is given in Table 2. The amounts of HBCD imported by Sweden for the last few years are given in Table 3.

1.4. Polybrominated biphenyls (PBBs)

In 1973, a commercial flame retardant containing PBBs was accidentally mixed into feed for dairy cattle, livestock and poultry in the state of Michigan, US (KEMI and WHO). The feed was used widely, leading to widespread PBB contamination of milk, meat and eggs and poisoning in animals. Over nine million people were exposed to PBBs from food. Because of this widespread exposure, research was funded to better understand the toxicology of PBBs, and poisoned animals and exposed humans have been studied as well. The effects of PBBs were found to be essentially the same as those seen for PCBs.

Technical hexabrominated biphenyl (HxBB) is banned in North America and in Europe. Technical decabrominated biphenyl (DeBB) production was terminated by the last producer in Europe in 2000.

2. Brominated flame retardant chemistry

Considerable work has been carried out to synthesize pure PBDE congeners for use as standards, for toxicology studies and in the identification of unknown substances in environmental samples. Within several research programs, a number of both unlabelled and radiolabelled PBDEs have been synthesized for these purposes (Hu; Jakobsson; Örn et al., 1996; Örn et al., 1998; Örn, 1997 and Marsh). This work is summarized in Bergman (1999) and in Table 4.

Table 4. Names and congener numbers of PBDEs synthesized

 (<1K)

2.1. Validation of synthetic substances

Using synthesized single congeners of some PBDEs, it has been possible to better quantify the components of the technical product Bromkal 70-5DE and to identify the previously unknown PeIBDE (2,2',4,4',6-PeBDE) in this product. According to previously published results, Bromkal 70-

5DE consisted of 41% 2,2',4,4'-TeBDE, 45% 2,2',4,4',5-PeBDE and 7% 2,2',4,4',6-PeBDE (Sundström and Hutzinger, 1976). Using the new standards, it has been found that the composition is actually 37%, 35% and 6.8% for these three PBDEs, respectively (Sjödin et al., 1998). Bromkal 70-5DE also contains 1.6% of 2,2',3,4,4'-PeBDE (BDE-85) and 3.9%, 2.5% and 0.41% of HxBDE-153, -154 (2,2',4,4',5,6'-HxBDE) and -138, respectively. A number of other standards that have been produced have also been analyzed in order to use them in identifying and quantifying more PBDEs in environmental samples on a congener-specific basis. This has now led to the commercial availability of congener-specific standards for many BDEs.

2.2. Physicochemical properties

PBDEs have low vapor pressures and are very lipophilic, with logK_{ow}s (octanol-water partitioning coefficients) in the range 5.9-6.2 for TeBDEs, 6.5-7.0 for PeBDEs, 8.4-8.9 for OcBDEs and 10 for DeBDE (Watanabe and Tatsukawa, 1990). Experimentally determined subcooled vapor pressures for several BDE congeners were found to be lower than for comparably chlorinated PCBs, and decreased with increasing number of bromines (Tittelmier and Tomy, 2000). Halogen substitution pattern influences vapor pressure such that congeners with bromine substitution in the *ortho* positions to the ether bond have higher vapor pressures (Wong et al., 2001). TBBPA has a logK_{ow} of 4.5 (WHO/ICPS, 1995). The dimethylated derivative of TBBPA (MeTA) has a logK_{ow} of 6.4, making it more lipophilic than the parent compound (Watanabe and Tatsukawa, 1990). The logK_{ow} for HBCD is 5.8 (IUCLID, 1996).

PBDEs are persistent, have low water solubility, high binding affinity to particles and a tendency to accumulate in sediments. HBCD also has low water solubility (IUCLID, 1996), and probably also has an affinity for particles and sediments.

2.3. Transformation/decomposition

Studies of TBBPA in sediments have shown that a dimethylated derivative is also found (Sellström and Jansson, 1995). The origin of this methylated TBBPA is not completely understood; however it probably has some use as a flame retardant (WHO/ICPS, 1997), but may also be due to methylation of TBBPA by microorganisms in the sediment (Watanabe et al., 1983). Recent laboratory experiments have found that TBBPA can be microbially metabolized in a two-step process: reductive debromination under anaerobic conditions to bisphenol-A, and aerobic mineralization of bisphenol-A by a gram-negative aerobic bacterium (Ronen and Abeliovich, 2000). The anaerobic step was carried out using sediment from a stream bed contaminated by chemical industry waste.

Previous studies indicate that DeBDE is debrominated by UV light and sunlight to lower brominated PBDE (to TrBDE with UV light and to TeBDE with sunlight) but it is not known if this happens in the environment (Norris; Norris and Watanabe; Ulrika Örn, Department of Environmental Chemistry, Stockholm University, personal communication).

More recent laboratory studies of the photolytic breakdown of DeBDE have shown that DeBDE in toluene and applied to silica gel is successively debrominated by UV light to lower brominated PBDE (down to TeBDEs) and that this occurs very rapidly (Sellström et al., 1998a). The half-life in toluene was less than 15 min. DeBDE-treated sand, exposed to UV light in the laboratory or to

sunlight outdoors for different time periods, showed that DeBDE is photolytically debrominated in the same manner as in solution and on silica gel, but that the debromination time course proceeds more slowly for both UV and sunlight exposure (half-life of 12 and 37 h, respectively). Similar results were obtained for sediment, with a half-life for DeBDE of 53 h for UV exposure and 81 h for sunlight exposure and with TeBDEs appearing at the longest exposure time (244 h). Results for soil samples treated with DeBDE and exposed to UV light in the laboratory indicated a half-life of 185 h and the debromination process was the same as that seen for all the other matrices tested. If this process is significant in the environment, it could lead to the formation of lower brominated BDEs, which are known to bioaccumulate.

TBBPA is also photolytically decomposed when exposed to UV light, both in the absence and presence of hydroxyl radicals (Eriksson and Jakobsson, 1998). The main breakdown product is 2,4,6-tribromophenol. A number of other decomposition products are also found and some of these have been tentatively identified as di- and tribromobisphenol A, dibromophenol, 2,6-dibromo-4-(bromoisopropylene)phenol, 2,6-dibromo-4-(dibromoisopropylene)phenol and 2,6-dibromo-1,4-hydroxybenzene.

3. Analytical methods for brominated flame retardants

In most analytical methods for brominated organic compounds, the sample is first extracted with an organic solvent. Lipids can be removed using sulfuric acid treatment or gel permeation chromatography methods. In some cases the extract needs to go through a further clean-up step using some form of column chromatography to remove interfering substances. The purified extract is then analyzed with gas chromatography using electron capture detection (ECD), mass spectrometry using the negative ions formed at chemical ionization (MS-ECNI) (Jansson; Jansson; Nylund; Sellstr and Sellstr) or high-resolution gas chromatography-mass spectrometry (HRGC-MS) (Sergeant; Alaee; Ikonomou; Ikonomou; Meironyt; Strandman; Strandman; Huwe; Luross; Ohta; Ryan; She; Stern and Thomsen). The higher brominated PBDEs have longer retention times and are often analyzed using a shorter GC column (Sellstr and Sellstr). For a more detailed review of analytical methods, see de Boer et al. (2000a).

TBBPA is separated from the neutral components by treatment with a basic water solution. The pH of this water solution is then adjusted to be acidic and TBBPA is extracted with an organic solvent. Before analysis, TBBPA is derivatized to its diacetylated derivative. Dimethylated TBBPA (MeTA) is analyzed with the PBDEs (Sellström and Jansson, 1995).

Hydroxylated PBDEs are separated from the neutral components by treatment with a basic water solution and then derivatized with diazomethane. Methoxy-PBDEs are found in the neutral fraction. The analysis is carried out using gas chromatography-mass spectrometry or GC-ECD (Asplund; Asplund and Haglund).

4. Toxicology

Thorough reviews of previous studies of the toxicology of PBBs, PBDEs and TBBPA can be found in the relevant WHO/IPCS reports (WHO; WHO and WHO) and in Darnerud and Darnerud for PBDEs.

4.1. Uptake, distribution, metabolism, excretion

4.1.1. Mammals

4.1.1.1. Lower brominated PBDEs

The distribution of ^{14}C-labelled 2,2',4,4'-tetrabromodiphenyl ether (BDE-47), 2,2',3,4,4'-pentabromodiphenyl ether (BDE-85), and 2,2',4,4',5-pentabromodiphenyl ether (BDE-99) has been studied in C57BL mice using whole-body autoradiography, as well as milk transfer studies during the neonatal period using liquid scintillation method to evaluate BDE-85 and BDE-99 (Darnerud and Risberg, 1998). The autoradiograph findings indicate that the uptake from the gastrointestinal tract was effective. Organs and tissues with high radioactivity concentrations were the fat depots, liver, adrenal and ovary, lung and (shortly after administration) the brain. At longer post-injection time, the apparent concentration in most tissues was considerably lower, and radioactivity was still present in white and brown fat depots. Studies in pregnant mice showed a low fetal uptake of the compounds. No significant difference in distribution between the three studied congeners was observed. Breast milk transport of the pentaBDE congeners was substantial and 30-40% of the administered single dose of radioactivity was found in the suckling offspring litter after 4 days. At the same time point, the plasma levels in the neonates were more than two times that of the mothers', although the absolute levels were low. The concentration in tissues and milk was in about the same range as that earlier observed for PCB congeners of a similar degree of halogenation, when administered in equimolar doses.

Uptake, distribution, metabolism and excretion of BDE-47 has been studied in rats and mice dosed orally with ^{14}C-labelled BDE-47 (Klasson; Örn, 1997 and Örn and Klasson-Wehler, 1998). In rats, 14% was excreted in feces and less than 0.5% in urine during 5 days. Of the amount excreted, 79% was the parent compound and 21% corresponded to metabolites. Treated rats retained 86% of the administered dose after 5 days and the highest concentrations were found in adipose tissue, where the ^{14}C corresponded to the parent BDE-47. All tissues analyzed contained parent BDE-47. Liver also contained low concentrations of five hydroxylated metabolites. Two of these were found in plasma (Örn and Klasson-Wehler, 1998).

In mice, 20% of the dose was excreted in feces and 33% in urine. Of the amount excreted, 15% was parent compound and 85% corresponded to metabolites. Treated mice retained 47% of the administered dose after 5 days and similar concentrations were found in both adipose tissue and liver. Covalently bound metabolites were found in liver (12% bound to macromolecules, 16% to lipids), in the lungs (28%) and kidneys (4%). Low concentrations of five hydroxylated metabolites were also observed in the liver. Three of these were seen in plasma as well (Örn and Klasson-Wehler, 1998). The conclusions drawn from this study were that BDE-47 was absorbed well by both the rat and mouse, but the rate of metabolism and excretion varied considerably.

Tissue disposition, metabolism and excretion of BDE-99 has been studied in bile-cannulated and uncannulated male rats dosed orally with ^{14}C-labelled BDE-99 (Hakk and Larsen). Feces was the major route of elimination in both groups (43% of the administered dose in uncannulated and 86% in cannulated rats after 72 h). Of the amount excreted in feces, more than 90% was parent BDE-99. Cumulative excretion of metabolites in both groups of rats was less than 1% of the administered dose

into urine and 3.7% into bile. In the uncannulated rats, 6.3% of the ^{14}C in urine was protein bound and was associated with alpha-2-microglobulin. In cannulated rats, 28-47% of the ^{14}C in bile was bound to an unidentified protein of 79 kDa. In the uncannulated rats, 39% of the administered dose was retained and the highest concentrations were found in adipose tissue, skin and adrenals. Covalently bound ^{14}C was observed in feces indicating the formation of reactive metabolic intermediates. Two monomethoxy pentabromodiphenyl ether metabolites and two de-brominated monomethoxy tetrabromodiphenyl ether metabolites were indicated in feces. Two monohydroxy pentabromodiphenyl ether metabolites, two dihydroxy pentabromodiphenyl ether metabolites and possibly two thio-substituted pentabromodiphenyl ether metabolites were characterized in bile.

Seventeen PBDE congeners (BDE-15, -28, -30, -32, -47, -51, -71, -75, -77, -85, -99, -100, -119, -138, -153, -166 and -190) were incubated individually with rat hepatic microsomes from rats treated with beta-naphthaflavone (NF), phenobarbital (PB) or clofibrate (CLOF) (Meerts and Meerts). The original congeners and the metabolites formed were then tested for their ability to compete with the thyroid hormone thyroxine (T_4) for binding to human transthyretin in vitro. A number of hydroxylated PCBs are structural analogues of T_4 and are known to compete with T_4 in this system. The structural requirements for binding to transthyretin are hydroxy-substitution in *para* or *meta* positions of one or both phenyl rings with adjacent halogen substitution (Lans, 1995). X-ray diffraction studies have shown that several phenolic organohalogen compounds bind with the hydroxy group in the central channel of the transthyretin molecule (Ghosh et al., 2000).

Results showed no competition with the parent compounds, but considerable potency for several of the metabolites, indicating the metabolism of PBDE to hydroxylated PBDE (see Section 4.2.1.1 for results). No binding competition was seen for several of the higher brominated PBDE such as BDE-138, -153, -166 and -190 after incubation with the microsomes. This may indicate that these congeners are not readily metabolized.

4.1.1.2. DeBDE

The uptake of ^{14}C-DeBDE has previously been shown to be low following oral administration to rats, and between 90% and 99% of the dose was eliminated in the feces and gut (Norris and El). A two-year feeding study of DeBDE in rats confirmed a low accumulation; however, the small portion that reached the adipose tissue, measured as the total bromine content, remained unaffected for 90 days of recovery (Kociba and Norris). When ^{14}C-DeBDE was injected intravenously, 74% of the dose was found in feces and gut contents after 72 h. Of the excreted material, 63% was metabolites and 37% was the parent compound (El Dareer et al., 1987). Longer half-lives may be evident in humans. In occupationally exposed workers, both BDE-183 and DeBDE (BDE-209) were detected in serum and the estimated half-lives of these were 86 days for BDE-183 and 6.8 days for BDE-209 (Hagmar et al., 2000b).

4.1.1.3. TBBPA

TBBPA fed to rats was eliminated primarily in feces (95%) and 1% was eliminated in urine within 3 days (WHO/ICPS, 1995). ^{14}C-labelled TBBPA was fed to rats with and without bile duct cannulation (Larsen et al., 1998). Bile, urine and feces were collected every 24 h for 3 days, after which the rats were killed and organs and tissues sampled. Three conjugated metabolites were found in the bile - a diglucuronide, a monoglucuronide and a glucuronide-sulfate ester. These represented 34%, 45% and

21% of the radioactivity excreted in bile during the first 24 h, respectively. In the cannulated rats, 71% of the dose was excreted in the bile within 72 h. In uncannulated rats, 95% of the ^{14}C-labelled TBBPA was excreted in the feces as TBBPA. However, there was a delay in fecal excretion. This is the result of enterohepatic circulation of TBBPA where the biliary metabolites are deconjugated and reabsorbed from the lower intestine, reconjugated and re-excreted in the bile. About 2% of the dose remained in the uncannulated rat after 72 h and highest levels were found in the large intestine (1%), small intestine (0.6%), lung (0.2%) and carcass (0.2%).

Uptake and distribution of ^{14}C-labelled TBBPA in pregnant rats after oral exposure on gestational days 10-16 has recently been studied (Meerts et al., 1999). The major portion of radioactivity was excreted in feces (79.8%). Only 0.83% of the total administered dose was found in the tissues of the dams and 0.34% in the fetuses. Highest maternal levels were found in the carcass (0.37%) and liver (0.26%).

In occupationally exposed workers, TBBPA has recently been determined to have an estimated half-life in serum of 2.2 days, indicating rapid turnover (Hagmar et al., 2000b).

4.1.1.4. HBCD

Rats fed a single oral dose of ^{14}C-labelled HBCD rapidly absorbed the substance (Yu and Atallah, 1980). HBCD was readily distributed in the entire body with highest concentrations found in adipose tissue, followed by liver, kidney, lung and gonads. The half-life was 2 h. HBCD was rapidly metabolized and 72% of the dose was eliminated via feces and 16% by urine within 72 h. Four metabolites were found but no information on their structures was given. HBCD absorption followed a two-compartment open model system, with the central compartment consisting of blood, muscle, liver, kidney and other non-adipose tissues, and the peripheral compartment consisting of fat tissue. Elimination from fat was slower than for the central compartment.

Rats fed HBCD daily for 5 days showed no urinary excretion of HBCD (Ryuich et al., 1983). Average daily fecal excretion was 29-37% of the administered amount. HBCD was found to accumulate in adipose tissue in this study. Experiments using a loop of the upper jejunum suggest that HBCD can be absorbed from the intestine.

4.1.2. Fish and shellfish

4.1.2.1. Lower brominated PBDE

A study of uptake, accumulation and excretion of BDE-47, -99 and -153 and several tri- through hexaCB congeners from water by blue mussels (*Mytilus edulis*) indicated that the uptake clearance rates were approximately 10 times higher for BDE-47 and -99 than for BDE-153 and the PCB congeners (Gustafsson et al., 1999). Several PCB congeners were included for comparison (tri-hexaCBs). Depuration rates were similar for all three PBDEs indicating no dependence on hydrophobicity, but were correlated to hydrophobicity for the PCBs.

In a dietary uptake study of TeBDE, PeBDE and HxBDE, pike were fed with rainbow trout that had been injected with a mixture containing BDE-47, -99 and -153 as well as selected PCB and PCN congeners (Burreau et al., 1997). The uptake efficiencies of all three PBDEs were high, with BDE-47

showing the highest uptake (more than 90% of the given dose) from the gastrointestinal tract. Uptake efficiencies for BDE-99 and -153 were 62% and 40%, respectively. The uptake efficiency of BDE-47 was higher than for the tri- to hexaCBs and the hexa- to octaCNs tested. The uptake efficiencies were higher than expected considering the size and lipophilicity of the compounds. It was concluded that uptake of these substances from the gastrointestinal tract may be facilitated by cotransport with lipids and/or proteins through a mediated or even active transport mechanism.

In a distribution study, several pike were fed rainbow trout previously injected with 5 μCi of ^{14}C-2,2',4,4'-TeBDE (BDE-47). Pike were killed after 9, 18, 36 and 65 days and whole-body autoradiography performed. The results showed accumulation of the labelled TeBDE in liver, gall bladder, kidneys, brain, chorion of the eye and perivisceral adipose tissue and along the spinal column (Burreau and Burreau).

Asplund and Asplund have found hydroxylated and methoxylated PBDE in Baltic salmon blood plasma, and Haglund et al. (1997) found methoxylated PBDE in ringed and grey seal, salmon and herring from the Baltic Sea. Kierkegaard et al. (1999b) found methoxy-BDE-47 in pike from Lake Bolmen, a freshwater lake. It is not clear what the source of these are but one possibility may be due to metabolism either in the organisms or by microorganisms. Natural production by invertebrates or algae cannot be ruled out.

4.1.2.2. DeBDE

A major argument made by flame retardant producers for the use of DeBDE is that the molecule is so large that it is not bioavailable and therefore will not be accumulated by living organisms. To determine if this was the case, uptake of DeBDE from the diet by rainbow trout was investigated by feeding 10 mg DeBDE/kg/day for 0, 16, 49 and 120 days (Kierkegaard et al., 1999a). One group was treated with clean food for 71 days after 49 days of exposure to study elimination. Some biological effects were also measured. The DeBDE concentrations in muscle ranged from <0.6 ng/g fresh weight after 0 days, to 38 (±14) ng/g fresh weight after 120 days. Corresponding liver concentrations were <5 and 870 (±219) ng/g fresh weight. A number of organic brominated substances, characterized as hexa- to nonaBDEs, increased in concentration with exposure length.

After the 71 day depuration period, DeBDE levels declined, but the levels of some of the lower brominated congeners were unaffected during the same period. This may indicate that DeBDE is metabolized via a reductive debromination process to the lower brominated diphenyl ethers and/or that lower brominated PBDEs present in the technical product are selectively absorbed. However, no HxBDEs were detected in the technical product and there was a pronounced shift in increasing peak heights for the first-eluting congeners in the fish compared to the technical product they were fed. The estimated uptake of DeBDE was 0.02-0.13% based on DeBDE concentrations in muscle and an estimation of the concentrations of the metabolites formed compared to the total dose administered. Liver body index (indicating increased liver weight) and plasma lactate levels increased in fish exposed for 120 days and in the depuration group. The number of lymphocytes was significantly lower after 120 days exposure compared to controls. DeBDE did not affect ethoxyresorufin-*O*-deethylase (EROD), ethoxycoumarin-*O*-deethylase (ECOD) or transketolase activity and no DNA adducts were seen.

4.1.2.3. TBBPA

TBBPA is rapidly taken up from water via the gills in fish. When fish are placed in clean water, the compound is rapidly eliminated (WHO/ICPS, 1995).

4.1.2.4. HBCD

The few available studies suggest that HBCD has a high bioaccumulation potential. In a study conducted in River Viskan, HBCD was found in both sediments and in pike (Sellström et al., 1998b), indicating that HBCD is bioavailable. It is probable that the levels found in pike are due to a combination of uptake via the gills and via food (gastrointestinal tract). In fathead minnow (*Pimephales promelas*) exposed to HBCD in water for 32 days (Veith et al., 1979), the bioconcentration factor after 2, 4, 8, 16, 24 and 32 days of exposure was estimated to be 18 100 (log BCF 4.26).

4.2. Toxicity/effects

More detailed reviews of the toxicology, including the toxicity and effects of PBBs, PBDEs and TBBPA, can be found in the relevant WHO/IPCS reports (WHO; WHO and WHO).

4.2.1. PBDEs

4.2.1.1. In vitro

A PeBDE technical product, Bromkal 70-5DE, has weak dioxin-like toxicity as measured in rat H-4-II E hepatoma cells (Hanberg et al., 1991). Using a recombinant H-4-II E rat hepatoma cell line having Ah-receptor-mediated expression of a luciferase reporter gene (the CALUX assay) (Aarts and Murk), a number of individual PBDE congeners have been tested for their potency to activate/deactivate the Ah receptor (Meerts et al., 1998a). In order to study antagonism, the same PBDE congeners were also tested in the presence of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Seven of the 17 PBDE congeners (BDE-32, -85, -99, -119, -153, -166, -190) tested showed ability to activate the Ah receptor. Potencies could only be determined for BDE-166 and BDE-190 and these are in the same range as the mono-*ortho* PCB congeners 105 and 118 (Sanderson et al., 1996). Some congeners such as BDE-85, -99 and -119 showed both agonist and antagonist activities depending on the concentration tested. Nine congeners, including BDE-15, -28, -47, -77 and -138, showed antagonist activities against TCDD. The observed antagonism may be due to competition between PBDEs and TCDD at the Ah-receptor level.

Studies of the potency of 17 PBDE congeners and their hydroxylated metabolites for competitive binding to human transthyretin in vitro have been carried out (Meerts and Meerts). Transthyretin is the thyroid hormone transport protein present in plasma and has a binding site for the thyroid hormone thyroxine (T_4). The parent compounds were incubated with rat hepatic microsomes from rats treated with beta-naphthaflavone, phenobarbital or clofibrate. The parent compounds and the metabolites formed from the different microsomal incubations were then tested for their ability to compete with T_4 for binding to transthyretin. The results are given in Table 5 and show that a number of metabolites were potent competitors for transthyretin. The parent compounds showed no competition. For example, after PB-microsomal incubation of 2,2',4,6'-tetraBDE (BDE-51), hydroxylated PBDE metabolites displaced T_4 from transthyretin with fairly high potency (Meerts and

Meerts). The results indicate that hydroxylated metabolites of PBDE may be potent competitors of T_4 and could disrupt normal thyroid hormone function in wildlife and humans if present.

Table 5. Inhibition of T_4-transthyretin binding in vitro by PBDE metabolites obtained after incubation with rat hepatic microsomes induced by phenobarbital (PB), beta-naphthaflavone (NF) or clofibrate (CLOF)

(<1K)

Inhibition potencies are given from the undiluted extract. ++ = 60% inhibition, + = 20% inhibition, - = 0-20% inhibition. *Source*: Meerts et al. (1998b).

Transthyretin carries T_4 in the plasma to the target tissues, where T_4 is then deiodinated to 3,3',5-triiodothyronine (T_3) (Fig. 2). T_3 then interacts with two subtypes of thyroid hormone receptors (THRs) designated alpha and beta. The T_3-THR complex can then bind to response elements on the DNA that regulate the transcription of thyroid hormone activated genes. T_4 can also interact with THR but has only about 10% of the potency of T_3. To determine the potency of hydroxylated PBDE to bind to THR-alpha and THR-beta, several brominated structural analogues of T_4 and T_3 were synthesized: 4-hydroxydiphenyl ether, 4-hydroxy-2',4',6'-tribromodiphenyl ether (III), 3-bromo-4-hydroxy-2',4',6'-tribromodiphenyl ether (IV) and 3,5-dibromo-4-hydroxy-2',4',6'-tribromodiphenyl ether (V) (Fig. 3) (Marsh et al., 1998).

(5K)

Fig. 3. Synthetic pathway and chemical structures of several hydroxylated PBDEs (Marsh et al., 1998).

The results showed that the highest affinity was found for the T_3 analogue (IV), with the T_4 analogue (V) showing about one-third of the affinity of the T_3 analogue. The affinity of analogue III was low and was lowest for 4-hydroxydiphenyl ether (Marsh et al., 1998). The brominated analogues had lower affinities than T_3 and T_4, probably because of the lack of the 4-carboxyl group. The results indicate that hydroxylated metabolites of PBDE may not only disrupt the normal transport of T_4 to target tissues, but may also be able to bind to the thyroid hormone receptors, thus influencing the regulation of thyroid hormone dependent genes within the cell nucleus.

Mitogen-induced DNA synthesis and immunoglobulin synthesis by human lymphocytes in vitro was examined after exposure to purified BDE-47 and -85. No effects on mitogen-induced proliferation or immunoglobulin synthesis were observed (Fernlöf et al., 1997). The results indicate that proliferation and immunoglobulin synthesis are insensitive to the direct action of PBDEs. Exposure to different PCB congeners (CB-77, CB-118, CB-153) also gave no effects.

BDE-47 was shown to induce a statistically significant increase in intragenic recombination when studied in one of two tested in vitro assays using mammalian cells (Helleday et al., 1999). This may indicate that BDE-47 can induce cancer via a non-mutagenic mechanism, similarly to other environmental contaminants such as DDT and PCB.

4.2.1.2. In mammals

Technical PBDE products are able to induce both phase I and phase II detoxification enzymes in the liver. Regarding the cytochrome P450 (CYP) mediated phase I metabolism, CYP1A1 and 1A2 are induced as shown by the increased activity of liver microsomal EROD after Bromkal 70 (a commercial pentaBDE) exposure in Wistar rats (von Meyerinck et al., 1990) and in H-4-II E cells (Hanberg et al., 1991). Other enzymes that are used as indicators of microsomal phase I activity were also induced by PBDEs (technical pentaBDE preparations in rats) including benzphetamine N-demethylation, *p*-nitroanisole demethylase, aryl hydrocarbon hydroxylase (AHH) and benzo(a)pyrene hydroxylase (Carlson; Carlson and von). Some of the enzymes were induced in a long-term oral administration study in rats at a concentration as low as ca 1 μmol/kg (technical pentaBDE), and the enzymes remained induced 30-60 days after termination of exposure (Carlson, 1980a). DeBDE seems to have low enzyme-inducing potency. However, as CYP1A1 and 1A2 are typically induced by halogenated dioxin-like compounds, possible contaminants with Ah-receptor binding affinity present in technical PBDE mixtures could be responsible for the enzyme induction seen in these studies.

In interaction studies of PBDE, PCB and chlorinated paraffins (CPs), microsomal enzyme activities were studied in rats (Hallgren and Darnerud, 1998). The results showed that the pure congener BDE-47 increased EROD and MROD activities only somewhat (to twice the control levels), indicating that this substance has a low CYP1A1/2-inducing activity. In the same study, PCB (Aroclor 1254) markedly induced EROD and MROD. Earlier results on a commercial PBDE mixture (Bromkal 70) showed a rather strong induction of EROD and MROD in rats, which suggests that the Bromkal mixture contained CYP 1A1/2-inducing substances, probably present as contaminants (unpublished studies in Darnerud and Sinjari, 1996).

Regarding other microsomal enzymes, PROD levels (which mirror CYP2B activities) were measured in rats exposed to BDE-47 (Hallgren and Darnerud, 1998). In this case the PROD levels were dose-dependently elevated up to 10 times in exposed animals, and the levels were about the same as those found after PCB exposure. As the microsomal enzyme induction results are indicative of operational metabolic systems, PBDE may therefore, at least to some extent, be transformed by CYP2B in the phase I metabolism step.

In studies on phase II induction, three different PBDE fractions, i.e. a low (24% tetra, 50% penta) and a high (45% hepta, 30% octa) brominated mixture, and the DeBDE congener only, were tested. Daily oral administrations (14 days, 0.1 mmol/kg body weight) of both mixtures, but not DeBDE, resulted in a long-lasting induction of uridine diphosphate glucuronyl transferase (UDPGT) activity in rats (Carlson, 1980b).

Short-term feeding studies using PeBDE in rats led to changes in the liver (increased weight, hepatocytomegaly) and thyroid (hyperplasia) (Great; Norris and El). No carcinogenicity studies have been performed for TeBDE or PeBDE.

The Bromkal 70-5DE product causes decreased thymus weight and increased liver/body weight ratios

in mice, and decreases in the thyroid hormone thyroxine in rats and mice (Fowles and Darnerud). Decreases in thyroxine were also seen when rats and mice were treated with the single congener BDE-47, but no effects on thyroid stimulating hormone were seen for BDE-47 or Bromkal 70 (Darnerud and Sinjari, 1996).

In subsequent studies the interactive effects of different organohalogen compounds (PCB, PBDE and CP) on thyroxine hormone levels and microsomal enzyme activities were tested (Hallgren and Darnerud, 1998). Female rats were orally exposed to single compounds or combinations daily during 14 days. The results show that PCBs (Aroclor 1254) and PBDEs (BDE-47) significantly reduce the T_4 levels in rats, in the actual exposure interval (6-18 mg/kg body weight/day), and that Aroclor 1254 results in the strongest effect, when administering the substances orally in isomolar concentrations. EROD and MROD, but to a lesser extent PROD and UDPGT, activites correlated to T_4 effects, which could indicate that glucuronidation of T_4 is not a major factor in explaining the observed decrease in T_4 plasma levels. Regarding the mixed BDE-47 + CP group, a synergistic decrease in free T_4 levels, and increase in EROD activity, was observed. As organisms are exposed to these environmental chemicals as mixtures, the observed interactive effects are of interest.

It is known that hydroxylated metabolites of PCB can compete with thyroxine for the binding site on the thyroxine-carrying protein transthyretin in plasma (Brouwer et al., 1990). The effects on the thyroid gland (hyperplasia, decreased thyroxine levels) seen with PBDE could also be due to effects of hydroxylated metabolites, which have been found as metabolites in mice, rats and fish. Some hydroxylated PBDEs have structural similarities to thyroxine. In preliminary experiments, metabolites formed in rat liver from BDE-47, -99 and -153 have been shown to be better competitors for the transthyretin binding site than the parent compounds, which indicates they could compete with thyroxine as well (Brouwer and Murk, personal communication, cited in Örn, 1997).

Short-term feeding studies with high doses of DeBDE in rats led to liver lesions and thyroid hyperplasia. Long-term exposure to DeBDE was also found to induce thyroid hyperplasia, hepatocellular and thyroid adenomas and carcinomas in mice (Great and Great). Workers producing DeBDE and decabromobiphenyl had a statistically significant increase in hypothyroidism (Bahn et al., 1980).

Immunotoxicity was studied after oral treatment with Bromkal 70-5DE or BDE-47 in rats and mice (Darnerud and Thuvander, 1998). In mice, BDE-47 caused reduced splenocyte number as reflected in decreased numbers of CD45R+, CD4+ and CD8+ cells in spleens. In mice treated with Bromkal 70-5DE, absolute numbers of double negative thymocytes were significantly lower than in controls and mice also showed reduced production of IgG. No effects were seen in rats. Thus, BDE-47 and Bromkal 70-5DE, which contains BDE-47, both seem to be immunotoxic in mice.

Studies have shown that there is a critical phase in neonatal mouse brain development when the brain is particularly susceptible to effects of low-dose exposure to toxic substances such as PCB, DDT, pyrethroids, organophosphates, paraquat and nicotine (Eriksson, 1997). This critical phase is known as the "brain growth spurt" (BGS) and disruption leads to persistent disruption in adult brain function. The BGS occurs at different time points in different mammalian species (Davison and Dobbing, 1968). In rats and mice it occurs in the first 3-4 weeks of life (neonatal period) whereas in humans it occurs during the third trimester of pregnancy and throughout the first 2 years.

To study possible neurotoxicity of brominated flame retardants, BDE-47, BDE-99 or TBBPA were administered orally to neonatal mice on day 10 (Eriksson et al., 1998). Doses administered are given in Table 6. Several tests of behavior, locomotion, activity and memory were performed with the treated individuals several months later. Results showed that BDE-47 and -99 both induced permanent aberrations in spontaneous motor behavior which worsened with age (Table 6). Similar effects have been seen in mice exposed neonatally to some *ortho*-substituted PCBs and co-planar PCBs in doses on the same molar basis (Eriksson; Eriksson; Eriksson and Eriksson). Neonatal exposure to BDE-99 also affected learning and memory functions in the adult animal. No effects were seen for TBBPA.

Table 6. Neurotoxicological effects seen in mice several months after administration of a single dose of specific PBDE congeners and TBBPA on day 10 after birth


(<1K)

Results from Eriksson et al., 1998. + indicates permanent aberrations; - indicates no effects.

In a follow-up study, Eriksson et al. (1999) investigated whether there is a critical time in neonatal mouse brain development for induction of the neurotoxic effects of BDE-99. One single oral dose of 8 mg/kg body weight (14 μmol/kg body weight) was administered to 3-day-, 10-day- and 19-day-old mice. Spontaneous behavioral tests were performed after 4 months. The mice exposed to BDE-99 on day 10 showed significant behavior aberration, as was previously seen, and mice exposed on day 3 showed similar aberration but to a lesser degree. The mice exposed on day 19 showed no significant change from the controls.

Uptake and retention of BDE-99 in the brain was also studied by administering ^{14}C-labelled BDE-99 to 3-day-, 10-day- and 19-day-old mice (Eriksson et al., 1999). The amounts of radioactivity found in the brain were measured at 24 h and 7 days after administration. The retention of BDE-99 was similar to what has been observed after neonatal exposure to CB-52, CB-153 and DDT (Eriksson, 1997). The retention of BDE-99 in mice exposed on day 3 indicates that the effects seen may be due to the amount still present in the brain on day 10. The neurotoxic effects seem to involve changes in the cholinergic system as mice given BDE-99 on day 10 and then challenged as adults with a low dose of nicotine behave completely the opposite of controls. From these studies, it was concluded that the window for permanent effects of BDE-99 and BDE-47 is day 10 in neonatal mice (Eriksson and Eriksson).

Female rats given BDE-47 orally for a period of two weeks were then killed and the choroid plexus of the brain removed, homogenized and incubated with ^{125}I-T_4 (Sinjari et al., 1998). Compared to controls, there was a dose-dependent reduction in the binding of ^{125}I-T_4 to the choroid plexus. In contrast, in vitro incubation of rat choroid plexus with BDE-47 revealed no competitive inhibition of labelled T_4 binding. This indicates that BDE-47 metabolites can cross the blood-brain barrier and bind to the choroid plexus T_4-binding sites. This in turn could cause the interference of T_4 transport to the brain, with risks for effects on neural development.

4.2.1.3. In fish

Microinjection of Bromkal 70-5DE in rainbow trout larvae led to weakly induced EROD activity (Norrgren et al., 1993). Three-spined stickleback fed Bromkal 70-5DE showed weakly induced liver EROD activity, fatty livers and a reduction in spawning success (Holm et al., 1993). Microinjection of BDE-47, BDE-85 or BDE-99 into newly fertilized rainbow trout eggs in an early life stage mortality bioassay showed no effects compared to 2,3,7,8-tetrachlorinated dibenzo-*p*-dioxin (Hornung et al., 1996). However, 3,3',4,4'-tetrabrominated biphenyl and 3,3',4,4',5,5'-hexabrominated biphenyl were 10-fold more potent than the identically chlorinated biphenyls.

Rainbow trout were fed food containing either BDE-47 or BDE-99 for 6 and 22 days to study biological effects. Both congeners were found to significantly inhibit EROD activity in the liver with BDE-47 being most powerful in this effect (Tjärnlund et al., 1998). A 35% reduction of GSH reductase was also observed after 22 days. Hematocrit and blood glucose also showed small but statistically significant changes after 6 days. No differences were seen in condition factor, liver somatic index, spleen somatic index, numbers of leukocytes, thrombocytes, granulocytes, lymphocytes or hemoglobin levels. When injected into fertilized fish eggs, no effects were seen on thiamine use.

4.2.2. TBBPA

4.2.2.1. In vitro

Studies of the potency of TBBPA for its competitive binding to human transthyretin in vitro have shown that TBBPA has the highest potency of all brominated and chlorinated substances tested so far. It is up to 10 times more potent than T_4 (Meerts et al., 2000). However, this effect was not seen in in vivo studies in pregnant rats, see below (Meerts et al., 1999).

TBBPA was tested for its activity in inducing intragenic recombination in two in vitro assays using mammalian cells but caused no effect (Helleday et al., 1999).

4.2.2.2. In mammals

TBBPA fed orally to mice and rats showed low or no effects on behavior, weight gain, mortality, organ abnormalities or hematology (WHO/ICPS, 1995).

The thyroid transport protein, transthyretin, may play a major role in delivering T_4 from the mother to the fetus across the placental barrier as well as across the blood-brain barrier, where it is converted to T_3, an essential hormone for normal brain development (Calvo and Southwell). TBBPA has been shown to competitively bind to human transthyretin in vitro with high affinity. To study the effects of TBBPA on thyroid hormone transport, including across the blood-brain barrier, pregnant rats were given ^{14}C-labelled TBBPA on days 10-16 of gestation (Meerts et al., 1999). Blood plasma thyroid hormone and thyroid stimulating hormone levels were measured as well as ^{125}I-T_4-competition binding on maternal and fetal plasma transthyretin. No effects were seen on total T_4, free T_4 or total T_3 levels in dams or fetuses. Thyroid stimulating hormone increased significantly in fetal plasma by 196% but no effect was seen in dams. No ^{14}C label was detected on transthyretin and there was no

shift in binding of ^{125}I-T_4, which would be expected if TBBPA had bound to transthyretin. Therefore, TBBPA was concluded to not bind to transthyretin in vivo. No selective accumulation of TBBPA-related activity was found in the fetal brain.

4.2.2.3. In fish

Bluegill sunfish exposed to TBBPA in water became irritated and exhibited abnormal swimming behavior. Rainbow trout exhibited irritation, twitching, erratic swimming, dark discoloration and labored respiration. Fathead minnow showed reduced survival of young at hatch and reduced survival and growth after 30 days (WHO/ICPS, 1995). There is no information regarding uptake, metabolism or effects in vitro, in mammals or in fish of the methylated derivative of TBBPA (MeTA).

4.2.2.4. In birds

In a study of possible estrogenic activity in quail and chicken embryos, TBBPA was injected at doses of 15 and 45 μg/g egg (Berg, 2000). No estrogenic effects were seen; however the high dose was found to be embryolethal.

4.2.3. HBCD

4.2.3.1. In vitro

The effects of HBCD on intragenic recombination were studied in two in vitro assays using mammalian cells (Helleday et al., 1999). HBCD caused statistically significant increases in recombination frequency in both test systems, indicating that it may induce cancer via a non-mutagenic mechanism, similarly to other environmental contaminants such as DDT and PCB.

4.2.3.2. In mammals

HBCD is not acutely toxic to rats or mice when given orally or in rats when inhaled. No acute dermal toxicity is seen in treated rabbits. Chronic exposure in rats leads to increased liver weights and, in one study, thyroid hyperplasia. Chronic exposure in female rats led to inhibited oogenesis (Zeller and Kirsch, 1969). In chronic oral studies in pregnant rats, HBCD was found to suppress maternal food consumption and increase maternal liver weight in the highest dose group (1% of diet), but no effects were seen on the offspring (IUCLID, 1996). The quality of most of these studies is, however, under question.

5. Environmental concentrations

5.1. Abiotic samples

5.1.1. Air

In 1979, DeBDE was identified in air particulates in the vicinity of plants manufacturing brominated flame retardants (Zweidinger et al., 1979). Watanabe and his coworkers found predominantly DeBDE in airborne dust from the Osaka region, in Japan (Watanabe et al., 1995). In samples from Taiwan and Japan in the vicinity of metal recycling plants, various tri-, tetra-, penta- and hexaBDEs were detected in air (Watanabe et al., 1992). Concentrations ranged from 23 to 53 pg/m^3 in Taiwan and

7.1 to 21 pg/m^3 in Japan.

Air samples collected from two sites in Sweden during 1990-1991, Ammarnäs in the northern mountains and Hoburgen on the southern tip of Gotland in the Baltic Sea, had quantifiable amounts of BDE-47, -99 and -100 and HBCD in both samples (Bergander and de). Total PBDE levels were approximately 1 and 8 pg/m^3 for Hoburgen and Ammarnäs, respectively. HBCD levels were 5.3 and 6.1 pg/m^3, respectively. Highest levels of BDE-47 were found on polyurethane foam plugs (gas phase) while higher levels of BDE-99 and -100 and HBCD were found on filters (particulate phase). No DeBDE was found, but the detection level for DeBDE was much higher than for the lower brominated PBDEs.

Air samples were collected at one rural site in southern England (Stoke Ferry) and one semirural site in northwestern England (Hazelrigg) during 1997 (spring, summer, autumn and winter) and analyzed for PBDEs (Peters and Peters). Detectable concentrations of tri- to heptaBDEs were found and the sum concentrations of BDE-47, -99 and -100 were 7-69 pg/m^3 at Hazelrigg and 6-58 pg/m^3 at Stoke Ferry (A. Kierkegaard, Stockholm University, personal communication). PBDE has also been measured in several archived air samples from the Arctic (Alert, NWT, Canada, and Dunai Island, eastern Siberia) taken between January 1994 and January 1995 (Alaee et al., 1999; M. Alaee, Department of Environment, NWRI, Canada, personal communication). The sum concentrations of several di- to hexaBDEs were 1-4 pg/m^3 at Alert most of the year, but 28 pg/m^3 in July 1994. Sum concentrations in air samples from Dunai were somewhat lower than at Alert, with the highest level also found in summer (7-8 pg/m^3). BDE-47 and -99 were the major congeners found.

Air samples collected in the US from urban, rural and remote shorelines of the Great Lakes all contained measurable amounts of BDE-47, -99, -100, -153 and -154 (Dodder et al., 2000). The predominant congeners were BDE-47 and -99. Highest concentrations were found near the city of Chicago and the total PBDE concentrations for all samples ranged from 6.9 to 77 pg/m^3.

Bergman et al. (1997) developed a sampling technique for sampling air particulates in the working environment. They determined a number of brominated flame retardants on air particulates in rooms containing computers and other electrical equipment. All particulate samples contained TBBPA, BDE-47 and BDE-99. The presence of these substances in air particulates shows that these substances are leaking into the indoor environment from electronic devices and therefore exposing humans.

In another study, air was sampled in an electronics dismantling plant, in an office with computers and outdoors (Bergman; Sj and Sj) and analyzed for several PBDEs as well as TBBPA. The highest concentrations of all substances were found in air from the electronics dismantling plant. Concentrations in the office were not detectable or 400-4000 times lower and not detectable concentrations were found for the outdoor air. Mean concentrations found in air at the dismantling plant were 2.5 pmol/m^3 (1250 pg/m^3) for BDE-47, 4.6 pmol/m^3 (2600 pg/m^3) for BDE-99, 6.1 pmol/m^3 for BDE-153, 26 pmol/m^3 for BDE-183, 38 pmol/m^3 (36 500 pg/m^3) for BDE-209 and 55 pmol/m^3 (29 900 pg/m^3) for TBBPA (Bergman and Sj). Samples were also taken near a plastics shredder at the dismantling plant to identify possible point sources (Sj and Sj). Concentrations of the PBDEs were found to be 4-10 times higher in the proximity of the shredder when compared to the air

samples at other sites in the dismantling plant.

5.1.2. Sewage sludge

Sewage sludge samples collected in 1988 from Ryaverket sewage treatment plant in Gothenburg, Sweden, were analyzed for PBDEs. Concentrations of BDE-47, -99 and -100 together were around 20-30 ng/g dry weight (Nylund et al., 1992) with BDE-47 and -99 present at similar levels. The proportions seen in sewage sludge are similar to those seen in the technical PeBDE product Bromkal 70-5DE. The levels were similar to those found in Germany by Hagenmaier et al. (1992), who found tri- to heptaBDEs in sewage sludge, with concentrations of Te- and PeBDEs (not designated which congeners) of 0.4-15 ng/g.

In 1988, a sewage sludge sample was collected from a treatment plant receiving leach water from a landfill where wastes from a plastics industry using TBBPA are placed (Klippan). For comparison, a second sewage sludge sample was collected from a treatment plant (Rimbo) having no known sources of TBBPA connected to it (Sellström and Jansson, 1995). The samples were analyzed for TBBPA and MeTA and results showed that MeTA was not detectable, but TBBPA levels were 56 and 31 ng/g dry weight. The samples were also analyzed for BDE-47, -99 and -100. The sum of these three PBDE congeners in the Klippan sample was 45 ng/g dry weight and for the Rimbo sample, 119 ng/g dry weight (Sellström, 1999).

Sewage sludge samples collected in 1997-1998 from sewage treatment plants in Stockholm, Sweden, were analyzed for TeBDE, PeBDE, DeBDE, HBCD and TBBPA (Sellstr and Sellstr). The results are given in Table 7. The concentrations of BDE-47, -99, -100 and -209 do not differ as much between plants as do those of TBBPA and HBCD.

Table 7. Mean concentrations of several PBDEs (n=4), HBCD (n=4) and TBBPA (n=2) in sewage sludge from three treatment plants in Stockholm in ng/g dry weight

 (<1K)

Effluent residues from several sewage treatment plants in the Netherlands contained median concentrations of BDE-47 of 22 ng/g dry weight and for BDE-209, 350 ng/g dry weight (de Boer et al., 2000b). BDE-99 and -153 concentrations were below the detection limits.

5.1.3. Sediment

Previous studies in Japan have found TeBDE, PeBDE, HxBDE and DeBDE in river sediments (Watanabe; Watanabe and Watanabe). Concentrations of TeBDE and PeBDE together were 21-59 ng/g dry weight. DeBDE was found in concentrations ranging from <25 to 11 600 ng/g dry weight (Environmental Agency Japan, 1991).

The upper layer of a sediment core collected in the southern Baltic Sea (Bornholm Deep) was analyzed for BDE-47, -99 and -100 and contained 0.52 ng/g dry weight (2.9 ng/g ignition loss) (sum of three congeners) (Nylund et al., 1992). In another study, 20 surficial sediment samples taken from

numerous sites in the Baltic Sea during 1993 were analyzed for BDE-47, -99 and -100 within a Helsinki Commission (HELCOM) sediment baseline study (Jonsson and Kankaanpää, 1999). Results showed low levels in all samples, from not detected to 5.4 ng/g ignition loss based on the sum of three congeners. BDE-47 and -99 dominated and were present in approximately equal concentrations.

In a study near a Swedish plastics industry using TBBPA, sediment samples were collected up- and downstream of the industry and these were analyzed for TBBPA and MeTA (Sellström and Jansson, 1995) as well as for BDE-47, -99 and -100 (Sellstr and Sellstr). TBBPA, MeTA and all three PBDE congeners were found in higher concentrations downstream of the plant than upstream (Table 8), indicating that the plastics industry was the most likely source for these substances.

Table 8. Concentrations (ng/g ignition loss) of TBBPA, MeTA and BDE-47, -99 and -100 in sediments taken upstream and downstream of a plastics industry (Sellström and Jansson, 1995)


(<1K)

Surficial sediment samples were collected in 1995 at eight sites along River Viskan, where numerous textile industries are located. These industries have used various brominated flame retardants in production of textiles. BDE-47, -99, -100 and -209 as well as HBCD were quantified in the sediments and the concentrations increased further downstream as more industries were passed (Sellström et al., 1998b). The concentrations of BDE-47, -99 and -100 together ranged from not detected to 120 ng/g ignition loss, BDE-209 ranged from not detected to 16 000 ng/g ignition loss and HBCD ranged from not detected to 7600 ng/g ignition loss. The lowest levels of the PBDEs and HBCD were found upstream of the industries. This is the first time HBCD has been found in environmental samples in Sweden.

A study of various contaminants in sediments collected from the mouths of major European rivers included several brominated flame retardants (Kierkegaard; van and Sellstr). Results are shown in Fig. 4. High levels of BDE-47 and -99 were found in two rivers in Great Britain (Humber and Mersey) and two in the Netherlands (sum of two congeners 1.61-13.1 ng/g dry weight). Highest 2,2',4,4',5,5'-HxBB levels were found in the Seine (France), three rivers in the Netherlands and the rivers Schelde (Belgium), Forth (Great Britain) and Ems (Germany) (range 0.013-0.056 ng/g dry weight). Levels of DeBB were highest in sediment from the Seine (2.4-3.9 ng/g dry weight). DeBDE (BDE-209) levels were highest in River Mersey (Great Britain), followed by the Schelde and River Liffey (Ireland) (range 34-1800 ng/g dry weight).


(13K)

Fig. 4. Mean concentrations (ng/g dry weight) of HxBB, DeBB, BDE-47, sum of BDE-99 and -100, and

BDE-209 in sediments from the mouths of several European rivers (Kierkegaard and Sellstr). SHW=Southampton, NZRTS100=North Sea (reference site), NWW=Rhine, WWZ=Wadden Sea, NZRNW2=Nordwijk, Netherlands, and NZBCS=North Sea, Belgium.

Allchin et al. (1999) carried out a survey of PBDE in sediments and fish (see Section 5.2.2.1) from several rivers and estuaries in the UK. Sediments were collected upstream and downstream of suspected sources including a manufacturer of PeBDE and OcBDE, several industries using PeBDE, several landfills receiving wastes suspected to contain PBDEs and a reference site.

The highest concentrations of BDE-47, -99, PeBDE (DE-71) and OcBDE (DE-79) were found in sediments around or downstream of the manufacturing site at Newton Aycliffe on River Skerne (Table 9). The highest concentrations of DeBDE (DE-83) were found downstream of a sewage treatment plant on River Calder, but high concentrations were also seen on River Skerne downstream of the manufacturing site. The general conclusions drawn from this study were that the PeBDE and OcBDE manufacturing plant at Newton Aycliffe is a major source of PBDE on River Tees/Skerne including the mouth of the river, which is 40 km downstream of the plant. Other sources are also implicated along other UK rivers although these could not always be identified. BDE-99 concentrations were similar or somewhat higher than those for BDE-47 in most sediments.

Table 9. Concentrations of BDE-47, -99, PeBDE quantified as the technical product DE-71, OcBDE quantified as the technical product DE-79 and DeBDE quantified as the technical product DE-83 in sediments from UK rivers (ng/g dry weight)

 (<1K)

Data from Allchin et al. (1999).

Sediment samples collected in 1999 from several sites in the Netherlands contained BDE-47, -99 and -209 (de Boer et al., 2000b). Concentration ranges were 0.3-7.1 ng/g dry weight for BDE-47, nd-5.5 ng/g dry weight for BDE-99 and nd-510 ng/g dry weight for BDE-209. Suspended particulate matter contained nd-9 ng/g dry weight BDE-47, nd-23 ng/g dry weight BDE-99 and nd-4600 ng/g dry weight BDE-209.

Sediment cores from a freshwater lake in Germany, the Wadden Sea and from Drammenfjord (Oslo Fjord) all contained measurable amounts of BDE-28, -47, -66, -99 and -100 (Zegers et al., 2000). The Drammenfjord and lake samples also contained BDE-153 and -154, and the Wadden Sea and lake samples contained BDE-209.

5.2. Biological and human samples

5.2.1. Terrestrial ecosystem

5.2.1.1. Birds

Muscle samples from juvenile starlings (*Sturnus vulgaris*) (3-4 weeks old) collected from four

Swedish sites were found to contain sum levels of BDE-47, -99 and -100 of 5.7-13 ng/g lipid (Sellstr and Sellstr). The congener pattern was similar to that of Bromkal 70-5DE. No geographical trends were apparent.

Chickens fed feed with or without dioxin-contaminated ball clay and chickens bought at a grocery store as a matrix blank were analyzed for PBDEs (Huwe et al., 2000). The congener profile was found to differ from that seen in fish and fish-eating animals; with BDE-99 being the dominant congener in all samples. The total PBDE concentrations (BDE-47, -99, -100, -153, -154, -183) in the store-bought chicken were 0.50 ng/g lipid weight, while in the ball clay exposed/unexposed chickens the concentrations ranged from 3.6 to 35.1 ng/g lipid weight. PBDE levels in the ball clay were at background levels. BDE-183 was detected in all samples but at low levels.

5.2.1.2. Mammals

PBDE levels (sum of BDE-47, -99 and -100) were determined in rabbit (*Oryctolagus cuniculus*) muscle, moose (*Alces alces*) muscle and reindeer (*Rangifer tarandus*) suet samples collected within the Swedish Environmental Monitoring Program. PBDEs were not detected in rabbit, and levels in moose and reindeer were low: 1.7 and 0.47 ng/g lipid weight, respectively (Jansson; Sellstr and Sellstr). Again, the congener pattern was similar to that of Bromkal 70-5DE. These levels are somewhat lower than those found in cow's milk from Germany (four samples), which ranged from 2.5 to 4.5 ng/g fat measured as Bromkal 70-5DE (Krüger, 1988).

5.2.1.3. Humans

Adipose tissue: Previously, DeBDE, as well as hexa-nonaBDE have been found in human adipose tissue samples from the US (Cramer and Stanley). The levels ranged from not detected to 1 ng/g fat for HxBDE, 0.001-2 ng/g fat for HpBDE, and not detected to 8 ng/g fat for OcBDE. NoBDE levels were estimated to exceed 1 ng/g fat and DeBDE levels were estimated to range between not detected and 0.7 ng/g fat. BDE-47 concentration in the adipose tissue of a 74-year-old Swedish male was found to be 8.8 ng/g lipid weight (Haglund et al., 1997). Human adipose tissue samples from 77 individuals in Sweden collected between 1995 and 1997 were analyzed for BDE-47 (Lindström et al., 1998). The means ranged from 3.8 to 16 ng/g lipid.

Adipose and liver tissue from two Swedish males were analyzed for several PBDEs (BDE-28, -47, -85, -99, -100, -153 and -154) (Meironyté Guvenius and Norén, 1999). The congener patterns in the two tissue types for each individual were similar. BDE-47, -99 and -153 were the predominant congeners with adipose BDE-47 concentrations of 2-2.4 ng/g lipid weight, BDE-99 concentrations of 1.6 ng/g lipid weight, BDE-100 concentrations of 0.1 ng/g lipid weight and BDE-153 concentrations of 1-1.3 ng/g lipid weight. The sum of the seven PBDEs in adipose tissue was 5 ng/g lipid weight and for liver, 6 and 14 ng/g lipid weight.

Adipose tissue samples from 10 randomly selected individuals in Finland were analyzed for BDE-47, -99 and -153 (Strandman et al., 1999). Mean concentrations were 7.3 ng/g fat for BDE-47, 2.2 ng/g fat for BDE-99 and 2.3 ng/g fat for BDE-153.

Adipose tissue samples from 13 individuals (three women, 10 men) from Tarragona, Spain, had mean BDE-47 concentrations of 1.36 ng/g lipid weight, BDE-99 was 0.42 ng/g lipid weight and BDE-153

was 1.83 ng/g lipid weight (Meneses et al., 1999).

Breast adipose samples collected in the late 1990s in northern California contained measurable amounts of BDE-47, -99 and -153 (She et al., 2000). Mean concentrations were 18 ng/g lipid weight for BDE-47, 4.9 ng/g lipid weight for BDE-99 and 2.2 ng/g lipid weight for BDE-153.

Blood: A number of brominated substances have been determined in 40 human blood plasma samples from Sweden. All samples were found to contain both TBBPA and PBDEs (Klasson Wehler et al., 1997). TBBPA levels were in the low ng/g range on a lipid weight basis based on semiquantitative analyses. Six PBDE congeners (tetra-hexaBDEs) were identified and quantified: BDE-28, -47, -66, -99, -100 and -153. Highest concentrations were found for BDE-47 and -99 and these made up 70% of the total PBDE concentration in plasma. The mean concentrations of PBDEs were 2.1±1.4 ng/g lipid weight.

Archived whole-blood samples from the German environmental specimen bank from 1985, 1990, 1995 and 1999 were analyzed and found to contain BDE-28, -47, -66, -85, -99, -100, -153 and -154 (Schröter-Kermani et al., 2000). BDE-47 was the major congener found and mean concentrations were 3.9 ng/g lipid weight in 1999. The mean total PBDE concentration was 5.6 ng/g lipid weight for 1999. The total PBDE concentrations were significantly lower in female blood samples.

In a study of workers at a computer disassembly plant, workers in a computerized office and cleaners (control), BDE-47, -153, -154 and -183 (2,2',3,4,4',5',6-HpBDE) as well as BDE-209 (DeBDE) were found in blood plasma for all three groups (Sjödin et al., 1999a). The median concentrations (sum of five congeners) were highest in the computer disassembly plant workers (26 ng/g lipid weight or 37 pmol/g lipid weight), next highest in the office workers (4.1 ng/g lipid weight or 7.1 pmol/g lipid weight) and lowest in the cleaners (3.3 ng/g lipid weight or 5.4 pmol/g lipid weight). The congener pattern was similar in the cleaners and the office workers, with BDE-47 as the dominant congener. However, the computer disassembly plant workers had highest median levels of BDE-183 (11 pmol/g lipid weight), followed by BDE-153 (7 pmol/g lipid weight), BDE-47 (5.9 pmol/g lipid weight) and BDE-209 (5 pmol/g lipid weight).

Stored serum samples collected from 12 US blood donors in 1988 were analyzed for PBDEs and found to contain BDE-47, -153, -183 and -209 (Patterson et al., 2000). Concentrations were comparable to those found in Swedish cleaners (see above).

Blood serum samples from 19 full-time computer technicians were analyzed and the serum concentrations of BDE-153, -183 and -209 were found to be approximately five times higher than in the cleaners and office workers in the above study (Hagmar et al., 2000a). Median concentration (sum of five congeners) was 10.6 pmol/g lipid weight. Highest concentrations were seen for BDE-153. Two OcBDE congeners and one NoBDE congener were also detected. Positive correlations were seen between fish consumption and serum concentrations of BDE-47, -153 and -183 and between computer time and BDE-153 and -183. TBBPA was detected in serum from four technicians.

BDE-47 was also determined in blood serum from persons with high fish intake or no fish intake to study the influence of diet on concentrations (Bergman and Sj). The high fish intake group had median BDE-47 concentrations of 4.4 pmol/g lipid weight (2.1 ng/g lipid weight), whereas the no fish intake group had median concentrations of 0.83 pmol/g lipid weight (0.40 ng/g lipid weight).

Breast milk: PBDE levels in human breast milk have been determined in 25 German mothers (Krüger, 1988). The levels ranged from 0.6 to 11 ng/g fat. In a recent study, Nor and Nor have performed a temporal trend study of PBDE in pooled breast milk samples from Swedish mothers in Stockholm (see Section 6.2.5). The PBDE level (sum of eight congeners) was 4 ng/g lipid in the 1997 sample.

PBDE levels were studied in breast milk obtained from primiparous mothers (n=39; 22-36 years old) from Uppsala county, Sweden (Darnerud et al., 1998a). The individual PBDE levels found in the breast milk were sums of the five most frequently found PBDE congeners (BDE-47, -99, -100, -153 and -154). The women also answered a questionnaire focusing on the present pregnancy, including symptoms, dietary and other habits (including smoking and alcohol consumption). Regression analysis was used to describe a possible relationship between PBDE levels in milk and some selected parameters from the questionnaire answers.

The observed mean value of the sum of eight PBDE congeners ($\sum$PBDE) was 4.4 ng/g fat whereas the median was 3.4 ng/g fat, which in part could be a consequence of a single, high peak value of 28.2 ng/g in the breast milk from one of the women. BDE-47 was the major congener in the breast milk, comprising ca 55% of $\sum$PBDE. Significant relationships were found between milk fat levels of $\sum$PBDE and smoking (p=0.001), and between milk fat levels of $\sum$PBDE and body mass index (p=0.014). However, the present study found no correlations between PBDE levels and the mother's age, computer usage frequency, consumption of fish (total or specifically fatty Baltic Sea fish), consumption of alcohol, place of residence during the mother's own childhood and adolescence (in a fishing village or not), or the birth weight of the child. However, the number of observations in this study may have been too few to reveal significant changes regarding these correlations.

New data from several other countries are now available. For Canada, the mean concentration for the sum of six congeners (BDE-28, -47, -99, -100, -153, -183) was 5.8 ng/g lipid weight for samples from Ontario and Quebec from 1992 (Ryan and Patry, 2000). Composite samples from 1992 representing four regions of Canada and one representing all Canadian provinces had sum concentrations ranging from 2.6 to 19 ng/g lipid weight, with the highest concentrations in the Maritimes region. Breast milk samples from Finland had sum concentrations (BDE-28, -47, -99, -153) ranging from 0.88 to 5.9 ng/g lipid weight (Strandman et al., 2000). In Japan, breast milk samples were found to contain sum concentrations (BDE-28, -47, -99, -100, -153, -154) ranging from 0.66 to 1.5 ng/g lipid weight (Ohta et al., 2000). Somewhat higher concentrations were seen in women with high consumption of fish (1.34 ng/g lipid weight) compared to those eating less fish (0.71 ng/g lipid weight). BDE-47 was the major congener in most of these samples but for Japan, BDE-153 levels were comparable in some samples.

5.2.2. Freshwater ecosystem

5.2.2.1. Fish and shellfish

Freshwater mussels (*Dreissena polymorpha*) were hung in nets at several locations in the Netherlands and then analyzed for BDE-47, -99, -153 and -209 (de Boer et al., 2000b). BDE-209 was below detection limits. Concentration ranges for the other congeners were 0.7-17 ng/g dry weight for BDE-47, 0.4-11 ng/g dry weight for BDE-99 and <0.1-1.5 ng/g dry weight for BDE-153.

Levels of BDE-47, -99 and -100 were determined in whitefish (*Coregonus* spp.) from Lake Storvindeln (pristine mountain lake in northern Sweden), Arctic char (*Salvelinus alpinus*) from Lake Vättern (heavily populated lake in south-central Sweden with numerous municipal and industrial point sources) and in trout (*Salmo trutta*) and pike (*Esox lucius*) from several sites along Dalslands Canal in west central Sweden (Jansson; Sellstr and Sellstr). Samples were collected between 1986 and 1988 and none of these sites have known point sources for PBDE. The whitefish sample contained the lowest levels, 26 ng/g lipid weight, whereas the Arctic char sample contained 520 ng/g lipid weight. BDE-47 was the predominant congener in both samples. The PBDE levels in pike on Dalslands Canal ranged from 180 to 210 ng/g lipid and in trout the range was 280-1200 ng/g lipid weight. The congener pattern was more similar to that of Bromkal 70-5DE, with similar amounts of both BDE-47 and -99. The levels are of the same order of magnitude as in the Arctic char indicating spread by diffuse sources. Levels of PBDE (sum of BDE-47, -99 and -100) in pike from Lake Bolmen (see Section 6.2.2) for the years 1987-1988 were 85-170 ng/g lipid weight, comparable to those from Dalslands Canal, and the congener pattern was similar as well (Kierkegaard et al., 1993).

In 1979 and 1980, high levels of TrBDEs-HxBDEs (950-27 000 ng/g lipid) were found in fish sampled along the Swedish river Viskan, where numerous textile industries are located (Andersson and Blomkvist, 1981). BDE-47 dominated the congener pattern (70-80% of total PBDE). These industries have used various brominated flame retardants in the production of textiles. No PBDEs were found in fish caught at the same sites in 1977. The high levels of BDE-47, -99 and -100 found were later confirmed in a study where fish caught from approximately the same locations as sampled in 1987 were analyzed (Sellstr and Sellstr). BDE-47 was the predominant congener (65-96% of total PBDE). Several different fish species were collected (pike, perch, bream, eel, tench, sea trout) in these studies. The large differences in PBDE concentrations that were found made it impossible to rule out species-specific differences in accumulation, thus making it difficult to draw conclusions about the location of the sources along the river.

New samples of pike as well as sediments (see Section 5.1.3) were collected along River Viskan in 1995 in order to search for sources. Pike samples were obtained at only four of the eight sites. BDE-47, -99, -100 and -209 (DeBDE) as well as HBCD were quantified in the fish. BDE-209 was found in trace amounts in a few fish. The concentrations of the other substances increased further downstream as more industries were passed (Sellström et al., 1998b). The concentrations of BDE-47, -99 and -100 together ranged from not detected to 4600 ng/g lipid weight, with BDE-47 again being the predominant congener (50-90%), and HBCD ranged from not detected to 8000 ng/g lipid weight. The lowest levels of the PBDEs and HBCD were found upstream of the industries. This is the first time HBCD has been found in environmental samples in Sweden.

In eels (*Anguilla anguilla*) from Dutch rivers and lakes (10 locations), levels of BDE-47 ranged from <20 to 1700 ng/g lipid and BDE-47 comprised 70% of the total PBDE (de Boer, 1990). Several species of freshwater fish from waters of North Rhine-Westphalia contained 18-983 ng PBDE/g lipid (Krüger, 1988).

In a more recent study, bream (*Abramis brama*) collected from several sites in the Netherlands had BDE-47 concentrations of 0.2-130 ng/g dry weight (de Boer et al., 2000b). The BDE-153 concentration range was <0.04-4.1 ng/g dry weight. BDE-99 was below detection limits.

Allchin et al. (1999) carried out a survey of PBDE in sediments (see Section 5.1.3) from several UK rivers and estuaries and fish from the estuaries. Plaice (*Pleuronectes platessa*), flounder (*Platichthys flesus*) and dab (*Limanda limanda*) were collected in the estuaries of rivers with suspected sources including a manufacturer of PeBDE and OcBDE, several industries using PeBDE, several landfills receiving wastes suspected to contain PBDEs and a reference site. The results are given in Table 10, and these support the conclusions drawn previously for sediments - that a major source is the manufacturing plant on River Tees. The predominant congener in fish is BDE-47, particularly where sediments are highly contaminated. This is similar to the situation found in Sweden along River Viskan. It is also interesting to note that OcBDE, quantified as the technical product DE-79, is bioavailable and found in relatively high concentrations in fish exposed via sediments.

Table 10. Concentrations of BDE-47, -100, PeBDE quantified as the technical product DE-71 and OcBDE quantified as the technical product DE-79 in fish from UK river estuaries (ng/g lipid weight)

 (<1K)

No DeBDE quantified as the technical product DE-83 was detected in any sample. Data from Allchin et al. (1999).

Loganathan et al. (1995) found TeBDE to HxBDE in carp (*Cyprinus carpio*) from Buffalo River, NY, an area around the Great Lakes showing environmental impairment. TeBDE dominated the congener pattern (94-96% of total PBDE) and TeBDE and PeBDE levels were 13-22 ng/g fresh weight. Asplund et al. (1999b) found tri- to hexaBDEs in steelhead trout (*Oncorhynchus mykiss*) from Lake Michigan sampled in 1995. The sum of BDE-47, -99 and -100 was 2700 ng/g lipid weight. Lake trout (*Salvelinus namaycush*) from several Great Lakes were also found to have di- to heptaBDEs with sum concentrations of 540 ng/g lipid weight for Lake Ontario, 240 ng/g lipid weight for Lake Huron and 140 ng/g lipid weight for Lake Superior (Alaee et al., 1999). Lake trout sampled from Lake Erie had 117 ng/g lipid weight (Luross et al., 2000). Differences were seen in congener profiles for the different lakes which may be due to variations in local sources combined with atmospheric transport.

In a recent study in Virginia, US, muscle samples from 253 fish samples representing 50 freshwater sites were collected and analyzed for PBDEs (Hale et al., 2000). Approximately 85% of the samples contained measurable concentrations of BDE-47, the predominant congener, and concentrations were greater than 1000 ng/g lipid weight at nine of the 50 sites. The highest total concentrations (up to 57 000 ng/g lipid weight) were seen in carp downstream of textile and furniture facilities. BDE-47 concentrations were higher than PCB-153 concentrations in 58% of the samples analyzed.

Fish were collected from two US lakes, Hadley Lake, IN, near a potential PBDE point source, and Lake of the Ozarks, MO, with no known sources (Dodder et al., 2000). Mean total PBDE concentrations (BDE-47, -99, -100, -153, -154) were higher in crappie (*Poxomis annularis*) and bluegill (*Lepomis macrochiras*) from Hadley Lake (1500 and 1900 ng/g lipid weight, respectively) than from Lake of the Ozarks (340 and 390 ng/g lipid, respectively). BDE-47, -99, -153 and -154 were found in Hadley Lake fish at similar concentrations and were the predominant congeners. BDE-47 was the predominant congener in fish from Lake of the Ozarks.

5.2.2.2. Birds

Muscle samples from ospreys (*Pandion haliaetus*) found dead in various parts of Sweden were pooled and analyzed for PBDE (Jansson; Sellstr and Sellstr). Ospreys feed on freshwater fish. The PBDE concentration was 2100 ng/g lipid (sum of BDE-47, -99 and -100) with BDE-47 dominating the congener pattern (86%). These high levels may reflect biomagnification and/or fish consumption along their migratory routes to Africa.

5.2.3. Marine ecosystem

5.2.3.1. Fish and shellfish

Marine mussels (*Mytilus edulis*) were hung in nets at several locations in the Netherlands and then analyzed for BDE-47, -99, -153 and -209 (de Boer et al., 2000b). BDE-153 and -209 were below detection limits. Concentration ranges for the other congeners were 0.9-4.3 ng/g dry weight for BDE-47 and 0.3-1.6 ng/g dry weight for BDE-99.

Hepatopancreas samples from Dungeness crab from the several sites on the Strait of Georgia, BC, Canada, were analyzed for di- to heptaBDEs (Ikonomou et al., 1999). BDE-47 was the major congener found and the sums of BDE-47 and -99 were approximately 100-350 ng/g lipid weight.

The sum of BDE-47, -99 and -100 in fall-caught herring (*Clupea harengus*) muscle from five sites along the Swedish coast ranged from 17 to 62 ng/g lipid, with BDE-47 being the dominant congener (Sellstr and Sellstr). Similarly, BDE-47 levels in different age groups of Baltic herring ranged from 3.2 to 27 ng/g lipid with the sum of BDE-47, -99 and -100 ranging from 3.2 to 32 ng/g lipid (Haglund et al., 1997). Lowest levels were in 2-year-old herring and highest levels were in 5-year-old herring. Haglund et al. (1997) found a similar trend for methoxy-PBDE in their Baltic herring samples. Strandman et al. (1999) also found increasing concentrations of BDE-47, -99 and -153 with age in Baltic sprat (*Sprattus sprattus*, age 3-13 years) but not in herring. BDE-47 was the major congener found and concentrations ranged from 7.6 to 24 ng/g lipid weight for 1- to 3-year-old sprat, 17 to 140 ng/g lipid weight for 3- to 13-year-old sprat and 7.6 to 24 ng/g lipid weight in the herring. Whole-body composites of herring were found to have BDE-47, -99 and -100 concentrations of 6.2, 0.6 and 0.8 ng/g lipid and sprat had 4.3, 0.7 and 0.8 ng/g lipid (Burreau et al., 1999). The levels found in Baltic herring are similar to BDE-47 levels of 8.4-100 ng/g lipid found by de and de in herring from three regions in the North Sea.

BDE-47, -99 and -153 levels in Baltic salmon (*Salmo salar*) muscle were 167, 52 and 4.2 ng/g lipid, respectively (Haglund et al., 1997). Methoxy-PBDEs were also found. In whole-body composites, BDE-47, -99 and -100 levels were 47, 7.2 and 6.3 ng/g lipid (Burreau et al., 1999). In another study, muscle, ripe eggs and blood plasma from Baltic salmon were analyzed for a range of organohalogen compounds including BDE-47, -99 and -100 (Asplund et al., 1999a). The levels found are shown in Table 11. Several hydroxylated and methoxy-PBDEs were also found. Methoxy-PBDEs were found in all samples at similar concentrations to the PBDEs. Several hydroxylated PBDEs were found in blood samples at 20-30% of the methoxy-PBDE levels.

Table 11. Mean concentrations of PBDEs in tissues from Baltic salmon (ng/g lipid weight)

 (<1K)

Results from Asplund and Asplund.

Cod (*Gadus morhua*) liver collected from three regions of the North Sea had sum levels of BDE-47 and -99 of 1.9-360 ng/g lipid (de and de). BDE concentrations in flounder from several sites in the Netherlands were 0.6-20 ng/g dry weight for BDE-47 and <0.01-4.6 ng/g dry weight for BDE-99 (de Boer et al., 2000b). BDE-153 and -209 were not detected.

Watanabe et al. (1987) found PBDE in several marine fish and shellfish samples in Japan. TeBDE and PeBDE concentrations were between 0.1 and 17 ng/g fresh weight with TeBDE being the major component in the samples. A mussel sample from Osaka Bay was also found to contain DeBDE. Recently, market fish from Japan were analyzed for PBDEs. Results showed highest total PBDE concentrations (BDE-28, -47, -99, -100, -153, -154) in salmon, cultured yellowtail and wild yellowtail muscle (46, 44 and 30.5 ng/g lipid weight, respectively) and lowest concentrations in yellowfin tuna (1.9 ng/g lipid weight) (Ohta et al., 2000). BDE-47 was the predominant congener in all samples. In another study in Japan, several fish species were analyzed for 15 BDE congeners (Hori et al., 2000). BDE-47 was the predominant congener and concentration ranges were 0.06-2.1 ng/g fresh weight.

5.2.3.2. Birds

Previously, Di- and TrBDE have been identified in black skimmer (*Rynchops nigra*) tissues and eggs in the US but no quantitative analysis could be performed due to lack of standards (Stafford, 1983). PBDE levels were measured in white-tailed sea eagle collected from the Baltic Sea and found to contain 350 ng/g lipid weight (Jansson et al., 1987). Common guillemots (*Uria aalge*) collected in 1979-1981 from the Baltic and North Seas contained 370 and 80 ng PBDE/g lipid (Jansson et al., 1987). Brunnich's guillemot (*Uria lomvia*) from Svalbard in the Arctic contained 130 ng PBDE/g lipid (Jansson et al., 1987). More recent results of PBDE analyses in guillemot eggs from the Baltic Sea are given in Section 6.2.3.

Cormorants (*Phalacrocorax carbo*) shot under license along the coast of England, UK, had sum PBDE concentrations (BDE-47, -99, -100, -153, -154) of 300-6400 ng/g lipid weight in liver (Allchin et al., 2000). BDE-47 was the predominant congener, followed by BDE-100 and then BDE-99.

Glaucous gulls (*Larus hyperboreus*) from Bear Island, Norway, collected in 1999 were found to contain 290-634 ng/g lipid weight of BDE-47 and, in one case, 160 ng/g lipid weight BDE-99 (Burkow et al., 2001).

5.2.3.3. Mammals

Several species of seal from several different sites have been analyzed for PBDE. Female grey seals (*Halichoerus grypus*) from the Baltic Sea collected in 1979-1985 contained 730 ng PBDE/g lipid in their blubber (sum of BDE-47, -99 and -100) (Jansson; Sellstr and Sellstr). Male Baltic grey seals had

280 ng/g lipid weight of sum BDE-47, -99 and -100 and male ringed seals (*Pusa hispida*) had 320 ng/g lipid weight (Andersson and Wartanian, 1992). Blubber from Baltic grey and ringed seals collected between 1981 and 1988 were found to contain 419 ng PBDE/ g lipid and 350 ng PBDE/g lipid (sum of BDE-47, -99 and -100), respectively (Haglund et al., 1997). Methoxy-PBDEs were also present in both species.

Female ringed seals collected in 1981 from Svalbard in the Arctic contained 40-51 ng PBDE/g lipid (Jansson; Jansson; Sellstr and Sellstr). Ringed seal from the Canadian Arctic had mean PBDE concentrations (di- to hexaBDEs) of 25.8 ng/g lipid weight (females) and 50 ng/g lipid weight (males) (Alaee et al., 1999). Ringed seal collected in 1996 from Holman Island, NWT, Canada (Arctic) had total PBDE concentrations of 2.4-4.9 ng/g lipid for males and 1.2-3.4 ng/g lipid for females, and for males, the concentrations increased with age (Ikonomou et al., 2000). Just as for many organochlorines, the lower levels in females indicate transfer to young.

Harbor seal (*Phoca vitulina*) from the Baltic Sea contained 90 ng PBDE/g lipid as compared to harbor seal from the North Sea, which contained 10 ng PBDE/g lipid (Jansson and Sellstr). Andersson and Wartanian (1992) found 230 ng PBDE/g lipid in harbor seals from the Swedish west coast (Skagerrak). Recently, harbor seal from the San Francisco Bay area have been analyzed for BDE-47, -99 and -153 (She et al., 2000). Mean concentrations were 1124 ng/g lipid weight for BDE-47, 107 ng/g lipid weight for BDE-99 and 50 ng/g lipid weight for BDE-153.

Blubber samples from three bottlenose dolphins (*Tursiops truncatus*) collected during a mass mortality event on the south Atlantic US coast in 1987-1988 contained 180-220 ng PBDE/g lipid (Kuehl et al., 1991). Bottlenose dolphins from the Gulf of Mexico were found to contain up to 8000 ng PBDE/g lipid (Kuehl and Haebler, 1995). de and de have found PBDE and PBB in the blubber of three sperm whales (*Physeter macrocephalus*), one minke whale (*Balaenaoptera acutorostrata*) and one whitebeaked dolphin (*Lagenorhynchus albirostris*) found stranded on the Dutch coast in early 1998. Several harbor seals were also sampled. Sperm whales feed in deep water and the stranded whales' stomachs were empty, indicating that the exposure occurred in the deep Atlantic via the food web. The levels found are given in Table 12. Analyses included BDE-209 (DeBDE) but levels were below detection limits in all samples.

Table 12. Levels of PBDE in blubber (ng/g lipid weight) from several marine mammals collected along the coast of the Netherlands

(<1K)

Results from de Boer et al. (1998b).

Long-finned pilot whale (*Globicephala melas*) from the Faeroe Islands in the north Atlantic were analyzed for 19 PBDEs (Lindström et al., 1999). Highest concentrations were found in young males and females (3000-3160 ng/g lipid) compared to adult females (840-1050 ng/g lipid) and males (1610 ng/g lipid). In a second study of long-finned pilot whales, a similar trend was seen with young animals having PBDE concentrations of 740 ng/g lipid weight, adult females having 230 ng/g lipid and adult males having 540 ng/g lipid (van Bavel et al., 1999).

Harbor porpoises (*Phocaena phocaena*) from British Columbia, Canada (Ikonomou et al., 2000), and from the coasts of England and Wales (Law et al., 2000) have recently been studied for PBDEs. The total PBDE levels (tri-hepta congeners) in the British Columbia samples were 350-2300 ng/g lipid weight with BDE-47 being the predominant congener (range 50-1200 ng/g lipid weight) (Ikonomou et al., 2000). Along the coasts of England and Wales, concentrations of total PBDE (sum of 13 congeners) ranged from 440 to 7670 ng/g lipid weight, and BDE-47 concentrations ranged from 227 to 6790 ng/g lipid weight (Law et al., 2000). The highest concentrations were found in a male porpoise stranded at Tynemouth in northeast England.

Beluga (*Delphinapterus leuca*) from the Canadian Arctic had mean PBDE concentrations (di- to hexaBDEs) of 81.2 ng/g lipid weight (females) and 160 ng/g lipid weight (males) (Alaee et al., 1999). Beluga sampled in 1997 from southeast Baffin (Cumberland Sound) had total PBDE concentrations of 15 ng/g lipid weight, with a BDE-47 concentration of 10 ng/g lipid weight (Stern and Ikonomou, 2000). The sum of BDE-47 and -99 concentrations in a killer whale (*Orca orcinus*) from British Columbia, Canada, was 100 ng/g lipid weight (Ikonomou et al., 1999).

The congener profile in all marine mammals studied shows highest concentrations for BDE-47.

5.3. Bioaccumulation, biomagnification

Bioconcentration factors determined in laboratory studies from water to Baltic blue mussels were found to be 1 300 000 for BDE-47, 1 400 000 for BDE-99 and 220 000 for BDE-153 (Gustafsson et al., 1999). These were higher than for the tri-hexaCB congeners that were also tested. In a field study in the Netherlands, bioconcentration factors were determined using blue mussels collected from several sites along the coast and in the Schelde estuary (Booij et al., 2000). Water concentrations were determined using semipermeable membrane devices. Maximum bioconcentration factors were log BCF of 9.0 for BDE-99 and -100 and approximately log BCF of 7.4 for BDE-28, 8.4 for BDE-47 and 8.2 for BDE-153.

When sediment concentrations were compared to those in pike collected at several of the same sites along River Viskan (see 5.1.3 and 5.2.2.1), high fish-to-sediment ratios were seen for BDE-47 (6.6-19), -99 (17), -100 (4.6-36) and HBCD (0.6-15) indicating that these are highly bioavailable (Sellström et al., 1998b).

Bioaccumulation has been studied in zebrafish fed with dried chironimid larvae treated with BDE-28, -47, -66, -85, -99, -100, -138, -153 and -154 (Andersson et al., 1999). Highest accumulation was seen for BDE-47 followed by BDE-28. BDE-100, -153 and -154 accumulated to a lesser extent. BDE-99 did not accumulate, which is not in agreement with the results of Gustafsson et al. (1999) in blue mussels or with uptake efficiency data seen in pike by Burreau et al. (1997).

Concentrations of PBDE in herring and their predators grey seal and guillemot, all collected in the same area of the Baltic Sea, have been compared to estimate potential biomagnification (Sellström, 1996). The herring were caught in the autumn of the same year as guillemot egg collection (1987). The grey seal sample was a pooled sample from eight females found dead in the area during 1979-1985. In a recent study, herring, sprat and salmon were collected from the Baltic Proper in 1998 (Burreau et al., 1999). These were analyzed for PBDEs (BDE-17 + 25, -28, -35, -47, -49, -66, -99, -

100 and -154) and nitrogen isotopes to study biomagnification. Salmon feed primarily on sprat and the lipid weight concentrations in the two species can be compared. In a complementary study, sprat, herring and salmon from the Baltic Sea and zooplankton, small herring, large herring and salmon from the Atlantic Sea (Iceland) were analyzed for several PBDEs (Burreau et al., 2000b). The calculated biomagnification factors for these studies are given in Table 13.

Table 13. Biomagnification factors of PBDE congeners in the the guillemot and grey seal (ng/g lipid weight) compared to herring (ng/g lipid weight) from the same area of the Baltic Sea (Sellström, 1996), for Baltic salmon compared to sprat (Burreau and Burreau), and Atlantic salmon compared to small herring (Burreau et al., 2000b)

 (<1K)

BDE-47 appears to biomagnify to the largest extent, which is in agreement with the results of uptake studies in fish showing highest assimilation efficiencies for BDE-47 from the gut compared to BDE-99 and -153 (Burreau et al., 1997). Burreau et al. (1999) also calculated *b* values (biomagnification potential) for all BDE congeners studied and found that these were all positive, meaning that all the studied congeners biomagnify. However, there were differences, with tetra- and pentaBDEs biomagnifying to a similar degree, the triBDEs biomagnifying somewhat less and the hexaBDE biomagnifying considerably less.

Osprey have higher concentrations than most of the freshwater fish analyzed in Swedish studies but no firm conclusions about biomagnification can be drawn due to their migratory habits.

(For an overview of this section see Table 14.)

Table 14. An overview of concentrations of several PBDEs, HBCD and TBBPA in environmental samples: air in pg/m^3; sewage sludge in ng/g dry weight; sediments in ng/g ignition loss; biota in ng/g lipid weight (unless otherwise stated)

 (<1K)

nd - not detected; dw - dry weight; fw - fresh weight.

6. Trends

6.1. Spatial trends

Results of PBDE analyses in surficial sediments from the Baltic Sea and the mouths of major European rivers indicates a gradient, with highest concentrations in southern Europe and lower levels in Scandinavia and the Baltic Sea (Kierkegaardand Sellstr). Results of PBDE analyses in freshwater fish in Sweden indicate that southern Sweden is more contaminated with PBDE than northern Sweden (Sellström, 1996). de and de found a clear spatial trend for PBDE in cod liver in the North Sea, with decreasing levels from south (22-360 ng/g lipid) to north (1.9-68 ng/g lipid). This was

attributed to major inputs from rivers in western Europe. A similar trend was seen for herring although the trend was not as clear due to their migration patterns (de Boer, 1990).

Fall-caught herring collected at five sites along the Swedish coast show that the lowest concentrations are found on the west coast (17 ng/g lipid) and the highest are found in the southern part of the Baltic Sea (62 ng/g lipid). The concentrations in the Baltic Sea then decrease from south to north up to the Bothnian Bay (30 ng/g lipid) (Sellstr and Sellstr). This spatial trend is almost identical to that found previously for PCB and DDT (NV, 1988).

A study of common guillemots collected in 1979-1981 from the Baltic and North Seas and Brunnich's guillemot from Svalbard in the Arctic indicated higher PBDE levels in the Baltic Sea (370 ng/g lipid) compared to the Arctic (130 ng/g lipid) (Jansson et al., 1987). Cormorant liver from the Rhine River delta have very high levels (28 000 ng/g fresh weight) (de Boer, 1990). Similarly, harbor seal from the Baltic Sea had higher levels (90 ng/g lipid) than than those in the northern North Sea (10 ng/g lipid) but highest levels were seen along the coast of the Netherlands (600-6000 ng/g lipid) (de Boer et al., 1998b). Low PBDE levels are generally seen in Arctic ringed seals (26-51 ng/g lipid) (Jansson and Alaee) compared to the Baltic Sea (320-350 ng/g lipid) (Andersson and Haglund).

Together, these results indicate that southern European coastal areas are most contaminated by PBDE, followed by the Baltic Sea. A gradient is also indicated in northern Europe with levels declining from south to north, with lowest levels in the Arctic. These results are similar to those seen for organochlorine contaminants such as PCB and DDT.

Data on spatial trends in North America are still sparse, but PBDE concentrations in harbor seal from northern California (She et al., 2000) and British Columbia are much higher than in ringed seal from Holman Island, NWT (Arctic) (Ikonomou et al., 2000) indicating similar north-south concentration gradients as seen in Europe.

6.2. Temporal trends

6.2.1. Dated sediment cores

At present, dated sediment cores are not available from different global locations to clearly establish the time period of first appearance of PBDEs in the different environments around the world. The best studied sediment core is a laminated sediment core collected from the southern part of the Baltic proper for analysis of a number of organochlorine contaminants as well as PBDEs (Nylund et al., 1992). The results provide a retrospective time trend from 1939 to 1987 (Fig. 5) and show that the PBDE levels (sum of BDE-47, -99 and -100) have increased, particularly after 1980. PBDE level in the sample from 1989 was 2.9 ng/g ignition loss.

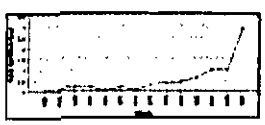
(8K)

Fig. 5. Concentrations of PBDE in different layers of a sediment core representing the years 1939-1987 (Nylund et al., 1992).

Analysis of sediment cores (dry weight basis) from Drammenfjord, Norway, the German freshwater lake Woserin and the Wadden Sea show that the lower brominated BDEs appear in the 1960s and BDE-209 appears about 10 years later (Zegers et al., 2000). Preliminary results from the core from Drammenfjord show increasing trends for BDE-47 from the 1940s and for BDE-99, -100, -153 and -154 from the 1950s up to 1999 (BDE-209 not yet analyzed). In the core from Lake Woserin, lower brominated BDE congeners appear in the late 1950s and increase until the late 1970s when they level off, just as BDE-209 first appears. The preliminary results from the Wadden Sea core also appear to show levelling off. Definite statements await final analysis of total organic carbon in these samples.

6.2.2. Fish

Pike are collected yearly from Lake Bolmen within the Swedish National Environmental Monitoring Program for contaminant analyses. Samples are also banked at the Environmental Specimens Bank at the Swedish Natural History Museum in Stockholm. A retrospective time trend for PBDEs was determined by analyzing BDE-47, -99 and -100 in pooled samples for most years between 1967 and 1990. For the years 1974, 1981, 1987 and 1991-1996, 9-11 individual muscle samples were analyzed for each year. The results show significantly increasing trends for the three congeners from 1967 to the early 1980s. The trend for BDE-47 is shown in Fig. 6. From 1982 to 1996, there are large between-year variations; however, there seems to be a tendency towards a fairly even trend level with no indication of decreasing levels (Kierkegaard and Kierkegaard). The predominant congener is BDE-47. The PBDE level (sum of BDE-47, -99 and -100) was 100 ng/g lipid weight for 1996. Based on the established time trend, yearly analysis of PBDEs in pike from Lake Bolmen is now included within the National Environmental Monitoring Program.

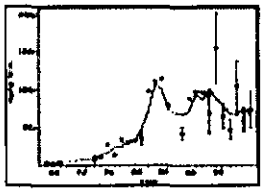
(5K)

Fig. 6. Concentrations of BDE-47 in Lake Bolmen pike (Kierkegaard et al., 1999b). The line represents a three-point running mean smoother ($p<0.001$). Circles represent arithmetic means with bars indicating 95% confidence intervals.

Methoxy-BDE-47 was also analyzed in the pike samples and the temporal trend for these is shown in Fig. 7. The concentrations show a significantly decreasing trend. The origin of this is not known. Biogenic sources from primary producers could be one explanation; however, from 1966 to 1997, this lake has had an increasing eutrophication trend (Kierkegaard et al., 1999b).

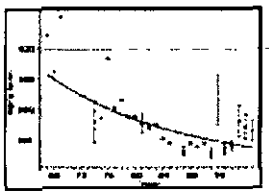
(4K)

Fig. 7. Concentrations of methoxy-BDE-47 in pike from Lake Bolmen (Kierkegaard et al., 1999b). The curve represents a log-linear regression line ($p<0.001$). Circles represent arithmetic means and bars indicate 95% confidence intervals.

A retrospective temporal trend study was performed using roach (*Rutilus rutilus*), which are collected yearly from Lake Krankesjön, a eutrophied lake, within the Swedish National Environmental Monitoring Program for contaminant analyses. Samples are also banked at the Environmental Specimens Bank at the Swedish Natural History Museum in Stockholm. A retrospective time trend for PBDEs was determined by analyzing BDE-47, -99 and -100 in individual samples for several years (1980, 1983, 1985, 1988, 1990, 1992, 1994 and 1996) (Kierkegaard et al., 1999b). The concentrations in roach were generally lower than in pike and there was considerable between-year variation as well as variation within years. No significant trend was detected. No methoxy-BDE-47 was detected.

A retrospective temporal trend study was performed using archived lake trout samples from Lake Ontario for the years 1978, 1983, 1988, 1993 and 1998 (Luross et al., 2000). The results show a dramatic increase in total PBDE concentrations over time (Fig. 8).

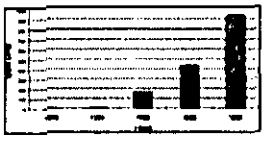
(10K)

Fig. 8. Mean concentrations of total PBDE in lake trout from Lake Ontario (Luross et al., 2000).

6.2.3. Guillemot eggs

Samples of guillemot eggs (St. Karlsö, Baltic Sea) are collected yearly within the Swedish National Environmental Monitoring Program. Samples are also banked at the Environmental Specimens Bank at the Swedish Natural History Museum in Stockholm. A retrospective time trend for PBDEs was determined by analyzing BDE-47, -99 and -100 in pooled egg samples from the specimen bank for most years between 1969 and 1990 (Sellstr; Sellstr and Sellstr). Samples from 10 individuals were analyzed for the years 1975, 1989 and 1992-1997. The results show significantly increasing trends for these three congeners from 1969 to the beginning of the 1990s. The results for BDE-47 and -99 are shown in Fig. 9. There is large between-year variation after 1990, but statistical analysis indicates that PBDE levels have declined for the period 1992-1997. The predominant congener is BDE-47 and the PBDE level (sum of BDE-47, -99 and -100) in 1997 was 190 ng/g lipid. Based on the established time trend, yearly analysis of PBDEs in guillemot eggs from St. Karlsö is now included within the National Environmental Monitoring Program.

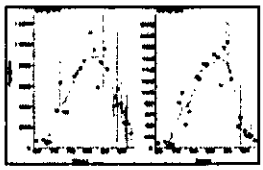
(6K)

Fig. 9. Concentrations of BDE-47 and -99 in guillemot eggs collected from St. Karlsö (Sellstr; Sellstr and Sellstr). Circles represent the arithmetic means and bars indicate 95% confidence intervals. The line represents a five-point running mean smoother (p<0.001).

A temporal trend for HBCD has also been studied in the same guillemot samples as were analyzed for PBDEs (Kierkegaard et al., 1999b). The trend shows an increasing tendency from 1969 to 1997.

6.2.4. Marine mammals

Archived samples of blubber from ringed seals from Holman Island, NWT, Canada, collected in 1981, 1991 and 1996 were analyzed in a retrospective temporal trend study (Ikonomou et al., 2000). The age-adjusted means for total PBDE concentrations increased from 1981 to 1996, from approximately 0.3 ng/g lipid weight in 1981 to 3.6 ng/g lipid weight in 1996.

A retrospective study of beluga from southeast Baffin, Canada, was performed using archived blubber samples from 1982, 1986, 1992 and 1997 (Stern and Ikonomou, 2000). Total PBDE concentrations increased significantly from 1982 to 1997 (Fig. 10). Increases for this time period were 6.5 times for BDE-47, 10.3 times for BDE-99, 7.9 times for BDE-100, 30.6 times for BDE-154 and 6.8 times for total PBDE.


(9K)

Fig. 10. Concentrations of total PBDE in beluga from southeast Baffin, Canada (Stern and Ikonomou, 2000). Figures represent age-adjusted least square means.

6.2.5. Human samples

Pooled human milk samples collected during the period 1972-1997 from native Swedish mothers living in the Stockholm region were analyzed for eight PBDE congeners (Fig. 11). All eight PBDEs were present in most samples and the predominant congener was BDE-47 (Meironyt and Meironyt).


(13K)

Fig. 11. Brominated diphenyl ethers in pooled samples of human milk, sampled 1972-1997 (Meironyté et al., 1998).

The results show an exponential increase of PBDEs in human breast milk from 1972 to 1997 with a doubling rate of 5 years (Nor and Nor) (Fig. 12). The PBDE level (sum of eight congeners) was 4 ng/g lipid in the 1997 sample. This time trend differs considerably from those of pike and guillemot collected in Sweden, which have levelled off or may be decreasing. This may indicate that the current exposure in humans may not be from just diet. Possible other routes of exposure could be the presence of brominated flame retardants in the work and home environment.

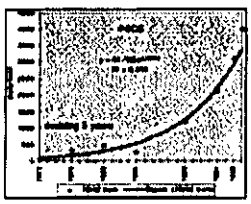
(19K)

Fig. 12. Concentrations of PBDE (sum of eight congeners) in pooled human breast milk samples from different time periods (Nor and Nor).

A retrospective study of archived human blood samples analyzed for the years 1985, 1990, 1995 and 1999 in Germany showed an increasing temporal trend (Schröter-Kermani et al., 2000). Total median PBDE concentrations increased from 3.1 ng/g lipid weight in 1981 to 4.7 ng/g lipid weight in 1999 (Fig. 13).

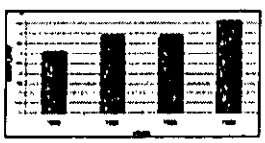
(15K)

Fig. 13. Median PBDE concentrations in blood samples from Germany (Schröter-Kermani et al., 2000).

7. Summary and conclusions

At present, inventories of environmental sources of PBDEs, HBCD and TBBPA have not been completed in Europe or North America. Sources are widely believed to include leaching of these chemicals from a wide range of plastics, electronic equipment and textiles. In the US and the UK,

sources also include industrial facilities that produce BFRs as well as consumer manufacturing facilities that use BFRs in a wide range of consumer products. An important research need is to better identify sources and to quantify emissions and sinks. The presence of PBDE in samples from living organisms in the Arctic and the presence of PBDE in air samples from Sweden, England, the US and Arctic Canada indicate long-range transport of PBDEs in air. The presence of BDE-47, -99 and -209, TBBPA and HBCD in sewage sludge indicates discharges to municipal sewage treatment systems, either from households, traffic and/or diffuse releases to the environment. Lower brominated PBDE have been found at low concentrations in sediments from the Baltic Sea but, together with DeBDE, in higher concentrations at the mouths of some European rivers, in several UK rivers and in the Swedish River Viskan.

Levels of BDE-47, -99 and -100 are low in mammals and birds at low trophic levels in the terrestrial ecosystem. Higher concentrations of these PBDE congeners have been found in biota samples (fish, birds, mammals) in aquatic and marine ecosystems. A spatial trend is apparent with highest levels in biota from the Netherlands coast followed by the Baltic Sea, with lower levels in the North Sea and lowest levels in northern Sweden and the Arctic. The spatial trend is very similar to that found for PCB and DDT. A similar spatial trend seems to be indicated along the Pacific coast of North America compared to the Canadian Arctic in marine mammals.

BDE-47 is the predominant PBDE in environmental samples collected from areas affected by general pollution (Lake Vättern, Baltic Sea, River Viskan, Great Lakes), whereas the congener pattern is more similar to the technical product Bromkal 70-5DE in sewage sludge, sediments and fish from background sampling sites. The highest concentrations of BDE-47 in Sweden are found in fish collected along River Viskan, where several textile industries are located. Even higher concentrations have been seen in the US near a furniture manufacturer (Renner, 2000). Sediments from River Viskan have also been found to contain DeBDE and HBCD, and fish also contain HBCD. High concentrations of especially BDE-47 are also found in fish-eating birds and mammals, possibly due to bioaccumulation and biomagnification since this congener has the highest bioavailability. In fish from the UK, highest concentrations of BDE-47, -99, OcBDE and DeBDE are found in estuaries of rivers with manufacturing plants or user sites upstream. In North America, highest concentrations of BDE-47, -99, -100, -153 and -154 are found in lakes that are heavily industrialized and near manufacturers and users.

Lower brominated PBDE, OcBDE and HBCD are bioavailable from sediment, as indicated by their presence in fish. Uptake from the gastrointestinal tract of rats, mice and fish is high for lower brominated PBDE. High rates of transfer are also seen in neonatal mice via breast milk. Species-specific differences are seen in metabolic capacity however. Metabolism seems to result in the formation of hydroxylated PBDE. HBCD is also rapidly absorbed from the gut in rats. Uptake from the GI tract of fish is low for DeBDE, but metabolic debromination to lower brominated PBDE may occur.

BDE-47, -99 and -100 biomagnify in fish-eating birds and mammals. BDE-47, -99 and -100 have been found in human and Baltic salmon blood. In general, considerably more is known about PBDE levels in European wildlife than in North America and elsewhere. With many more studies planned or currently underway in Canada, Japan and the US, our understanding of PBDEs is likely to improve considerably over the next few years.

PBDEs have been found in human adipose tissue, blood and breast milk. Higher brominated PBDEs, including DeBDE, have been found in human adipose tissue and in blood. Highest DeBDE

concentrations were found in people working at a computer disassembly facility. DeBDE is also bioavailable in fish.

Several hydroxylated and methoxylated PBDE (Te- and PeBDE) have been found in salmon, herring, ringed seal and grey seal from the Baltic Sea and methoxy-BDE-47 in freshwater pike. The origin of these is not known.

Several lower brominated PBDEs, including BDE-47, -99 and -100, have been shown to activate and inhibit the Ah receptor. Bromkal 70-5DE, a PeBDE product, has been shown to induce Ah-receptor-mediated liver enzymes such as EROD, in vitro as well as in vivo in rats and rainbow trout. BDE-47 and -99 have been shown to decrease EROD activity in rainbow trout liver however. Hydroxylated PBDE and TBBPA are potent competitors for transthyretin, the plasma protein responsible for transporting thyroid hormones in the plasma. Brominated structural analogues of thyroxine and T_3 also interact with thyroid hormone receptors. Rats and mice treated with Bromkal 70-5DE or BDE-47 had decreased thyroxine levels as well as changes in immune response. Oral administration of BDE-47 or BDE-99 to neonatal mice on day 10 induced permanent aberrations in spontaneous motor behavior which worsened with age. Neonatal exposure to BDE-99 also affected learning and memory functions in the adult animal.

Therefore, these substances have a potential to induce/down-regulate liver enzyme production, negatively influence the regulation of the thyroid hormone system and induce immunotoxicity. They also induce neurotoxicity when administered at a sensitive period of brain growth.

Based on levels of PBDE in fatty Baltic Sea fish and available toxicological studies, the Nordic Council of Ministers performed a risk assessment for human consumption, and recommended a no-effect level (NOEL) of 2 mg/kg body weight/day (Darnerud et al., 1998b). This was later revised based on new toxicological data to a lowest-observed-adverse-effect level (LOAEL) of 1 mg/kg body weight/day (Darnerud et al., 2001).

The time trends studied all indicate increased levels of PBDEs in environment since the 1970s. Some differences are seen between Europe and North America. In North America, temporal trends in lake trout from Lake Ontario, ringed seal and beluga from the Canadian Arctic all indicate steady and continuing increases in PBDE concentrations, with no indications of levelling off. The trends in Baltic guillemot indicate that levels of BDE-47, -99 and -100 have begun to decline in the Baltic Sea since voluntary withdrawal of use in a number of countries, but that levels of HBCD are increasing. The temporal trend in pike from Lake Bolmen, Sweden, and the sediment cores from Lake Woserin and the Wadden Sea indicate a levelling off of PBDE levels. The temporal trend in the Drammenfjord sediment core indicates continuous increases and the Swedish human breast milk trend indicates that levels are increasing exponentially, doubling every 5 years. Temporal trends in human blood from Germany also indicate continued increases in concentrations with time. These differences may reflect differences in exposure routes. Lake Bolmen receives its input of contaminants from long-range transport in air. The Baltic Sea is affected by both long-range transport as well as direct releases of contaminants into the environment from cities and agriculture. These results may indicate that humans are exposed to these substances not just from the diet, but also from current exposure to electronic appliances and textiles containing brominated flame retardants in the home and work environment. The continuous increasing trends in North America may be more reflective of the fact that PeBDE technical products are still being used to a larger extent than in Europe.

PBDE, TBBPA and HBCD are present in the environment. They are taken up by living organisms,

and lower brominated PBDE biomagnify. TBBPA and PBDE and/or their metabolites have been shown to be biologically active. Levels of PBDE seem to be increasing, and the trend in humans in particular indicates that this increase may be rapid. This could lead to levels high enough in wildlife and/or humans to cause effects. The results presented here indicate that brominated flame retardants may be a new "PCB problem". BFRs are in widespread use and only a limited ban of PeBDE technical products within the EU in the next few years is pending. There is therefore an urgent need to fill the following research gaps in order to perform adequate risk assessments so that further remedial actions can be taken.

With the availability of congener-specific standards, it is imperative that physicochemical properties of specific congeners be determined in order to model and predict their environmental behavior. Pure standards are lacking for many BDE congeners making it impossible to identify and quantify many BDEs found in environmental samples. Efforts are needed to synthesize more congeners, both unlabelled and radioactively labelled, for quantification and identification purposes as well as for experimental studies including physicochemical properties, degradation rates, toxicokinetics and toxicology.

Toxicokinetic data is very limited, both as to the substances tested (BDE-47, -99, -153 primarily) and the animal species used (rats, mice, fish). There is some data for BDE-209 and TBBPA, but very little for HBCD. There is no data for other BDE congeners. There is little toxicokinetic data for humans and there have been no toxicokinetic studies in birds or in more ecotoxicologically relevant mammals. Some BDE congeners may be metabolized to biologically active hydroxylated BDEs, but DeBDE may be debrominated to lower brominated BDEs. More toxicokinetic studies of specific compounds are therefore needed and more species need to be used to understand species-specific differences in accumulation and metabolism. More experimental studies of bioconcentration and bioaccumulation are also needed in order to understand the higher uptake efficiencies and accumulation behavior of some brominated substances as compared to organochlorines.

Only technical products (PBDEs, TBBPA and HBCD) and a few BDE congeners have been tested for in vitro toxicity in a few systems. In vivo studies in rats and mice support the in vitro results obtained for liver enzymes and thyroid effects, but have so far only been carried out for technical products and BDE-47. No carcinogenicity studies have been carried out for the few substances studied so far for intragenic recombination. Earlier toxicity studies of HBCD are of poor quality and new studies need to be carried out. In vivo studies also indicate that PBDEs may be immunotoxic and BDE-47 and -99 have been shown to cause permanent neurobehavioral changes. There is very little data on possible reproductive effects and no information on possible second-generation effects of these substances. There is therefore an urgent need to perform more in-depth toxicity studies on more BDE congeners, including the higher brominated congeners as well as HBCD and TBBPA. Much of the data implicates hormone disruption and further studies of other hormonal systems are also needed. The metabolites/substances responsible for thyroid effects need to be identified and studied further. The results for TBBPA, showing strong TTR binding in vitro but not in vivo, need to be followed up for BDE congeners that also are potent in vitro TTR binders. More BDE congeners need to be tested for intragenic recombination or other types of non-mutagenic carcinogenicity. Neurotoxicity seems to be one of the more sensitive effects and more congeners, particularly more highly brominated BDEs, should be tested. The mechanism for neurotoxicity needs to be elucidated.

The database on environmental levels and trends needs to be expanded geographically as little data is available outside of northern Europe and Japan, and more BDE congeners, including higher brominated congeners, and other BFRs need to be included. More data for all matrices are needed -

air, sewage sludge, sediments, soil, biota from higher trophic levels in the terrestrial environment, such as birds of prey, piscivorous birds and mammals known to be hard hit by other organohalogens in aquatic and marine environments and higher trophic level predators in the marine environment. More efforts are needed to determine the environmental occurrence and behavior of DeBDE, the most widely used PBDE technical product, and its possible photolytic breakdown products.

Temporal trend studies of lower brominated BDEs in the environment indicate differences in inputs at different geographical sites. More temporal trend studies are needed on an international basis to better understand this and to aid modellers in determining fluxes. No temporal trends have been carried out in the terrestrial environment. There are no temporal trends for TBBPA or higher brominated BDEs (Hx-DeBDE), and only one for HBCD. It is imperative that archived material be analyzed for as many BFRs are possible in order to quickly establish temporal trends for these as well. Analysis of more sediment cores globally is also a priority. Monitoring programs should include as many of these BFRs as possible in order to follow trends in the future. Correlation studies need to be carried out to see if adverse effects are already occurring due to high BFR concentrations in some species.

More research is needed on levels and temporal trends in humans. At present, it is not possible to properly quantify human exposure as we do not know the exposure routes for these substances in humans. We know virtually nothing about concentrations of these substances in different types of food. A few studies indicate that fish intake may be important for human levels of BDE-47; others do not support this. Occupational exposure via electronic equipment may be important for levels of some higher brominated BDEs. There is thus an urgent need to quantify intake via different types of food, via exposure from flame-retarded electronic equipment in the workplace, textiles, furniture, home environments, etc. The exposure routes may be different for different BFRs and this is also important to determine. Exposure routes for nursing infants and children are also important to establish. The increasing temporal trends seen in humans need to be confirmed in other countries and monitored closely. If possible, studies should be made attempting to link temporal trends with production/use trends in different regions to try to explain the differences that are currently seen globally.

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Polybrominated Diphenyl Ethers: Occurrence, Dietary Exposure, and Toxicology

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Polybrominated diphenyl ethers (PBDEs) are used as flame retardants in plastics (concentration, 5–30%) and in textile coatings. Commercial products consist predominantly of penta-, octa-, and decabromodiphenyl ether mixtures, and global PBDE production is about 40,000 tons per year. PBDEs are bioaccumulated and biomagnified in the environment, and comparatively high levels are often found in aquatic biotopes from different parts of the world. During the mid-1970–1980s there was a substantial increase in the PBDE levels with time in both sediments and aquatic biota, whereas the latest Swedish data (pike and guillemot egg) may indicate that levels are at steady state or are decreasing. However, exponentially increasing PBDE levels have been observed in mother's milk during 1972–1997. Based on levels in food from 1999, the dietary intake of PBDE in Sweden has been estimated to be 0.05 µg per day. Characteristic end points of animal toxicity are hepatotoxicity, embryotoxicity, and thyroid effects as well as maternal toxicity during gestation. Recently, behavioral effects have been observed in mice on administration of PBDEs during a critical period after birth. Based on the critical effects reported in available studies, we consider the lowest-observed-adverse-effect level (LOAEL) value of the PBDE group to be 1 mg/kg/day (primarily based on effects of pentaBDEs). In conclusion, with the scientific knowledge of today and based on Nordic intake data, the possible consumer health risk from PBDEs appears limited, as a factor of over 10^6 separates the estimated present mean dietary intake from the suggested LOAEL value. However, the presence of many and important data gaps, including those in carcinogenicity, reproduction, and developmental toxicity, as well as additional routes of exposure, make this conclusion only preliminary. Moreover, the time trend of PBDEs in human breast milk is alarming for the future. *Key words:* brominated, diet, environmental levels, exposure, flame retardant, human, organohalogen compounds, toxicity. — *Environ Health Perspect* 109(suppl 1):49–68 (2001). *http://ehpnet1.niehs.nih.gov/docs/2001/suppl-1/49-68darnerud/abstract.html*

The presence of persistent man-made chemicals in our environment is not a new problem. However, it was not until the beginning of the 1960s that environmental pollutants aroused debate and concern. Since then, a large number of chemicals have been identified in environmental samples, and the time trends of their concentrations have been the subject of continuous interest. Apart from the heavy metals, the group of chlorinated hydrocarbons includes many pollutants regarded as major environmental problems, e.g., the well-known polychlorinated biphenyls (PCBs), polychlorinated dibenzodioxins (PCDDs) and dibenzofurans (PCDFs), the two latter groups generally called dioxins. The PCBs and dioxins and their harmful effects on nature and man have been extensively reviewed. Today the effects of these substances are quite well known, although their mechanism(s) of action remains largely unsolved. The toxicity of these chemicals and their presence in certain food items, mostly of animal origin, have resulted in introduction of dietary restrictions and recommendations by food administrations in different countries. Continuous monitoring of the environmental levels of these chemicals has shown a decreasing trend in their occurrence over the last 10 years or more in many Western countries.

There are significant amounts of other chemicals in our environment that we know less about, and one such group is the brominated flame retardants. Even today, limited amounts of data are available on these compounds, with regard to their presence and levels in various products, environmental levels, transformation products, disposition, and toxic effects. Earlier overviews have compiled the present knowledge about brominated flame retardants (*1–7*).

Polybrominated diphenyl ethers (PBDEs) constitute an important group of brominated flame retardants. The compounds are mostly found in ready-made plastic products. PBDEs are used in large quantities worldwide and are persistent in the environment, possibly because the compounds are in the technosphere and will be released into the environment for years to come. Over the last 10–15 years, there have been indications of increased environmental and human levels of these compounds, although the levels are still lower than those for PCBs and DDT. Therefore, it is important to summarize the present state of knowledge about the environmental occurrence, human exposure, and toxicity of PBDEs to assess health consequences from the present and future use of this group of brominated compounds. This article reviews risks from PBDEs, with special emphasis on dietary risks.

Chemical and Physical Properties of PBDEs

The general chemical formula of a PBDE is $C_{12}H_{(9-0)}Br_{(1-10)}O$, with the sum of H and Br atoms always equal to 10. Structure formulas are given in Figure 1.

The theoretical number of possible congeners is 209 and is divided into 10 congener groups (mono- to decabromodiphenyl ethers). However, compounds with less than four bromine atoms are generally not found in commercial PBDE products. The number of PBDE congeners used in commercial products, and thus found in environmental samples, is quite small compared to the number of PCB congeners commonly found. PBDE congeners are often numbered according to the International Union of Pure and Applied Chemistry (IUPAC) system originally designed for PCBs (*8*). Commercial PBDEs are quite resistant to physical, chemical, and biologic degradation. The boiling point of PBDEs is between 310 and 425°C and their vapor pressure is low at room temperature. PBDEs are lipophilic, and their solubility in water is low, especially for the higher brominated compounds. The *n*-octanol–water partition coefficient, log P_{ow}, ranges between 4.3 and 9.9. Physical properties are summarized in Table 1.

Analytical Methods

Extraction methods normally used for environmental (biologic and sediment) samples are batch or Soxhlet extraction. Different methods of cleanup are used depending the nature of other compounds analyzed and the type of analytic method. Among these procedures are sulfuric acid cleanup and different types of column separations (e.g., silica gel, aluminum oxide, and gel permeation chromatography). Recently supercritical fluid extraction also has been described (*9*). Gas

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chromatography (GC) analysis is normally done on capillary columns with methyl or methyl plus 5% phenyl packing substrates. Detection is made on an electron capture detector (mass spectrometry–electron impact ionization or mass spectrometry–electron capture negative ionization). The high-brominated diphenyl ethers with longer retention times are analyzed using a shorter GC column. More details on PBDE analysis are given in International Programme on Chemical Safety (IPCS) (*2*) and by Sellström (*10,11*). See also Örn (*12*), Haglund et al. (*13*), and Sellström et al. (*14*) for further details.

Pure PBDE-congeners are needed to unequivocally identify a PBDE congener. Örn et al. (*15,16*) have described the synthesis and characterization of tetra-, penta-, and hexa-bromodiphenyl ethers. Recovery experiments on tetraBDE (DE-47) and pentaBDEs (DE-99 and DE-100) show that acceptable recoveries are made in fish (111–114%) and in sediment (106–140%) (*14*).

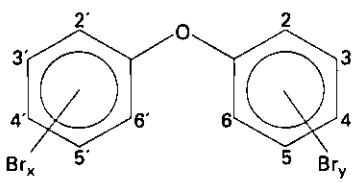

Figure 1. General structural formula of PBDEs.

In experimental studies, it is also beneficial to use synthesized PBDE congeners of high purity to circumvent impurity problems. In commercial PBDEs the presence of contaminants might otherwise affect the results of studies. Consequently, it is important to know the impurity profiles of the PBDE preparations being studied. Up to now, such data are often missing. One type of contaminants that could be found are the PBDDs and PBDFs. These compounds could be formed during heating of PBDE mixtures, but the toxic 2,3,7,8-substituted compounds seem to be produced in minute amounts (*17,18*).

Production and Use of PBDEs

Commercial PBDEs are synthesized by bromination of diphenyl ethers under conditions resulting in mixtures of brominated diphenyl ethers. The commercial products predominantly consist of penta-, hepta-, octa-, and decabromodiphenyl ethers. Chemically, the pentabromo product is a mixture primarily of tetra and penta congeners, and the octabromo product consists mainly of hepta and octa congeners (Table 2). Consequently, almost no data are available on mono-, di-, tri-, hexa-, and nonadiphenyl ethers. The number of different congeners found in each commercial product is relatively small. The composition of commercial brominated diphenyl ethers is given in Table 2.

PBDEs are used only for flame retardant purposes. The rationale for using brominated compounds as flame retardants is based on the ability of halogen atoms, generated from the thermal decomposition of the bromoorganic compound, to chemically reduce and retard the development of fire. Factors favoring the use of PBDEs are therefore the high bromine content (which means good flame-retardant properties), thermal stability, and relatively low cost. They are used as additive flame retardants at concentrations of 5–30% in many different polymers, resins and substrates, and common plastics, including acrylonitrile butadiene styrene and high impact polystyrene (*19*). Additive flame retardants leach and escape from the finished polymer product more easily than reactive flame retardants. Examples of products containing flame retardants, and especially PBDEs, include many components of electronic devices, e.g., cabinets for and circuit boards in personal computers and television (TV) sets and various other products (electrical cables, switches and capacitors), building materials, and textiles (Figure 2). The technical decaBDEs have the widest industrial use. More details about the use of PBDEs in various resins or polymers and the applications of these PBDE-containing resins are given in Tables 4 and 5 in IPCS (*2*).

The annual world production of flame retardants is roughly 600,000 metric tons, of which about 60,000 tons are chlorinated and 150,000 are brominated compounds. Of the brominated products, about one-third contain tetrabromobisphenol A (TBBP-A) and derivatives, another third contain various bromines, including polybrominated biphenyls (PBBs), and the last third contain PBDEs (*20*).

In 1990, global production of PBDE was 40,000 tons per year, of which approximately 10% was commercial penta-, 15% octa-, and 75% decaBDEs (*21*). The global production figures have stayed at approximately the same levels for more than 10 years, but there has been a shift in use toward the higher brominated preparations. Consequently, the use of

Table 1. Physical properties for some PBDE congener groups.[a]

	TetraBDE	PentaBDE	OctaBDE	DecaBDE
Chemical formula	$C_{12}H_6OBr_4$	$C_{12}H_5OBr_5$	$C_{12}H_2OBr_8$	$C_{12}OBr_{10}$
Molecular mass	485.8	564.8	801.5	959.2
Vapor pressure (Pa)	$2.7–3.3 \times 10^{-4}$ (20°C)	$2.9–7.3 \times 10^{-5}$ (20°C)	$1.2–2.7 \times 10^{-7}$ (20°C)	$< 1 \times 10^{-4}$ (25°C) 670 (306°C)
Melting point (°C)	79–82 (BDE-47)	92 (BDE-99) 97–98 (BDE-100)	~ 200	290–306
Boiling point (°C)	—	> 300°C (decomposition)	—	(Decomposition)
Water solubility (µg/L)	—	0.0009 (20°C)	—	(20–30 ?)
Log K_{ow}	5.9–6.2	6.5–7.0	8.4–8.9	10

—, no information given; ?, unreliable value.
[a]Data from IPCS (*2*), Sellström (*10,11*), and OECD (*20*).

Table 2. Composition of commercial PBDEs.[a]

	Commercial product			
Congener group	TetraBDE[b] (%)	PentaBDE (%)	OctaBDE (%)	DecaBDE (%)
Unknown	7.6			
TriBDE		0–1		
TetraBDE[c]	41–41.7	24–38		
PentaBDE[d]	44.4–45	50–62		
HexaBDE[e]	6–7	4–8	10–12	
HeptaBDE[f]			43–44	
OctaBDE			31–35	
NonaBDE			9–11	0.3–3
DecaBDE[g]			0–1	97–98

[a]Data from IPCS (*2*). [b]No longer commercially produced. [c]Including 2,2',4,4'-tetrabromodiphenyl ether (BDE-47). [d]Including 2,2',3,3',4-pentabromodiphenyl ether (BDE-82), 2,2',3,4,4'-pentabromodiphenyl ether (BDE-85), 2,2',4,4',5-pentabromodiphenyl ether (BDE-99), and 2,2',4,4',6-pentabromodiphenyl ether (BDE-100) [e]Including 2,2',4,4',5,5'-hexabromodiphenyl ether (BDE-153) and 2,2',4,4',5,6'-hexabromodiphenyl ether (BDE-154). [f]Including 2,2',3,4,4',5',6-heptabromodiphenyl ether (BDE-183) [g]Including 2,2',3,3',4,4',5,5',6,6'-decabromodiphenyl ether (BDE-209)

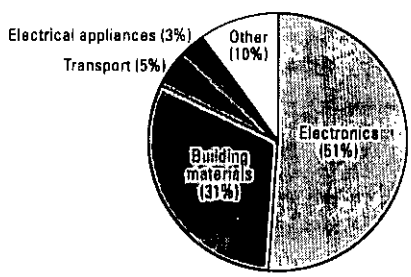

Figure 2. The relative amounts of flame retardants (all types) used in different sectors. Data from Pijnenburg et al. (*7*).

decaBDE today is even more prevalent. According to IPCS (*2*), eight manufacturers of PBDEs are located in the Netherlands, France, Great Britain, Israel, Japan, and the United States. In the European Union (EU), the total import and production of PBDEs was approximately 11,000 tons in 1989, of which roughly 4,000 tons were produced (*12*). No pentaBDEs are produced in the EU. In the United States the commercial production or import of PBDEs (penta-, octa-, and decaBDEs) was greater than 1,500 tons per year during the 1990s (*1*).

It is difficult to assess the quantities of PBDEs used and consumed in different countries, as the compounds are imported primarily as additives in manufactured products. For example, in Sweden the Chemicals Inspectorate has estimated that in 1993 the import of pure PBDE chemicals to Sweden was 17 tons. However, an additional 20 tons were imported in semimanufactured products. At least 150 tons were supposedly imported in TV sets and at least the same amount in personal computers (*6*). Consequently, the total import of PBDEs to Sweden was approximately 400 tons per year, or about 1% of world production.

PBDEs: International and National Legislation and Restrictions

The need for restrictions on certain PBDEs in different types of plastics and textiles is currently being discussed within the EU. Consequently, the use of certain brominated flame retardants [PBB and tris(2,3-dibromopropyl) phosphate] in textiles has already been banned. Within the EU Existing Substances Programme, documents are currently being produced that separately assess penta-, octa-, and decaBDEs.

An Organisation for Economic Co-operation and Development (OECD) document proposes precautions for each type of PBDEs. These include recommendations to stop the use of certain compounds (mainly tetra- and pentaBDEs), as well as to limit occupational exposure (*20*). Also the Paris Commission for the Prevention of Marine Pollution is working toward restricting and phasing out PBDEs and PBBs.

An example of a national restriction is the Swedish government's intention to ban PBDEs and PBBs in products sold on the Swedish market (*22*).

PBDEs in the Environment

Sources of PBDEs in the Environment

PBDEs are used as flame retardants in a wide range of products, and waste from these products is probably the main source of PBDEs in the environment. The waste is either incinerated as municipal waste or deposited in landfills. Although specific data are missing, incineration is thought to be an important route of release of PBDEs into the environment. No study on leaching of PBDEs from landfills is available, but PBDE-containing products are widespread, and leaching may be an important long-term pathway of contamination. PBDEs are discharged into the environment through sewage, as indicated by analysis of sewage sludge from various countries. Volatilization of PBDEs into the surrounding air from electrical components and other products during their lifetime can also be significant.

Apart from anthropogenic sources, PBDE-related brominated compounds also appear to be formed by nature and have been detected in certain marine sponges (*23,24*). These compounds structurally resemble hypothetical phenolic metabolites of PBDE congeners.

Environmental Levels

PBDEs have been detected in environmental samples from aquatic environments and organisms. On a congener basis, the levels of PBDEs in these samples (such as fatty fishes) could be similar to those of PCBs, but the levels of total PBDEs are lower because fewer congeners of PBDEs are present in technical mixtures and in the environment. Only a limited number of PBDE analyses have been conducted on terrestrial environments.

Little data are available about the environmental levels of nona-, hepta-, and hexaBDEs. These compounds probably exist in the environment at lower concentrations than other PBDEs, since nona-, hepta-, and hexaBDEs are impurities of commercial deca-, octa-, and/or pentaBDE preparations. The formation of these compounds as a result of degradation of higher BDs is also possible.

The following review of environmental levels of PBDEs is a selection of important observations and is not intended to be a definitive listing of all studies performed. For recent reviews of environmental levels of PBDEs and other organobrominated compounds, see de Boer et al. (*25*) and de Wit (*26*).

Abiotic samples. **Air.** In air samples from the vicinity of recycling plants in Japan and Taiwan various tri-, tetra-, penta-, and hexaBDEs were detected in the range of 23–53 pg/m^3 (Taiwan) and 7.1–21 pg/m^3 (Japan) (*27*). In Swedish air samples from remote sites with no known local source of contamination, the total PBDE levels were approximately 1–8 pg/m^3. Whereas the tetraBDE level was higher in the gas phase, the pentaBDE level was higher in the particle fraction, in agreement with the respective vapor pressures of the two PBDE homologs (*28*).

Sewage sludge. PBDEs with 3–7 bromine atoms were identified in German sewage sludge samples (*29*). Pooled samples of sewage sludge from a Swedish sewage treatment plant (Gothenburg) in 1988 were found to contain 15 and 19 ng of 2,2′,4,4′-tetraBDE (BDE-47) and 2,2′,4,4′,5-pentaBDE (BDE-99) per g ignition loss, respectively (ignition loss: the carbon content in sample, measured as the loss in sample weight after incineration) (*30*). The sum of the levels of BDE-47 and BDE-99 were considerably higher, i.e., 100–190 ng/g dry weight, in recent sludge samples from three Swedish sewage treatment plants (*31*). The Swedish sludge data in Nylund et al. (*30*) found a BDE-47+99 level of 21 ng/g, calculated on dry weight basis. Sellström and coworkers (*31*) found that levels of BDE-209 were even higher—160–260 ng/g dry weight.

Water and sediments. DecaBDE, octaBDE, or hexaBDE were not found in water samples taken from more than 200 river, estuarine, and marine waters collected in Japan in 1977 and 1987–1989 (limit of detection 0.04–2.5 µg/L) (*32*). This is probably due to the very low water solubility of PBDEs. However, decaBDE, octaBDE, hexaBDE, pentaBDE, and tetraBDE were found in samples of river, estuarine, and marine sediments. DecaBDE was found at concentrations of < 25–11,600 µg/kg dry weight, while the range for the other compounds was from below the limit of detection up to 70 µg/kg sediment dry weight. The highest concentrations were found in contaminated rivers.

PBDE levels in sediment layers from the Baltic Sea (Bornholm Deep) decreased with increasing depth (*10,30*). At 5 mm deep, the levels of BDE-47 and BDE-99 were 1.6 and 1.1 µg/kg ignition loss, respectively. Below 40 mm, almost no pentaBDE was detected, whereas the concentration of tetraBDE was 0.1 µg/kg. In one situation, river samples collected upstream and downstream pinpointed the PBDE effluent source, a factory manufacturing flameproof textiles (*14*). For the sum of the three congeners BDE-47, BDE-99, and 2,2′,4,4′,6-pentaBDE (BDE-100), the concentration in sediment varied from below detection to 120 ng/g ignition loss, whereas BDE-209 (the full-brominated congener) had a peak concentration of 12,000 ng/g. In addition, the peak concentration of another brominated flame retardant, hexabromocyclododecane (HBCD), was 7,000 ng/g ignition loss. All three analyzed compounds or compound groups had the highest levels at the same sampling location.

Analysis of sediments from a number of European estuaries revealed high concentrations of BDE-209 in some rivers in the United Kingdom (e.g., River Mersey, 1,700 ng/g dry weight) and Belgium (River Schelde, 200 ng/g dry weight), whereas most samples showed levels below 20 ng/g dry weight (*31*).

BDE-47+99 levels were always considerably lower than those of BDE-209.

Biota. **Aquatic organisms.** In 1981, fish from the Swedish River Viskan were analyzed for PBDEs (*33*). The maximum levels in pike were 27 mg/kg lipid in muscle and 110 mg/kg lipid in liver. The water system was at that time receiving effluent water from nearby textile factories. Later analysis of PBDEs and HBCD in fish from the River Viskan indicated lower PBDE levels (maximum PBDE levels 4.6 mg/kg lipid; sum of BDE-47, BDE-99, and BDE-100) (*14*). Concomitant analysis of sediment samples showed that tetra- to pentaBDEs and HBCD are much more bioavailable than the full-brominated BDE-209.

A number of species from Swedish fauna were analyzed for the presence of PBDEs (sum of BDE-47, BDE-99, and BDE-100) from 1979 to 1988 [(*34–36*); Table 3]. The highest levels were found in fish from waters with known or suspected local sources of contamination. However, fish from other sampling spots also contained measurable levels. It is evident that animals (herring and seal) from the Baltic Sea contain higher levels of PBDEs than the same or similar species from other waters.

Herring collected during 1985 from three North Sea regions and from the Straits of Dover (between Holland and United Kingdom) contained an average of 8.4–100 µg BDE-47/kg lipid. Eels from Dutch rivers and lakes (10 locations) contained from < 20 to 1,700 µg BDE-47/kg lipid (*37*). Freshwater fish of various species from the waters of North-Rhine Westfalia contained 18–983 µg PBDE/kg lipid, and Baltic and North Sea fish 12–57 and 1–120 µg/kg lipid, respectively, quantified with Bromkal 70-5DE as a reference (*38*). In fish and shellfish samples from Osaka Bay, Japan, measurable levels of PBDEs were occasionally found (*32*). Concentrations ranging from 0.1–14.6 µg/kg wet weight were observed for tetra-, penta-, hexa-, and decaBDE, respectively. PBDEs were detected in bottlenose dolphins at levels up to 8 mg/kg lipid (*39*). PBDEs have also been found in the blubber of pilot whales caught off the coast of the Faroe Islands from 1994 to 1996 (*40*). Nineteen tetra- to hexaBDEs were identified in the pilot whales at mean total levels of about 1–3 mg/kg lipid, depending on sex and age. BDE-47 and BDE-99 accounted for about 70% of total PBDEs.

Haglund and co-workers (*13*) reported PBDE levels in fish and seals caught in the Baltic Sea. In herring (different age groups) and in salmon muscle, the BDE-47 levels were 3.2–27 and 167 µg/kg lipid weight, respectively. These BDE values in herring are lower than those earlier analyzed by Janssson and Sellström and their co-workers (*35,36*). PBDEs and other organohalogen compounds were found in sea-run Baltic salmon from the River Dalälven (total PBDE levels about 300 µg/kg lipid) (*41*). The presence of methylated and phenolic PBDE derivates at levels similar to those of the major PBDE congeners was also reported (*13,41*). Strandman and co-workers (*42*) showed that the total PBDE (BDE-47, BDE-99, BDE-153) levels in Baltic herring were 13–24 µg/kg lipid weight and in sprat 22–149 µg/kg lipid weight. In this study, analysis of different age groups of Baltic fish suggests an age-related accumulation of PBDEs in the body fat (Figure 3). PBDE concentrations (sum of BDE-47, BDE-99, and BDE-153) showed an age-related increase in 1- to 3-year-old Baltic herring, as well as in 3- to 13-year-old sprat (*42*). In sprat, the accumulation seemed to level off at 8 years of age. Also, Haglund et al. (*13*) found an age-related PBDE uptake (Figure 3).

Recent studies from the American continent have shown relatively high levels of PBDEs in certain samples from the aquatic environment. Canadian samples of crab, fish, and marine mammals all contained PBDEs, and the highest levels were found in porpoise from British Columbia waters (peak value, 1,400 µg BDE-47/kg lipid) (*43*). PBDE levels in fish from the Great Lakes were analyzed in two studies. One study (*44*) found total PBDE levels of 135–545 µg/kg lipid in Great Lakes lake trout, and the other (*45*) found about 3,000 µg PBDE/kg lipid in Lake Michigan steelhead trout. On a wet weight basis, the Lake Michigan fish contained almost 6 times more PBDE (41 µg/kg wet weight) than the Baltic salmon (7 µg/kg fresh weight).

Terrestrial/avian organisms. The osprey (*Pandion haliaetus*), a bird that feeds exclusively on fish, is the only nonaquatic species listed in Table 3 that has relatively high PBDE levels (*36*). In addition, eggs from the fish-eating guillemot contain relatively high levels of PBDEs. Apart from these species, samples from all other nonaquatic avian or terrestrial wild-living species (e.g., moose, reindeer) showed low or nondetectable PBDE levels.

Table 3. Swedish PBDE levels in sediment and biota, 1979–1988.[a]

Sample	No. of samples[b]	2,2',4,4'-tetraBDE[c]	2,2',4,4',5-pentaBDE[b]	Unknown pentaBDE[b]	Sampling year
Aquatic organisms[d]					
Whitefish, freshwater	35	15	7.2	3.9	1986
Arctic char, Lake Vättern	15	400	64	51	1987
Herring, Skagerrack	100	59	9.8	4.7	1987 (April)
Herring, Baltic proper	60	450	46	32	1987 (June)
Herring, Baltic proper	10 (in)	38	17	6	1987 (Sept)
Herring, Bothnian Sea	100	82	27	14	1986 (Nov)
Bream, River Viskan[c]	2 (in)	250, 750	2.3, 2.4	11, 37	1987
Perch, River Viskan[c]	2 (in)	2,200	380	230	1987
	24 000	9,400	3500		
Pike, River Viskan[c]	2 (in)	2,000	78,	170	1987
	6 500	1,100	640		
Pike, Dalsland Canal	9 (2 x p)	94–98	60–74	25–36	1988
Trout, Dalsland Canal	22 (in, p)	120–460	64–590	37–150	1988
Grey seal, Baltic Sea	8 (p)	650	40	38	1979–1985
Ringed seal, Svalbard	7 (p)	47	1.7	2.3	1981
Terrestrial/avian organisms[d]					
Rabbit	15 (p)	< 1.8	< 0.3	< 0.2	1986
Moose	13 (p)	0.8	0.6	0.2	1985–1986
Reindeer	31 (p)	0.2	0.3	< 0.1	1986
Starling, young	4 (in)	2.7–7.8	2.3–4.2	0.6–1.1	1988
Osprey	35 (p)	1,800	140	200	1982–1986

Abbreviations: p, pooled sample; in, individual sample.
[a]Data from Jansson et al. (*34,35*) and Sellström (*36*). [b]Concentrations in µg/kg lipid weight (sediment: µg/kg ignition loss). [c]Effluents from industry using flame retardants reach the Viskan-Häggån river systems.. [d]Muscle samples taken for analysis, except for reindeer (suet) and seal (blubber)

Trend Studies

Time trend. A retrospective time trend of PBDE levels was constructed (*10,46*) through laminated sediment cores collected in the Bornholm Deep (southern part of the Baltic Proper). In contrast to other environmental pollutants such as PCBs and DDT, PBDEs

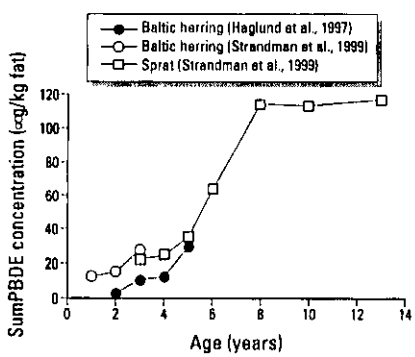

Figure 3. Age-related accumulation of PBDEs (sum of BDE-47, -99 and -153) in Baltic herring and sprat. Data from pooled sample analyses in Haglund et al. (*13*) and Strandman et al. (*42*).

were detected quite late in the cores, but from the late 1970s on, the levels increased exponentially (Figure 4). This strong increase in PBDE levels has continued to the most recent part of the study (1988–1989). This is in contrast to other environmental contaminants, the levels of which have decreased or remained unaltered in newly formed sediment layers (*47*).

The total concentrations of BDE-47, BDE-99, and an unidentified PBDE in guillemot eggs (from Stora Karlsö in the Baltic Sea) increased from 158 µg/kg lipid in 1970 to 1,211 µg/kg lipid in 1989 (*36,48*). However, subsequent egg analyses suggest a somewhat decreasing trend from 1990 and later (*11*). The trend analysis is hampered by large annual variations in PBDE concentrations, which may be explained in part by the small number of eggs ($n = 10$) collected per year (*10*). Concentrations of another brominated flame retardant, HBCD, increased significantly in the eggs over the entire time period (1968–1997) (*49*).

Studies using sample-bank specimens of pike muscle from fish in Lake Bolmen in southern Sweden showed increasing concentrations of PBDEs from 1974 to 1991 (from about 10 to 100 ng BDE-47/g lipid) (*10*). Since 1991, the trend in PBDE levels in pike is more difficult to interpret but seems stagnant (*49*). Levels of the methoxylated derivative of BDE-47 (MeO-BDE-47) decreased in in pike during the time period (*49*).

Eel samples from the Rhine and Meuse Rivers revealed decreasing PBDE time trends levels during 1983–1993, whereas PBDE levels increased in the River Roer eel during the same time period (*37,50*).

Thus, time trend studies during the 1970s and 1980s show that PBDE levels in biota generally differ from those of most other organohalogenated environmental chemicals: PBDE levels increase or remain constant, whereas levels of most other persistent compounds decrease. Time trends in recent years are often difficult to follow because of small amount of samples and/or lack of data.

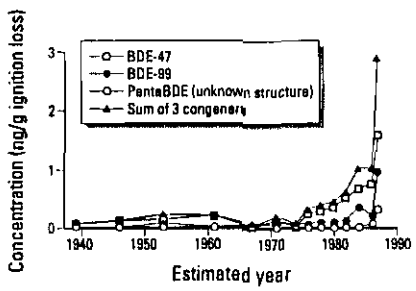

Figure 4. Time trend study of a laminated sediment core from the Baltic Sea (Bornholm Deep). Data from Nylund et al. (*30*).

In addition to environmental samples, mothers' milk has shown a strong increase in PBDE levels during 1972–1996 (*51*) (Figure 5) (see "Human Exposure to PBDEs").

Spatial trend. Few studies have been designed to study spatial or regional trends in environmental occurrence of PBDEs. However, de Boer (*52*) studied levels of BDE-47 in livers of cod caught from various locations in the North Sea. A distinct spatial trend was observed, with decreasing levels from the southern to the northern part of the North Sea (southern NS; 170 µg/kg, central NS; 54 µg/kg; northern NS, 26 µg/kg; mean values, all on wet weight basis). Other organohalogenated compounds such as sumDDT, exhibited the same type of spatial patterns. In the same study, the levels of BDE-47, the PBDE congener found at highest concentrations, remained at a relatively constant level during the period of sample collection. This trend was different from those of several organochlorine compounds that, with the exception of DDT, showed decreasing time trends in the same samples.

Bioaccumulation and Biomagnification

Although limited data are available, existing information strongly suggests that PBDEs are globally transported and distributed in the environment in a manner similar to PCBs. Consequently, they are probably of minor importance in terrestrial systems but may reach levels of concern in aquatic environments. In addition, PBDEs are persistent and have very low water solubility, high binding affinity to particles, and tendencies to accumulate in sediments. The presence of PBDEs in biota is likely to be because of their high lipophilicity and resistance to degradation.

Available data indicate that the higher brominated compounds (heptaBDEs and above) do not bioaccumulate to a significant degree, possibly because of a low uptake in organisms. However, in experimental studies the observation time may have been too short to detect a slow uptake. The uptake of the lower brominated PBDEs in the biota is more significant.

Increasing levels of the full-brominated BDE-209 were observed in rainbow trout during dietary exposure to this congener (*53*). Exposure of rainbow trout under static conditions to BDE-209 in water, however, did not result in measurable concentrations in muscle, skin, or viscera after 48 hr (*54*). The very low solubility of BDE-209 in water makes the latter study of questionable relevance. Bioconcentration factors of < 4 for BDE-209, < 2 for octaBDE, < 4 for heptaBDE, and < 4 for hexaBDE were determined in a bioaccumulation study in carps exposed to a mixture of hexa-, hepta-, nona-, and decaBDE for 8 weeks (probably present in thewater) (*55*). In contrast, a bioconcentration factor of > 10,000 was found in a study with carp exposed to 10 or 100 µg pentaBDE/L for 8 weeks (probably a commercial mixture, i.e., a mixture of a tetraBDE and two pentaBDE congeners) (*55*). Also, components of commercial "tetraBDE" (41% BDE-47; 45% BDE-99, 7% hexa- and 7–8% unknown PBDEs) are considered accumulative (*6*). An inverse relation was shown between the uptake efficiency and number of bromine atoms in pike when tetra (BDE-47), penta (BDE-99), and hexa (BDE-153) congeners were studied (*56*). The uptake efficiency of BDE-47 was over 90% and thus the highest of all the studied organohalogens.

Comparison of PBDE concentrations at different trophic levels of aquatic ecosystem suggests that PBDEs have a biomagnification potential in the food chain. PBDE levels in Baltic herring, salmon, and seals (*13*), and those in guillemot eggs and osprey (*36*) are highly indicative of biomagnification in the Baltic ecosystem (Figure 6). In a recent study, Burreau et al. (*57*) calculated biomagnification potentials for a number of PBDE congeners on the basis of sprat, herring, and salmon

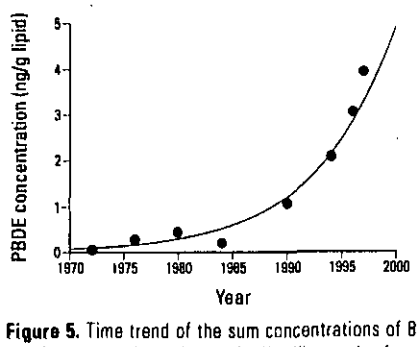

Figure 5. Time trend of the sum concentrations of 8 PBDE congeners in pooled mother's milk samples from Swedish mothers living in the Stockholm region. Data from Norén and Meironyté (*51*).

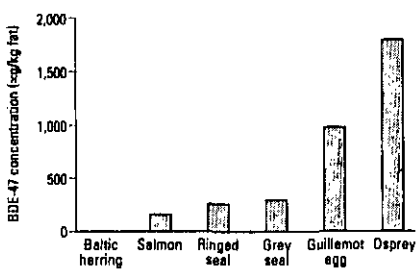

Figure 6. Concentration of the most abundant PBDE congener, BDE-47, at different trophic levels of the Baltic food chain: Baltic herring (muscle), salmon (muscle), ringed seal (blubber) and grey seal (blubber) (*13*), as well as guillemot (egg) and osprey (muscle) (*36*). Samples were collected between 1981 and 1991 along the Swedish coastline of the Baltic. The value for guillemot eggs prepresents the mean of six different analyses from 1982 to 1989.

data. All PBDE congeners analyzed were biomagnified. The tetra- and pentaBDEs were biomagnified to approximately the same degree, the triBDEs slightly less and the hexaBDEs considerably less. Preliminary data indicate that the tri-, tetra- and pentaBDEs are biomagnified more efficiently than any PCB congener.

It can be concluded, therefore, that PBDEs bioaccumulate in biota and that the extent of accumulation is inversely related to the degree of bromination. Available data indicate biomagnification of PBDEs in the food chain.

Environmental Transformation

Small amounts of data are available on transformation of PBDEs in the environment. Most studies have been done with decaBDE and mono/diBDEs.

Incineration. Incineration of waste containing PBDEs may result in formation of PBDDs and PBDFs (*17,18*). These compounds are structurally similar to the corresponding chlorinated compounds, more toxic than PBDEs, and very persistent in the environment. Formation of these compounds depends largely on combustion conditions. In a modern waste incinerator with well-controlled burning conditions, emission of brominated dioxins and furans is very low. In a Swedish study (*58*) on incinerator plants with well-controlled combustion conditions and efficient flue gas cleaning, no unacceptable environmental risk was found to be associated with combustion of PBDE-containing materials. On the other hand, uncontrolled fires at waste disposal sites may lead to formation and release of PBDDs and PBDFs into the environment (*2*).

Photodegradation. Watanabe and Satsukawa (*59*) showed that debromination of decaBDE, dissolved in organic solvents, occurs in ultraviolet (UV) light and sunlight, leading to formation of lower brominated BDEs and various PBDFs with 1–6 bromine atoms. Formation of PBDFs seems to occur only from low-brominated diphenyl ethers, but not directly from decaBDE. Photodegradation of decaBDE dissolved in water was also shown to occur, but no lower-brominated diphenyl ethers were detected among the degradation products (*54,60*). Sellström and co-workers (*61*) studied the photodegradation of decaBDE in various matrices (toluene, silica gel, sand, soil, and sediment). They showed that the time course for decaBDE debromination and formation/debromination of lower-brominated diphenyl ethers was rapid in toluene, whereas the degradation process in other matrices was considerably slower (half-life of decaBDE exposed to UV light in toluene and sand was 15 min and 12 hr, respectively).

Microbiologic degradation. No biotransformation products of BDE-209 were detected in sediment samples after incubation for 4 months (*6*). For other flame-retardant PBDEs, no data are available. On the other hand, low-brominated diphenyl ethers, not used as flame retardants, are more likely to be biodegradable. Microbiologic degradation of mono- and diBDEs has been demonstrated, but these congeners were not used as a carbon source for the bacteria (*62–64*). Even though BDEs with less than four bromine atoms are not used as flame retardants, they have been detected in the environment, possibly a result of degradation of higher-brominated diphenyl ethers (DEs). Thus, on the basis of limited data, microbial degradation of PBDEs seems to depend on the degree of bromination, and the full-brominated congener seems to be resistant to microbial degradation.

PBDEs in the Environment: Summary and Conclusions

PBDEs have been detected in air samples, even from remote areas. Analyses of organisms from terrestrial ecosystems indicate low levels of PBDEs but considerably higher levels are found in aquatic environments. In sediments the higher-brominated compounds are prevalent, but in biota these congeners are normally below the limit of detection. This indicates that the bioaccumulation of highly brominated PBDEs (especially decaBDE) is low. Microbial degradation of decaPBDE is negligible, whereas photodegradation of decaBDE may generate lower-brominated PBDEs. Thus, the lower brominated PBDEs, tetraBDEs, and pentaBDEs predominate and accumulate in biota. The uptake effieciency of BDE-47 in fish is very high, but an increase in PBDE bromination will gradually decrease uptake. Accumulation of PBDEs in fish appears to be age-related. The highest concentrations are found in top predators of aquatic ecosystems, suggesting the biomagnification potential of these compounds. Tetra- to hexaBDEs are probably the principal congeners to which humans are exposed via food. On a congener basis, the levels of PBDEs in these samples could be similar to those of the more frequent PCB congeners, but because fewer congeners are present, total PBDE levels are lower than total PCB levels. Observed spatial differences in environmental PBDE levels may reflect emissions from regional or point sources. Series of samples from sediments, guillemot eggs from the Baltic, and banked samples of pike from a Swedish lake have been used for time-trend analyses of PBDE levels. Until the late 1980s, PBDE levels in both sediment and biota generally showed an increasing trend. However, the most recent biologic samples (1990 and onward) indicate somewhat lower PBDE levels, although the variation is large. Thus, continuation of the time trend studies is important.

Human Exposure to PBDEs

As pointed out previously, the environmental fate of PBDEs appears similar to the fate of other persistent environmental pollutants, PCBs, for example. On the basis of several years of PCB monitoring, it has been established that the main route of human exposure to PCBs is via food. Food of animal origin with high fat content, e.g., fatty fish, meat, and dairy products are major contributors to dietary exposure. Because of the similarity between PCBs and PBDEs in their environmental distribution, attention also should be focused on these food items when assessing exposure to PBDEs. For another persistent organic pollutant, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), as much as 98% of human exposure is suspected to occur through consumption of foods of animal origin (*65*). However, until recently very few estimates of human dietary PBDE intake have been made. In addition, little is known of other routes of human exposure and their importance in total human exposure to PBDEs.

PBDE Levels in Food

Fish. Several fish species consumed by man have been analyzed for PBDEs. Large variations in PBDE levels observed in freshwater fish may result from widely varying degrees of contamination. PBDE levels in fish sampled in Swedish lakes and rivers (pike, perch, bream, and trout) varied from 26 to 36,900 μg/kg lipid (*14,33,36*). In German freshwater fish, the levels varied between 18 and 983 μg/kg fat (*2,38*), and in eel from Dutch waters the variation in BDE-47 levels was < 20–1,700 μg/kg lipid (*52*). Herring (North Sea, Baltic Sea), salmon (Baltic Sea), and cod liver (North Sea) have been analyzed in studies of marine and brackish-water fish (*13,36,37,41,52*). Baltic herring contained 24–528 μg/kg lipid, whereas Swedish west coast herring contained 17–74 μg/kg lipid (Swedish herring analyses: sum of three congeners) and the North Sea herring 9–100 μg BDE-47/kg lipid (*36,37*). Levels in Baltic herring are generally higher than those in Atlantic herring. A similar difference was shown earlier for PCBs (*66*), which reflects, among other things, the limited water exchange between this inland sea and the ocean.

Dairy products. PBDE levels of 2.5–4.5 μg/kg milk fat, determined to be Bromkal 70-5DE, have been reported in cows' milk in Germany (*38*). Levels in mixed dairy products from 1999 Swedish market basket samples (sum of levels of BDE-47, -99, -100, -153, and -154), however, are considerably lower, with a mean value of 0.36 μg/kg milk fat (*67*).

Mothers' milk. PBDE levels in mothers' milk have been studied in a survey of 25

German mothers (*38*). Levels varied between 0.6–11 μg/kg (i.e., 600–1,100 ng/kg) milk fat.

Darnerud and co-workers (*68*) studied 39 primiparous women from Uppsala county, Sweden, and analyzed the five most frequently found PBDE congeners in their milk (BDE-47, -99, -100, -153, and -154). The mean value was 4.4 μg/kg lipid and the range 1.1–28.2 μg/kg. Milk PBDE levels correlated positively and significantly with mothers' smoking habits and body mass index. However, no correlation was seen between PBDE and mothers' ages, consumption of fish, places of residence during childhood or adulthood, birth weights, or frequency of using computers.

In another Swedish study, a time trend was created using stored samples of breast milk collected from 1972 to 1997 (*51*). The mean PBDE level (sum of eight different congeners; predominantly BDE-47) in milk from 1997 was 4 μg/kg, quite similar to the above report by Darnerud et al. (*68*). Concentrations of PBDEs in milk showed a strong time-dependent increase, with a doubling in levels within 5 years (Figure 4). In contrast, tissue levels of most halogenated aromatic hydrocarbons of concern (e.g., PCB, DDT) were declining. Thus, future levels of PBDE in milk may eclipse those of PCB if trends continue.

PBDE levels detected in mothers' milk are, on a lipid basis, generally within the same range as those observed in human adipose tissue samples from the United States and human serum samples from Sweden (see "PBDE Levels in Humans").

PBDEs from other food groups. Except for the low levels of PBDEs found in samples from animals living in the wild (*36*) (Table 3), little data are available on PBDE levels in meat and eggs. However, preliminary data from mixed meat products from Sweden (market-basket samples) revealed a mean level of 0.36 μg/kg fat (*67*). The mean level in Swedish eggs (from the same study) was 0.42 μg/kg fat.

Estimation of PBDE Exposure

From food. Darnerud et al. (*69*), using primarily Nordic data, estimated the exposure of PBDEs from food in a report to the Nordic Council of Ministers (*69*). Their estimates were based on the upper range of total PBDE levels in herring caught in the Baltic Sea (*36*), i.e., 528 μg/kg lipid (of which 450 μg/kg was BDE-47 and the remainder two pentaBDEs). The total PBDE intake can be estimated by assuming a similar relative intake from different dietary sources, as described earlier in a Swedish estimation for PCBs (*66*). Consequently, according to this very approximate calculation, the total PBDE intake for the Nordic consumer would be 0.2–0.7 μg/day.

A Swedish intake estimate was made recently (*67*) on the basis of PBDE levels (BDE-47, -99, -100, -153, and -154) in market basket samples from 1999. Provisions from eight grocers in four major Swedish cities were received and divided into selected groups. Within these groups, each food item was added in an amount determined from per capita consumption statistics. Analyses of the homogenates were performed and sumPBDE levels were obtained by addition of congener levels, assuming that levels below the limit of quantification (LOQ) were equal to one-half of this limit. The total PBDE intake was obtained by adding intake from the fish, meat, dairy products, eggs, fats/oils, and pastry food groups (i.e., the product groups assumed to contribute most to total intake). Using this method, total PBDE intake in Sweden was estimated to be 51 ng/day. Almost half total intake originated from fish products; meat, dairy products, and fat/oils contributed approximately 15% each. Total PBDE intake was 4–14 times below estimates performed earlier but should be a more accurate description of the intake, as it is based of a more complete set of occurrence-level data. However, this estimate should be supplemented by studies from other countries and by using other methods of estimating intake to obtain a more complete picture of the intake of PBDEs from food.

From mothers' milk. The most recent human milk data come from two Swedish surveys (*51,68*). The mean PBDE levels in these two studies are almost identical, giving a total mean value of 4.2 μg/kg milk fat. To estimate the PBDE intake in infants, consumption data from Ahlborg and co-workers (*70*) were used. The calculated PBDE intake using these predictions (3.7% fat in milk; consumption of 0.7 L milk daily for 6 months) is 0.11 μg/day from mothers' milk, or 20 μg in 6 months.

Other possible routes of exposure. It appears obvious that sources other than food significantly influence total PBDE intake in humans. There is, however, almost a complete lack of data on these other sources of human exposure. Emission of PBDEs from electronic apparatuses like computers and TV sets, especially from warm devices after prolonged use, are suspected. In an experiment with a TV set, Ball et al. (*71*) detected PBDEs in air drawn from a warm TV. In Darnerud's previously cited study (*68*) on PBDE milk levels from primiparous women, however, no correlation was found between frequency of computer usage and PBDE milk levels. Because of these conflicting results, the use of computers and TV sets as sources for human exposure should be further investigated.

Sjödin et al. (*72*), in a recent publication, monitored the occupational exposure to PBDEs. Subjects working at a Swedish dismantling plant had significantly higher serum levels of all studied PBDE congeners compared to both hospital cleaners and office clerks using computers (see "PBDE Levels in Humans"). It was concluded that exposure from daily computer work in an office does not appear to increase serum PBDE levels.

PBDE Levels in Humans

PBDEs were detected (up to 5 μg/kg dry weight) in 2 of 40 hair samples from American citizens living in an area where PBDEs are manufactured, according to DeCarlo (*73*).

In two studies from the United States (*74,75*), PBDEs were detected in human adipose tissue samples collected in 1987. The presence of PBDEs was demonstrated in all samples (48 composite samples from 865 individuals). These studies also showed a large variation in PBDE levels between individuals (hexaBDE, not detected–1 μg/kg lipid; heptaBDE, 3 ng–2 μg/kg lipid; octaBDE, not detected–8 μg/kg fat; no congener specification was presented).

PBDE levels in adipose tissue were reported in three Swedish studies. In a male Swede 74 years of age, a level of 8.8 μg BDE-47/kg lipid was reported (*13*). In the second study (*76*), adipose tissue from 77 individuals of both sexes, 28–85 years of age, some of whom were cancer patients, were analyzed. In persons without malignancies, the mean BDE-47 level was 5.1 μg/kg lipid, but in patients with non-Hodgkin lymphoma (NHL) it was 13 μg/kg lipid. (According to the authors a connection between PBDEs and NHL cannot be excluded.) In the third Swedish study, levels of several PBDEs in adipose and hepatic tissue from two male subjects (66 and 78 years of age) were reported (*77*). The mean levels of BDE-47 in these two types of tissue were 2 and 2–5 μg/kg lipid, respectively. In a Finnish study (*42*), the PBDE levels (sum of BDE-74, -99, and -153) in adipose tissue from randomly selected individuals (36-84 years of age) ranged from 6.2 to 22 μg/kg lipid.

Studies on human blood have demonstrated that 2,2′,4,4′-tetraBDE is the major PBDE congener (of six congeners analyzed) in serum samples from Swedish blood donors (n = 40; sampling year 1996) (*78*). The mean serum level of sumPBDE was 2.1 ± 1.4 μg/kg lipid, which is approximately two orders of magnitude lower than that of sumPCBs.

Results from analysis of PBDEs in Swedish breast milk are presented above ("PBDE Levels in Food") (*51,68*). PBDE levels in lipids are in the same range as those for adipose tissue and blood from Swedish individuals.

In a study on Swedish dismantling plant workers, levels of five PBDE congeners [BDE-47, BDE-153, BDE-154, BDE-183 (2,2′,3,4,4′,5′,6-heptaBDE), and BDE-209] were measured in serum (*72*). Among the dismantling workers hepraBDE congener

BDE-183 was the most abundant at the mean concentration of 8 μg/kg lipid, and also the mean concentration of BDE-209 was as high as 5 μg/kg lipid. The sumPBDE levels in groups of hospital cleaners, computer clerks, and electronics dismantlers were 3, 4, and 26 μg/kg lipid weight, respectively. Therefore, occupational exposure in dismantling plants may contribute significantly to human exposure to PBDEs. Nevertheless, PBDE levels of the dismantling workers are still rather low compared to PCB serum levels (26 μg sumPBDE and 270 μg PCB-153 per kg lipid, respectively).

Human Exposure to PBDEs: Summary and Conclusions

Sufficient data are available on PBDE levels in fish, whereas less is known about PBDE levels in other major food groups or about possible differences in food levels between countries or regions.

A recent intake study based on a complete set of Swedish occurrence (market basket) data from 1999 found PBDE intake from food to be 51 ng/day. The intake from mothers' milk during lactation, using data from Swedish studies, was estimated to be 110 ng/day. These estimates should be supplemented by studies from other countries and by using other methods to estimate intake to obtain a more complete picture of the intake of PBDEs from food.

Analytic data on human blood, adipose tissue, and milk have confirmed that PBDEs are also present in the human body. The major route of human exposure appears to be via food, although other routes of exposure cannot be excluded. Workers exposed to PBDEs during computer dismantling were shown to have significantly higher blood levels of several PBDE congeners than nonexposed subjects. Available data generally suggest that current levels of PBDE are more than an order of magnitude lower than those of PCBs. However, attention must be paid to exposure of infants from continously increasing levels of PBDEs in mothers' milk.

Toxicokinetics

Experimental Animals

Absorption and elimination/retention. Oral administration of ^{14}C-labeled decaBDE (BDE-209) to rats resulted in fecal excretion of > 90% of the dose 2 days after administration. Elimination in urine and in expired air was less than 1% over a period of 16 days, and accessible data seem to suggest minimal absorption of decaBDE from the gastrointestinal tract (*54,60,79*). In a carcinogenicity study (*80*), ^{14}C-labeled decaBDE was given in the diet to rats to quantify decaBDE absorption from the gastrointestinal tract. Results indicated that for all dose levels (250–50,000 mg/kg) more than 99% of the radioactivity recovered was eliminated in the feces within 72 hr. Urinary excretion accounted for about 0.01% or less of the dose; tissue levels of radioactivity were near the limit of detection, and in the liver, the radioactivity was confirmed as decaBDE. Similarly, El Dareer and co-workers (*81*) showed that < 1% of the dose of a radiolabeled decaBDE given orally to rats was found in tissues (about half in the liver) after 24 hr.

In a 2-year accumulation study, rats were maintained on diets providing up to approximately 1.0 mg technical decaBDE/kg body weight (bw) per day (of which 77.4% was the decaBDE congener, 21.8% nonaBDE, and 0.8% octaBDE). Selected tissues were analyzed for total bromine content. In most tissues (serum, liver, kidneys, skeletal muscle, and testes) the bromine contents were not above background, but in the adipose tissue bromine concentration was 3-fold that of controls (0.1 mg/kg bw/day). Regarding elimination, the moderate bromine accumulation in the adipose tissue remained unaffected during 90 days of recovery, whereas bromine was cleared from the liver within 10 days of recovery (*82,83*).

These data suggest that the bioaccumulation potential of decaBDE is low, but the retention in body fat may be pronounced. In comparison, the bioaccumulation of an octabromobiphenyl (belonging to the PBB group) was much more pronounced (*79*). Possible metabolism and/or debromination of decaBDE may interfere with the results.

Von Meyerink and co-workers (*84*) studied the retention of different fractions of Bromkal 70 in perirenal fat from rats. Bromkal 70 (mainly pentaBDE) was given as a single oral dose of 300 mg/kg bw, and the rats were killed after 1–10 weeks. Concentrations of the different PBDEs were analyzed by high pressure liquid chromatography, and gas chromatography–mass spectroscopy was used to confirm the degree of bromination (the stereochemistry of the individual compounds was not elucidated). Half-lives for two hexaBDE congeners were 50 and 105 days, respectively, and for two different penta congeners, 42 and 25 days. Half-life of tetraBDE(s) was 19–30 days, depending on sex of the subject. Half-life was shorter in males than in females.

In a bioaccumulation study in pike (*Esox lucius*) (*56*), 12 different halogenated diaromatic compounds, including three PBDE congeners, were administered in feed. Gastrointestinal bioavailability was 95% for BDE-47 (highest for all compounds studied), 60% for BDE-99, and 40% for BDE-153, indicating a clear decrease in uptake with increasing bromination.

Generally, lower-brominated PBDEs appear to be accumulated in biota to a greater extent than higher-brominated compounds. This is also evident from comparison of the Bromkal 70-5DE composition with the PBDE pattern in herring and seal: concentrations of tetraBDE (BDE-47) are increased in biota in relation to other congeners. Conversely, the relative concentrations were the same in Bromkal 70-5DE and in samples from herbivorous mammals in Sweden. According to Zitko (*85*), compounds with low bromine content are bioconcentrated more strongly from water than compounds with high bromine content. For example, PBBs with more than six bromine atoms were scarcely bioconcentrated; similar conditions may apply for PBDEs. In contrast, half-lives of PBDEs in adipose tissue of rats increase with the degree of bromination (*84*). It can be concluded that absorption of PBDEs, which favors lower-brominated congener groups, is an important determinant of bioaccumulation, at least in aquatic environments. Elimination of PBDE, on the other hand, may be slower for higher-brominated compounds, although they are less accumulative. However, data are sparse and somewhat contradictory.

Distribution. Tissue distribution of ^{14}C-decaBDE was studied in rats 16 days after a single oral dose (*54,60,79*). Radioactivity was observed only in the adrenal glands (0.01% of administered dose) and in the spleen (0.06% of administered dose) but not in any other tissue studied.

In the above-mentioned high-dose experiment with oral ^{14}C-decaBDE (*80*), the liver contained about 0.5 and 0.016% of the dose 24 and 72 hr, respectively, after dosing. Radioactivity extracted from the liver was found to be mainly the unchanged component. Traces of the label were found in the kidneys, spleen, lung, brain, muscle, fat, and skin. Distribution data after intravenous (iv) injection of labeled decaBDE indicate that feces collected during this period and the contents of the intestines altogether contained 74% of the dose, suggesting considerable biliary excretion. Radioactivity was also detected in the liver, kidneys, and lungs, and at lower concentrations in muscle, skin, and fat.

In a comparative study on radiolabeled BDE-47 in rats and mice (*12,16*), an effective absorption and accumulation in adipose tissue was shown. The study also indicated a marked species difference in degree of retention. In rats 86% of the oral dose (approximately 30 μmol/kg bw=14.6 mg/kg bw) was retained in the body within 5 days, compared to only 47% in mice. Radioactivity was about 3 times higher in fat than in liver in rats, whereas in mice the levels in the two tissues were comparable.

Metabolism. In studies by Norris and coworkers (*54,60,79*), rats were injected iv with ^{14}C- decaBDE, after which the radioactive

compounds in feces were analyzed. Of the excreted fecal label, 63% were unidentified metabolite(s) of decaBDE and 37% were unchanged decaBDE. El Dareer and coworkers (*81*) obtained similar results. They suggested that extensive metabolism of the orally administered compound might take place in the gastrointestinal tract.

Studies with ^{14}C-BDE-47 in rats and mice revealed five hydroxylated PBDE metabolites in feces and tissues, although BDE-47 was the major compound detected (*12,16*). A marked difference was found in urinary excretion between the mouse and rat. Within 5 days, rats excreted < 1% and mice excreted 33% of the dose in urine (the corresponding figures for fecal excretion, including nonabsorbed material, were 14 and 20%, respectively). The label in the mouse urine was highly hydrophilic and labile, and no specific metabolites could be identified. No debromination products of PBDEs were found. Thus, the metabolism of BDE-47 seems limited, at least in rats, but there are marked species differences in rodents in metabolism and excretion of this and probably other PBDE congeners.

Limited metabolism and excretion of another PBDE congener, BDE-99 (a pentaBDE), was observed in rats (*86*). Small amounts of mono-hydroxylated metabolites of penta- and tetraBDE were detected in feces, which indicates *in vivo* debromination. Mono- and dihydroxy pentaBDEs as well as two thio-substituted pentaBDEs were detected in bile.

Methoxylated PBDE congeners have been identified in Swedish fish and seal samples (*13*). These compounds may have been formed by *in vivo* methylation of hydroxylated PBDE metabolites. On the other hand, microbial formation or other biogenic sources are also possible.

In a toxicokinetic study (*53*), rainbow trout were administered technical decaBDE (containing small amounts of nona- and octaBDE) in the diet. PBDEs levels in muscle were monitored during a depuration period in clean water. The analysis revealed decreasing levels of decaBDE, whereas levels of BDE-153 increased continuously during the depuration period. The results indicate metabolic debromination of decaBDE to BDE-153 in fish.

Microsomal enzyme induction. PBDEs are able to induce both phase I and phase II xenobiotic metabolizing enzymes. Regarding the cytochrome P450 (CYP)-mediated phase I metabolism, CYP1A1 and CYP1A2 were induced, as indicated by the increased activity of liver microsomal 7-ethoxyresorufin *O*-deethylase (EROD) activity after Bromkal 70 exposure in Wistar rats (*84*) and in H-4-II cells (*87*). The other enzymes used as indicators of microsomal phase I activity, benzphetamine *N*-demethylase, *p*-nitroanisole demethylase, arylhydrocarbon hydroxylase (AHH), and benzo[*a*]pyrene hydroxylase, were also induced by PBDEs (technical pentaBDE preparations in rats) (*84,88,89*). Some of the enzymes were induced in a long-term oral administration study at doses as low as approximately 1 µmol/kg/day, and the enzyme activities continued 30–60 days after termination of the exposure (*89*). The full-brominated decaBDE, however, appears to have low enzyme-inducing potency.

The effects of BDE-47 and Aroclor 1254 on microsomal enzyme induction was studied in Sprague-Dawley rats (*90*). Induction of EROD and 7-methoxyresorufin-*O*-dœthylase activities by BDE-47 was limited, in contrast to the marked induction (> 100-fold) in Aroclor-exposed animals. The effect on the pentoxyresorufin analog, known to describe CYP2B activity, was, on the other hand, almost the same after treatment with both BDE-47 and Aroclor 1254. If induction capacity is indicative of metabolic pathways of these compounds, the CYP2B enzymes may be important in the metabolism of PBDEs.

In a recent study using a recombinant rat hepatoma cell line H4IIE with a luciferase reporter gene, several PBDE congeners acted as Ah-receptor agonists (*91*). In this model potencies of the agonists were comparable to those of some mono-*ortho* PCBs. Some PBDE congeners also had antagonistic effects on TCDD-induced luciferase production.

Halogenated dioxinlike compounds typically induce CYP1A1 and 1A2. Therefore, enzyme induction may be due to impurities with Ah-receptor binding affinity present in technical PBDE mixtures. It was shown that PCDF impurities at concentrations < 1% could completely account for the observed EROD activity of all except one of 29 tested polychlorinated diphenyl ether (PCDE) congeners (*92*). This study demonstrated that in studies with halogenated DEs, it is important to control the presence of potent dibenzofuran and dibenzodioxin impurities, which even at low concentrations can account for considerable biologic activity attributed to the relatively high dosages required for DEs. The outcome of this study is in agreement with structural considerations suggesting that the nonplanarity of halogenated DEs results in a low binding affinity to the Ah receptor (*93*). Nevertheless, some studies have shown that pure PCDEs indeed are weak inducers of microsomal EROD and AHH activities in a congener- and conformation-specific manner (*94–97*).

In studies on phase II induction, three different PBDE fractions were tested; i.e., low (24% tetra, 50% penta) and high (45% hepta, 30% octa) brominated mixtures, and the decaBDE congener only. After daily oral administrations (14 days, 0.1 mmol/kg bw), both of the mixtures, but not the decaBDE, resulted in long-lasting induction of uridine diphosphate glucuronyltransferase (UDPGT) activity in rats (*88*).

Human Data

As mentioned previously, PBDEs are found in human blood and tissues and in human breast milk. Data show that PBDEs are also absorbed and retained in man and that BDE-47 is the most abundant congener in most cases. Today, data are too limited to estimate the degree of human bioavailability and bioaccumulation. However, Sjödin (*98*) estimated the elimination half-lives of certain PBDE congeners in humans. Calculations were based on blood levels taken before and after vacation of workers in an electronics dismantling plant (*72*). By this method, the estimated half-lives of BDE-183 and BDE-209 were set to 86 days and 6.8 days, respectivdy. The levels of BDE-47 and BDE-153 were also determined in the same manner, but because no clear decrease during vacation could be observed, the half-lives could not be estimated.

These results suggest that the elimination half-lives of PBDEs increase with degree of bromination. This suggestion contrasts with results in rats, where the reverse seems to be true, at least regarding tetra- to hexaBDEs (*84*). Results on elimination of PBDEs increase with degree of bromination in humans are quite different than those found for PBBs and PCBs, where increases in halogenation generally extend elimination half-lives.

Toxicokinetics: Summary and Conclusions

DecaBDE is minimally absorbed from the gastrointestinal tract of mammals because of its relatively high-molecular mass, and therefore it is unlikely to bioaccumulate. In fish, however, uptake of decaBDE and subsequent debromination to lower-brominated PBDEs has been demonstrated. There is no information on uptake or elimination of octaBDEs in mammals. OctaBDEs are found in human adipose tissue and in aquatic sediments, but bioaccumulation is not likely to be substantial because of low absorption. PentaBDEs are found in biota, sediment, and sewage sludge samples. They are likely to be persistent and to bioaccumulate, and a bioconcentration factor of > 10,000 has been determined for carp. A commercial tetraBDE mixture accumulates and is persistent in many organisms in the environment.

Penta- and tetraBDEs are quite persistent in the environment. Some mammalian uptake and elimination studies suggest effective absorption and only moderate retention. Considerable species differences have been reported in metabolism and excretion of the tetraBDE congener BDE-47. The rat appears

to have a much slower metabolism and (urinary) excretion than the mouse. Formation of hydroxylated, thiol-substituted as well as debrominated metabolites of BDE-47 and BDE-99 has been demonstrated in rats. Methoxylated PBDE analogs have been found in samples from fish and seal. Possibly they are formed by *in vivo* methylation of hydroxylated PBDE metabolites or by microbiologic transformation.

Toxicology

No complete toxicologic evaluation is currently available on any of the commercially available PBDE mixtures or on any individual congener. Most studies have been performed using commercial PBDEs that are more or less unspecified mixtures of congeners and isomers. Consequently, a limited amount of data is available about congener-specific effects. An important question concerning PBDEs is whether because of their structural similarity to the highly toxic polyhalogenated aromatic hydrocarbons, such as polyhalogenated biphenyls, dibenzo-*p*-dioxins, and dibenzofurans, PBDE effects could occur by the same mechanism(s) of toxicity. It is also important to determine if contaminants in technical PBDE mixtures exert toxic effects, and if so, through which mechanism(s).

In this section, we examine available toxicity data on PBDEs. On the basis of existing data, a lowest-observed-adverse-effect level (LOAEL) value for the whole PBDE group is proposed. The collected data are used in the next section, "Toxicological Evaluation."

Experimental Studies in Animals

Toxicologic information on PBDEs is based on both published studies and research reports made available recently by the chemical industry (*2,7*). A summary of the data from studies on the acute toxicity, irritation, sensitization, genotoxicity and other short-term effects of PBDEs is presented in Table 4. Available

Table 4. Summary of the acute toxicity, irritation, sensitization, genotoxicity, and porphyrinogenic activity of PBDEs.

Study	PBDE	Test system	Result	Reference
DecaBDE				
Acute toxicity	DecaBDE (commercial)			
Oral LD_{50}		Rat, f (Sprague-Dawley)	> 2 g/kg bw	(*2,54,79*)
		Rat, m (Spartan)	> 5 g/kg bw	(*2*)
Dermal LD_{50}		Rabbit, m+f /New Zealand white)	> 2 g/kg bw (exposure for 24 hr)	(*2*)
Inhalation LC_{50}			> 48,2 mg/l (exposure for 1 hr) Rat, m+f (Spartan)	(*2*)
Skin irritation	DecaBDE (commercial)	Rabbit, m+f (New Zealand white)	No irritation - slight erythema and edema (on abraded skin)	(*2,54,79*)
Chloracnegenic activity	Saytex 102[a]	Rabbit, m+f (New Zealand white)	Negative	(*2*)
	DecaBDE (commercial)	Rabbit (not specified)	Negative	(*2*)
Eye irritation	Saytex 102	Rabbit, m+f (New Zealand white)	Transient redness and chemosis	(*2,54,79*)
Skin sensitization	DecaBDE (commercial)	Human volunteers	Negative	(*2,54,79*)
Genotoxicity				
Mutagenicity	HFO 102[b]	*S. typhimurium*	Negative	(*2*)
	Not specified	*S. cerevisiae*	Negative	(*2*)
	DecaBDE (commercial)	Mouse lymphoma L5178Y cells	Negative	(*2*)
Chromosomal effects	DecaBDE (commercial)	Chinese hamster ovary cells	Negative	(*2*)
	DecaBDE (commercial)	Rat bone marrow *in vivo*	Negative	(*2,54,79*)
Porphyrinogenic activity	DecaBDE (commercial)	Chick embryo liver cells	Negative	(*100*)
OctaBDE				
Acute toxicity				
Oral LD_{50}	Not specified	Rat (not specified)	> 28 g/kg bw	(*2*)
	OctaBDE (commercial)	Rat, m (Charles River CD)	> 5 g/kg bw	(*2*)
Dermal LD_{50}	OctaBDE (commercial)	Rabbit, m+f (albino)	> 2 g/kg bw (exposure for 24 hr)	(*2*)
Inhalation LC_{50}	OctaBDE (commercial)	Rat, m+f (Charles River CD)	> 60 mg/L (exposure for 1 hr)	(2)
	Saytex 111[c]	Rat (not specified)	> 50 mg/L	(*2*)
Skin irritation	OctaBDE (commercial)	Rabbit, m+f (albino)	No irritation - slight erythema	(*2*)
Eye irritation	OctaBDE (commercial)	Rabbit, m+f (New Zealand white)	No irritation - slight redness	(*2*)
Genotoxicity	OctaBDE (commercial)			
DNA damage		WI-38 human fibroblasts	Negative	(*2*)
Mutagenicity		*S. typhimurium*	Negative	(*2*)
		S. cerevisiae	Negative	(*2*)
Chromosomal effects		CHO cells	Negative	(*2*)
Porphyrinogenic activity	OctaBDE (commercial)	Chick embryo liver cells	Strong porphyrinogenic effect	(*100*)
PentaBDE				
Acute toxicity				
Oral LD_{50}	PentaBDE (commercial)	Rat, m (Charles River CD)	0.5 – 5 g/kg bw	(*2*)
		Rat, m+f (Wistar)	m: 7.4 g/kg bw	(*2*)
Dermal LD_{50}		Rabbit, m+f (New Zealand white)	> 2 g/kg bw (exposure for 24 hr)	(2)
Inhalation LC_{50}		Rat, m+f (Charles River CD)	> 200 mg/L (exposure for 1 hr)	(2)
Genotoxicity				
Mutagenicity	PentaBDE (commercial)	*S. typhimurium*	Negative	(*2*)
		S. cerevisiae	Negative	(*2*)
Porphyrinogenic activity	DE-71[d]	Rat, m+f (Sprague-Dawley CD)	Highly increased concentrations of porphyrins in liver and urine after oral dosing at 100 mg/kg bw/day for 13 weeks.	(*2*)

Abbreviations: f, female; m, male.
[a]Saytex 102 is a technical decaBDE product. [b]HFO 102 is a technical decaBDE product. [c]Saytex 111 is a commercial mixture containing pentaBDE, 0.2%; hexaBDE, 8.6%; heptaBDE, 45%; octaBDE, 33.5%, nonaBDE, 11.2%; and decaBDE, 1.4% (*2*). [d]DE-71 is primarily a mixture of tetra-, penta-, and hexaBDE containing low levels of tri- (> 1%) and heptaBDE (< 2%) (*2*).

information on subchronic and chronic toxicity, carcinogenicity, and teratogenicity is given in Table 5.

Acute toxicity and local irritation. In general, the acute toxicity of PBDEs (administered orally, dermally, or by inhalation) is low (Table 4). The low toxic potency of fully brominated BDE-209 is likely to be explained by poor absorption after oral administration (*54,60,79,80*). Available data are not suitable for use in even limited structure–toxicity correlations. PentaBDEs appeared to be more toxic after oral administration than octa- and decaBDEs, as they caused clear signs of toxicity and mortality primarily at high doses (oral LD_{50} in rats 0.5–5 g/kg or 7.4 g/kg, depending on the study; Table 4). The clinical signs were

Table 5. Summary of the repeated dose toxicity, carcinogenicity and special toxicity studies on PBDEs.

Study	Species (strain)	PBDE (purity)	Dosage (mg/kg bw/day)	Effects (dose level for observed effect)	Reference
DecaPDE					
Subacute toxicity (14 days)	Mouse ($B6C3F_1$)	DecaBDE (99%)	In diet: 0, 3,000, 7,500 or 15,000[a]	No compound-related effects	(*80*)
	Rat (Fischer 344/N)	DecaBDE (99%)	In diet: 0, 500, 2,000 5,000 or 10,000[a]	No compound-related effects	(*80*)
Subacute toxicity (28 days)	Rat (Charles River CD)	DecaBDE (commercial)	In diet: 0, 10, or 100[a]	No compound-related effects	(2)
(30 days)	Rat (Sprague-Dawley)	DecaBDE (77.4%), NonaBDE (21.8%), OctaBDE (0.8%)	In diet 0, 8, 80, or 800	Thyroid - hyperplasia (≥ 80); liver - enlarged (≥ 80); kidney - hyaline degeneration (≥ 80); hematocrit and red cell count - decreased (800)	(2,*54,79*)
Subchronic toxicity (13 weeks)	Mouse ($B6C3F_1$)	DecaBDE (> 97%)	In diet: 0, 465, 930, 1,875, 3,750, or 7,500[a]	No compound-related effects	(*80*)
	Rat (Fischer 344/N)	DecaBDE (> 97%)	In diet: 0, 310, 620, 1,250, 2,500, or 5,000[a]	No compound-related effects; liver weights not recorded, in subsequent studies; liver - enlarged (≥ 2,500)	(*80*)
Chronic toxicity, carcinogenicity (103 weeks)	Mouse ($B6C3F_1$)	DecaBDE (94–99%)	In diet: 0, 3,200, or 6,650 (males), 0, 3,760, or 7,780 (females)	Liver - hypertrophy, granulomas, adenomas, carcinomas (in males ≥ 3,200); thyroid - follicular cell hyperplasia, adenomas, carcinomas (≥ 3,200)	(*80*)
	Rat (Fischer 344/N)	DecaBDE (94–99%)	In diet: 0, 1,120, or 2,240 (males), 0, 1,200, or 2,550 (females)	Liver - adenomas (≥ 1,120), thrombosis, degeneration (in males 2,240); pancreas - acinar cell adenomas (in males 2,240); spleen - hyperplasia (in males 2,240; lymphoid tissue - hyperplasia (in males 2,240)	(*80*)
OctaBDE					
Subacute toxicity (28 days)	Rat (Charles River CD)	OctaBDE (commercial)	In diet: 0, 10, or 100[a]	Liver - enlarged, cytoplasmic eosinophilic round bodies (≥ 10); thyroid - hyperplasia? (100)	(2)
	Rat (Charles River CD)	OctaBDE (commerical)	In diet: 0, 10, 100, or 1,000[a]	Liver - hepatocytes enlarged with cytoplasmic eosinophilic round bodies (≥ 10), whole liver enlarged (≥ 100), hepatocytes vacuolized with focal necrosis (1,000)	(2)
Subchronic toxicity (13 weeks)	Rat (Charles River CD)	OctaBDE (commercial)	In diet: 0, 10, 100, or 1,000[a]	Liver - enlarged, cytoplasm granular (≥ 10), hepatocytes vacuolized, hyaline intracytoplasmic inclusions, hyperplastic nodules (≥ 100), accentuated lobulation, yellowish mottling, brownish discoloration, focal necrosis, centrilobular fibrosis, pigmented Kupfer cells (1,000); thyroid - enlarged (≥ 100), cellular changes (1,000); kidney - enlarged, cortical regenerative tubules, a single case of tubular nephrosis (1,000); hemoglobin, hematocrit, erythrocyte count - decreased (1,000); blood glucose - decreased (1,000); urine - orange discoloration (1,000); bw gain - decreased (≥ 100)	(2)
PentaBDE					
Subacute toxicity (28 days)	Rat (Charles River CD)	PentaBDE (commercial)	In diet: 0, 10, or 100[a]	Liver - enlarged, cytoplasmic eosinophilic round bodies (≥ 10); thyroid - follicular hyperplasia? (≥ 10)	(2)
Subchronic toxicity (90 days)	Rat (Sprague-Dawley CD)	DE-71[b]	In diet: 0, 2, 10, or 100	Serum thyroxin -decreased (≥ 10); thyroid hyperplasia - (≥ 10); liver - enlarged, hepatocytomegaly (≥ 10); liver and urine porphyrins - increased (100); serum cholesterol - increased (100); food consumption - decreased (in females 100); bw - decreased (100)	(2)
Special single dose toxicity	Mouse (C57BL/6)	DE-71[b]	By gavage: 0, 0.8, 4, 20, 100, or 500	Serum thyroxin - decreased (≥ 0.8?)	(*104*)
Special repeated dose toxicity (14 days)	Mouse (C57BL/6)	DE-71[b]	By gavage: 0, 18, 36, or 72	Serum thyroxin - decreased (≥ 18)	(*104*)
	Mouse (C57BL/6	Bromkal 70-5 DE[c]	By gavage: 0, 18, or 36	Serum thyroxin - decreased (≥ 18) Liver somatic index - increased (36)	(*130*)
	Rat (Sprague-Dawley)	Bromkal 70-5 DE[c]	By gavage: 0, 18, or 36	Serum thyroxin - decreased (≥ 18) Liver somatic index - increased (36)	(*130*)
TetraBDE					
Special repeated dose toxicity (14 days)	Mouse (C57BL/6)	2,2',4,4'-TetraBDE (> 98%)	By gavage: 0 or 18	Serum thyroxin - decreased (18) Liver somatic index - increased (18)	(*130*)
	Rat (Sprague-Dawley)	2,2',4,4'-TetraBDE (> 98%)	By gavage: 0, 1, 6, or 18	Serum thyroxin - decreased (18)	(*90*)

?, it was not clear for the authors of the original report whether or not the compound was compound related.
[a]The daily dose (in mg/kg bw/day) is estimated based on dietary PBDE concentrations and the generally used average daily food consumption of 10 g/day in young rats, 20 g/day in older rats, and 3 g/day in mice. [b]DE-71 is primarily a mixture of tetra-, penta-, and hexaBDE containing low levels of tri- (> 1%) and heptaBDE (< 2%) (2). [c]Bromkal 70-5 DE is a commercial mixture containing about 60% pentaBDE and 40% tetraBDE (2).

reduced growth, diarrhea, piloerection, reduced activity, clonic persistent tremors of forelimbs, red staining around eyes and nose, and continuous chewing. Acute toxicity of diBDE (purity and isomer profile not specified) has only been studied in mice after intraperitoneal (ip) administration (*2,99*). The ip LD_{50} value of 125 mg/kg clearly was lower than those reported for the higher-brominated congeners in rats after oral administration. This difference, however, does not conclusively reflect a higher potency of diBDE; it may have resulted from different routes of administration and differences in species sensitivity.

Skin and eye irritation studies with octa- and decaBDEs revealed only slight or no local irritation in rabbits (*2,54,79*). DecaBDE was not chloracnegenic in rabbits (*2*). In human volunteers decaBDE did not cause skin irritation or skin sensitization (*2,54,79*).

Subacute/subchronic toxicity. Subacute/subchronic oral toxicity studies in rats have been carried out with deca-, octa- and pentaBDE preparations (Table 5). In general, effects were less pronounced with decaBDE than with the other two congener groups. Target organs were liver, kidney, and thyroid gland. These organs were generally enlarged with or without some degenerative changes. In addition, hepatocellular necrosis was observed in both oral and inhalation toxicity studies with octaBDEs. Toxic effects were noted at daily dietary doses of 10 mg/kg bw and above for penta- and octaBDE. DecaBDE was effective at 80 mg/kg bw in one study, whereas another study showed effects only at much higher doses or no effects at all (*54,79*).

Porphyrinogenic activity. Chronic hepatic porhyria is a condition characterized by a liver disorder resulting in production and excretion of excess porhyrins. A commercial octaBDE preparation (10 µg/mL) was strongly porphyrinogenic in cultured chick embryo liver cells after incubation for 24 hr (*100*). Exposure of rats to a commercial pentaBDE in the diet (100 mg/kg bw/day) for 13 weeks resulted in highly increased liver and urine concentrations of porphyrins [unpublished data, cited in IPCS (*2*)]. It is interesting to note that octa- and pentaBDEs share the porphyrinogenic activity of dioxins, PCBs, and PBBs (*101–103*).

Immunotoxicity. In a study by Fowles and co-workers (*104*), immunologic (and other) effects of the PBDE preparation DE-71 [a mixture of tetra-, penta-, and hexaBDE with low levels of tri- (< 1%) and heptaBDE (< 2%)] were monitored using sheep erythrocyte (SRBC) plaque-forming cell response and natural killer (NK) cell activity. C57BL/56 mice were orally exposed to single DE-71 doses of up to 500 mg/kg, or to subcronic daily doses of 72 mg/kg bw over a 14-day period, totaling 1,000 mg/kg bw. Suppression of the anti-SRBC response was seen only in the mice exposed subchronically; this exposure also resulted in decreased thymus weight. NK cell activity was not altered.

Immunotoxic potential of Bromkal 70-5DE (a commercial mixture containing about 60% of pentaBDE and 40% of hexaBDE) was compared with that of a commercial PCB preparation—Aroclor 1254—in Sprague-Dawley rats and C57BL mice (*105*). The animals were given the preparations at 10–36 mg/kg bw/day for 14 days by oral gavage. Mice were also exposed to BDE-47 and PCB-105. The study showed that the exposure to PCBs induces certain immunologic alterations in both species, but signs of immunotoxicity after PBDE exposure were observed only in mice. The reduction in splenocyte numbers in mice was markedly decreased after BDE-47 exposure (18 mg/kg/day). Moreover, Bromkal 70 treatment at 36 mg/kg resulted in decreased production of IgG antibodies from pokeweed-stimulated splenocyte cultures *ex vivo*.

In an *in vitro* study on cultured human lymphocytes, the effects of two pure PBDE congeners [BDE-47 and BDE-85 (2,2′,3,4,4′-pentaBDE)] and three PCBs [2,3,3′,4,4′-pentaCB (PCB-105), 2,3′,4,4′,5-pentaCB (PCB-118), and 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB-153)] on cell proliferation were studied (*106*). None of the chemicals significantly altered the proliferative response compared with control cells. Not even the PCBs affected the lymphocyte proliferative response, although PCBs are commonly known to be immunotoxic *in vivo*. The known discrepancies between *in vivo* and *in vitro* effects of polyhalogenated aromatic hydrocarbons on immunologic parameters may be a result of indirect effects of these compounds on immune function *in vivo*, e.g., via possible Ah receptor-mediated hormone interactions (*107*). Consequently, if PBDEs and PCBs affect the immune system via the same mechanism(s), this method may have limited relevance for predicting the immunologic effects of PBDEs *in vivo*.

The immunosuppressive potencies of eight isomers of tetra-, penta-, or hexachlorinated DEs were determined using the mouse splenic plaque-forming cell (PFC) response to sheep red blood cell antigens (*95*). The isomers dose dependently decreased the number of PFCs, with ED_{50} values between 0.19 and > 151 mg/kg (given as a single ip dose). The immunotoxic effect appears to be highly isomer specific, as there were remarkable differences between different isomers of penta- and hexachlorinated DEs.

Reproductive and developmental toxicity. Reproductive toxicity of PBDEs has been studied using commercial deca-, octa-, and pentaBDE preparations and Saytex 111, a commercial mixture of PBDEs containing several congeners from penta- to decaBDE, of which the hepta- and octaBDEs are most abundant (45 and 34% of all congeners, respectively) (Table 6). One of the studies is a rat reproductive performance study with decaBDE. All other studies are teratogenicity studies, and only Saytex 111 has been studied in both rats and rabbits.

Effects of decaBDE on reproductive performance were studied in male and female Sprague-Dawley (Spartan) rats given commercial decaBDE in the diet in doses of 0, 3, 30, or 100 mg/kg bw/day (*2,54,79*). Group sizes were 20 males and 40 females (control group), 10 males and 20 females (the low- and mid-dose group) and 15 males and 30 females (the highest-dose group). Treatment began 60 days before mating and continued throughout gestation and lactation. No treatment-related changes were reported in reproductive performance or maturation of pups.

In a teratogenicity study with Sprague-Dawley (Spartan) rats, commercial decaBDE was given in doses of 0, 10, 100, or 1,000 mg/kg bw/day by oral gavage on gestational days 6–15; fetuses were collected by cesarean section on gestational day 21 (*2,54,79*). No maternal toxic effects in terms of clinical signs, body weight gain, food consumption, or liver weights were observed. Similarly, the number of corpora lutea, positions and numbers of fetuses *in utero*, individual fetal weights, crown–rump lengths, and sex ratios were not affected by the treatment. However, significantly increased incidences of resorption were observed at the lower dose levels but not at the 1,000 mg/kg bw/day dose. In the absence of numeric as well as historical control data, the possibility of embryolethality cannot be ruled out. No external abnormalities were observed in fetuses, but soft tissue and skeletal examinations revealed an increased number of litters with pups with subcutaneous edema and delayed ossification of normally developed bones of the skull at the dose level of 1,000 mg/kg bw/day. Analysis of maternal and fetal livers for bromine concentration (reflecting liver concentration of decaBDE) showed significantly increased concentrations only in maternal livers at the 1,000 mg/kg bw/day dose. Although this study is inadequately reported, it suggests that decaBDE is not teratogenic but clearly fetotoxic at dose levels not maternally toxic.

Teratogenicity of a commercial octaBDE preparation was studied in rats (strain and number of animals not specified) receiving the test compound by gavage at 0, 2.5, 10, 15, 25, or 50 mg/kg bw/day on gestational days 6–15 (*2*). Reduced maternal body weight gain and slightly increased cholesterol levels, but no histopathologic changes in livers or kidneys were observed at the 50 mg/kg bw/day. These

maternal effects were associated with marked fetal toxicity as indicated by increased numbers of late resorptions, significantly reduced mean fetal weights, severe generalized edema (anasarca), reduced ossification of the skull, and various unossified bones. In addition, developmental variations such as bent limb bones and bent ribs were reported at the 50 mg/kg bw/day dose. No treatment-related effects were observed at 15 mg/kg bw/day or lower, but the findings at 25 mg/kg bw/day were not discussed. Therefore, the no-observed-effect level (NOEL) for maternal toxicity is 25 mg/kg bw/day and for fetal effects 15 mg/kg bw/day (*2*).

Teratogenicity of the commercial Saytex 111 mixture was studied in four groups of 25 Charles River (Sprague-Dawley) rats (*63*). They were administered corn oil suspensions of the test substance by gavage at 0, 2.5, 10, or 25 mg/kg bw/day on gestational days 6–15. Fetuses were examined on day 20 for gross visceral and skeletal abnormalities. The test substance was found to be more toxic to the fetuses than to the dams. It resulted in a dose-dependent reduction of fetal body weight at the two highest dose levels. At 25 mg/kg/day it also increased the number of early and late resorptions, delayed skeletal ossification, and induced fetal malformations such as enlarged heart and rear limb malformations (type of malformation not specified).

Teratogenicity of Saytex 111 was also studied in groups of 26 New Zealand white rabbits by the Dow Chemical Company (*108*). The rabbits were administered the test substance by gavage at 0, 2, 5, or 15 mg/kg bw/day on gestational days 7–19, and the fetuses collected on gestational day 28. Approximately half of the fetuses in each litter were randomly assigned for soft tissue examination. In addition, all fetuses were examined for skeletal alterations. Maternal body weight showed a dose-dependent decrease that was statistically significant only at 15 mg/kg bw/day (93% of control weight). Also, the absolute and body weight-related liver weights were increased at this dose level. One rabbit at 5 mg/kg bw/day and two rabbits at 15 mg/kg bw/day delivered their litters prior to gestational day 28. In addition, one rabbit at 15 mg/kg bw/day was killed after exhibiting signs of abortion. This animal had multiple resorption sites in the uterus. Except for these animals, the number of resorptions was not affected by the treatment. Signs of fetal toxicity were slight (nonsignificant) decreases in fetal body weights at 5 and 15 mg/kg bw/day and increased incidences of delayed ossification of the hyoid, dental process (at 5 mg/kg/day only), and sternebrae at 2, 5, and 15 mg/kg bw/day (statistically significant only at 15 mg/kg bw/day). Treatment-related fetal variants included increased incidences of retrocaval ureter and fused sternebrae at all dose levels of Saytex 111, with the maximum incidence at 5 mg/kg/day (statistically significant). These variants were absent from the concurrent

Table 6. Summary of the reproduction and developmental toxicity studies on PBDEs.

Study	Species (strain)	PBDE (purity)	Dosage (mg/kg bw/day)	Effect (dose level for observed effect)	Reference
DecaBDE					
Reproductive performance	Rat (Sprague-Dawley)	DecaBDE (77.4%), NonaBDE (21.8%), OctaBDE (0.8%)	In diet: 0, 3, 30, or 100	No compound-related effects	(*2,54,79*)
Teratogenicity	Rat (Sprague-Dawley)	DecaBDE (77.4%) NonaBDE (21.8%) OctaBDE (0.8%)	By gavage: 0, 10, 100, or 1000	Fetal toxicity - resorptions (100), subcutaneous edema, delayed ossification (1,000)	(*2,54,79*)
OctaBDE					
Teratogenicity	Rat (not specified)	DE-79	By gavage: 0, 2.5, 10 15, 25 ,or 50	Fetal toxicity - late resorptions, weight decreased, anasarka, bent limp bones, reduced ossification, bent ribs (50); maternal toxicity - bw decreased, serum cholesterol increased (50)	(*2*)
Teratogenicity	Rat (Charles River Crb: COBS CD (SD) BR)	Saytex 111[a]	By gavage: 0, 2.5, 10, or 25	Fetal toxicity - weight decreased (≥ 10, resorptions delayed ossification (25); fetal variations/ malformations - enlarged heart, rear limb malformation (25)	(*63*)
	Rabbit (New Zealand white)	Saytex 111[a]	By gavage: 0, 2, 5, or 15	Fetal toxicity - delayed ossification (≥ 2), weight decreased (≥ 5), resorptions? (15); fetal variations/ malformations - retrocaval ureter (≥ 5), fused sternebrae (5); maternal toxicity - bw gain decreased, liver enlarged (15)	(*108*)
PentaBDE					
Teratogenicity	Rat (not specified)	PentaBDE (commercial)	By gavage: 0, 10, 100, or 200	Maternal toxicity - bw gain decreased (≥ 100), fetal toxicity - weight decreased (200)	(*2*)
Special developmental toxicity	Mouse (NMRI)	2,2', 4,4',5-PentaBDE	By gavage: 0, 0.8, or 12 on postnatal day 10	Developmental neurotoxicity - impaired habituation (locomotion, rearing, total activity) in adulthood; worsens with aging; impaired learning and memory functions (12)	(*111*)
Special developmental toxicity	Mouse (not specified)	2,2',4,4',5-PentaBDE	By gavage: 0 or 8 on postnatal day 3, 10, or 19	Developmental neurotoxicity - impaired spontaneous motor behavior in adulthood; nicotine-induced hypoactivity (8)	(*113*)
Special developmental toxicity	Rat (not specified)	DE-71[b]	By gavage: 0, 1, 10, or 30 to dams on gestational day 6–postnatal day 21	Offspring toxicity - serum thyroxin decreased (≥ 1) Maternal toxicity - serum thyroxin decreased (30)	(*132*)
TetraBDE					
Special developmental toxicity	Mouse (NMRI)	2,2',4,4'-TetraBDE	By gavage: 0, 0.7, or 10.5 on postnatal day 10	Developmental neurotoxicity - impaired habituation (locomotion, rearing, total activity) in adulthood; worsens with aging (10.5)	(*111*)

[a]Saytex 111 is a commercial mixture containing pentaBDE, 0.2%; hexaBDE, 8.6%; heptaBDE, 45%; octaBDE, 33.5%; nonaBDE, 11.2%; and decaBDE, 1.4% (*2*). [b]DE-71 is primarily a mixture of tetra-, penta, and hexaBDE containing low levels of tri- (> 1%) and heptaBDE (> 2%) (*2*).

controls, but they were reported to have occurred at relatively high frequencies among some historical controls. This fact and the lower incidence at 15 mg/kg bw/day compared to 5 mg/kg bw/day led the authors (*108*) to consider these variants spontaneous. Nevertheless, we consider that the results indicate a PBDE-induced increase in the incidence of fetal variations. Isolated incidences of fetal malformations were observed in all groups including controls, but they were not considered to represent a teratogenic effect. We concluded that Saytex 111 caused fetal toxicity and variants at maternally nontoxic dose levels.

A teratogenicity study with a commercial pentaBDE preparation was carried out in rats (strain and number of animals not specified) (*2*). The test compound was suspended in corn oil and given by gavage at 0, 10, 100, or 200 mg/kg/day on gestational days 6–15. Maternal body weight gain was decreased at 100 and 200 mg/kg/day, and a slight (nonsignificant) reduction of fetal body weight was observed at 200 mg/kg/day.

Treatment of pregnant mice and rats with certain pure PCDE congeners on gestational days 6–15 resulted in embryolethality as indicated by the decreased number of litters or pups born to dams administered the 100 mg/kg/day dose (*109*). In mice, the most potent congeners were 2,3′,4′,6-tetraCDE and 2,2′,4,5,6′-pentaCDE, but also 2,2′,4,4′,5,6′-hexaCDE and 2,2′,4,4′,5,5′-hexaCDE caused a slight but statistically significant effect. The other five congeners studied did not show any signs of embryotoxicity at dose levels up to 100 mg/kg/day. In rats, 2,3′,4′,6-tetraCDE, 2,2′,4,5,6′-pentaCDE and 2,2′,4,4′,5,6′-hexaCDE were studied; all were embryotoxic in terms of number of pups born or pup weight. No structural hallmarks in the PCDE molecule related to embryotoxicity could be established. However, a common feature of the congeners causing prenatal mortality was at least two chlorine substituents at the *ortho* position (although not all congeners with this property caused prenatal mortality). Induction of CYP enzymes did not correlate well with the ability of the congeners to cause embryotoxicity.

Reproduction and developmental toxicity studies showed that in general fetuses are more sensitive to PBDEs than mothers, and that the increased incidence of developmental variants is a frequent fetal effect. Although it is known that maternal toxicity can influence fetal ossification (*110*), the fetal effects seem to appear at lower doses than those indicative of maternal toxicity (manifested as decreased bw gain and increased liver weight in some cases).

Neurotoxicity. The developing central nervous system is a potential target for toxicity of PBDEs. Neonatal mice given a single oral dose of BDE-47 (10.5 mg/kg bw) or BDE-99 (12.0 mg/kg bw) on postnatal day 10 (coincident with the rapid brain growth period) had permanent impairment of spontaneous motor behavior in adulthood (*111*). The latter congener also affected learning and memory functions. Further studies with BDE-99 confirmed these permanent behavioral effects (*112,113*). Furthermore, studies with ^{14}C-BDE-99 revealed concentrations in the brains of 10-day-old mice similar to those observed for other substances (DDT and certain PCB congeners) that induced the same type of behavioral effects in earlier studies. The neurodevelopmental toxicity of PBDEs appears to involve changes in the cholinergic system and may also be related to altered thyroid homeostasis. The latter hypothesis is based on the fact that brain development is highly dependent on thyroid hormones (*114*). PCBs and PCDDs that, like PBDEs, alter the thyroid hormone homeostasis have been suggested to disrupt brain development, resulting in permanent neurologic damage (*115,116*).

Genotoxicity. The genotoxic potential of commercial grade deca-, octa-, and pentaBDEs has been examined in several, mostly unpublished, studies (*2*). Mutagenicity tests carried out on four strains of *Salmonella typhimurium* with a technical product of decaBDE (HFO-102) and commercial decaBDE with and without metabolic activation were negative. Similarly, studies in eukaryotic cells utilizing yeast (*Saccharomyces cerevisiae*) and the TK locus of the mouse lymphoma cell line L5178Y with and without metabolic activation were negative. Commercial decaBDE did not induce chromosomal aberrations or sister chromatide exchanges in Chinese hamster ovary cells with or without metabolic activation. Results were also negative in cytogenetic examination of bone marrow cells from parent rats exposed to decaBDE (at dose levels up to 100 mg/kg/day in a reproduction study) and their weanlings (*54,79*).

A commercial octaBDE preparation was found to be negative in unscheduled DNA synthesis assay in the human fibroblast cell line WI-38 with and without metabolic activation. It neither induced mutations in *S. typhimurium* or *S. cerevisiae* nor caused sister chromatide exchanges in Chinese hamster ovary cells with or without metabolic activation. Mutagenicity studies with a commercial pentaBDE preparation in four strains of *S. typhimurium* and in *S. cerevisiae* with and without metabolic activation were all negative (*2*).

In two assays for intragenic recombination at an endogenous mammalian cell locus, lower-brominated DEs (2-monobromoDE and 3,4-dibromoDE) as well as other brominated flame retardants (TBBP-A and HBCD) significantly increased the recombination frequency (*117*). The possible role of this type of increased intragenic recombination in human diseases remains to be clarified.

In conclusion, these studies, although not complete state-of-the-art genotoxicity test batteries, suggest that none of the PBDEs examined are genotoxic. However, the possible increase in intragenic recombination, as observed for the low-brominated DEs, should be noted.

Carcinogenicity. Rodent carcinogenicity bioassays have been carried out only for decaBDE (*2,118*). A mouse study and a rat study have been reported in the National Toxicology Program (NTP) report (*80*) (Table 4), and a rat study of limited value has been conducted by the Dow Chemical Company (*83*).

In the NTP mouse study (*80*), decaBDE (purity 94–99%; no brominated dioxins or furans found) mixed in the diet was given to groups of 50 male and 50 female B6C3F$_1$ mice for 103 weeks; all survivors were killed at 112–113 weeks of age. The concentration of decaBDE in the diet was 0, 25, and 50 g/kg. The average daily exposure to decaBDE was estimated to be 3,200 and 6,650 mg/kg bw/day in low- and high-dose males, respectively, and 3,760 and 7,780 mg/kg bw/day in low- and high-dose females. Body weight development and survival of decaBDE-treated mice were comparable to those in controls. Liver granulomas were observed in low-dose males and liver hypertrophy in low- and high-dose males. Significantly increased combined incidence of hepatocellular adenomas and carcinomas was observed in male mice (8/50 in controls, 22/50 in low-dose, and 18/50 in high-dose males; trend not significant), whereas the combined incidences of thyroid follicular cell adenomas and carcinomas in males (0/50 in controls, 4/50 in low-dose, and 3/50 in high-dose males) and females (1/50 in controls, 3/50 in low-dose, and 3/50 in high-dose females) were increased only nonsignificantly. Furthermore, follicular cell hyperplasia was increased at both dose levels in males and females.

In a study by the Dow Chemical Company (*83*), groups of 25 male and 25 female Sprague-Dawley rats were given "decaBDE" (containing decaBDE 77.4%, nonaBDE 21.8%, and octaBDE 0.8%) in the diet for 100–105 weeks. The dose levels were 0, 0.01, 0.1, and 1 mg/kg/day. The treatment did not have any influence on survival rates, appearance, body weights, feed consumption, hematology, urinalysis, or organ weights. There were no other discernible toxic effects, and no significant differences in number of rats developing tumors between the groups. The International Agency for Research on Cancer (IARC) Working Group pointed out that the dose levels were very low (*2*).

In the NTP rat study (*80*), groups of 50 male and 50 female Fischer 344/N rats

received decaBDE (purity 94–99%; no brominated dioxins or furans found) mixed in the diet for 103 weeks, all survivors were killed at 111–112 weeks of age. The concentration of decaBDE in the diet was 0, 25, and 50 g/kg diet, and the estimates for average daily doses of decaBDE were 1,120 and 2,240 mg/kg bw/day in low- and high-dose males, respectively, and 1,200 and 2,550 mg/kg bw/day in low- and high-dose females, respectively. Body weights of the decaBDE-treated rats were not significantly different from those of controls. After week 75 survival rates of the treated groups were lower than those of controls. In high-dose males, thrombosis and degeneration of the liver, fibrosis of the spleen, and lymphoid hyperplasia were observed. The incidences of neoplastic nodules of the liver (adenomas) were significantly increased in both males (1/50 in controls, 7/50 in low-dose, and 15/49 in high-dose males; $p < 0.001$, incidental tumor test for trend) and females (1/50 in controls, 3/49 in low-dose, and 9/50 in high-dose females; $p = 0.002$, incidental tumor test for trend). However, no differences in incidences of hepatocellular carcinomas were detected among the groups. Significantly increased incidences of acinar cell adenomas of the pancreas was observed in males (0/49 in controls, 0/50 in low-dose, and 4/49 in high-dose rats; $p = 0.017$, incidental tumor test for trend). Additionally, high incidences of mononuclear cell leukemia were observed in treated and control rats of both sexes.

These studies have led to a conclusion that there is limited evidence for carcinogenicity of decaBDE in experimental animals, and that decaBDE is not classifiable as to its carcinogenicity to humans (Group 3) (*118*). The lack of genotoxicity suggests that the mechanism of the possible carcinogenicity of decaBDE would be epigenetic.

Ah receptor-mediated effects. Polyhalogenated aromatic hydrocarbons exert many different mechanisms of toxicity. One they share in common, however, may be the Ah receptor (AhR) binding effects (*96*). The best characterized and one of the most sensitive of the AhR-mediated phenomena is the induction of isoenzymes CYP1A1 and CYP1A2, but the role of AhR in mediating toxic effects is less understood. Recent studies in AhR-deficient mice, however, strongly suggest that at least TCDD-induced thymic atrophy and the most characteristic liver lesions would be mediated by the AhR (*119*).

Several studies have shown that PBDEs may induce several microsomal enzyme activities. One of these, EROD, is a marker of AhR binding. In a study by von Meyerinck and co-workers (*84*) the same levels of EROD induction were observed after exposure of rats to pentaBDE as after Aroclor 1254 treament (both given as a single dose of 300 mg/kg bw). However, Hallgren and Darnerud (*90*) observed a much weaker induction by BDE-47 than Aroclor 1254 when given orally to rats in isomolar doses.

The AhR agonist and antagonist activities of a series of 17 PBDE isomers were recently studied using a recombinant H4IIE rat hepatoma cell line with a luciferase reporter gene, the CALUX-assay (*91*). Seven of the congeners induced luciferase expression, whereas nine congeners decreased TCDD-induced luciferase expression. Thus, some pure PBDE congeners acted via the AhR signal transduction pathway as antagonists, agonists, or both. Their potencies, however, were approximately six orders of magnitude lower than that of TCDD but comparable to those of some mono-*ortho* PCBs such as PCB-105 and PCB-118 (*120*). The above studies suggest that PBDEs may have certain AhR binding activities, although results deviate. However, it seems less likely that the AhR would have any major role in mediating toxic effects of pure PBDEs.

Effects on thyroid hormones. Several studies have demonstrated that exposure to dioxins and related compounds are associated with complex alterations in thyroid function (*121–123*). These alterations include two different basic mechanisms. First, there is increased elimination of thyroid hormones, especially thyroxin (T_4), primarily because of induced activity of UDPGT in liver, which leads to accelerated hepatic clearance of T_4. This has been reflected consistently in decreased serum levels of total and free T_4. Alterations of 5′-deiodinase activities may also change serum and tissue levels of thyroid hormones (*124*). Second, many organohalogen compounds structurally resemble thyroid hormones and therefore compete for binding to thyroid hormone receptors and transporter proteins (*121,125,126*). In fact, halogenated DEs appear to bind to the thyroid hormone receptor with higher affinity than planar ligands do (*93*), and therefore PBDEs are likely to be more potent thyroid agonists (or antagonists) compared with many other classes of organohalogen compounds. Hydroxylated PBDE metabolites are of particular interest because thyroid hormones are also hydroxyhalogenated DEs. Hydroxylated PBDE congeners 4′-OH-1,3,3′,5-tetraBDE and 4′-OH-1,3,3′,5,5′-pentaBDE that theoretically show the highest structural similarity with triiodothyronin (T_3) and T_4, respectively, have the highest binding affinities to the thyroid hormone receptors (*127*). The binding affinities, however, were about two to three orders of magnitude lower than those of the endogenous ligands T_3 and T_4. Another potentially significant property of hydroxylated PBDE metabolites is their ability to disrupt the transportation of thyroid hormones by displacing them from the thyroxin plasma transporter, transthyretin (TTR). A preliminary study revealed that several microsomal PBDE metabolites (not identified) are potent competitors of T_4 binding to human TTR (*128*). Interestingly, metabolism of BDE-47 by phenobarbital-induced microsomes (mainly CYP2B) resulted in a strong increase in TTR binding, whereas β-naphthoflavone-induced microsomes (mainly CYP1A1) did not. Binding to TTR has been shown to be a general property for hydroxylated metabolites of PCBs and of other organohalogen compounds (*123,129*).

Recent studies have shown that PBDEs share the general property of several organohalogen compounds in lowering the serum total and free T_4 in mice and rats (*90,104,130*). Significantly decreased serum T_4 concentrations were found in C57BL/6 mice treated orally with a commercial pentaBDE mixture (DE-71), both after exposure to a single dose of 0.8–500 mg/kg bw and after daily exposure for 14 days to total doses of 250–1,000 mg/kg bw, i.e., 18–71 mg/kg bw/day (*104*). Similarly, Sprague-Dawley rats and C57BL/6 mice given daily oral doses of Bromkal 70 for 14 days at total dose levels of 0, 250, or 500 mg/kg bw (0, 18, or 36 mg/kg bw/day) showed significant dose-dependent decreased levels of plasma total T_4 (*130*). Rats proved to be more sensitive than mice. The plasma thyroid-stimulating hormone concentrations were largely unaffected. The pure congener BDE-47 was equally or more potent in decreasing serum total T_4 than the commercial pentaBDE in mice suggesting that this effect is not caused by impurities of the technical grade PBDE mixture. In addition, the technical grade PCB mixture Aroclor 1254 (containing about 5% pentaCB) and the pure congener PCB 105 (a mono-*ortho* PCB with some AhR binding affinity) were slightly more potent than the PBDEs in causing these effects in mice. In continued studies on Sprague-Dawley rats, daily oral doses of BDE-47 for 14 days resulted in significantly decreased serum levels of free T_4 levels at 18 mg/kg bw/day and gave a NOEL for this effect of 6 mg/kg bw/day (*90*). Moreover, BDE-47 induced a synergistic effect with technical preparations of PCBs or chlorinated paraffins in decreasing T_4 levels.

In addition to the decreased T_4 observed after treatment with lower brominated DEs, indirect evidence suggests that decaBDE may also affect thyroid function. Increased incidences of thyroid follicular cell hyperplasia as well as slightly increased incidences of follicular cell adenomas and carcinomas were observed in decaBDE-treated mice in the NTP carcinogenicity bioassay (*80*). Furthermore, workers

exposed to decaBDE, other PBDEs, and PBBs had increased prevalence of primary hypothyroidism, although serum analysis did not confirm the exposure to decaBDE (*131*).

In a recent preliminary report, exposure of pregnant rats to DE-71 from gestational day 6 to postnatal day 21 at 0, 1, 10, or 30 mg/kg bw/day was shown to decrease serum T4 dose dependently in dams, fetuses, and offspring (*132*). In the offspring the decreases were 25, 50, and 70% at 1, 10, and 30 mg/kg bw/day, respectively, on postnatal days 4 and 14, but levels recovered to the control levels by postnatal day 36. Thyroid toxicity was also observed in rat pups prenatally exposed to polychlorinated DEs (*133*). Significantly decreased serum T4 levels were observed in 16-day old pups given three PCDE congeners (2′,3,4,6′-tetraCDE, 2,2′,4,5,6′-pentaCDE, or 2,2′,4,4′,5,5′-hexaCDE) at maternal dose levels of 25–100 mg/kg/day on gestational days 6–15. The maximum decrease generally had been observed at the lowest dose level, and therefore the NOEL could not be determined. At lower doses the T_4 decrease was shown in absence of any other toxic effect, but the highest dose level (100 mg/kg bw/day) caused embryolethality as the number of litters and the number of pups were highly reduced. Decreased maternal T_4 was observed only after treatment with the tetra and penta congeners at 100 mg/kg/day. Serum T_3 levels were unaffected in both pups and dams. The decrease in T_4 levels did not correlate with the ability of the congener to induce CYP enzymes.

Collectively, these studies demonstrate that thyroid homeostasis is a sensitive target of PBDEs. Because the development of the central nervous system is highly dependent on thyroid hormones, alterations in thyroid homeostasis may result in permanent neurobehavioral defects (*114,115*). It may also be the primary cause of neurodevelopmental toxicity of PBDEs. Therefore, altered thyroid function, especially during development, is likely to be among the most important end points of toxicity in the risk assessment of PBDE.

NOELs and LOAELs. Data from effects observed in experimental animals administered PBDEs orally have been compiled in Table 7. In this table, the NOELs and the LOAELs of the various studies are given. In general, the NOEL values are considerably higher for the decaBDE group than for the lower-brominated technical PBDE preparations and pure BDE-47. The most sensitive end point of toxicity, as indicated by the LOAEL values, was the decrease in serum T_4 concentration. A LOAEL value of 0.8 mg/kg bw after a single dose of Bromkal DE-71 was observed in adult mice (*104*). In this study, however, a dose–response relationship was absent. In a recent preliminary report (*132*), a LOAEL value of 1 mg/kg bw/day was reported in rat offspring exposed to DE-71 *in utero* and lactationally. A LOAEL of 2 mg/kg bw/day for fetal toxicity was found after exposure to the Saytex 111 mixture in rabbits (*108*). In adult rats exposed to DE-71 for 90 days, a NOEL value of 2 mg/kg bw/day was determined for decreased serum T_4, thyroid hyperplasia, and hepatocytomegaly (*2*). Moreover, a NOEL of 1 mg/kg bw/day for decaBDE (containing 77.4% decaBDE, 21.8% nonaBDE, and 0.8% octaBDE) was derived from the Dow Chemical Company carcinogenicity study on rats (*83*). This dose level was the highest dose and it did not cause any toxicity. A general limitation of the studies on the technical PBDEs is that the purity was not always determined and the contaminants present were not evaluated for their possible modulation of the observed effect. Considering this, the studies on the pure BDE-47 congener (> 98% pure) are of value, as they exclude the contribution of possible contaminants to the observed thyroxin-lowering effect, shown at the dose of 18 mg/kg bw (*90,130*). Also, in a preliminary report, extended studies by Eriksson and co-workers on neurobehavior showed effects of the single congener BDE-99 at 8 mg/kg bw (*113*).

Toxicity in Humans

DecaBDE is the only PBDE for which limited human data are available. Skin sensitization potential of decaBDE (Dow Chemical Company, Midland, Michigan USA); containing 77.4% decaBDE, 21.8% nonaBDE, and 0.8% octaBDE) was studied in 50 volunteers (*54,79*). A 5% suspension of decaBDE in petrolatum was applied to the skin 3 times per week for 3 weeks, followed by a challenge treatment 2 weeks after the last application. No skin sensitization responses were observed during the study. Another skin sensitization study was performed on 80 male and 120 female volunteers exposed to two batches of decaBDE (purity not stated) (*2*). The volunteers were treated with nine induction patches at 2-day intervals and the test substance was kept in contact with skin for 24 hr. The induction regimen was followed by a period of 12 days without treatment, after which a new skin site was used for a 24-hr challenge patch. Skin reactions were observed at 24 and 48 hr after removal of the challenge patch. The study revealed no evidence of skin sensitization with either batch of decaBDE.

Workers exposed during manufacture to PBBs and PBDEs, including decaBDE, were reported to have higher-than-normal prevalencies of primary hypothyroidism and significant reductions in conducting velocities in sensory and motor neurons but no other neurologic or dermatologic changes (*131*). It was not possible to conclude whether these changes could be attributed to PBB or PBDE exposure, but no decaBDE could be detected in serum of the exposed workers.

Four epidemiologic studies have been conducted on workers at facilities where flame-retardant polymers were extruded (*2*). The workers were potentially exposed to brominated flame retardants, including PBDEs and in some cases also to polybrominated PBDDs and PBDFs. These studies found no adverse effects attributable to exposure to these chemicals.

Toxicology: Summary and Conclusions

Limited amounts of data are available about the toxicity of PBDEs. Most of the studies have been carried out using technical- or commercial-grade PBDEs, the purity of which has been known in several cases, but the isomer composition unknown. Moreover, often no data are available about possible halogenated dioxinlike impurities. A general concern about toxicity data is that many studies are documents that are difficult to obtain and may not have been subjected to examination by independent referees. Therefore, because the quality of these studies cannot be verified, their relevance in some cases may be questionable.

Available data suggest that the acute toxicity of PBDEs is low, they have at most only slightly irritating properties, and they are not skin sensitizers. No severe signs of toxicity were observed in subacute and subchronic toxicity studies. Target organs were the liver, kidney, and thyroid gland, which were enlarged and/or showed mainly minor histopathologic changes. Different PBDEs seemed to have similar toxicologic profiles, but in most studies decaBDE was less potent than the other congeners. PBDEs were not genotoxic in short-term tests. In carcinogenicity studies with decaBDE (at very high doses), increased incidences of hepatocellular and thyroid adenomas and carcinomas were observed in mice, and increased incidences of hepatocellular adenomas and acinar cell adenomas of the pancreas were observed in rats. Reproduction toxicity studies revealed increased sensitivity of pregnant animals and fetuses to PBDEs. Except for pentaBDE, toxic effects on fetuses were observed already at dose levels not toxic to mothers. Fetal toxicity was sometimes associated with low incidences of developmental variants. Neurobehavioral effects were seen in adult mice after single relatively low oral doses of tetra- and pentaBDEs were given neonatally during the sensitive brain growth period. Humans occupationally exposed to PBBs and PBDEs were found to have hypothyroidism and decreased conduction velocity of sensory and motor neurons, but the association of these effects with exposure to PBDEs was equivocal.

Available evidence suggests that in spite of certain structural similarity to dioxins, PBDEs are weak agonists of the AhR. In fact, observed AhR effects (e.g., CYP1A1 induction) may be at least partly attributed to their highly potent polyhalogenated dioxin or furan impurities. This coupled with the lack of obvious dioxinlike toxic effects suggests that the primary mechanisms of toxicity of PBDEs are different from those of dioxins. However, it appears likely that halogenated DEs and their hydroxylated metabolites bind effectively to transport proteins for thyroid hormones, and that alteration of thyroid homeostasis by this and perhaps also other mechanisms may represent an important and characteristic mechanism of toxicity of PBDEs.

The most sensitive end points of toxicity determining LOAEL and NOEL values were the hepatomegaly with cytoplasmic eosinophilic "round bodies," fetal and maternal toxicity, and disturbances of thyroid homeostasis. Also, the recently discovered neurotoxic effect of several PBDE congeners at relatively low levels should be noted. These effects and their significance therefore should

Table 7. NOEL and LOAEL values of orally administered PBDEs in different toxicity studies.

Study	Species	PBDE (purity)	NOEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Effect	Reference
DecaBDE						
Subacute toxicity (14 days)	Mouse	DecaBDE (99%)	15,000	—	—	(80)
	Rat	DecaBDE (99%)	10,000	—	—	(80)
Subacute toxicity (28 days)	Rat	DecaBDE (commercial)	100	—	—	(2)
(30 days)	Rat	DecaBDE (77.4%), nonaBDE (21.8%), octaBDE (0.8%)	8	80	Thyroid - hyperplasia Liver - enlarged	(54,79)
Subchronic toxicity (13 weeks)	Mouse	DecaBDE (> 97%)	7,500	—		(80)
	Rat	DecaBDE (> 97%)	5,000	—		(80)
Chronic toxicity, carcinogenicity	Mouse	DecaBDE (94–99%)	—	3 200	Liver - hypertrophy , granulomas adenomas, carcinomas; thyroid - follicular cell hyperplasia, adenomas, carcinomas	(80)
	Rat	DecaBDE (94–99%)	—	1 120	Liver - adenomas	(80)
Reproductive performance	Rat	DecaBDE (77.4%), nonaBDE (21.8%), octaBDE (0.8%)	100	—		(54,79)
Teratogenicity	Rat	DecaBDE (77.4%), nonaBDE (21.8%), octaBDE (0.8%)	— 1,000	10 —	Fetal toxicity Maternal toxicity	(54,79)
OctaBDE						
Subacute toxicity (28 days)	Rat	OctaBDE (commercial)	—	10	Liver - hepatocytes enlarged, cytoplasmic eosinophilic round bodies	(2)
Subchronic toxicity (13 weeks)	Rat	OctaBDE (commercial)	—	10	Liver - enlarged	(2)
Teratogenicity	Rat	DE-79	15 25	50 50	Fetal toxicity Maternal toxicity	(2)
Teratogenicity	Rat	Saytex 111[a]	2.5 25	10 —	Fetal Toxicity Maternal toxicity	(63)
	Rabbit	Saytex 111[a]	— 5	2 15	Fetal toxicity Maternal toxicity	(108)
PentaBDE						
Subacute toxicity (28 days)	Rat	PentaBDE (commercial)	—	10	Liver - enlarged, cytoplasmic eusinophilic round bodies; thyroid - follicular hyperplasia?	(2)
Subchronic toxicity (90 days)	Rat	DE-71[b]	2	10	Serum thyroxin - decreased, thyroid hyperplasia; liver - enlarged, hepacytomegaly	(2)
Teratogenicity	Rat	PentaBDE (commercial)	100 10	200 100	Fetal toxicity Maternal toxicity	(2)
Special development toxicity (maternal) exposure on GD 6-PND 21)	Rat	DE-71[b]	— 10	1 30	Offspring toxicity: serum thyroxin decreased Maternal toxicity: serum thyroxin decreased	(132)
Special single dose toxicity	Mouse	DE-71[b]	—	0.8	Serum thyroxin - decreased	(104)
Special repeated dose toxicity (14 days)	Mouse	Bromkal 70-5DE[c]	—	18	Serum thyroxin - decreased	(130)
	Rat	Bromkal 70-5DE[c]	—	18	Serum thyroxin - decreased	(130)
Special developmental toxicity, single dose on PND 10	Mouse	2,2',4,4',5-PentaBDE	0.8	12	Developmental neurotoxicity	(111)
Special developmental toxicity, single dose on PND 3, 10, or 19	Mouse	2,2',4,4'5-PentaBDE	—	8	Developmental neurotoxicity	(113)
TetraBDE						
Special repeated dose toxicity (14 days)	Mouse	2,2',4,4'-TetraBDE	—	18	Serum thyroxin - decreased	(130)
	Rat	2,2',4,4'-TetraBDE	6	18	Serum thyroxin - decreased	(90)
Special developmental toxicity, single dose on PND 10	Mouse	2,2',4,4'-TetraBDE	0.7	10.5	Developmental neurotoxicity	(111)

Abbreviations: GD, gestational day; PND, postnatal day.
[a]Saytex 111 is a commercial mixture containing pentaBDE, 0.2%; hexaBDE, 8.6%; heptaBDE, 45%; octaBDE, 33.5%; nonaBDE, 11.2%; and decaBDE, 1.4% (2). [b]DE 71 is primarily a mixture of tetra-, penta, and hexaBDE containing low levels of tri- (> 1%) and heptaBDE (< 2%) (2). [c]Bromkal 70-5DE is a commercial mixture containing about 60% of pentaBDE and 40% of tetraBDE (2)

be addressed in further toxicologic studies with PBDEs.

Toxicologic Evaluation

PBDEs are accumulating in sediment and biota and appear to bioconcentrate at least in aquatic ecosystems. Relatively high levels could be found in fatty fish, Baltic herring, for example. However, analyses of terrestrial mammals reveal low PBDE levels. This may imply that for humans exposure to PBDEs directly from the environment is low (except for occupational exposure), but exposure my occur indirectly through the intake of food from aquatic ecosystems. An area of concern is the increasing exposure of infants to PBDEs during lactation because of increasing PBDE levels in mothers' milk. Also, the possibility of long-term exposure from electronic equipment such as computers should however not be ignored. Data on PBDE levels in human tissues and fluids suggest an increasing time trend, although levels are still considerably lower than those of PCBs.

Data on rodents suggest that elimination of PBDEs from the body is species dependent (*urinary excretion of BDE-47 is much higher* in mice than in rats). No clear differences in body distribution between different congeners is seen in mice. Hydroxylated PBDE metabolites were detected in the mouse and the rat. The toxic potency of these metabolites is unknown, but it is suggested that hydroxylated PCB metabolites mimic both thyroid hormones and estrogens. There is evidence that debromination of PBDEs to lower brominated DEs occurs in rodents and fish.

As nonplanar compounds, PBDEs do not bind to the AhR with high affinity or exert dioxinike toxicity. Part of the observed limited CYP1A1 induction may be explained by potent halogenated dioxin and furan impurities which, even if present only at low concentrations, may become significant in exerting these effects.

Effects on thyroid function, experimentally observed primarily as T_4 hypothyroidism, appear to be sensitive end points of PBDE toxicity. Interestingly, primary hypothyroidism *has also been reported in humans after possible* occupational exposure. Other effects include hepatotoxicity, developmental neurotoxicity, and embryotoxicity as well as maternal toxicity during gestation. Fetal effects observed in several studies were delayed ossification of bones, bent ribs and bones, and decreased fetal weight. Although not severe, such effects if found at relatively low exposure levels are cause for concern. Currently there are no reports about effects of PBDE exposure on the human fetus/offspring. However, the possibility of these effects on humans should be considered.

The NOEL values for fetal effects were found at < 2 to 15 mg/kg bw/day (commercial octa- and pentaBDEs). A LOAEL of 8 mg/kg bw was seen in mice subjected to neurotoxicity tests (BDE-99). Also, the effects on liver and thyroid hormones occurred at relatively low doses (NOEL 2–18 mg/kg bw/day). Effects on thyroid hormones were observed at an even lower dose, 0.8 mg/kg bw (but in the absence of dose–response correlation), by Fowles and co-workers (*104*). In a recent preliminary study in rats, exposure to a commericial PBDE mixture resulted in a LOAEL of 1 mg/kg bw based on thyroid hormone effects. Consequently, if the most sensitive end points are chosen (although partly based on a preliminary report on a technical pentaBDE), a LOAEL value of 1 mg/kg/day is reasonable for compounds or mixtures belonging to the PBDE group. Using an extrapolation factor of 10, a NOEL of 0.1 mg/kg/day could be suggested for the PBDEs. More studies are needed, however, to provide a better basis for risk assessment of PBDEs and for producing a more solid LOAEL and NOEL.

The preliminary estimated dietary PBDE intake of 51 ng/day equals 0.7 ng/kg/day for a *person weighing 70 kg. Comparison of the* estimated dietary intake with the presented LOAEL value of 1 mg/kg/day results in a safety factor of > 10^6 with regard to the mean PBDE exposure from food. However, special dietary habits or other types of PBDE exposure may decrease this safety factor considerably.

Data Gaps and Future Research Needs

It is important to know the purity and impurity profiles of PBDEs used for kinetic and toxicity studies; these data should always be available. To avoid impurity problems, studies using isomer-specific pure congeners should be encouraged.

Further studies on PBDE time trends are needed to confirm and extend earlier observations. Results from ongoing studies are important in assessing resource needs for future PBDE studies. It is currently known that certain PBDE congeners accumulate in the environment, where others do not, but the reason *for this discrepancy is not understood.* Therefore, environmental transformation studies are needed to examine, for example, the degradation of decaBDE to penta- and tetraBDEs. It is also important to collect more data on human exposure, especially through food, but also through other routes.

There still are large gaps in data concerning PBDE toxicity. General toxicity studies should be performed on specific congeners of importance in terms of environmental levels and bioaccumulation, congeners such as BDE-47, -99, -100 and -153. There are also large data gaps in the field of toxicokinetics, i.e., the metabolism of PBDEs, metabolites formed, and enzymes involved. The following characteristic and critical end points of PBDE toxicity should be studied further: mechanisms of thyroid toxicity, possible effects on other hormonal systems, and reproduction and developmental toxicity. The latter should concentrate especially on multigeneration studies and peri- and postnatal toxicity and include neurotoxicity models. It is also important to perform cancer bioassays on the tetra- and pentaBDEs, as these PBDE groups are bioavailable and are often found in higher concentrations in the environment than decaBDE. Finally, studies on structure–activity relationships and interactions (including synergism, antagonism, and interactions with other halogenated aromatic hydrocarbons) of PBDE are important for the toxicologic evaluation of PBDEs.

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Toxic effects of brominated flame retardants in man and in wildlife

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Abstract

Brominated flame retardants (BFRs) are ubiquitous industrial chemicals, and many of them are produced in large volumes. Due to this fact, several BFRs are found in quantifiable levels in wildlife, as well as in humans. However, we are still lacking information on the effects of BFR in wildlife and, especially, in man. This review summarises the biological effects of polybrominated diphenyl ethers (PBDEs), tetrabromobisphenol A (TBBPA) and derivates, hexabromocyclododecane (HBCD) and polybrominated biphenyls (PBBs), however excluding other aspects such as environmental levels. These BFR groups were selected because of a large volume production (PBDEs, TBBPA and derivates), and availability of some toxicity data in spite of much lower production volumes (HBCD and PBBs). In addition, the increase in levels of PBDEs in human (breast milk) and wildlife samples during later time made it especially interesting to include this BFR group. *PBDEs:* The commercial PBDE products predominantly consist of so-called penta-, octa- and decabromodiphenyl ether products. Each product consists of a rather narrow range of congeners and is named after the dominating congener as regards the bromination pattern. Generally, the PentaBDEs seem to cause adverse effects at the comparably lowest dose, whereas much higher doses were needed for effects of the DecaBDEs. The critical effects of PentaBDEs are those on neurobehavioural development (from 0.6 mg/kg body weight) and, at somewhat higher dose, thyroid hormone levels in rats and mice, of OctaBDEs on fetal toxicity/teratogenicity in rats and rabbits (from 2 mg/kg body weight), and of DecaBDEs on thyroid, liver and kidney morphology in adult animals (from 80 mg/kg body weight). Carcinogenicity studies, only performed for DecaBDEs, show some effects at very high levels, and IARC (1990) evaluates DecaBDEs not classifiable as to its carcinogenicity to humans. *TBBPA:* The toxicity of TBBPA in the experimental in vivo studies is suggested to be low. In most reported studies, only doses in g/kg body weight were effective, but at least one study suggested renal effects at around 250 mg/kg body weight. Although difficult to include and interpret in a quantitative risk assessment, the in vitro effects on immunological and thyroid hormones, as well as binding to erythrocytes should be noted. Before a solid standpoint could be reached on TBBPA toxicity additional studies must be performed. This statement is even more valid regarding the TBBPA derivates, where there is an almost complete lack of toxicity data. *HBCD:* Also in the case of HBCD, relevant toxicity studies are lacking. Based on the present animal studies, a critical effect is seen in the liver and on thyroid hormones (LOAEL 100 mg/kg body weight/day). However, in a recent short paper behavioural effects in mice pups were observed already at 0.9 mg/kg body weight, and behavioural effects may be a sensitive endpoint for HBCD, as well as for other BFRs. *PBBs:* Due to the Michigan accident in 1973–1974, many toxicity studies on PBBs are available. The critical experimental effects are those on reproduction and carcinogenicity, and a NOAEL of 0.15 mg/kg body weight/day could be suggested based on the cancer effects. In man no unequivocal effects have been observed, although in some studies neurological and musculoskeletal symptoms were suggested. Based on the carcinogenic effects in animals, a human TDI of 0.15 μg/kg body weight has been presented.

To conclude, the toxicity data are almost entirely based on experimental models. There are differences among the BFR groups, as well as within these groups, both regarding type of toxic effect and at what dose it appears. As BFRs will continue to appear both in industrial applications and, even if the production has ceased, in our environment, there is a continued need for effects studies on BFRs.

Keywords: Brominated flame retardants; Man; Wildlife

1. Overview

Brominated flame retardants (BFRs) are ubiquitous chemicals with large and global industrial use, and many of them are still produced in large volumes. Due to this fact, several BFRs are found in quantifiable levels in wildlife as well as in humans (e.g. De Wit, 2002). However, we still know little about the effects of BFRs in wildlife and in man. Among the BFRs, the best environmental and human risk assessment data are available for polybrominated biphenyls (PBBs) and polybrominated diphenyl ethers (PBDEs). The interest for PBBs stems mainly from the contamination incident in Michigan 1974, where PBBs by accident were

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given to farm animals. As a result, individuals living on affected farms and consumers of contaminated farm products were exposed to these compounds for months before the mistake was discovered. Regarding PBDEs, the high production volume and the structural resemblance to other well-known environmental contaminants such as polychlorinated biphenyls (PCBs) are two main reasons for environmental and health concern. Several BFRs are accumulated in biota, and in many cases the highest levels in wildlife are found in the aquatic environment. In the PBDE example, water-living mammals (e.g. whales), fatty fishes and/or fishes near point sources of contamination could reach very high levels. PBDEs and occasionally also other BFRs are found in human breast milk and adipose tissues in several western countries, and the PBDE levels in both biota and in breast milk have increased in later time. This article will give a brief overview of the effects that have been observed or proposed to be related to BFR exposure, and will focus on data relevant for human risk assessment.

2. Definition of study area

BFRs are produced in a volume of ca. 150000 tons/year (OECD, 1994). Of the total BFR volume produced, about one-third consist of PBDEs, another third consist of tetrabromobisphenol A (TBBPA) and derivatives, and the last third contain various other brominated compounds, two examples being PBBs and hexabromocyclododecane (HBCD). In this last group, IPCS have registered over 30 brominated compounds with flame-retardant usage (IPCS, 1993). Of these, most of them have an aromatic structure, but also aliphatic, cycloaliphatic, heterocyclic and bromophosphorous compounds are listed. However, the majority of the compounds in the latter group are used, if at all, in small quantities and little is known of their possible effects in biological systems. Because of earlier usage and the Michigan contamination accident, the PBBs are rather well studied. As regards the HBCD, high levels (compared to other BFRs) have been found in aquatic biota from polluted areas (e.g. Sellström et al., 1998).

This article summarises the biological effects of PBDEs, TBBPA and derivatives, PBBs and HBCD. The decision for selecting these BFR compounds for review was made based on large volume production (PBDEs, TBBPA and derivates), and availability of exposure and toxicity data in spite of much lower production volumes (PBBs and HBCD). In addition, the increase in levels of PBDEs in human (breast milk; Meironyté Guvenius, 2002) and wildlife (Ikonomou et al., 2002) samples during later time made this BFR group of special interest. It should be noted that only data on the *toxic effects* of the mentioned substances will be discussed in this chapter, and not environmental levels, toxicokinetic properties, or some other aspects of them. Moreover, there will be a focus on effects that are of importance for *human risk assessment*, i.e. mainly mammalian toxicity studies, and nonmammalian effect data will be reviewed in less detail. Lastly, the present compilation will not try to include all the published articles in this area, but will primarily concentrate on those critical studies that will be of main use in the risk assessment of these chemicals. In case of enzyme induction, it is doubtful if these effects could be considered as adverse, and microsomal enzyme induction will therefore not be considered to be an important critical effect in the risk assessment of PBDEs.

3. Data search methods

In this review, I have compiled data and used references from the available IPCS documents for each flame retardant group (PBDE—IPCS, 1994a; TBBPA—IPCS, 1995; PBB—IPCS, 1994b).The KEMI draft document on HBCD (KEMI, 2002) has also been very valuable. In addition, other available reviews on flame retardants (Darnerud et al., 2001; Peltola and Ylä-Mononen, 2001; De Wit, 2002; Hardy, 2002) have been used. New studies have been found by the selected searching in useful databases, e.g. the National Library of Medicine PubMed Service and iNFOTRIEVE®.

4. PBDE

The commercial PBDE products predominantly consist of so-called penta-, octa- and decabromodiphenyl ether products. Each product consists of a rather narrow range of congeners and is named after the dominating congener as regarding the bromination pattern. PBDE congeners are often identified by the IUPAC nomenclature originally invented for denomination of PCB congeners (Ballschmiter and Zell, 1980). Today, decabromodiphenyl ether (Deca-BDE) is the largest product on the market and makes up over 80% of the total production of PBDEs, whereas pentabromodiphenyl ether (PentaBDE) and octabromodiphenyl ether (OctaBDE) products constitute about 12% and 6%, respectively, of the total PBDE production (www.bsef.com; De Wit, 2002). Examples of products containing PBDEs are various plastic components in electronic devices (e.g. cabinets, circuit boards, cables, switches, capacitors) and in cars and in building materials, and textiles.

Most of the toxicological effect studies in experimental systems have used the commercial PBDE mixtures, but there are also examples of studies performed on single congeners. A general problem with the use of commercial PBDE product mixtures, as well as with many other commercial organohalogen products, is the comparably low purity of the mixture and the lack of knowledge on the nature of possible interfering compounds. Another problem is the possibility of transformation of PBDEs during combustion, etc. into other products of which some

are more toxic than the original products (e.g. brominated dibensofurans and dioxins). Transformation as well as toxicokinetic aspects will however not be dealt with in this particular review.

Below are summaries of the data on toxicity and similar effects (e.g. enzyme induction) from studies on commercial PentaBDE (tetra- to hexa-congeners), OctaBDE (hexa- to nona-congeners) and DecaBDE (nona- and deca-congeners) products. Many of these results are described more in detail in earlier reviews. The reader is referred to these reviews, or further to the original articles, to obtain more details (e.g. IPCS, 1994a; Darnerud et al., 2001; Peltola and Ylä-Mononen, 2001; De Wit, 2002; Hardy, 2002).

4.1. Effects in mammals and related experimental systems

4.1.1. PentaBDE

PentaBDE gave a low acute toxicity in experimental animals (rats, rodents) and the oral LD-50 in rat was in the range 0.5–5 g/kg body wt. (IPCS, 1994a). The clinical signs were reduced growth, diarrhoea, piloerection, reduced activity, tremors of forelimbs, red staining around eyes and nose, and continuous chewing. The porphyrogenic activity was relatively high according to studies in which the concentration of porphyrins increased considerably after oral dosing with the commercial pentaBDE product DE-71 (mixture of tetra-, penta- and hexaBDE) at 100 mg/kg body wt./day for 13 weeks (IPCS, 1994a). No mutagenic potency was observed in Ames test models using several different *Salmonella* strains with and without microsomal activation (IPCS, 1994a).

After repeated dosage with PentaBDE products, several morphological effects were observed, such as changes in hepatic and thyroid size and histology. In two studies of commercial PentaBDE mixtures in rats, these effects appeared at the 10 mg/kg body wt. dose (Great Lakes Chem.; IPCS, 1994a). Immunological effects were suggested in mice after exposure to commercial DE-71 mixture (72 mg/kg body wt. for 14 days); suppression of the anti-SRBC response was observed, as well as a decreased thymus weight (Fowles et al., 1994). The PBDE congener BDE 47 markedly reduced the splenocyte numbers in mice (C57BL) after daily oral administrations of 18 mg/kg body wt. for 14 days (Darnerud and Thuvander, 1999). In the same study, Bromkal 70 reduced IgG antibody production from pokeweed-stimulated mouse splenocycte cultures ex vivo, whereas no immunological effects were seen in rats.

Commercial PeBDEs affected thyroid hormone homeostasis, and both technical products and pure tetra- and penta-congeners (the latter at doses of 10–18 mg/kg body wt./day for 2 weeks) produced effects on serum thyroxin levels in rats and mice (Great Lakes Chem., IPCS, 1994a; Hallgren et al., 2001; Zhou et al., 2001; Hallgren and Darnerud, 2002). In one study on mice, effects on thyroxin were observed already at single dose of 0.8 mg/kg (Fowles et al., 1994), although with lack of dose–response effects relationship.

Microsomal enzyme induction was seen in several studies after administration of pentaBDE products. Induction of several CYP isozymes was indicated by use of substrates for specific enzymes, and increases in EROD, MROD and PROD activities were suggested at BDE 47 doses from 6 to 10 mg/kg body wt./day (Zhou et al., 2001; Hallgren and Darnerud, 2002). In a rat hepatoma cell line (H4IIE), some of the pure PBDE congeners acted via the AhR signal transduction pathway as agonists, antagonists, or both. Their potencies were approximately six orders of magnitude lower than that of TCDD but comparable to those of some mono-ortho PCBs such as PCB 105 and PCB 118 (Sanderson et al., 1996). In studies by Chen et al. (2001), pure PBDE congeners and commercial PBDE mixtures had Ah receptor binding affinities 10^{-2} to 10^{-5} times that of 2,3,7,8-TCDD. Interestingly, binding affinities for PBDEs could not be related to the planarity of the molecule, possibly because of sterical reasons.

A commercial PentaBDE gave rise to maternal and fetal toxicity (chiefly manifested as a weight decrease) in rats at rather high doses (100 and 200 mg/kg body wt., respectively, on gestation days 6–15) (BFRIP; IPCS, 1994b). The effects on thyroid hormones, mentioned above in adult animals after PBDE exposure, could also be seen in offspring to PBDE-dosed dams: In rat pups to DE-71-exposed dams, decreased thyroxine levels were seen at 10 mg/kg body wt. upon gavage on gestational day 6 to postnatal day 21 (Zhou et al., 2002). The effects were seen at PND 4 and 14, but a reduction in thyroxin was also seen in fetuses on GD 20. At lower doses (from 0.6 to 0.8 mg/kg body wt.), the pure pentaBDE congener BDE 99 caused impaired habituation and impaired learning and memory functions in mice pups after oral administration (to pups) at day 10 after partus (Eriksson et al., 2001). At a higher dose, BDE 47 also caused similar effects. Branchi et al. (2002) showed similar effects on spontaneous motor activity in mice after perinatal exposure to PBDE 99 (from 0.6 mg/kg body wt., GD 6-PND 21). Also in adult mice, behavioural effects were observed, following neonatal exposure to BDE 99 during a critical period (Eriksson et al., 2002a).

No cancer study has been performed on PentaBDE.

4.1.1.1. Critical effects of PentaBDEs. For PentaBDEs, the critical effects among the available studies seem to be developmental neurotoxicity and, generally at somewhat higher doses, altered thyroid hormone homeostasis. Regarding the neurotoxicity in mice, no clear mechanism could be defined but effects of the PentaBDEs both via thyroid hormone disruption and directly on signal transmission in brain have been discussed. For example, PBDEs as well as other BFRs, were capable to induce cell death of cerebellar granule cells in culture (Reistad et al., 2002). PBDEs were also shown to release arachidonic acid in cerebellar granule cells via a phospholipase A_2 pathway, and PLA_2 has been associated with learning and memory (Kodavanti and Derr-Yellin, 2002). As concerns the thyroid hormone effects

observed in the rodent model, a suggested mechanism is binding of PBDE metabolites to the thyroxin-transporting protein TTR, thereby decreasing the thyroxin levels in the blood and peripheral organs (Brouwer et al., 1988, 1998). An additional explanation to the hormonal effects is that thyroxin will be degraded faster due to PBDE-induction of the phase II enzyme UDP-GT (Bastomsky, 1974; Zhou et al., 2001) and therefore excreted at a higher speed. The thyroid effects seem to occur at lower PBDE exposure levels during early developmental stages. During these stages, thyroid hormones play an important role in the development of vital organs, including the brain.

To summarise, the LOAEL value for PentaBDE could be set to 0.6–0.8 mg/kg body wt., based on the most sensitive effect observed, neurobehavioural effects during early development. However, it is not known what these observations of altered spontaneous activity in neonatal mice means in terms of human risk assessment. Also, gaps in our knowledge make this evaluation only preliminary. For example, the lack of carcinogenicity data is unsatisfactory, and more should be learnt about reproductive and immunological effects. For the future, such studies should be given high priority, especially as the tetra- and pentabrominated congeners in most cases are the main PBDE congeners found in biota and in man.

4.1.2. OctaBDEs

Similar to the pentaBDEs, the acute toxicity of the OctaBDEs preparations is low. The dermal (rabbit) and oral (rat) LD-50 values range between >2 and >28 g/kg body wt. (IPCS, 1994a). Low skin and eye irritation are reported (IPCS, 1994a). Although being a nonmammalian model, the porphyrinogenic potential was reported strong in cultured chick embryo liver cells after incubation at 10 μg/ml for 24 h (Koster et al., 1980). Regarding genotoxicity, OctaBDE caused no effects, either in *Salmonella* mutagenicity tests or in unscheduled DNA synthesis assay (IPCS, 1994a). Moreover, no effect on sister chromatic exchange in Chinese hamster ovary cells was observed.

In subacute toxicity studies in rats with a commercial OctaBDE, morphological effects on the liver (enlarged with eosinophilic round bodies) were observed at the 10 mg/kg body wt. level (estimated from dietary OctaBDE concentrations) (Great Lakes Chem.; IPCS, 1994a). Effects on the thyroid as well as on the liver (necrosis) was observed at higher levels. A subchronic (13 weeks) toxicity study in rats revealed a similar liver effect at 10 mg/kg body wt. and at higher doses effects also sere observed in the thyroid, kidney and haemological system. Similarly to PentaBDEs, no carcinogenicity study has been reported on OctaBDEs.

In the rat, fetal toxicity was seen after administration of OctaBDE preparations (DE-79 and Saytex 111) by gavage to the mother (IPCS, 1994a; US EPA, 1986). The fetal effects, namely late resorptions, weight decrease, reduced ossification, bent ribs and limp bones, and rear limb malformations, started to appear at 10–25 mg/kg body wt., whereas maternal toxicity (weight decrease, effects on cholesterol levels) were seen at a higher dose. In rabbits, similar foetal toxic effects were seen after Saytex 111 administration, however in this case effects were seen at lower doses; delayed ossification occurred at 2 mg/body wt., and weight decrease and fused sternebrae at 5 mg/kg body wt. Also in the rabbit, maternal toxicity was observed at OctaBDE doses higher than those giving fetal effects (maternal body weight gain decrease and liver enlargement at 15 mg/kg body wt.) (Breslin et al., 1989; commented in Darnerud et al., 2001).

4.1.2.1. Critical effects of OctaBDEs. Morphological effects in the liver of adult rats were seen after dietary OctaBDE administration at 10 mg/kg body wt. Higher doses also affected the thyroid gland, kidney and the haemological system. Fetal toxicity of OctaBDE was manifested as weight decrease, reduced ossification, bent ribs and limp bones/fused sternebrae, in the rat and the rabbit, and in the rabbit these effects began to appear at 2 mg/kg body wt. (Breslin et al., 1989). The maternal effects in the rabbit started at 15 mg/kg body wt.

Thus, it could be concluded that OctaBDE toxicity is first seen at early developmental stages, and that the effects are suggested to appear at 2 mg/kg body wt. (=LOAEL) in rabbits, and at somewhat higher doses in the rat. The correspondence in effects in the rat and the rabbit study increase the impact of these results. As noted for the PentaBDEs, no carcinogenicity study has been performed on OctaBDEs, and there is also a lack of data on many other endpoints.

4.1.3. DecaBDE

The acute toxicity of DecaBDE products is low, oral and dermal LD-50 values varying between 2 and 5 g/kg body wt. (IPCS, 1994a; Norris et al., 1975a,b). Skin irritation and chloracnegenic activity were negative, whereas a transient eye irritation (redness and chemosis) was observed after Saytex 102 application. Studies of mutagenicity and chromosomal aberration were all negative. The porphyrogenic activity was also negative.

In the subacute/subchronic studies performed, effects of DecaBDE were first seen at 80 mg/kg body wt. (thyroid hyperplasia, liver enlargement and hyalin degeneration in kidney in rats; dose estimated from dietary intake), whereas hematocrit and red cell count were affected first at 800 mg/kg body wt. (IPCS, 1994a; Norris et al., 1975a,b).

In chronic toxicity studies (103 weeks) in rats and mice, the tumour incidence was observed after DecaBDE (commercial, 94–99% pure) exposure via the diet (NTP, 1986). At the doses (ca. 1200 and 2500 mg/kg body wt./day) given in rats, a dose-related increase in hepatic adenomas and an increase in pancreatic adenomas at the high dose were observed. In mice, the combined incidence of hepatocellular adenomas and carcinomas was increased, although not dose-related (doses ca. 3500 and 7000 mg/kg body wt./day).

Also, the combined incidence of thyroid gland follicular-cell adenomas and carcinomas was slightly increased. In an additional cancer study in rats, no effects were observed (Kociba et al., 1975). Regarding the latter study, the IARC Working Group pointed out that the dose levels (corresponding to 0–1 mg/kg body wt./day) were very low.

No reduction in reproductive performance was seen after DecaBDE exposure in the diet (DecaBDE product: 77% decaBDE, 22% nonaBDE, 1% octaBDE). In a teratogenicity study in rats, the same DecaBDE substance was given by gavage at gestation days 6–15; at 100 mg/kg body wt. resorptions were observed, and at 1000 mg/kg body wt. foetal subcutaneous edema and delayed ossification were registered (both studies: IPCS, 1994a; Norris et al., 1975a,b).

4.1.3.1. Critical effects of DecaBDE. The effects of DecaBDE products in mammalian models seem rather modest. Effects are first seen in a subacute study in rats at about 80 mg/kg body wt. (=LOAEL) (thyroid hyperplasia, liver enlargement and hyaline degeneration in kidney). At 100 mg/kg body wt., foetal resorptions were registered in rats.

The carcinogenicity study of DecaBDE in rats and mice is important, being the only study of tumour induction performed on PBDE. In this study, adenomas and carcinomas are indeed observed, but at very high doses (1200 mg/kg body wt./day and above). Hypothetically, another dosing regimen (narrower dosing intervals) might have resulted in carcinogenic effects at lower doses.

4.2. Effects in other animals/in wildlife

Effects of PBDEs have been reported in algae, invertebrates and in fish. In fish, Bromkal 70-5 DE induced CYP enzymes (EROD), generated fatty liver and reduced spawning success (Holm et al., 1993). Hornung et al. (1996) tested pure PBDE congeners on fish eggs (by microinjection) and saw no effects. In a study on rainbow trout, BDE-47 and -99 were given in the feed to rainbow trout (Tjärnlund et al., 1998). The result was a reduction in GSH reductase, haematocrit and blood glucose values. The effects of the congener BDE-47 on the calanoid *Acartia tonsa* were studied by Breitholz et al. (2001). In the 48-h acute toxicity test and larval developmental test, the 2- and 5-days LC-50 values were 2.4 and 0.013 mg/l, respectively. In addition, in reports not generally available, toxic effects of a tetra- to hexa-BDE mixture induced toxicity in a *Daphnia* test model. The NOEC values in a 48-h acute toxicity test and a 21-day life-cycle study were in both cases about 5 μg/l (CITI, 1882; Dottar and Kreuger, 1998; both in Peltola and Ylä-Mononen, 2001).

4.3. Effects in man

Few studies have been performed where effects of PBDE have been studied in humans.

In two separate studies, the skin sensitisation potential of commercial DecaBDE products was followed in human volunteers (Norris et al., 1975a,b; IPCS, 1994a). Neither of these studies revealed any evidence of skin sensitisation.

Four epidemiologic studies have been performed in working places where flame retardants were used (IPCS, 1994a). The workers were potentially exposed to brominated flame retardants, including PBDEs and possibly also to PBDDs and PBDFs; the quality of the studies was not assessed. According to these studies, no adverse effects could be related to exposure to these chemicals. In one occupational study, where workers were exposed to PBBs and PBDEs during manufacture, higher-than-normal prevalency of primary hypothyroidism and significant reductions in conducting velocities in sensory and motor neurons were reported (Bahn et al., 1980). Apart from these findings, no other neurologic or dermatologic changes were seen. No decaBDE could be detected in the serum of exposed workers, and it could not be concluded that the observed effects were caused by the PBB and PBDE exposure.

In a study of male fish-eaters from the Baltic region, consuming 0–32 meals/month, a number of hormones were measured in the blood and compared to the levels on some selected environmental contaminants, including the PBDE congener BDE 47 (Hagmar et al., 2001). After adjustment for age, there was a significant association (negative correlation) between plasma BDE-47 and TSH. The authors stated that some significant correlation will occur from pure chance, and it was concluded that high consumption of organohalogen-polluted fish may not appear to affect plasma levels concentrations of pituitary, thyroid, or testosterone hormone levels in male adults.

4.4. Levels in man

Apart from these few reported effect studies, many observations of PBDE levels in human have been made. In occupationally exposed workers, PBDEs were detected, and significantly higher levels were recorded in workers exposed to these compounds during computer dismantling (Sjödin et al., 1999; Thomsen et al., 2001) and rubber manufacturing (Thuresson et al., 2002) and in computer technicians (Jakobsson et al., 2002). However, also in humans with no occupational exposure, detectable PBDE levels in serum have been observed (Sjödin et al., 2000; Van Bavel et al., 2002; Petreas et al., 2002; Lee et al., 2002). PBDEs have been detected also in adipose tissues (Meironyté Guvenius et al., 2001; Choi et al., 2002; Covaci et al., 2002; Crhova et al., 2002; She et al., 2002). In several studies, PBDEs have been found in breast milk from the mothers representing the general population (Meironyté et al., 1999; Lind et al., 2003; Ohta et al., 2002; Ryan et al., 2002), and very high PBDE levels were registered in a pooled breast milk sample from USA (Päpke et al., 2001). However, human PBDE levels, and more specifically time

trends and spatial trends in breast milk, will be discussed in other parts of this issue.

4.5. General PBDE conclusion

To conclude, exposure to PBDEs gives rise to adverse effects in experimental in vivo models, and depending on type of product different effects are seen, occurring at varying dose levels. Generally, the technical PentaBDE products seem to cause effects at the comparably lowest dose, whereas much higher doses were needed for effects of the DecaBDEs. Indeed, DecaBDES are generally considered to have the lowest toxicity of these three compound groups. The critical effects of PentaBDEs are those on neurobehavioural development and, although somewhat less sensitive, thyroid hormones in offspring (from 0.6 to 0.8 and 6 to 10 mg/kg body wt., respectively), whereas OctaBDEs primarily give rise to fetal toxicity/teratogenicity in rats and rabbits (from 2 mg/kg body wt.) and DecaBDEs cause certain morphological effects in the thyroid, liver and kidney of adult animals (from 80 mg/kg body wt.). Carcinogenicity studies on decaBDE, revealing some effects at high very high doses, have resulted in an IARC classification stating limited evidence for carcinogenicity of decaBDE in experimental animals (IARC, 1990). The overall IARC evaluation says decaBDE is not classifiable as to its carcinogenicity to humans (Group 3). We know very little about human effects of PBDEs, and have to base our risk assessment chiefly on animal models. What we know is that humans in general, at least in Western countries, are exposed to PBDEs and that human tissues contain measurable PBDE levels. If these levels are high enough to cause adverse human effects is unknown, but we know that the lowest body weight-related dose levels that cause effects in animals are much higher than available estimations of human dietary intake. Thus, a factor of 10×6 differs between these two exposure levels, based on Nordic and Canadian human intake data (Darnerud et al., 2000; oral information from Dr. Ryan, Canada and Dr. Kiviranta, Finland, at the BFR Workshop in Stockholm 2001). However, we know very little of PBDE toxicokinetics in man, and the actual margin of safety may be much smaller if based on body burden levels or concentrations in target organs. Also other data gaps in our knowledge of PBDE toxicity make this conclusion preliminary. Toxicity gaps that should be filled are, e.g. the carcinogenic potential of other PBDE compounds than DecaBDEs, more data on reproductive and immunologic effects, the mechanism of PentaBDE neurotoxicity and the possible relation between the observed thyroxin effects and other endpoints of toxicity. Another area where data is missing and which has purposely been omitted in this review is the degradation of PBDEs to lower brominated PBDE or other bromoorganic compounds. This transformation could in some case produce products with a higher toxic potency than the initial compounds.

5. Tetrabromobisphenol A (TBBPA)

5.1. Effects in mammals and related experimental systems

The acute oral toxicity of TBBPA for laboratory animals is low. The oral LD-50 for the rat and mouse and rabbit was >5 and 10 g/kg body wt., and the dermal LD-50 in rabbits was >2 g/kg body wt. (IPCS, 1995). TBBPA was not irritating and gave no sensitization reaction in animals tests, and only upon dermal exposure on abraded skin up to 2500 mg TBBPA/kg body wt. a slight skin erythema was seen in rabbits (Goldenthal et al., 1979; IPCS, 1995). After a single-dose TBBPA, moderate microsomal enzyme induction was observed in liver (Gustafsson and Wallén, 1988).

TBBPA was not mutagenic in various studies with *Salmonella typhimurium* strains, with metabolic activation by an S9 mix of Aroclor-induced rats and hamsters (IPCS, 1995). TBBPA caused no effect on induction of intragenic recombination in two in vitro mammalian cell assays (Helleday et al., 1999). In an in vitro model for immunotoxicity, TBBPA and also TBBPA bisallylether reduced CD25 (IL-2 receptor-alfa-chain), an inducible receptor chain essential for proliferation of activated T cells. The immunosuppressive effect was suggested not to be mediated via the Ah receptor (Pullen and Thiegs, 2001).

TBBPA was shown to be an effective binder to human transthyretin (TTR) in vitro, with ca. 10 time higher potency than thyroxin, the natural ligand (Meerts et al., 2000). In the same system, pentabromophenol also bound effectively to TTR (7 times T4). On the other hand, PBDE congeners as such did not compete with T4 for TTR binding, but were active only after microsomal biotransformation to their hydroxylated counterparts. When Meerts et al. (1999) studied the effects of TBBPA in an in vivo model, no effect of this compound on thyroid hormones in pregnant mice could be seen (see below).

An in vitro study of TBBPA on the function of biological membranes resulted in haemolysis of human erythrocytes and uncoupling of the oxidative phosphorylation in rat mitochondria, suggesting that TBBPA primarily alters the permeability of biological membranes (Inouye et al., 1979; IPCS, 1995). In study on the disposition of TBBPA in rats, it was seen that the level of ^{14}C-TBBPA radioactivity was 10 times higher in erythrocytes than in plasma 72 h after the administration (Szymanska et al., 2001). The authors suggest that the erythrocyte labelling represents TBBPA metabolite(s).

In several subacute or chronic exposure studies (oral, dermal, inhalation) of TBBPA in rats, no effects were observed (including body weight, haematology, clinical chemistry, urinalysis, organ weights, and gross and microscopic examination). In the dermal study, only slight erythema was noticed in rabbits. In an oral 90-day study on mice, 700 mg/kg body wt. did not cause any detectable adverse effects, whereas 2200 mg/kg body wt. resulted in decreased body weight, increased spleen weight, and re-

duced concentration of red blood cells, serum proteins, and serum triglycerides (Tobe et al., 1986; IPCS, 1995). In a study on rats, which were orally administered TBBPA in daily doses up to 250 mg/kg body wt. for 1 to 4 weeks, certain parameters suggest a slight renal impairment (significantly increased elimination of renal epithelial remnants in urine) (Frydrych and Szymanska, 2001). However, the results of this study are somewhat difficult to interpret. No carcinogenicity or long-term toxicity studies on TBBPA were reported.

In two teratogenicity studies in rats, no teratogenic effects were observed. However, in one study, three of five dams died in the highest (10 g/kg body wt. during day 6–16 during gestation) dose group (IPCS, 1995). In another study, TBBPA was given to pregnant rats on days 10–16 of gestation (Meerts et al., 1999), and effects on thyroid hormones (incl. TSH), and on competitive binding to fetal and maternal transthyretin (TTR) were studied. No effect was seen on T4 and T3 levels in dams and fetuses, whereas TSH levels were significantly increased in fetuses but not in dams. Result from the ^{125}I-T4-binding study showed no shift in binding, suggesting no TBBPA-related binding to TTR. Consequently, TBBPA was concluded not to bind to TTR in vivo. The difference between in vitro and in vivo effects (a strong effect observed of TBBPA on TTR in vitro) may have several explanations, and may well include toxicokinetic factors; a rapid excretion of TBBPA would prevent the formation of high enough plasma levels to have effects on the T4–TTR complex.

5.2. Effects in other animals/in wildlife

In fish, the acute 96-h LC-50 values of TBBPA for three species of fish was about 0.5 mg/l. Effects observed during the experiments were irritation, twitching and erratic swimming (bluegill sunfish, rainbow trout) and reduced survival and reduced growth of young individuals (fathead minnow) (IPCS, 1995). In effect studies in marine and freshwater algae, TBBPA was toxic in some marine species. TBBPA affected the reproduction of *Daphnia magna* and was toxic to Mysid shrimps (LC-50 about 1 mg/l).

TBBPA had no estrogenic activity in quail and chicken embryos, but resulted in embryolethality at high doses (Berg et al., 2001).

5.3. Effects in man

In humans, no skin irritation of sensitisation was observed in 54 human volunteers (Dean et al., 1978a; IPCS, 1995). Apart from that, no epidemiological or other data on effects of TBBPA of humans are available.

5.4. TBBPA derivates

Concerning TBBPA derivates, very few toxicity studies are available, but there is also a lack of other important data concerning their physical and chemical properties, production and use, environmental transport, etc. Because of this lack of data, these compounds were not evaluated by IPCS (1995). The TBBPA derivates (mentioned in IPCS, 1995) are dimethylether, dibromopropylether, bis(allylether), bis(2-hydroxyethyl ether), brominated epoxy oligomer, and carbonate oligomers. Where acute toxicity data were present, the derivates had only weak effects. Mutagenicity tests (*S. typhimurium* strains without and with metabolic activation) were mostly negative but in one case, TBBPA dibromopropylether, positive in *S. typhimurium* strains TA 100 and TA 135 (IPCS, 1995). However, using the same substance the results of an unscheduled DNA synthesis and an in vitro sister chromatid exchange were negative.

5.5. TBBPA conclusion

The toxicity of TBBPA in the tested experimental systems is suggested to be low. In most of the reported mammalian studies, only doses in gram/kg body wt. were effective. However, studies by Frydrych and Szymanska (2001) suggested that lower doses (250 mg/kg body wt. and lower) could result in a slight renal impairment in rats, although the study was difficult to interpret and should be repeated. Some of the in vitro studies could also be of interest in risk assessment aspects, and both the immunosuppressive effect, the in vitro binding to TTR (a T4 transporter in blood) and the binding to the erythrocytes should be noted. Before a definite standpoint could be reached on TBBPA toxicity, additional toxicity studies must be performed, including those on reproductive, immuno-, neurobehavioural and kidney toxicity. Because of the lack of toxicity data on the TBBPA derivates, little can be concluded on the possible risk from exposure to these compounds.

6. HBCD

Hexabromocyclododecane (HBCD) is an additive flame retardant used in the polymer and textiles industries. The major use of HBCD is in polystyrene, which is largely used in insulation panels and blocks for building constructions. HBCD could reach the environment by these products being incinerated, recycled or dumped at waste sites.

HBCD is reported to be absorbed from the gastrointestinal tract and it could be hypothesised that food intake is the largest single source of human exposure to HBCD. In experimental studies, HBCD has been found in several organs after oral administration, and the substance accumulates in adipose tissue after administration. Several (unidentified) metabolites are also shown in experimental studies (Yu and Atallah, 1980; KEMI, 2002).

6.1. Effects in mammals and related experimental systems

Toxicological effects of HBCD have been investigated in several studies, of which many are internal reports. According to KEMI (2002), the acute toxicity have been studied after dermal (rabbit), oral, (rat and mouse) and inhalation (rat) exposure. In the dermal studies, essentially no effects were seen after application of up to 20 g/kg body wt. In the oral studies, no increased death rate could be observed, and the LD-50 was defined to >6400 mg/kg body wt. in mice and >10 000 mg/kg body wt. in rats (EPA, 1990a; Wilson and Leong, 1977; both in KEMI, 2002). In some studies, at higher levels the animals showed some toxic signs, i.e. hypoactivity, corneal opacity, ptosis, and diarrhoea, as well as some decrease in body weight gain. After inhalation, a slight dyspnea was seen in rats at 200 mg/l air (Wilson and Leong, 1977; KEMI, 2002). To conclude, the acute toxicity of HBCD seems low.

In a number of eye and skin irritation studies on rabbits and guinea pigs, the substance was concluded not to be irritative or corrosive to the skin, and a very mild eye irritant. However, two of three studies showed that HBCD induced a dose-related sensitisation at higher doses after intra-dermal and topical application on guinea pigs. Mutagenicity studies (Ames test and in vitro test of chromosomal aberrations) showed no mutagenic potency of HBCD.

Regarding repeated dose toxicity of HBCD, two 28-day and two 90-day studies have been presented (Chengelis, 1997; Zeller and Kirsch, 1969, 1970; Chengelis, 2001; KEMI, 2002). In all these studies, the liver was defined as a target organ, and the effects observed were increased liver weight, microfoliar hyperplasia, and lipoid phanerosis. Based on the 90-day study by Chengelis et al., a LOAEL of 100 mg/kg body wt. was proposed based mainly on increased liver weight. In the same study, serum concentrations of thyroid hormones (T4 and TSH) were also affected at the 100 mg/kg dose. In addition, in the 28-day study by Zeller and Kirsch (1969) thyroid hyperplasia and inhibition of oogenesis was seen, although at higher doses (effects seen at 500 and 2500 mg/kg body wt. dose, respectively).

In an 18-month study carcinogenicity on mice, a large number of organs and tissues were monitored for possible tumours or neoplastic changes (Kurokawa et al.; cited in KEMI, 2002). Certain changes were seen in the liver only, and both gross findings (liver nodules) and histopathology (necrosis, fatty infiltration, altered foci and hepatocellular tumour) were generally most pronounced in the medium-dosed group (130 mg/kg body wt.; compared to 13 and 1300 mg/kg body wt., and control). In spite of inconsistencies in the dose–effect relationship in the cancer study, available data suggest that the carcinogenic risk of HBCD should not be overlooked. However, the lack of mutagenic effects suggests an epigenetic factor behind the increased tumour incidence, and an effect threshold could thus be proposed. From the tabulated data of the carcinogenicity study on mice, one of the observed effects seems to occur more frequent already at the 13 mg/kg-dose, although no statistical evaluation was presented. At the highest dose, 1300 mg/kg body wt., the levels of all these parameter decreased. To conclude, the carcinogenic potency of HBCD on the liver should not be disregarded but several factors (lack of strict dose–effect relationship, lack of effects in female mice, the studied mice strain, B6C3F1, is a sensitive strain for development of liver neoplasms) make this study questionable as a tool for carcinogenicity assessment.

In a 28-day study on reproductive effects of HBCD, high doses were shown to inhibit oogenesis in rats (Zeller and Kirsch, 1969; KEMI, 2002). In the males, no effects of high HBCD doses were observed on testes and epididymis. Based on the oogenesis effects, a NOAEL was set to 2500 mg/kg body wt./day. Studies on the developmental toxicity in rats, administering HBCD on days 0–20 of gestation in doses up to 750 mg/kg body wt./day showed a slight suppression of maternal food intake, and a certain increase in maternal liver weight. Based on these effects, a maternal NOAEL of 75 mg/kg body wt./day was obtained. However, no foetal abnormalities were seen and the number of live-born was unaltered. To conclude, available HBCD studies indicate a low foetotoxicity and teratogenicity, but that there is a need for further studies.

In a recent extended abstract, Eriksson et al. (2002b) exposed neonatal NMRI mice for HBCD on day 10, as a single oral gavage dose (0.9 or 13.5 mg/kg body wt.). At a later timepoint (3 months), behavioural studies were conducted in which locomotion, rearing, and total activity were recorded. The mice in the exposed groups were initially less active but became at later measurement periods more active than the control groups, and these effects were dose–response related. Based on this short paper, a preliminary LOAEL of 0.9 mg/kg body wt. may be set for these effects.

6.2. Effects in other animals/in wildlife

In general, toxic effects in the aquatic systems tested were hard to find: No toxic effects were seen in algae, in Daphnia (short-term test), and in fish. However, in a chronic study on Daphnia (21 days of exposure) the LOEC value was quite low (5.6 μl/l) (Dottar and Kreuger, 1997).

6.3. Effects in man

No human data are available except for a patch test in which patches with 10% HBCD were applied for 48 h, with no skin reactions on any subject (EPA, 1990b; KEMI, 2002).

6.4. HBCD conclusion

To summarise the HBCD data, there is a lack of relevant studies of high quality that could form a basis for a risk assessment for this compound. In a human test, HBCD

induced no skin reactions in a patch test. Based on available animal data, the critical effects are found in the liver: There was an increased liver weight and hepatic "lipoid phanerosis" in a 90-day feeding study on rats (LOAEL 100 mg/kg body wt./day), and effects on thyroid hormones were observed at the same dose level. Due to the many shortcomings of the carcinogenicity study on mice by Kurokawa et al., this study is of limited value and must be repeated. However, mutagenicity studies are negative which suggest that the carcinogenic effect, if any, has an epigenetic mechanism. In a recent short paper, a behavioural study in mice showed effects already at 0.9 mg/kg body wt., when HBCD was given on day 10 and testing was performed at 3 months after birth. Thus, behavioural effects may be a sensitive endpoint for HBCD, as have been shown for other flame retardant substances. Other types of behavioural tests should therefore be conducted on HBCD.

7. PBB

Polybrominated biphenyls (PBB) are identical to their chlorinated counterpart PCB, except for type of halogen atom present in the molecule. Thus, theoretically PBB should have a similar pattern of toxicity compared to PCB, apart from the change in effects that the chlorine–bromine substitution brings about. Consequently, the planar PBBs are most toxic (as they bind to the Ah receptor), whereas mono-ortho congeners are intermediate and di-ortho congeners least toxic. Indeed, 3,3′,4,4′,5,5′-hexabromobiphenyl was found to be the most toxic PBB congener in several systems, but this congener is present in low concentrations in technical PBB mixtures. Generally, the number of congeners in commercial mixtures is smaller in comparison to PCB, and most of the reported studies have been performed with use of the commercial mixture FireMaster (FM), or with similar hexabromobiphenyl mixtures. The cause for the comparably large number of studies on FM is the accident in Michigan 1973–1974, when FM was inadvertently added to animal feed. FM contained (in average) 60–80% 2,2′,4,4′,5,5′-hexaBB, 12–25% 2,2′,3,4,4′,5,5′-heptaBB, and smaller amounts of lower brominated compounds (IPCS, 1994b). Therefore, when nothing else is stated, in the present text the term PBB is equal to FM/hexaBB. Today, with the recent closure of the decaBB production in France, the PBB production in the world has ceased.

Much of the toxicological data gathered on PBB up to the early 1990s is compiled in IPCS health criteria document on PBB (IPCS, 1994b).

7.1. Effects in mammals and related experimental systems

The acute toxicity of commercial PBB mixtures is low (LD-50>1–21 g/kg body wt.) in rats, rabbits and quails, following oral or dermal administration (rats: e.g. Gupta and Moore, 1979). Generally, repeated dosing induced toxic effects at a lower dose compared to single (bolus) dosing. Effects, including death, were delayed after PBB (hexabromobiphenyl) administration, and signs of toxicity include reductions in feed consumption. Thus, a "wasting syndrome" is developed as an early indication of toxicity, and at death, the loss in body weight can be 30–40%. These effects are not seen in the few studies performed on octa- and deca-BB.

Eye and skin irritation tests/sensitization tests gave no, or only mild, reactions with use of the OctaBB and DecaBB preparations. Various assays for the detection of mutagenicity or genotoxicity generally failed to show any effect with individual PBB congeners or commercial mixtures. However, in some tumour-promotion models, FM and certain pure PBB isomers were effective, but the results from different models were somewhat contradictory.

Rhesus monkeys are among the species most sensitive to FM, and at long-time exposures of 1.3–300 mg FM/kg feed, they developed a number of symptoms, including weight loss, clinical chemistry changes, hair loss, skin lesions, oedema, etc. (Allen et al., 1978). In rats, repeated PBB (OctaBB and FM) exposure to low doses increased the liver weight, whereas a decrease in thymus weight was seen after FM exposure. Histopathologic changes in these organs were also noted. The morphological effects of PBB were most prominent in the liver, and were shown as liver enlargement, hepatocyte swelling and vacoulation, proliferation of ER and single-cell necrosis (e.g. Gupta et al., 1983). Histopathological effects were also seen in thymus, and it seems to be more toxic PBB congeners that give rise to the most severe effects, observed as body, thymus (decrease) and liver (increase) weight chances as well as morphological changes in liver and thymus. Atrophy of the thymus is a frequent observation following PBB exposure. In the liver, induction of mixed function oxidase enzymes has been much studied, and FM is considered to be a mixed-type inducer of hepatic microsomal enzymes.

PBBs interact with the endocrine system, and exposure resulted in decreases in serum T3 and T4 in rats and pigs (Byrne et al., 1987; Werner and Sleight, 1981). Vitamin A levels were shown to be strongly influenced by the PBB exposure, and also steroid hormone levels were reported to be altered (e.g. Bonhaus et al., 1981). PBB resulted in porphyria in rats and mice at 0.3 mg/kg body wt. (NOEL 0.1 mg/kg body wt./day) (e.g. Gupta et al., 1983).

In in vivo long-term toxicity studies, the liver was shown to be the principal site of tumour formation after PBB exposure. The incidence of hepatocellular carcinoma was increased in both rats and mice receiving PBB (FM and technical NonaBB) in doses from 0.5 and 5 mg/kg body wt./day for 2 years and 18 months, respectively (NTP, 1993; Momma, 1986; IPCS, 1994b).

Adverse effects of PBB on reproduction, such as resorptions and decrease viability of offspring, were observed in many species, and in the mink they were seen at a dietary

concentration of 1 mg/kg (Aulerich and Ringer, 1979). In Rhesus monkeys, decreases in the viability of the offspring were observed following a 12.5-month exposure via the diet, corresponding to an approximate daily dose of 0.01 mg/kg body wt. (Allen et al., 1979).

7.2. Effects in other animals/in wildlife

In short-term tests, the immobilization of *D. magna* by decabromobiphenyl was investigated. The EC-50 (24 h) value was reported to be 66 mg/l, but could be questioned because of low solubility of the substance (Atochem, 1990; IPCS, 1994b).

In hens, egg production and hatchability were affected at 30 mg PBB/kg feed (Ringer and Polin, 1977). In the same species, PBB resulted in increased mortality and reduced growth rates (Cecil and Bitman, 1978), and embryonic death. Japanese quail seemed somewhat less sensitive to these effects.

7.3. Effects in man

Human effects have been studied mainly in two separate epidemiological studies (reviewed in IPCS, 1994b), in which a number of endpoints were studied, e.g. cutaneous effects, liver function, porphyrin production, neurological effects, immune function and pediatric aspects. In the Michigan study (Michigan Department of Public Health), there were no general pattern of differences between "contaminated" and "noncontaminated" farms, and no unusual abnormalities of a number of examined organs or tissues were noticed. When comparing groups with different levels of exposure, there was no positive association between serum concentration of PBB and reported symptoms/disease frequencies. In the other study, from Wisconsin (Environmental Science Laboratory), the incidence of symptoms was greater and the greatest differences were in the classification of neurological and musculoskeletal symptoms. In spite of that the two studies could be interpreted somewhat differently, both studies showed that there were no positive dose–response relationship between the PBB levels in serum and adipose tissue, and the prevalence of symptoms. In one case, neurological performance tests gave a negative correlation between serum PBB levels and performance test scores, and subtle neuropsychological effects in the offspring to exposed individuals were also suggested; however, these effects have been questioned by other experts. In a recent study, the cancer risk in the MDPH cohort was followed 1973–1993 (Hoque et al., 1998). The authors found a serum PBB dose–response-related cancer risk of the digestive system, after adjustment for several possible confounders, and also the risk for lymphoma showed a dose–response relation. However, there was no increased overall cancer risk related to higher PBB serum levels.

7.4. PBB conclusion

Results from animal studies have shown that the reproductive effects in monkeys and the carcinogenic effects are those seen at the lowest administered dose. In the 2-year NTP carcinogenicity study, a daily dose of 0.15 mg/kg body weight did not result in any adverse effect, whereas higher doses induces tumours. Thus, a NOAEL of 0.15 mg/kg body wt./day could be suggested on the basis of this study. As PBB probably cause cancer by an epigenetic mechanism, an uncertainty factor (1000) was used to obtain a tolerable daily intake; this will result in a TDI of 0.15 μg/kg body wt. (IPCS, 1994b). This could be compared to an estimated daily intake for adults in the general population, 2 ng PBB/kg body wt., and for infants receiving human milk, 10 ng/kg body wt. (IPCS, 1994b).

8. General conclusion

In this article, I have only discussed the toxic effects of the compounds and not the exposure. However, it must always be remembered that the risk for health effects from exposure to chemical compounds, in this case brominated flame retardants, is a combination of the intrinsic toxic potential of and the actual (target tissue) exposure for the compound.

The BFR compounds/compound groups discussed above do in most cases belong to a similar structural group, i.e. they contain two aromatic bromine-containing rings (with the exception of HBCD). Indeed, this structural resemblance also affects the toxic potential, and several congeners bind to the dioxin (or Ah) receptor. This is for instance the case with the hexabromobiphenyl (3,3′,4,4′,5,5′ -HBB). In practice, these "highly toxic" congeners play a little role, as they often are present in low levels. However, in the case of HBB the observed carcinogenicity could very well be Ah-receptor mediated. Other similarities in effects of the BFRs, that could in some cases be structure related, are the neurobehavioral effects, the effects on thyroid hormone homeostasis and the effects on the liver. There are however also rather distinct differences between the members of the BFR groups, and TBBPA seems to have a comparatively low toxic potential, compared to the other groups. There are also differences within each BFR group, regarding both target organs/tissues and effective dose, and one example is the PBDEs. The differences could partly be caused by the differences in uptake, distribution and elimination, parameters that will not be discussed in this presentation.

Lastly, there are several and important data gaps that have to be filled before we could perform solid risk assessments of the different members of the BFR. These gaps include, among other, carcinogenicity, neurotoxicity, immunotoxicity and reproduction toxicity studies. As stressed in the Summary session of the Dioxin 2002 Conference in Barcelona, effect studies are highly needed.

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A review on human exposure to brominated flame retardants—particularly polybrominated diphenyl ethers

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Abstract

Brominated flame retardants (BFRs) have been and are still heavily used as additive or reactive chemicals in polymers and textiles. Only a few of the BFRs have been assessed in human subjects with a major data set on internal exposures to polybrominated diphenyl ethers (PBDEs). Increasing PBDE levels have been observed in mothers' milk from Sweden as well as in blood from Germany and Norway. The levels are in general lower than PCB levels. However, the PBDE concentrations found in the North Americans are considerably higher compared to European subjects. The PBDEs are dominated by 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47). Decabromodiphenyl ether (BDE-209) is reported both in the general population and in occupationally exposed persons showing the bioavailability of this high molecular weight compound. While the lower and medium brominated diphenyl ethers are persistent, BDE-209 has a fairly short half-life of approximately 2 weeks. Tetrabromobisphenol A (TBBPA) is readily eliminated in humans showing a half-life of about 2 days. Still, TBBPA is accumulated in humans but a continuous exposure to this BFR is required to maintain a certain level in the human subject. TBBPA has not been detected in the general population but in people exposed at work. The current review addresses human exposure routes and levels of BFRs.
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Keywords: Human; Brominated flame retardants; Polybrominated diphenyl ethers

1. Background

Flame retardants (FRs) are incorporated into potentially flammable materials, such as plastics, rubbers and textiles, to slow down and/or inhibit the initial phase of a developing fire. Thus, FRs perform an important service in our modern society by reducing the number of fires and limiting the consequences of fires that do develop. Common applications of FR chemicals include the plastic housings of electronic appliances and in printed circuit boards as well as in upholstery and construction materials (OECD, 1994; WHO, 1994b). Historically, during the second half of the 17th century inorganic compounds, such as ammonium salts, were used for protecting textiles and property of the famous French king, Ludwig XIV. The need for flame retardants increased with the inventions of polymers and modern materials. New fire retarding compounds were developed, including inorganic compounds as well as organohalogen chemicals, organophosphate esters and less common nitrogen containing compounds (WHO, 1997, 1998). These chemicals are divided into two major groups: reactive and additive FRs. Reactive FRs are covalently bonded into the polymer matrix and these chemicals pose a different exposure problem than additive chemicals which form no chemical bonds with the materials.

FRs are a diverse group of industrial chemicals, which include more than just PBDEs. Chemical and physical properties for selected brominated flame retardants (BFRs) are summarized in Table 1; their chemical structures are shown in Fig. 1. Approximately 75 different chemicals have been used as FRs (WHO, 1997). Reactive BFRs are mixed with the plastic before polymerization to form covalent bonds and become a part of the polymer matrix. Additive BFRs, however, are mixed with the polymer. This makes additive FRs much more likely to leach out of goods and products during their lifetime. Polybrominated diphenyl ethers (PBDEs) (WHO, 1994b), polybrominated biphenyls (PBBs) (WHO, 1994a) and hexabromocyclododecane (HBCDD) (WHO, 1997), cf. Fig. 1, are examples of common

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Table 1
Chemical and physical properties for some selected brominated flame retardants, and for comparison 2,2',4,4',5,5'-hexachlorobiphenyl

Compound (abbreviation)	Formula	Molecular mass (amu)	Vapor pressure (Pa)	log K_{ow}	pK_a	Literature reference
Brominated flame retardants (BFRs)						
Tetrabromodiphenyl ether (tetraBDE)	$C_{12}H_6Br_4O$	485.8	$(2.6–3.3) \times 10^{-4}$, 25 °C	5.9–6.2		Watanabe and Tatsukawa, 1989
Pentabromodiphenyl ether (pentaBDE)	$C_{12}H_5Br_5O$	564.8	$(2.9–7.3) \times 10^{-5}$, 25 °C	6.5–7.0		Watanabe and Tatsukawa, 1989
Hexabromodiphenyl ether (hexaBDE)	$C_{12}H_4Br_6O$	643.6	$(4.2–9.4) \times 10^{-6}$, 25 °C	6.9–7.9		Watanabe and Tatsukawa, 1989
Octabromodiphenyl ether (octaBDE)	$C_{12}H_2Br_8O$	801.5	$(1.2–2.2) \times 10^{-7}$, 25 °C	8.4–8.9		Watanabe and Tatsukawa, 1989
Decabromodiphenyl ether (decaBDE)	$C_{12}Br_{10}O$	959.2	$<10^{-4}$, 20 °C <100, 250 °C 270, 278 °C 670, 300 °C	10		WHO, 1994b
Hexabromobiphenyl (hexaBB)	$C_{12}H_4Br_6$	627.6	8.0×10^{-6}, 25 °C	7.5		Watanabe and Tatsukawa, 1989
Decabromobiphenyl (decaBB)	$C_{12}Br_{10}$	943.2	$<1.3 \times 10^{-9}$, 25 °C	8.6		Watanabe and Tatsukawa, 1989
1,2-Bis(2,4,6-tribromophenoxy) ethane (BTBPE)	$C_{14}H_8Br_6O_2$	687.6	not available	8.9		Watanabe and Tatsukawa, 1989
Tetrabromobisphenol A (TBBPA)	$C_{15}H_{12}Br_4O_2$	543.9	<100, 20 °C	4.5–5.3[a]	7.7 and 8.5	WHO, 1995
Other organohalogen substances (OHS)						
2,2',4,4',5,5'-Hexachlorobiphenyl (CB-153)	$C_{12}H_4Cl_6$	360.9	7.0×10^{-4}, 25 °C	7		WHO, 1993

[a] pH not given.

additive FRs. Tetrabromobisphenol A (TBBPA) can be used as either an additive or a reactive FR (WHO, 1995). Organophosphate compounds have also been used as flame retardants (WHO, 1998). Inorganic compounds, such as antimony oxide (Sb_2O_3), are commonly used in combination with BFRs and organophosphate FRs, due to a synergistic effect that enhances the flame retarding effect (Price, 1989). The amount of FRs in any one product depends on the type of application and need for fire protection. In polymeric materials, between 5% and 30% by weight, has been reported to consist of FR chemicals (WHO, 1994b).

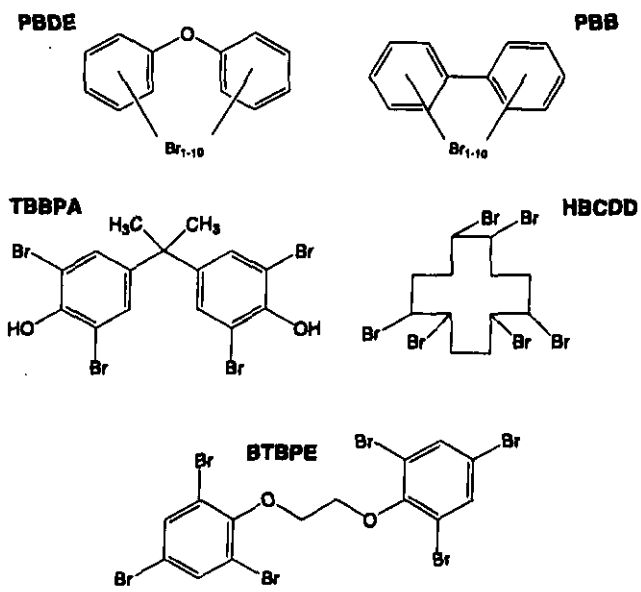

Fig. 1. Structural formulas of common chemicals applied as flame retardants. PBDE, polybrominated diphenyl ethers; PBB, polybrominated biphenyls; TBBPA, tetrabromobisphenol A; HBCDD, hexabromocyclododecane; BTBPE, 1,2-bis(2,4,6-tribromophenoxy) ethane.

1.1. Commercial production of PBDEs

PBDEs are commercially produced with three degrees of bromination, i.e., pentaBDE, octaBDE, and decaBDE, indicating the average bromine content. Brominated aromatic compounds are commercially produced by catalyzed direct bromination. Since this method is nonselective, mixtures of homologues and isomers are formed; theoretically 209 PBDE congeners can be formed. Commercially produced PBDE mixtures contain a limited number of PBDE congeners and are less complex than the corresponding technical polychlorinated biphenyl (PCB) mixtures depending on major steric hindrance from the large bromine atom. Commercially produced pentaBDE for example is dominated by two major congeners, representing ~ 70% in total of the product by weight (Sjödin et al., 1998).

The global demand for PBDEs has been estimated to be close to 70,000 metric tonnes in 1999 (13%, 6% and 81% were produced as penta-, octa- and decaBDE, respectively) (Bromine Science and Environmental Forum, 2000). The percentage of the world wide demand used in North America in 1999 corresponded to 98% for pentaBDE, while the corresponding figures for octa- and decaBDE were 36% and 44%, respectively (Bromine Science and Environmental Forum, 2000). These numbers can be compared to data from

1992 showing an estimated global demand of 40,000 metric tonnes annually for PBDE of which 10% was penta, 15% was octa and 75% was decaBDE (WHO, 1994b). These data, although limited, show a considerable increase of PBDE use worldwide and a substantial use of the pentaBDE mixture in North America.

1.2. PBDE environmental levels

PBDEs (produced since the 1970s) were first reported as an environmental contaminant in the River Viskan, in the early 1980s (Andersson and Blomkvist, 1981). Since then, they have been found in most environmental compartments, including aquatic and terrestrial ecosystems. These include sediments (Watanabe et al., 1987; Yamamoto et al., 1997; Sellström et al., 1998, 1999; Sellström, 1999), fish (Sellström et al., 1993; Asplund et al., 1999a,b; Dodder et al., 2002; Hale et al., 2002; Luross et al., 2002), mussels (Booij et al., 2002) and Guillemot eggs (Sellström, 1999) as well as Rein Deer (Sellström et al., 1993). Although most published data originate from the Scandinavian countries, PBDE levels appear to be much higher in North America than in Europe, at least in certain areas, such as the Great Lakes. This is illustrated by the sum of sPBDE (6 tri- to hexaBDE congeners quantified) concentrations of 3000 ng/g lipid weight (l.w.) in Steelhead trout (Asplund et al., 1999b) and sPBDE (6 tetra- to hexaBDE congeners quantified) of 2440 ng/g l.w. in Salmon (Manchaster-Neesvig et al., 2002) taken from Lake Michigan. These results are in contrast to Salmon caught in the Baltic Sea, which have been reported to have a sPBDE (6 tri- to hexaBDE congeners quantified) concentration of 180 ng/g l.w. (Asplund et al., 1999b). Furthermore, data on herring gull eggs collected at several locations in the Great Lakes region show a rapidly increasing trend of tetra- to hexaBDEs (Norstrom et al., 2002). The sum PBDE level in herring gull eggs collected at Shelter Island in Lake Huron was reported to be 23, 142 and 633 ng/g (wet weight), for the years 1981, 1990 and 2000, respectively, which corresponds to a doubling in concentration every 5 years (Norstrom et al., 2002). By contrast, the sum PBDE (BDE-47, -99 and -100) level in guillemot eggs collected at Stora Karlsö in the Baltic Sea has been reported to be 190 ng/g l.w. (23 ng/g wet weight) in 1997 (Sellström, 1999). Commercial production of PBDEs ceased some 20 years ago in the Great Lakes region. Within the United States, current commercial production of PBDEs is localized in Arkansas (Hardy, personal communication).

1.3. Other BFRs of environmental concern

TBBPA is a phenolic, weakly acidic and hydrophobic compound (pH dependent), cf. Table 1. The worldwide demand for (TBBPA) and its derivatives has been estimated to be approximately 120,000 tonnes annually (Bromine Science and Environmental Forum, 2000). The primary use of TBBPA is as a reactive FR in epoxy and polycarbonate resins. These polymers are typically used in, among other things, printed circuit boards and various electronic equipment. Approximately 10% of total TBBPA produced is used as an additive FR in acrylonitrile–butadiene–styrene (ABS) resins as well as in high impact polystyrene (WHO, 1995).

TBBPA has been reported in river sediments in Japan in 1983 (Watanabe et al., 1983) and later in river sediments in Sweden (Sellström and Jansson, 1995). TBBPA has further been identified and quantified in workers occupationally exposed to BFRs (Hagmar et al., 2000), cf., occupational exposure below. TBBPA has, to our knowledge, not hitherto been reported in human foodstuffs.

Polybrominated biphenyls (PBBs) (Fig. 1) represent a class of BFRs that are no longer commercially produced. The last known commercial production of decaBB ended in France in year 2000 (De Poortere, personal communication). Commercial production of hexaBB in the US ended in 1974 following an accident in the state of Michigan were animal feeds were contaminated by a hexaBB product, called Fire Master BP-6, cf., accidental exposure below.

Another BFR, HBCDD (Fig. 1) is mainly used in polystyrene foams and in back coating layers of textiles as well as in high impact polystyrene. HBCDD has been identified in sediments and fish from contaminated rivers (Sellström et al., 1998).

Reports about 1,2-bis(2,4,6-tribromophenoxy) ethane (BTBPE), cf. Fig. 1, in the scientific literature are scarce, no reliable information about production volumes or environmental levels is available. BTBPE was reported in air samples collected in the vicinity of a manufacturing plant in 1976 and 1977 (Zweidinger et al., 1977). More recently, BTBPE was identified in indoor air at an electronics recycling plant (Sjödin et al., 2001). However, it was not detected in the workers blood, cf., occupational exposure below.

2. Human exposure to BFRs

2.1. Routes of exposure

BFRs make their way to human populations primarily via food intake in cases when the compounds are persistent enough to be biomagnified in the food web similarly to other persistent chemicals as has been shown for BDE-47 in humans consuming large quantities of Baltic Sea fish (Sjödin et al., 2000). This means that fatty fish from contaminated areas are a major source (Sjödin et al., 2000; Sjödin, 2000) and mother's milk is a source (Meironyté et al., 1999; Meironyté Guvenius, 2002) for the nursing child. Nonpersistent BFRs, such as TBBPA, other phenolic BFRs and esters, do not biomagnify hence leading to direct exposures via inhalation being the predominant route of exposure. However, the exposures are only of significance if there is a continuous (persistent) exposure

of the subject. Also, the presence of persistent BFRs in ambient air in indoor work and/or home environments may cause a nonnegligible exposure of these chemicals in humans as discussed below. Dermal uptake of BFRs seems less likely; at least this is not believed to be a major route of exposure.

2.2. General population

It has been estimated (in Sweden) that 50% of the overall PCB intake, occurs via intake of various kinds of fish (Darnerud et al., 1995). The remaining exposure to PCBs occurs through consumption of meat and diary products. This is most likely the case for PBDEs as well, as indicated by a cohort in Sweden showing a strong association between consumption of Baltic Sea fish and serum PBDE levels. Swedes who did not consume fish had a median PBDE level of 0.4 ng/g l.w. (<0.1–2.5; 10–90 percentile) compared to 2.2 ng/g l.w. (0.96–5.7; 10–90 percentile), for the high consumers of fish who ate between 12 and 20 meals of fatty Baltic Sea fish per month (Sjödin et al., 2000). Interestingly, when analyzing the correlation of an individual's age versus PBDE concentration (Fig. 2), there was no significant relationship with age even though an increase in overall PBDE concentration was observed with increased consumption of fish. On the other hand, a significant correlation with age was found for 2,2′,4,4′,5,5′-hexachlorobiphenyl (CB-153) for groups with moderate to high consumption of fatty Baltic Sea fish. CB-153 represents a stable and persistent environmental contaminant, which has been present in the Baltic Sea environment since the 1960s (Bignert et al., 1998). The lack of a correlation with age for PBDEs may indicate that levels of PBDEs are increasing in the Scandinavian environment and in the fish consumed. Time trend data from the Scandinavian countries support this assumption showing an increasing PBDE concentration in humans and wildlife, cf. temporal PBDE trends below.

2.3. Human levels and trends

Time trend studies of PBDEs have been performed by a few authors using different matrices, i.e., breast milk (Meironyté et al., 1999; Meironyté Guvenius, 2002), whole blood (Schröter-Kermani et al., 2000) and serum (Thomsen et al., 2002). The results from these studies are summarized in Table 2 and Fig. 3. As seen in Fig. 3, the concentration of BDE-47 (the dominating congener in non-occupationally exposed subjects) increases in the general European population from the early 1970s until the mid-1990s. Interestingly, one of these studies (Meironyté Guvenius, 2002) shows a decrease in concentration starting in the latter part of the 1990s. Finding a decreasing trend of PBDEs in Sweden is encouraging news and is further supported by data on Guillemot eggs from Stora Kalsö in the Baltic proper (Sellström, 1999), which also show decreasing levels of PBDEs. However, the trend-line on Guillemot eggs starts

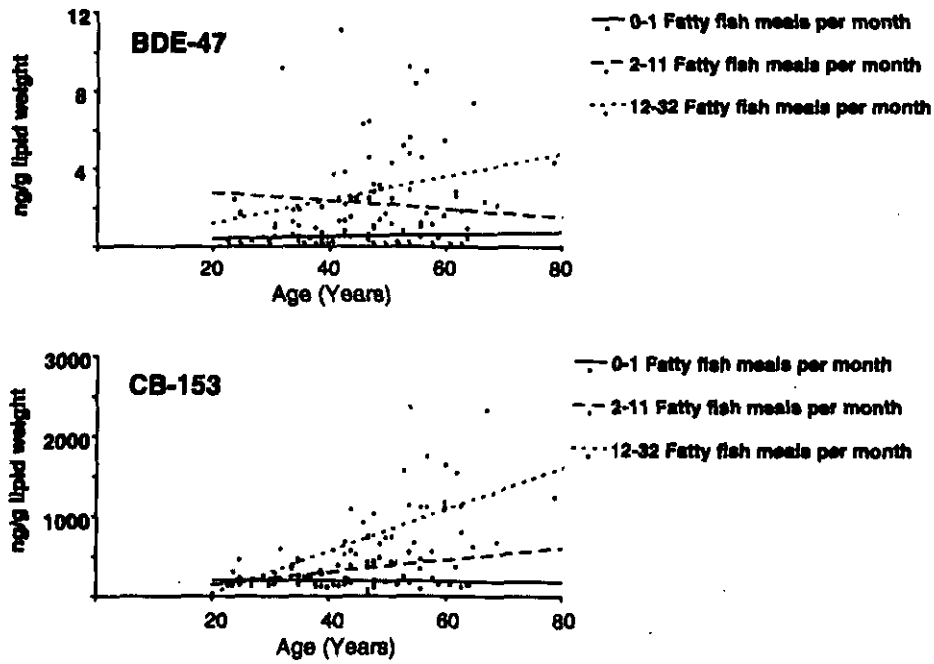

Fig. 2. Plasma levels (ng/g lipid weight) of 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) and 2,2′,4,4′,5,5′-hexachlorobiphenyl (CB-153) in relation to age in three groups of men with different consumption of fatty fish from the Baltic Sea; none or low (0–1 meals/month), moderate (2–11 meals/month) and high (>12 meals/month). Spearman's rank correlation coefficients and levels of significance are $R_s = -0.03$, $p > 0.5$; $R_s = 0.00$; $p > 0.5$; and $R_s = 0.28$, $p = 0.10$ for BDE-47; and $R_s = 0.00$, $p > 0.5$; $R_s = 0.22$, $p = 0.20$; and $R_s = 0.71$, $p < 0.001$ for CB-153 for low, moderate and high consumption, respectively. Data from Sjödin et al. (2000).

Table 2
Concentrations of polybrominated diphenyl ethers (PBDEs) in human tissues, expressed as ng/g lipid weight (l.w.), and when available for the level of 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB-153)

Year	Tissue	Location	Age	Sex (*n*)	BDE-47	BDE-99	BDE-100	BDE-153	BDE-154	PCB-153	Reference
			Mean[a]		Mean(range)	Mean(range)	Mean(range)	Mean(range)	Mean(range)	Mean(range)	
Belgium											
2000	Adipose	Antwerp	–	– (20)	1.5(0.54–4.7)	0.29(nd–1.6)	0.48(nd–1.5)	2.5(nd–4.7)	–	–	Covaci et al., 2002
Canada											
1992	Milk	Ontario and Quebec	–	F (10)	3.4(0.31–19)	1.2(0.10–5.6)	0.44(0.07–2.1)	0.41(0.14–1.3)	–	60(28–130)	Ryan and Patry, 2000
2002[b]	Milk	British Columbia		F (20)	18	4	5	13	1		Ryan et al., 2002
Finland											
1994–1998	Milk	–	34[c]	F (11)	1.3(0.3–4.3)	0.39(0.14–0.94)	–	0.39(0.19–0.72)	–	–	Strandman et al., 2000
Germany											
1985	Blood	Germany	20–30[d]	M (8)	3.4(2.3–3.4)	–	–	–			Schröter-Kermani et al., 2000
1985	Blood	Germany	20–30[d]	F (8)	2.8(1.3–2.6)	–	–	–			Schröter-Kermani et al., 2000
1985	Blood	Germany	20–30[d]	F/M (16)	3.1(2.5–2.9)						Schröter-Kermani et al., 2000
1990	Blood	Germany	20–30[d]	M (10)	4.8(2.3–8.0)	–	–	–			Schröter-Kermani et al., 2000
1990	Blood	Germany	20–30[d]	F (9)	2.3(0.88–4.1)	–	–	–			Schröter-Kermani et al., 2000
1990	Blood	Germany	20–30[d]	F/M (19)	3.6(2.6–5.0)						Schröter-Kermani et al., 2000
1995	Blood	Germany	20–30[d]	M (10)	3.5(1.8–3.8)	–	–	–			Schröter-Kermani et al., 2000
1995	Blood	Germany	20–30[d]	F (9)	3.9(1.9–6.1)	–	–	–			Schröter-Kermani et al., 2000
1995	Blood	Germany	20–30[d]	F/M (19)	3.7(2.9–4.4)						Schröter-Kermani et al., 2000
1999	Blood	Germany	20–30[d]	M (10)	4(2.5–4.8)	–	–	–			Schröter-Kermani et al., 2000
1999	Blood	Germany	20–30[d]	F (10)	3.8(2.5–4.1)	–	–	–			Schröter-Kermani et al., 2000
1999	Blood	Germany	20–30[d]	F/M (20)	3.9(3.4–4.7)						Schröter-Kermani et al., 2000
Norway											
1977[e]	Serum	–	40–50	M (34)	0.25	0.09	nd	0.10	nd		Thomsen et al., 2002
1981[e]	Serum	–	40–50	M (17)	0.32	0.13	0.08	0.18	0.22		Thomsen et al., 2002
1986[e]	Serum	–	40–50	M (24)	0.41	0.13	0.12	0.14	0.26		Thomsen et al., 2002
1990[e]	Serum	–	40–50	M (20)	0.89	0.24	0.13	0.27	0.23		Thomsen et al., 2002
1995[e]	Serum	–	40–50	M (19)	1.4	0.33	0.32	0.52	0.50		Thomsen et al., 2002
1999[e]	Serum	–	40–50	M (29)	1.5	0.31	0.35	0.59	0.35		Thomsen et al., 2002

Sweden											
1972[e]	Milk	–	27–28[a]	F	0.06	nd	nd	0.01	nd	215	Lundén and Norén, 1998; Meironyté et al., 1999
1976[e]	Milk	–	27–28[a]	F	0.18	0.04	0.05	0.02	0.01	197	Lundén and Norén, 1998; Meironyté et al., 1999
1980[e]	Milk	–	27–28[a]	F	0.28	0.09	0.04	0.03	0.01	152	Lundén and Norén, 1998; Meironyté et al., 1999
1984[e]	Milk	–	27–28[a]	F	0.49	0.08	0.06	0.05	0.02	124	Lundén and Norén, 1998; Meironyté et al., 1999
1990[e]	Milk	–	27–28[a]	F	0.81	0.15	0.06	0.10	0.04	116	Lundén and Norén, 1998; Meironyté et al., 1999
1994[e]	Milk	–	27–28[a]	F	1.5	0.26	0.09	0.15	0.02	93	Meironyté et al., 1999; Norén and Meironyté, 2000
1996[e]	Milk	–	27–28[a]	F	2.1	0.41	0.15	0.24	0.01	83	Meironyté et al., 1999; Norén and Meironyté, 2000
1997[e]	Milk	–	27–28[a]	F	2.3	0.48	0.42	0.46	0.05	73	Meironyté et al., 1999; Norén and Meironyté, 2000
1998[e]	Milk	–	30	F	2.3	0.60	0.31	0.47	0.02	59	Meironyté Guvenius, 2002
1999[e]	Milk	–	31	F	2.0	0.43	0.24	0.54	0.04	50	Meironyté Guvenius, 2002
2000[e]	Milk	–	30	F	1.7	0.23	0.22	0.45	0.02	47	Meironyté Guvenius, 2002
United States of America											
1988	Serum	Illinois	–	M (12)	0.63(<0.4–24)[c]	0.32(<0.2–3.7)[c]	0.17(<0.1–2.4)[c]	0.96(0.08–2)[c]		69(7.6–940)	Sjödin et al., 2002
late 90s	Adipose	California Bay	47	F (23)	33(7–200)	11(2.2–72)	9.1(0.77–61)	16(1.5–200)	17(2.9–71)		She et al., 2002
1997[e]	Milk	New York State	F	F (19)[f]	83	30	16	9.1	1.4		Betts, 2002
2000[e,g]	Milk	Austin and Denver	–	F (?)	130	28	24	15	1.9		Päpke et al., 2001

Available data has been subdivided according to country and region.

BDE-47, 2,2′,4,4′-tetraBDE; BDE-99, 2,2′,4,4′,5-pentaBDE; BDE-100, 2,2′,4,4′,6-pentaBDE; BDE-154, 2,2′,4,4′,5,5′-hexaBDE; 2,2′,4,4′,5,6-hexaBDE, BDE-154.

[a] F = female; M = male.

[b] Data from figure.

[c] Median age given.

[d] Range given.

[e] Pooled samples.

[f] Illinois, USA.

[g] Austin and Denver, USA.

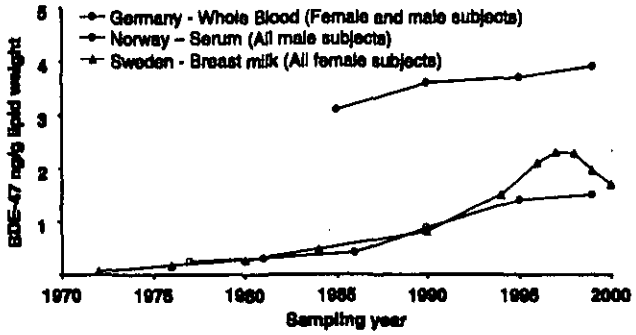

Fig. 3. Published time trends of 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) in Europe using different matrices, i.e., breast milk (Sweden) (Meironyté et al., 1999; Meironyté Guvenius, 2002), serum (Norway) (Thomsen et al., 2002) and whole blood (Germany) (Schröter-Kermani et al., 2000). Lipid adjusted values (ng/g lipid weight) are used to minimize matrix differences.

to decrease in the mid-1980s, indicating a potential lag-time of a decade before a positive effect in the environment was translated to decreasing levels in the human population.

The results from several studies originating from North America are shown in Fig. 4 (Ryan and Patry, 2000; Sjödin et al., 2002; Päpke et al., 2001; Betts, 2002; She et al., 2002). These studies were never intended to be a determination of temporal trends. Different matrices and locations in North America were used but when comparing data on a l.w. basis the assumption can be made that differences in the matrices are minimized. As shown in Fig. 4, it may be speculated that the concentrations of PBDEs are increasing with time also in North America. Strikingly, the concentrations are higher in the U.S. samples, representing humans from North America, than in the European populations studied so far. The levels in breast adipose tissue taken from women living in San Francisco Bay area (She et al., 2002) in 2000 are almost two orders of magnitude higher than what has been reported in human milk from Sweden (Meironyté et al., 1999). The last data point originating from Canada in 2002 (Ryan et al., 2002) is much lower than what is determined for other locations in North America during proceeding years. No firm conclusions from this observation can be drawn since the amount of data is still limited. Further information on variation between different locations in North American as well as studies designed to assess time trends are needed before any conclusions can be drawn.

The reason for the apparent higher concentrations in North America is unknown. One explanation may be that the main use of the commercial pentaBDE is within the North American continent (Bromine Science and Environmental Forum, 2000). This product is the one in which the congeners most commonly found in humans and wildlife are present.

It must be pointed out that most studies have included only low to medium brominated diphenyl ethers thus not including any results on decaBDE even though these studies are in progress. DecaBDE is present in human subjects without known exposures to this major PBDE product. Levels of BDE-209 (decaBDE) have been reported to range from <0.3 to 9.3 ng/g l.w., in the Swedish general population (Sjödin et al., 1999; Thuresson et al., 2002).

2.4. Dietary exposure

Recently, it has been reported that PBDEs are present in chickens originating from North America. However, the levels in poultry (1.8–39 ng/g l.w. for the sum of tri- to decaBDE (Huwe et al., 2002)) are still much lower than what is reported in fish (Asplund et al., 1999b; Manchaster-Neesvig et al., 2002). Nevertheless, poultry may be a contributing factor to PBDE exposure for people with a low dietary intake of fish. Tri- to hexaBDE levels in spinach, potato and carrot have been reported in Japan to be 134, 47.6 and 38.4 ng/g fresh weight (Ohta et al., 2002), respectively. The same article also reported 63.4 ng/g in pork, 16.2 ng/g in beef and 6.25 ng/g in chicken.

The total daily intake of PBDEs has been estimated to be 44 ng/day in Canada by compiling PBDE concentration data from 40 common commercial food items and food consumption data (Ryan and Patry, 2001).

Relatively high levels of PBDEs have been found in sewage sludge destined for land-applications, i.e., biosolids, in North America. Eleven samples of biosolids destined for land-application had levels of 1100–2290 ng/g of tetra- to hexaBDE and <75 to 9160 ng/g of decaBDE (Hale et al., 2001). A potential route of human exposure to PBDEs is the application of biosolids in agricultural areas; this route of exposure should be investigated further. Over half of the 6.9 million tonnes of biosolids generated in 1998 by U.S. wastewater treatment plants were land-applied (United States Environmental Protection Agency, 1999).

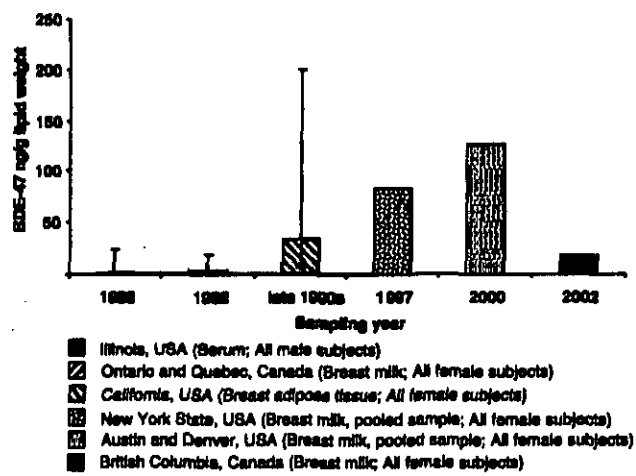

Fig. 4. Published levels of 2,2',4,4'-tetrabromodiphenyl ether in various matrices from North America, presented in relation to year of sample collection. Lipid adjusted values (ng/g lipid weight) are used to minimize matrix differences. Error bars indicate the range of data from Betts (2002), She et al. (2002), Sjödin et al. (2002), Päpke et al. (2001), Ryan and Patry (2000) and Ryan et al. (2002).

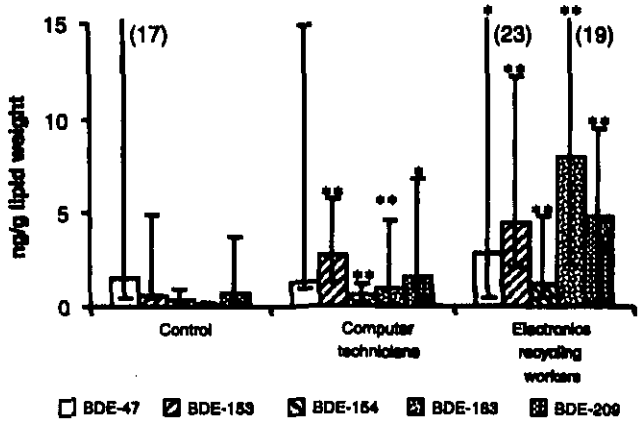

Fig. 5. Median concentration of polybrominated diphenyl ethers (PBDEs) in three occupational groups in Sweden: hospital cleaners (control; $n=20$), computer technicians ($n=19$) and electronics dismantling workers ($n=19$). Error bars indicate range, maximum value given in parenthesis when off scale. Levels of significance (Mann–Whitney U-test) as compared to control group are indicated; $^{*}p \leq 0.05$, $^{**}p \leq 0.001$. BDE-47, 2,2′,4,4′-tetraBDE; BDE-153, 2,2′,4,4′,5,5′-hexaBDE; 2,2′,4,4′,5,6′-hexaBDE (BDE-154); BDE-183, 2,2′,3,4,4′,5′,6-heptaBDE; BDE-209, 2,2′,3,3′,4,4′,5,5′,6,6′-decaBDE. Data from Sjödin et al. (1999) and Jakobsson et al. (2002).

2.5. Occupational exposure

In Sweden, occupational exposure to PBDEs has been identified among electronics recycling personnel (Sjödin et al., 1999), technicians responsible to repair and maintain computers (Jakobsson et al., 2002), cf. Fig. 5, workers adding decaBDE during rubber manufacturing (Thuresson et al., 2002), and employees working at facilities manufacturing electric cables using the same rubber (Thuresson et al., 2002).

The recycling workers performed manual dismantling of computers as well as other electronic devices such as television sets, stereos and machinery such as mail sorting equipment. This equipment was dismantled with the aid of pneumatically operated power tools, which mobilizes large quantities of the dust accumulated within the electronic equipment. The dismantled materials from computers, monitors and television sets were sorted into several fractions: plastics, metals, circuit boards and picture tubes. Respirators were not worn at the plant with the exception of a single employee operating a shredder used for grinding plastics into small pieces (1–5 cm^2). Air concentration measurements inside the plant (Sjödin et al., 2001) revealed much higher concentrations of BDE-183 and BDE-209 in the air (19 and 36 ng/m^3, respectively) than those determined in common office environments (0.0082 and 0.083 ng/m^3 for BDE 183 and BDE-209, respectively). These findings demonstrate that there was occupational exposure at the recycling plant to BDE-183 and BDE-209 (Fig. 5) and to a lesser extent exposure to BDE-47, BDE-153 and BDE-154. These findings are consistent with the known uses of PBDEs (pentaBDE mainly in polyurethane foam and upholstery, while the main uses of octa- and decaBDE is in plastics). A similar exposure situation has been identified at an electronics recycling facility in the Stockholm area, Sweden (Hovander et al., 2001).

Although levels of PBDEs in the recycling workers were elevated above the controls, the levels are still lower than PCB-153 (a most persistent PCB congener). The median levels for the sum of PBDEs and CB-153 were determined to be 26 and 270 ng/g l.w., respectively (Sjödin et al., 1999).

Air concentration measurements at the electronics recycling plant also revealed surprisingly high concentrations of BTBPE (20 ng/m^3), TBBPA (30 ng/m^3) and 2,2′,3,3′,4,4′,5,5′-decabromobiphenyl (BB-209; 36 ng/m^3) in the electronics dismantling area. Follow-up studies also revealed the presence of TBBPA (Hagmar et al., 2000; Sjödin, 2000) and BB-209 (Sjödin, unpublished) in serum drawn from workers engaged in electronics recycling. Interestingly, BTBPE was not detected in the workers serum (Sjödin, unpublished). This may be a result of a rapid metabolism of BTBPE which has been indicated in rats given BTBPE orally (Nomeir et al., 1993).

Occupational exposure has also been studied for a limited number of electronic recycling workers ($n=5$) in Norway (Thomsen et al., 2001) (Fig. 6). Although BDE-209 was not included among the reported data, the exposure seems to be less pronounced in this occupational setting. It is possible that this is due to the fact that respirators were used by these workers.

Another study of workers repairing and maintaining computers, at a hospital in Sweden (Jakobsson et al., 2002) showed significant exposure to PBDEs, as illustrated in Fig. 5. These findings indicate that exposure to PBDEs is not only limited to dismantling of electronic goods but also encompasses repair and maintenance work on computers

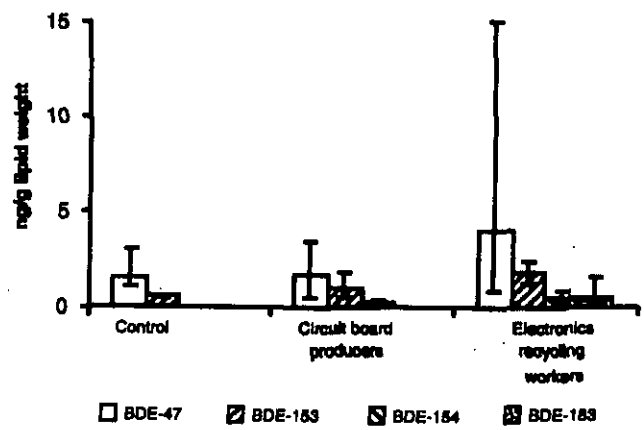

Fig. 6. Average concentration of polybrominated diphenyl ethers (PBDEs) in three occupational groups in Norway: laboratory personnel (control; $n=5$), circuit board producers ($n=5$) and electronics recycling workers ($n=5$). Error bars indicate range. Only levels of BDE-153 were found to be significantly different when comparing the group of electronics dismantlers and the control group ($p=0.05$). Data from reference Thomsen et al. (2001).

which significantly increases the number of people with potential exposure to PBDE. Worth noting is the similarity in the congener pattern found in recycling plant workers and in computer technicians, indicating that the routes of exposure are similar although resulting in lower absolute serum concentrations for the computer technicians.

A possible route of occupational exposure is inhalation of particulate matter, i.e., dust, originating from the aged electronic devices, which is then mobilized by human intervention. This assumption is consistent with air concentration measurements in which the vast majority of PBDEs were found in the particulate fraction of air as opposed to the semivolatile fraction (Sjödin et al., 2001).

2.6. Half-lives of BFRs in humans

The half-lives of higher brominated PBDEs and TBBPA has also been estimated in the Swedish workers engaged in the recycling process (Hagmar et al., 2000; Sjödin, 2000) by repeated sampling of blood during the workers vacation. The half-life estimated for BDE-209 was in the range of a week, while the corresponding figure for BDE-183 was estimated to be 3 months (Hagmar et al., 2000; Sjödin, 2000). The half-life of BDE-209 is short and hence indicates a quite rapid metabolism of BDE-209. The BDE-209 metabolites are still not known even though some indications of their identities have been reported (Mörck and Klasson Wehler, 2001).

Also, the half-life of TBBPA is short, estimated to be 2 days (Hagmar et al., 2000; Sjödin, 2000), which is not surprising since TBBPA is a phenol which is rapidly conjugated and subsequently excreted (Hakk et al., 2000), although, evidence for enterohepatic circulation has been reported.

2.7. Accidental exposure

The only known accidental exposure to BFRs occurred in 1974 in the state of Michigan (Fries, 1985). The flame retardant "Fire Master BP-6", consisting of polybrominated biphenyls (PBBs), was discovered to have been accidentally exchanged for a dairy feed additive (magnesium oxide) at a manufacturing plant. This accident lead to a widespread contamination of animal feeds, livestock and food products. A large number of cattle, pigs and chickens had to be slaughtered as a consequence of the contamination. Estimates of the amount of PBBs introduced into the feeds ranged from 225 to 450 kg, although, it could have been more (Reich, 1983). The accident resulted in high PBB serum concentrations being detected in Michigan dairy farmers. No PBBs were detected in residents from the neighboring state of Wisconsin (Bekesi et al., 1978). Among the reported symptoms were: tiredness, fatigue, loss of appetite, weight loss, pain and swelling of joints and abdominal pain (Anderson et al., 1978b). Effects on liver function were also observed (Anderson et al., 1978a). This accident resulted in the cessation of production of hexabromobiphenyl inside the US while octabromobiphenyl and decabromobiphenyl continued to be produced until 1979 (WHO, 1994a).

Recently, surprisingly high levels of BB-153 were detected in archived serum samples collected in Illinois in 1988 (Sjödin et al., 2002). A median level of 12 ng/g l.w. (range 2.6–53) was reported in these samples which was significantly higher than the levels of PBDEs found in the same samples. Blood samples taken from Michigan residents in the same year still contained detectable levels of BB-153 even though the use of PBBs had been banned soon after the accident (Blanck et al., 2000). The long median half-life of BB-153 (29 years in humans) (Blanck et al., 2000) indicates that the samples drawn in Illinois could still have detectable levels of BB-153 in 1988 even if the individuals were exposed in 1973 or 1974. On the other hand, the Illinois residents could also have been exposed through various consumer materials impregnated with fire retarding PBBs.

3. Conclusion

At least one order of magnitude higher levels of PBDEs have been found in the North American population as compared to European populations. Concentrations of PBDEs in Steelhead trout and salmon from the Great Lakes region were also significantly higher than Salmon taken from the Baltic Sea. These results call for intensified research to determine the routes of exposure within the North American general population. Traditionally, exposure to organohalogen compounds has been primarily through the consumption of contaminated food. However, in the case of PBDEs that are currently being used in our modern indoor environment, the exposure situation may be much more complex; sources such as inhalation and dermal exposure may also need to be taken into account. There may also be subgroups within the general population that are at an increased risk of PBDE exposure in much the same way as the electronics recycling workers in Sweden. Limited data on BDE-209 is a major data gap. Similarly, there are no data on other BFRs in humans which is a major drawback when trying to assess human exposure to BFRs.

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Comparisons of PBDE composition and concentration in fish collected from the Detroit River, MI and Des Plaines River, IL

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Abstract

Polybrominated diphenyl ethers (PBDEs) were identified in fish collected from the Detroit River, MI and Des Plaines Rivers, IL. In the Detroit River fish, carp and large mouth bass, the congener patterns were dominated by the 2,2',4,4'-tetrabromo (BDE-47) congener; however, in Des Plaines River carp the dominant isomers were the hepta-bromo congeners BDE-181 and BDE-183 and lesser amounts of another heptabromo congener, BDE-190, and two hexabromo congeners, BDE-154 and BDE-153. Three possible sources exist for these less-commonly identified PBDE congeners: (a) waste discharge from manufacturing or discarded products near the river, (b) public owned treatment work (POTW) effluents which constitute more than 75% of the flow in the Des Plaines River, (c) or formation of these congeners by debromination of in-place deposits of decabromodiphenyl ether. Average concentration totals (sum of concentrations for seven of the dominant PBDE congeners) were similar on a wet weight bases for the carp (5.39 ng/g wet weight) and large mouth bass (5.25 ng/g) in the Detroit River samples; however, the bass were significantly higher, $\rho = 0.01$, when compared on a lipid basis (bass-163 ng/g vs. carp-40.5 ng/g lipid weight). Some of the PBDE congeners were positively correlated with increasing lipid levels in both fish species. Average total PBDE concentrations in the carp from the Des Plaines River (12.48 ng/g wet weight) were significantly higher, $\rho = 0.01$, than in carp from the Detroit River. The residues were isolated using standard organochlorine methods for fish and analyzed using gas chromatography/mass spectrometry-negative chemical ionization methods.
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Keywords: Polybrominated diphenyl ethers; Carp; Detroit River; Des Plaines River; Large mouth bass

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are an emerging environmental contaminant that appears to be ubiquitous in the environment (Alaee in Renner, 2000a; Strandberg et al., 2001) and magnify in fish tissue (Alaee et al., 1999; Hale et al., 2001a,b; Manchester-Neesvig et al., 2001). The majority of PBDEs so far identified in the environment are composed predominantly of the tetrabromo congener, BDE-47 (2,2',4,4'-tetrabrominated dipheyl ether) and lower levels of two pentabromo congeners, BDE-99 (2,2',4,4',5-pentabromodiphenyl ether) and BDE-100 (2,2',4,4',6-pentabromodiphenyl ether). Usually the hexa- and heptabromo forms are only minor components in environmental samples, except at locations near known sources of contamination, e.g. a contaminated river in Sweden (Sellström et al., 1998)

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and a former manufacturing site in Indiana (Dodder et al., 2002). The typical distribution of congeners found in biological samples has been likened to the composition in the "pentabromodiphenyl commercial product" by Strandberg et al. (2001). BDE-47 typically provides 50–60% of the total mass; and BDE-99 accounts for 35–40%; while the other congeners account for less than 10% of the total. Swedish researchers have been studying the environmental occurrence of PBDEs for several years (de Boer, 2000). A clear trend towards increasing levels with time has been discovered in mother's milk samples in Swedish women (Norén and Meironyté, 2000). While Canadian researchers have been collecting data on PBDE levels in fish for several years (Great Lakes and coastal waters near Vancouver) (Sergeant et al., 1998; Alaee et al., 1999; Ikonomu et al., 2002; Luross et al., 2002), the US has only recently begun to monitor the levels in fish (Loganathan et al., 1995; Hale et al., 2001a,b; Johnson and Olson, 2001; Manchester-Neesvig et al., 2001; Dodder et al., 2002).

PBDEs are used in commerce for several applications; they are especially noted for their fire retarding properties. Hale et al. (2001b) revealed a largely overlooked source arising from the use of the pentabromodipenyl commercial product as an additive in upholstery foams. Another important source of release is their use in plastic components of computers and televisions, especially circuit boards and other electronic components and as a flame retardant in textiles. Increasing levels that are apparent in the environment have been attributed to several causes including increased disposal of outdated electronic equipment and volatile losses from products in use (Danish Environmental Protection Agency in Renner (2000b)). One newer area of speculation is the possible contribution of lower brominated PBDEs to the environment by the debromination of the decabromodiphenyl form, BDE-209, which is the most heavily used product in commerce (Kierkegaard et al., 1999). Strandberg et al. (2001) has suggested that photolytic degradation of congener BDE-209 to lower brominated forms may be taking place.

This study was carried out to determine the levels and patterns of PBDE congeners in fish collected near suspected areas of high release, particularly effluent-dominated waterways in industrialized areas of the Great Lakes.

2. Methods

The fish analyzed in this study were obtained in May and June of 1999 from two major river systems, the Detroit River, MI, and the Des Plaines River, IL. These sites were selected because they have high levels of industrial and municipal effluents contributing to their flow. Carp and large mouth bass were collected from the

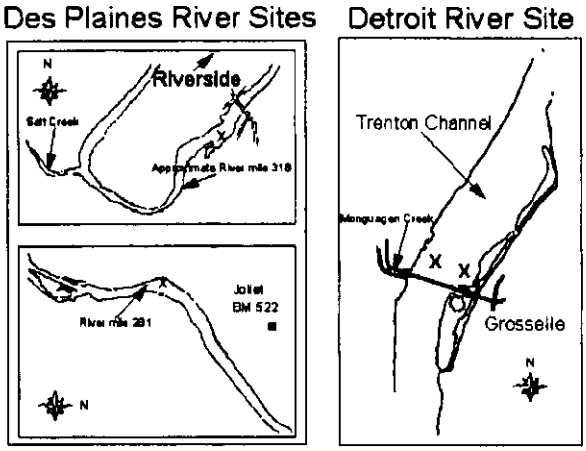

Fig. 1. Sampling sites (X) at collection locations.

Detroit River in the western branch of the Trenton Channel close to the southern tip of Grosse Ile, Fig. 1. Carp were obtained from two locations on the Des Plaines River, the upper site was near Riverside, IL (approx. river mile 318) and the lower site was near Joliet, IL, MP 281, Fig. 1. The fish were collected by electro-shocking. The stunned fish were netted, held in live tanks until return to shore where each individual fish was weighed, measured and subjected to external and internal examinations. For storage, the fish were wrapped in aluminum foil, placed in 4 mm thick polyethylene bags, and maintained chilled on dry ice until delivery to the laboratory where they were frozen at −30 °C and held until homogenized for contaminant analysis.

The entire fish was homogenized and subsamples of the homogenate were placed into certified chemically cleaned I-Chem® jars (TM 300 series), capped and stored frozen until prepared for extraction. At extraction, tissue was thawed but kept cold and rehomogenized. Surrogate standards (PCB #65, 166) were added to a 10 g subsample and then mixed with 40 g of sodium sulfate (previously dried by heating to 140 °C overnight). The mixture was stirred until it was dry and free-flowing. Contaminants were extracted by column elution methods (Schmidt, 1997a,b) using 10% ethyl acetate in petroleum ether, solvent exchanged into ethyl acetate and brought to a final volume of 10 ml. A volume of sample extract, equivalent to 1 g tissue, was pipetted into preweighed aluminum drying pans for computing the percent extractable lipids.

An aliquot of each sample was loaded onto a 52 cm × 1.27 cm i.d. gel permeation column (OI Analytical Envirobeads® S-X3 select) and cleared of lipid interferences using 1.48 ml/min ethyl acetate as the eluting solvent. The first 41 ml fraction of eluant, containing the lipids, was discarded. The following 62 ml were collected, 1 ml of iso-octane was added and this

fraction was concentrated to 1 ml. To remove remaining trace amounts of lipids, the concentrated sample was eluted with 7% ethyl acetate in hexane through a clean-up column packed with Florisil® and 0.1 ml of 4.5 M sulfuric acid. Extracts were concentrated to 1 ml and internal standards were added (PCBs #136 and #204) (Schmidt, 1997b). A preliminary fractionation test was performed to verify that PBDEs eluted with the pesticide fraction and no interference was observed from brominated biphenyls.

2.1. GC/MS/NCI analysis

An HP 5973 GC/MS was utilized for analyte separations and quantification. It was equipped with a DB-5 gas chromatographic column, 30 m × 0.25 mm i.d., 0.25 μm film thickness, obtained from J&W Scientific. Helium was used as the carrier gas, and methane was used as the MS-reagent gas. The column temperature was programmed as follows: initial setting was 80 °C (held for 2 min), then increased at 10 °C/min to 300 °C (held for 10 min). The injector temperature was 280 °C, the interface was 280 °C, and the temperature of the ion source was 150 °C. PBDEs were quantified using bromide ions ($M/z = 79.81$). The detection limits (ng/g fresh weight) for the respective analytes are as follows: 0.30 for BDE-47; 0.25 for BDE-99, BDE-100, BDE-153, BDE-154; and 0.2 for BDE-181, BDE-183 and BDE-190.

2.2. Quality assurance

In parallel with the above analyses, blanks (corn oil) and performance check fish were analyzed with each sample batch (e.g. this same check fish sample has been extracted by our laboratory with every organochlorine batch of fish that is run since the mid 1990s (Chernyak et al., 1998) and PBDEs were added to the list of organochlorine analytes in 2000). Linearity, drift check (repeat analysis of a standard injected every fifth sample), and spike recovery analyses using the check fish were also carried out. Spike recovery was 95–110%, surrogate recoveries were 90%, blanks were clear of interferences, and drift checks and individual BDE-congener precision checks were all below 10%. PCB, toxaphene and pesticide standards were also run using these methods; no interferences from these analytes were detected.

3. Results and discussion

During initial examination of the samples for PBDE the various fish extracts were screened against a standard which contained 40 different PBDEs ranging from 1 bromo up to 7 bromo-substituted diphenyl ethers, Cambridge Isotope standard # EO-4980. From this initial screening those peaks were selected for exact quantification that represented more than 5% of the total. The quantities of individual PBDE congeners in the each individual bass (Table 1) and carp (Table 2) are provided along with statistical data summarizing their average per-site concentrations and relative abundances.

PBDE totals: The average total concentrations (wet weight basis) in carp and bass from the Detroit River are very similar; e.g. bass, 5.25 and 5.39 ng/g in the carp. If concentrations in these two fish species are expressed on a lipid-normalized basis, there was a greater average level of PBDE in the bass ($\rho = 0.01$), 163 (±64) ng/g lipid than the carp, 40.7 (±8.2) ng/g lipid. There was a weak correlation of lipids with level of total PBDE in the large mouth bass; and this was especially true for the female fish, $R^2 = 0.673$. This correlation was even stronger when only congener BDE-47 was considered in these female fish, $R^2 = 0.88$. There was no correlation of lipid content vs. PBDE levels for the Carp at any location except for the lower Des Plaines site. Here, there was a positive correlation between lipid in female carp and the two heptabromodiphenyl ethers (BDE-181 ($R^2 = 0.75$) and BDE-183 ($R^2 = 0.70$)). There was no clear correlation of PBDE with weight or length for any of the fish and at any of the sites. There was a significant difference ($\rho = 0.01$) in average total concentration of PBDEs in fish collected from the Des Plaines (total PBDE 12.48 (±4.2) ng/g wet weight) vs. those collected in the Detroit River (total PBDE 5.4 (±0.62) ng/g wet weight). These differences are accounted for almost entirely by the higher levels of the higher homologues (153, 154, 181, 183 and 190) in the Des Plaines fish, both at the upper and lower locations. The lower Des Plaines River had the highest average level of these higher brominated congeners Table 2 (Fig. 2). There was little difference in the percent composition for the two Des Plaines River sites, respectively, the heptabromo congener for the upper vs. the lower site were, 55.7% and 48.4% and the hexabromo congener was 20.1% and 21.4%. Since the lower site was about 36 miles farther downstream of the upper location, it would seem there are multiple sources of the PBDE releasing these higher homologue patterns into the Des Plaines River.

The average total concentration of PBDEs in all of these fish are low compared to measurements in the literature. Hale et al. (2001b) reported that over one-half of the fish sampled from two major Virginian watershed had >100 μg/kg wet weight BDE-47 which was the major congener found. Johnson and Olson (2001) found that total PBDE concentrations ranged from 1.4 ng/g (wet weight) in rainbow trout from a remote location to 1.25 ng/g collected in an urbanized area of the Spokane River; however, most of their remaining data were in the 20–300 ng/g wet weight range. Dodder et al. (2002) reported levels ranging from 6.9 to 18 ng/g wet weight for various fish species, smelt, blue gills, crappie from

Table 1
Concentration PBDE's by individual congener amounts in large mouth bass collected from the Detroit River near Grosse Isle, MI

ID	Weight (g)	Length (mm)	Lipid (%)	Sex	Congener breakdown (ng/g wet weight)							Total PBDEs wet weight	Total PBDEs lipid basis (ng/g lipid weight)
					BDE-47	BDE-99	BDE-100	BDE-153	BDE-154	BDE-181	BDE-183		
54	784	355	5.2	M	1.94	0.3	0.33	0.49	0.4	0.32	0.36	4.14	86
55	315	432	5.0	M	2.7	0.93	0.75	0.68	0.56	0.32	0.36	6.3	126
56	261	342	3.5	M	1.68	0.55	0.53	0.4	0.3	0.15	0.11	3.72	106
57	192	414	3.7	M	1.59	0.32	0.34	0.33	0.33	0.15	0.19	3.25	86
59	1450	352	3.8	M	4.23	0.41	0.41	0.56	0.56	0.36	0.28	6.81	179
60	625	408	5.8	M	6.11	0.92	0.75	0.56	0.56	0.36	0.33	9.59	152
61	668	276	2.0	F	2.25	0.3	0.33	0.4	0.48	0.39	0.36	4.51	251
62	280	265	3.1	F	4.37	0.33	0.32	0.35	0.33	0.15	0.12	5.97	166
64	1263	342	3.6	F	6.22	0.35	0.33	0.33	0.3	0.28	0.35	8.16	221
65	577	334	2.3	F	0.92	0.23	0.26	0.26	0.3	0.1	0.11	2.18	91
68	689	266	1.6	F	0.96	0.33	0.33	0.35	0.44	0.23	0.34	2.98	213
70	1267	233	1.4	F	0.65	0.76	0.66	0.61	0.59	0.36	0.22	3.85	275
Percent composition					53.4	9.1	8.5	8.4	8.2	5.0	5.0		
Average					2.80	0.48	0.45	0.44	0.43	0.26	0.26	5.25	163
%RSD					70.1	52.8	40.3	29.9	27.3	39.1	40.2	2.00	40.7

background locations and higher levels (65 ng/g wet weight) in fish collected from a location near a PBDE manufacturing facility. Dodder et al. (2002) also analyzed two carp fillet from a site near a manufacturing facility and found concentration ranging from 6.2 to 20 ng/g wet weight. Loganathan et al. (1995) found carp in the Buffalo River to have levels from 13 to 23 ng/g for total PBDEs. Manchester-Neesvig et al. (2001) found congener BDE-47 averaged 52 ng/g wet wt. for coho salmon collected from Lake Michigan. Asplund et al. (1999) reported values on a lipid basis for Steelhead that would yield wet weight values of 53 ng/g, and for Baltic salmon this would be 21 ng/g. None of the fish studied here contained single congener amounts approaching these values.

The positive relationships between increasing lipid levels and increasing amounts of PBDEs which were observed in the large mouth bass in the Detroit River is not surprising since PBDEs belong to the class of compounds which are known to biomagnify and these compounds tend to accumulate to higher levels in lipid reserves (Burreau et al., 1997). The fact that this relationship was strongest for the female fish may have something to do with the fact that these fish were all in spawning stage and ripe with lipid-rich egg masses. Burreau et al. (2000) also showed that BDE-47 has the highest biomagnification potential and this supports our observation that the lipid correlation improved when this congener was plotted separately. The positive relationship between lipid amounts and increasing levels of the two heptabromodiphenyl ethers, BDE-181 and BDE-183, that was observed only in female carp and only at the Des Plaines site is a bit more puzzeling. Especially since Burreau et al. (2000) determined that the biomagnification potential for hexaBDEs is considerably less than what they found for the tetra- and pentaBDEs. While Burreau and associates (1999) did not test for the biomagnification of heptabromo BDEs, it could be assumed that this congener would have an even lower biomagnification potential than the hexaBDEs because of its higher bromine substitution. Why the female carp at this location showed a positive lipid correlation that was not displayed by the other congeners, seems to contradict Burreau's findings. The fact that only females exhibited this positive correlation, however, probably has something again to do with the fact that these fish were in spawning stage.

PBDE congener patterns: The relative proportions of the seven congeners (BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, BDE-181, and BDE-183) which were present in the carp and large mouth bass collected at the Detroit River site were very close between the two species. They were 53–56% for congener BDE-47; vs. 18% for the sum of the two pentabromo congeners (BDE-100 and BDE-99); and 17% for the hexabromo congeners (BDE-154 and BDE-153) (Tables 1 and 2). This pattern is very typical of those reported by other researchers. For example Dodder et al. (2002) observed a similar composition of PBDEs in fish representing background levels for their collections and Manchester-Neesvig et al. (2001) reported the relative amounts of PBDE congeners in Lake Michigan salmon as 56% tetra congener, 21%

Table 2
Concentration of PBDE's by congener amounts in carp collected from the Detroit River, MI, and the Des Plaines River, IL

ID	Weight (g)	Length (mm)	Lipid (%)	Sex	Conger composition (ng/g wet weight)								Total PBDE wet weight	Total PBDE lipid basis (ng/g lipid weight)
					BDE-47	BDE-99	BDE-100	BDE-153	BDE-154	BDE-181	BDE-183	BDE-190		
Detroit River, from mouth of Rouge River and near Grosse Isle, MI														
33	2892	659	12.3	F	3.7	0.55	0.5	0.47	0.47	0.26	0.23	<dl	6.18	50.2
34	2244	530	13	M	2.21	0.42	0.44	0.38	0.4	0.21	0.23	<dl	4.29	33.0
35	2899	510	13.6	M	2.82	0.49	0.48	0.49	0.49	0.23	0.25	<dl	5.25	38.6
36	5572	530	13.6	F	2.62	0.48	0.44	0.49	0.43	0.21	0.27	<dl	4.94	36.3
37	2776	663	11.6	F	2.75	0.47	0.46	0.46	0.47	0.27	0.25	<dl	5.13	44.2
38	2581	568	11.1	M	3.52	0.53	0.5	0.43	0.45	0.24	0.28	<dl	5.95	53.6
39	3136	548	16.5	M	2.44	0.47	0.45	0.47	0.45	0.26	0.25	<dl	4.79	29.0
40	1853	577	14.8	F	2.92	0.56	0.53	0.49	0.45	0.25	0.26	<dl	5.46	36.9
43	3078	461	16.8	F	3.35	0.54	0.49	0.51	0.49	0.2	0.24	<dl	5.82	36.6
44	3082	617	12.6	M	3.66	0.52	0.48	0.46	0.44	0.28	0.25	<dl	6.09	48.3
Percent composition					55.6	9.33	8.85	8.63	8.42	4.47	4.66	na		
AVG					3.0	0.50	0.48	0.47	0.45	0.24	0.25	na	5.39	40.7
%RSD					17.6	8.79	6.18	8.00	6.07	11.48	6.36	na	11.5	19.7
Des Plaines River (Lower) downstream of Joliet, IL														
96	2457	540	3.4	M	3.9	0.52	0.45	1.21	2.56	4.21	3.28	2.2	18.33	482
97	1433	451	5.64	M	1.93	0.49	0.44	0.82	0.6	3.32	3.1	0.42	11.12	188
98	2251	532	4.75	M	3.62	0.54	0.47	1.14	1.51	4.17	2.48	3.14	17.07	335
99	3942	634	11.0	F	3.88	0.51	0.44	1.33	2.65	2.63	2.63	0.37	14.44	132
100	1502	467	9.21	F	1.76	0.48	0.43	0.47	1.62	2.66	2.52	2.51	12.45	128
101	2283	551	2.27	M	3.35	0.53	0.43	1.26	2.57	2.95	2.83	1.14	15.06	685
103	1691	480	4.26	M	2.52	0.52	0.45	1.02	2.02	4.51	3.54	2.65	17.23	401
105	1257	423	22.5	F	1.37	0.47	0.45	0.95	2.73	3.73	4.11	2.36	16.17	73.5
106	1086	419	4.47	F	1.53	0.44	0.44	0.93	0.94	2.7	2.52	0.28	9.78	200
107	1561	437	5.76	F	1.54	0.45	0.44	0.94	1.66	1.92	2.92	2.43	12.3	189
Percent composition					17.6	3.44	3.08	7.00	13.1	22.8	20.8	12.2		
AVG					2.54	0.50	0.44	1.01	1.89	3.28	2.99	1.75	14.4	281
%RSD					41.2	6.9	2.6	24.9	39.7	25.9	17.7	61.9	20.0	68.3
Des Plaines River (Upper) near Riverside, IL														
87	2823	590	11.3	M	1.12	0.48	0.47	0.52	0.72	1.21	1.15	0.91	6.58	52.2
88	2823	590	13.5	M	1.29	0.49	0.48	0.64	0.74	1.33	1.13	0.95	7.05	47.3
92	4388	575	3.75	F	0.72	0.48	0.48	0.38	0.54	1.01	1.02	0.88	5.51	134.4
93	5987	680	14.4	F	2.21	0.53	0.51	1.11	1.92	2.22	1.94	1.12	11.56	79.2
Percent composition					17.4	6.4	6.3	8.6	12.8	18.8	17.1	12.6		
AVG					1.34	0.50	0.49	0.66	0.98	1.44	1.31	0.97	7.68	78.3
%RSD					47.2	4.81	3.57	47.8	64.6	37.1	32.4	11.1	34.8	107.5

penta and 10.2% hexa. It however should be recognized that these researchers may not have looked for any homologues higher that the 154 congener.

In comparing the PBDEs compostion in carp at the two locations, Des Plaines River vs. the Detroit River, it is clear that different congeners were present in fish collected at the Des Plaines River site (Fig. 2). Rather than congener BDE-47 being the major congener in the Des Plaines collections, congeners BDE-183 and BDE-181 were predominant, e.g. 20% and 22% respectively of the total PBDE concentration. Furthermore congener BDE-190, also a heptabromo congener like BDE-183 and BDE-181, was measured here at 12.4% of the total amount of PBDE. This congener was not even found in the Detroit fish samples (detection limit 0.2 ng/g). The likeliest explanation for the occurrence of these congeners in fish obtained from the Des Plaines River would be discharges from a manufacturing facility(ies) that use products containing these congeners or discharges from a waste facility(ies) that may be leaking these components.

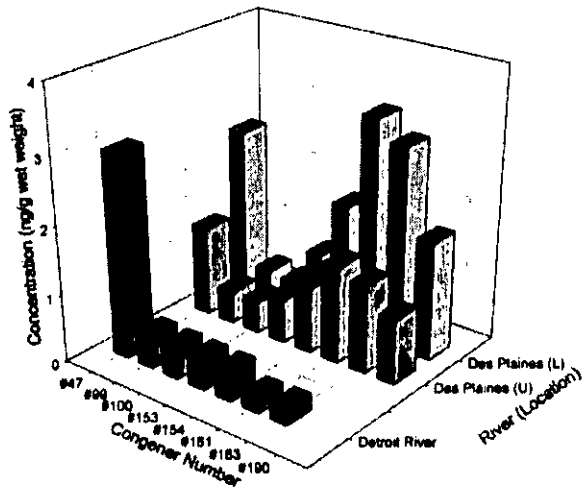

Fig. 2. Average congener-specific PBDE concentrations in carp collected from the Detroit River, MI and the Des Plaines River, IL.

Office and business equipment housings are often made with a polymeric acrylonitrile butadiene styrene material that is composed 12–18% by weight of commercial octaBDE (OECD, 1994); and these octaBDE mixes contain mostly BDE-183 (Sjödin, 2000). Three of these octaBDE commercial products were also determined by Sjödin to contain lesser amounts of three octaBDEs, a nonabrominated dipheyl ether and BDE-153 and BDE-154. There are other technical PBDE products besides the octaBDE that have been reported to contain higher levels of congeners other than BDE-47. For example one particular pentaBDE, technical mix (DE-71®), was characterized by both Sjödin (2000) and Dodder et al. (2002) to contain higher levels of the BDE-99 congener than the BDE-47 congener and lesser amounts of BDE-100, BDE-153 and BDE-154. This technical mix added to the octaBDE mix discussed above could possibly account for at least the BDE-183, BDE-153, BDE-154 and BDE-100 congeners that we found in the Des Plaines carp.

Possible sources for the other heptabromodiphenyl ethers, BDE-181 and BDE-190, that were found in the carp in the Des Plaines River are less obvious, especially since no commercial products have been documented as containing major quantities of these congeners. Some researchers have postulated that debromination of decaBDE (a major product used in commerce (Renner, 2000b)) can result in the occurrence of lower brominated congeners in the environment. Kierkegaard et al. (1999) noticed a progressive increase in concentration of specific hepta and octaBDEs after feeding rainbow trout a commercial decabromobiphenyl ether mixture, the deca congener itself, BDE-209, however, did not bioaccumulate. The assumption by these researchers was that these congeners were resulting either from metabolic processes acting on the BDE-209 or efficient absorption of those congeners that are present as minor impurities in the deca product relative to the others. Perhaps the high occurrence of congeners 181 and 183 in our samples is arising due to active metabolism of deposits of BDE-209 that are residing in sediments of the Des Plaines River.

Another explanation also exists for the unusual pattern of higher congeners observed in Des Plaines carp vs. those in the Detroit River. The Des Plaines River receives much higher contributions of POTW effluents than does the Detroit River and these effluent loads could be the source of these PBDE residues. For example it has been reported that under low-flow conditions in the Detroit River, POTWs contribute about 1% to the flow; while during normal flow for the lower Des Plaines River, 75% of the river flow comes from POTWs and during low-flow this amount is 100%. Furthermore, the flow in the lower Des Plaines River principally originates from the Chicago area which is largely due to alteration in this river system that were made to direct sewage effluent away from Lake Michigan (Howe, Personal communication). Analysis of POTW effluents for PBDEs and careful determination of their congener makeup might help answer this question. Without further studies on this problem, it is only possible to speculate as to which of these processes or combinations of processes are truly causing higher levels of hexa- and heptabromodiphenyl ether congeners to occur in the Des Plaines fish. Analysis of sediments could assist in this investigation by identifying possible sources; however these data were not obtained as part of this study.

Dodder et al. (2002) also found congener patterns in fish that were different than the normal patterns observed in most studies where the BDE-47 congener predominates (de Witt, 2002). In the Dodder and coworker study, the highest congener levels were contributed by two hexabrominated congeners BDE-153 and BDE-154 (47%). Their explanation was that these nontypical congener patterns were caused by proximity of the fish to a known source that originated from a former PBDE manufacturing facility near Hadley Lake, Indiana. They also examined sediments near their collection sites and identified patterns similar to those in their fish extracts.

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Exponential Increases of the Brominated Flame Retardants, Polybrominated Diphenyl Ethers, in the Canadian Arctic from 1981 to 2000

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A suite of 37 polybrominated diphenyl ether (PBDE) congeners and all of the homologue groups from mono- to deca-brominated were determined in ringed seal (*Phoca hispida*) blubber collected from subsistence hunts in the Canadian Arctic in 1981, 1991, 1996, and 2000. Total PBDE (ΣPBDE) concentrations have increased exponentially over this period in male ringed seals aged 0–15 years. Penta- and hexa-BDEs are increasing at approximately the same rate (t_2 = 4.7 and 4.3 years, respectively) and more rapidly than tetra-BDEs (t_2 = 8.6 years) and tri-BDEs (t_2 = ∞) in this age/sex grouping. In contrast to declining PBDE concentrations since 1997 in human milk from Sweden, ΣPBDE concentrations in arctic ringed seals continue to increase exponentially similar to worldwide commercial penta-BDE production. PBDE congener profiles in male ringed seals aged 0–15 years from 1991 to 2000 also differ significantly from other aquatic organisms and semipermeable membrane devices collected from temperate coastal regions of British Columbia. While PBDE concentrations are 50 times lower than those of mono-ortho and non-ortho PCBs, and ~500 times higher than PCDD/Fs, our data indicate that, at current rates of bioaccumulation, PBDEs will surpass PCBs to become the most prevalent organohalogen compound in Canadian arctic ringed seals by 2050.

Introduction

Polybrominated diphenyl ethers (PBDEs) are flame retardants used in a wide range of materials. Three commercial mixtures are widely used (penta-BDE, octa-BDE, and deca-BDE), making up 14%, 6%, and 80%, respectively, of the estimated 1999 worldwide PBDE production of 67 000 tonnes (*1*). These products are not pure, and the suffixes indicate the average degree of bromination with 209 different congeners possible for mono- through deca-BDE. Only recently have multiresidue sample processing and tandem gas chromatography with high-resolution mass spectrometry (GC–HRMS) techniques been developed to identify the majority of congeners (*2*).

PBDEs are also members of a broader chemical class termed polyhalogenated aromatic hydrocarbons (PHAHs), which also include polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs), among others. PHAHs have been shown to enter Arctic marine food webs via atmospheric transport from the industrialized regions of North America, Europe, and Asia (*3*). Local PBDE usage is not expected to be important in extreme northern environments. Biotransformation of PBDEs is also thought to be relatively slow (*4, 5*), leading to their accumulation in lipid-rich regions of biota. Toxicological studies show that while the acute toxicity of commercial penta-, octa-, and deca-BDE mixtures to aquatic life is low (LD_{50} > 1 g·kg^{-1}) (*6*), individual congeners, and their hydroxylated metabolites, may act as endocrine disruptors (*7–9*); hence, their environmental behavior is of concern.

Temporal trends for PBDEs suggest that while concentrations were increasing in sediments (*10*), guillemot eggs (*11*), and human milk (*12, 13*) from the mid-1970s to as late as 1997, these levels appear to be leveling off or declining in the industrial regions of Europe. However, there are few temporal studies of PBDEs from other regions of the world, including North America. A study examining PBDE concentrations in herring gull (*Larus argentatus*) from the highly industrialized Great Lakes region of North America also showed increasing levels over the period from 1981 to 2000. However, there was less evidence of a recent decline in PBDE concentrations than that observed in Europe, with increased concentrations in each sequential sampling year except for 1988/1989 and 1999/2000 (*14*). These studies indicate that PBDE concentrations in the environment have increased substantially since large-scale PBDE production began in the early 1970s. On the other hand, no previous studies have examined the temporal patterns of PBDEs in remote areas such as the polar regions.

To help elucidate the environmental fate of PBDEs, we determined the concentrations of 37 individual BDE congeners plus all of the homologue groups from mono- through deca-brominated in ringed seals sampled from Holman Island in the Canadian Arctic. The exponentially increasing concentrations of PBDEs in Arctic biota over the past two decades and unique congener profiles of these compounds indicate the presence of an environmental problem and demonstrate the value of, and need for, further investigations into congener-specific analytical methods, environmental transport processes, and toxicology.

Experimental Section

Sample Collection and Preparation. Blubber samples (10–250 g) were taken from the mid-dorsal region of ringed seals (*Phoca hispida*) collected from Holman Island, Northwest Territories, Canada (70°44′N, 117°43′W) and captured during subsistence hunts between mid-March and early June of 1981, 1991, 1996, and 2000. Blubber samples were wrapped in solvent-washed aluminum foil and frozen at −20 °C (domestic freezer) until analysis. Length, girth, and sternal blubber thickness of the seals were measured following capture. Sex and reproductive status were recorded, and canine teeth were removed for age determination.

Samples of Dungeness crab (*Cancer magister*), English sole (*Pleuronectes vetulus*), and harbor porpoise (*Phocoena phocoena*) were collected from pristine reference sites (Gardener Channel and Bamfield), principle harbors (Vancouver, Victoria, Esquimalt, and Prince Rupert), and near pulp and paper mills (Howe Sound, Crofton, Prince Rupert, Kitimat and Fraser River Delta) on the west coast of British Columbia, Canada (*15*). Hepatopancreases of Dungeness crabs (six composites of 2–6 organisms) collected with crab

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traps from 1993 to 1995 and livers of English sole (14 composites of 1−13 organisms) collected by trolling in 1992 and 2000 were removed and stored at −20 °C until analysis. Single blubber samples were also taken from five stranded harbor porpoises (10−50 g) from 1991 to 1993 and stored at −20 °C until analysis.

Semipermeable membrane device (SPMD) samplers were prepared using the protocols discussed in detail previously (*16*). The SPMDs were placed in perforated 20-L plastic food buckets, three SPMDs to one bucket, prior to immersion in the water column. The food buckets were anchored using heavy chain, and the containers were attached to log booms or pilings with rope. Care was taken to avoid attachment to creosoted timbers, which are abundant in the lower Fraser River. Seven SPMDs were deployed in the Fraser River near Vancouver, BC, Canada (population, 2 000 000), from August 6 to September 30, 1996, at a low-tide depth of 2−3 m for a total exposure time of 55 days (*15*). One SPMD was located at MacMillan Island, near Fort Langley, which is 28.5 km upstream from the city of New Westminster and the industrial activities in the lower Fraser River. However, this site is still subject to tidal influences and possible upstream transport of contaminants. It is assumed that the majority of the observed PBDEs at this site will arise from atmospheric deposition or transport from the more remote regions of British Columbia drained by the Fraser River.

The remaining six SPMDs were located in the lower Fraser River west of the city of New Westminster. Three SPMDs were located on the highly industrialized north arm of the Fraser River. One of these SPMDs was situated ~1 km west of where the Fraser River bifurcates into the north and south arms. Other north arm SPMDs were deployed at the railway bridge to Mitchell Island, approximately halfway between separation of the north and south arms and where the north arm discharges into the Strait of Georgia, and near the office of the North Fraser Harbor Commission (NFHC). The NFHC sample was located where the north arm bifurcates ~6 km east of discharge into the Strait of Georgia. Three further SPMDs were deployed on the less industrialized south arm of the Fraser River between New Westminster and where the south arm discharges into the Strait of Georgia. One site was approximately 1 km downstream of the bifurcation into the north and south arms in Annacis Channel near a muddy beach. The other two south arm SPMD sites were downstream on the south bank of the Fraser River near Chatterton Chemicals and at Purfleet Point at the southwest corner of Annacis Island, 3 km below a major sewage treatment outfall. In addition to the SPMDs deployed in the water column, another set of SPMDs was exposed to ambient air during the deployment at the sampling sites (~0.5 h) to serve as field blanks and to reveal possible atmospheric contamination. These field blanks were processed and analyzed in the same manner as other SPMD samples.

Sample Extraction and Cleanup Procedures. All organic solvents used were pesticide residue analysis grade (Caledon Laboratories, Ltd., Georgetown, ON, Canada). Anhydrous, granular sodium sulfate (Mallinckrodt Baker, Inc., Paris, KY) was baked at 450 °C at least overnight and cooled to room temperature in a desiccating chamber before use. Biobeads S-X3 (Bio-Rad Laboratories Ltd., Mississauga, ON, Canada) were swelled in 1:1 CH_2Cl_2/hexane for a minimum of 24 h. Neutral silica (100−200 mesh) and neutral alumina (Mallinckrodt Baker, Inc.) were activated, at least overnight, at 200 °C and cooled in a desiccating chamber over anhydrous calcium sulfate. Acidic and basic silica were prepared by mixing 25 g of concentrated H_2SO_4 (ACS grade; BDH Inc., Toronto, ON, Canada) with 50 g of neutral silica and 14 g of 1 N NaOH (ACS grade; BDH Inc.) with 40 g of neutral silica, respectively. A carbon fiber column contained 300 mg of glass filter paper pieces (124 mm P100 prefilter; Nucleopore Corp., Pleasanton, CA) mixed with 25 mg of PX-21 carbon (BP Amoco Chemicals, Naperville, IL). The internal and performance standards containing ^{13}C labeled bromo- and chlorodiphenyl ethers (BDEs and CDEs, respectively) were purchased from Cambridge Isotope Laboratories (CIL; Andover, MA). Native compounds used to prepare the quantitation standards (see the following discussion for individual congeners analyzed) were also purchased from CIL.

Blubber, tissue, and hepatopancreatic samples (approximately 0.2−2 g of blubber and 5−10 g of tissue) were spiked with internal standards (1 ng of [^{13}C]-3,3′,4,4′-tetrachlorodiphenyl ether (CDE77), 2 ng of [^{13}C]-2,3,3′,4,4′,5-hexachlorodiphenyl ether (CDE156), and 3 ng of [^{13}C]-2,2′,3,3′,4,4′,5,5′-octachlorodiphenyl ether (CDE194)) and ground with 50 g of sodium sulfate until a free-flowing mixture was attained. Samples were then transferred quantitatively to an extraction column with rinses of 1:1 CH_2Cl_2/hexane and eluted with 250−350 mL of 1:1 CH_2Cl_2/hexane at ~5 mL·min^{-1}. Procedures on the extraction of SPMD samples are given in detail in our previous work (*16*). The extracts of all matrixes were reduced by rotary evaporation to 1 mL followed by further cleanup.

Sample cleanup took place in three stages. In the first step, aliquots were passed through a multilayer silica column packed with successive layers of silica gel (basic, neutral, acidic, neutral) and eluted with 60 mL of CH_2Cl_2/hexane (1:1). The second cleanup step was via a neutral alumina-activated column capped with anhydrous sodium sulfate. Once the sample was loaded to the column, the column was washed with 30 mL of hexane followed with 60 mL of 1:1 CH_2Cl_2/hexane elution to recover the analytes of interest. Eluants from the alumina column were concentrated to less than 10 μL and spiked with a ^{13}C-labeled method performance standard (1 ng of [^{13}C]-3,3′,4,4′-tetrabromodiphenyl ether (BDE77)) prior to congener-specific PBDE analyses by GC−HRMS. If PCB and PCDD/F analyses were also required from the same sample, then the eluant concentrate collected from the alumina column was fractionated with an automated high-performance liquid chromatography (HPLC) system utilizing a column (5 mm i.d. × 7.5 cm length) packed with a 1:12 mixture of an activated carbon/filter paper homogenate. The four fractions collected from this system were first individually analyzed for the desired target analytes (i.e., PCBs and PCDD/Fs), and subsequently, all four were combined and spiked with the corresponding method performance standard (1 ng) prior to GC−HRMS analysis. Details on the solvents, composition of cleanup columns, and conditions used in all the cleanup and fractionation steps are given elsewhere (*17*).

Lipid contents for blubber, tissue, and hepatopancreatic samples were determined as follows. Aliquots (~2−5 g) of each sample were weighed and then transferred quantitatively to a mortar with 100 g of anhydrous sodium sulfate. The mixture was ground until homogeneous and transferred to a glass extraction column packed with glass wool. Samples were eluted with 100 mL of 1:1 CH_2Cl_2/hexane, and the eluant was reduced to ~1 mL by rotary evaporation and dried in a 40 °C vented oven for several hours. Once a consistent weight was achieved, samples were cooled in a desiccator over anhydrous calcium sulfate and their weights recorded. Percent lipid was calculated using the following equation: % lipid = (mass of lipid/mass of sample) × 100%.

All samples (including lipid determinations) were processed in batches of 12, which consisted of a procedural blank, an in-house certified reference sample, and nine real samples out of which one was analyzed in duplicate. The recoveries of the ^{13}C-labeled PCDE surrogates ranged from 40% to 120%, within the allowable limits. Congener concentrations presented below are corrected for percent recovery of the internal standards.

Instrumental Analysis and Parameters. Analyses of clean PBDE extracts were analyzed by GC–HRMS using a VG-Autospec high-resolution mass spectrometer (Micromass, Manchester, U.K.) equipped with a Hewlett-Packard model 5890 series II gas chromatograph and a CTC A200S autosampler (CTC Analytics, Zurich, Switzerland). The GC was operated in the splitless injection mode, and the splitless injector purge valve was activated 2 min after sample injection. The volume injected was 1 μL of sample plus 0.5 μL of air. Either a 15-m high-temperature DB-5-HT (0.25 mm i.d. × 0.1 μm film thickness; used for analysis of BDE209) or a standard 30-m DB-5 column (0.25 mm i.d. × 0.25 μm film thickness; used for all other analytes) from J&W Scientific (Folsom, CA) was used with UHP He as the carrier gas at a constant head pressure of 25 psi to maintain a linear velocity of 35 cm·s^{-1}. The temperature program used under constant pressure (~80 kPa for either column) for the DB-5-HT was as follows: hold at 100 °C for 1 min; 2 °C·min^{-1} to 140 °C; 4 °C·min^{-1} to 220 °C; 8 °C·min^{-1} to 330 °C; and hold 1.2 min. For the 30-m DB-5 column, the temperature program was as follows: hold at 100 °C for 2 min; 4 °C·min^{-1} to 320 °C; and hold 2.5 min. All sample injections were performed using the CTC A200S autosampler. The splitless injector port, direct GC–MS interface, and the MS ion source were maintained at 300, 270, and 310 °C, respectively.

The high-resolution MS was a sector instrument of EBE geometry coupled to the GC via a standard Micromass GC–MS interface. For all analyses, the MS was operated under positive EI conditions with the filament in the trap stabilization mode at 600 μA, an electron energy of 39 eV, and perfluorokerosene used as the calibrant. The instrument operates at 10 000 resolution, and data were acquired in the selected ion monitoring (SIM) mode for achieving the maximum possible sensitivity. Two or more isotopic ions of known relative abundance were monitored for each molecular ion cluster representing a group of isomers, as were two for each of the ^{13}C-labeled surrogate standards. Under SIM conditions, the two most abundant isotopes representing the parent ion (M^+) were monitored for all of the MoBDE and DiBDE congeners and the TeBDE congener BDE77. For all other homologues (TrBDEs through HeBDEs), the two most intense isotopes representing the $(M - 2Br)^+$ fragment were monitored. BDE209 was also monitored but not reported because the levels measured were those of the procedural blanks. The PBDE congeners analyzed in this study were as follows: 2-BDE1; 3-BDE2; 4-BDE3; 2,6-BDE10; 2,4-BDE7; 3,3′-BDE11; 2,4′-BDE8; 3,4-BDE12; 3,4′-BDE13; 4,4′-BDE15; 2,4,6-BDE30; 2,4′,6-BDE32; 2,2′,4-BDE17; 2,3′,4-BDE25; 2′,3,4-BDE33; 2,4,4′-BDE28; 3,3′,4-BDE35; 3,4,4′-BDE37; 2,4,4′,6-BDE75; 2,2′,4,5′-BDE49; 2,3′,4′,6-BDE71; 2,2′,4,4′-BDE47; 2,3′,4,4′-BDE66; 3,3′,4,4′-BDE77; 2,2′,4,4′,6-BDE100; 2,3′,4,4′,6-BDE119; 2,2′,4,4′,5-BDE99; 2,3,4,5,6-BDE116; 2,2′,3,4,4′-BDE85; 2,2′,4,4′,6,6′-BDE155; 2,2′,4,4′,5,6′-BDE154; 2,2′,4,4′,5,5′-BDE153; 2,2′,3,4,4′,6′-BDE140; 2,2′,3,4,4′,5-BDE138; 2,3,4,4′,5,6-BDE166; 2,3,3′,4,4′,5,6-BDE190; and 2,2′,3,3′,4,4′,5,5′,6,6′-BDE209. Only the following 13 congeners where 30% of the sample values were above the method detection limit (MDL) are reported here: BDE15; BDEs28/33 (coeluting congeners); BDE75; BDE49; BDE47; BDE66; BDE100; BDE119; BDE99; BDE155; BDE154; and BDE153. The total PBDEs (ΣPBDE) are the sum of these 13 congeners.

Compounds were identified only when the GC–HRMS data satisfied all of the following criteria: (1) two isotopes of the specific congeners were detected by their exact masses with the mass spectrometer operating at 10 000 resolving power or higher during the entire chromatographic run, (2) the retention time of the specific peaks was within 3 s to the predicted time obtained from analysis of authentic compounds in the calibration standards, (3) the peak maxima for both characteristic isotopic ions of a specific congener coincided within 2 s, (4) the observed isotope ratio of the two ions monitored per congener were within 15% of the theoretical isotopic ratio, and (5) the signal-to-noise ratio resulting from the peak response of the two corresponding ions was 3 for proper quantification of the congener. Concentrations of identified compounds and their MDLs were calculated by the internal standard isotope-dilution method using mean relative response factors (RRFs) determined from calibration standard runs made before and after each batch of samples was analyzed.

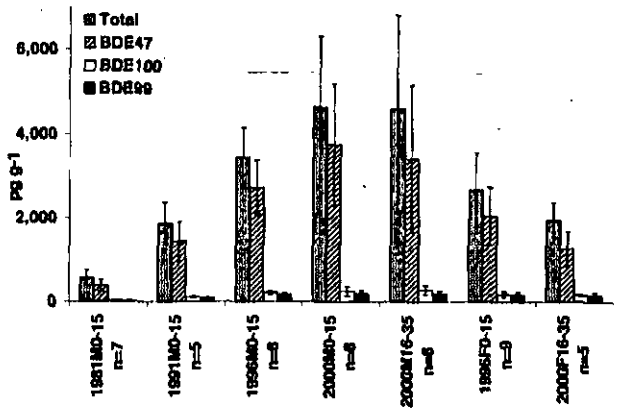

FIGURE 1. Levels of PBDEs in ringed seal (*Phoca hispida*) blubber taken from Holman Island, Northwest Territories. Labels (e.g., 1981M0–15 *n* = 7) indicate year of sampling, sex, age range in years, and sample size, respectively.

Data Analysis. Data compilation and analysis were performed using Microsoft Excel XP and SPSS, version 10.0. Concentrations of total PBDEs and all congeners reported individually, as well as total PCBs and PCDD/Fs levels, are in picograms of analyte per gram of sample and are lipid normalized for intercomparison. Error bars always indicate 95% confidence limits on the mean. As no significant relationships were observed between age and concentrations within any sample group, data were not age-normalized using ANCOVA. Differences between sampling groups were investigated using single factor ANOVA. The exponential relationship in ΣPBDE between 1981 and 2000 in male seals aged 0–15 years shown in Figure 1 was confirmed by transforming ΣPBDE concentrations to their common logarithms and performing linear regression (slope ≠ 0, $p = 10^{-10}$, $R^2 = 0.85$; residuals evenly distributed with no curvature). Insets in Figure 4 (see later) show averaged congener profiles for each sampling group (as percent in ΣPBDE). Congener profiles of PBDEs were selectively normalized using an established method to minimize closure of the data set (*18*). Also included in the plot is a Bromkal Mix which was constructed using the relative 1999 production values of the commercial penta-BDE mixture (Bromkal 70-5DE) and octa-BDE mixture (Bromkal 79-8DE) (*1*).

Results and Discussion

Temporal Trends of PBDEs. Mean concentrations of ΣPBDE in male ringed seals aged 0–15 years have increased exponentially ($p = 10^{-10}$, $R^2 = 0.85$; residuals evenly distributed with no curvature) by more than an order of magnitude (572–4622 pg·g^{-1}) between 1981 and 2000 (Figure 1). Also shown in this figure are levels of the three most prevalent PBDE congeners (BDEs 47, 99, and 100) of which only BDEs 47 and 100 have increased in much the same exponential fashion as that of ΣPBDE. While BDE 99 increased exponentially in a manner similar to ΣPBDE and BDEs 47 and 100 from 1981 to 1996, the 2000 samples show that the increasing levels of BDE 99 over this earlier period have since slowed

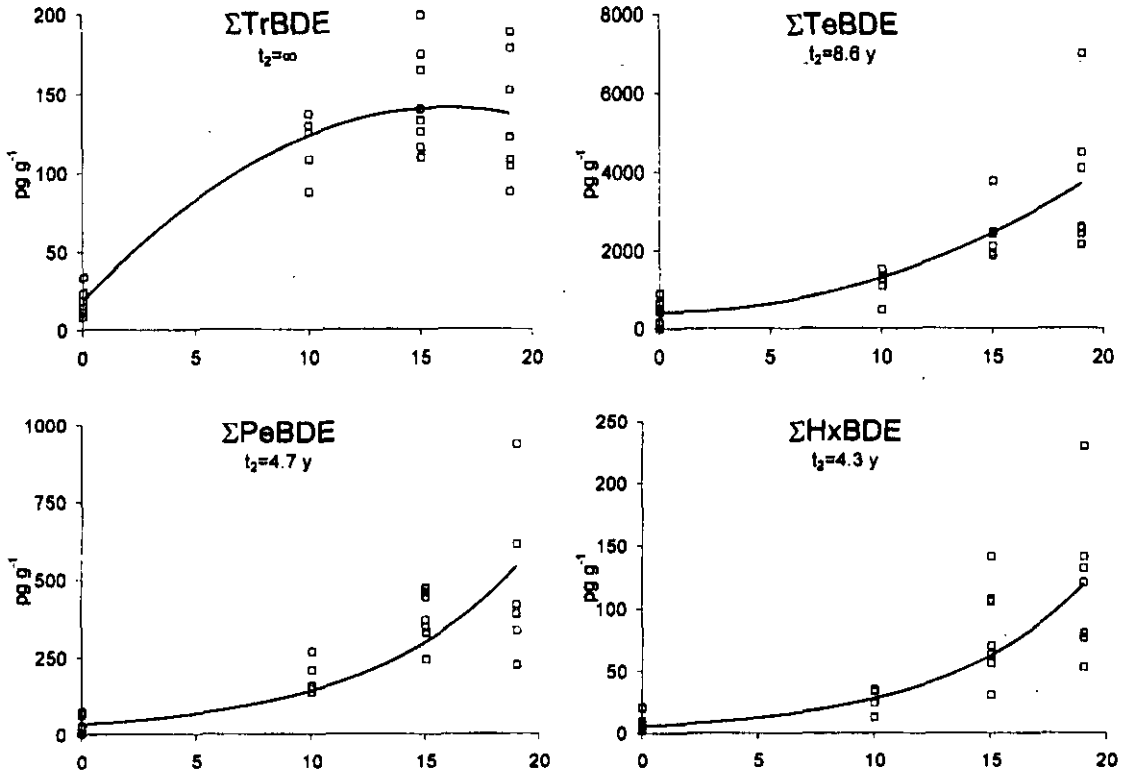

FIGURE 2. Levels of the four major PBDE homologue groups for male ringed seals aged 0−15 years: Tr = tri-, Te = tetra-, Pe = penta-, Hx = hexa-, and BDE = bromodiphenyl ether. Values on *x* axis are years since 1981 (e.g., 10 = 1991).

considerably. ΣPBDE levels for the 2000 male seals aged 16−35 years and female seals aged 0−15 years (in 1996) and 16−35 years (in 2000) are shown for comparison.

For male seals aged 0−15 years, mean ΣPBDE levels in 1981, 1991, and 1996 differ significantly (572, 1863, and 3437 pg·g^{-1}, respectively; $p = 0.01$). Because of the large variation in the 2000 samples (mean = 4622 pg·g^{-1}), there is no significant difference between the ΣPBDE levels for 1996 and 2000 ($p = 0.06$), although the log-transformed data was significantly increasing through 2000. For these samples, we examined the temporal trends of both individual congeners and homologue groups. Homologue group totals from 1981 to 2000 are shown in Figure 2. While levels of TrBDEs have not changed significantly since 1991 and appear to be stabilized or declining, levels of TeBDEs, PeBDEs, and HxBDEs have all increased since 1981. Using the best-fit lines shown in Figure 2, doubling times (t_2, time required for 2000 levels to increase by a factor of 2) for each homologue group are reported in the inset subtitles. These rates of increase show that TeBDEs appear to be increasing at approximately one-half of the rate (t_2 = 8.6 years) of PeBDEs (t_2 = 4.7 years) and HxBDEs (t_2 = 4.3 years), suggesting that the congener profile in these samples is changing over time. Such findings contrast with data from herring gull eggs in the Great Lakes region, where TeBDEs and PeBDEs are increasing approximately twice as fast (t_2 = 2.5−2.9 years) as HxBDEs (t_2 = 3.4−5.9 years) (*14*). As shown in the Supporting Information, temporal trends of the individual congeners are more complex than the homologue group totals. The five major congeners (BDEs 47, 99, 100, 153, and 154) appear to be driving the exponential increases of both ΣPBDE and their respective homologue groups.

Juvenile seals acquire much of their residue burdens during lactation, while in adult males, PHAH levels increase as some function of age, as accumulation from food is more rapid than degradation and excretion processes (*19*−*23*). Age patterns are more complex in female seals as a large proportion of pollutant residues are lost during lactation (*19*).

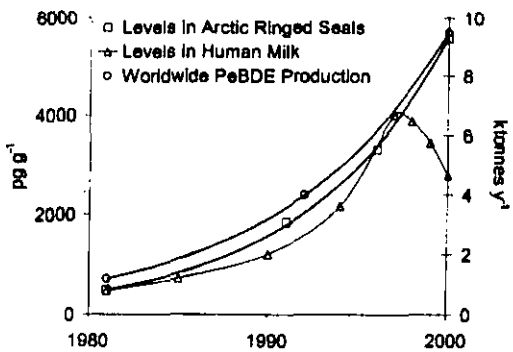

FIGURE 3. Comparison of PBDE levels in ringed seals from the Canadian arctic, PBDE levels in human milk from Sweden, and worldwide commercial penta-BDE (PeBDE) production.

No difference in PBDE levels (both total and of individual congeners, $p = 0.98$ for ΣPBDE) were observed between younger (0−15 years) and older (16−35 years) male seals in 2000, suggesting that recent PBDE accumulation dominates potential historic accumulation for the older seals. This observation is consistent with the exponential increase from 1981 to 2000 in that levels are increasing at a rate such that both young and old individuals have similar PBDE burdens. Similarly, male seals in 1996 and 2000, aged 0−15 and 16−35 years, respectively, have higher PBDE levels than similar age grouping in females for the same sampling year. These results suggest that females are "off-loading" PBDEs during lactation in a manner similar to other PHAHs.

Comparison to Worldwide Levels and Commercial Production. The exponential increases of ΣPBDE we observe in ringed seals from the Canadian arctic correlate well with production of the commercial penta-BDE mixture (e.g., Bromkal 70-5DE) over the same time period (Figure 3). It is evident that both commercial penta-BDE production (*1*, *24*) and ΣPBDE levels have increased exponentially since 1981. Penta-BDE production in 1981 was estimated from the

production values reported in Japan (*24, 25*), assuming that Japan's consumption of penta-BDE and deca-BDE as a percentage of total worldwide production remained approximately constant between 1981 and 1994. The ΣPBDE levels in ringed seals are composed primarily of TeBDEs and PeBDEs (see Figure 1), and both these homologue groups (as well as that of HxBDEs) have been shown to be increasing exponentially over the same period (see Figure 2). Conversely, while ΣPBDE levels in human milk from Sweden increased sharply from 1981 to 1997, levels have since decreased (*12, 13*). This reduction in human milk ΣPBDE burdens may reflect regulatory measures in Europe to halt or limit commercial penta-BDE production (*12, 26*). However, ΣPBDE levels in the arctic remain 1−2 orders of magnitude lower than that reported for ringed seals in industrialized regions (*5*).

The higher PBDE concentrations in Arctic mammals versus humans from an industrialized center (i.e., Stockholm) until the mid-1990s demonstrate surprisingly efficient atmospheric transport to, and bioaccumulation in, remote regions. That ΣPBDE levels in ringed seals correlate with worldwide commercial penta-BDE production (which is used primarily in North America (*12, 26*)) suggests that these compounds are still being rapidly transmitted to the Canadian arctic in large quantities. Some atmospheric transport patterns to the Holman Island region during the summer months originate or pass over the industrialized regions of North America and Asia, rather than via Europe, which is the favored pattern in winter (*27*) when atmospheric transport of PBDEs is expected to be at a minimum because of their low vapor pressures. Hence, we do not observe a reduction in ΣPBDE levels in the ringed seals after 1996 following the enactment of the European penta-BDE restrictions because their ΣPBDE burdens appear to originate from North America. The correlation between commercial penta-BDE production and ringed seal ΣPBDE levels also suggests rapid transport of these compounds to polar regions, as has been reported for other halogenated aromatics (*27, 28*). This relatively rapid transport supports our belief in a North American "source" of these compounds, because if the source was primarily European, we would expect to observe a similar decline as was seen in Sweden following 1996.

Spatial and Temporal Congener Profiles. The congener profiles for ringed seals sampled in the present study, as well as for Dungeness crab (*Cancer magister*), English sole (*Pleuronectes vetulus*), harbor porpoise (*Phocoena phocoena*), and SPMDs from British Columbia, Canada, and resulting principal component analysis (PCA) are presented in Figure 4. Also included in the figure is a technical PBDE mixture composed of the commercial penta-BDE (Bromkal 70-5DE) and octa-BDE (Bromkal 79-8DE) mixtures according to their relative 1999 production rates (*1*). While deca-BDE (BDE209) was monitored in all samples, the levels of this fully brominated congener were those of the procedural blanks (162−236 pg·g^{-1}). Deca-BDE (with >90% BDE209) is the major commercial PBDE mixture in production; however, its log $K_{ow} \approx 10$ and vapor pressure ($P^{o}_{L,298K}$) = 1.3×10^{-12} Pa (*29*) may significantly hinder long-range transport and help explain why we do not observe this congener in the Arctic. The 1981M0−15 group was not included as the levels of all congeners other than BDEs 47, 99, 100, 153, and 154 were at or near the detection limits for the 1981 samples and hence skewed their positions on the PCA plot.

While values of the three predominant congeners (BDE47, BDE99, and BDE100) are widely reported in the literature and shed valuable insights into absolute levels of PBDE contamination, a full congener profile is necessary to highlight the differences due to source inputs, environmental fractionation, and weathering. In addition, the development of toxic equivalence factors (TEFs) for PCBs and PCDD/Fs demonstrated the need to ascertain individual congener contributions to total contaminant loading; a similar requirement can be expected for PBDEs. This approach has proven necessary in dealing with other PHAHs such as PCDD/Fs (*30*) where TEFs for individual congeners can range over several orders of magnitude, making the determination of minor, but more toxic, congeners critically important.

Figure 4 shows a strong correlation between geographic distance from industrialized regions and distance from the commercial mixture on the PCA plot. Harbor porpoise, English sole, Dungeness crab, and the SPMD samples, while being approximately equidistant from the commercial mixture, are geographically located much nearer large urban centers than the ringed seal, which is also much farther away on the PCA plot. The unique congener patterns of the SPMD, sole, and porpoise highlight the point that congener patterns in aquatic biota do not necessarily reflect those in the local water column, with differences in congener patterns for species occupying the same locale. As noted in the Supporting Information, most individual congeners displayed no significant differences as a percent of ΣPBDE among the various possible ringed seal groupings (e.g., sex, age, sampling year) either between or within sampling years. However, some notable exceptions exist that suggest congener profiles may be changing over time, suggesting a changing source composition of PBDEs over the past two decades. For male seals aged 0−15 years, the contribution of BDE153 to ΣPBDE has increased since 1991 (0.3−1.6%, $p = 0.02$), while some lower brominated congener contributions have decreased (BDE66, 1.3−0.7%; BDE28/33, 6.7−3.3%; $p = 0.03$). As well, male seals aged 16−35 years in 2000 have a significantly lower contribution of BDE47 than their younger (0−15 years) counterparts (71.4% vs 80.4%, $p = 0.02$). This reduced contribution has apparently been made up for by BDE49, which increases from 2.9% in the 0−15 years group to 7.4% in the 16−35 years group. Because BDE49 cannot come from the same hexa-BDE precursor as BDE47 (vide infra), this suggests that older seals may have been exposed to a different HxBDE source than younger seals.

Biotic patterns in general reveal a predominance of tetra- and penta-BDEs, with little or no contribution from hepta- through octa-BDEs. Deca-BDE environmental weathering, uptake, and metabolism do not appear to explain the congener patterns observed in aquatic biota (*31*). Hence, it is of interest to investigate the source of the unique PBDE profiles we observe in the ringed seals. Several physicochemical parameters are expected to influence the congener profiles in environmental samples: the octanol−water (K_{ow}) and octanol-air (K_{oa}) partition coefficients, vapor pressures (P^{o}_{L}) and saturated water concentrations (S_w), and rates of environmental degradation for individual congeners. Ringed seals are depleted in the contribution of PBDEs with ≥5 bromine atoms as compared to the Bromkal Mix and samples from temperate, industrialized regions. This is understood in terms of the physicochemical properties given previously which show a decreasing preference for the aqueous and atmospheric phases as the level of bromination increases (*29, 32, 33*). Hence, we observe an "atmospheric distillation" similar to that reported for other PHAHs (*34*).

In addition to the use of physicochemical properties, biological processes may also contribute significantly to the congener profile we observed in biota. While an effective molecular cross-section of 9.5 Å was proposed as limiting for biological uptake across membranes (*35*), subsequent studies demonstrated relatively high dietary uptake efficiencies for BDEs 47, 99, and 153 despite effective molecular cross-sections >9.5 Å for BDEs 99 and 153 (*32*). Both uptake clearance rate coefficients and bioaccumulation factors (BCFs) are notably high for BDEs 47 and 99 (~13 000 mL·g^{-1} dry weight) and are greater than those observed for PCBs

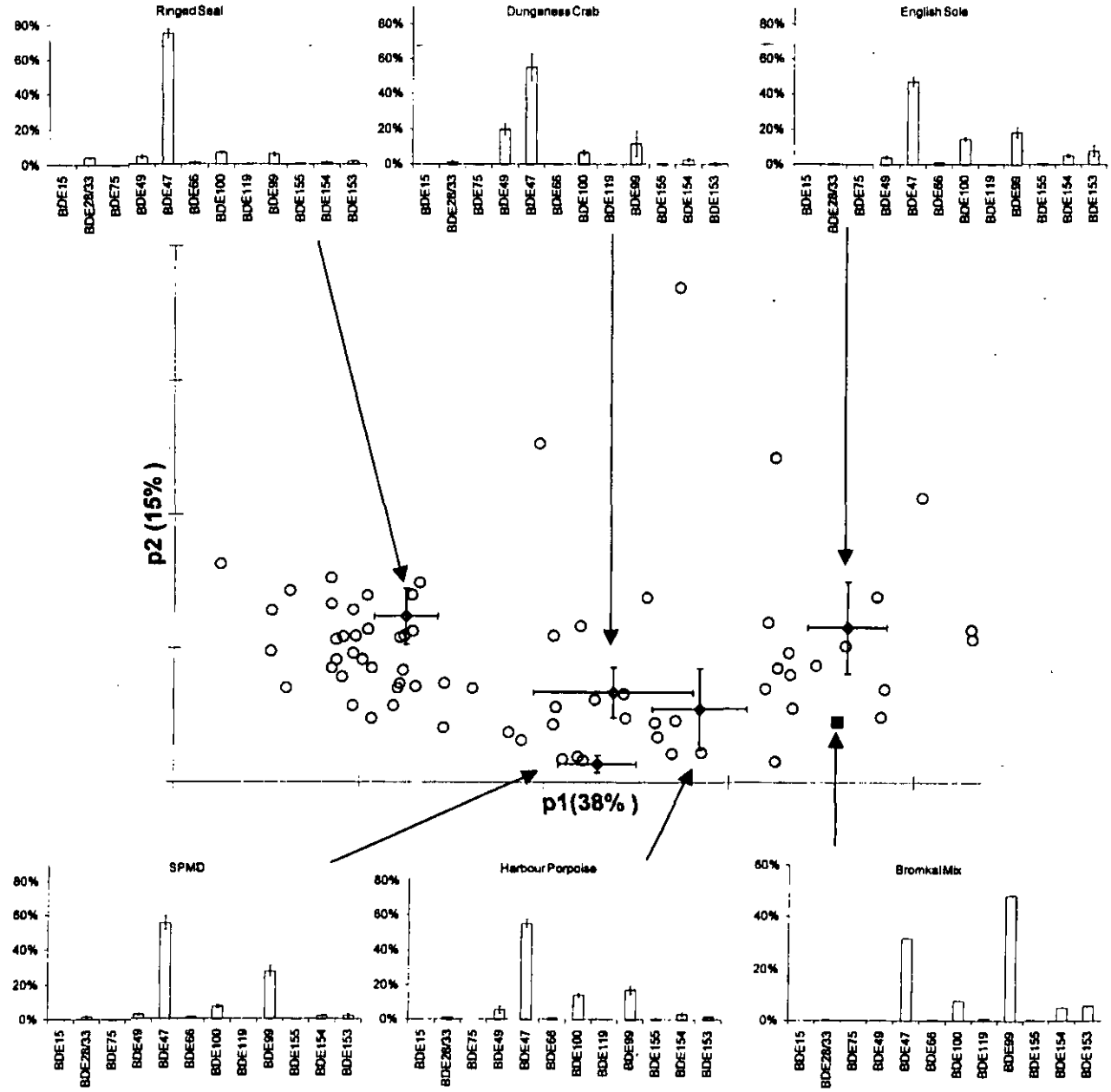

FIGURE 4. Principal components plot of PBDE congener profiles for Dungeness crab, English sole, and harbor porpoise from pristine and industrial marine environments in British Columbia, SPMDs in the Fraser River near Vancouver, BC, and ringed seals from Holman Island. Insets show averaged congener profiles for each sampling group (as percent in ∑PBDE). Also included in the plot is a Bromkal Mix, which was constructed as noted in the text.

(1100−2700 mL·g^{-1} dry weight). Elimination of BDEs 99 and 153 is more rapid than predicted based on log K_{ow} values, indicating that these congeners may accumulate to a lesser extent than other congeners (*36*). This may also help explain why congener patterns are depleted in BDEs 99 and 153 relative to the Bromkal Mix.

The notable absence of BDE119 in our samples suggests that environmental exposure to the commercial octa-BDE mixture is negligible. Examining the structures of the major congeners found in our samples (BDEs 47, 49, 99, 100, 153, and 154) demonstrates that environmental debromination by either metabolic, photolytic, or other abiotic processes, if taking place, leads to two major congener pathways: BDE154 → BDE99 → BDE47 and BDE153 → BDE100 → BDE49. These pathways are not interconvertible unless a bromine is transferred from one position to another, a highly unusual process in photochemical- or microbial-mediated dehalogenation (*37*). BDE119 cannot enter either pathway because of its unique substitution patterns.

Comparison of PBDE Levels with PCBs and PCDD/Fs. Mono-ortho and non-ortho PCBs (MO + NO PCBs: 149 000−174 000 pg·g^{-1}) and PCDD/F (8.6−14.6 pg·g^{-1}) levels in male ringed seals aged 0−15 years from the Canadian arctic have remained approximately constant since 1981 (Figure 5). These results are consistent with other Arctic studies which show declining or stabilized PCB levels (*20, 38*). In contrast, ∑PBDE levels have increased exponentially (572−4281 pg·g^{-1}) over this period. Older male seals (16−35 years) from the 2000 sampling group have higher levels of MO + CP PCBs than their younger counterparts (0−15 years; 302 000 vs 150 000 pg·g^{-1}). However, PBDE levels between these two groupings are not significantly different (4575 vs 4281 pg·g^{-1}). In female seals from 1996 and 2000, MO + CP PCB levels are much lower in the 16−35 years age group from 2000 (43 000 pg·g^{-1})

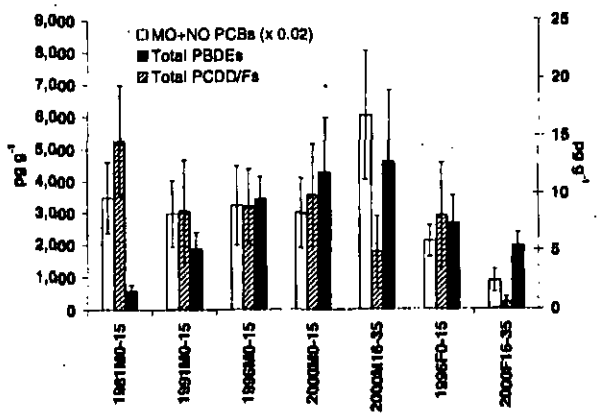

FIGURE 5. Levels of mono-ortho and non-ortho PCBs (MO + NO PCBs), total PBDEs, and total PCDD/Fs in ringed seals from the Canadian arctic. PCB levels are multiplied by 0.02 to allow for suitable representation with PBDEs on the left y axis. PCDD/F levels are on the right y axis.

than the 0−15 years age group from 1996 (105 000 $pg \cdot g^{-1}$). Conversely, PBDE levels between these groups have only declined slightly (2650 vs 1948 $pg \cdot g^{-1}$). This suggests that PBDEs may be more resistant to depuration and lactation off-loading to young than for PCBs. Assuming PBDE production and transport to the Arctic continues at its present pace while ΣPCB levels remain constant, we calculate that ΣPBDE levels will surpass those of ΣPCB by 2050 and become the most prevalent organohalogen contaminant in the Arctic.

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Supporting Information Available

PBDE concentrations and congener profiles for each sampling group. This material is available free of charge via the Internet at http://pubs.acs.org.

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Polybrominated diphenyl ethers in whitefish from Swiss lakes and farmed rainbow trout

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Abstract

A method for trace analysis of polybrominated diphenyl ethers (PBDE) in fish based on gas chromatography/electron ionization high resolution mass spectrometry (GC/EI-HRMS) was developed, and levels of PBDE were determined in whitefish (*Coregonus* sp.) from eight Swiss lakes and in rainbow trout (*Oncorhynchus mykiss*) from four Swiss fish farms. PBDE concentrations (sum of PBDE congeners BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, and BDE-183) in filet from whitefish between 36 and 165 ng/g lipid weight (lw) were found, corresponding to wet weight (ww) concentrations of 1.6–7.4 ng/g ww. PBDE contents in filet from farmed rainbow trout were significantly lower than in wild whitefish (12–24 ng/g lw, 0.74–1.3 ng/g ww), and the PBDE congener patterns were different for both species (a higher BDE-47 to BDE-99 ratio for farmed rainbow trout compared to wild whitefish was found). Whitefish PBDE levels [ng/g lw] correlate better with the surface/volume ratio of the respective lakes ($r^2 = 0.70$) than with other lake properties such as catchment area (size or number of inhabitants) or residence time, suggesting atmospheric deposition as an input pathway for PBDE. Based on an average daily consumption of 20 g whitefish (Switzerland) with a PBDE content of 7.4 ng/g ww (highest PBDE concentration detected in this study), a maximum daily intake of 0.15 μg PBDE was estimated (0.026 μg/day for farmed trout). This number corresponds to the lower end of the estimate for the total PBDE intake of the Nordic consumer of 0.2–0.7 μg/day.

Keywords: Flame retardants; PBDE; Fish; Human exposure; Atmospheric deposition

1. Introduction

Polybrominated diphenyl ethers (PBDE) are a class of flame retardants for which ubiquitous occurrence and increasing levels in the environment have been observed (Renner, 2000; Betts, 2002a). PBDE are released into the environment mainly from plastic materials and textiles where they are used as flame retardants. In plastics treated with flame retardants, they account for 5–30% of the total weight (Darnerud et al., 2001). Brominated flame retardants are very successful on the global market, they have become the second largest additives used by the plastics industry (Tullo, 2000). The worldwide demand for brominated flame retardants in 1999 was about 204 000 metric tons, including about 67 000 metric tons of PBDE (Renner, 2000). Most technical PBDE products are mixtures of various brominated diphenyl ethers (congeners). Pentabromodiphenyl ether (PeDBE) is a mixture of tetra- to hexabromodiphenyl ethers. Octabromodiphenyl ether (OcBDE) contains hexa- to nonabromodiphenyl ethers (mainly heptabromodiphenyl ether), and decabromodiphenyl ether (DeBDE) consists to 97–98% of the congener BDE-209 (decabromodiphenyl ether). Theoretically, there are 209 different

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PBDE congeners (mono- to decabromodiphenyl ether). Usually, only a subset of all congeners is analyzed, including the commercially available congeners BDE-28 (2,4,4′-tribromodiphenyl ether), BDE-47 (2,2′,4,4′-tetrabromodiphenyl ether), BDE-99 (2,2′,4,4′,5-pentabromodiphenyl ether), BDE-100 (2,2′,4,4′,6-pentabromodiphenyl ether), BDE-153 (2,2′,4,4′,5,5′-hexabromodiphenyl ether), BDE-154 (2,2′,4,4′,5,6′-hexabromodiphenyl ether), and BDE-183 (2,2′,3,4,4′,5′,6-heptabromodiphenyl ether).

Although PBDE exhibit typical characteristics of persistent organic pollutants (POP), they have not yet been added to the POP list of the United Nations Environment Programme (UNEP). Similar to dioxins and PCB, brominated flame retardants have been shown to accumulate in the food chain, and they can be found in samples from all over the world (de Wit, 2002). Brominated flame retardants were even detected in whales living in deeper Atlantic waters (de Boer et al., 1998). A recent study from North America reports PBDE levels up to 200 ng/g based on lipid weight in human milk (Betts, 2002b). According to Norén and Meironyté (2000), levels of PBDE in human milk have doubled every five years between 1972 and 1999, while levels of other organochlorine compounds have decreased to various extent. Recently, endocrine disruption properties have been discovered for flame retardants such as PBDE (de Boer et al., 1999) and tetrabromobisphenol A (TBBPA) (Meerts et al., 2000). Endocrine disrupting effects triggered by these and other chemicals are discussed to be responsible for various disorders and the diminution of fish populations.

The objective of this study was to develop an up-to-date analytical method for trace analysis of PBDE in fish tissue, which is an excellent material to assess environmental levels as well as human exposure to these compounds. PBDE were determined in pooled whitefish (*Coregonus* sp.) samples originating from eight Swiss lakes. Whitefish is an abundant zooplanktophagous species and a popular food fish (Riccardi, 2000). Typical prey of whitefish are *Daphnia* and *Bythotrephes* (Riccardi, 2000), setting *Coregonus* sp. on a medium trophic level (Vander Zanden et al., 1999). Based on these data, correlations between PBDE levels in fish and the properties of the respective lakes were explored. In order to assess human exposure through farmed fish as well, rainbow trout (*Oncorhynchus mykiss*) from four Swiss fish farms were analyzed, and daily PBDE intake through human consumption of farmed fish was estimated, too.

2. Experimental

2.1. Samples

Whitefish (*Coregonus* sp.) from eight different Swiss lakes (Lake Geneva, Lake Greifen, Lake Biel, Lake Lucerne, Lake Zürich, Lake Neuchatel, Lake Constance and Lake Thun), and farmed rainbow trout (*Oncorhynchus mykiss*) from four different Swiss fish farms collected by the fishery and hunt authorities were selected for analysis. Whole fish or fish filet were frozen (bags were previously checked for absence of PBDE residues) and sent to the laboratory (storage at −20 °C). Each pooled whitefish sample represents 10 individuals (in two cases only five individuals). Weight, length, and lipid content are listed in Table 1. Farmed rainbow trout were 18 months old (standard length 30 cm, standard weight 300 g), and samples were based on three individuals (see Table 2). Due to the low PBDE levels detected in the farmed rainbow trout samples compared to wild fish from Swiss lakes, the commercial fish feed used by the four fish farms was not analyzed for PBDE.

2.2. Extraction and clean up

Fish filets were separated from the skin with a ceramic knife (parts of the samples were used for trace metal analysis) and the skin was scraped off to collect the lipids under the skin. Filets (male and female fish in a 1:1 ratio) were cut, crushed into small pieces, and homogenized manually. An aliquot of 100–150 g of the homogenate was suspended in about 300 ml of ultra-pure water and mixed thoroughly. The fine suspension was transferred quantitatively into a 2000 ml separation funnel and extracted with ethanol, diethyl ether and *n*-pentane, according to Fürst et al. (1989). No difference in lipid yield between liquid–liquid extraction and soxhlet extraction (*n*-hexane/acetone 1:1) could be observed. The lipid content was determined gravimetrically, and an aliquot of 1 g of fish oil was used for clean up and analysis. After spiking the sample with the internal standard ($^{13}C_{12}$ labeled BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-183, 5–25 ng per congener, Cambridge Isotope Laboratories, Andover, MA, USA), the sample was diluted with 2 ml of *n*-hexane and treated with 1 ml of concentrated sulfuric acid. The suspension was stirred rigorously for one minute on a vortex shaker and spinned down in a centrifuge for one minute at 5000 rpm. It was verified based on experimental treatment of a BDE reference solution that sulfuric acid treatment does not induce any losses of the PBDE congeners. The *n*-hexane phase was collected with a Pasteur pipette, and the acidic suspension was reextracted with *n*-hexane, twice. The colorless or slightly amber *n*-hexane extract was further purified by chromatography through a multilayer silica gel column containing 4 g of acidic silica (30% concentrated sulfuric acid), 2 g of basic silica (23% 1 M sodium hydroxide) and 3 g of neutral silica (10% water), followed by a column containing 12.5 g basic alumina (Alumina B1, activated at 600 °C for 16 h). The multilayer silica gel column was eluted with 90 ml *n*-hexane. The basic

Table 1
Data for whitefish (*Coregonus* sp.) samples taken from Swiss lakes

Site	Lake Geneva		Lake Greifen		Lake Biel		Lake Lucerne		Lake Zürich		Lake Neuchatel		Lake Constance		Lake Thun	
Lake surface [km^2]	582.4		8.45		39.3		114		88.4		217.9		539		48.4	
Lake volume [km^3]	89		0.148		1.24		11.8		3.77		13.98		48.53		6.44	
Human population	850 000		100 000		970 000		168 000		330 000		273 000		1 448 000		94 300	
Individuals	10		5		10		10		5		10		10		10	
Length [cm]	40.3 ± 1.5		40.4 ± 1.5		30.7 ± 1.1		21.9 ± 1.1		34.1 ± 1.0		33.4 ± 2.5		32.3 ± 2.5		30.8 ± 1.3	
Weight [g]	552 ± 43		640 ± 102		259 ± 21		76 ± 8.3		390 ± 29.4		304 ± 91		283 ± 59		237 ± 37	
Lipids [%]	4.9		4.1		5.8		2.4		1.9		6.8		4.3		5.6	
	Lipids (lw) [ng/g]	Wet (ww) [ng/g]	Lipids (lw) [ng/g]	Wet (ww) [ng/g]	Lipids (lw) [ng/g]	Wet (ww) [ng/g]	Lipids (lw) [ng/g]	Wet (ww) [ng/g]	Lipids (lw) [ng/g]	Wet (ww) [ng/g]	Lipids (lw) [ng/g]	Wet (ww) [ng/g]	Lipids (lw) [ng/g]	Wet (ww) [ng/g]	Lipids (lw) [ng/g]	Wet (ww) [ng/g]
BDE-28	0.82	0.040	3.4	0.14	0.93	0.054	0.89	0.022	1.3	0.024	0.83	0.056	0.59	0.026	0.24	0.014
BDE-47	26	1.3	96	3.9	75.9	4.4	56	1.4	56	1.0	41	2.8	32	1.4	19	1.0
BDE-99	13	0.62	52	2.1	39	2.3	46	1.1	25	0.5	20	1.3	15	0.64	12	0.69
BDE-100	2.5	0.12	9.1	0.37	7.1	0.41	10	0.24	4.5	0.08	4.0	0.28	2.9	0.123	2.5	0.14
BDE-153	0.62	0.030	2.1	0.085	2.2	0.13	2.9	0.071	1.1	0.020	0.82	0.056	0.83	0.036	0.72	0.041
BDE-154	0.80	0.039	2.4	0.10	2.4	0.14	4.8	0.12	1.3	0.024	1.2	0.081	0.77	0.033	1.1	0.065
BDE-183	0.20	0.0097	0.088	0.0036	0.16	0.0094	0.37	0.0090	0.093	0.0017	0.059	0.0041	0.075	0.0033	0.085	0.0048
Sum PBDE	44	2.2	165	6.7	128	7.4	121	3.0	89	1.6	68	4.6	52	2.3	36	2.0

Table 2
Data for rainbow trout (*Oncorhynchus mykiss*) samples taken from Swiss fish farms

Site	Farm A		Farm B		Farm C		Farm D	
Lipid content [%]	6.2		4.1		6.5		7.3	
	Lipids (lw) [ng/g]	Wet (ww) [ng/g]	Lipids (lw) [ng/g]	Wet (ww) [ng/g]	Lipids (lw) [ng/g]	Wet (ww) [ng/g]	Lipids (lw) [ng/g]	Wet (ww) [ng/g]
BDE-28	0.29	0.018	0.65	0.027	0.46	0.030	0.26	0.019
BDE-47	8.5	0.53	16	0.67	14	0.89	9.3	0.68
BDE-99	1.6	0.10	3.1	0.13	2.8	0.19	1.9	0.14
BDE-100	1.2	0.075	2.9	0.12	2.0	0.13	1.2	0.085
BDE-153	0.18	0.011	0.44	0.018	0.33	0.022	0.20	0.015
BDE-154	0.10	0.0060	0.82	0.034	0.60	0.039	0.36	0.027
BDE-183	0.033	0.0020	0.093	0.0038	0.021	0.0013	0.020	0.0015
Sum PBDE	12	0.74	24	1.0	20	1.3	13	0.97

alumina column was first eluted with 60 ml of a mixture of 2% dichloromethane in *n*-hexane, and subsequently the PBDE were eluted with 80 ml of a mixture of 50% dichloromethane in *n*-hexane (Covaci et al., 2002). The cleaned extract was reduced to about 1 ml using a rotary evaporator (40 °C and 300 mbar) and finally concentrated to 10 μl under a gentle stream of nitrogen at 40 °C. Finally, 5 ng of the recovery standard $^{13}C_{12}$-BDE-126 (Cambridge Isotope Laboratories, Andover, MA, USA) was added, and the volume was adjusted to 70 μl by adding *n*-nonane.

2.3. Instrumental analysis

Gas chromatography/electron ionization high resolution mass spectrometry (GC/EI-HRMS) was carried out on a MAT 95 (Thermo Finnigan MAT, Bremen, Germany) mass spectrometer coupled to a Varian 3400 gas chromatograph (Walnut Creek, CA, USA) equipped with an A200S auto sampler (CTC Analytics, Zwingen, Switzerland). An aliquot of 3 μl was injected in splitless mode (splitless time 60 s) at an injector temperature of 300 °C and at an initial oven temperature of 150 °C. After 1 min, the temperature was ramped at 10 °C/min to 210 °C and at 6 °C/min to 300 °C. For the GC separation, a fused silica capillary column Rtx-5 Sil MS (Restek Corporation, Bellefonte, PA, USA) of 30 m length, 0.25 mm i.d. and 0.10 μm film thickness was used. Helium at 100 kPa served as carrier gas. For maximum sensitivity, the transfer line was set to 300 °C, and the capillary was introduced directly into the ion source, which was held at 280 °C and operated at an electron energy of 60 eV (previously optimized). The mass spectrometer was tuned to a resolution of 8000. The two most abundant signals in the ion cluster $(M\text{-}2\ Br)^+$ were recorded in single ion monitoring (SIM) mode. For the recovery standard $^{13}C_{12}$-BDE-126, the molecular ions $(M)^+$ m/z 563.6218 and m/z 565.6199 were recorded, since their abundance was higher than $(M\text{-}2\ Br)^+$. Typical retention times were between 8:25 min for BDE-28 (elution temperature 219 °C) and 19:00 min for BDE-183 (elution temperature 282 °C). The ions at m/z 245.9680 and m/z 247.9661 were selected for the detection of BDE-28. BDE-47 was measured using m/z 323.8786 and m/z 325.8766. BDE-99 and BDE-100 were detected using m/z 403.7871 and m/z 405.7852. For BDE-153 and BDE-154, m/z 481.6976 and m/z 483.6957 were chosen. BDE-183 was recorded using m/z 561.6061 and m/z 563.6042. The isotope labeled internal standards were measured on their corresponding masses. All congeners except BDE-154 (a $^{13}C_{12}$-BDE-154 standard was not available) were quantified based on their corresponding $^{13}C_{12}$-labeled analogues used as internal standards. BDE-154 was quantified using the $^{13}C_{12}$-BDE-153 internal standard. PBDE concentrations were reported as the sum of the PBDE congeners BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, and BDE-183. Sample concentrations were reported in ng/g on a wet weight basis (ww) and on a lipid weight basis (lw).

2.4. Analytical aspects and quality management

Analytical method development included the determination of detection limits, method blanks, reproducibility and recoveries of the internal standards (see Table 3). Detection limits based on a signal to noise ratio of 3:1 were 0.014–0.033 ng/g lw. Method blanks (calculated based on a full clean up and analysis without sample) were all below the detection limit, except for BDE-47 (0.057 ng/g lw) and BDE-99 (0.024 ng/g lw). Reproducibility was between 1.4% and 10% for the individual congeners and 2.4% for the sum of the congeners. Average recoveries of the internal standards were between 70% and 95%.

Table 3
Analytical data on detection limits, method blanks, reproducibility, and recovery

	Detection Limit[a]		Method Blank[b]		Reproducibility[c] [%]	Average recovery [%]
	Lipids (lw) [ng/g]	Wet (ww) [ng/g]	Lipids (lw) [ng/g]	Wet (ww) [ng/g]		
BDE-28	0.030	0.0015	<0.030	<0.0015	10	70
BDE-47	0.033	0.0016	0.057	0.0029	1.4	79
BDE-99	0.018	0.0009	0.024	0.0012	4.1	91
BDE-100	0.015	0.00077	<0.015	<0.00077	8.2	89
BDE-153	0.014	0.00068	<0.014	<0.00068	4.0	95
BDE-154	0.014	0.00068	<0.014	<0.00068	6.6	n/a[d]
BDE-183	0.017	0.00087	<0.017	<0.00087	9.0	93
Sum			0.17[e]	0.0086	2.4	

Levels below the detection limit are labeled with "<".
[a] Based on a signal to noise ratio of 3:1.
[b] Calculated for a typical sample (filet, 20 g ww, 5% fat content).
[c] Average from four samples, each measured twice (clean up and analysis).
[d] No $^{13}C_{12}$-BDE-154 standard was available. BDE-154 was quantified based on $^{13}C_{12}$-BDE-153.
[e] Values below detection limit set equal to detection limit.

3. Results and discussion

3.1. PBDE in whitefish from Swiss lakes

PBDE were detected in all whitefish (*Coregonus* sp.) samples taken from eight Swiss lakes (see Table 1). Lipid contents of the pooled samples ranged from 1.9% to 6.8%. PBDE levels in lipids expressed as the sum of BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, and BDE-183 were between 36 and 165 ng/g lw, corresponding to a wet weight (ww) concentration of 1.6–7.4 ng/g ww. No correlation exists between lipid content and PBDE lipid levels, as already shown by Hale et al. (2001). The concentration range of our data set, which is based on pooled samples, extends over one order of magnitude and is in the same range as data reported for lake trout from Lake Erie (117 ± 37 ng/g lw), reported by Luross et al. (2002). Johnson and Olson (2001) report total PBDE concentrations in fish tissue samples from selected locations in Washington State between 29 ng/g lw (rainbow trout) and 8390 ng/g lw (Mountain whitefish, values calculated from ng/g ww and lipid content). Near known and suspected point sources, PBDE levels up to 27 000 ng/g lw (Andersson and Blomkvist, 1981) and 2400 ± 600 ng/g lw were found (Dodder et al., 2002). Even higher total PBDE concentrations (up to 47 900 ng/g lw) were detected by Hale et al. (2001) in carp (*Cyprinus carpio*) from the Hyco river (Virginia, USA).

The highest PBDE concentrations in our study were found in whitefish from Lake Greifen (165 ng/g lw), and the lowest concentrations were detected in whitefish from Lake Thun (36 ng/g lw). The congener pattern is similar for all whitefish samples (see Fig. 1). It is dominated by BDE-47, followed by BDE-99 and BDE-100. Congeners BDE-153 and BDE-154 are found in similar concentrations, just below the levels of BDE-100. BDE-183 could be detected in trace amounts, only. This pattern is related to the congener pattern of a typical PeBDE product, such as Bromkal 70-5DE (Sjödin et al., 1998). However, the ratio of BDE-47 to BDE-99 detected in the whitefish samples is about 2, while Bromkal 70-5DE shows a ratio of about one for these two congeners. The congener patterns of our samples are similar to the results reported by Dodder et al. (2002) for their background fish samples from the Northeastern United States. Other authors report partly different congener patterns, showing in contrast to our samples, about equal amounts of BDE-99 and BDE-100 (Manchester-Neesvig et al., 2001; Hale et al., 2001).

3.2. PBDE in farmed rainbow trout

PBDE were also detected in all rainbow trout (*Oncorhynchus mykiss*) samples taken from four Swiss fish farms (see Table 2). PBDE levels in lipids expressed as the sum of BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, and BDE-183 between 12 and 24 ng/g lw (0.74–1.3 ng/g ww) were found. Lipid contents of the pooled samples ranged from 4.1% to 7.3%. Compared to the levels measured in wild whitefish (36–165 ng/g lw, 1.6–7.4 ng/g ww), concentrations of PBDE in farmed rainbow trout were significantly lower. In a study on farmed and wild salmon, Easton et al. (2002) found consistently higher levels of PBDE, PCB and other organic pollutants in farmed salmon than in wild salmon. They report average PBDE levels in wild salmon from the Pacific coast of 2.7 ng/g lw (0.178 ng/g ww, 6.5% lipids), while an average PBDE level of 18 ng/g lw (2.668 ng/g ww, 14.8% lipids) was measured for farmed salmon.

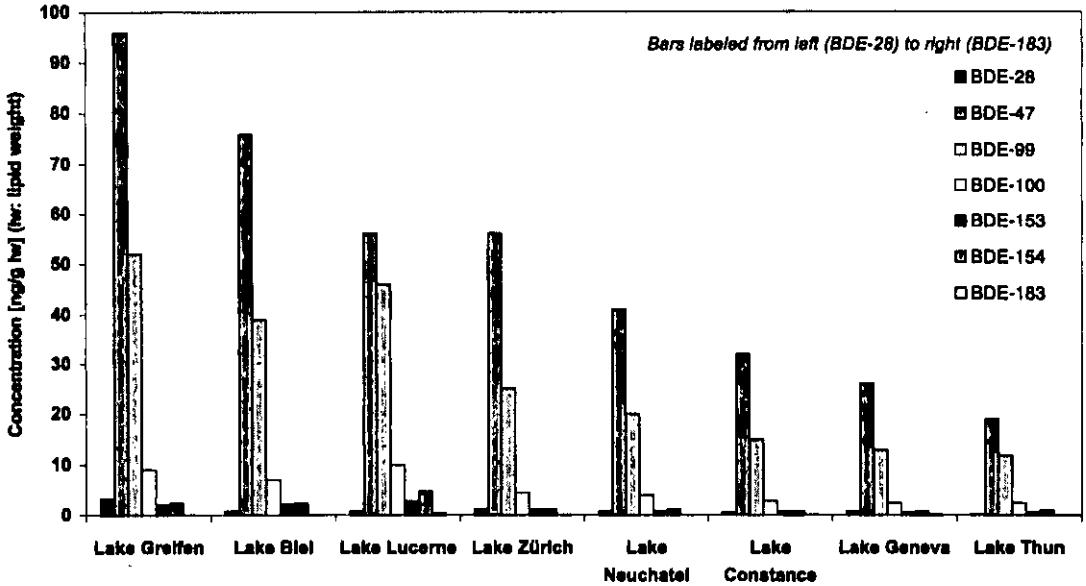

Fig. 1. Levels [ng/g lw] of BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, and BDE-183 in whitefish (*Coregonus* sp.) from eight Swiss lakes (sequence within each sample from left to right: BDE-28 to BDE-183).

The PBDE congener distribution patterns for farmed rainbow trout are shown in Fig. 2. The congener pattern is similar for all samples. It is dominated by BDE-47, followed by BDE-99 and BDE-100. Congeners BDE-153 and BDE-154 are found in similar concentrations, clearly below the level of BDE-100. BDE-183 could be detected in trace amounts, only. Compared to the PBDE congener pattern for wild whitefish, the ratio BDE-47 to BDE-99 is significantly higher. The congener pattern for farmed Swiss fish compares well to the pattern reported for salmon samples from Lake Michigan (Manchester-Neesvig et al., 2001). The low relative concentration of BDE-99 found for the farmed rainbow trout samples compared to the wild whitefish samples might be due to factors such as feed (commercial fish feed versus natural feed), species differences in PBDE uptake (rainbow

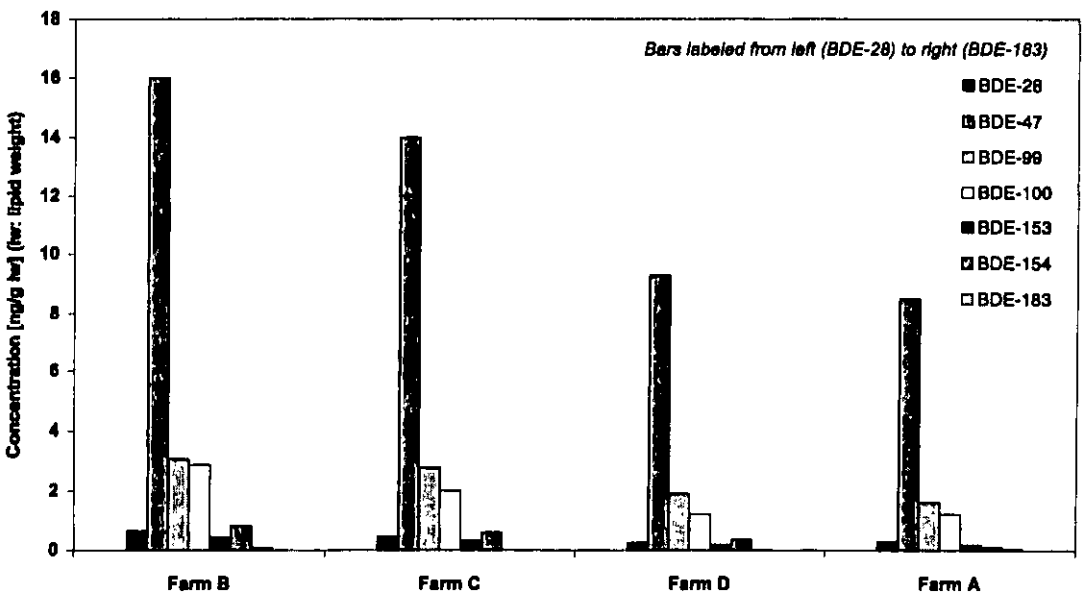

Fig. 2. Levels [ng/g lw] of BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, and BDE-183 in rainbow trout (*Oncorhynchus mykiss*) from four Swiss fish farms (sequence within each sample from left to right: BDE-28 to BDE-183).

trout versus whitefish) and differences in metabolism and trophic level (differences in bioaccumulation between lake trout and whitefish originating from Lake Siskiwit have been reported by Swackhamer and Hites (1988)).

3.3. Atmospheric deposition of PBDE

All lakes investigated in our study are situated in populated areas, and none of them can be considered as a background site. Since total PBDE levels are low compared to sites near known point sources of PBDE (Andersson and Blomkvist, 1981; Dodder et al., 2002), we assume that no major point source is present and that the PBDE content in our fish samples is mainly caused by diffuse contamination, such as atmospheric deposition (atmospheric deposition is a major input of organochlorines to aquatic systems, see Swackhamer and Hites (1988)). Without attempting a detailed analysis based on sediment, water column, and biota concentrations, correlation of PBDE levels in whitefish with lake parameters such as volume, surface, and number of inhabitants were investigated (data see Table 1). If atmospheric deposition (assumed to be constant throughout Switzerland) is a major input pathway for PBDE, and under the assumption that elimination rates are similar for all lakes, high PBDE levels in fish from shallow lakes (i.e. high surface/volume ratio) would be expected. Fig. 3 shows that whitefish PBDE levels [ng/g lw] and surface/volume ratio [km^{-1}] of the respective lakes do indeed correlate ($r^2 = 0.70$), suggesting atmospheric deposition as an input pathway for PBDE. At a given atmospheric deposition rate of PBDE (assumed to be constant), lakes having large surface/volume ratios (low depth at a given surface) are expected to show higher PBDE concentrations than lakes with a small surface/volume ratio. Compared to its surface/volume ratio, whitefish from Lake Lucerne showed considerably higher PBDE levels in whitefish than all other lakes which allows some speculation about the presence of local PBDE source in this case. Additionally, the BDE-47 to BDE-99 ratio is also lower for the samples from Lake Lucerne compared to all other lakes (see Fig. 1). If adjusted for fish size (average length was only 21.9 cm), the deviation of the Lake Lucerne sample from the line generated from fish from the other lakes would be expected to be even larger and further strengthen the suggestion of a local source. A final conclusion on the presence of a local source, however, will need further investigation, since this data is based on a single composite sample (ten individuals). If Lake Lucerne is omitted from the data set, the correlation coefficient (whitefish PBDE levels versus surface/volume ratio) is close to one ($r^2 = 0.96$). The correlation between the PBDE concentration and the ratio of human population (inhabitants in the catchment area) and lake volume, however, was less pronounced ($r^2 = 0.59$), as were other correlations between PBDE concentrations and lake

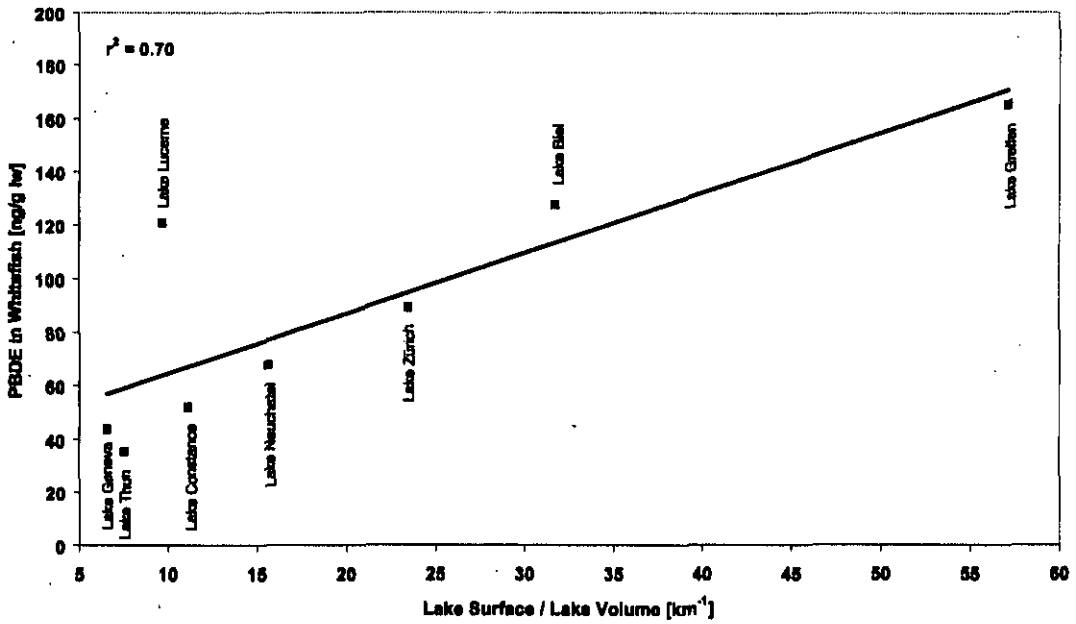

Fig. 3. Surface/volume [km^{-1}] ratio of each lake versus the levels [ng/g lw] (sum of BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, and BDE-183) in whitefish (*Coregonus* sp.).

properties. Based on these data, wastewater effluents might represent only a minor source of PBDE input to the lakes investigated.

3.4. Human exposure of PBDE through fish consumption

The increased use of PBDE as flame retardants (Renner, 2000), their rapidly rising concentrations in human milk (Betts, 2002a,b), and their potential for triggering endocrine disrupting effects are important reasons to estimate human exposure through these contaminants. Next to exposure to PBDE through flame retarded products (textiles, plastics), PBDE are efficiently taken up by consumption of fish. In a study on nursing women in Japan, Ohta et al. (2002) report a strong positive relationship between PBDE concentrations in human milk and dietary intake of fish and shellfish. Based to the results of our study, human exposure to PBDE through consumption of Swiss fish was estimated. In some parts of Switzerland (Lake Geneva region), up to 70% of the population eats fish 1–2 days a week (BFS, 1998). Based on an average daily consumption of 20 g fish (Grüter et al., 1998) from Lake Biel with a PBDE content of 7.4 ng/g ww (highest PBDE concentration detected in this study), a maximum daily intake of 0.15 μg PBDE was estimated (0.026 μg/day for Swiss farmed trout). This number corresponds to the lower end of the estimate for the total PBDE intake of the Nordic consumer of 0.2–0.7 μg/day reported by Darnerud et al. (2001). Based on the current knowledge on PBDE, they consider the lowest observed adverse effect level (LOAEL) of the PBDE group to be 1 mg/kg/day (preliminary value due to data gaps including carcinogenicity, reproduction and developmental toxicity as well as additional routes of exposure). In the light of continued use of PBDE in large quantities, monitoring levels of these compounds will continue to be an important issue.

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Polybrominated diphenyl ethers in influents, suspended particulate matter, sediments, sewage treatment plant and effluents and biota from the Netherlands

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"Capsule": *Suspended particulate matter is an important carrier for higher brominated diphenylethers in the aquatic environment.*

Abstract

Polybrominated diphenyl ethers (PBDEs) have been determined in 133 samples of suspended particulate matter (SPM), sediments, sewage treatment plant (STP) influents and effluents, fish and mussels from various locations in The Netherlands, as a part of a large Dutch national study on estrogenic contaminants in the aquatic environment (LOES project). Some PBBs were also analysed but not found in any of the samples at detectable levels. PBDEs and PBBs were included in this study because indications of long term effects on the balance of endocrine systems were found in the literature. High concentrations of decaBDE (up to 4600 μg/kg dry weight) were found in SPM from the Western Scheldt. These levels are possibly related to spillage during use of PBDEs in industries upstream the river Scheldt in Belgium. SPM was identified as an important carrier for higher brominated diphenyl ethers in the aquatic environment. DecaBDE was not found at detectable levels in flounder, bream and mussels. The bioaccumulation of decaBDE in these fish and shellfish samples is apparently limited. Lower brominated PBDE congeners (tetra/penta) were also found in the Western Scheldt as well as in the Rhine delta and the river Meuse, but in much lower concentrations than the decaBDE. In contrast with decaBDE, the tetra and pentaBDEs were found in biota. It was concluded that at least a small part of the PBDE can pass STPs.

Keywords: Polybrominated diphenyl ethers; Suspended particulate matter; Sewage treatment plants; Sediment; Fish

1. Introduction

Today, 470,000 tons of bromine are produced annually, used in water purification, health care, agriculture, cars and photography (Anon., 2000). However, the most important use of bromine is in flame retardants. Flame retardants are chemicals which, added to materials, inhibit or suppress the combustion process. They are being used in electronic equipment, upholstered furniture, construction materials, and textiles. Thirty-nine percent of all flame retardants are based on bromine (Anon., 2000). Some of the most important brominated flame retardants are: polybrominated diphenyl ethers (PBDEs, composition of various techhnical mixtures given in Table 1), hexabromocyclododecane (HBCD), and tetrabromobisphenol-A (TBBP-A).

On 30 January 2001, the European Commission has issued a proposal to ban the production and use of PentaBDE (Dungey, 2001). Polybrominated biphenyls (PBBs) have mainly been used in the USA (de Boer et al., 2000). The production of the main mixture, hexabromobiphenyl (Firemaster BP-6), ceased in 1974, after the Michigan disaster (WHO, 1994). The production in Europe has been limited to decabrominated biphenyl (decaBB), but PBBs have been imported from the USA (Brinkman and de Kok, 1980). The decaBB production, which since 1977 only took place in France, has been terminated a few years ago (de Boer et al., 2000; Spiegelstein, 2000).

PBDEs have been detected in the environment since the late 1970s (Andersson and Blomkvist, 1981; de Boer, 1989, Sellström et al., 1990; Pijnenburg et al.,

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Table 1
Composition of technical mixes of polybrominated diphenyl ethers (PBDEs)

PBE-mix	Percentage of mixture						
	tetraBDE	pentaBDE	hexaBDE	heptaBDE	octaBDE	nonaBDE	decaBDE
PentaBDE	33.7	54.6	11.7				
OctaBDE			5.5	74.9	3.6	13.9	2.1
DecaBDE						3	97

Source: Spiegelstein, 2000.

1995, Allchin et al., 1999, Rahman et al., 2001; Hale et al., 2001; de Wit, 2002). More recently, more attention is given in the scientific literature to the environmental occurrence of PBDEs. PBDEs were found to be present in sperm whales and a wider range of marine mammal species which is an indication for the presence of these compounds in deeper waters of the ocean (de Boer et al., 1998). PBDEs were also found in relatively high concentrations in marine mammals from the North Sea (de Boer et al., 1998; Lindstrøm et al., 1999). In addition to that, increasing trends of BDE47 and BDE99 concentrations in Swedish human milk were presented (Norén and Meironyté, 1998, 2000), although the most recent data showed that these curves have past the top (Meironyté Guvenius and Norén, 2001) and are declining. The PBDE patterns observed in human milk and in marine mammals and fish mainly consist of 2,4,2′,4′-tetra BDE (BDE47) together with smaller amounts of some other tetra, penta and hexa BDEs. PBDEs were also found in several estuaries in Europe, among which those of the rivers Scheldt in The Netherlands and Mersey and Tees in the UK (Allchin et al., 1999; Anon., 1997). In these rivers also relatively high levels (up to mg/kg dry weight) decaBDE were found. Environmental levels of PBBs were reported after the Michigan disaster (WHO, 1994). PBBs have also been reported in fish from German rivers (WHO, 1994) and in North Sea and Baltic fish (Jansson et al., 1993).

The present study was carried out as a part of the Dutch national study on estrogenic contaminants in the aquatic environment (LOES project) (Vethaak et al., 2002). PBDEs and PBBs were included in this study because indications of long term effects on the balance of endocrine systems were found in the literature (de Boer et al., 2000). Other contaminants included in this project were phthalates, alkyl phenols, bisphenol-A and several hormones (Vethaak et al., 2002). The LOES project also included a large biological effect part, focussed on the observation of possible estrogenic effects in fish sampled at the same locations. The objective of this study was to screen the Dutch aquatic environment for concentrations of PBDEs and PBBs. Therefore, fish, mussels, sediment, and suspended particulate matter (SPM) samples of the most important rivers and estuaries were analysed for these compounds. In addition, SPM samples of a number of sewage treatment plants (STP) influents and effluents were analysed for PBDEs and PBBs to estimate if these compounds were able to pass STPs.

2. Methods

The following PBDEs were determined:

47	2,4,2′,4′-tetraBDE
85	2,3,4,2′,4′-pentaBDE
99	2,4,5,2′,4′-pentaBDE
138	2,3,4,2′,4′,5′-hexaBDE
153	2,4,5,2′,4′,5′-hexaBDE
209	decaBDE

The BDEs 47 and 99 are the main compounds in the technical Penta-mix. The BDEs 138 and 153 are also present in the Penta-mix, in lower percentages. decaBDE is the main compound (97%) of the technical Deca-mix. BDE 85 was available as a standard, but does not represent a specific technical mixture. Some other important representatives of the technical mixtures such as BDE100 (of the Penta-mix) and BDE183 (of the Octa-mix) were not available at the time of the design of this programme.

SPM samples were obtained from surface waters in the Netherlands and from sewage treatment plant influents and effluents and industrial wastewater by (flow-through) centrifugation (effluent and surface waters) or filtration (influent) of the water samples. Centrifugation took place at 20,000 rpm until 200 g of material was collected. Sediment samples were obtained by pooling nine sub-samples, taken by a Van Veen grab sampler from a square of ca. 100 m^2 per location. All sediment samples were sieved (<63 μm). Flounder (*Platichthys flesus*) samples were taken by beam trawling and bream (*Abramis brama*) samples by small bottom trawls. The flounder and bream were filleted and the fillets of 25 fishes per location were pooled. Marine mussels (*Mytilus edulis*) and freshwater mussels (*Dreissena polymorpha*) were hanged out in small nets for 6 weeks at

the specific locations, after which they were recollected. Depuration was not applied. The mussels were taken out of the shell and pooled, ca. 100 g mussel flesh per sample. In total 133 samples were analysed. The number of samples per sample type and per location is given in Table 2. The samples were taken during three sampling periods: April, July and September 1999.

The STP influent samples were filtered over Whatman filters (GF/C particle retention 1.2 μm). The filtered volume was 4 l. STP effluent samples could only be taken at a few selected locations, due to the high costs of the required centrifuge, which was used to centrifuge the samples on the spot, and its transport to the various locations. The SPM and STP effluent residues, biota and sediments were mixed with sodium sulphate, allowed to dry for 3 h or overnight (dependent of the volume, <6 g sample: 3 h) and Soxhlet extracted for 12 h with hexane/acetone (3:1, v/v, 70 °C) (de Boer et al., 2001). All solvents used were nanograde quality, and were obtained from Promochem, Wesel, Germany. The extracts were concentrated on a rotary evaporator and dissolved in 2 ml of dichloromethane. The extracts were cleaned by gel permeation chromatography (GPC) over two Polymer Laboratories (PL) gel columns (300 × 25 mm, pore size 10 μm), using dichloromethane at 10 ml/min. The collected fraction was 18–23 min. The fraction was concentrated under nitrogen, dissolved in iso-octane and further purified by shaking with sulphuric acid. After separation of the iso-octane phase, the sulphuric acid phase is washed twice with pentane to extract all PBEs. Finally, the pentane/iso-octane mixture was concentrated under nitrogen to 2 ml (iso-octane) and eluted over a 1.6 g silica gel column (2% deactivated) with 11 ml iso-octane and 10 ml 20% diethylether in iso-octane. This sequential elution results in both a high recovery and a clean sample. The fractions were combined and concentrated to 1 ml (iso-octane), after addition of a syringe standard (2,3,5,6,3′-pentachlorobiphenyl (CB112). An external standard solution of all PBDEs analysed was subjected to the entire method to determine the recoveries. All results reported are corrected for recovery. Amber glassware was used throughout the entire project, while direct sunlight or other UV light entrance in the laboratory was blocked by installing UV filtering foils at the windows and UV filter plates under the fluorescent lights. This was necessary to prevent possible degradation of decaBDE. The final analysis was carried out by GC/MS, using electron capture negative ionisation (ECNI) as ionisation technique (MSD transferline 290 °C, source temperature 200 °C, quadrupole temperature 106 °C, electron energy 70 eV with methane (3.25 ml/min) as a reagent gas). Initially (period 1), a 25 m CP Sil 8 column [internal diameter (i.d.) 0.25 mm, film thickness 0.25 μm] was used for the determination all PBDEs. However, due to a co-elution of BDE153 with tetrabromobisphenol-A (TBBP-A), BDE153 could not be determined properly in a number of samples taken in period 1. In addition, the decaBDE peak at a 25 m column occasionally varied in shape due to degradation as a consequence of too long exposure to elevated temperatures in the GC oven. Substitution of this 25 m column by a 50 m (i.d. 0.25 mm, film thickness 0.25 μm) CP Sil 8 column enabled the determination of BDE153 and provided a maximum resolution for the determination of all other BB and BDE congeners.

GC conditions: Oven 90 °C, 3 min → 210 °C, 30 °C/min, 20 min 210 °C → 290 °C, 5 °C/min. Injection was pulsed splitless and the carrier gas was helium.

A 15 m (i.d. 0.25 mm, film thickness 0.25 μm) CP Sil 8 column provided good conditions for the determination of decaBDE. The maximum oven temperature during the decaBDE analysis was 300 °C, the injector temperature was 275 °C. The peak identification was based on retention time and the recognition of the Br^- ion (m/z 79/81). For decaBDE, the mass fragments 486.7 and 488.7 were used for additional identification. Detection limits were calculated as three times the noise level of the chromatogram. The limit of determination was set by the lowest concentration of the multi-level (6 point) calibration curve. Further details of the analysis have been reported before (de Boer et al., 2001).

The total lipid contents of the biota samples were determined by a chloroform/methanol extraction according to Bligh and Dyer (1959). Dry weights of sediment and SPM samples were determined after heating at 105 °C for 24 h.

Table 2
Sampling scheme

Sample type	Number of samples	Number of locations
SPM	44	18
Sediment	22	17
Flounder and bream	35	20
Mussels (freshwater + marine)	16	16
STP influent/effluent	13	9
Industrial wastewater	3	3

3. Results and discussion

The PBDEs 85 and 138 were generally below the detection limits (<1–<0.1 μg/kg dw). An overview of the concentration ranges found for the BDEs 47, 99, 153 and 209 is given in Table 3.

3.1. SPM

All PBDE concentrations in SPM and sediments are expressed on a dry weight basis (Figs. 1–3). Markedly

Table 3
Summary of PBDE concentrations in SPM, sediment, biota and waste water residues (μg/kg dw)

Sample type	BDE 47	BDE 99	BDE 153	BDE 209
SPM	2.2 (<0.2–9)[a]	2.4 (<0.1–23)	<0.6 (<0.1–9.7)	71 (<9–4600)
Sediment[b]	1.1 (0.3–7.1)	0.6 (<0.2–5.5)	<0.7 (<0.1–5)	22 (<4–510)
Flounder	0.9 (0.6–20)	0.2 (<0.01–4.6)	0.1 (<0.02–<1)	<0.9 (<0.2–<6)
Bream	16 (0.2–130)	0.1 (<0.01–<0.8)	0.9 (<0.04–4.1)	<5 (<0.03–<21)
Marine mussels	1.2 (0.9–4.3)	0.5 (0.3–1.6)	<0.1 (<0.1–<0.2)	<4 (<4–<5)
Freshwater mussels	1.8 (0.7–17)	1.4 (0.4–11)	<0.9 (<0.1–1.5)	<23 (<4–<34)
STP influent[c]	2.3 (<0.1–68)	5.2 (0.3–33)	<0.9 (<0.02–<5)	24 (<0.5–330)
STP effluent[d]	22 (11–35)	<1 (<1–<1)	<5 (<0.4–<7)	350 (310–920)
Industrial wastewater	0.4 (<0.1–68)	6.6 (0.3–66)	<1 (<0.02–2.6)	45 (<0.5–200)

[a] Median and range are given for each BDE and each sample type.
[b] <63 μm fraction.
[c] Particulate matter filtered from influent.
[d] Particulate matter centrifuged from effluent.

high concentrations of decaBDE (up to 4600 μg/kg dw.) were found in SPM from the Western Scheldt (Fig. 3). A clear decreasing trend was observed from the east part of the river near Antwerp (locations 4–9–7) towards sea. The pattern found suggests a relationship between the PBDE concentrations and the textile industry in Antwerp, in which decaBDE is used. The local bromine industry in Terneuzen (location 9), half way the Dutch part of the Western Scheldt, may also have influenced the PBDE levels, but its impact seems to be small compared to the PBDE plume coming from Antwerp. Clear variations in PBDE concentrations in SPM were found between the three sampling periods. It is evident that variations due to variable discharges are rather observed in the SPM than in sediments or biota which both show a more integrative picture. The highest BDE47 and 99 concentrations were found in SPM from the Haringvliet (river Rhine delta, location 1) with 5.2–9 and 4–12 μg/kg dw, respectively (Figs. 1 and 2). The BDE47 and 99 concentrations at two other locations in the Rhine delta, Nieuwe Waterweg and Rotterdam harbour were also high. Given the extremely low solubility of higher brominated PBDEs in water and the high PBDE concentrations found in these SPM samples, SPM is clearly identified as an important carrier for higher brominated diphenyl ethers in the aquatic environment. This is the first report on PBDEs in SPM, no other data on PBDE concentrations in SPM could be found in the literature. SPM is apparently a good matrix for monitoring the momentary concentration of PBDEs in the aquatic environment. However, both sampling and pre-treatment of SPM samples are rather expensive and laborious.

3.2. Sediments

All concentrations shown in Fig. 4 are on a dry weight basis. Sieving of the sediment to <63 μm has caused a certain normalisation, but small differences in PBDE concentrations due to differences in organic carbon content per location may still occur. The decaBDE pattern in sediment of the Western Scheldt (locations 4–9–7) was similar to that found in SPM: higher levels in the east part (up to 510 μg/kg dw), decreasing down to 110 μg/kg dw at Terneuzen and <2.9 μg/kg dw (all <63 μm fractions) at Vlissingen (Fig. 4). Again, the input from Antwerp appears to be more important than a supposed contribution from the bromine industry at Terneuzen. Although there is a considerable tidal movement, which could move a possible PBDE plume from Terneuzen to the east, it is unlikely that the higher PBDE levels in the most eastern part of the Western Scheldt would only have been caused by such a tidal influence. The sediment samples were only taken in period 3 (September). The decaBDE concentrations in the river Rhine were the second highest after the Western Scheldt, with 84 μg/kg dw in sediment from Lobith (German border, location 3) and 220 μg/kg dw in SPM from Lobith. The highest BDE47 and 99 concentrations in sediment were found at this location (7.1 and 5.5 μg/kg dw, respectively). The decaBDE concentrations in sediments are among the highest reported until now (de Boer et al., 2000). DecaBDE levels up to 1,700 μg/kg dw. have been reported in the river Mersey, (UK) (Anon., 1997). Much lower decaBDE concentrations were reported in Danish sediments with a maximum level in Copenhagen harbour of 21.5 μg/kg dry weight (Platz and Christensen, 2001). The decaBDE concentration in North Sea sediment off the Western Scheldt was 32 μg/kg dry weight (<63 μm fraction) (Klamer et al., 2001), which corresponds with the results of this study. Other decaBDE concentrations in North Sea sediments along the Dutch coast were slightly lower, and decreased further as sampling locations were further away from the coast (Klamer et al., 2001). The BDEs 47, 99 and 153 concentrations in SPM, sediments and biota are not as high as the highest concentrations reported in the literature (de Boer et al., 2000). The high

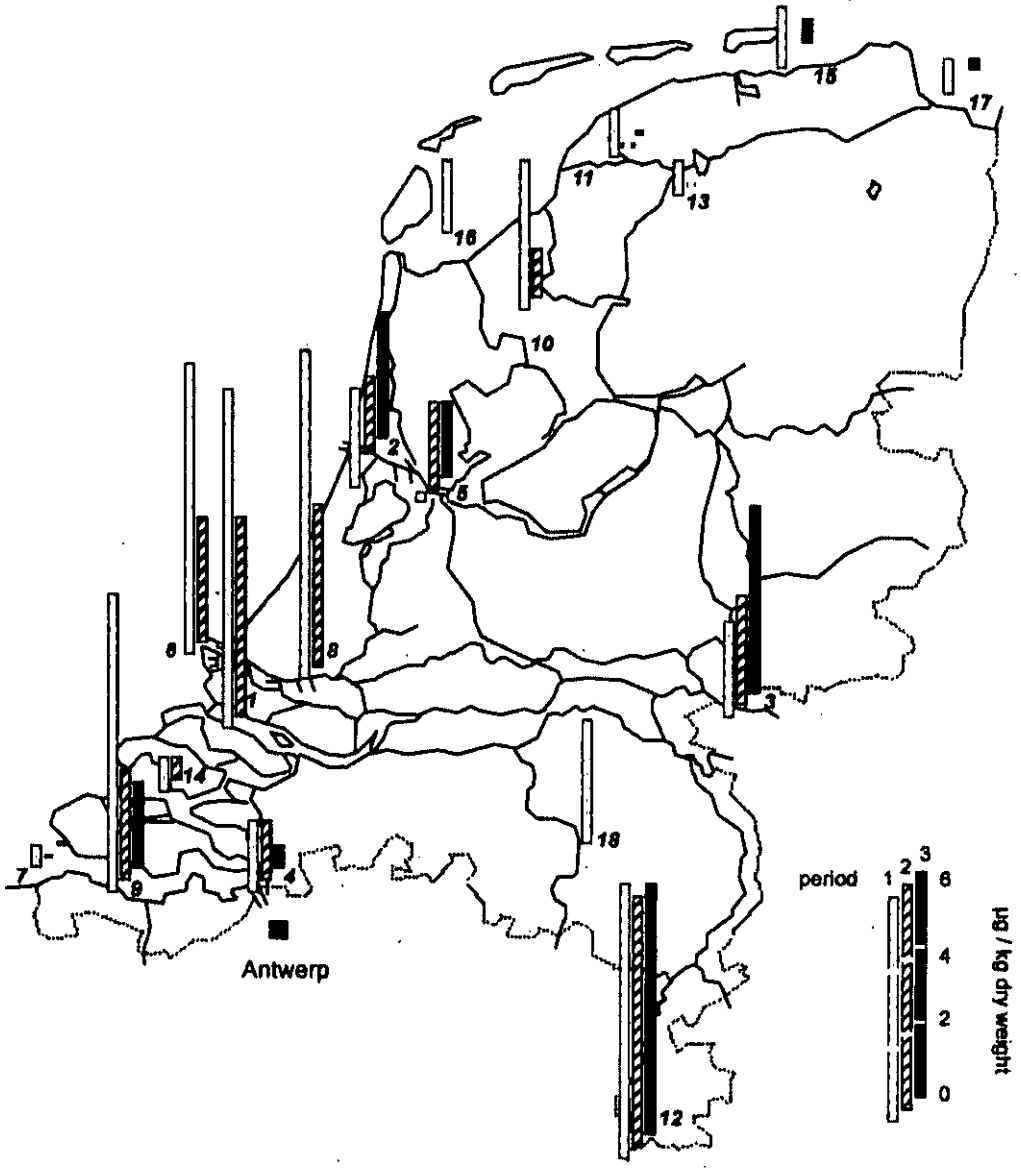

Fig. 1. BDE47 concentrations in suspended particulate matter in μg/kg dw; locations (in The Netherlands): 1. Haringvliet (west), 2. IJmuiden, 3. Rhine (Lobith, border), 4. Western Scheldt (Schaar van Ouden Doel), 5. Amsterdam harbour, 6. Rotterdam harbour (Splitsingsdam), 7. Western Scheldt (Vlissingen), 8. Nieuwe Waterweg (Maassluis), 9. Western Scheldt (Terneuzen), 10. IJssel Lake (Vrouwenzand), 11. Koudevaart (St. Annaparochie), 12. Meuse (Eijsden, border), 13. Bergumer Lake (Bergum), 14. Eastern Scheldt (Hammen), 15. Wadden Sea (Dantzig Gat), 16. Wadden Sea (Den Oever), 17. Ems Dollard (Bocht van Watum), 18. Dommel (near Eindhoven), 19. North Sea (Noordwijk), 20. North Sea (Oestergronden), 21. Meuse (Borgharen), 22. Apeldoorns Canal (Epe), 23. Amsterdam (Westpoort).

decaBDE concentrations in sediment emphasize the need for a thorough understanding of the fate of this compound. Dietary uptake and degradation of decaBDE in fish has been reported to occur in an aquarium experiment (Kierkegaard et al., 1999), but should be confirmed for prevailing conditions in the aquatic environment prior to drawing further conclusions. If a complete absence of degradation of decaBDE would appear from further studies, this should be ensured for the long term, because even the slighest formation of lower brominated diphenylethers from these high levels of decaBDE in the sediment studied could cause serious environmental damage, even after periods of tens of years.

3.3. Fish and mussels

None of the fish samples contained decaBDE in measurable concentrations (<0.2–<21 μg/kg dw) (Tables 4 and 5). In two marine mussel samples (Western Scheldt, Vlissingen, and Wadden Sea) decaBDE was found at a level of 5 μg/kg dw (Table 6). However, these mussels had not been depurated after sampling and the concentrations found are very likely due to decaBDE which

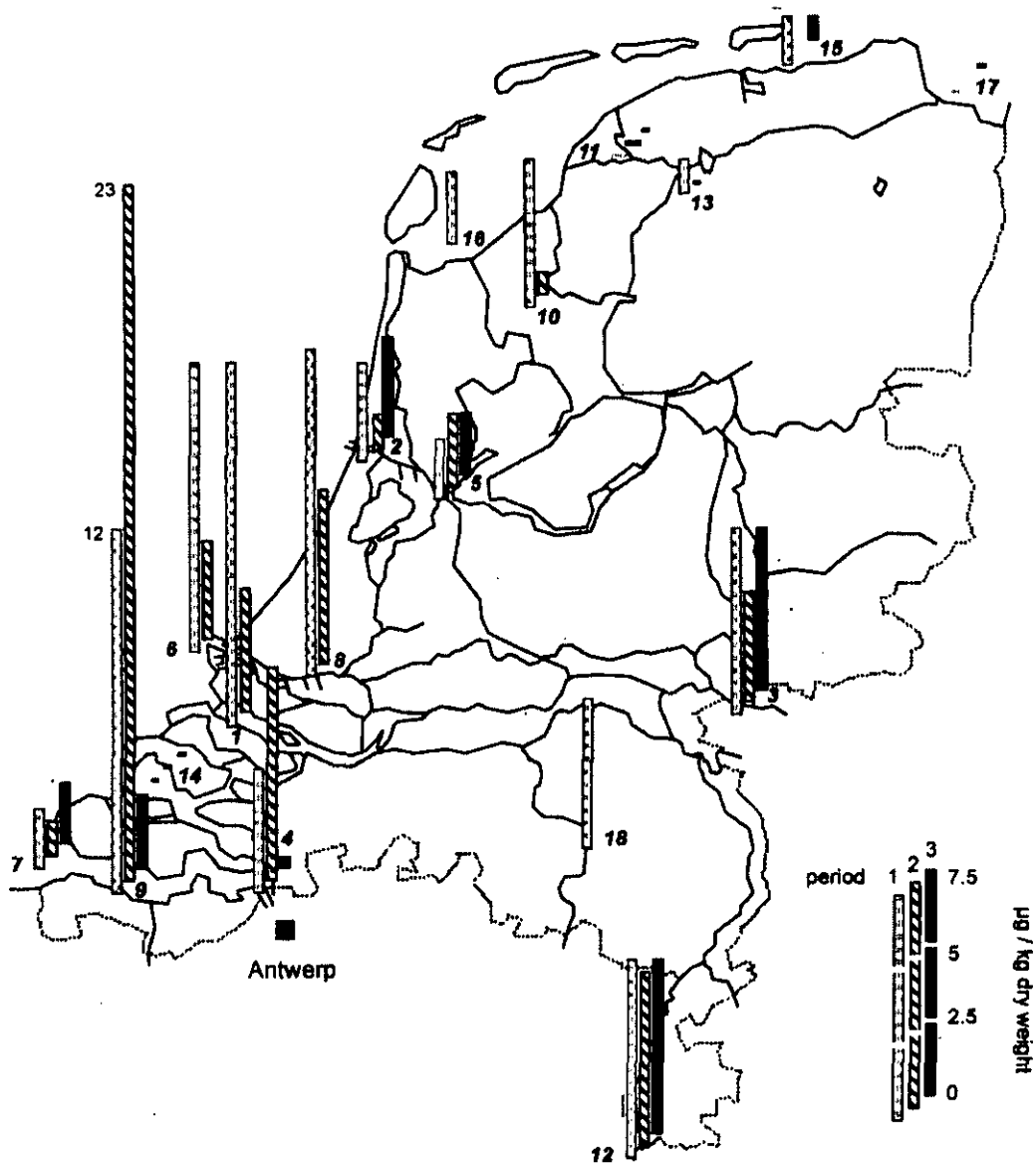

Fig. 2. BDE99 concentrations in suspended particulate matter in µg/kg dw (locations see Fig. 1).

is present in small particles in the gut of the mussels (Booij et al., 2000). BDE 47 is clearly higher in the fish samples than BDE99, which is often around the detection limits (ca. <0.1 µg/kg dw). Such a selective bioaccumulation of BDE47 has been reported before (de Boer et al., 2000). In mussels little difference is found between BDE47 and BDE99 concentrations, similarly to the pattern in SPM (Table 7). This difference in pattern between fish and mussels can be explained by a lower biotransformation capacity of mussels for BDE99 or a limited uptake of BDE99 by fish. In sediment and SPM samples BDE47 is normally slightly higher than BDE99. Relatively high BDE47 concentrations were found in bream from the rivers Meuse, Rhine and Dommel (locations 12–3–18): 110, 90 and 130 µg/kg dw, respectively. A perch sample from south Sweden contained an exceptionally high concentration of 24,000 µg/kg lipid weight BDE 47 (Sellstrøm et al., 1993). Much lower PBDE concentrations were found in herring near Karlskrona and other places along the Swedish coast, with maximum levels of 18 µg/kg for BDE47, 7.1 µg/kg for BDE99 and 3.3 µg/kg for BDE100, all on a lipid weight basis with a mean lipid content of 2% (Nylund et al., 2001). The BDE47 concentrations found in bream from the rivers Meuse (Eijsden), Rhine (Lobith) and Dommel can be considered as relatively high (ca. 600 µg/kg lipid weight), when compared to background concentrations such as found at the locations Koudevaart (St. Annaparochie, no 11) and Bergumermeer (location 13) (Table 4). Because of these elevated levels in bream, the median values given in Table 3 for bream are higher than in flounders which originated from the generally more clean marine locations. Clear differences in BDE concentrations in bream are observed between

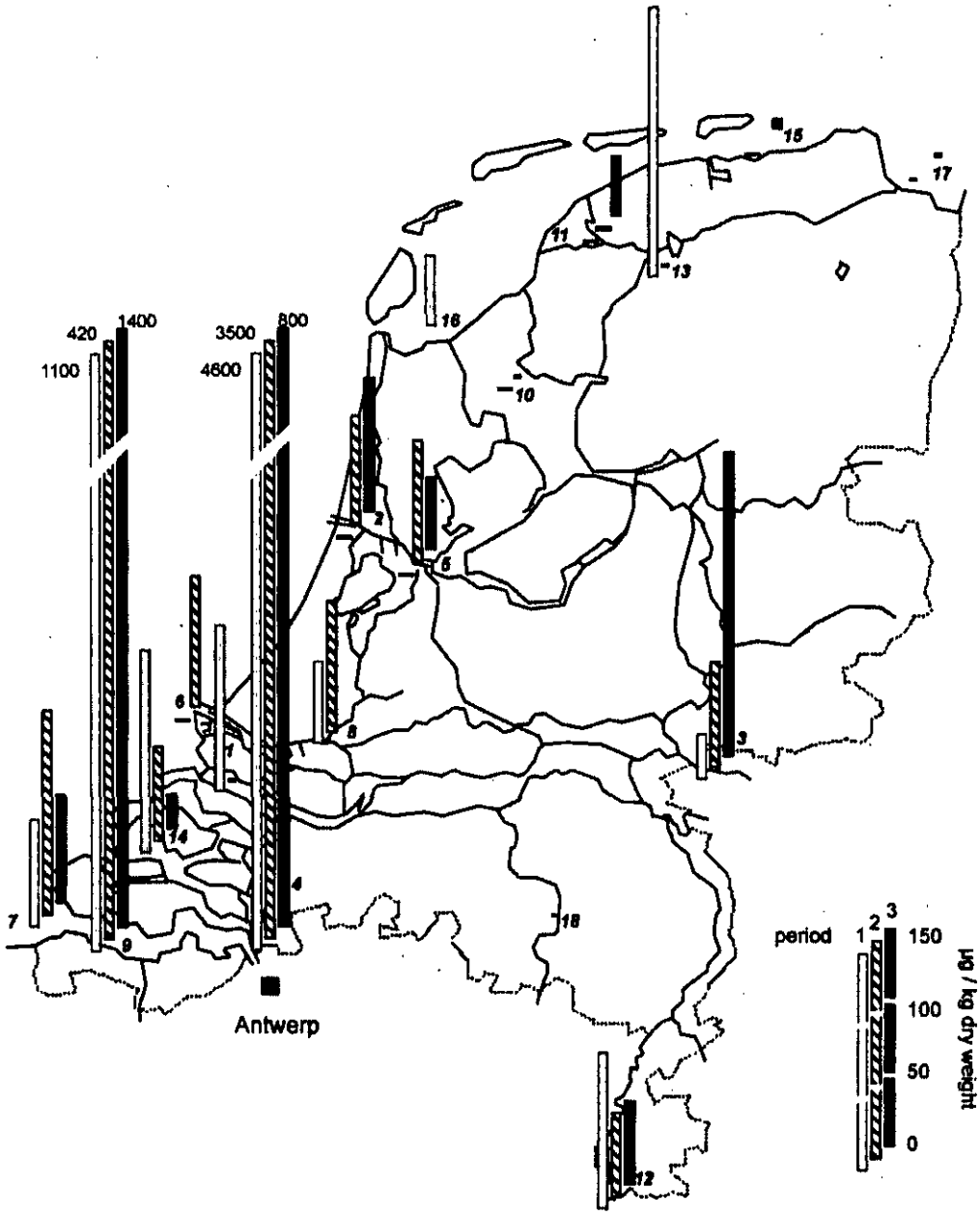

Fig. 3. DecaBDE concentrations in suspended particulate matter in μg/kg/dw (locations see Fig. 1).

the two periods. The higher concentrations were found at the end of the summer (period 3), after which the bream had built up more fat (and thus more contaminants on a dry weight basis, while just after spawning (period 1) the bream is more lean and has lost some of the contaminants during spawning.

3.4. STP in- and effluent

Relatively high decaBDE concentrations were found in some STP samples (Table 8). The effluent of the Eindhoven STP contained 920 μg/kg dw decaBDE, whereas the influent contained 72 μg/kg dw. Clearly, decaBDE can pass STPs. The higher concentrations in the effluent can be explained by a different way of sampling. The influents were delivered at the laboratory as water samples and were filtered over Whatman filters (GF/C, particle size 1.2 μm). The effluents were delivered as particulate matter residues, obtained after centrifugation on the spot, immediately after sampling. Possibly, these centrifugated samples have contained a higher proportion of fine particles, which contained higher concentrations of PBDEs. Large particles could also settle in the STP process, resulting in enrichment of the smaller particles in the effluent. Variation in the PBDE flux through the STP may also have played a role, because the influent and effluent samples were taken at the same moment. The retention time of the

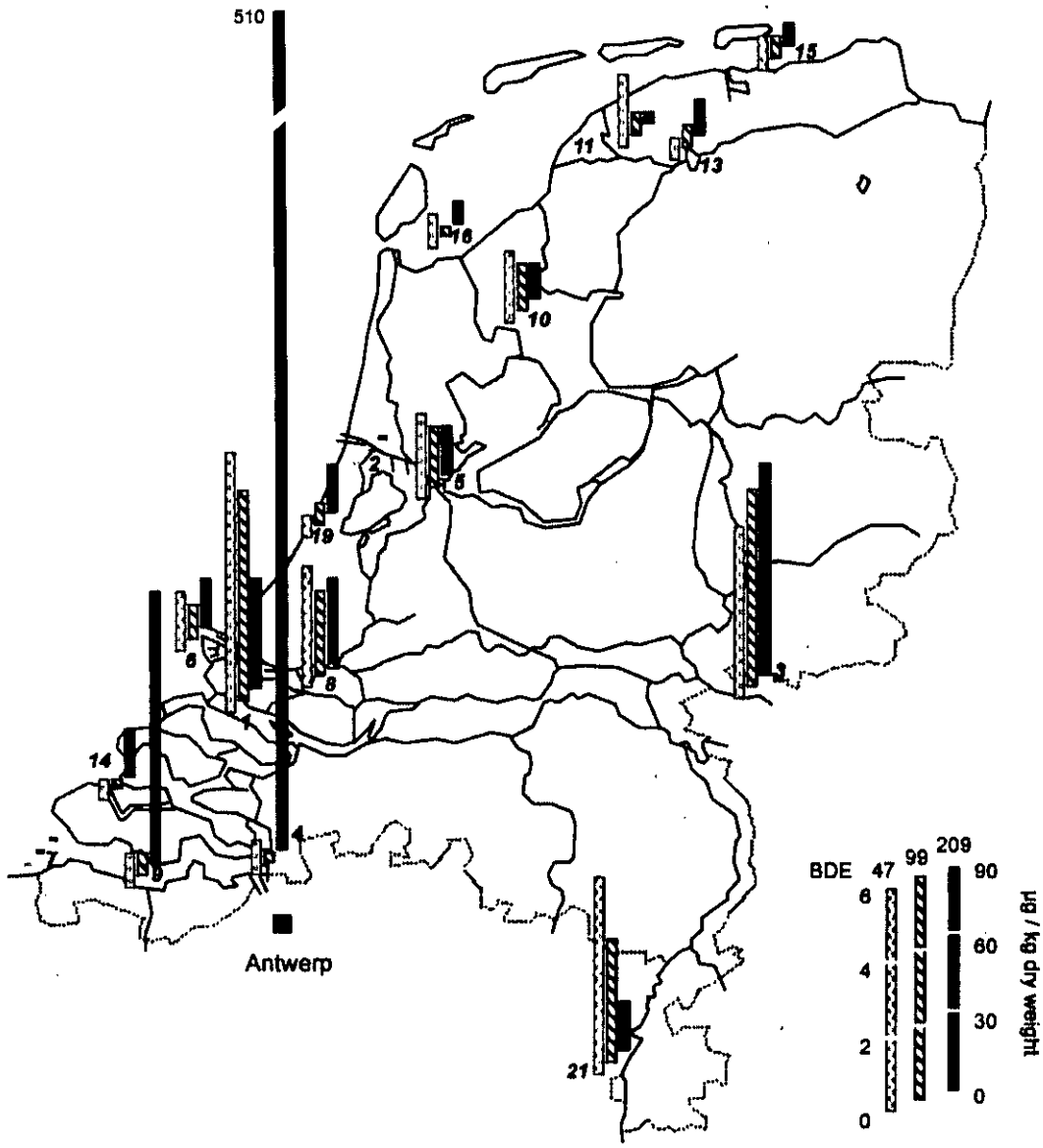

Fig. 4. Concentrations of BDEs 47, 99 and 209 in sediment in µg/kg/dw (locations see Fig. 1).

waste water in the STP was not taken into account, but more than one sample per day was taken to prepare a composed sample. In an Amsterdam STP 310 µg/kg dw decaBDE was found in the effluent, whereas 110 µg/kg dw was found in the influent. It should be taken into account that regularly, ca. each month, the SPM in the influent to which PBDEs are bound, is removed from the STP as the primary sludge, together with the secondary sludge, which is produced during the treatment of the waste water. The fraction of SPM passing the STP is not more than ca. 2.5%. Three sludge samples [Ameland (2x) and St. Annaparochie] showed substantial decaBDE concentrations (up to 190 µg/kg dry weight), as well as substantial concentrations of other BDEs (up to 40 µg/kg dry weight for BDE47) (Table 8b,c). Most of the sewage sludge in the Netherlands is burned in specific sludge incinerators. In other countries, sewage sludge may be deposited in landfills (Hale et al., 2001). That procedure may cause a delayed input of PBDEs into the environment through contaminated leachate water. Incineration at sufficiently high temperatures under well-controlled conditions will destroy the PBDEs, but less well-controlled incineration may result in the formation of limited quantities of brominated dibenzo-*p*-dioxins and furans and bromochlorodibenzo-*p*-dioxins and furans (Vehlow et al., 2000; Sakai et al., 2001).

Up to 200 µg/kg dw decaBDE was found in particles filtrated from industrial waste waters. The concentrations of the other PBDEs in STP in- and effluents and industrial waste water residues were in most cases considerably lower than the decaBDE concentrations (Table 2). No other data on PBDEs in STP in- and

Table 4
PBDEs in fillets of bream

Location	No.	% DW	BDE47	BDE85	BDE99	BDE138	BDE153	decaBDE
(a) Period 1 (μg / kg dry weight)								
Amsterdam	5	22.7	4.4	0.2	<0.05	<0.02	<0.4	<0.06
Eijsden (Meuse)	12	22.2	9.1	0.2	<0.02	<0.02	<0.4	<0.4
Haringvliet	1	20.1	16	0.2	<0.06	<0.02	<0.7	0.3
Lobith (Rhine)	3	23.7	37	0.2	<0.1	<0.02	1.3	<0.03
Vrouwenzand (IJssel Lake)	10	25.0	13	0.2	<0.03	<0.02	1.0	<0.8
Bergumermeer, Bergum	13	19.7	0.2	0.013	<0.01	<0.02	<0.04	<0.4
Koudevaart, St Annaparochie	11	20.1	0.6	0.02	<0.01	<0.06	0.04	
(b) Period 3 (μg / kg dry weight)								
Amsterdam	5	24.4	19	<0.08	0.2	<0.2	0.9	<5.5
Eijsden (Meuse)	12	22.8	110	<0.08	<0.1	<0.2	2.9	<5.8
Haringvliet	1	19.4	80	0.07	<0.5	<0.2	<3.2	<5.9
Lobith (Rhine)	3	16.3	90	<0.06	<0.2	<0.2	<2.5	<5.1
Bergumermeer, Bergum	13	19.7	1.9	<0.07	<0.04	<0.2	0.1	<0.8
Vrouwenzand (IJssel lake)	10	20.3	22	<0.1	<0.8	<0.3	<1.6	<8.9
Apeldoorns Canal (Epe)	22	20.5	15	<0.03	<0.09	<0.07	0.4	<21
Dommel, near STP Eindhoven	18	23.5	130	<0.09	<0.6	<0.2	4.1	<6.3

Table 5
PBDEs in fillets of flounder

Location	No.	% DW	BDE47	BDE85	BDE99	BDE138	BDE153	decaBDE
(a) Period 1 (μg / kg dry weight)								
Amsterdam (North Sea Canal)	5	19.0	1.0	0.02	<0.2	<0.02	<0.04	<0.4
Schaar v Ouden Doel (Western Scheldt)	4	19.0	0.9	0.04	<0.03	<0.03	<0.06	<0.4
Vrouwenzand (IJssel Lake)	10	18.0	1.0	0.01	<0.1	<0.02	<0.06	<0.4
Noordwijk (North Sea)	19	18.7	0.9	0.04	<0.04	<0.02	<0.05	<0.3
Oestergronden (North Sea)	20	18.7	0.4	0.02	<0.02	<0.02	<0.02	<0.4
Den Oever (Wadden Sea)	16	15.8	2.1	0.03	<0.01	<0.02	0.1	<0.2
Maassluis (Nieuwe Waterweg)	8	15.3	4.3	0.06	<0.08	<0.02	0.1	<0.4
Vlissingen (Western Scheldt)	7	19.5	0.7	0.01	<0.01	<0.02	<0.02	<0.4
(b) Period 3 (μg / kg dry weight)								
Dantziggat (Wadden Sea)	15	19.3	<1.5	<0.05	0.8	<0.09	<0.1	<5.9
Hammen (Eastern Scheld)	14	23.3	0.6	<0.1	0.1	<0.2	<0.2	<0.9
Schaar v Ouden Doel (Western Scheldt)	4	18.3	7.5	0.04	0.7	<0.1	0.9	<3.2
Vlissingen (Western Scheldt)	7	38.1	1.0	<0.03	<0.05	<0.08	<0.09	<0.5
Amsterdam Westpoort	23	21.7	5.8	<0.08	0.3	<0.2	<0.3	<0.9
Noordwijk (North Sea)	19	21.7	1.9	<0.06	0.2	<0.2	<0.1	<1.1
Bocht v Watum (Ems Dollard)	17	20.7	0.9	<0.06	0.1	<0.2	0.1	<1.1
Amsterdam	5	16.2	3.6	<0.06	0.3	<0.2	0.2	<1.1
IJmuiden (North Sea Canal)	2	17.5	3.2	<0.09	0.3	<0.2	<0.2	<1.3
Vrouwenzand (IJssel Lake)	10	18.9	1.4	<0.08	0.2	<0.2	<0.1	<1.1
Den Oever (Wadden Sea)	16	20.8	5.2	<0.02	0.8	<0.07	<0.3	<2.3
Maassluis (Nieuwe Waterweg)	8	16.2	20	<0.1	4.6	<0.01	<1.3	<3.6

Table 6
PBDEs and PBBs in marine mussels, period 3 (μg / kg dry weight)

Location	No.	DW%	BDE47	BDE85	BDE99	BDE138	BDE153	decaBDE
Reference (Eastern Scheldt)	14	19.8	0.9	0.2	0.3	<0.1	<0.08*	<3.8
Splitsingsdam (Rotterdam harbour)	6	17.6	4.3	<0.2*	1.6	<0.1	<0.2*	<4.1
Noordwijk (North Sea)	19	16.8	3.6	0.05	1.2	0.1	<0.2*	<4.3
Bocht v Watum (Ems Dollard)	17	17.9	1.2	<0.2*	0.6	<0.1	<0.07*	<3.7
Vlissingen (Western Scheldt)	7	18.9	1.3	<0.2*	0.5	<0.1	<0.2*	4.9
Dantziggat (Wadden Sea)	15	21.9	1.1	<0.1*	0.4	<0.1	<0.08*	4.9

Table 7
PBDEs in freshwater mussels, period 3 (μg / kg dry weight)

Location	No.	% DW	BDE47	BDE85	BDE99	BDE138	BDE153	decaBDE
Reference (IJssel Lake)		4.0	<1.0	<0.4	<0.6	<1.0	<0.3	<5.9
Haringvliet	1	3.4	5.6	<0.1	5.0	<1.0	1.5	<30
Lobith (Rhine)	3	2.9	5.6	<0.5	4.2	<1.1	<0.9	<34
Vrouwenzand (IJssel Lake)	10	4.0	0.7	<0.3	0.5	<0.8	<0.2	<23
Bergumermeer, Bergum	13	4.8	0.8	<0.2	0.4	<0.5	<0.1	<15
Eijsden (Meuse)	12	4.1	7.0	<0.2	5.8	<1.0	1.0	<12
Koudevaart, St Annaparochie	11	5.5	<0.8	<0.3	<0.5	<0.7	<0.7	<4.3
Dommel, near STP Eindhoven	18	3.6	17	<0.5	11	<0.9	<1.1	<30
Amsterdam	5	3.9	1.8	<0.3	1.4	<0.9	1.2	<31

Table 8
PBDEs in waste water

Location	% DS	BDE47	BDE85	BDE99	BDE138	BDE153	decaBDE
(a) Period 1 (μg / kg dry weight)							
STP Amsterdam Westpoort effl	9.1	14	<1.0	18	<1.1	<4	310
STP Eindhoven, effl	15.1	25	<1.3	28	<0.3	<7	920
STP Oostermeende, infl	100	2.2	<0.1	5.4	<0.9	<0.08	1.1
Textile factory Nijverdal infl	100	0.4	<0.03	6.6	<0.2	<1	45
Industry Genemuiden, infl	100	<0.1	<0.04	0.3	<0.03	<0.02	<0.5
STP Amsterdam Westpoort infl	100	3.4	<0.2	3.6	<0.2	<0.9	110
STP Ameland, infl	100	1.7	<0.1	2.1	<0.1	<0.1	15
STP St Annaparochie, infl	100	0.9	<0.1	0.9	<0.03	<0.3	<20
Industry Almere, infl	100	68	<0.9	33	<0.3	2.6	200
STP Eindhoven, infl	100	2.3	<0.1	1.7	<0.08	<0.07	24
(b) Period 2 (μg / kg dry weight)							
STP Ameland, sludge	6.1	9.5	<0.4	11	<2	<2.2	<180
STP St Annaparochie, sludge	12.1	11	<0.7	14	<0.8	<2.6	190
STP St Annaparochie, infl	100	9.3	<0.3	5.2	<1.0	1.0	24
STP Ameland, infl	100	16	<0.6	10	<0.7	<2.1	140
(c) Period 3 (μg / kg dry weight)							
STP Ameland, sludge	2.6	40	<0.7	38	4.0	4.8	8.6
STP Ameland, infl	6.8	16	<0.4	15	1.5	<1.6	330
STP St Annaparochie, infl		1.3	<0.04	1.1	<0.05	<0.2	7.6
STP Oostermeende, infl		0.7	<0.02	0.5	<0.04	<0.1	2.7
STP Eindhoven, effl	10.2	35	<1.1	29	<2.1	<5.0	350

effluents could be found in the literature. Further research on the PBDE balance in STPs is recommended.

3.5. PBBs

The PBBs 15, 49, 52, 101, 153, 169 and 209 were also analysed but not found in any of the samples. The detection limits for most PBBs were between <1 and <0.1 μg/kg dry weight (dw), but for BB209 the detection limits were generally between <1 and <10 μg/kg dw. This result is in agreement with the negligible PBB production in Europe over the past decades.

3.6. Comparison with other contaminants

During the LOES project other potential endocrine disrupting contaminants have been analysed at the same locations. Table 9 gives an indicative overview of concentrations of other contaminants found at the same locations in the various matrices. The results show that nonylphenol and ethoxylates are more important in terms of concentration than the PBDEs, although decaBDE concentrations in the Western Scheldt are of the same order of magnitude. Bisphenol-A and the hormones estradiol and ethinylestradiol are present at a much lower level. Bisphenol-A and nonylphenol and ethoxylates are wider distributed over the various environmental compartments including the water phase, whereas the PBDEs are mainly found in SPM, sediments and biota, or, in the case of decaBDE, almost exclusively in SPM and sediments. PCBs were not analysed during the LOES study. However, there is a wealth of PCB data in The Netherlands. Those data suggest that PCBs in fish and sediment are generally

Table 9
Overview of PBDE, nonylphenol and ethoxylates and bisphenol-A concentrations in water, SPM and bream from The Netherlands found during the LOES project

Compound	Surface water(ng/l)	SPM (μg/kg dw)	Bream (μg/kg dw)
ΣBDE[a]	–	0.9–18	4.6–39
DecaBDE	–	9–4,600	0.03–<21
Nonylphenol + ethoxylates	0.7–3	60–4,400	680–2,000
Bisphenol-A	0.012–0.2	12–42	1.9–6

[a] Sum of BDEs 47, 85, 99, 100, 138, 153.

higher than the PBDE concentrations, again with the exception of decaBDE in the Western Scheldt.

4. Conclusions

SPM was found to be an important carrier for higher brominated diphenylethers in the aquatic environment. High decaBDE concentrations (up to 4600 μg/kg dw) were found in SPM from the Western Scheldt, most likely related to spills during the use of decaBDE in the textile industries along the river Scheldt in Antwerp and further upstream. A similar decaBDE pattern is found in the sediment of the Western Scheldt with maximum values up to 510 μg/kg dw. The presence of decaBDE in SPM and sediments calls for further studies on the stability or possible degradation of this compound. DecaBDE was not found in flounder, bream, freshwater and marine mussels, which means that if it would bioconcentrate in these biota, it would only be at marginal levels.

The BDE47 and 99 concentrations in SPM, sediments and biota from various riverine locations such as in the rivers Rhine and Meuse, indicate a relation with the—possibly former—industrial use of pentaBDE, including a possible use in German mining.

Relatively high PBDE concentrations (up to 920 μg decaBDE/kg dw) were found in STP influents and effluents. The removal of decaBDE and other PBDEs by STPs is apparently only partially effective. A more detailed study is required to establish a mass balance for STPs, taking into account that the major part of organic material from the influent is removed through the sewage sludge.

In further studies, the selection of PBDEs should be extended with the congeners 100, 154, 183 and possibly also 28, whereas the congeners 85 and 138 are of little value.

The levels of PBDEs in the Dutch aquatic environment are, apart from decaBDE, generally lower than other contaminants such as PCBs and nonylphenol and ethoxylates.

PBBs were not found in measurable concentrations. Although the toxicity of PBBs may be different from that of PBDEs, it is unlikely that PBBs will be very important as a contaminant in the Dutch aquatic environment.

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Levels and trends of polybrominated diphenylethers and other brominated flame retardants in wildlife

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Abstract

In this paper, we review the available data for polybrominated diphenylethers (PBDEs) and other flame retardants in wildlife, with the exception of fishes from Europe and North America which are covered in more detail elsewhere. More data are available for PBDEs than for other compounds, and these show that some of these compounds have become widely distributed in the environment, [redacted] Most available data relate to birds and their eggs and marine mammals, but the results of two food web studies are also included. The detection of PBDEs in pelagic marine mammals which feed in deep offshore waters, including baleen whales, indicate that these compounds have found their way into deep-water, oceanic food webs as well as the coastal/shallow sea examples described in detail. In the North Sea study, the most marked increase in lipid-normalised concentrations of six BDE congeners occurred during transfer from predatory fish to marine mammals. In the St. Lawrence Estuary study, marked differences in the ratios observed between species suggested that some fish species may be able to metabolise BDE99.

[redacted]

Keywords: Flame retardants; Polybrominated diphenylethers; PBDEs; Wildlife; Temporal trends

1. Introduction

Bromine-based flame retardant formulations are applied annually to over 2.5 million tons of polymers, and approximately 70 brominated flame retardant chemicals account for a global consumption of over 300,000 tonnes per annum (Arias, 2001). Further details of the products, their applications, patterns of use and environmental sources are given elsewhere in this volume (Alaee et al., 2003). In environmental studies, the major emphasis to date has been on the polybrominated diphenylethers (PBDEs), and in particular those congeners which derive from the penta-mix formulation. [redacted]

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PBDE formulations within the European Union, which concluded that one (the penta-mix) should be banned, and that a further test programme should be undertaken to provide additional data for the octa-mix and deca-mix (predominantly decabromodiphenyl ether, BDE209) (Dungey, 2001). Subsequently, EU technical experts have recommended that precautionary restrictions on the marketing and use of these formulations are also needed in order to protect the environment. This action was recommended because of evidence of bioaccumulation in wildlife, and uncertainty over their potential to degrade to form persistent and bioaccumulative breakdown products (Environmental Data Services (ENDS), 2002). Attention in Europe, therefore, is now switching to those compounds which are still widely used, and in particular tetrabromo bisphenol-A (TBBP-A) and hexabromocyclododecane (HBCD).

In this paper, we review the available data for wildlife, with the exception of fishes from Europe and North America, which are covered in more detail elsewhere (Boon et al., 2002a; Hale et al., 2003).

2. Polybrominated diphenylethers

A considerable number of studies of PBDEs in wildlife have been undertaken since the mid-1980s, when Jansson et al. (1987) first indicated that these compounds were present in samples collected remote from local sources and so may have become ubiquitous environmental contaminants.

2.1. PBDEs in birds

2.1.1. Cormorants from England and Wales

Cormorants (*Phalocrocorax carbo*) are piscivorous birds which are common on European coasts and are also becoming increasingly common at inland sites. They exploit a range of fish species according to locality and season, often concentrating on locally dominant species. Liver samples from birds shot under license during the 1996–1997 and 1999–2000 winter periods (October to March) were collected within a study investigating the impact of cormorants on commercial aquaculture and recreational fisheries. Subsequent analysis of feathers from these birds has shown that they were feeding almost entirely on freshwater prey at the time of shooting (Bearhop et al., 1999), although this does not preclude migration to and from marine areas at other times. The liver samples were analysed for a suite of 14 tri- to heptabromodiphenylether congeners (Allchin et al., 2000; Law et al., 2002). Σ14BDE concentrations ranged from 1.8 to 140 μg kg^{-1} wet weight, and BDE47 contributed 24–100% of the BDEs present. The highest concentration was found in an immature female bird shot in Hampshire. Few other data are available for comparison. One cormorant sampled in the Rhine delta in the Netherlands in 1981 yielded liver concentrations of 25,000 and 4000 μg kg^{-1} wet weight for BDE47 and BDE99, respectively, both more than two orders of magnitude higher than the highest values for these congeners observed in the UK study (de Boer, 1990).

2.1.2. Birds and bird's eggs from Europe

Although comparatively few data are available, it seems that concentrations of PBDEs are relatively low in muscle tissue of birds from terrestrial ecosystems (e.g. starlings; Σ3BDE concentrations 5.7–13 μg kg^{-1} lipid weight), as is the case for herbivorous mammals (reindeer and moose) (Σ3BDE concentrations 0.5–1.7 μg kg^{-1} lipid weight) all from Sweden (Sellström, 1999). However, PBDE concentrations in eggs of peregrine falcons (*Falco peregrinus*) from northern and southern Sweden are high (Sellström et al., 2001). In this study, wild falcons were found to have higher concentrations of PBDEs than captive birds fed on chickens. BDE209 was found in 18 of 21 eggs analysed (concentration range 28–430 μg kg^{-1} lipid weight). BDE183 (often taken as indicative of the presence of the octa-mix) was found in all 52 eggs, with much higher concentrations in the two wild populations (range 56–1300 μg kg^{-1} lipid weight) than in the captive falcons (range 6–19 μg kg^{-1} lipid weight). The concentrations of other prominent BDE congeners in the wild populations were: 15–3800 μg kg^{-1} lipid weight for BDE47; 110–9200 μg kg^{-1} lipid weight for BDE99; 77–5200 μg kg^{-1} lipid weight for BDE100; 270–16000 μg kg^{-1} lipid weight for BDE153 and 50–4400 μg kg^{-1} lipid weight for BDE154. The pattern of BDE congeners observed in peregrine falcon eggs is different to that seen in other biota samples. Compared to eggs of guillemots (*Uria aalgae*) from the Baltic Sea (Sellström, 1999), the falcon eggs contained several of the more highly brominated congeners. Dominant congeners in falcon eggs were BDE153 and BDE99, as compared to BDE47 in guillemot eggs and other marine biota. Concentrations of the five dominant congeners listed above were also much higher in falcon eggs than in guillemot eggs. Similar results were reported by Herzke et al. (2001), with eggs from peregrine falcons, merlins (*Falco columbarius*) and gyrfalcons (*Falco rusticolus*) from Norway showing a higher proportion of BDE99 and BDE153 than of BDE47. Eggs from sparrowhawk (*Accipiter nisus*), goshawk (*Accipiter gentilis*), white-tailed sea eagle (*Haliaeetus albicilla*) and osprey (*Pandion haliaetus*) showed a predominance of BDE47. Summed mean BDE concentrations ranged from 18 to 732 μg kg^{-1} wet weight (in the gyrfalcon and sparrowhawk, respectively). Blood plasma samples taken from peregrine falcons, goshawks, sparrowhawks and white-tailed sea eagles collected from Germany in 1998–1999 have also been analysed for a range of BDE congeners (BDE47, BDE66, BDE85, BDE99, BDE100, BDE153 and BDE154) (Lepom, unpublished data). With the exception of BDE66 and BDE85, all congeners were found at detectable concentrations in all samples. Summed BDE concentrations ranged from 1 to 400 μg l^{-1}, with higher concentrations (factors of 10–100 times) in adults

than nestlings and with the maximum concentrations being found in the sparrowhawk. Again, peregrine falcons showed BDE153 as the dominant congener, whilst for goshawk and sparrowhawk it was BDE47.

Initially, these results seem to indicate the influence of habitat and food specialisation on the BDE congener patterns in birds of prey, perhaps suggesting that birds feeding in terrestrial environments and on other birds may be more highly exposed to the higher brominated BDE congeners than marine species. However, the differences in BDE congener patterns observed between *Accipiter* (*A. nisus*, *A. gentiles*) and *Falco* (*F. rusticolus*, *F. columbarius*, *F. peregrinus*) species, all feeding on small- and medium-sized birds, cannot be adequately explained in this way. In spite of similar feeding habits, the differences in BDE congener pattern between, e.g. peregrine falcon and sparrowhawk, are much more pronounced than those observed between sparrowhawk and fish-eating species (see Fig. 1).

Herzke et al. (2003) determined concentrations of two PBDE congeners in liver and intestinal contents of glaucous

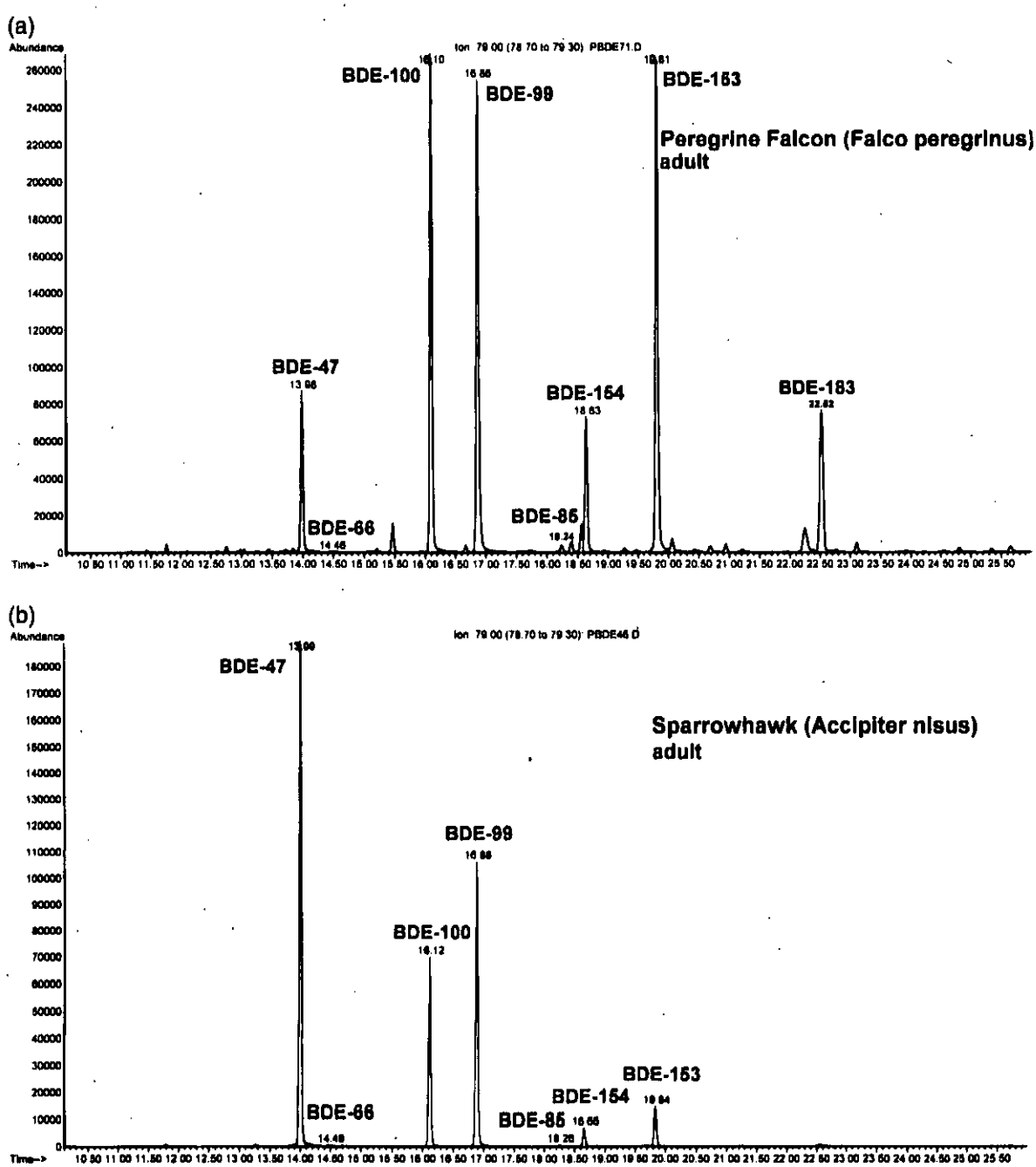

Fig. 1. Mass chromatograms (m/z = 79 Da) showing BDE congener patterns in (a) peregrine falcon and (b) sparrowhawk from Germany.

gulls (*Larus hyperboreus*) collected from Bear Island in the Arctic Ocean in 1999. Bear Island is an important breeding site for these seabirds inhabiting the Barents Sea. The glaucous gull is a generalist feeder, belonging to the scavenger–predatory species. Important components of their diet include eggs and chicks of other seabirds, fish, crabs, and amphipods, but they also eat seal blubber and garbage. BDE47 was found in the livers at concentrations between 0.5 and 22 μg kg^{-1} wet weight, and BDE99 from not detected to 7.9 μg kg^{-1} wet weight. The intestinal contents were more highly contaminated than the liver samples, and qualitative GC/MS analysis of these indicated the presence of BDE100, BDE153, BDE190 as well as several unidentified congeners. A number of other flame retardant compounds, including five polybrominated biphenyl congeners and HBCD, were not detected in any samples.

2.1.3. Bird's eggs from North America

The herring gull (*Larus argentatus*) is a year-round resident species in the lower Great Lakes area, and its diet consists of alewife (*Alosa pseudoharengus*) and rainbow smelt (*Osmerus mordax*), which are abundant prey fish in the Great Lakes. Consequently, this species is ideal for biomonitoring of persistent organic contaminants. Since the mid 1970s, as part of their monitoring programme, Canadian Wildlife Services has been systematically collecting herring gull eggs from a number of colonies in the Great Lakes region on an annual basis and archiving them. In this study, pooled homogenates of herring gull eggs from various locations in the Great Lakes were analysed to determine the spatial and temporal distribution of PBDEs. A detailed description of this study including materials and methods used is provided in Norstrom et al. (2002) and Moisey et al. (2001).

PBDEs (tetra- to hexa-BDEs analysed as isomer groups) were detected in samples from 15 locations throughout the Great Lakes region, with concentrations ranging between 16,500 μg kg^{-1} lipid weight for Big Sister Island in Lake Michigan and 1830 μg kg^{-1} lipid weight for Port Coulbourne in Lake Erie. The concentrations of PBDEs were highest in Lake Michigan, followed by Lakes Ontario, Superior, Huron, and Erie. Similar results have been reported in lake trout from Lake Ontario, Superior, Huron and Erie (Luross et al., 2002). The BDE congener distribution found in herring gull eggs is similar to that seen in other aquatic biological matrices. BDE47 is the major congener followed by BDE99, BDE100, BDE153, BDE154, BDE28 and BDE183, respectively. These congeners represent over 96% of the total BDEs found in these samples.

2.2. PBDEs in a North Sea food web

In a study of accumulation in North Sea food webs (Boon et al., 2002a), the concentrations of individual BDE congeners were determined in the invertebrate species whelk (*Buccinum undatum*), sea star (*Asterias rubens*), and hermit crab (*Pagurus bernhardus*), the fish species herring (*Clupea harengus*), whiting (*Merlangius merlangus*) and cod (*Gadus morhua*), and the marine mammal species harbour seal (*Phoca vitulina*) and harbour porpoise (*Phocoena phocoena*). These species are all important representatives of different trophic levels of the North Sea food web. All six major BDE congeners detected (BDE28, BDE47, BDE99, BDE100, BDE153 and BDE154) are most prevalent in the commercial penta-BDE formulation. The concentrations of BDE47, for example, ranged from 2.6 to 118 μg kg^{-1} lipid weight in the invertebrates; from 7.6 to 307 μg kg^{-1} lipid weight in fish liver; and from 57 to 9250 μg kg^{-1} lipid weight in harbour seal blubber. No evidence was found for the occurrence of the octa-BDE formulation in the North Sea food web, as its dominant congener, BDE183, was not detected in any samples. BDE209, the major congener (>97%) of the deca-BDE formulation, could not be unambiguously determined in any biota samples. Since BDE209 is often the main BDE congener found in sediments from the area, the main reason for the low concentrations observed in biota from the North Sea seems to be its relatively low bioaccumulation potential. This may be due either to a low uptake rate for this very large molecule (BDE209 has a high log K_{ow} of 9–10, cf. 6.2 for BDE47, and so will be predominantly particle-bound) or a relatively rapid excretion after biotransformation. Laboratory studies have demonstrated that when the commercial octa-mix formulation (which contains BDE209) is offered to Atlantic salmon in an oil capsule placed in the stomach, BDE209 does enter the liver (Boon et al., 2002b). To enter the liver, BDE209 had to cross the intestinal biomembrane. In contrast to lower brominated congeners, BDE209 was transported less efficiently to fillet (muscle and skin) and brain.

Since all invertebrates investigated are sentinel species, they are highly representative for the area of capture. The highest lipid-normalised concentrations of BDEs in the invertebrates occurred near the mouth of the River Tees in NE England (546 μg kg^{-1} lipid weight in sea stars for BDE47). The geographical distribution of the BDEs could be explained by transport following the residual currents in the area. The direction of these currents differs between the summer and the winter season as a result of the presence or absence of vertical summer stratification in the deeper waters to the north of the Dogger Bank. Summer stratification results in the development of a density-driven bottom water current formed after the onset of vertical stratification of the water column in May, which leaves the UK coast near Flamborough Head and moves towards the Dogger Bank. In winter, the residual currents run in a more southerly direction and follow the UK coastline towards the southern bight of the North Sea. The distribution pattern of the PCBs and *p,p'*-DDE in the invertebrates was entirely different from that of the BDEs, as was also the case for CBs and BDEs in cormorants and porpoises from the UK (Law et al., 2002). This reflects the differences in production and use of these materials, as the use of these organochlorine compounds in

Western Europe peaked during the 1960s and 1970s, followed by their manufacture being prohibited more than 20 years ago. In contrast, the major production and use of the penta-BDE formulation has occurred more recently.

The higher trophic levels of the North Sea food web were represented by the predatory gadoid fish species whiting and cod, and the marine mammal species harbour seal and harbour porpoise. The lipid-normalised levels of the six major BDE congeners in fish were similar to the levels found in the invertebrates, but a biomagnification step in concentrations of generally more than an order of magnitude occurred from gadoid fish to marine mammals, marking the transition from gill-breathing to lung-breathing animals. Mean concentrations of, for example, BDE47 in blubber of harbour porpoise and harbour seal were 1331 and 1328 $\mu g/kg^{-1}$ lipid weight, respectively; whilst in liver of herring, cod and whiting the mean concentrations were 30, 133 and 70 g kg^{-1} lipid weight, respectively. In the invertebrates sampled (sea star, hermit crab, whelk and shrimp) mean BDE47 concentrations were 22, 38, 10 and 37 $\mu g/kg^{-1}$ lipid weight, respectively. Based on the limited number of samples available, no differences could be observed between harbour seal and harbour porpoise.

The results observed in three species of sentinel invertebrates from a network of stations covering a major part of the North Sea basin showed the estuary of the River Tees to be a major source for tri- to hexa-BDEs. The Tees had also been identified as a major source for PBDEs during an earlier study, with a manufacturing plant upstream and high concentrations being observed in fish and sediments from the estuary and Tees Bay (Allchin et al., 1999).

2.3. PBDEs in the St. Lawrence Estuary food web

Located downstream of the Great Lakes–St. Lawrence River, the St. Lawrence Estuary receives the waters from one of the most industrialised regions of the world. As a result, the marine organisms of the St. Lawrence Estuary food web are directly exposed to the contaminants transported by these fluvial inputs. A bioaccumulation study of PBDEs was performed using a variety of marine organisms from the St. Lawrence Estuary food web, which includes two marine mammal species (beluga whales and harbour seals) that are permanent residents of St. Lawrence Estuary (Lebeuf et al., in preparation (a)). All organisms were collected during the 1999–2000 time period, allowing the comparison of BDE concentrations in different organisms with minimal bias due to the temporal changes of BDE concentrations in organisms (Lebeuf et al., 2001, in preparation (b); Lebeuf and Trottier, 2001; Luross et al., 2001, 2002). Concentrations of individual BDE congeners and of the summed congeners (expressed as Σ10BDE; the sum of BDE28, BDE47, BDE49, BDE66, BDE99, BDE100, BDE153, BDE154, BDE155 and BDE183) were determined in 13 biological species including benthic and pelagic invertebrates, pelagic and demersal fish, and marine mammals (Table 1).

The levels of Σ10BDE in the examined species varied from approximately 0.2 to more than 500 $\mu g\ kg^{-1}$ wet weight, a range exceeding three orders of magnitude. The lowest concentrations were found in invertebrates, whereas the highest concentrations were found in the blubber of marine mammals. The concentrations of Σ10BDE in the examined fish species (mainly liver or lipid-rich muscle) varied within about one order of magnitude. The mean concentrations of Σ10BDE were not statistically different between male harbour seals and both male and female beluga whales. These results differ from the generally higher levels of contamination (e.g. PCBs) found in male beluga whales compared to male harbour seals (Bernt et al., 1999).

Marked differences in the ratios of BDE47 to BDE99 were observed among the examined biological species of

Table 1
Mean concentrations ($\mu g\ kg^{-1}$ wet weight) of selected BDE congeners and the sum of those determined in marine organisms collected during the 1999–2000 time period from the St. Lawrence Estuary, Canada[a]

Species	Common name	Tissue	n	Sample	Lipid (%)	BDE47	BDE99	BDE100	Σ10BDE
Maldane sarsi	Maldane worm	whole	3	pool	1.0	0.31	0.38	0.12	0.93
Nereis virens	Nereis worm	whole	2	pool	0.8	0.18	0.19	0.10	0.47
Pandalus borealis	Shrimp	muscle	3	pool	0.9	0.17	0.02	0.03	0.24
Calanus sp.	Zooplankton	whole	5	pool	16	0.66	0.46	0.13	1.5
Osmerus mordax	Rainbow smelt	liver	4	pool	5.4	22	1.0	5.8	34
Clupea harengus harengus	Atlantic herring	liver	3	pool	3.4	7.2	1.6	1.2	13
Microgadus tomcod	Atlantic tomcod	liver	6	pool	21	121	77	36	274
Anguilla rostrara	American eel	muscle	5	individual	24	65	1.7	15	101
Hippoglossoides platessoides	American plaice	liver	3	individual	7.7	10	0.3	1.9	17
Pleuronectes putmani	Smooth flounder	liver	3	pool	5.5	30	0.4	5.8	41
Reinhardtius hippoglossoides	Greenland halibut	muscle	3	individual	7.3	6.4	0.8	2.9	13
Reinhardtius hippoglossoides	Greenland halibut	liver	2	individual	19	8.2	0.9	1.0	12
Phoca vitulina	Harbour seal (M)	blubber	4	individual	92	445	121	54	709
Delphinapterus leucas	Beluga whale (F)	blubber	8	individual	88	231	91	52	471
Delphinapterus leucas	Beluga whale (M)	blubber	7	individual	92	258	100	59	493

Σ10BDE: the sum of BDE28, BDE47, BDE49, BDE66, BDE99, BDE100, BDE153, BDE154, BDE155 and BDE183.

[a] Lebeuf et al., in preparation (a).

the St. Lawrence Estuary food web. BDE47/99 ratios in some fish species such as rainbow smelts, American eels, American plaice and smooth flounders were much higher than in other organisms, whereas the BDE47/100 ratios were similar among all the examined biological species. The depletion in BDE99 concentrations relative to BDE47 in some fish samples suggests that some marine organisms, but not the marine mammal species studied, possess the capacity to eliminate this specific congener. These differences in BDE47/99 ratios among marine organisms of the St. Lawrence food web also provide some basic information on the prey–predator relationships. For instance, the relatively low BDE47/99 ratios in the examined marine mammals of the St. Lawrence Estuary indicates that fish species such as rainbow smelts, American eels, American plaices and smooth flounders form only a minor part of their diet.

2.4. PBDEs in marine mammals

A suite of 14 tri- to heptabromodiphenyl ether congeners were determined in 60 harbour porpoises (*P. phocoena*) stranded or bycaught around England and Wales during the period 1996–2000 (Law et al., 2002). Concentrations of the summed congeners (Σ14BDE) in blubber ranged from not detected (in a single individual) to 6900 μg kg^{-1} wet weight (7670 μg kg^{-1} on a lipid basis). The major congeners present were generally BDE47, BDE99 and BDE100, as found for fish in other studies (Kierkegaard et al., 1999). BDE47 contributed 39–88% of the total, with no apparent relation to the total burden. Concentrations of both BDE47 and Σ14BDE in a porpoise foetus were approximately 60% of those seen in its mother, indicating transplacental transfer of these compounds. Further transfer from mother to offspring would also be expected to occur during suckling, and, other factors such as location relative to inputs being equal, we would expect to see lower concentrations of BDEs in reproducing female porpoises than in males of similar age. A possible relationship between summed concentrations of chlorobiphenyls and BDEs was investigated, but no correlation was observed (r^2 value for linear regression 0.003). A single porpoise stranded in the Netherlands in 1990 had concentrations of 830 and 79 μg kg^{-1} wet weight, respectively, for BDE47 and BDE99 (de Boer and Dao, 1993). Five male porpoises from the coast of British Columbia, Canada, yielded blubber ΣBDE concentrations of 300 to 2300 μg kg^{-1} expressed on a lipid basis (Ikonomou et al., 2000).

Σ13BDE concentrations were also determined in the blubber of other cetacean species, including pelagic species feeding in deep offshore waters (Law et al., 2002). These ranged from 38 μg kg^{-1} wet weight (61 μg kg^{-1} on a lipid basis) in a fin whale (a baleen whale: *Balaenoptera physalus*) to 9410 μg kg^{-1} wet weight (12,700 μg kg^{-1} on a lipid basis) in a white-beaked dolphin (*Lagenorhyncus albirostris*). BDEs were detected and quantified in all the species studied, and Table 2 lists the concentrations of the four major congeners found (BDE47, BDE99, BDE100 and BDE153) and the sums of the 13 congeners determined for each sample. Similarly high concentrations to those seen in the white-beaked dolphins analysed in this study have also been reported for another single white-beaked dolphin stranded on the Dutch coast of the North Sea, in which the concentrations of BDE47 and Σ3BDE (congeners BDE47, 99 and 100) were 5500 and 7700 μg kg^{-1} wet weight, respectively (de Boer et al., 1998).

Summed concentrations of three BDE congeners (Σ3BDE; BDE congeners 47, 99 and 100) determined by de Boer et al. (1998) in the blubber of three sperm whales (*Physeter macrocephalus*) stranded on the coast of the Netherlands ranged from 78 to 136 μg kg^{-1} wet weight, and they also found 122 μg kg^{-1} in the blubber of a minke whale (*Balaenoptera acutorostrata*). Three other sperm whales stranded around the North Sea showed concentrations of a similar order (Table 3).

Σ19BDE concentrations in blubber of male and female long-finned pilot whales (*Globicephala melas*) from the Faroe Islands ranged from 690 to 2400 μg kg^{-1} wet weight (840 to 3160 μg kg^{-1} on a lipid basis) (Lindström et al., 1999). Higher concentrations were found in young specimens of both sexes than in adults, which the authors ascribed to lactational transfer of BDEs during the suckling of young. Table 3 includes data for three small cetaceans

Table 2
Concentrations of selected BDE congeners and the sum of those determined in cetaceans stranded around England and Wales (μg kg^{-1} wet weight)[a]

Species		Lipid (%)	BDE47	BDE99	BDE100	BDE153	Σ13BDE
White-sided dolphin	*Lagenorhyncus acutus*	77	33	21	13	14	192
White-beaked dolphin	*Lagenorhyncus albirostris*	46	2480	622	539	40	3790
White-beaked dolphin	*Lagenorhyncus albirostris*	74	5780	1480	1930	113	9410
Striped dolphin	*Stenella coeruleoalba*	39	162	77	53	22	450
Common dolphin	*Delphinus delphis*	71	121	99	38	25	353
Risso's dolphin	*Grampus griseus*	81	631	393	176	31	1400
Long-finned pilot whale	*Globicephala melas*	46	163	51	20	9.0	319
Fin whale	*Balaenoptera physalus*	62	13	12	5.0	nd[b]	38
Minke whale	*Balaenoptera acutorostrata*	26	47	13	5.0	nd	99
Sowerby's beaked whale	*Mesoplodon bidens*	56	62	27	nd	nd	172

[a] CEFAS (unpublished data).
[b] nd: not detected.

Table 3
Concentrations of selected BDE congeners in blubber of marine mammals (μg kg^{-1} wet weight)[a]

Species		Location	Year	Lipid (%)	BDE47	BDE99	BDE100	BDE153	Σ14BDE
Common seal	*Phoca vitulina*	Eastern England	1991	67	560	329	65	36	990
Common seal	*Phoca vitulina*	Eastern England	1991	47	822	115	51	68	1070
Common seal	*Phoca vitulina*	NE England	1994	82	1780	101	93	18	2020
Grey seal	*Halichoerus grypus*	NE England	1990	74	366	26	12	5.9	415
Grey seal	*Halichoerus grypus*	NE England	1990	89	488	27	10	nd[b]	525
Grey seal	*Halichoerus grypus*	SW England	1991	70	422	71	16	8.7	527
Grey seal	*Halichoerus grypus*	Wales	1992	82	524	169	94	92	1080
Grey seal	*Halichoerus grypus*	Wales	1994	82	291	nd	22	16	343
Grey seal	*Halichoerus grypus*	Wales	1995	8	126	10	7.9	27	182
Grey seal	*Halichoerus grypus*	Wales	1997	30	138	11	11	6.9	176
Caspian seal	*Phoca caspica*	Azerbaijan	1997	77	11	nd	nd	nd	11
Caspian seal	*Phoca caspica*	Azerbaijan	1997	74	15	nd	nd	nd	15
Caspian seal	*Phoca caspica*	Azerbaijan	1997	76	nd	nd	nd	nd	nd
Caspian seal	*Phoca caspica*	Azerbaijan	1997	65	nd	nd	nd	nd	nd
Bottlenose dolphin	*Tursiops truncatus*	Australia	1995	61	20	nd	nd	nd	49
Bottlenose dolphin	*Tursiops truncatus*	Australia	1996	80	152	nd	15	nd	167
Melon-headed whale	*Peponocephala electra*	Australia	1996	16	20	4.8	5.7	nd	36
Sperm whale	*Physeter macrocephalus*	Orkney Islands	1994	64	67	nd	nd	nd	67
Sperm whale	*Physeter macrocephalus*	Netherlands	1995	55	263	nd	nd	nd	263
Sperm whale	*Physeter macrocephalus*	Netherlands	1995	47	35	nd	nd	nd	35

[a] CEFAS (unpublished data).
[b] nd: not detected.

(two bottlenose dolphins *Tursiops truncatus* and a melon-headed whale *Peponocephala electra*) from Queensland, Australia, sampled during 1995–1996. All show low concentrations of BDEs, with Σ14BDE concentrations ranging from 36 to 167 μg kg^{-1} wet weight.

The blubber of 11 stranded harbour seals (*P. vitulina*) from San Francisco Bay collected during 1989–1998 yielded Σ3BDE (BDE47, BDE99 and BDE153) concentrations ranging from 66 to 1990 μg kg^{-1} wet weight (67–7140 μg kg^{-1} on a lipid basis) (She et al., 2000). In this study, BDE47 contributed 62–94% of the summed concentration of the three congeners. Comparative data are available for blubber of harbour seals and grey seals (*Halichoerus grypus*) from England and Wales (Table 3). Three harbour seals sampled during 1991–1994 yielded BDE47 and Σ14BDE concentrations of 560–2780 and 990–2020 μg kg^{-1} wet weight, respectively, whilst seven grey seals sampled during 1990–1997 showed ranges of 126–524 and 176–1080 μg kg^{-1} wet weight, respectively, for the same parameters. In contrast, only BDE47 was detected in two of four Caspian seals from Azerbaijan sampled in 1997, the maximum concentration being 15 μg kg^{-1} wet weight (Table 3).

2.5. PBDEs in frogs

In order to compare the environmental fate of PBDEs and PCBs, common frogs (*Rana temporaria*) were collected along a ~1500-km-long latitudinal transect of the Scandinavian Peninsula and their livers sampled and analysed (Ter Schure et al., 2002). BDE47 was detected in >90% of the individuals, and BDE99 in <50%. Concentrations of BDE47 ranged from 123 ± 100 ng kg^{-1} wet weight ($n=24$) at Lund, the most southerly sampling site, to 26 ± 10 ng kg^{-1} wet weight ($n=27$) at Kiruna, the most northerly sampling site and inside the Arctic circle. The liver concentrations of both BDEs and CBs were negative functions of latitude, and this trend was similar to that demonstrated earlier for freshwater fish, which indicated that those in the south (i.e., closer to the source area) were more contaminated with BDEs than those from the north (Sellström, 1996). The concentrations of BDE47 and total PCB (the sum of CB52, CB153, CB183, CB201 and CB206) were correlated ($r^2=0.48$, $n=177$) and the authors concluded that the environmental fate of these two classes of compounds is analogous. This close correlation is, however, in marked contrast to the essentially non-existent relationship seen in porpoises from the UK and described above (Law et al., 2002), and more comparative data are needed for other species and locations before the parallels can be fully elucidated and explained. Given current concerns over the reported global declines in frog populations, Ter Schure et al. recommended further study of the distribution and toxic effects of PBDEs in amphibians.

2.6. PBDEs in mussels

A recent study of PBDEs in the aquatic environment in the Netherlands included the determination of six BDE congeners (BDE47, BDE85, BDE99, BDE138, BDE153 and BDE209) in 16 samples of marine and freshwater mussels from 16 different locations (de Boer et al., 2003). Only BDE47 and BDE99 could be detected in both sets of samples, at concentrations ranging from 0.7 to 17 and 0.3 to 11 μg kg^{-1} dry weight, respectively. Concentrations of BDE153 ranged from <0.1 to 1.5 μg kg^{-1} dry weight in the fresh-

water mussels. There are few other data with which to compare these, but Allchin et al. (1999) reported concentrations for three BDE congeners in a single sample of mussels from the Wash, eastern England, whose rivers drain mainly agricultural land. The concentrations found were: BDE47, 3.5 μg kg^{-1} wet weight; BDE85, 2.0 μg kg^{-1} wet weight; and BDE99, 3.9 μg kg^{-1} wet weight (these represent concentrations of 194, 111 and 217 μg kg^{-1}, respectively, on a lipid basis). A sample of periwinkles (*Littorina littorea*) from the River Tweed (a relatively clean river in NE England, with no known sources of PBDEs and used as the study reference site) yielded concentrations for the same three BDE congeners of 1.9, 1.5 and 1.8 μg kg^{-1} wet weight, respectively (73, 58 and 69 μg kg^{-1} on a lipid basis).

3. Other flame retardants

3.1. Birds

Polybrominated biphenyls have not been analysed as frequently as PBDEs, although they were determined in the study of Norwegian birds of prey conducted by Herzke et al. (2001). Summed PBB concentrations in eggs of white-tailed sea eagle, peregrine falcon and goshawk (mean 40 μg kg^{-1} wet weight) were up to an order of magnitude lower than those for PBDE, whilst only a few eggs of golden eagle (*Aquila chrysaetos*), eagle owl (*Bubo bubo*), gyrfalcon and merlin showed measurable concentrations of PBB (maximum 20 μg kg^{-1} wet weight). Tetra- and hexabrominated congeners dominated the PBB profiles. They were not detected in any of the samples from the initial UK survey of flame retardant compounds (Allchin et al., 1999). PBBs were imported into Europe from the USA, but European production of PBBs was limited to the manufacture of BB209 in France, and this has now ceased (Law and Allchin, 2001; de Boer et al., 2003).

HBCD was found in guillemot eggs from the Baltic Proper at a mean concentration of 124 μg kg^{-1} lipid weight, but with no clear time trend (Lundstedt-Enkel et al., 2001). HBCD and TBBP-A are currently being determined in a range of biota samples (including molluscs, crab, fish, cormorant, harbour seal and harbour porpoise) from estuaries and marine waters around the North Sea, and these data will be reported elsewhere (Morris et al., in preparation).

4. Time trends in concentration

4.1. PBDEs in beluga whales from the St. Lawrence Estuary

A geographically distinct population of beluga whales (*Delphinapterus leucas*) inhabits the St. Lawrence Estuary, Canada. During the last century, this isolated beluga population declined to about 80–90% of its estimated size of 5000 animals in the early 1900s, in part due to hunting pressure. Although the St. Lawrence Estuary belugas were given the status of endangered species from the Canadian government in 1980 and hunting was prohibited, the population has failed to recover. Previous studies on organochlorine contaminants in St. Lawrence beluga adipose tissues reported elevated levels of PCBs and DDT-related compounds, which were generally 20–30-fold higher than in other beluga populations from the Canadian Arctic and sub-Arctic waters (Muir et al., 1990, 1996; Béland et al., 1993).

A retrospective temporal trend study of polybrominated diphenyl ethers (PBDEs) was performed using samples of stranded beluga whales collected between 1988 and 1999 on the shore of the St. Lawrence Estuary (Lebeuf et al., 2001, in preparation (b); Lebeuf and Trottier, 2001). PBDE concentrations (expressed as Σ10BDE; the sum of BDE28, BDE47, BDE49, BDE66, BDE99, BDE100, BDE153, BDE154, BDE155 and BDE183) were determined in blubber samples of 26 female and 28 male beluga whales aged at least 9 years. Three time periods, defined as 1988–1990, 1992–1995 and 1997–1999, were considered for statistical analysis and data from animals collected within these time periods were grouped accordingly (Table 4). The results show a significant increase of Σ10BDE levels in both female and male beluga whales during the 1990s. No statistical differences were observed in either lipid content or age of belugas for each time period, within sexes. The mean concentrations of Σ10BDE were not significantly different between males and females for both 1992–1995 and 1997–1999 time periods but were lower in females in 1988–1990. The same results were obtained with or without lipid normalisation of the BDE data. This lack of difference in PBDE concentrations between sexes was taken to indicate the very recent increase in the concentrations of these compounds in the animals. Between the 1992–1995 and 1997–1999 time periods (i.e. 5 years), the average BDE concentrations have more than doubled in the blubber of belugas. Apparently, the females did not exhibit lower

Table 4
Mean concentrations (μg kg^{-1} wet weight) of selected BDE congeners and the sum of those determined in blubber of female and male beluga whales found stranded over selected time periods on the shore of the St. Lawrence Estuary, Canada[a]

	n	Lipid (%)	Age (years)	BDE47	BDE99	BDE100	Σ10BDE
Male							
1988–1990	6	86	22	16	7	3	45
1992–1995	7	94	22	79	29	17	174
1997–1999	15	87	18	204	71	45	377
Female							
1988–1990	6	89	20	11	5	2	26
1992–1995	6	94	22	87	32	18	172
1997–1999	14	92	21	253	108	60	496

Σ10BDE: the sum of BDE28, BDE47, BDE49, BDE66, BDE99, BDE100, BDE153, BDE154, BDE155 and BDE183.

[a] Lebeuf et al., in preparation (b).

blubber concentrations due to the transfer of a portion of their PBDE burden to their offspring. This is probably due to the relatively recent and fast increase in inputs of these compounds to the St. Lawrence Estuary, such that to date PBDEs have tended to be predominantly accumulated by both male and female belugas, rather than be eliminated.

Concentrations of BDEs in male beluga whales from the St. Lawrence Estuary collected in 1997–1999 were more that 25 times higher than in those collected in 1997 from the SE Baffin area and Cumberland Sound (Stern and Ikonomou, 2000a). The concentrations of BDEs in beluga whales from the St. Lawrence Estuary were also four and seven times higher than those observed in male and female beluga whales from the Canadian Arctic, respectively (Alaee et al., 1999). BDE47 represented on average about 50% of Σ10BDE, whereas the sum of BDE47, BDE99, BDE100, BDE154, and BDE153 represented more than 85% of Σ10BDE. This pattern, characterised by a strong dominance of only a few congeners (particularly BDE47) is typical for marine mammals (de Wit, 2002).

4.2. Marine mammals in the Canadian Arctic

Marine mammals are exploited by many communities in the Canadian Arctic. These animals occupy high trophic levels in marine food webs and so accumulate (relatively) high concentrations of persistent organohalogens (AMAP Assessment Report, 1998; CACAR, 1997).

4.2.1. Beluga whales

Temporal trends of PBDEs over the 15-year period from 1982 to 1997 and over a 12-year period from 1989 to 2001, in eastern and western Canadian Arctic beluga, respectively, have been reported by Stern and Ikonomou (2000a,b, 2001). In the eastern Arctic, blubber tissues were collected from male beluga in the Clearwater Fjord area (1982 and 1986) and the southern coastline (1992 and 1997) of Cumberland Sound. Tissues from the western Arctic animals were collected from male Kugmallit Bay beluga (1995 and 2001) and from animals trapped in the Husky Lakes (1989). In the Cumberland Sound animals, BDE47 is the predominant congener, followed by BDE99, BDE100, and BDE154. As was observed for Holman ringed seal (Ikonomou et al., 2002), blubber tissues for Canadian Arctic beluga are depleted in the contribution of BDEs with a bromine content greater than four atoms when compared both to the Bromkal formulation and to biotic samples collected from the more highly industrialised mid-latitude regions. This result is consistent with the known physical–chemical characteristics and the persistence of BDEs (Harner, 2001; Tittlemeier and Tomy, 2001; Tittlemeier et al., in press; Wong et al., 2001) and with other halogenated aromatic contaminants such as PCBs (Breivik et al., 2002a,b). Mean concentrations of CB153 (2,2′,4,4′,5,5′-PCB) in the 2001 Kugmallit Bay and 1997 Cumberland Sound animals were 52- and 49-fold higher, respectively, than the BDE47 concentrations measured in the corresponding animals (Stern et al., in preparation).

Relationships between log [PBDE] and age for selected congeners and ΣPBDE were investigated for animals from the eastern and western Arctic. Significant negative correlations were observed in the Cumberland Sound samples collected from animals in 1992 and 1997 and in the 2001 samples from the Kugmallit Bay animals. In contrast, a strong positive correlation was generally observed for major organochlorine compound groups such as PCBs and toxaphene in the same animals for all collection periods (Stern et al., in preparation). This difference between PBDEs and OCs could reflect the fact that older animals have been exposed to PBDEs for only a portion of their lives. Recent PBDE accumulations in Arctic beluga, therefore, dominate over potential historical accumulation in the older animals. This observation is consistent with the increase of PBDE concentrations in beluga since the mid-1980s. An alternative explanation might be that beluga have a greater capacity to metabolise and eliminate these compounds compared to PCBs.

Means (standard deviation) and a summary of ANCOVA results used to assess the effects of year to year collections (temporal trends), age and age × year interactions (homogeneity of the slope between age and [PBDE]) on BDE congener and major homologue group concentrations in blubber from male eastern and western Arctic beluga are shown in Tables 5 and 6, respectively. All univariate analyses were performed with lipid-normalised $\log_{10}$ transformed data in order to adjust for skewness in the data. Differences between collection years were examined with paired comparisons of age-adjusted least squared mean concentrations (SAS Institute 1989–1996). Only results for animals older than 2 years of age were included in the analysis of covariance because of the large variations in concentrations seen in younger animals (Stern et al., 1994). Since 1982, the levels of the major PBDE homologue groups and BDE congeners in the Cumberland Sound animals have increased significantly. For example, concentrations of ΣPBDE and BDE47 increased by 6.8- and 6.5-fold, respectively, over this 15-year time period. These results almost certainly reflect the increase in production and industrial usage of PBDEs worldwide (de Boer et al., 2000; BSEF, 2000; Ikonomou et al., 2002). Between 1982 and 1997, the contributions of the tri- and tetra-BDE homologue groups to ΣPBDE have declined by 7% and 4%, respectively. Conversely, the penta- and hexa-BDE contributions have increased by 3% and 8%, respectively. This change in the Cumberland Sound beluga PBDE composition is most likely to have been driven by the shift in composition of commercial PBDE formulations to higher brominated mixtures (WHO, 1994; de Boer et al., 2000).

Over the 6-year period from 1989 to 1995, the levels of the penta- and hexa-BDE homologue groups and all major congeners in the western Arctic beluga, like the Cumberland sound animals, have increased significantly. Increases

Table 5
Means (standard deviation) and summary of ANCOVA results used to assess the effects of year to year collections (temporal trends), age and age × year interactions (homogeneity of the slope between age and [PBDE]) on PBDE congener and major homologue group concentrations in blubber from male Cumberland Sound beluga. All concentrations are in ng kg^{-1} wet weight[a]

	Mean (SD)				Age (Pr)	Age × year (Pr)	R^2 (model)	Age-adjusted mean		Factor
	1982	1986	1992	1997				1982	1997	
n	8	15	11	17	–	–	–	8	17	–
Age	9.7 (5.4)	5.5 (1.7)	8.4 (5.2)	14 (5.4)	–	–	–	–	–	–
Lipid (%)	90 (2.4)	91 (2.4)	86 (2.4)	90 (4.1)	–	–	–	–	–	–
BDE47	1450 (280)	2830 (2220)	4,220 (4930)	9700 (4260)	<0.0001	0.7414	0.70	1550	10,100	6.5 ↑
BDE49	91 (28)	107 (73)	153 (136)	420 (246)	<0.0001	0.9177	0.66	96	393	4.1 ↑
BDE99	89 (58)	346 (851)	451 (928)	726 (448)	0.0117	0.3714	0.64	79	812	10.3 ↑
BDE100	194 (38)	394 (339)	633 (662)	1550 (977)	<0.0001	0.5618	0.75	205	1620	7.9 ↑
BDE119	17 (12)	17 (7.2)	30 (21)	80 (44)	<0.0001	0.0084	0.77	–	–	–
BDE154	28 (5.6)	91 (79)	272 (423)	9132 (881)	<0.0001	0.3641	0.84	29	886	30.6 ↑
TrBDE	225 (98)	250 (199)	552 (157)	534 (186)	<0.0001	0.8060	0.57	232	524	2.3 ↑
TeBDE	1550 (298)	2980 (2320)	9490 (5030)	10,300 (4430)	<0.0001	0.7510	0.71	1670	10,800	6.5 ↑
PeBDE	304 (92)	767 (1210)	2400 (1480)	2350 (1440)	0.0011	0.6081	0.70	315	2520	8.0 ↑
HxBDE	31 (7.8)	208 (391)	1190 (656)	1440 (1430)	0.0003	0.7198	0.80	31	1320	42.2 ↑
ΣPBDE	2110 (383)	4200 (4080)	13,600 (6790)	14,600 (7090)	<0.0001	0.7236	0.72	2270	15,400	6.8 ↑

Significant increase (↑) or decrease (↓) in the adjusted means of individual congeners and groups ($p < 0.05$).
[a] Stern and Ikonomou (unpublished data).

ranged from 1.5-fold for BDE47 to 5.7-fold for BDE153. Overall, ΣPBDE concentrations increased by 1.3-fold. Age-adjusted mean concentrations could not be determined for the 2001 collection because age × year interactions were found to be significant. In the Cumberland Sound beluga, the greatest increase in BDE concentrations occurred over the 6 years between 1986 and 1992.

4.2.2. Ringed seal

Blubber samples were collected from ringed seal (*Phoca hispida*) located in Holman Island in the Northwest Territories (Ikonomou et al., 2002). All samples were collected by hunters during their subsistence hunts between mid-March and early June of 1981, 1991, 1996 and 2000. Length, girth, and sternal blubber thickness of the seals were measured following capture. Sex and reproductive status were recorded, and the canine teeth were removed for age determination.

Mean concentrations of ΣBDE in male ringed seal, ages 0–15 years, increased exponentially by almost an order of magnitude (572–4620 ng kg^{-1}) between 1981 and 2000 (Ikonomou et al., 2002). Of the three most prevalent congeners (BDE47, BDE99 and BDE100) only BDE47 and BDE100 increased in the same exponential fashion as

Table 6
Means (standard deviation) and summary of ANCOVA results used to assess the effects of year to year collections (temporal trends), age and age × year interactions (homogeneity of the slope between age and [PBDE]) on PBDE congener and major homologue group concentrations in blubber from male western Arctic beluga. All concentrations are in ng kg^{-1} wet weight[a]

	Mean (SD)			Age (Pr)	Age × year (Pr)	R^2 (model)	Age-adjusted mean[b]		Factor[c]
	1989	1995	2001				1989	1995	
n	12	9	11	–	–	–	–	–	–
Age[b]	14 (7.7)	15 (4.8)	15 (5.7)	–	–	–	–	–	–
Lipid (%)	90 (3.7)	93 (1.8)	93 (3.5)	–	–	–	–	–	–
BDE47	5810 (2260)	8760 (2450)	8770 (4780)	0.5539	0.8791	0.29	6060	9100	1.50 ↑
BDE49	3450 (1530)	3120 (1610)	1290 (877)	0.0513	0.2390	0.24	3560	2940	1.21
BDE99	595 (266)	1030 (281)	1710 (991)	0.8735	0.5817	0.38	598	1080	1.81 ↑
BDE100	603 (229)	1140 (300)	1480 (902)	0.7068	0.9669	0.51	624	1200	1.92 ↑
BDE119	8.1 (2.8)	20 (8.8)	35 (12)	0.0060	0.3378	0.72	8.3	20	2.40 ↑
BDE153	28 (19)	118 (41)	357 (195)	0.4313	0.8691	0.58	22	124	5.70 ↑
BDE154	110 (43)	381 (109)	634 (397)	0.1674	0.5098	0.83	112	403	3.60 ↑
TrBDE	232 (99)	276 (77)	305 (87)	0.0748	0.9593	0.24	233	291	1.25
TeBDE	10,100 (3940)	12,500 (3910)	10,700 (5970)	0.2416	0.8026	0.15	10,600	12,800	1.20
PeBDE	1210 (48)	2190 (567)	3280 (1900)	0.8773	0.7945	0.46	1240	2300	1.86 ↑
HxBDE	165 (69)	594 (179)	1190 (669)	0.2448	0.4989	0.81	166	626	3.76 ↑
ΣPBDE	11,700 (4440)	15,500 (4400)	15,500 (8520)	0.3638	0.9317	0.20	12,300	16,100	1.32 ↑

[a] Stern and Ikonomou (unpublished data).
[b] Comparison with the 2001 results could not be made as significant age × year interactions were observed for all major homologue groups (see Fig. 1).
[c] Significant increase (↑) or decrease (↓) in the adjusted means of individual PBDE congeners and major homologue groups ($p < 0.05$).

ΣPBDE. While BDE99 concentrations increased in a similar manner to those of BDE47 and BDE100 between 1981 and 1996, the increase slowed considerably over the 4-year period from 1996 to 2000. Mean ΣBDE levels in 1981, 1991, 1996 and 2000 were 572, 1860, 3440 and 4620 ng kg^{-1}, respectively. 1981, 1991 and 1996 levels differed significantly from one another. However, because of the large variation in the 2000 samples, no significant difference was observed between the ΣBDE levels measured in the 1996 and 2000 samples. No significant difference in the levels of ΣBDE or individual congeners were observed between younger (0–15 years) and older (16–35) male seals in 2000. As was found for the beluga, these results suggest that the recent PBDE accumulation dominates potential historic accumulation for the older seals.

Doubling times were calculated for major homologue groups and showed that the tetra-BDEs appear to be increasing at approximately one-half of the rate (t_2=8.6 years) of penta-BDEs (t_2=4.7 years) and hexa-BDEs (t_2=4.3 years). These results indicate that the congener profiles are changing over time. Factor increases of BDE47, BDE99 and BDE100 in ringed seal over the 5-year period between 1991 and 1996 are similar to those observed in the western Arctic beluga over the 6-year period between 1989 and 1995. An exponential increase in PBDE levels in ringed seals between 1981 and 2000 was shown to closely parallel worldwide production of the penta-mix (Ikonomou et al., 2002).

4.2.3. Guillemot eggs from the Baltic Proper

Guillemot eggs collected at Stora Karlsö in the Baltic Proper between 1969 and 1997 were analysed for three BDE congeners (BDE47, BDE99 and BDE100) (Sellström, 1999). Guillemots are one of the few bird species that are resident in the Baltic all year round, feeding on sprats and herrings with females producing a single egg each year. This study indicated that BDE concentrations increased from the 1970s to the 1980s, peaking around the middle of that decade, followed by a rapid decrease. Sellström noted that this was not in agreement with a study on pike from Lake Bolmen (southern Sweden) which showed an initial increase in concentrations from the 1960s, levelling off at the beginning of the 1980s. Subsequently, data for guillemot eggs from Stora Karlsö taken between 1996 and 2000 were studied by Lunstedt-Enkel et al. (2001). Mean concentrations of the congeners determined ranged from 1.4 μg kg^{-1} lipid weight for BDE153 to 128 μg kg^{-1} lipid weight for BDE47. No statistically significant time trend could be found in this case, suggesting that the decline noted by Sellström has slowed. Kierkegaard et al. (1999) have studied concentrations of HBCD in guillemot eggs from Stora Karlsö taken between 1969 and 1997, and demonstrated a significant increase in concentrations over the entire time period.

4.2.4. Temporal trends in herring gull eggs from North America

Recent data indicate that the levels of PBDEs in herring gull eggs from the Great Lakes region have increased significantly during the past two decades. Moisey et al. (2001) observed a 60-fold increase in the concentration of PBDEs in gull eggs from the Great Lakes. Similar trends were observed in other environmental compartments of the Great Lakes, such as in lake trout from Lake Ontario. Luross et al. (2001) reported that the levels of PBDEs have increased by 300-fold over the past 20 years in these fish. Such trends are analogous to those observed in marine mammals from the Arctic and from California. Stern and

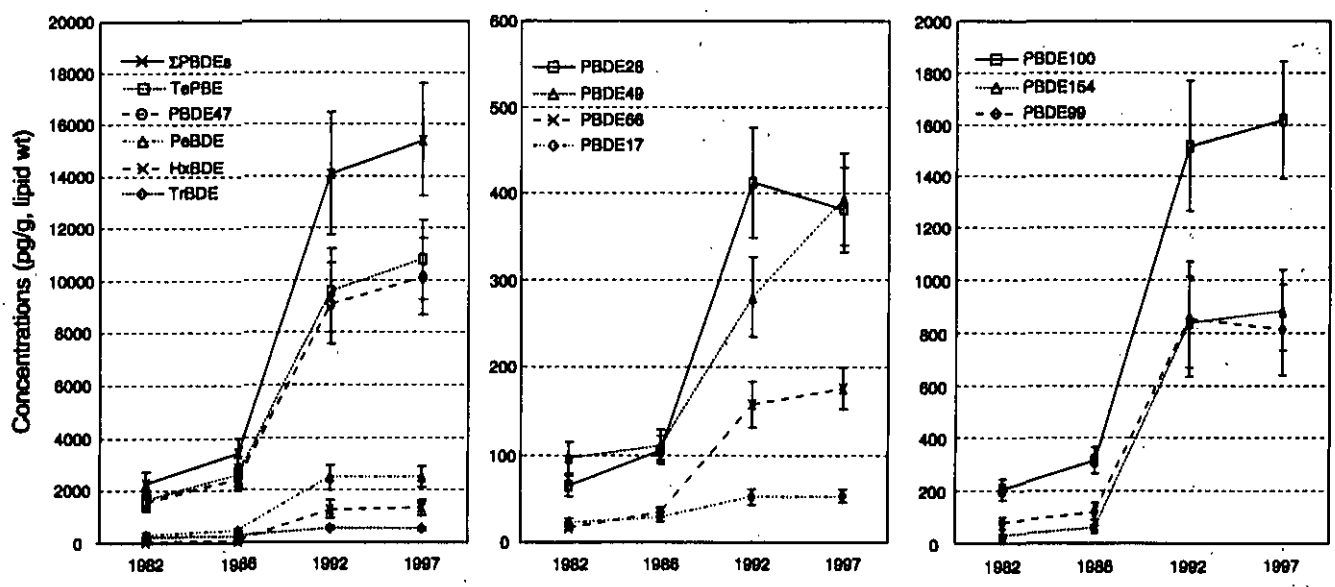

Fig. 2. Temporal trends of lipid normalised (age-adjusted) concentrations of major PBDE homologue groups and congeners in blubber samples collected from male Cumberland Sound beluga (1982–1997).

Ikonomou (2000a,b) reported a 75-fold increase in the concentration of PBDEs in belugas from Baffin Island between 1982 and 1997. Ikonomou et al. (2002) observed a 10-fold increase in the concentration of PBDEs between 1981 and 2000; and She et al. (2002) reported a 65-fold increase in the concentration of PBDEs in harbour seals from California between 1988 and 2000.

5. Conclusions

Tetra- to hexa-BDEs are widely distributed in wildlife, and are found in animals from the Arctic region as well as close to their sources, which are predominantly in the industrialised temperate regions. Such temporal trends as have been determined tend to show rapid rises over the last two to three decades, in line with the growth in production and use of these compounds. These trends have now slowed in many cases. Declines could be expected in European and future for many BDE congeners following the cessation of manufacture and use of the penta-mix in the EU, although these are not yet clearly apparent in environmental samples. However, the rapidly rising concentrations seen currently in Northern Canada (Fig. 2) could be expected to continue for some time, reflecting continued production and use of the penta-mix formulation in North America (>95% of the world total) and the impact of long-range atmospheric transport. Because of the rapid rises in BDE concentrations in marine mammals, care should be taken when comparing concentrations in samples collected during different time periods. In addition, the large variability seen in these data can be explained at least in part by the significant changes in concentration with time.

If the occurrence of BDE183 is taken as indicative of the presence of the octa-mix, it is concluded that this mixture is either not present (North Sea), or present in relatively low levels compared to the penta-mix (Great Lakes/Canadian Arctic), in aquatic ecosystems. In contrast, the relatively high levels of BDE183 in peregrine falcons from Scandinavia and Germany (Fig. 4) indicates that the octa-mix might be more widespread in at least some terrestrial food webs.

Two important questions regarding the environmental fate of decabromodiphenylether (BDE209) remain to be definitively answered. Firstly, what is its true potential for bioaccumulation? Secondly, are there circumstances under which it can debrominate in the environment to yield less highly brominated congeners?

There is currently a huge need for information on the distributions, fate and effects of TBBPA and HBCDD, to feed into risk assessments for these compounds, and studies addressing all of these topics are encouraged. It will be instructive to see how rapidly the environmental concentrations of these flame retardant compounds increase as a result of their increasing use, both to inform the regulatory process regarding future approval of novel compounds, and to allow identification of the dominant sources and pathways following release. We expect initial data for these two flame retardant compounds to become available during 2003.

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Trace Contaminants in a Polybrominated Biphenyl Fire Retardant and a Search for These Compounds in Environmental Samples

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As a result of the accidental contamination of a cattle feed supplement in 1973 with a hexabromobiphenyl fire retardant, Firemaster FF-1, large numbers of cattle and other farm animals in Michigan became severely ill and many of the animals died (ROBERTSON and CHYNOWETH 1975, KAY 1977). Cattle mortality remained high in certain areas of the State, although polybrominated biphenyls (PBBs) in the animal tissues had decreased below the levels considered as significant from a human health standpoint by State and Federal regulatory authorities (CARTER 1976). The present study was undertaken with a view to determining if other brominated organic compounds of a more toxic nature than PBBs were present in Firemaster FF-1 or in environmental samples which may have been contaminated with Firemaster FF-1.

EXPERIMENTAL

Florisil chromatography was used as a first step for the isolation of polar compounds from Firemaster FF-1 (Michigan Chemical Co., St. Louis, Michigan). The procedure was a scaled down version of a similar step developed by VOS et al. (1970) and BOWES et al. (1973) for the separation of chlorinated dibenzofurans from polychlorinated biphenyls (PCBs). A 35 mg sample of Firemaster FF-1 was dissolved in 20 ml hexane and added to 30 g of suitably activated Florisil in a 15 mm i.d. column. The column was eluted with: (1) 100 ml hexane; (2) 75 ml 5% diethyl ether/hexane; and (3) 75 ml 25% diethyl ether/hexane. The third fraction was concentrated to 2 ml and then subjected to chromatography on a neutral alumina column (200 mm X 6 mm) followed by a microFlorisil column (40 mm X 6 mm). These two column separations were carried out by following the conditions described in a neutral procedure for the isolation of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) from bovine tissues (O'KEEFE et al. 1978). The complete neutral cleanup procedure of O'KEEFE et al. (1978) was used for the separation of polar brominated organic compounds from *a trace mineral salt* (38 g), feed scrappings from a dairy cattle barn (14 g), bovine eye fat (10 g) and bovine liver (40 g).

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Diazomethane was prepared by the method of ARNDT (1947). Reaction with Firemaster FF-1 was then accomplished by adding a 1 mg sample of the fire retardant in 1 ml hexane to 3 ml of an 0.5 M diazomethane solution in diethyl ether. After 1 hr at room temperature the solution was evaporated to dryness and taken up in a small volume of benzene in preparation for analysis by mass spectrometry.

All analyses were conducted with an AEI MS9 mass spectrometer. Samples were introduced into the mass spectrometer by capillary constricted glass tubes attached to a direct insertion probe (BAUGHMAN and MESELSON 1973 a, b). The mass spectral standard compound, perfluorotributylamine (PCR, Inc.), was used for peak reference in both the low resolution and high resolution analyses. Spectra from dual ion monitoring in the high resolution mode were recorded after signal averaging or direct readout on a UV oscillographic recorder (BAUGHMAN and MESELSON 1973a, b). Low resolution spectra were recorded by the latter technique.

RESULTS AND DISCUSSION

(1) Firemaster FF-1

Low resolution (≈1,000) mass spectral analysis from m/e 200 to 850 showed that Firemaster FF-1 consisted of over 60% hexabromobiphenyl together with tetrabromobiphenyl, pentabromobiphenyl and heptabromobiphenyl. Bromobenzene compounds also appeared to be present although it was difficult to identify them positively because of the overlapping of their molecular ion clusters with those of the brominated biphenyl series. Two series of brominated compounds with signals appearing at 52 and 32 mass units lower than the corresponding brominated biphenyls were also recorded on the mass spectra. The identity of these compounds is not known at present. Some of the Firemaster solution was treated with diazomethane, a reagent which is known to methylate hydroxyl groups. There was no difference between the low resolution mass spectra from the diazomethane treated Firemaster and the untreated Firemaster. These results indicate that compounds such as phenoxy phenols and hydroxy biphenyls are not present to any significant extent in the Firemaster FF-1. Chlorinated derivatives of the former can serve as precursors of dibenzo-p-dioxins (RAPPE and NILSSON 1974) and halogenated derivatives of the latter could possibly give rise to the formation of dibenzofurans (NILSSON and RENBERG 1974).

In order to separate polar compounds from the major biphenyl components in the fire retardant, a 35 mg sample of Firemaster FF-1 was subjected to a multicolumn cleanup procedure. Although PBB compounds still predominated in low resolution mass spectra, two new brominated compounds containing 5 and 6 bromine atoms appeared in the spectra with molecular weights 26 mass

units less than the corresponding PBBs. The molecular weights suggested that the compound were brominated naphthalenes and in fact chlorinated naphthalenes have been identified by VOS et al. (1970) and BOWES et al. (1973) in commercial preparations of PCBs. To provide additional evidence for the presence of naphthalenes, high resolution (9,000) peak matching was carried out for the principal isotopic ion (m/e 602) of the molecular ion cluster for hexabromonaphthalene. The spectrum, recorded on a signal averaging computer, had a peak at m/e 601.519 (Figure 1).

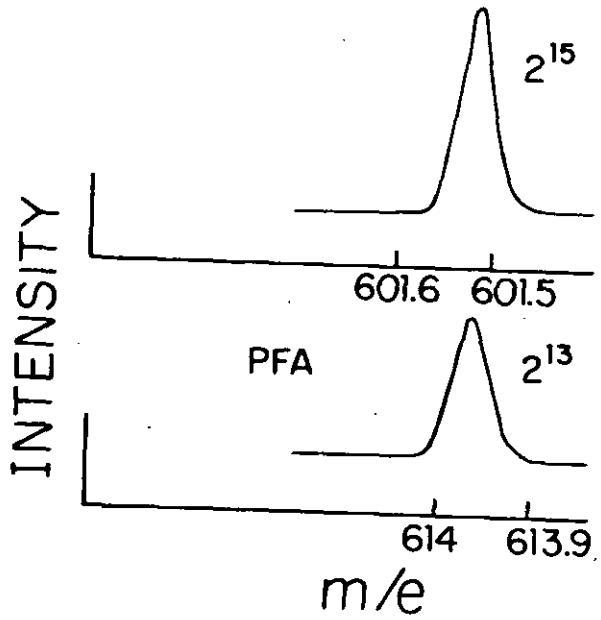

FIGURE 1. Analysis by dual ion direct probe high resolution (9,000) mass spectrometry of a cleaned up extract from Firemaster FF-1.

The theoretical value for hexabromonaphthalene ($C_{10}H_2Br_6$) is m/e 601.51945. When the peak matching controls were adjusted to monitor ions at m/e 602 and 604, the relative intensity of these ions (604/602) was found to be 0.76 (theoretical 0.73).

Since a standard sample of hexabromonaphthalene was unavailable. it was calculated that there was approximately 25 parts per million (ppm) of hexabromonaphthalene in Firemaster FF-1, by comparison to a standard sample of 2,3,7,8-tetrabromodibenzofuran (TBDF).

This compound volatilizes into the mass spectrometer source at the same temperature (290°C) as hexabromonaphthalene, and there is a strong probability that molecular ions are produced from the two compounds at approximately the same percentage of the total ion current. The material in the glass tubes was dissolved in benzene and cleaned up a second time on the alumina and microFlorisil columns. Over 60% of the hexabromonaphthalene was recovered and therefore it appeared that the cleanup method provided for a quantitative recovery of bromonaphthalenes. High resolution mass spectra were also recorded for the ions at m/e 521.611 and 523.609, the two major isotopic ions expected from a pentabromonaphthalene compound. The ion intensity ratio, 522/524 was found to be 1.06 (calculated 0.98). When TBDF was used as an external reference compound it was calculated that there was approximately 1 ppm of pentabromonaphthalene in the Firemaster FF-1.

Firemaster BP-6, an alternative formulation of Firemaster FF-1 without calcium silicate to prevent caking, has recently been analyzed by gas chromatography-mass spectrometry (HASS et al. 1978). Hexa- and pentabrominated naphthalenes were also identified in this study at levels of 70 ppm and 150 ppm respectively. These compounds were eluted in a nonpolar fraction from a Florisil column using a higher ratio of sample to adsorbent (100 mg/g vs 1 mg/g) and different eluents than in the present study. The differences in levels of brominated naphthalenes reported in each study may possibly result from obtaining samples from different production batches of PBB.

In a preliminary toxicological evaluation, HASS et al. (1978) found by means of rabbit ear skin tests that the naphthalene fraction and the unfractionated Firemaster BP-6 had some acnegenic potential. Since the materials were applied at equal concentration levels it was concluded that PBB compounds had probably produced the toxic effects. However it has been demonstrated that cattle inadvertantly exposed to chlorinated naphthalenes develop hyperperatosis and other symptoms (GREGORY et al. 1954) which are very similar to those prevalent in PBB exposed cattle (KAY 1976).

The high toxicity of certain chlorinated dibenzodioxins and dibenzofurans has been confirmed by many reports in the literature. Dibenzodioxins would not be expected to form readily in a mixture of biphenyl compounds, but chlorinated dibenzofurans have been detected in commercial PCBs (BOWES et al. 1975, VOS et al. 1970, NAGAYAMA et al. 1976). Single ion monitoring of the most abundant molecular ion isotopes was carried out by high resolution mass spectrometry for tetra-, penta-, and hexabromodibenzofurans in Firemaster FF-1. No detectable traces (>0.5 ppm) of these compounds could be identified under the conditions used in the present study. HASS et al. (1978) also did not find brominated dibenzofurans

or brominated dibenzodioxins in Firemaster BP-6 at similar limits of detection.

(2) Bovine tissues and feed materials.

Samples of a mineral salt containing magnesium oxide, feed scrappings from a barn, and PBB contaminated[1] eye fat from two dairy cows and liver from one of the cows were subjected to a neutral cleanup procedure previously developed for the isolation of TCDD from bovine tissues (O'KEEFE et al. 1978). Low resolution mass spectral analysis failed to reveal any brominated organic compounds in the cleanedup extracts. In additional analyses of the liver and one eye fat sample (Cow #1), the m/e 602 ion of hexabromonaphthalene was monitored at high resolution but no signals were found in this mass region (Figure 2).

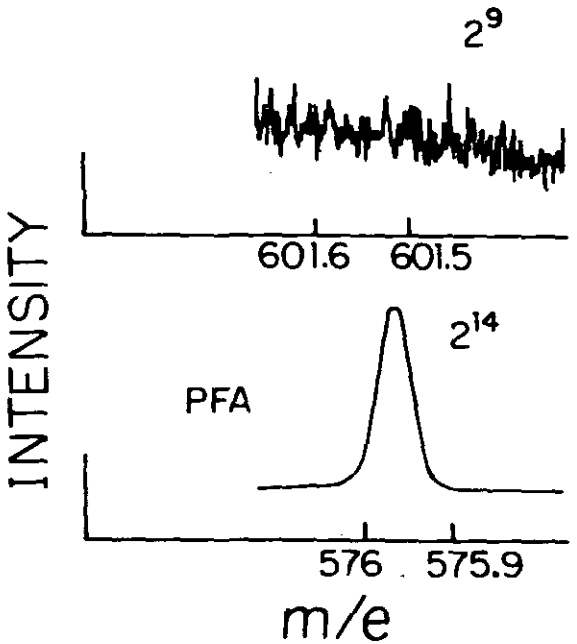

FIGURE 2. Analysis by dual ion direct probe high resolution (9,000) mass spectrometry of a cleanedup extract from bovine eye fat (Cow #1).

[1]Levels of PBB compounds were determined by other laboratories using gas-liquid chromatography with the following results: eye fat cow #1, 0.08 ppm; eye fat cow #2, 0.46 to 1.69 ppm.

Although brominated naphthalenes were not detected in the environmental samples analyzed in this study, it is possible that these compounds could be detected with a mass spectrometer operating under more sensitive conditions. If in fact the possible contamination of environmental samples by brominated naphthalenes is based on the levels of brominated naphthalenes found in Firemaster FF-1, a detection limit of at least 2 parts per trillion would be required to determine their presence in the eye fat sample. However, at the time the mass spectrometer had a detection limit for TBDF of approximately 2 ng using dual ion monitoring under high resolution conditions. Since subsamples for analysis were generally equivalent to a 2 g fraction of the original sample prior to cleanup, this would lead to a detection limit of only 1 part per billion for brominated aromatic compounds in the environmental samples.

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Do flame retardants threaten ocean life?

Brominated flame retardants are important in modern life. They are used at relatively high concentrations in electronic equipment such as computers and television sets, in textiles, cars and in many other applications. Here we show that two groups of these flame retardants, polybrominated biphenyls (PBBs) and polybrominated diphenyl ethers (PBDEs), are present in sperm whales, which normally stay and feed in deep water, indicating that these compounds have reached deep ocean waters.

The most frequently used brominated flame retardants are tetrabromobisphenol-A, hexabromocyclododecane, PBBs and PBDEs, which are produced globally at an estimated 150,000 tonnes a year[1–3]. Although these compounds are similar in behaviour and toxicity to well-known environmental contaminants such as polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT), they have not been banned. Humans may directly absorb PBBs and PBDEs when they are emitted from electronic circuit boards and plastic computer and TV cabinets[4], and there is also an environmental problem. Because of their high lipophilicity (log K_{ow}>6, where K_{ow} is the octanol–water partition coefficient) and resistance to degradative processes, PBBs and PBDEs are expected to bioaccumulate easily[5].

We determined the PBB and PBDE concentrations in 13 marine animals (from 4 species) from coastal seas and the Atlantic Ocean. The sperm and minke whales and whitebeaked dolphin were all stranded alive at the Dutch coast; the juvenile seals were found shortly after they died. We studied the resistance of PBDEs and PBBs to oxidative metabolism by using the cytochrome P450 enzyme system in an *in vitro* bioassay with hepatic microsomes from a whitebeaked dolphin, a sperm whale and a harbour seal. We tested genotoxicity in a mutatox assay.

We found most of the PBBs and PBDEs analysed in both sperm whales and the other samples (Table 1). The total PBDE concentrations in sperm whale blubber (around 100 μg kg^{-1}) were about 50-fold higher than the total PBB concentrations in the blubber of the same animals (around 2 μg kg^{-1}). The presence of these xenobiotic compounds in sperm whales indicates that the compounds have reached deep ocean waters, as sperm whales are not usually found in shelf seas. Females do not migrate north of a latitude of 45° N (northern Spain); males occur as far north as northern Norway, Iceland and Greenland. At this latitude, sperm whales hunt in waters of depths 400–1,200 m or more.

Postmortem investigations of the same sperm whales showed a complete absence of food in the alimentary tracts and evidence of weight loss and blubber reduction[6]. Thus it is likely that the PBBs and PBDEs in the blubber and liver of the sperm whales came from deep-water organisms of the oceanic food chain. PCBs are reported to flux at 1.6 μg m^{-2} yr^{-1} to a depth of 3,200 m in the North Atlantic Ocean (Sargasso Sea)[7], showing that organic contaminants can be transported to deeper waters fairly quickly.

We found relatively high PBDE concentrations in a whitebeaked dolphin (>7 mg kg^{-1}) and in harbour seals (>1 mg kg^{-1}) (Table 1), which had been feeding in the North Sea and the Wadden Sea. PBDE levels in dolphins and seals indicate that ongoing industrial production of PBDEs may create an environmental problem similar to that caused by PCBs, which have been found at concentrations up to 128 mg kg^{-1} in marine mammals[8].

The *in vitro* biotransformation tests confirm that the two classes of brominated flame retardant are very persistent, even more so than PCBs. Concentrations of BB 15 (4,4′-dibromobiphenyl) decreased by about 80% in the tests, but none of the other PBBs and PBDEs listed in Table 1 showed any indication of biotransformation.

Both classes of compound did not show a genotoxic response in the mutatox assay at concentration ranges of 0.07–900 ng ml^{-1} (PBDEs) and 0.002–9 μg ml^{-1} (PBBs), neither as the parent compound nor after incubation with induced rat liver microsomal fractions ('S9'). However, PBBs can promote the carcinogenicity of other compounds[9] and PBBs and PBDEs are both listed as compounds that can affect the regulation of thyroid and steroid hormones[5,10].

There may be several reasons for the absence of the decabrominated congeners BB 209 and BDE 209 in all wildlife samples. They may disappear (and possibly degrade to lower brominated congeners) because of

Table 1 Concentrations of bromobiphenyls and bromodiphenyl ethers in marine wildlife samples

Species	Tissue	Fat content* (g per kg)	PBB† concentrations (μg per kg wet weight)							PBDE† concentrations (μg per kg wet weight)			
			15	49	52	101	153	188	209	47	xy‡	99	209
Sperm whale 1	Blubber	722	0.06	0.24	0.40	0.91	1.9	<0.1	<0.5	95	15	28	<6
Sperm whale 2	Blubber	234	0.04	0.13	0.21	0.40	0.73	0.06	<0.3	58	8.1	15	<3
Sperm whale 3	Blubber	317	0.07	0.20	0.36	0.70	1.1	<0.1	<0.4	61	7.5	10	<5
Sperm whale 2	Liver	23	<0.01	<0.01	<0.01	0.83	18	<0.04	<0.3	2.7	0.54	0.91	<3
Whiteb. dolphin	Blubber	980	0.2	7.5	4.1	8.3	13	<0.2	<0.9	5,500	1,200	1,000	<10
Whiteb. dolphin	Liver	27	<0.01	0.06	0.03	0.74	19	<0.02	<0.1	22	6.8	9.0	<1
Minke whale	Blubber	140	0.11	0.27	0.24	0.54	0.82	<0.02	<0.1	88	11	23	<1
Harbour seal 1	Blubber	244	<0.05	34	5.7	9.3	61	12	<1	1,200	110	160	<15
Harbour seal 2	Blubber	983	<0.05	3.1	2.3	1.4	18	<0.2	<1	1,200	100	40	<10
Harbour seal 3	Blubber	722	<0.05	3.0	0.52	1.1	13	<0.1	<1	280	18	140	<10
Harbour seal 2	Liver	35	<0.01	0.10	0.06	0.62	1.5	<0.02	<0.1	21	0.93	0.85	<2
Harbour seal 3	Liver	51	<0.01	0.10	0.03	0.04	0.82	<0.01	<0.1	12	0.33	5.1	<1
Harbour seal 4	Liver	30	<0.01	0.90	0.14	0.44	13	<0.02	<0.1	20	0.07	0.53	<2
Mackerel	Muscle	152	0.01	0.01	0.01	<0.01	0.04	<0.03	<0.2	5.4	1.8	1.9	<2

* Total lipid contents are according to ref. 11, except for seal 1 blubber and seal 3 blubber and liver, for which extractable lipid contents are shown.
† Numbering of PBB and PBDE congeners is according to the PCB numbering system of ref. 12.
‡ xy-PBDE is pentabrominated BDE with unknown structure.

chemical or microbial degradation (weathering), or they may be taken up at reduced rates because it is difficult for their relatively large molecules to cross cell membranes. More research is required to understand the environmental behaviour of the decabrominated compounds, which, despite being increasingly produced, have not yet been found in aquatic organisms.

The presence of PBBs and PBDEs in sperm whales, the high levels of particularly PBDEs in seals and dolphins, and the ongoing industrial production of these compounds suggest that an environmental problem may be on its way.

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Density of states reflects diameter in nanotubes

Dresselhaus[1] asked recently why scanning tunnelling microscopy and spectroscopy experiments[2,3] find a similar number of peaks, in the density of states near the Fermi level, for a range of semiconducting single-wall carbon nanotubes with roughly the same diameters. After all, semiconducting achiral zigzag single-wall nanotubes can have translational unit cells more than an order of magnitude smaller than those of chiral semiconducting single-wall nanotubes with a similar diameter, so they might be expected to have fewer but sharper peaks in their density of states[1]. We show that all semiconducting single-wall nanotubes with similar diameters should have a similar density of states near the Fermi level, independent of translational unit cell size or chiral angle.

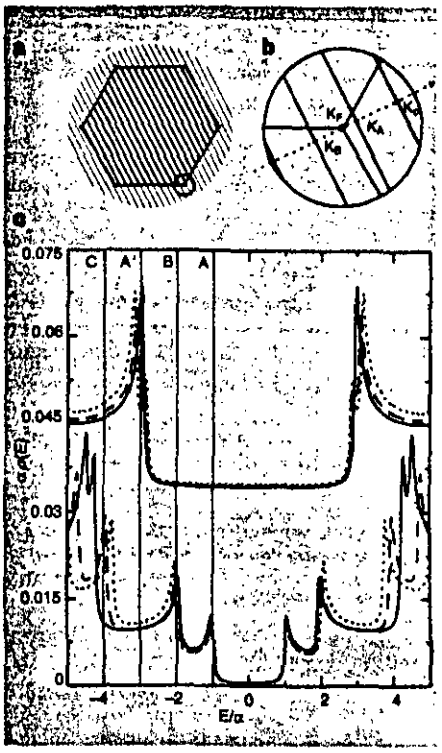

Figure 1 Density of states in nanotubes. **a**, Hexagonal central Brillouin zone of graphene. Parallel lines depict allowed states for (11,9) SWNT. Circle at bottom right encloses region of states near ϵ_F. **b**, Expanded depiction of allowed states near ϵ_F, with dotted line parallel to R, k_F corner of hexagon with energy ϵ_F, and k_A, k_B and k_C special points of closest approach to k_F of three nearest allowed-state lines in reciprocal space. **c**, DOS per carbon, $\rho(E)$, as a function of energy in units of $\alpha = |V_{pp\pi}|d/D$ computed using the full graphene dispersion relation $\epsilon_\pm(k) = \pm|V_{pp\pi}|(3+2\cos(\mathbf{k}\cdot\mathbf{R}_1)+2\cos(\mathbf{k}\cdot\mathbf{R}_2)+2\cos(\mathbf{k}\cdot(\mathbf{R}_1-\mathbf{R}_2)))^{1/2}$ for six different SWNTs with similar diameters, D, for a range of chiral angles, ϕ, and number of atoms in the translation unit cell, N_T. The average D for these six tubes is $\bar{D}$ = 1.33 nm for d = 0.142 nm with all D varying less than 3% from $\bar{D}$. Also, α = 0.29 eV for $V_{pp\pi} = -2.7$ eV and $D = \bar{D}$. All DOS are broadened by a lorentzian of width 0.01α. Shown are the DOS for the (17,0) [solid line; D = 1.33 nm, ϕ = 0°, N_T = 68], (13,6) [dashed line; D = 1.32 nm, ϕ = 18°, N_T = 1,132] and (11,9) [dotted line; D = 1.36 nm, ϕ = 27°, N_T = 1,204] semiconducting SWNTs. Vertical lines A, B and C (corresponding to k_A, k_B, and k_C in Fig. 1b) are peak positions in the occupied semiconducting DOS predicted using $\epsilon_-(k) = -\frac{3}{2}|V_{pp\pi}|d|k - k_F|$ approximately valid for $|\epsilon_- - \epsilon_F| \ll \frac{3}{2}|V_{pp\pi}|$. Also shown are the DOS for the (10,10) [solid line; D = 1.36 nm, ϕ = 30°, N_T = 40], (14,5) [dashed line; D = 1.34 nm, ϕ = 15°, N_T = 1,164] and (16,1) [dotted line; D = 1.29 nm ϕ = 3°, N_T = 1,092] metallic SWNTs offset upward by 0.03. Vertical line A' is the first peak position in the occupied metallic DOS predicted from the approximate theory. Curvature introduces a small gap at ϵ_F in the non-armchair metallic tubes which is probably smeared out in the experimental results[2,3].

A single-wall nanotube (SWNT) can be constructed by rolling up a single sheet of graphite (graphene) along one of its two-dimensional lattice vectors $\mathbf{R} = n_1\mathbf{R}_1 + n_2\mathbf{R}_2$ to form an (n_1, n_2) nanotube with diameter $D = |\mathbf{R}|/\pi$. To a first approximation, the allowed electron states of the SWNT are the graphene bands satisfying the periodic boundary conditions $\mathbf{k}\cdot\mathbf{R} = 2\pi m$, with m being an integer.

Graphically, these allowed $\mathbf{k}$ values lie along the parallel lines in Fig. 1a, which are perpendicular to $\mathbf{R}$ with an interline spacing of $2/D$. The Fermi level, ϵ_F, is given by states at the corner points of the hexagonal Brillouin zone in Fig. 1a. So, neglecting the effects of curvature, nanotubes are either metallic or semiconducting depending on whether or not $\mathbf{k}_F\cdot\mathbf{R} = 2\pi m$, where $\mathbf{k}_F = 4\pi(\mathbf{R}_1 - \mathbf{R}_2)/9d^2$ and d is the carbon–carbon bond distance[4].

The two-dimensional dispersion relation of the occupied states of graphene is given to lowest order in $d|\mathbf{k} - \mathbf{k}_F|$ by $\epsilon_-(\mathbf{k}) = -\frac{3}{2}|V_{pp\pi}|d|\mathbf{k} - \mathbf{k}_F|$, where $V_{pp\pi}$ is the nearest-neighbour ppπ interaction[5]. Hence, near ϵ_F, $\epsilon_-(\mathbf{k})$ is radially symmetric about the point k_F located by $\mathbf{k}_F$ and decreases with increasing $|\mathbf{k} - \mathbf{k}_F|$.

For semiconducting SWNTs, the point of closest approach to k_F in any allowed line therefore yields to good approximation a local maximum in the one-dimensional band structure as long as $d|\mathbf{k} - \mathbf{k}_F| \ll 1$, leading to a van Hove singularity and a divergence in the occupied density of states (DOS) near ϵ_F. These special points also yield peaks in the unoccupied DOS because the dispersion relation for the unoccupied states is given by $\epsilon_+(\mathbf{k}) = -\epsilon_-(\mathbf{k})$. The special point on the line closest to k_F (labelled as k_A in Fig. 1b) also yields the SWNT band gap[5,6]. This point is separated from k_F by a distance $|\mathbf{k}_A - \mathbf{k}_F| = \frac{2}{3D}$, one-third the interline spacing — a result easily confirmed by projecting $\mathbf{k}_A - \mathbf{k}_F$ along R. So k_A generates peaks in the DOS near $\epsilon_\pm(\mathbf{k}_A) = \pm|V_{pp\pi}|d/D$ and a band gap near $2|V_{pp\pi}|d/D$ when $\frac{2d}{3D} \ll 1$.

Incrementing by the interline spacing, $2/D$, the next two special points yield peaks in the DOS near $\epsilon_\pm(\mathbf{k}_B) = 2\epsilon_\pm(\mathbf{k}_A) = \pm 2|V_{pp\pi}|d/D$ when $\frac{4d}{3D} \ll 1$, and peaks near $\epsilon_\pm(\mathbf{k}_C) = 4\epsilon_\pm(\mathbf{k}_A) = \pm 4V_{pp\pi}\,d/D$ when $\frac{8d}{3D} \ll 1$. Changing chirality at a given SWNT diameter effectively rotates the lines of allowed states around k_F without changing the distances between the points along the lines and k_F. Therefore the DOS of two semiconducting SWNTs with differing chiral angles but similar diameters are similar in the vicinity of ϵ_F because $\epsilon_\pm(\mathbf{k})$ is radially symmetric about k_F. A parallel analysis shows that all metallic SWNTs with similar diameters have similar DOS near ϵ_F within the graphene sheet model but with peaks close

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Potential role of fire retardant-treated polyurethane foam as a source of brominated diphenyl ethers to the US environment

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Abstract

Five tetra- to hexabrominated diphenyl ether (BDE) congeners (BDE-47, -99, -100, -153 and -154) are the most frequently reported in wildlife and humans. The commercial penta-BDE product, used predominantly to flame-retard polyurethane foam, consists primarily of these same congeners. In 1999, North American demand accounted for 98% of the total global penta-market of 8500 metric tons. Frogs, housed with flame retardant-treated polyurethane foam as a dry substrate, accumulated 10,100 μg/kg (wet weight) of the above BDEs. Crickets kept therein as food contained 14,400 μg/kg. The crickets are believed to have browsed directly on the foam and, in turn, were consumed by the frogs. BDE congener composition in all three matrices matched that of the penta-commercial product. Similar congeners were also observed in soil and stream sediments collected near a polyurethane foam manufacturing plant. Summed concentrations of BDE-47, -99 and -100, the dominant congeners observed in these samples, ranged from < 1 to 132 μg/kg (dry weight basis). Sunfish fillets obtained from a nearby, off-site pond contained a total of 624 μg/kg (lipid basis). Sewage treatment plant (STP) sludge exhibited these same congeners at 1370 μg/kg (dry weight). BDE-209, the fully brominated congener predominant in the commercial deca-BDE product, was also present at 1470 μg/kg. While no known polyurethane foam manufacturers discharged to this plant, the distribution pattern of the low brominated congeners in the sludge matched that of the penta-product. After four weeks of exposure to ambient outdoor conditions, the surface of flame-retarded polyurethane foam became brittle and began to disintegrate. Subsequent dispersal of these penta-containing foam fragments may be one mechanism by which these BDEs reach the environment.

Keywords: Bioaccumulation; Frog; Sewage sludge; Insect; Sediment; Soil

1. Introduction

Brominated diphenyl ethers (BDEs) are widely used as additives to flame retard flammable polymers. While consumption of BDE formulations has recently decreased in some European nations, due to potential adverse health and environmental effects, overall global demand for these products has increased, reaching 67,125 metric tons in 1999 (Renner, 2000a). BDEs appear to be persistent organic pollutants (POPs) and some congeners are highly bioaccumulative, as a consequence they have emerged as contaminants of global concern (de Boer et al., 1998). BDE congeners with four to six bromines have been detected in biota from remote areas (de Boer et al., 2000), indicating possible long-range transport. Some studies have indicated increasing levels in the North American environment (e.g. Luross et al., 2000). Exponential increases in BDE concentrations in human breast milk over time in Sweden have

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2. Methods

African running frogs (*Kassina senegalensis*) were maintained in the laboratory in anticipation of future pesticide exposure studies. Frogs were held in tanks containing approximately 1 cm of water. Pieces of flexible polyurethane furniture foam were provided to serve as dry "island" substrates for the frogs, a common herpetological husbandry technique. Frogs were provided food in the form of small crickets. Those crickets not immediately eaten sought refuge on the foam. Frogs were sacrificed and analyzed in their entirety, as described below, to establish backgrounds levels of halogenated contaminants. Foam and crickets were also analyzed.

To assess whether BDEs could be released from foam manufacturing operations, sampling was conducted in 2000 at a polyurethane foam production facility located in the US mid-Atlantic region. Foam production at the 16-acre facility began in 1981 and ceased in 1997, due to health concerns related to atmospheric releases of methylene chloride and toluene diisocyanate. Surface soil samples were taken adjacent to the property. In addition, the interior of a disassembled exhaust stack from the foam production building was sampled with a linen swab. Associated dust was rinsed with methylene chloride (Burdick & Jackson, Muskegon, MI) into a glass vessel. Surficial sediments and bluegill sunfish (*Lepomis macrochirus*) were also collected from a small pond, located in a downwind residential area about 250 m from the foam production building. Surficial sediments from two small creeks exiting the plant site were also obtained.

Since fish in the lower Dan River had previously been found to contain significant burdens of BDE congeners containing four to six bromines (Hale et al., 2000), sludge was collected from the regional sewage treatment plant (STP) discharging to this area. The sludge was taken directly from the dewatering press. No known polyurethane foam manufacturers discharged to this facility, although a plastics processor using the deca-product was a contributor.

Fish, soil, sediment and sludge samples were lyophilized prior to extraction. Foam was cut into pieces and analyzed directly. Frogs and crickets were minced and dried by mixing with activated sodium sulfate. A surrogate standard was added (PCB-30, -65, -121 and -204; Ultra Scientific, North Kingstown, RI) to all samples to monitor recoveries. The congener naming convention described by Schulz et al. (1989) was used for the PCBs and a similar scheme applied to the BDEs. Blanks were run concurrently with the samples to assess possible laboratory contamination. Total organic carbon (TOC) measurements on the sediments, soils and the sludge were performed using platinum catalyzed combustion at 680 °C, followed by nondispersive infrared detection (TOC-5000 Shimadzu Scientific Instruments, Columbia, MD).

Samples were subjected to enhanced solvent extraction (Dionex ASE 200, Sunnyvale, CA) using two 5-min extraction cycles with methylene chloride at 100 °C and 1000 psi, followed by a 60% vessel flush. Lipids in biota were estimated by evaporation of a fraction of the resulting extract to constant weight. Extracts were first purified on an Envirosep® size-exclusion column (Phenomenex, Torrance, CA), eluted with methylene chloride. The resulting fraction of interest was further purified on 2 g, silica gel, solid-phase extraction columns (EnviroPrep®, Burdick & Jackson). Columns were initially eluted with 3.5 ml hexane and then 6.5 ml of 60:40 hexane:methylene chloride. The latter fraction contained the halogenated compounds of interest.

Following solvent exchange to hexane, halogenated compounds in the purified extracts were separated on a gas chromatograph (GC; Varian 3400, Sugar Land, TX), equipped with a 60 m DB-5 column (J&W Scientific, Folsom, CA; 0.25 μm film, 0.32 mm ID). Carrier gas was He and injections were made in the splitless mode. Initial column temperature was held at 90 °C for 2 min and then programmed to 320 °C at 4 °C/min, where it was held for 10 min. BDE-209 may degrade if subjected to high temperatures for extended periods (Kierkegaard et al., 1999). Therefore, the sludge extract was also analyzed on a 15 m DB-5 column of the same film thickness and diameter. Initial column temperature was held 2 min at 80 °C, ramped at 15 °C/min to 320 °C and held for 3 min, then increased at 15 °C/min to 350 °C for 3 min.

Tetra- to hexabrominated diphenyl ethers, PCBs and organochlorine pesticides were initially screened and quantitated in all fish and sediments by halogen selective electrolytic conductivity detection (ELCD). The STP sludge sample was examined for these BDEs, as well as BDE-209. Pentachlorobenzene was used as an internal standard. Data were corrected based on PCB-204 recoveries, generally greater than 80%. BDEs were also confirmed in selected samples by GC, with the column described above, and full scan electron ionization mass spectrometry (MS), on a Varian 4D ion trap (Sugar Land, TX) or a Finnigan Incos XL quadrupole (ThermoQuest, San Jose, CA). BDE standards used included individual congeners (BDE-47, -99, -100, -153 and -154 obtained from Cambridge Isotope Laboratories, Andover, MA; BDE-209 from Fluka Chemie, Buchs, Switzerland) and a commercial penta-BDE mixture (DE-71, Great Lakes Chemical, West Lafayette, IN).

3. Results and discussion

As noted, frogs were originally analyzed to establish baseline organochlorine pesticide levels for future

Table 3
Concentrations of BDEs in soil, sediment and fish collected near a polyurethane foam manufacturing facility

Sample	BDE-47 (μg/kg dry)	BDE-99 (μg/kg dry)	BDE-100 (μg/kg dry)	Total BDEs (μg/kg dry)	Total BDEs (μg/kg TOC or lipid basis)
Soil near foam production building	31.6	41.2	3.15	76.0	33,600
Soil downwind of foam plant	8.11	4.75	0.77	13.6	2160
Soil downwind of foam plant	<0.1	<0.1	<0.1	ND	ND
Sediment from stream leaving foam plant	6.37	9.47	1.34	17.2	22,300
Sediment from stream leaving foam plant	<0.1	<0.1	<0.1	ND	ND
Sediment from stream leaving foam plant	36.2	86.3	9.01	132	20,500
Pond sediment	0.50	<0.1	<0.1	0.50	63.1
Pond sediment	<0.1	<0.1	<0.1	ND	ND
Bluegill from pond – fillet	18.1	6.18	2.78	27.1	624
Bluegill from pond – remainder	44.9	12.5	4.75	62.2	709
Sewage sludge	544	725	266	1540	6620

Totals provided are for the sum of BDE-47, -99 and -100. BDE-209 was only detected in the sludge, but at 1470 μg/kg. Fish from the river to which the STP discharged had previously been found to contain high burdens of tetra- to hexabrominated diphenyl ethers (Hale et al., 2000).

reduction strategies were mandated by EPA in 1997 (US EPA, 1996). High methylene chloride and toluene diisocyanate emissions were documented from this particular foam plant. Coincidentally, we also use methylene chloride at 100 °C to extract BDEs from our environmental samples. Thus, atmospheric releases of the penta-constituents have likely occurred in the past during manufacture of flame retardant-treated foam.

BDEs were also detected in two of three surficial bed sediments from two small streams exiting the plant site. In these sediments the relative BDE-47 contributions were lower than those of BDE-99 (Table 3). Total BDEs (in these cases BDE-47, -99 and -100) ranged from < 0.3 to 132 μg/kg (dry weight basis). BDE-153 and -154 were below detection in most of these samples. The pond examined did not receive direct surface water contributions from the manufacturing site. BDE-47 was detected (0.5 μg/kg) in one of the two pond sediments. Again TOC contents of these samples were low. Lipid normalization equalized BDE concentrations in different body compartments of bluegill sampled from this pond. Muscle contained 624 μg/kg total BDEs, while the remaining tissues exhibited 709 μg/kg (lipid basis). These concentrations are intermediate between those reported by Dodder et al. (2000) in sunfish collected 1.3 km from a US industrial facility involved in flame retardant research and development and those in fish from a lake distant from known point sources. In the present study, BDE-47 was the major congener detected in the bluegill, similar to most published reports of residues in aquatic organisms (de Boer et al., 2000). This was also the case for the Dodder et al. (2000) "background" fish. These researchers found elevated contributions from penta- and hexabrominated congeners in the sunfish collected near the industrial research and development facility. This pattern probably was due to exposure to more brominated mixtures. Constituents of these formulations exhibit lower bioaccumulation potentials and sorb more strongly to sedimentary organic matter in the environment than BDE-47 and -99, the dominant congeners in the penta-mixture. Log K_{ow} values of 6.0 and 6.8 have been reported for BDE-47 and -99, respectively, in the same range as the most bioaccumulative PCBs (Burreau et al., 1997; de Boer et al., 2000). In contrast, the log K_{ow} of BDE-209 has been estimated at 9.97 (de Boer et al., 2000). No foam manufacturing facilities were apparent in the area where elevated BDE burdens in Virginia fishes were previously noted (Hale et al., 2000). This, in concert with the relatively modest levels of BDEs in the environment surrounding the foam plant examined here, suggest alternative sources may be responsible for those observations.

Hydrophobic wastewater constituents preferentially partition to high organic particles in STP waste streams and thus may concentrate in resulting sewage sludges. Analysis of these sludges may serve as a useful indicator of societal contaminant discharges. In this context, sludge was obtained from a STP operating in the area where the elevated BDE burdens were previously observed in Virginia fishes (Hale et al., 2000). The sludge contained 1370 μg/kg of tetra- to hexabrominated diphenyl ethers (sum of BDE-47, -99, -100, -153 and -154; dry weight basis) and a TOC content of 23.2%. The chromatographic pattern of these congeners matched that of the penta-commercial product used in foam (Fig. 1). While this supports the view that penta-treated foam may be a source of BDEs to the environment, no foam manufacturing facilities were reported to be dischargers to this STP. Concentrations of these same congeners in European sludges are generally an order of magnitude lower (Sellstrom et al., 1999; de Boer et al., 2000). This is likely reflective of the 40-fold greater consumption of the penta-product in North America.

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Polybrominated Diphenyl Ethers: Neurobehavioral Effects Following Developmental Exposure

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Abstract

Polybrominated diphenyl ethers (PBDEs), a class of widely used flame retardants, are becoming widespread environmental pollutants, as indicated by studies on sentinel animal species, as well as humans. Of particular concern are the reported increasingly high levels of PBDEs in human milk, as should be given that almost no information is available on their potential effects on developing organisms. In order to address this issue, studies have been conducted in mice and rats to assess the potential neurotoxic effects of perinatal exposure to PBDEs (congeners 47, 99, 153 and the penta-BDE mixture DE-71). Characteristic endpoints of PBDE neurotoxicity are, among others, endocrine disruption (e.g. decreased thyroid hormone levels), alteration in cholinergic system activity (behavioral hyporesponsivity to nicotine challenge), as well as alterations of several behavioral parameters. In particular, the main hallmark of PBDE neurotoxicity is a marked hyperactivity at adulthood. Furthermore, a deficit in learning and memory processes has been found at adulthood in neonatally exposed animals. Some of neurotoxic effects of PBDEs are comparable to those of polychlorinated biphenyls (PCBs), though the latter class of compounds seems to exert a stronger toxic effect. Available information on PBDE neurotoxicity obtained from animal studies and the possibility of neonatal exposure to PBDEs via the mother's milk suggest that these compounds may represent a potential risk for neurobehavioral development in humans.

Keywords: PBDE; PCB; Neurobehavioral development; Mice; Perinatal exposure; Brominated flame retardants

INTRODUCTION

Polybrominated diphenyl ethers (PBDEs), are an important group of flame retardants, used worldwide in large quantities in polymers, especially in ready-made plastic products. They are persistent compounds that appear to have an environmental dispersion similar to that of polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT). In contrast to the well studied developmental neurotoxicity of PCBs, there is almost no information on the potential neurotoxicity of PBDEs. Their levels in the environment are still lower than those of PCBs or DDT. However, over the past 25 years, while other organohalogens have decreased in concentration, PBDEs have been detected at increasing frequency and amount in various wildlife species and in human tissues (de Boer et al., 1998b; Law et al., 2002; Luross et al., 2002; Meironyte et al., 1999; She et al., 2002). In particular, a breast-milk monitoring program in Sweden has shown

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that, during the course of 20–30 years, organochlorines decreased to the half of the original concentration in a range of 4–17 years period, while PBDE levels doubled each 5 years (Noren and Meironyte, 2000), though in the very last years their milk concentration is declining (Meironyte and Noren, 2001). Numerous studies have documented that exposure to chemicals during vulnerable developmental phases often inflicts more important toxic consequences than exposure to mature nervous system (Rice and Barone, 2000; Selevan et al., 2000). The presence of increasing quantities of PBDEs in human milk samples during the last decade, is thus already a matter of concern.

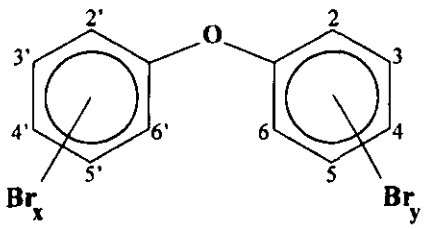
In this paper, we review the present state of knowledge on PBDE neurotoxicity following developmental exposure, in order to illustrate the potential adverse consequences of PBDE exposure on human health.

CHEMICAL AND PHYSICAL PROPERTIES OF PBDEs

The general chemical formula of PBDEs is $C_{12}H_{(0-9)}$-$Br_{(1-10)}O$ and the theoretical number of possible congeners is 209 (Fig. 1), though the number of PBDE congeners used in commercial products is limited. For instance, PBDE compounds with less than four bromine atoms are not found in commercial products (Darnerud et al., 2001). PBDEs are numbered using the IUPAC numbering system (Ballschmiter et al., 1993), and are divided into 10 congener groups (mono- to decabromodiphenyl ethers). PBDEs, as PCBs, have low vapor pressure at room temperature and high lipophilicity. Moreover, PBDEs are characterized by a high resistance to physical, chemical and biologic degradation, by a boiling point between 310 and 425 °C and by a low solubility in water, especially for the higher brominated compounds (for further details, see Darnerud et al., 2001; de Wit, 2002).

Three major commercial mixtures of the PBDEs are produced, which vary in the degree of bromine substitution on the aromatic rings (Darnerud et al., 2001; Hardy, 2002): (i) decabromodiphenylether (deca-BDEs) composed of about 98% deca-BDEs and 2% nona-BDEs; (ii) octabromodiphenylether (octa-BDEs) composed roughly of 10% hexa-BDEs, 40% hepta-BDEs and 30% octa-BDEs and the remainder nona- and deca-BDE; (iii) pentabromodiphenylether (penta-BDEs) comprised of roughly 40% tetra-BDE, 45% penta-BDE and 6% hexa-BDE congeners. The commercial PBDE mixtures generally contain fewer congeners than do commercial PCB mixtures (e.g. roughly 80 congeners in the Aroclor 1254 mixture; Schultz et al., 1989; Sjodin, 2000; Sjodin et al., 1998). They are used in large quantities as flame-retardant additives in polymers, especially in the manufacture of a variety of electrical appliances (TV sets, computer circuit boards and casings) and of building materials. PBDEs are also used in foams and upholstery furnishings, in the interiors of vehicles, and in drapery textiles (Darnerud et al., 2001; de Wit, 2002; Hale et al., 2001; Hardy, 2002; WHO, 1994).

Polybrominated diphenyl ethers



Polychlorinated biphenyls

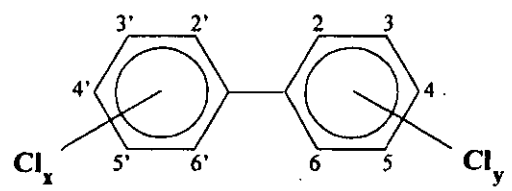


Fig. 1. General structural formula of PBDEs and PCBs.

HUMAN EXPOSURE

The fully brominated deca-BDE congeners are poorly absorbed and rapidly eliminated, thus their bio-accumulative potential is low (el Dareer et al., 1987; Norris et al., 1975). Extensive testing of these compounds has indicated their toxicity, which appears to be different from that of other classes of PBDEs (Darnerud et al., 2001; Hardy, 2002; McDonald, 2002). In contrast, lower molecular weight congeners, tetra- to hexa-BDEs, appear to be almost completely absorbed, slowly eliminated and highly bio-accumulative (de Wit, 2002; Orn and Klasson-Wehler, 1998). The main route of human exposure to PBDEs, especially of the lower brominated congeners, has been suggested to be via food (Alaee et al., 1999; Darnerud et al., 2001; Wenning, 2002).

High levels of PBDEs have been found in food of animal origin, particularly fish. In a recent study, Ohta et al. (2002) have determined PBDE levels in fish meat and in other food products, such as vegetables. PBDEs

were found in spinach, potatoes and carrots at concentrations of 134, 47.6 and 38.4 pg/g fresh weight, respectively. The higher PBDE concentration found in spinach, a leafy vegetable, compared to root vegetables, such as potato and carrot, might be due to PBDE contamination in air. The concentrations of PBDEs in pork, beef and chicken were 63.6, 16.2 and 6.25 pg/g fresh weight, respectively. Interestingly, concentrations of PBDEs found in both vegetables and meat, were significantly lower than the concentration found in fish, which ranged between 17.7 and 1720 pg/g fresh weight.

Sources other than food also seem to influence total PBDE intake in humans. A possible source is represented by contaminated airborne dust particles. In air samples from computer halls and from office rooms with computers, PBDEs, strongly bound to particulate matter, have indeed been detected (Sjodin, 2000).

PBDEs have been found in human breast-milk and in other human tissues, such as adipose tissue and blood. Several studies have reported high levels of PBDEs in human adipose tissue and concentration of these compounds was found to be dependent to the country where samples have been collected. The average tetra-, penta- and hexa-BDE levels (ng/g lipid) were, respectively, 1.4, 0.9 and 1.8 in Spain (Meneses et al., 1999), 2.8 and 1.95 (hexa-BDE were not reported) in Israel (de Boer et al., 1998a), 7.3, 2.2 and 2.3 in Finland (Strandman et al., 2000), 1.4, 0.8 and 1.5 in Belgium (Covaci et al., 2002) and 33.3, 9.8 and 16.3 in California, USA (She et al., 2002). The very high PBDE levels in human adipose tissue samples collected in California may be partially explained by the California State requirements against fire danger which may favor the use of PBDEs (She et al., 2002).

The high degree of inter-individual variability observed in the concentration of PBDEs in human adipose tissue samples (Hardell et al., 1998; She et al., 2002) may be due to different factors related to exposure, including diet (e.g. fish consumption; Darnerud et al., 2001; Jakobsson et al., 2002; Ohta et al., 2002), occupation (Jakobsson et al., 2002; Sjodin et al., 1999, 2001), age (She et al., 2002), and use of consumer products (WHO, 1994). For example, recent studies have shown that workers at a computer disassembly plant had elevated serum levels of several PBDE congeners (especially BDE 47 and 209; Hagmar et al., 2000; Sjodin et al., 1999). In particular, their mean PBDE serum concentration was of 37 pmol/g lipid weight, about six times higher than the one reported among hospital cleaners and computer clerks, considered as controls (Sjodin et al., 1999). However, it is worthy of notice that these PBDE levels are at least two orders of magnitude below the PBDE levels found in breast-milk samples from Sweden women, suggesting that this is likely a minor route of exposure compared to food or breast-milk (Darnerud et al., 2001).

Developmental Exposure

A concern of PBDE contamination via food is the increasing exposure of children, and in particular of nursing infants, to these chemicals. Various studies have evidenced that PBDEs are present in human breast-milk (Betts, 2002; Hooper and McDonald, 2000; Noren and Meironyte, 2000), and that the most abundant congeners in the samples studied are the penta-BDE 47, 99 and 153 (Darnerud et al., 2001; Jakobsson et al., 2002; Meironyte et al., 1999; Ohta et al., 2002).

On the basis of data on human milk collected in Sweden in 1999, assuming that a 2–3-month-old infant weighing about 5 kg consumes 700 ml breast-milk per day, the average daily intake of PBDEs via milk can be estimated at 50–100 ng per day (Meironyte et al., 1999). A first breast-milk monitoring program in Sweden has shown that the concentration of certain PBDE isomers (BDE 47, 99, 100, 153 and 154) in human breast-milk have increased exponentially from 1972 to 1997, reaching a level that could possibly represent a risk for child safety (Meironyte et al., 1999; Noren and Meironyte, 2000). Even if a follow-up study conducted in Sweden from 1998 to 2000 have evidenced a decrease in the PBDE concentration in human milk, probably due to the phase out of PBDEs in Sweden (Meironyte and Noren, 2001), new data from several countries such as Canada, Finland and Japan have confirmed an increasing presence of PBDEs in human milk (Ohta et al., 2002; Ryan and Patry, 2000; Strandman et al., 1999). Recently, preliminary data have shown that PBDE levels in the breast-milk of North American women are rising at an exponential rate doubling every 5 years and body burden of Americans and Canadians are the highest in the world, in the range of 50–200 ng/g lipid, i.e. around 10–40 times greater than the highest levels reported for women in Sweden (Betts, 2002; Ryan and Patry, 2000).

Another Swedish study (Darnerud et al., 1998) has evidenced that PBDE levels in milk correlated positively and significantly with mothers' smoking habits and body mass index, while no correlation was found between PBDE levels and consumption of fish and frequency of using computers. Nevertheless, Ohta et al.

(2002) have found a strong positive relationship between total PBDE levels in breast-milk of nursing Japanese women and the frequency of fish consumption. PBDE concentrations in breast-milk at 1 month after delivery ranged from 668 pg/g fresh weight, in women with small consumption of fish, to 2840 pg/g fresh weight, in women with high consumption of fish.

THE POTENTIAL RISKS OF PBDEs FOR HUMAN HEALTH

Evidence from animal models suggest that exposure to some PBDEs results in induction/down-regulation of liver enzyme and increase in liver weight (Darnerud et al., 2001; Hardy, 2002; McDonald, 2002). Moreover, treatment-related changes were reported in rodent reproductive performances (Branchi et al., 2002; Darnerud et al., 2001) and gestational exposure to PBDEs significantly increase incidence of resorption and subcutaneous edema and delayed ossification of normally developed bones of the skull in pups (WHO, 1994).

Some in vitro evidence is available for estrogenic activity of PBDEs (Meerts et al., 2001; Thayer et al., 2000) and animal studies have shown a carcinogenicity of deca-BDE compounds (National Toxicology Program, 1986), but more studies are needed to determine whether low-dose exposures to PBDE have estrogenic and carcinogenic activity in humans or other species. One of the most sensitive end-points of PBDE toxicity observed in animal bioassays appears to be the effects on thyroid function, such as an induction of thyroid hyperplasia (National Toxicology Program, 1986; WHO, 1994) and alteration of thyroid hormone metabolism (Fowles et al., 1994; Hallgren et al., 2001; Zhou et al., 2001, 2002). In particular, PBDE are reported to mainly affect thyroxine (T4) levels by means of an increased T4 catabolism via up-regulation of hepatic uridinediphosphoglucuronosyl transferase activity (Hallgren et al., 2001; Zhou et al., 2001). Another system affected by PBDEs is that of the cytochromes P-450 1A1 and 2B, as suggested by in vitro studies revealing increased ethoxyresorufin-*O*-deethylase and pentoxyresorufin-*O*-deethylase activities, respectively (Chen et al., 2001; Zhou et al., 2001).

Pregnant women are particularly sensitive to thyroid hormone disruption and developing fetuses and infants are especially responsive to small changes in thyroid hormone (Glinoer, 1997; Morreale de Escobar et al., 2000). Several studies have shown that relatively small decreases in maternal serum T4, free T4 or other indicators of thyroid abnormalities can have a negative impact in intelligence and psychomotor skills of children (Haddow et al., 1999; Morreale de Escobar et al., 2000; Pop et al., 1999).

Furthermore, exposure to low doses of PBDEs during critical phases of brain development (Rice and Barone, 2000; Selevan et al., 2000), such as during gestation and/or during the neonatal period (by ingestion of mother's milk) in the mouse, has been shown to induce irreversible changes in adult brain function (Branchi et al., 2002; Eriksson et al., 2001). The mechanism by which these chemicals produce neurotoxic effects remains unclear. However, recent rodent studies showed that developmental administration of PBDEs (congeners 99 and 153) affects the cholinergic system at adulthood (Viberg et al., 2002a, 2002b), which may be mediated throughout the thyroid system (Porterfield, 2000).

At present, much remains uncertain regarding the toxicity of PBDEs in humans, but nearly all individuals are exposed to low-doses of PBDEs, as shown by tissue monitoring data, so the potential health impact should include assessment at the population level (de Wit, 2002). In particular, pregnant women and developing fetuses and infants should be viewed as sensitive population for exposure to PBDEs.

ANIMAL MODELS TO INVESTIGATE NEUROBEHAVIORAL TOXICITY

The study of behavioral changes following developmental exposure to possible neurotoxic agents employs animal models for both practical reasons and obvious ethical constraints of human experimentation. This approach consists of monitoring the development of the nervous system through the assessment of selected neurobehavioral endpoints appropriate for each maturational phase (Branchi and Ricceri, 2002; Cory-Slechta et al., 2001; Cuomo et al., 1996; Spear, 1990). Animal models allow one to analyze developmental changes in response to different concentrations of a specific compound or to different time schedules of its administration, identifying dose–response relationships and critical phases of susceptibility. Furthermore, laboratory rodents can be successfully used for extended follow-up of treatment effects in a relatively short time-span, since they mature rapidly (they reach puberty around postnatal day 35 and sexual maturation at 60 days; (Venerosi et al., 2002).

The extrapolation from animals to humans remains an open question in the field of neurotoxicology/behavioral

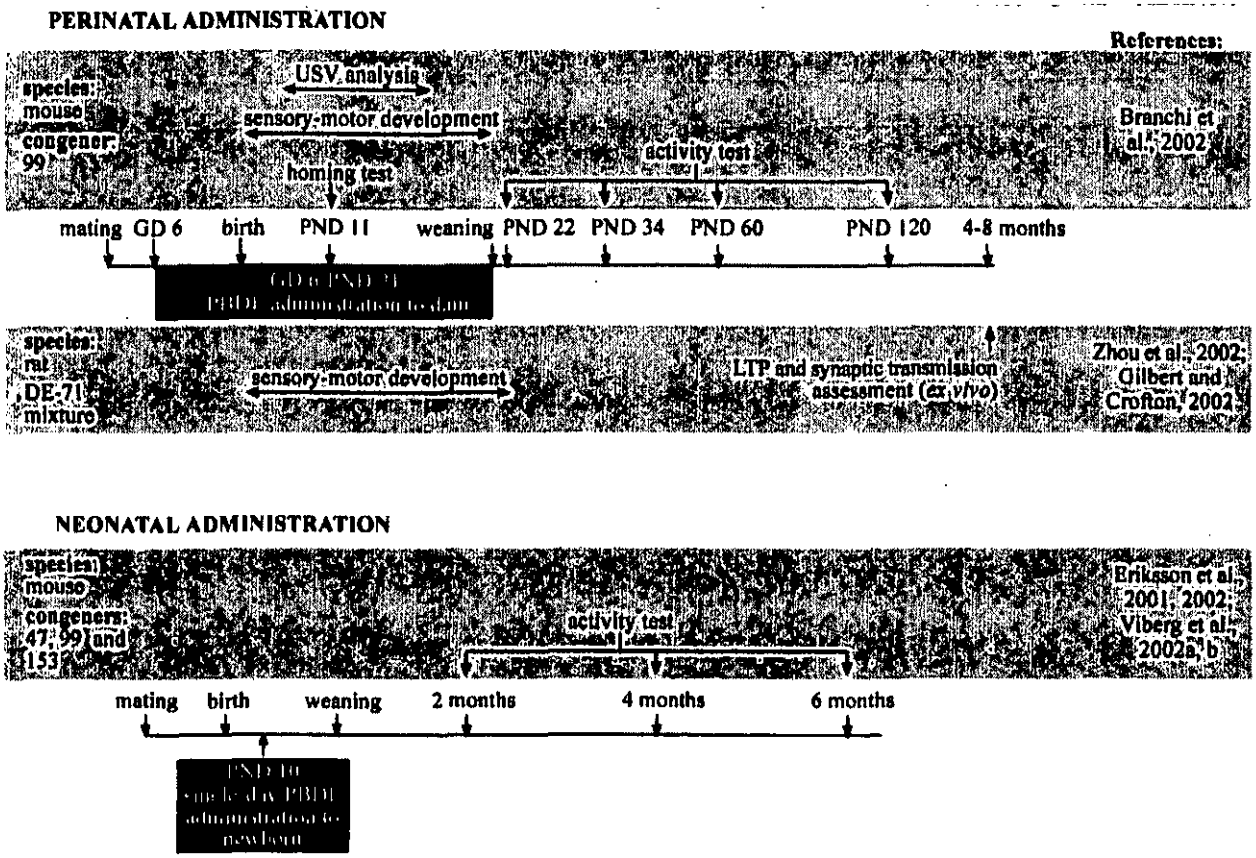


Fig. 2. Experimental designs and neurobehavioral tests used for investigating PBDE developmental neurotoxicity in mice and rats.

teratology (Winneke, 1992). Nevertheless, since the mid-seventies, a number of studies have clearly indicated that both physical anomalies and neurobehavioral abnormalities observed in humans following exposure to toxicants are mirrored in animal models (Rice et al., 1996; Vorhees et al., 2001). In particular, with regard to functional categories (sensory, motivational, cognitive, motor function, social behavior), a close agreement among human and laboratory animals in the developmental toxicity of various neurotoxic agents has been reported (Stanton and Spear, 1990). Animal studies have confirmed the neurobehavioral toxicity of several environmental pollutants, such as lead (Rice et al., 1996), methylmercury (Burbacher et al., 1990; Rice, 1996), and polychlorinated biphenyls (Tilson et al., 1990), as well as of pharmacological agents, including benzodiazepines (Kellogg et al., 1998) or anticonvulsants (Adams et al., 1990; Laviola et al., 1994).

A careful neurobehavioral assessment helps in identifying specific toxic effects on the central nervous system (CNS) as well as non-specific effects such as alterations of neuroendocrine regulations, hypo- or hyper-thermia, or alterations in nutritional status that may also indirectly have an impact on CNS development (Spear, 1990). Behavioral teratology tests can show subtle and/or transient changes early in life which may have long-term consequences for the organism. An agent, even if in the absence of specific effects on the CNS, might interfere with complex interaction of the CNS with other systems such as the endocrine and immune systems. The resulting behavioral disorders may not be evident during early postnatal life, but may emerge later, even at adulthood or during senescence (Isaacson and Spear, 1984).

A limited number of studies have been carried out to investigate the potential toxic effects of perinatal exposure to PBDEs on neurobehavioral development. For clarity, a synoptic review of the relative study designs and of the main results obtained is shown in the diagrams presented in Fig. 2 and Tables 1 and 2.

EFFECTS OF PERINATAL EXPOSURE TO PBDEs ON DEVELOPMENT

Reproductive Parameters

Studies in either rats or mice have not shown important adverse consequences on reproductive parameters upon exposure to PBDEs in utero and/or immediately

Table 1
Neurobehavioral effects of perinatal administration of different PBDE compounds in mice and rats

Age phase	Test	Effects	References
Early developmental phase (PND 22)	Sensory-motor development	Delay in screen climbing response in BDE 99 perinatally exposed mice. The 30 mg/kg per day dose group showed a ~2 days delayed maturation of this response. No alteration has been found in other somatic/behavioral parameters	Branchi et al. (2002), Zhou et al. (2002), Taylor et al. (2002)
	Ultrasonic vocalizations	No effect	Branchi et al. (2002)
	Homing test	No effect	Branchi et al. (2002)
Late developmental phase (PND 22-2 month)	Spontaneous behavior	PND 34: hyperactivity and impaired habituation in BDE 99 perinatally exposed mice. Subjects exposed to 6 mg/kg per day locomoted more during the second part of the test compared with controls	Branchi et al. (2002)
Adulthood (>2-month)	Spontaneous behavior	2-month-old mice: hyperactivity and impaired habituation in both perinatally exposed mice (BDE 99, 0.6 and 6 mg/kg per day doses), as well as neonatally exposed mice (BDE 47, 99 or 153; dose range: 0.7–12 mg/kg). Altered thigmotaxis in BDE 99 perinatally exposed subjects (6 mg/kg per day) 4-month-old mice: BDE 99 perinatally exposed animals showed an activity profile only slightly different from that of controls. 0.6 and 6 mg/kg per day doses groups displayed a significantly lower level of locomotion than controls during the second part of the 60 min test. Neonatal exposure resulted in permanent aberrations in activity profile (BDE 47, 99 or 153; dose range: 0.7–12 mg/kg) 6-month-old mice: neonatal exposure to BDE 153 resulted in permanent aberrations in activity profile (dose range: 0.9–9 mg/kg)	Eriksson et al. (2001), Branchi et al. (2002), Viberg et al. (2002b)
	Water maze learning	BDE 99 or 153 neonatally exposed mice (dose range: 0.9–12 mg/kg) showed a learning impairment in a water maze task	Eriksson et al. (2001), Viberg et al. (2002b)
	Cholinergic system	BDE 99 neonatally exposed mice (12 mg/kg) showed an altered response to nicotine challenge (hyporesponsivity). BDE 153 neonatally exposed mice (9 mg/kg) showed a decrease of specific [^{3}H]-alpha-bungarotoxin binding sites in the hippocampus	Eriksson et al. (2001), Viberg et al. (2002a, 2002a)
	Synaptic transmission	No effect	Gilbert and Crofton (2002)

after birth. Body weight gain of pregnant females, pregnancy duration, proportion of successful deliveries and pup sex-ratio were not affected by perinatal PBDE treatment (DE-71 or BDE 99) in either mice or rats (Branchi et al., 2002; Zhou et al., 2002). However, in a mouse study, litter viability was found to be slightly affected perinatal exposure to BDE 99, with the 6 mg/kg per day dose significantly reducing the number of newborn pups. Since the number of pups was evaluated on PND 1, the investigators suggested that some pups could have been born dead and then cannibalized, or their reduced viability could have induced cannibalism in the mothers (Branchi et al., 2002).

Somatic and Neurobehavioral Development

Mice and rats are altricial rodents: pups are born in highly immature condition, with eyes and ears closed, after a short pregnancy (gestation lasts around 20 days, depending on the strain and species). At birth, they are

Table 2
General effects of perinatal administration of different PBDE compounds in mice and rats

Parameters	Effects	References
Reproductive parameters	No effects on maternal body weight gain, pregnancy duration, proportion of successful deliveries and sex ratio. BDE 99 perinatal exposure (6 mg/kg per day) affected litter viability reducing the number of newborn mice. Such effect was not found in the rat	Branchi et al. (2002), Zhou et al. (2002)
Body weight	No effects	Eriksson et al. (2001, 2002), Branchi et al. (2002), Zhou et al. (2002)
Liver-to-body weight ratio	Perinatal exposure to DE-71 produced a transient increased liver-to-body weight ratio from PND 4 to PND 14 in rats (10 and 30 mg/kg per day) but they recovered to control levels by PND 36	Zhou et al. (2002)
Thyroidal hormones levels	Perinatal exposure to DE-71 reduced serum thyroxine in a dose-dependent manner on from GD 20 to PND 14 in rats (10 and 30 mg/kg per day). A total recover to control levels by PND 36. Triiodiothyronine has not been found to be affected	Zhou et al. (2002)
Hepatic enzymes activity	Perinatal exposure to DE-71 resulted in significant increases in hepatic EROD, PROD, and UDPGT activity from GD 20 to periadolescence (10 and 30 mg/kg per day)	Zhou et al. (2002)

EROD: ethoxyresorufin-*O*-deethylase; PROD: pentoxyresorufin-*O*-deethylase; UDPGT: uridinediphosphoglucuronosyl transferase.

able to crawl, to get attached to a nipple and to suckle, while they need close body contact with the mother since they are not able to thermoregulate. Several reflexes and behavioral responses appear at successive postnatal steps in parallel with somatic changes, progressively expanding pup sensory and motor capabilities (Bignami, 1996). The subsequent, more complete, behavioral repertoire increases the ability of the pup to procure food and fluid, in particular after eye opening which occurs around the end of the second postnatal week (Fox, 1965). The time of onset of selected somatic changes and the time of first appearance and gradually complete maturation of various reflexes and responses shows a remarkable regularity, providing an effective tool to assess possible neurobehavioral developmental alterations (Bignami, 1996; Fox, 1965).

No alteration in postnatal body weight gain was found following perinatal exposure to BDE 99 and to the PBDE mixture DE-71 (Branchi et al., 2002; Zhou et al., 2002). Furthermore, tests of sensory-motor development failed to evidence gross alterations in PBDE perinatally-exposed mice (congener 99; Branchi et al., 2002) and rats (mixture DE-71; Taylor et al., 2002; Zhou et al., 2002). An effect of perinatal BDE 99 treatment was evidenced only in the screen climbing response in which the ability to climb a vertical screen using both fore- and hindpaws is measured. Mice exposed to 30 mg/kg per day BDE 99 had a ~2-day delay in maturation of this response (Branchi et al., 2002).

Rodent pup ultrasonic vocalizations, whistle-like sounds in the frequency range between 30 and 90 kHz, provide a useful model to study the ontogeny of emotionality (Branchi et al., 2001). The emission pattern of these vocalizations can also reveal subtle effects of pharmacological treatments and in recent years the adverse effects of drug treatment on ultrasonic calls has been extensively tested (Cuomo et al., 1996; Winslow and Insel, 1991). Marked alterations in ultrasonic vocalizations were observed in animals exposed to behavioral teratogens such as methylmercury (Adams et al., 1983), carbon monoxide (Di Giovanni et al., 1993) and other compounds (Venerosi et al., 2002). However, the ontogenetic profile of ultrasound emission rate in BDE 99 exposed mice was similar to that found in controls (Branchi et al., 2002).

The homing test exploits the strong tendency of the immature pup with closed eyes to maintain body contact with the dam and the siblings. It requires adequate sensory (olfactory) and motor capabilities as well as associative and discriminative capabilities that allow the pup to become imprinted by the mothers odor, to remember it, and to choose it among others (Alleva et al., 1985; Bignami, 1996). No significant differences between control and BDE 99 treated mice were observed in homing performance assessed on PND 11 both for distance traveled (crossings) and latency to reach the scent area (Branchi et al., 2002).

Activity Profile

In altricial species, like the rat and the mouse, the activity level is low until PND 10 or more, but it increases rapidly around or shortly after the end of the second postnatal week (i.e. in relation with eye and ear opening). The typical adult-like habituation pattern, i.e. the reduction of activity during a test of at least 30 min duration, emerges only several days later (around the time of weaning). Since this phenomenon provides useful information on the ontogeny of several CNS neurotransmitter systems, such as the dopaminergic GABAergic and cholinergic ones, activity should be tested at different developmental ages (Alleva and Bignami, 1985; Bignami, 1996; Bignami et al., 1992; Campbell and Mabry, 1973; Gruen et al., 1990; Laviola and Alleva, 1990; Robbins, 1977; Spear, 1990).

On PND 22, the activity profile of BDE 99 treated mice did not differ from controls. However, a clear hyperactivity appeared on PND 34, when mice exposed to the 6 mg/kg per day dose showed significantly higher activity levels during the last 20 min of the test. At this age, BDE 99 treatment also affected rearing behavior—another endpoint that can be considered an index of activity—that was displayed more frequently by treated animals. In particular, the 0.6 and 6 mg/kg per day dose groups displayed more rearings than controls. For any of the behavioral points analyzed, no sex differences were found (Branchi et al., 2002). It is worthy of notice that a similar alteration of locomotor activity profile during development, with normal activity around PND 22 and an appearance of hyperactivity around PND 34, has been reported in perinatal hypothyroid rats, suggesting that an alteration of thyroid function may be involved in this behavioral alteration (Goldey et al., 1995). Other studies on models of hypothyroidism in rats reported an hyperactivity profile displayed at the young-adult phase (Akaike et al., 1991; Hendrich et al., 1984).

LONG-TERM EFFECTS OF PERINATAL EXPOSURE TO PBDEs

Activity Profile

The data collected so far show that the main behavioral effect of perinatal PBDE exposure is a marked alteration of activity profile at adulthood. Prolonged perinatal PBDE administration (congener 99) to dams, as well as neonatal single-day (PND 10) administration (congeners 47 or 99), induced hyperactivity in young-adult mice. At 2 months of age, BDE 99 treated animals showed high levels of locomotor activity, especially in the last 40 min of a 60 min activity test, with a reduced habituation profile compared to controls. Rearing behavior was also higher in treated mice than in controls, especially in the last 30 min of the test (Branchi et al., 2002). Furthermore, at 2 months of age, thigmotaxis was less pronounced in treated animals than in controls, evidencing a less marked fearful response in treated mice; animals from the 6 mg/kg per day dose group spent significantly less time than controls near the arena walls (Branchi et al., 2002). The neonatal single-day exposure to BDE 47, 99 or 153 caused permanent changes in spontaneous behavior in 2-month-old mice. In particular, hyperactivity and decreased habituation were observed during a 30 min test in treated mice compared to controls (Eriksson et al., 2001; Viberg et al., 2002b).

It is of special interest that at adulthood, the effects of a prolonged perinatal administration to dams and neonatal single-day administration produced different effects on activity levels. At 4 months of age, the locomotor activity profile of BDE 99 perinatally-treated animals was only slightly different from that of controls, with the 0.6 and 6 mg/kg per day dose groups displaying a significantly lower level of locomotion than controls during the last 20 min of a 60 min test session. In contrast, animals exposed neonatally to a single dose of BDE 47, 99 or 153 showed an hyperactivity profile and a decreased habituation capability compared to controls (Eriksson et al., 2001). Furthermore, in these animals changes in spontaneous behavior were more pronounced with increasing age.

Comparison of the data collected by Eriksson et al. and Branchi et al. (Fig. 2) suggests that different exposure profiles produce different behavioral alterations in the developing mouse. Eriksson et al. (Eriksson et al., 2001, 2002; Viberg et al., 2002a) found that a single PBDE administration directly to the newborn (congener 99 or 47) on PND 10 disrupts spontaneous behavior in mice in a dose-dependent fashion. Furthermore, this functional alteration appeared to be permanent and also to worsen with age. Results from Branchi et al. (2002) showed instead that a prolonged perinatal BDE 99 exposure, involving administration of this compound to dams from gestational day 6 to PND 21, produced transient a effect on activity profile, characterized by an inverted dose–response relationship, the majority of the effects occurring at the 6 and/or 0.6 mg/kg per day dose and not at the 30 mg/kg per day dose. This effect is markedly reduced around

4 months of age. A possible explanation for this difference is that exposure to high BDE 99 doses during both prenatal and postnatal periods could trigger a compensation phenomenon in the developing organism, as in the cases of prolonged exposure to high doses of other compounds, such as lead (Gilbert et al., 1999). Alternatively, the transient effects and the inverted dose–response relationship may be due to a prepartum selection, whereby the most vulnerable subjects died during gestation in the high BDE 99 dose groups. The more resistant surviving individuals would thus be less sensitive to the toxic insult and show only subtle and transient behavioral alterations during the developmental phase. This hypothesis would be in agreement with litter viability data, which indicated that pregnant mice exposed to high BDE 99 doses tended to give birth to a reduced number of pups (Branchi et al., 2002).

It is noteworthy that the two exposure profiles may provide different but, in both cases, important information on the potential neurotoxicity of PBDEs. It has been widely reported that acute and chronic exposure may reveal different mechanisms of neurotoxicity (Costa, 1988). Furthermore, a direct administration to neonate may show different effects of these compounds when compared with the administration through the mother, since in the latter case PBDE metabolites (e.g. hydroxylated PBDEs) may play a more important role (Meerts et al., 2001; Meerts et al., 2000). However, the prolonged perinatal exposure may represent a model more relevant for humans, since human infant exposure, particularly during early ontogeny, occurs mainly during gestation and through lactation (Hooper and McDonald, 2000). As in humans, a substantial breast-milk PBDE transfer has been shown to occur in mice: 30–40% of an administered single dose of radioactive compound (congeners 99 and 85) has been found in the suckling offspring after 4 days. Furthermore, the PBDE plasma levels in the neonates were more than twice those found in the dams, though the absolute levels were low (de Wit, 2002).

Cognitive Abilities

Cognitive abilities are impaired by developmental exposure to several pollutants such as methylmercury, PCBs, lead and others (Bignami, 1996; Burbacher et al., 1990; Rice et al., 1996). One of the most used behavioral procedure to assess cognitive abilities is the Morris water maze, in which rats or mice can learn to orient and swim rapidly to an invisible escape platform using distant cues (Morris, 1984). The ability to solve this task seems to be markedly dependent on the hippocampal function (Morris et al., 1982).

Neonatal single-day administration of BDE 99, but not of BDE 47, was found to impair cognitive abilities in the Morris water maze test. No learning deficits were found during the acquisition phase of the test; however, mice exposed to BDE 99 (12 mg/kg) were significantly impaired in finding the new location of the platform during the reversal phase (Eriksson et al., 2001). These data suggest a specific impairment in behavioral flexibility to cope with changes in the learning context. Recent data show an alteration of learning and memory processes following neonatal administration of BDE 153, with treated mice being impaired in locating the platform in a Morris water maze (Viberg et al., 2002b). Tests of fear-conditioning performed on rats perinatally exposed to the DE-71 mixture revealed a dose-dependent decrease in cue- but not context-based performance in male offspring tested as adults (Taylor et al., 2003).

In agreement with the fear-conditioning data, suggesting a learning impairment amygdala- but not hippocampus-dependent (Anagnostaras et al., 2001; Frankland et al., 1998), no alterations of synaptic transmission or long-term potentiation in the dentate gyrus of hippocampus were found in rats perinatally exposed to DE-71 (Gilbert and Crofton, 2002). In vitro studies have shown that in cerebellar granule cells, DE-71 (but not DE-79, an octa-BDE mixture) stimulated the release of [^{3}H]arachidonic acid ([^{3}H]AA), identified as a second messenger involved in synaptic plasticity, by a phospholipase A_2(PLA_2)c/iPLA_2-dependent mechanism, whose activity has been associated with learning and memory (Kodavanti and Derr-Yellin, 2002).

Altered Behavioral Response to Nicotine Challenge

Interesting data have emerged from the analysis of pharmacological reactivity assessed by a nicotine challenge. An injection of nicotine (80 μg) at adulthood has been shown to induce an altered behavioral response in mice exposed to BDE 99 (8 mg/kg) on PND 10. As expected, nicotine induced an increased activity profile in control animals, while BDE 99-treated animals showed a marked hypoactivity after the pharmacological challenge (Viberg et al., 2002a). These results would suggest that alterations of cholinergic functions may be involved in the behavioral changes found in mice exposed to BDE 99.

BDE 153 has also been found to affect the cholinergic system. At 6 months of age, mice neonatally (PND 10) exposed to this compound (9 mg/kg), showed a decrease of specific [^{3}H]-alpha-bungarotoxin binding sites in the hippocampus (Viberg et al., 2002b).

PBDEs AND PCBs: COMPARABLE STRUCTURES LEAD TO SIMILAR EFFECTS?

PBDEs and PCBs have a comparable structure (Fig. 1) and their effects on neurobehavioural profile show similarities but also important differences, suggesting that their actions do not always overlap.

In adult animals, these two classes of polyhalogenated aromatic hydrocarbons exert in some cases similar toxicities. For example, both the PBDE mixture Bromkal 70-5 DE (constituted by penta- and tetra-BDEs), and the PCB mixture Aroclor 1254, cause an induction of cytochrome P-450 (Hanberg et al., 1991; von Meyerinck et al., 1990). PBDEs, especially the hydroxylated metabolites (Darnerud et al., 2001), as well as hydroxylated and laterally substituted PCBs, exert a disrupting effect on thyroid function, reducing circulating thyroxine (Hallgren et al., 2001; Porterfield, 2000). Both PBDEs and PCBs stimulate the release of [^{3}H]AA by a PLA_2c/iPLA_2-dependent mechanism (Kodavanti and Derr-Yellin, 2002). However, PCBs appear to exert a stronger effect on most of these parameters: when comparing these two classes of compounds, PCBs cause larger alterations at similar doses and are effective at lower doses than PBDE (Darnerud et al., 2001; Hallgren et al., 2001; von Meyerinck et al., 1990).

While the effects of perinatal exposure to PCBs have been widely studied (for reviews, see Eriksson, 1997; Faroon et al., 2001; Rice, 2000; Storm et al., 1981; Tilson et al., 1990; Tilson and Kodavanti, 1998), only a limited number of studies has focused on the effects of PBDEs. This lack of data allows only a preliminary, and certainly not exhaustive, comparison of neurobehavioral effects of developmental exposure to these compounds. It has been reported that both PBDEs and PCBs exert a disrupting effect on thyroid function when administered during the perinatal phase (Crofton et al., 2000; Morse et al., 1996; Zhou et al., 2002). Both classes of compounds induce a clear hyperactivity profile when administered on PND 10 (Eriksson, 1997; Eriksson et al., 2001). Furthermore, they produce a similar response to a nicotine challenge, suggesting that both compounds, administered on PND 10, affect the cholinergic system in a similar way (Eriksson, 1997). As seen in adult mice, PCBs seems to exert a stronger effect than PBDEs following perinatal exposure (Branchi et al., 2002; Eriksson et al., 2001). These results suggest that these two classes of pollutants could share some of the same mechanisms of action (Hallgren et al., 2001). However, important differences in the effects of PBDEs and PCBs in adult mice, following prolonged perinatal administration, have also been found. For example, while PCB-exposed animals display hyperactivity from early developmental phase to adulthood, PBDE-exposed animals are hyperactive only until the young-adult phase, and later recover and show a trend toward hypoactivity at adulthood (Branchi et al., 2002). Furthermore, perinatal exposure to PCB, but not PBDE, has been shown to produce in rats a decrement in the magnitude of evoked LTP and an increase in the train intensity required to induce LTP (Gilbert and Crofton, 2002; Gilbert et al., 2000).

CONCLUSIONS

Because of the high production volume of PBDEs and their accumulation in the environment, and the still limited knowledge of their potential neurotoxic/behavioral teratogenic effects (alone or in combination), more investigations are indeed warranted. In particular, more extensive studies using animal models are required in order to better characterize the neurotoxic effects of perinatal administration to PBDEs. In particular, learning and motor function should be assessed, since these behavioral endpoints are likely to be affected by thyroid hormone level alteration (Porterfield, 2000; Zoeller and Crofton, 2000; Zoeller et al., 2002). Furthermore, examination of the possible physiological or biochemical processes in the central nervous system that may be responsible for the changes in the behavioral effects observed would be of interest. In particular, neurochemical pathways and neural population which are targets for other structurally similar classes of compounds, such as PCBs (Costa, 1998; Costa et al., 2001), should be considered. In vitro data suggest that PBDEs (DE-71) could stimulate the release of [^{3}H]AA by a PLA_2c/iPLA_2-dependent mechanism (Kodavanti and Derr-Yellin, 2002) and affect the mitogen-activated protein kinase cascade, a critical mediator of cell development and neuronal plasticity (Mundy et al., 2002). Furthermore, similarly to PCBs (Kodavanti et al., 1995), BDE 99 has been shown to cause activation of protein kinase C in glial and neuronal cells in vitro (F. Madia and L.G. Costa, unpublished observations).

In conclusion, perinatal PBDE exposure produces neurobehavioral alterations in mice and rats. Since several studies clearly indicated that neonates may be exposed to PBDEs, particularly through maternal milk (Hooper and McDonald, 2000; Meironyte et al., 1999; Ohta et al., 2002), this calls for concern of the risk of adverse effects on neurobehavioral development in humans.

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Developmental Exposure to Brominated Diphenyl Ethers Results in Thyroid Hormone Disruption

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The objective of the current study was to characterize the effects of DE-71 (a commercial polybrominated diphenyl ether mixture containing mostly tetra- and penta-bromodiphenyl ethers) on thyroid hormones and hepatic enzyme activity in offspring, following perinatal maternal exposure. Primiparous Long-Evans rats were orally administered DE-71 (0, 1, 10, and 30 mg/kg/day) in corn oil from gestation day (GD) 6 to postnatal day (PND) 21. Serum and liver samples obtained from dams (GD 20 and PND 22), fetuses (GD 20), and offspring (PNDs 4, 14, 36, and 90) were analyzed for circulating total serum thyroxine (T_4) and triiodothyronine (T_3), or hepatic microsomal ethoxy- and pentoxy-resorufin-*O*-deethylase (EROD and PROD), and uridine diphosphoglucuronosyl transferase (UDPGT) activity. There were no significant effects of treatment on maternal body weight gain, litter size, or sex ratio, nor were there any effects on any measures of offspring viability or growth. Serum T_4 was reduced in a dose-dependent manner in fetuses on GD 20 (at least 15%) and offspring on PND 4 and PND 14 (50 and 64% maximal in the 10 and 30 mg/kg/day groups, respectively), but recovered to control levels by PND 36. Reduction in serum T_4 was also noted in GD 20 dams (48% at highest dose), as well as PND 22 dams (44% at highest dose). There was no significant effect of DE 71 on T_3 concentrations at any time in the dams or the offspring. Increased liver to body weight ratios in offspring were consistent with induction of EROD (maximal 95-fold), PROD (maximal 26-fold) or UDPGT (maximal 4.7-fold). Induction of PROD was similar in both dams and offspring; however, EROD and UDPGT induction were much greater in offspring compared to dams (EROD = 3.8-fold; UDPGT = 0.5-fold). These data support the conclusion that DE-71 is an endocrine disrupter in rats during development.

Key Words: **brominated diphenyl ether; development; thyroid hormone; hepatic enzyme activity; endocrine disrupter; rat.**

Polybrominated diphenyl ethers (PBDEs), produced commercially as mixtures, are commonly used as flame retardants for various consumer products including electronic equipment. Global production of PBDEs is approximately 40,000 tons per year (IPCS, 1994; Darnerud *et al.*, 2001). Increasing concentrations in environmental samples and human breast milk (Meironyte *et al.*, 1999; Sellstrom *et al.*, 1993; Stern and Ikonomou, 2000; She *et al.*, in press) have focused worldwide attention on the potential health effects of PBDEs (Darnerud *et al.*, 2001; Hooper and McDonald, 2000; McDonald, in press). The PBDE congeners found in biota and human samples are predominately 2,2′,4,4′-tetraBDE (IUPAC: BDE-47), followed by 2,2′,4,4′,5-pentaBDE (BDE-99) and 2,2′,4,4′,6-pentaBDE (BDE-100) (Kierkegaard *et al.*, 1999; Lindstrom *et al.*, 1999; Meironyte *et al.*, 1999; Strandman *et al.*, 1999).

Recent evidence from animal models suggests that exposure to some PBDEs results in disruption of thyroid hormone homeostasis (for reviews see Darnerud *et al.*, 2001; Hooper and McDonald, 2000; McDonald, in press). Studies in both rats and mice showed that exposure to 2,2′,4,4′-tetraBDE as low as 18 mg/kg/day for 14 days decreased circulating thyroxine (T_4) concentrations (Darnerud and Sinjari, 1996; Hallgren and Darnerud, 1998). Short-term exposures to some commercial PBDE mixtures such as DE-71 and Bromkal 70 (consisting mainly of tetra- and penta-BDE) induced hypothyroxinemia in both rats and mice (Darnerud and Sinjari, 1996; Fowles *et al.*, 1994; IPCS, 1994; Zhou *et al.*, 2001). Furthermore, both commercial mixtures and BDE-47 have been demonstrated to induce both phase I (ethoxyresorufin-O-deethylase [EROD] and pentoxyresorufin-O-deethylase [PROD]), and phase II metabolic enzyme activity (uridinediphosphate-glucuronosyltransferase [UDPGT]) (Carlson, 1980a,b; Fowles *et al.*, 1994; Hallgren and Darnerud, 1998; von Meyerinck *et al.*, 1990; Zhou *et al.*, 2001). T_4 glucuronidation by phase II UDPGT enzymes in liver has been suggested as one of the mechanisms contributing to circulating T_4 depletion by PBDEs and other polyhaloge-

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nated aromatic hydrocarbons (PHAHs) (Brouwer *et al.*, 1998; Hallgren and Darnerud, 1998; Zhou *et al.*, 2001).

Normal thyroid hormone homeostasis is essential for development of many organs including the brain (Chan and Kilby, 2000; Dussault and Ruel, 1987). Chemicals that disrupt thyroid hormone systems during pregnancy may have profound adverse impact on the normal development process of the brain (Brouwer *et al.*, 1998; Porterfield, 2000; Porterfield and Hendrich, 1993; Zoeller and Crofton, 2000). In humans, developmental hypothyroidism leads to a characteristic deafness and mental retardation (Boyages and Halpern, 1993). Hypothyroxinemia during fetal and/or postnatal periods, even when serum triiodothyronine (T_3) concentration is normal, can lead to permanent functional abnormalities in children (e.g., Haddow *et al.*, 1999; Larsen, 1982; Morreale de Escobar *et al.*, 2000). Brouwer *et al.* (1998) proposed that thyroid hormone disruption induced by PHAHs might be at least partially responsible for neurochemical and behavioral changes observed in laboratory animal studies. Adverse neurobehavioral effects were found in neonatal mice exposed developmentally to single PBDE congeners (Eriksson *et al.*, 1998, 1999; Viberg *et al.*, 2000).

Considering that the rapid increase in PBDEs in human breast milk (Meironyte *et al.*, 1999) suggests an increasing risk of developmental exposure, and previous data from animal exposures indicate an adverse effect of PBDEs on thyroid hormone homeostasis (Darnerud and Sinjari, 1996; Fowles *et al.*, 1994; IPCS, 1994; Zhou *et al.*, 2001), the purpose of the present study was to characterize the disruptive effects on thyroid hormones of developmental exposure to a commercial PBDE mixture (DE-71) in both dams and offspring. Induction of hepatic enzyme activity, EROD, PROD, and UDPGTs, were also examined to help characterize possible biochemical mechanisms for thyroid hormone disruption. EROD and PROD activity were monitored as biomarkers of aryl hydrocarbon (Ah)-type and phenobarbital (Pb)-type induction of UDPGT isoforms.

METHODS AND MATERIALS

Animals. All animal procedures were approved in advance by our facility's Institutional Animal Care and Use Committee. Time-pregnant Long-Evans female rats, approximately 80–90 days of age, were obtained from Charles River Laboratories, Inc. (Raleigh, NC) on GD 2, and were allowed 4 days acclimation in an American Association for Accreditation of Laboratory Animal Care-approved animal facility prior to being treated. Dams were housed individually in plastic cages (45 × 24 × 20 cm) with sterilized pine shavings as bedding, which was changed twice a week except on the day of parturition (i.e., GD 21). They were maintained at 21 ± 2°C with 50 ± 10% humidity on a 12 light:12 dark (0600–1800 h) photoperiod, with free access to food (Purina Rodent Chow, Barnes Supply Co., Durham, NC) and tap water *ad libitum*.

On GD 21, dams were checked for the number of pups delivered at 0800, 1000, 1200, 1500, and 1700 h, and pups were aged as PND 0 on the date of birth. All nonpregnant rats were euthanized. On PND 4, 7, 14, and 21, offspring were counted, sexed, and group-weighed by sex. Average pup weight by sex was calculated by dividing the group weight by the number of pups. In addition, body weights were recorded on PNDs 36 and 90, prior to tissue collection. Litters were culled to either 8 or 10 pups per litter with the number of pups kept similar, to the degree possible within 1 or 2 pups, throughout the preweaning period. Pups were checked daily for eye opening (pups with at least one eye open) from PNDs 11 through 18. Pups were weaned on PND 21, and housed by gender in groups of 2 or 3 per cage.

Chemicals and treatment. DE-71 (penta-BDE, lot 7550OK20A) was generously supplied by the Great Lakes Chemical Corporation (West Lafayette, IN). DE-71 is a mixture that consists primarily of tetra and penta congeners (see Sjodin, 2000). The stock DE-71 solution (300 mg/ml) was prepared by mixing the compound with corn oil and sonicating it for 30 min at 40°C. The desired dosing solutions (1, 10, or 30 mg/ml) were obtained by serial dilution with corn oil.

Dams were assigned to treatment groups in a semirandom, weight-balanced fashion before being treated. Body weights of dams were recorded and dosing volumes adjusted on a daily basis. They were orally dosed, via gavage, with DE-71 (0, 1, 10, or 30 mg/kg/day) from GD 6 through PND 21, except for PND 0 (day of birth) when dams were left undisturbed. The dams (GD 20 and PND 22) and offspring (GD 20 and PND 4, 14, 36, and 90) were decapitated for collection of trunk blood. Liver samples were removed immediately and frozen in liquid nitrogen. Serum was obtained after clotting whole blood on ice for approximately 1.5 h, followed by centrifugation at 2500 rpm at 4°C for 20 min. Due to the limited amount of samples collected for GD 20, PND 4, and PND 14 pups, serum or liver samples within a litter were pooled. For pups at ages PND 36 and PND 90, 1 male and 1 female pup per litter were randomly sampled for body weight measurement and collection of serum and liver samples. All serum and liver samples for each age point were obtained from a minimum of 8 litters, and were stored at −80°C until analysis for thyroid hormone (T_4 and T_3) concentrations and hepatic enzyme (EROD, PROD, and UDPGT) activity.

Thyroid hormone assay. Serum total concentrations of T_4 and T_3 were measured as previously described (Goldey and Crofton, 1998; Goldey *et al.*, 1995). Serum total T_4 and T_3 were measured in duplicate by using standard radioimmunoassay kits (Diagnostic Products Corp., Los Angeles, CA). Intra-assay and interassay coefficients of variance for the assays were below 10%. Since T_4 concentrations for GD20 fetuses were below 10 ng/ml, a standard curve ranging from 2.5 to 120 ng/dl, and double volume of serum samples (50 μl) were used. The sensitivity for our T_4 assay was 2.99 ng/ml, which resulted in 90.49% binding. Therefore, any result below this limit of quantitation (LOQ), i.e., above 90.49% specific binding, was recorded as 2.99 ng/ml. T_3 was not assayed in serum from GD20 fetuses, due to the limited sample volumes available.

Hepatic enzyme activity assay. Liver microsomal fractions were prepared as described previously (DeVito *et al.*, 1993). Microsomal protein concentrations were determined using the Bio-Rad protein assay kit (Bio-Rad, Richmond, CA) with bovine serum albumin as the standard. Hepatic microsomal EROD activity (a marker for CYP1A1 activity) and PROD activity (a marker of CYP2B activity) were assayed using the method of DeVito *et al.* (1993). All substrate concentrations were 1.5 nM. Both EROD and PROD values were calculated as pmol resorufin per mg protein per min, or per 30 min for GD 20 fetuses (all data corrected to per min rate). PROD and UDPGT activity were not measured for samples obtained from GD 20 fetuses, due to limited available sample.

Hepatic microsomal T_4-UDPGT activity was assayed as described in Zhou *et al.* (2001). Briefly, 100 μl microsomes (2 mg protein per ml Tris/HCl buffer) were incubated at 37°C with purified, radiolabeled T_4, 6-n-propyl-2-thiouracil (PTU), and UDPGA (or no UDPGA for blank) over a 30-min period. The reaction was stopped by addition of ice-cold methanol followed by centrifugation and mixing the supernatant with HCl. The formed glucuronyl T_4 (T_4-G), separated by chromatography on lipophilic sephadex LH-20 columns, was counted on the gamma-counter. The UDPGT activity was calculated as pmol T_4-G per mg protein per min.

Data analysis. All statistical analyses were performed on SAS© 6.12 (SAS Institute, Inc., Cary, NC). The litter was the statistical unit for all analyses. Analysis of variance (ANOVA) was used to analyze for effects of treatment and interactions. If there was more than one independent variable, significant interactions were followed by step-down ANOVA tests for each independent

TABLE 1
Reproductive Parameters for Long-Evans Rats following Perinatal Exposure to DE-71

Parameters	Control	1 mg/kg/day	10 mg/kg/day	30 mg/kg/day
Number of animals	47/38	47/39	55/48	55/45
Gestation length	21.3 ± 0.09	21.4 ± 0.09	21.4 ± 0.08	21.3 ± 0.07
Litter size at birth	12.5 ± 0.51	12.4 ± 0.45	12.6 ± 0.38	12.4 ± 0.45
Sex ratio at birth	1.10 ± 0.13	1.08 ± 0.12	1.11 ± 0.11	1.30 ± 0.18
Viability index	93.2 ± 0.03	99.7 ± 0.01	93.7 ± 0.03	99.4 ± 0.1

Note. For gestation length, litter size, sex ratio, and viability index, the data are presented as group means ± SE. Number of animals, number of dams dosed/number of dams with live births. Gestation length, day 0 of presumed gestation to the day the first birth was observed. Liter size, number of live pups on day of birth. Sex ratio at birth, number of female pups/number of male pups at PND1. Viability index, % pups that survived to PND 4 (before being culled).

variable (e.g., treatment and age). When more than one reading for each litter was obtained (i.e., body weights for male and female samples from the same litter) then a nested design was used, that is to say litter was nested under treatment. Repeated-measures ANOVAs were applied to data on dam body weights, offspring body weights (for PND 4, 7,14, and 21) and eye opening. Gestation and lactation body weight data for dams were analyzed with separate repeated-measures (day) ANOVAs because of the lack of lactation data for animals killed on GD 20. Body weights of dams were inadvertently not recorded on PND 1, and thus no data were used from this age in data analyses. Postweaning offspring body weights (i.e., from PNDs 36 and 90) were analyzed with a two-way ANOVA, with time and dose as independent variables, and litter nested under treatment. For eye opening data, only data from days 14 to 18 were analyzed due to an absence of eye opening in all groups prior to day 14. For significant effects of treatment, Duncan's Multiple Range test was used for mean contrast comparisons. The fetal T_4 data were analyzed with the Kruskal-Wallis test followed by a Dunn Multiple Comparison test (due to the lack of homogeneity of variance, see also Results section). A significance level of 0.05 was used for all statistical tests.

Benchmark dose (BMD) estimates were determined for alterations in thyroid hormones and hepatic enzyme activity using the U.S. EPA Benchmark Dose Software (BMDS, V 1.3). For each endpoint a BMD was estimated using the data from the age or time point that demonstrated the greatest potency and efficacy (see all figures and Table 1). The EROD and PROD data, as well as the T_4 and UDPGT data for neonates data were fit with the Hill model, as this function best describes the biological response. The T_4 data and UDPGT data from the dams were fit using the power model and a second-order polynomial, respectively, due to a lack of significant fit for the Hill model. The benchmark effect levels were set at 20% decreases for the thyroid hormone data and 50% increases for the liver enzyme data (Zhou *et al.*, 2001). The BMDLs (lower-bound confidence limit) were calculated as the 95% lower confidence interval for the BMD.

RESULTS

Reproductive Parameters

There was no evidence of any treatment-related effects of developmental exposure to DE-71 on any reproductive parameter. No treatment-related effect was detected for gestation length, litter size, or sex ratio ($F(3,138) < 1.04$, $p > 0.3760$; Table 1), nor did treatment affect the viability index ($F(3,138) = 2.26$; $p < 0.0838$; Table 1).

Body and Organ Weights

No evidence of treatment-related effects were found for maternal (Fig. 1A) or offspring body weights (Fig. 1B). For body weights of dams, repeated-measures analysis showed neither a treatment-by-age interaction during gestation ($F(42,451) = 0.83$, $p < 0.7549$) or lactation ($F(57,287) = 0.98$, $p < 0.5186$). Nor was there any main effect of treatment

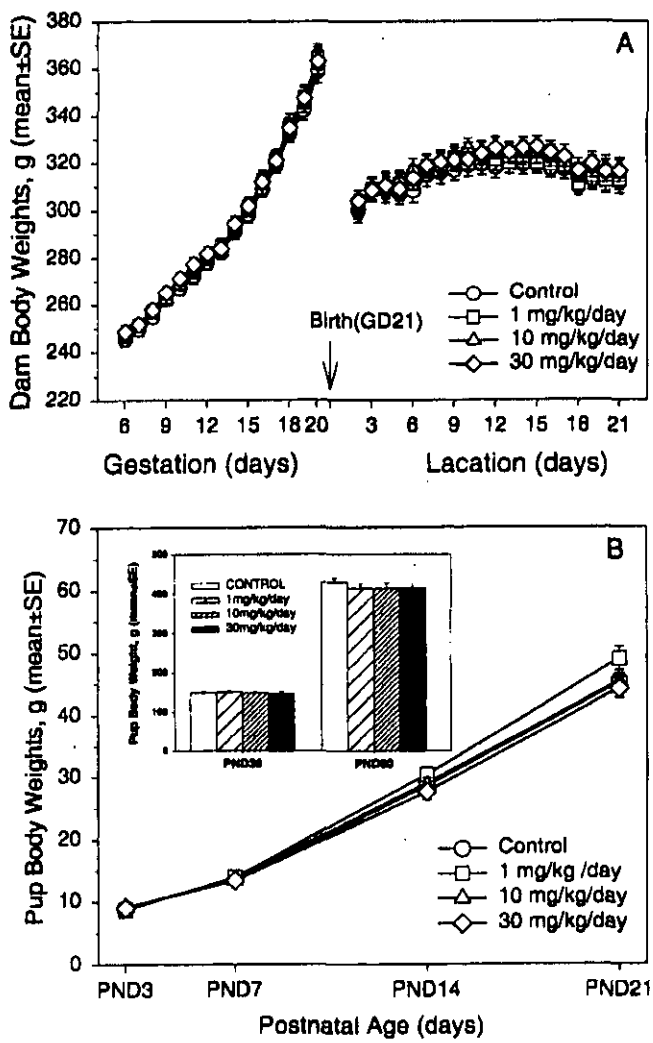

FIG. 1. Lack of effect of perinatal exposure to DE-71 on body weights of dams (A) and offspring (B). Data are presented as group means ± SE; symbols with no apparent error bars have error estimates hidden by the symbol.

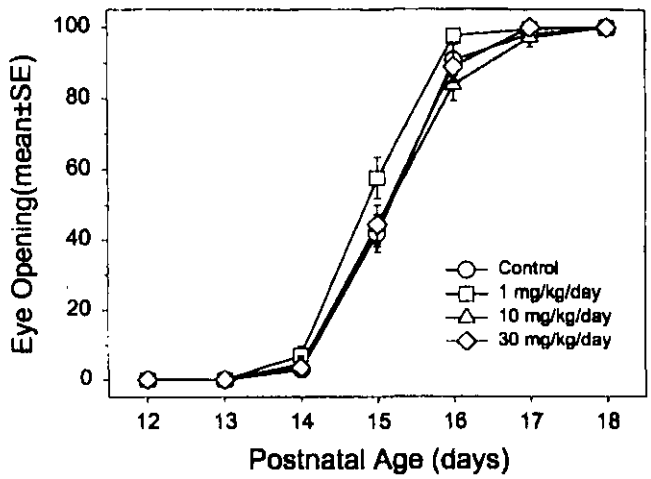

FIG. 2. There was a lack of effect of DE-71 on eye opening, determined as the percentage of all pups within a litter with at least one eye open at each age. Data are presented as group means ± SE; symbols with no apparent error bars have error estimates hidden by the symbol.

(gestation: $F(3,165) = 0.22$, $p < 0.8853$; lactation: $F(3,114) = 0.27$, $p < 0.8438$). Consistent with the overall changes in body weight due to pregnancy, there were main effects of age during both gestation ($F(14,152) = 402.22$, $p < 0.0001$) and lactation ($F(19,96) = 37.16$, $p < 0.0001$; Fig. 1). For preweaning offspring body weights, there were no significant interactions of treatment with any other variables (all F values < 1.44, p values > 0.2445), nor was there a main effect of treatment ($F(3,41) = 0.94$, $p < 0.4296$). Consistent with postnatal growth, there was a main effect of age ($F(3,39) = 618.37$, $p < 0.0001$). There were no significant interactions of treatment with any other variables (all F values < 0.42, p values > 0.6963), nor was there a main effect of treatment ($F(3,70) = 0.25$, $p < 0.8620$). There was a significant interaction of gender and age ($F(1,70) = 313.63$, $p < 0.0001$) that was consistent with age-related increases in body weight (Fig. 1), and more so in males than in females (body weight data not shown by gender).

Eye opening was first observed on PND 14, and all groups showed 100% eye opening by PND 18 (Fig. 2). Repeated measures ANOVA revealed no main effect of treatment on eye opening ($F(4,118) = 1.99$, $p < 0.1191$), nor at any age by treatment interaction ($F(12,304) = 0.82$, $p < 0.6263$). There was a highly significant main effect of age ($F(4,115) = 1734.49$, $p < 0.0001$) reflecting the normal ontogeny of eye opening (Fig. 2).

Exposure to DE-71 caused an increase in liver weight in both pregnant and lactating dams. There was a significant treatment-related increase in liver-to-body weight ratio in the 30 mg/kg/day group at both ages compared to controls (Fig. 3A). This increased ratio was due to an increase in liver weights of approximately 8% in the high dose, relative to controls. This was confirmed by a main effect of treatment ($F(3,81) = 3.18$, $p < 0.0282$), but no treatment-by-age interaction ($F(3,81) = 0.34$, $p < 0.7951$). There was also a main effect of age ($F(1,81) = 129.84$, $p < 0.0001$) that reflects an

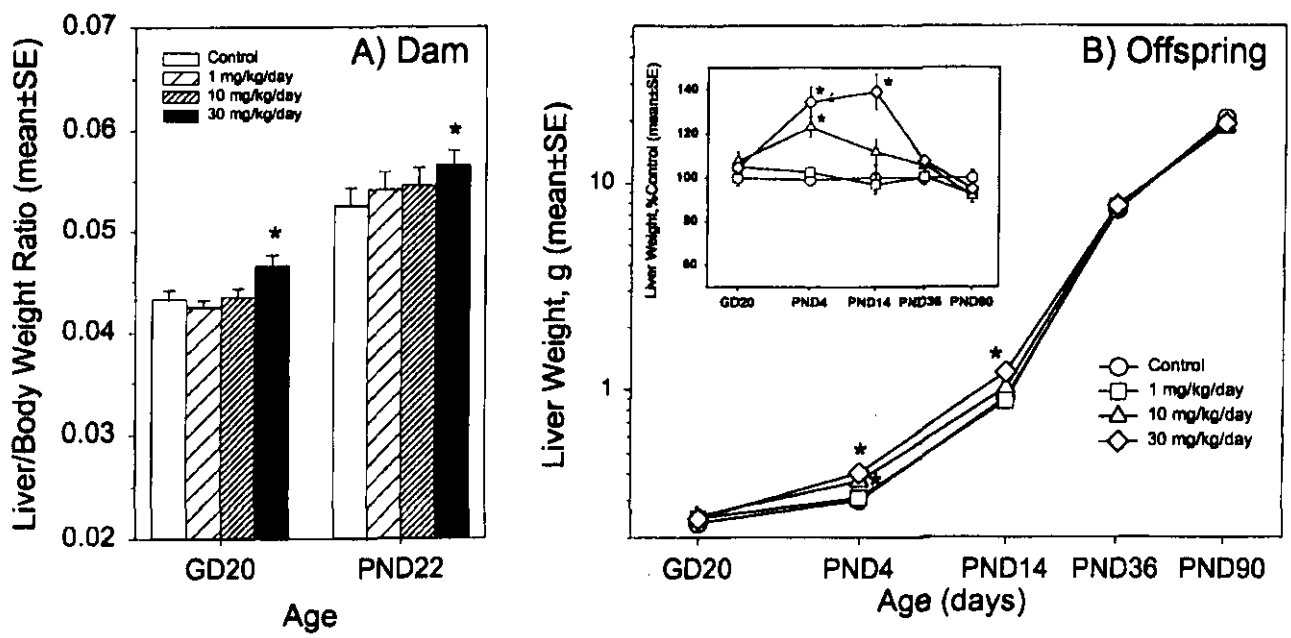

FIG. 3. Developmental exposure to DE-71 significantly increased liver-to-body weight ratios in dams (A), and significantly increased liver weights in offspring during the early postnatal period (B). Inset: data from panel B expressed as a percent of daily control means. Data are presented as group means ± SE; symbols with no apparent error bars have error estimates hidden by the symbol. *Significantly different from the respective age control, $p < 0.05$.

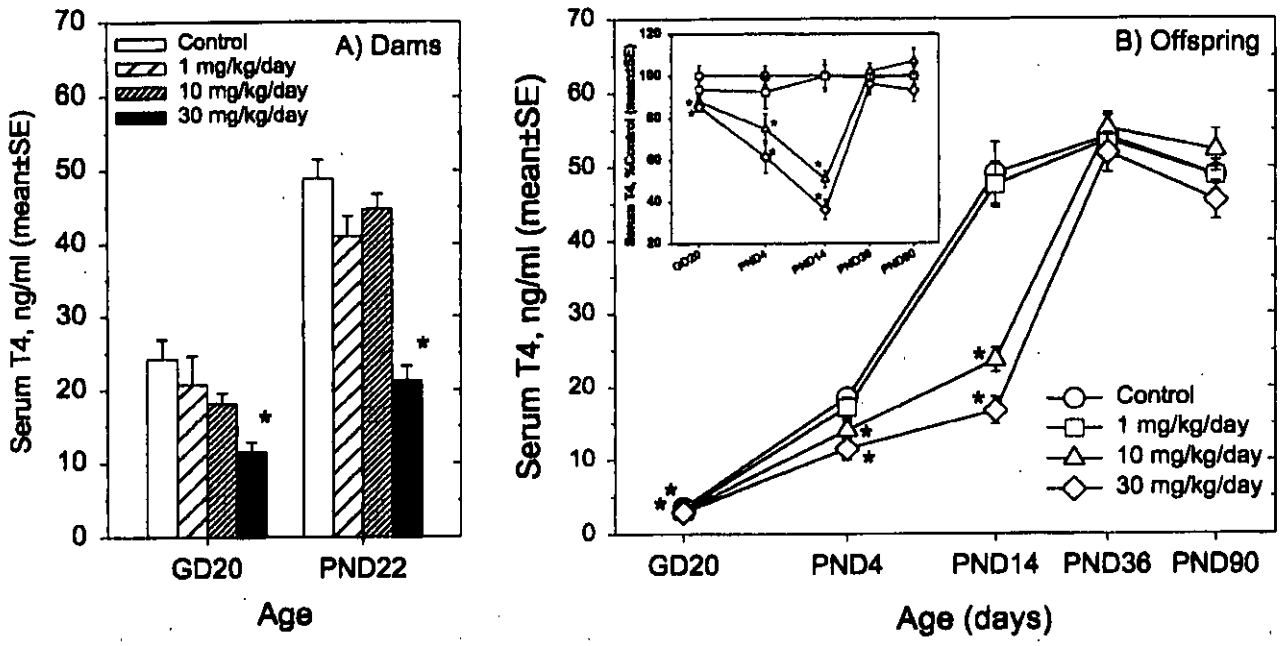

FIG. 4. Serum total T_4 concentrations of dam, fetus, and offspring exposure to DE-71 during gestation and lactation. T_4 concentrations were decreased in dams at the highest dose (A), and fetuses and offspring at the middle and high doses (B). Inset: data from panel B expressed as a percent of daily control means. There was no detectable effect on serum total T_3 concentrations in dams or offspring (data not shown). Data are presented as group means ± SE; symbols with no apparent error bars have error estimates hidden by the symbol. *Significantly different from the respective age control, $p < 0.05$.

overall increase in liver-to-body weight ratios from GD 20 to PND 22 (Fig. 3A).

Maternal exposure to DE-71 caused dose-dependent increases in liver weights in offspring during the early preweaning period that returned to control levels by PND 36 (Fig. 3B). This increase was much greater than that seen in the dams. Maximal increases in offspring were 35% and 39% above controls at PND 4 and PND 14, respectively (compared to 8% increases in the dams). Statistically, these conclusions were supported by a significant treatment-by-age interaction ($F(12,203) = 5.25$, $p < 0.0001$), a main effect of treatment ($F(3,12) = 19.28$, $p < 0.0001$), and an effect of age ($F(4,12) = 13.68$, $p < 0.0001$). Step-down ANOVAs at each age indicated significant effects of treatment on PND 4 ($F(3,51) = 13.93$, $p < 0.0001$) and PND 14 ($F(3,50) = 9.52$, $p < 0.0001$). Results of mean contrast comparisons at each age sampled are illustrated in Figure 3B.

Thyroid Hormones

Perinatal maternal exposure to DE-71 caused a decrease in serum total T_4 in dams, fetuses, and offspring (Fig. 4). The effects in the dam were present during both gestation and lactation. On GD 20 there was a significant decrease only in the high dose (48% relative to controls). On PND 22 there again was only a significant decrease in the high-dose group (44% relative to controls). These inferences were supported by a significant treatment-by-age interaction ($F(3,83) = 4.30$, $p < 0.0071$) that resulted from a slightly larger dose effect on PND 22 and a high overall serum concentration of T_4 on PND 22 (Fig. 4A). For dams, there were main effects of treatment at GD 20 ($F(3,47) = 4.23$, $p < 0.0099$) and PND 22 ($F(3,36) = 27.37$, $p < 0.0001$). Results of mean contrast comparisons at each age sampled are illustrated in Figure 4A.

The effects of maternal exposure to DE-71 on T_4 concentrations in fetuses and offspring were age-dependent. Fetal serum concentrations of total T_4 in the 10 and 30 mg/kg/day groups were significantly decreased compared to controls ($p < 0.05$). However, the extent of this decrease is uncertain. The concentration of serum total T_4 in the control fetuses was approximately 3.5 ± 0.2 ng/dl. Because the LOQ for T_4 was 2.99 ng/dl, this resulted in an assay that was only able to detect a maximal reduction of ~15% relative to the control group. The ratio of the number of samples below the LOQ over the total number of samples per group were: controls, 3/12; 1 mg/kg/day, 7/12; 10 mg/kg/day, 11/12; and 30 mg/kg/day, 12/12. All samples for all other ages were above the LOQ. On PND 4 and PND 14, significant dose-dependent decreases were observed in the 2 highest dose groups with maximal decreases of 40% on PND 4 and 66% on PND 14. Serum total T_4 concentrations returned to control levels by PND 36 and remained unaffected on PND 90. These conclusions were supported by a significant interaction of treatment and age ($F(12,229) = 11.23$, $p < 0.0001$). Step-down ANOVAs by age revealed significant effects of treatment on PND 4 ($F(3,50) =$

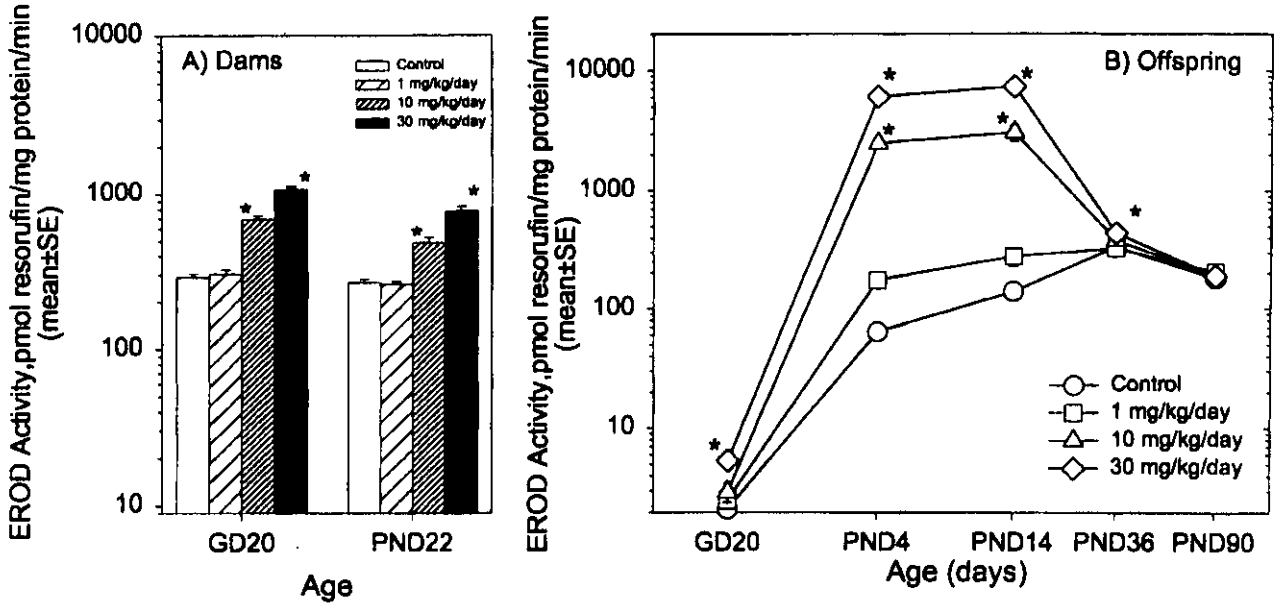

FIG. 5. Developmental exposure to DE-71 caused an increase in hepatic microsomal EROD activity for dams, fetuses, and offspring. Data are presented as group means ± SE; symbols with no apparent error bars have error estimates hidden by the symbol. *Significantly different from the respective age control, $p < 0.05$.

6.61, $p < 0.0008$) and PND 14 ($F(3,49) = 29.41$, $p < 0.0001$). There was a significant main effect of age ($F(4,219) = 377.37$, $p < 0.0001$) that reflected normal age-related increases in T_4 concentrations. For the fetal T_4 data, there was a main effect of treatment ($\chi^2(3) = 16.94$, $p < 0.0007$). Results of the mean contrasts are illustrated in Figure 4B.

There were no treatment-related effects of developmental DE-71 exposure on serum total T_3 concentrations in either the dams or the offspring (data not shown). There were no significant main effects of treatment, nor any interactions of treatment and age for either dams or pups (all $p > 0.5$). There was a significant effect of age in dams ($F(1,83) = 7.35$, $p < 0.0081$) that reflects a slightly lower (~10%) concentration of T_3 in the PND 22 dams (88.75 ± 5.3 ng/dl) compared to GD 20 dams (98.72 ± 3.7 ng/dl). There was also a significant effect of age in offspring ($F(3,159) = 214.57$, $p < 0.0001$) that reflects an age-related increase in serum total T_3 (in controls: 23.89 ± 3.33 ng/dl at PND 4; 84.27 ± 6.38 ng/dl at PND 14; 119.08 ± 5.06 ng/dl at PND 36; and 109.79 ± 5.22 ng/dl at PND 90).

Hepatic Enzyme Activity

Maternal exposure to DE-71 resulted in significant increases in hepatic EROD, PROD, and UDPGT activity in both dams and offspring (Figs. 5, 6, and 7).

Hepatic EROD activity was slightly increased in dams on GD 20 compared to PND 22 (Fig. 5A). There were dose-dependent increases in EROD activity of 2.4-fold and 3.7-fold on GD 20, and 1.8-fold and 2.9-fold on PND 22, in the 10 and 30 mg/kg/day groups, respectively. These conclusions were confirmed statistically with a significant treatment-by-age interaction ($F(3,81) = 7.95$, $p < 0.0001$), and significant effects of treatment on GD 20 ($F(3,45) = 134.92$, $p < 0.0001$) and PND 22 ($F(3,36) = 58.43$, $p < 0.0001$). There was no effect of the 1 mg/kg/day dose on EROD activity.

Hepatic EROD activity in fetuses and offspring was increased as a result of maternal exposure to DE-71 (Fig. 5B). Fetal EROD activity was significantly increased 2.5-fold in the high-dose group on GD 20. Offspring EROD activity was significantly increased, 39-fold and 95-fold, on PND 4 and 20-fold and 57-fold on PND 14 in the 10 and 30 mg/kg/day groups, respectively. There was a much smaller, yet significant, increase of 0.5-fold in the high-dose group on PND 36. There were no treatment-related changes in EROD activity on PND 90. These effects were confirmed by a significant treatment-by-age interaction ($F(12,219) = 100.99$, $p < 0.0001$) and significant main effects of treatment on GD 20 ($F(3,26) = 7.32$, $p < 0.0010$), PND 4 ($F(3,49) = 219.61$, $p < 0.0001$), PND 14 ($F(3,48) = 137.40$, $p < 0.0001$), and PND 36 ($F(3,40) = 3.96$, $p < 0.0146$). There was also a main effect of age ($F(4,219) = 227.15$, $p < 0.0001$) that resulted from the large treatment-related increases at the younger ages, as well as increases in basal activity in control samples as a function of age (Fig. 5B). Results of mean contrast comparisons at each age sampled are illustrated in Figure 5B.

Hepatic PROD activity was increased slightly more in dams on PND 22 compared to GD 20 (Fig. 6A). There were dose-dependent increases in PROD activity of 9-fold and 19-fold on GD 20, and 9-fold and 24-fold on PND 22 in the 10 and 30 mg/kg/day groups, respectively. These conclusions were confirmed statistically with a significant treatment-by-age interac-

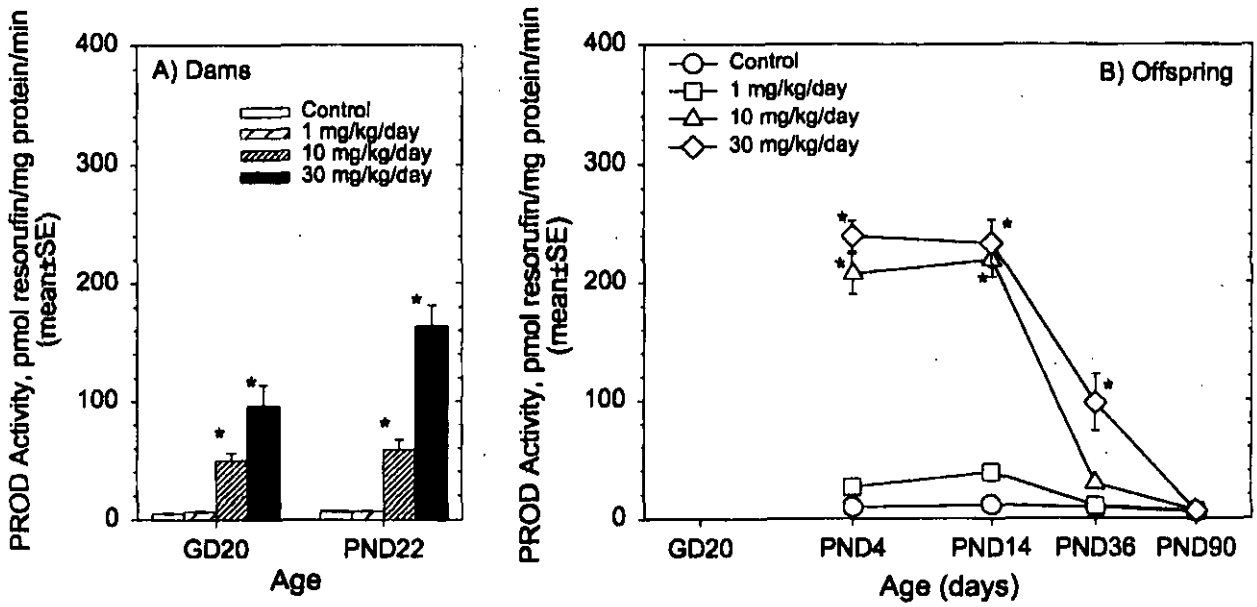

FIG. 6. Developmental exposure to DE-71 caused an increase in hepatic microsomal PROD activity for dams and offspring. Data are presented as group means ± SE; symbols with no apparent error bars have error estimates hidden by the symbol. *Significantly different from the respective age control, $p < 0.05$.

tion ($F(3,83) = 5.87, p < 0.0011$), and significant main effects of treatment on GD 20 ($F(3,45) = 24.52, p < 0.0001$) and PND 22 ($F(3,36) = 56.49, p < 0.0001$). There was no effect of the 1 mg/kg/day dose on PROD activity.

Hepatic PROD activity in offspring was increased as a result of maternal exposure to DE-71 (Fig. 6B). Offspring PROD activity was significantly increased by 21- and 26-fold on PND 4 and 19- and 21-fold on PND14, in the 10 and 30 mg/kg/day groups, respectively. There was a significant increase of 10-fold in the high-dose group on PND36. There were no treatment-related changes in PROD activity on PND 90. These effects were confirmed by a significant treatment-by-age inter-

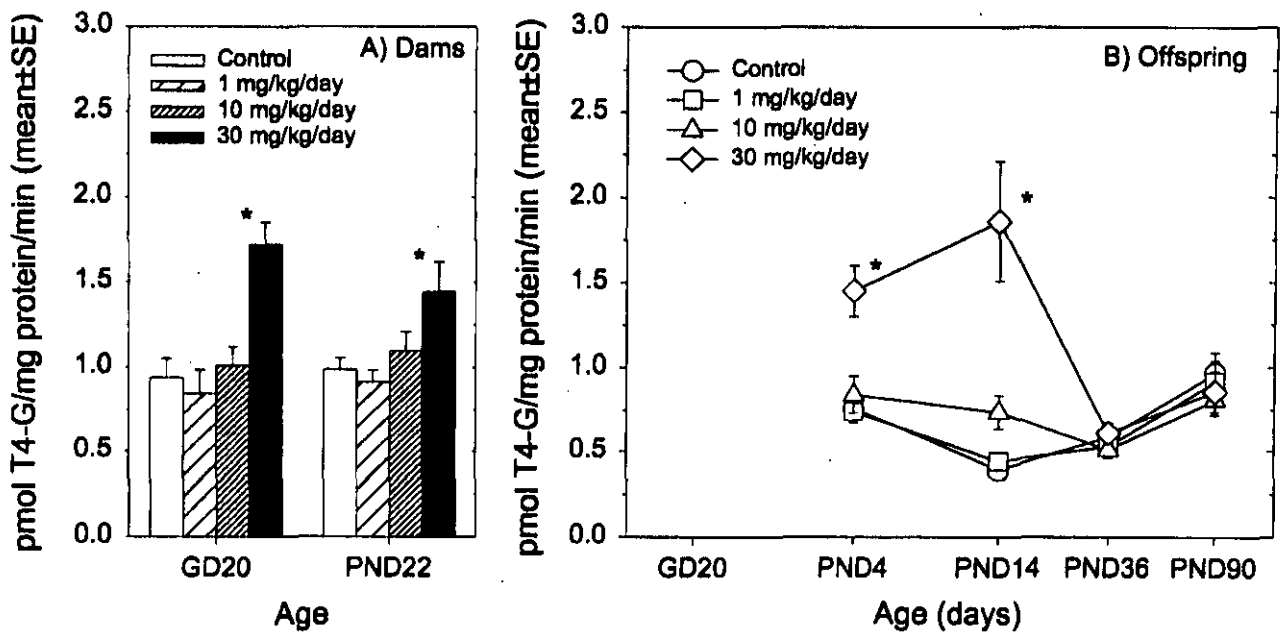

FIG. 7. Developmental exposure to DE-71 caused an increase in hepatic microsomal T_4-UDPGT activity in dams and offspring. Data are presented as group means ± SE; symbols with no apparent error bars have error estimates hidden by the symbol. *Significantly different from the respective age control, $p < 0.05$.

action ($F(9,193) = 40.64$, $p < 0.0001$) and significant main effects of treatment on PND 4 ($F(3,49) = 125.90$, $p < 0.0001$), PND 14 ($F(3,48) = 81.59$, $p < 0.0001$), and PND 36 ($F(3,40) = 11.89$, $p < 0.0001$). There was also a main effect of age ($F(3,193) = 158.79$, $p < 0.0001$) that resulted from the large treatment-related increases at the younger ages. In contrast to EROD activity, PROD activity did not vary significantly as a function of age in control samples (Fig. 6B). Results of mean contrast comparisons at each age sampled are illustrated in Figure 6B.

The effects of perinatal maternal exposure to DE-71 on hepatic UDPGT activity, measured as T_4 glucuronidation, is illustrated in Figure 7. Exposure to DE-71 caused increases in the rate of glucuronidation of T_4 in both dams and offspring. In dams, there was a similar increase of about 1.6-fold in UDPGT activity, only in the high-dose groups, on both GD 20 and PND 22 (Fig. 7A). There were no effects on UDPGT activity detected in the two lower doses. These conclusions were confirmed statistically by a nonsignificant treatment-by-age interaction ($F(3,55) = 1.04$, $p < 0.3839$), and a significant main effect of treatment ($F(3,55) = 13.52$, $p < 0.0001$). There was no main effect of age ($F(1,55) = 0.05$, $p < 0.8190$) reflecting a similar basal activity level on both days.

Hepatic UDPGT activity in offspring was increased as a result of maternal exposure to DE-71 (Fig. 7B). Offspring UDPGT activity was significantly increased 1.9-fold and 4.7-fold on PNDs 4 and 14, respectively, in the 30 mg/kg/day group. There was no significant effect of any lower doses on PNDs 4 or 14. There were no effects of exposure on PND 36 or PND 90. These effects were confirmed by a significant treatment-by-age interaction ($F(9,146) = 5.89$, $p < 0.0001$) and significant main effects of treatment on PND 4 ($F(3,44) = 10.65$, $p < 0.0001$) and PND14 ($F(3,50) = 14.17$, $p < 0.0001$). There was also a main effect of age ($F(3,193) = 158.79$, $p < 0.0001$) that resulted from the large treatment-related increases at the younger ages. There was a small decrease in basal UDPGT activity in control offspring on PND 14 (0.39 ± 03 pmol T_4-G/mg protein/min) compared to PND 4 (0.75 ± 0.06), PND 36 (0.59 ± 0.07) and PND 90(0.89 ± 0.12). Results of mean contrast comparisons at each age sampled are illustrated in Figure 7B.

NOELs and model estimates for BMDs and BMDLs are shown in Table 2. The relationship for NOELs and BMDs vary by endpoint. These variations are likely due to the effects of data variability and dose spacing on the NOEL estimates. Based on visual inspection of the data, BMD estimates appeared to be better approximations of potency. BMD estimates for all endpoints were lower for neonates compared to dams. BMDs for EROD and PROD were up to an order of magnitude lower in neonates when compared to dams, whereas there was only a 2–4-fold difference for T_4 and UDPGT (Table 2).

TABLE 2
NOEL, BMD, and BMDL Estimates for the Effects of Developmental Exposure to DE-71

	Neonate			Dam		
Response	NOEL	BMD	BMDL	NOEL	BMD	BMDL
Serum T_4	1	2.36	0.94	10	6.13	4.03
EROD	1	0.43	0.31	1	4.01	2.4
PROD	1	0.48	0.36	1	3.8	1.66
UDPGT	10	5.50	3.41	10	21.05	11.22

Note. All values are in mg/kg/day. Data for neonatal T_4 and UDPGT are from PND 14; neonatal EROD and PROD data are from PND 4. Data for dams from GD 20, except PROD from PND 22.

DISCUSSION

Developmental exposure to DE-71 caused a significant reduction in serum T_4 in both dams and offspring. This exposure regimen did not affect dam or offspring body weights, nor did it alter sex ratio, litter size, or postnatal survival. Hepatic enzyme activity (EROD, PROD, and UDPGT) among PBDE-treated dams and offspring were significantly increased compared to controls, but the magnitude of the increase was higher among the offspring relative to the dams. Following cessation of exposure, there was a full recovery in T_4 and UDPGT on PND 36, while hepatic EROD and PROD activity had not completely returned to control levels until PND 90. Consistent with increased hepatic metabolic activity were increased liver weights in both dams and offspring.

The current study demonstrated a perinatal hypothyroxinemia following DE-71 developmental exposure. DE-71 caused dose- and time-dependent reductions in serum total T_4 concentrations in fetal and postnatal rats (GD 20, PND 4, and PND 14), with a maximal reduction of 66% occurring at the highest doses on PND 14. There was a complete recovery in hypothyroxinemia in rats on PND 36, 15 days after cessation of lactation exposure. It is important to note that the large number of samples below the LOQ suggest that the magnitude of reduction in T_4 during the fetal period is uncertain and may be underestimated. The effect of DE-71 on T_4 was less pronounced in dams than in offspring. This is reflected in lower NOEL (10-fold) and BMD (2-fold) values in the offspring compared to dams and indicates that the offspring are more sensitive to the effects of DE-71 than pregnant dams. Serum total T_3 was not affected by DE-71 in either dams or offspring at any time point sampled.

The effects of DE-71 reported here, decreases in T_4 with no significant changes in T_3, are consistent with previous reports on the effects of PBDE exposures, and extends those findings to include effects in the dam, fetus, and developing offspring. Norris (1975) first reported the thyrotoxic effects of PBDE compounds in a 30-day exposure in adult rats to octa- and deca-BDE mixtures, which resulted in thyroid hyperplasia.

Fowles *et al.* (1994) first showed that T_4 was decreased in mice exposed for 14 days to 18, 36, or 72 mg/kg/day of DE-71. Darnerud and Sinjari (1996) demonstrated decreased total plasma T_4 in both rats and mice exposed for 14 days to 18 or 36 mg/kg/day of Bromkal 70. These same authors also exposed mice to 18 mg/kg/day of BDE-47 and found a 31% decrease in total plasma T_4. Hallgren and Darnerud (1998) found decreases in both total and free plasma T_4 with no increase in TSH following a 14-day exposure of female rats to 18 mg/kg/day BDE-47. In a 90-day study, T_4 concentrations were decreased, but T_3 concentrations were not altered in rats administered doses as high as 100 mg/kg/day DE-71 in the diet (IPCS, 1994). This is consistent with a previous 4-day exposure study in weanling rats (Zhou *et al.*, 2001), where there was no significant reduction in T_3 at doses up to 30 mg/kg/day. Rosiak *et al.* (1997) reported that maternal exposure to individual chlorinated diphenyl ether congeners (2,2',4,4',5,5'-hexachlorodiphenyl ether; 2,2',4,5,6'-pentachlorodiphenyl ether) depressed T_4 in dams during gestation and in preweaning offspring. These chlorinated diphenyl ether congeners did not affect serum T_3 or TSH concentrations in maternal or juvenile rats. The mechanism(s) responsible for the lack of effects of DE-71 on serum T_3 are currently unknown. Possible mechanisms include increased tissue-specific conversion of T_4 to T_3 due to increased deiodinase activity (Raasmaja *et al.*, 1996). Alternatively, there may be an increased hepatic catabolism and clearance of T_4, and not T_3. Recent work by Hood and Klaassen (2000) has demonstrated that Aroclor 1254 (A1254), a polychlorinated biphenyl mixture, decreases serum T_4 and induces glucuronidation of T_4, but does not alter serum T_3 concentrations or T_3 glucuronidation (see also discussion below). In general, the data presented herein clearly demonstrate that PBDEs adversely impact circulating concentrations of T_4.

The data in this paper do not show a clear relationship between serum T_4 depletion and induction of the T_4-UDPGT activity. Significant reduction in T_4 was observed in doses as low as 10 mg/kg/day for dams at GD 20 and PND 22, and for offspring at PNDs 4 and 14. However, only the 30 mg/kg/day treatment groups showed significant induction of T_4-UDPGT activity. One explanation for the inconsistency between T_4 concentrations and UDPGT activity is that the T_4-UDPGT assay may be a better biomarker for the isoforms of UDPGT that are induced by Ah-receptor agonists compared to phenobarbital-like agonists (Craft *et al.*, 2001). A second possible explanation for this lack of correlation is that PBDE congeners and metabolites, as well as structurally similar PCBs, are known to displace T_4 from transthyrethin (TTR), the major protein that transports thyroid hormones in rats and mice (Chauhan *et al.*, 2000; Cheek *et al.*, 1999; Meerts *et al.*, 2000). While the exact role this mechanism plays in the regulation of serum concentrations of T_4 is unknown, displacement of serum T_4 could lead to increased glucuronidation and a consequent lowered serum concentration of T_4. PBDEs may also have direct effects on the thyroid gland (Brouwer *et al.*, 1998). However, previous studies (Darnerud and Sinjari, 1996; Zhou *et al.*, 2001) found no evidence of increased thyroid stimulating hormone (TSH). Previous work with other PHAHs, such as A1254 and some chlorinated diphenyl ethers, have also failed to find any upregulation of TSH during development (Goldey *et al.* 1995; Morse *et al.*, 1996; Rosiak *et al.*, 1997). This indicates a lack of activation of the hypothalamic-pituitary-thyroid feedback process normally found with direct acting thyrotoxicants (Capen, 1997; DeVito *et al.*, 1999). Interestingly, long-term exposure studies to the deca-BDE have found small increases in the rate of thyroid hyperplasia and neoplasia (Norris *et al.*, 1975; NTP, 1986). However, no long-term cancer bioassays have been conducted on the tetra-, penta-, or octa-BDEs. A combination of the above mentioned mechanisms might be ultimately responsible for the difference between measured increases in UDPGT activity and decreases in serum T_4 concentrations.

Developmental exposure to DE-71 resulted in increased hepatic metabolic activity in dams, fetuses, and offspring. DE-71 exposure resulted in increased EROD, PROD, and UDPGT activity in dams during both gestation and lactation, with the amount of induction fairly similar at both time points. There was a slightly larger increase in EROD induction on GD 20 (3.7-fold) compared to PND 22 (2.9-fold) and a slightly higher PROD induction on PND 22 (24-fold) compared to GD 20 (19-fold). There was no statistically significant effect of age for UDPGT induction (1.6-fold). It is unlikely that the small difference in EROD activity prenatally versus postnatally is biologically significant. These data suggest that the level of induction of hepatic metabolizing enzymes, as measured by EROD, PROD, and UDPGT activity, is relatively similar at the end of pregnancy and the end of lactation. This is the first report of increased hepatic Phase-1 and Phase-2 activity by PBDEs in pregnant animals. Previous reports have found increased liver weights in pregnant rabbits exposed to Saytex 111, a commercial mixture consisting mostly of hepta- and octa-BDEs (Breslin *et al.*, 1989). Norris *et al.* (1975) reported no effect of a deca-BDE mixture on liver weights in dams from a rat teratology study.

Developmental exposure to DE-71 resulted in increased hepatic microsomal enzyme activity at both fetal and early postnatal time periods. EROD and PROD activity were increased on PND 4, PND 14 and PND 36, with activity returning to control levels by PND 90. UDPGT activity was increased on PND 4 and PND 14, and recovered to control levels by PND 36. Consistent with increased hepatic metabolic activity were increased liver weights in the offspring. Important to note was that EROD was also increased on GD 20. This supports a conclusion of significant fetal exposure to DE-71 and/or metabolites. Liver weights in fetuses were not statistically different. These effects are consistent with a number of previous reports on the effects of PBDE exposure in weanling or adult rats and mice. Carlson (1980a; 1980b) showed that both 14- and 90-day exposures to penta- and otca-BDE mixtures increased hepatic benzo-[*a*]-pyrene and p-nitroanisole metabolism. von Meyerinck *et al.* (1990) found increased

EROD and benzphetamine N-demethylation activity in hepatic tissue from mice treated for 14 days with Bromkal 70. Increased EROD and PROD activity were found in mice exposed to DE-71 for 14 days, but only increased PROD was found following acute exposure (Fowles *et al.*, 1994). More recently, Hallgren and Darnerud (1998) reported increased EROD (3-fold) and PROD (14-fold) activity in rats after 14 days of exposure to BDE-47.

The increase in hepatic metabolic capacity has a number of important implications for the toxicity of PBDEs. First, PBDE mixtures have been suggested to be either solely phenobarbital-type inducers, such as DE-71 and DE-79 (Carlson, 1980a), or mixed-type inducers (i.e., phenobarbital and dioxin-like substances) of xenobiotic metabolism such as Bromkal 70 (von Meyerinck *et al.*, 1990) or DE-71 (Zhou *et al.*, 2001). The present data support the conclusion that DE-71 is a mixed-type inducer in both pregnant rats and offspring during the early postnatal period. Second, the effects of DE-71 on fetal EROD activity following maternal exposure, together with decreased fetal T_4 concentrations, suggest placental transfer and fetal exposure to DE-71 congeners and/or metabolites. This is consistent with data from maternal exposure to other PHAHs such as dioxins and PCBs. Third, a comparison of maternal/fetal and maternal/offspring ratios of EROD activity suggests a much greater postnatal exposure to DE-71 components or their metabolites. EROD activity has been suggested as a biomarker of exposure to Ah-active compounds (Lagueux *et al.*, 1999; Sewall *et al.*, 1995; Whyte *et al.*, 2000). EROD activity was induced to a fairly similar degree in dams and fetuses on GD 20 (3.7-fold for dams, and 2.5-fold for fetuses). In contrast, induction of EROD activity was much greater in the postnatal offspring (95-fold) compared to the postnatal dam (2.9-fold). Thus, it is interesting to speculate whether there was a much greater magnitude of exposure to the postnatal offspring compared to both the fetus and the dam. Greater postnatal exposure to offspring via lactation has been demonstrated previously where compounds like TCDD and PCBs are transferred placentally to the fetus in limited quantities compared to the amount delivered via lactation (Crofton *et al.*, 2000; Masuda *et al.*, 1978; Takagi *et al.*, 1986; Vodicnik and Lech, 1980). Confirmation of this hypothesis will require developmental toxicokinetic studies of PBDEs.

Lastly, there were neither significant adverse effects on dam or offspring body weight, nor effects on postnatal survival, sex ratio at birth, or litter size. These data indicate that DE-71, at the dosages examined, did not produce overt toxicity in either dams or offspring. These findings were consistent with other studies on commercial penta-BDE (IPCS, 1994). Commercial penta-BDE, as high as 100 mg/kg in the diet during gestation and lactation, had no effects on the number of pregnancies or on survival and weight of the neonates. For pregnant rats given commercial penta-BDE from GD 6 through 15, inhibition of maternal body weight gain only occurred above doses of 100 mg/kg.

In conclusion, developmental exposure to DE-71 reduced circulating T_4 concentrations and induced hepatic EROD, PROD, and UDPGT activity in both dams and offspring. The T_4-depleting effects of DE-71 are likely to involve multiple mechanisms of action. These data demonstrate that DE-71 is an endocrine disrupter in rats during development.

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Teratogenic Effects of 2,3,7,8-Tetrabromodibenzo-*p*-dioxin and Three Polybrominated Dibenzofurans in C57BL/6N Mice[1]

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Teratogenic Effects of 2,3,7,8-Tetrabromodibenzo-*p*-dioxin and Three Polybrominated Dibenzofurans in C57BL/6N Mice. BIRNBAUM, L. S., MORRISSEY, R. E., AND HARRIS, M. W. (1991). *Toxicol. Appl. Pharmacol.* **107**, 141–152. Brominated flame retardants involved in many industrial uses contain polybrominated dibenzo-*p*-dioxins (PBDDs) and dibenzofurans (PBDFs) as contaminants. The levels of these contaminants can be dramatically increased by combustion. These chemicals are closely related in structure to the polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs), of which 2,3,7,8-tetrachloridibenzo-*p*-dioxin (TCDD) is the most toxic isomer. TCDD and related PCDFs are potent mouse teratogens inducing cleft palate and hydronephrosis at doses below those at which overt maternal and embryo/fetal toxicity occurs. This study examines the teratogenic effects of 2,3,7,8-tetrabromodibenzo-*p*-dioxin (TBDD), 2,3,7,8-tetrabromodibenzofuran (TBDF), 1,2,3,7,8-pentabromodibenzofuran (1PeBDF), and 2,3,4,7,8-pentabromodibenzofuran (4PeBDF) in C57BL/6N mice treated on gestation day (gd) 10 and examined on gd 18. Pregnant dams were treated with 0–4000 μg of each congener per kilogram body weight in 10 ml corn oil/kg. Dose selection was based on the relative toxicity of the chlorinated isomers. Maternal toxicity and developmental toxicity were assessed, and the hard palate and kidney, the target organs for the teratogenic effects of TCDD and related compounds, were examined for structural abnormalities. While the maternal liver weight increased at all dose levels examined for all four compounds, there was no evidence of any maternal toxicity. Embryo/fetal mortality was increased only at ≥500 μg TBDF/kg, while fetal weight increased in a dose-related manner following exposure to TBDD and TBDF. All compounds produced hydronephrosis (HN) at doses below that at which cleft palate (CP) occurred. The incidence of HN was significantly increased above background levels at the following doses (μg/kg): TBDD, 3; TBDF, 25; 1PeBDF, 500; 4PeBDF, 400. The LOELs (μg/kg) for CP were: TBDD, 48; TBDF, 200; 1PeBDF, 4000; 4PeBDF, 2400. The cleft palate incidence for all four brominated compounds and TCDD could be fit to a common slope, compatible with the concept that these chemicals all exert their teratogenic effects through a common mechanism. The potency of these chemicals, relative to TCDD as 1 for the induction of cleft palate, is TBDD, 0.24; TBDF, 0.10; 1PeBDF, 0.004; and 4PeBDF, 0.005. Previous studies from our laboratory had determined that the chlorinated dibenzofuran isomers had relative potencies of 0.05 (TCDF), 0.03 (1PeCDF), and 0.09 (4PeCDF). Thus, bromination decreases the teratogenic activity of TBDD relative to TCDD and of both 1- and 4PeBDF relative to the chlorinated isomers. However, substitution of bromines for chlorines increases the potency of TBDF relative to TCDF. Thus, while these brominated isomers induce hydronephrosis and cleft palate as do their chlorinated congeners, their relative potencies have been altered.

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Brominated flame retardants are a potential source of polybrominated dibenzo-*p*-dioxins (PBDDs) and dibenzofurans (PBDFs) in the environment (Donnelly *et al.*, 1989). Thermolysis or pyrolysis of compounds containing brominated diphenyl ethers and biphenyls can result in the formation of complex mixtures of PBDDs and PBDFs (Buser, 1986). In addition, PBDFs are present as contaminants in brominated diphenylether flame retardant formulations and in plastics containing them (U.S. EPA, 1988). This could lead to exposure in the occupational setting as well as to general environmental exposure. Although few data regarding the actual environmental occurrence of PBDDs and PBDFs exist, their structural similarity to the chlorinated congeners make them important subjects of toxicological evaluation. In fact, recent studies have indicated that the toxic effects of two of these chemicals, 2,3,7,8-tetrabromodibenzo-*p*-dioxin (TBDD) and 2,3,7,8-tetrabromodibenzofuran (TBDF), are similar to those observed for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the most toxic representative of this class. Using both the rabbit ear bioassay for detecting acnegic activity and the acute oral exposure in the rat, Pinkerton *et al.* (1989) observed the same spectrum of toxicologic responses as with TCDD, but suggested that TBDD and TBDF were approximately 10- to 100-fold less active. A similar conclusion was reached by Ivens *et al.* (1989), who examined the subchronic toxicity of TBDD compared to that of TCDD in rats.

One of the most characteristic effects of TCDD is its production of developmental malformations in the mouse. TCDD is a potent teratogen, causing cleft palate and hydronephrosis at doses where there is no overt maternal or fetal toxicity (Neubert *et al.*, 1973). No other soft tissue or skeletal terata are produced (Birnbaum *et al.*, 1989). At higher doses, atrophy of the thymus and spleen becomes evident. This pattern of response has been used diagnostically to determine whether a compound is TCDD-like in its activity (Miller and Birnbaum, 1986). For example, 2,3,7,8-tetrachlorodibenzofuran (TCDF) (Weber *et al.*, 1985), 3 PCDFs (Birnbaum *et al.*, 1987a), and 2,3,4,5,3',4'-hexachlorobiphenyl (Birnbaum *et al.*, 1987b) were TCDD-like in their teratogenic effects. In contrast, perfluorodecanoic acid, a long-chain, totally fluorinated carboxylic acid that causes many of the same classic signs of acute toxicity as does TCDD, such as thymic atrophy and wasting, was not teratogenic in the mouse (Harris and Birnbaum, 1989). In addition, the relative teratogenic potency of those compounds which fall into the same class can be compared. In fact, the relative teratogenic potency of this class of compounds has been used in determining the toxic equivalency factors used by various regulatory agencies (EPA, 1989).

Four brominated compounds were used in these studies: TBDD, TBDF, 1,2,3,7,8-pentabromodibenzofuran (1PeBDF), and 2,3,4,7,8-pentabromdibenzofuran (4PeBDF) (Fig. 1). The teratogenic activity of these compounds was examined in the mouse to determine if they produced the same spectrum of abnormalities as the chlorinated congeners and to determine their potency relative to each other and to TCDD.

MATERIALS AND METHODS

Chemicals. TBDD was synthesized by AccuStandard (Boston, MA), while TBDF, 1PeBDF, and 4PeBDF were

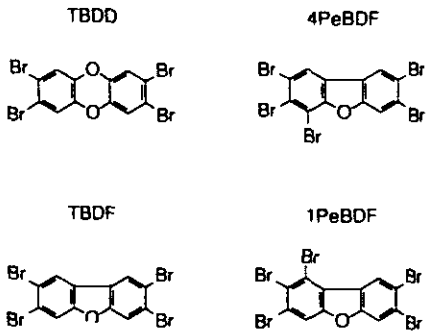

FIG. 1. Chemical structures.

obtained from Cambridge Isotope Laboratories (Woburn, MA). All four chemicals had purity >98% as determined by GC-MS. The structures of the compounds were confirmed by proton NMR using deuterated chloroform and solvent blanks at the Research Triangle Institute (Research Triangle Park, NC). For dosing, the brominated compounds were dissolved in reagent-grade acetone and mixed with corn oil (Mazola brand); the acetone was then removed by evaporation *in vacuo*. Dosing solutions were prepared by dilution from concentrated stocks which were analyzed by GC/ECD: TBDD, 19.2 μg/ml (78% of target); TBDF, 400 μg/ml (96% of target) and 20 μg/ml (88% of target); 1PeCDF, 400 μg/ml (91% of target); 4PeCDF, 160 μg/ml (97% of target) and 400 μg/ml (109% of target). Because of the potential for photodecomposition of these compounds (Neupert, *et al.*, 1988), all of the neat chemicals were handled under reduced light and all solutions were stored in the dark in amber bottles or covered with foil.

Animals. All mice were C57BL/6N from the Frederick Cancer Research Center (Frederick, MD). Female mice were received at 6–8 weeks of age and acclimated for 2 weeks before mating with proven males from our breeding colony. Two females per male were housed together overnight; the presence of a vaginal plug the next morning led to the designation of gestation day 0 (gd 0). The plug-positive females were ear-tagged for identification, weighed, and housed five per cage. They were provided with food (NIH 31) and water *ad libitum*, and held under conditions of constant temperature (22 ± 1°C), humidity (50 ± 10%), and lighting (12/12 hr light/dark cycle). On gd 10 the mice were weighed to confirm pregnancy; the pregnant dams were randomly assigned to the various treatment groups.

Treatment. Pregnant mice were treated by gavage with 10 ml corn oil/kg body wt on the afternoon of gd 10. Control mice received the corn oil vehicle. Doses (μg/kg) ranged from 0 to 192 for TBDD and 0 to 4000 for TBDF, 1PeBDF, and 4PeBDF. The dams were checked daily for signs of toxicity. On gd 18, the mice were weighed and killed by decapitation. Gravid uterine weight was determined. The positions of the fetuses were recorded, viability was determined, and the fetuses were immediately removed. The number and location of dead fetuses and resorptions were also recorded. Weights of the maternal liver, empty uterus, and individual fetuses were measured. Maternal weight gain was defined as the absolute gain in weight of the dam between gd 10 and 18 minus the weight of the uterine contents. Live fetuses were examined for gross external abnormalities and then chilled. Fetuses were fixed in Bouin's solution for 4–5 days, and then stored in alcohol. Palates were examined by cutting between the upper and lower jaws. To assess hydronephrosis, fetal kidneys were sliced longitudinally and examined under a dissecting microscope. Severity of the kidney lesions was scored according to Birnbaum *et al.* (1987a): normal (0), mild (+1), moderate (+2), or severe (+3). No other soft tissues were examined nor was any skeletal staining performed since studies with the related chlorinated compounds have not revealed any other structural malformations (Birnbaum *et al.*, 1987).

Data analysis. The litter was considered the basic experimental unit. The significance of dose–response trends was determined by Jonckheere's test. Kruskal–Wallis nonparametric analysis of variance procedures was used to assess the overall effects. Whenever a significant trend was noted, pairwise comparisons were made using the Mann–Whitney *U* test. Fisher's exact test was used when the incidence in the control group was zero. Probit analyses (Weber *et al.*, 1985) were based on the proportion of affected fetuses where probit (incidence rate) = $a + b$ (ln dose). The data for TCDD used in the probit analysis were from Birnbaum *et al.* (1989). Previous studies have demonstrated the utility of the probit model for exploring dose-response relationships in teratogenicity studies in general (Fabro *et al.*, 1982) and for the induction of cleft palate in particular (Biddle, 1978).

RESULTS

Initial dose selections were based on two factors: the relative teratogenicity of the related chlorinated isomers, TCDD, TCDF, 1PeCDF, and 4PeCDF (Birnbaum *et al.*, 1987b); and the suggestion that bromination might decrease the toxicity of this class of compounds (Pinkerton *et al.*, 1989; Mason *et al.*, 1987).

TBDD (Table 1). Pregnant mice were dosed with 0–192 μg TBDD/kg body wt. There was no effect of TBDD on maternal weight gain. The lowest dose used, 3 μg/kg, resulted in a significant increase in the liver/body weight ratio. This was due to an increase in the absolute liver weight (data not shown). The relative liver weight continued to increase up to 96 μg/kg. No effect of TBDD was observed on fetal mortality, although the number of live fetuses did show a decreasing trend. Fetal body weight also tended to increase as the dose was raised. However, the biological significance of this increase at doses of 12 and 24 μg/kg is questionable since the control body weight in this experiment was lower than that usually observed (see controls for other compounds).

TBDD induced both hydronephrosis and cleft palate at doses where there was no maternal toxicity, as evidenced by maternal weight gain, or fetal toxicity. While the incidence of cleft palate is not significantly in-

TABLE 1

DEVELOPMENTAL TOXICITY OF TBDD

	Dose (μg/kg[a])								
	0	3	6	12	24	48	96	192	ANOVA
Numbers of litters	20	15	18	17	17	11	11	14	
Maternal effects									
Weight gain (g)[b]	1.5 ± 0.2[c]	1.6 ± 0.3	1.7 ± 0.2	1.2 ± 0.2	1.3 ± 0.2	1.5 ± 0.3	1.5 ± 0.2	1.5 ± 0.2	NS
Liver/body weight (×100)	6.7 ± 0.1	7.0 ± 0.1*	7.1 ± 0.1**	7.4 ± 0.1***	7.4 ± 0.1***	7.2 ± 0.1***	8.0 ± 0.1***	7.7 ± 0.2***	$p < 0.001$
Fetal effects									
Weight[d]	1.10 ± 0.03	1.12 ± 0.02	1.17 ± 0.03	1.18 ± 0.02*	1.18 ± 0.02**	1.28 ± 0.05**	1.24 ± 0.04*	1.26 ± 0.04**	$p < 0.001$
No. Live[d]	8.3 ± 0.5	8.7 ± 0.6	7.9 ± 0.6	7.7 ± 0.5	8.4 ± 0.4	7.5 ± 0.7	6.9 ± 0.8	7.2 ± 0.6	$p < 0.05$
% Mortality[d]	11.8 ± 2.8	6.1 ± 1.6	13.9 ± 4.2	10.6 ± 3.4	7.6 ± 1.3	12.2 ± 6.4	16.9 ± 4.7	21.1 ± 4.5	NS
Cleft palate									
No. litters affected	0	0	0	0	1	3	11	14	
Incidence[d]	0	0	0	0	0.65 ± 0.65	11.51 ± 6.47*	87.46 ± 6.26***	100.00***	$p < 0.001$
Affected fetuses/ total fetuses	0/166	0/130	0/143	0/126	1/143	9/82	70/76	101/101	
Hydronephrosis									
No. litters affected	3	9	8	16	17	11	11	14	
Incidence[d]	4.2 ± 3.0	15.9 ± 5.4*	16.3 ± 5.9	92.5 ± 3.1***	95.9 ± 2.4***	100.0***	100.0***	100.0***	$p < 0.001$
Affected fetuses/ total fetuses	5/166	17/130	21/143	115/126	137/143	82/82	76/76	101/101	
Severity[d]	0.03 ± 0.02	0.09 ± 0.03**	0.11 ± 0.05	1.14 ± 0.11***	1.44 ± 0.11***	1.98 ± 0.11***	2.05 ± 0.06***	2.12 ± 0.07***	$p < 0.001$

[a] 10 ml corn oil/kg, gd 10.
[b] Difference in maternal body weight between gd 10 and 18 minus gravid uterus weight.
[c] Mean ± SEM.
[d] Per litter.
* $p < 0.05$ vs controls.
** $p < 0.01$ vs controls.
*** $p < 0.001$ vs controls.

creased until 48 μg/kg, hydronephrosis is significant at the lowest dose studied, 3 μg/kg. For both end points the dose–response relationship is very steep, with a doubling in dose (for cleft palate from 48 to 96 μg/kg; for hydronephrosis from 6 to 12 μg/kg) resulting in a nearly maximal response. Examination of the severity of hydronephrosis reveals that the extent of the lesion also increases dramatically between 6 and 12 μg/kg, but then continues to rise so that the average kidney would be moderately affected.

TBDF (Table 2). Because TCDF has been reported to have approximately 1/20–1/30 of the teratogenic potency of TCDD (Weber *et al.*, 1985; Birnbaum *et al.*, 1987b), initial studies were conducted at doses of 0–4000 μg TBDF/kg, i.e., doses that were 20 times higher than those used for TBDD. Even at a dose of 250 μg/kg, all of the fetuses had cleft palate. Therefore, doses from 0 to 200 μg/kg were studied. The data from these two sets of experiments were pooled since there was no difference observed in the controls. Maternal weight gain was significantly elevated only at the highest dose of TBDF, 4000 μg/kg. This was due to subcutaneous edema. Relative liver weight was increased even at the lowest dose tested, 25 μg/kg, and continued to increase with increasing dose. This was due to an absolute increase in liver weight. Embryo/fetal toxicity was evident at doses of TBDF ≥ 500 μg/kg. Fetal mortality and live fetal body weight increased while the number of live fetuses decreased with increasing dose. In fact, at doses of >2000 μg/kg, there were few live fetuses. At 3000 μg TBDF/kg, the few survivors were edematous.

The induction of cleft palate and hydronephrosis occurred at doses below that at which maternal and fetal toxicity occurred. While cleft palate was not significant at 100 μg TBDF/kg, all litters were affected at 200 μg/kg. Hydronephrosis affected all litters at the lowest dose studied, 25 μg/kg, but the severity at this dose was still low (0.4). Doubling of the dose to 50 μg/kg resulted in an incidence per litter of nearly 100% and a mild to moderate degree of severity. Even where toxicity was evident (≥500 μg/kg), the average severity for hydronephrosis was still moderate.

1PeBDF (Table 3). Pregnant mice were treated with seven dose levels ranging from 0 to 4000 μg 1PeBDF/kg, based on the results of previous studies indicating that the chlorinated isomer, 1PeCDF, was approximately 1/30 as teratogenic as TCDD (Birnbaum *et al.*, 1987b). Maternal toxicity, as evidenced by an increase in maternal weight gain, was not observed. The increase in absolute liver weight was reflected in the increase in the liver to body weight ratio observed at the lowest dose studied, 250 μg/kg. Prenatal treatment with 1PeBDF had no effect on fetal toxicity, as noted by the lack of increase in fetal mortality or fetal body weight or a decrease in the number of live fetuses.

Cleft palate and hydronephrosis were induced by 1PeBDF in this study even though no maternal or fetal toxicity was observed. The incidence of cleft palate was first significant at 3000 μg/kg. Even at the highest dose used, 4000 μg/kg, all of the litters were not affected, although the incidence per litter was close to 50%. Hydronephrosis was first significantly increased at 500 μg/kg, but the average severity was low (0.2). Doubling the dose to 1000 μg 1PeBDF/kg increased the incidence to nearly 100%, and produced a mild lesion. Moderate severity was not reached until the highest dose, 4000 μg/kg, was used.

4PeBDF (Table 4). Since 4PeCDF has been reported to be approximately 1/10 as teratogenic as TCDD (Birnbaum *et al.*, 1987b), dams were treated with 4PeBDF at doses from 0 to 1600 μg/kg. At 1600 μg 4PeBDF/kg, only 1 litter in 10 had any fetuses with cleft palate. Therefore, additional studies were conducted at doses of 2400 and 4000 μg/kg. There were no effects on maternal weight gain at any dose studied. Relative liver weight was increased at the lowest dose examined, 25 μg/kg, due to an increase in absolute liver weight. The liver/body weight ratio continued to increase with increasing dose. There were no significant ef-

TABLE 2

DEVELOPMENTAL TOXICITY OF TBDF

	Dose (μg/kg[a])											
	0	25	50	100	200	250	500	1000	2000	3000	4000	ANOVA
Numbers of litters	22	10	10	9	12	9	12	11	7	10	7	
Maternal effects												
Weight gain (g)[b]	1.9	2.2	2.2	2.2	1.5	1.0	1.1	2.2	2.5	2.1	2.3	
	± 0.2[c]	± 0.3	± 0.3	± 0.3	± 0.2	± 0.3*	± 0.4	± 0.3	± 0.4	± 1.1	± 1.4*	$p < 0.01$
Liver/Body Weight (×100)	6.8	7.5	8.1	8.2	8.2	8.4	8.7	8.2	9.0	9.4	9.5	
	± 0.1	± 0.2**	± 0.1***	± 0.1***	± 0.1***	± 0.1***	± 0.2***	± 0.4*	± 0.1***	± 0.3***	± 0.2***	$p < 0.001$
Fetal Effects												
Weight[d]	1.17	1.16	1.12	1.21	1.18	1.20	1.27	1.20	1.19	1.32	1.24	
	± 0.03	± 0.02	± 0.04	± 0.04	± 0.02	± 0.06	± 0.04*	± 0.02	± 0.05	± 0.07	± 0.15	$p < 0.01$
No. live[d]	8.7	9.0	8.2	7.8	8.7	7.9	7.3	3.7	1.3	1.2	0.6	
	± 0.5	± 0.3	± 0.6	± 0.9	± 0.5	± 0.5	± 0.6*	± 0.8***	± 0.5***	± 0.3***	± 0.3***	$p < 0.001$
% Mortality[d]	7.3	5.9	11.7	8.3	4.9	11.0	21.8	52.6	81.6	87.2	93.3	
	± 2.8	± 2.1	± 3.0	± 4.7	± 2.8	± 3.3	± 5.0**	± 10.3***	± 6.6***	± 3.0***	± 3.6***	$p < 0.001$
Cleft palate												
No. litters affected	0/22	0/10	0/10	2/9	12/12	9/9	12/12	8/8	5/5	8/8	3/3	
Incidence[d]				8.63	79.30		99.17					
	0	0	0	± 5.79	± 6.73***	100.00***	± 0.83***	100.0***	100.0***	100.0***	100.0***	$p < 0.001$
Affected fetuses/total fetuses	0/191	0/90	0/82	7/70	82/104	71/71	88/89	41/41	9/9	12/12	4/4	
Hydronephrosis												
No. litters affected	6/22	10/10	10/10	9/9	12/12	9/9	12/12	8/8	5/5	8/8	3/3	
Incidence[d]	3.8	53.1	97.0									
	± 1.3	± 9.6***	± 2.1***	100.0***	100.0***	100.0***	100.0***	100.0***	100.0***	100.0***	100.0***	$p < 0.001$
Affected fetuses/total fetuses	6/191	48/90	79/82	70/70	104/104	71/71	89/89	41/41	9/9	12/12	4/4	
Severity[d]	0.02	0.41	1.49	1.90	2.00	1.90	1.99	1.99	2.03	2.05	2.17	
	± 0.01	± 0.08***	± 0.15***	± 0.07***	± 0.09***	± 0.04***	± 0.02***	± 0.02***	± 0.03***	± 0.04***	± 0.17**	$p < 0.001$

[a] 10 ml corn oil/kg, gd 10.
[b] Difference in maternal body weight between gd 10 and 18 minus gravid uterus weight.
[c] Mean ± SEM.
[d] Per litter.
* $p < 0.05$ vs controls.
** $p < 0.01$ vs controls.
*** $p < 0.001$ vs controls.

TABLE 3

DEVELOPMENTAL TOXICITY OF 1PeBDF

	Dose (μg/kg[a])							
	0	250	500	1000	2000	3000	4000	ANOVA
Numbers of litters	5	10	7	11	9	11	11	
Maternal effects								
Weight gain (g)[b]	1.3 ± 0.2[c]	0.4 ± 0.5	1.0 ± 0.4	1.4 ± 0.5	1.2 ± 0.4	1.0 ± 0.3	1.4 ± 0.5	NS
Liver/body weight (×100)	6.7 ± 0.5	8.1 ± 0.2*	8.1 ± 0.2*	7.9 ± 0.3*	8.6 ± 0.1**	8.8 ± 0.2**	9.0 ± 0.2**	$p < 0.001$
Fetal effects								
Weight[d]	1.20 ± 0.06	1.11 ± 0.02	1.18 ± 0.04	1.17 ± 0.03	1.25 ± 0.04	1.18 ± 0.04	1.21 ± 0.03	NS
No. live[d]	8.2 ± 0.9	7.1 ± 0.9	8.7 ± 0.4	8.1 ± 0.7	8.1 ± 0.8	8.2 ± 0.5	7.2 ± 0.7	NS
% Mortality[d]	9.4 ± 2.7	23.3 ± 7.2	6.2 ± 3.1	4.1 ± 1.8	16.1 ± 6.9	7.5 ± 2.3	17.0 ± 6.8	NS
Cleft palate								
No. litters affected	0/5	0/10	0/7	0/11	1/9	3/11	9/11	
Incidence[d]	0	0	0	0	1.59 ± 1.59	15.19 ± 9.54	47.96 ± 12.38**	$p < 0.001$
Affected fetuses/total fetuses	0/41	0/71	0/61	0/89	1/73	12/90	36/79	
Hydronephrosis								
No. litters affected	1/5	6/10	7/7	9/11	9/9	11/11	11/11	
Incidence[d]	2.2 ± 2.2	29.3 ± 10.4	93.6 ± 3.3**	99.1 ± 0.9***	100.0***	98.7 ± 1.3***	100.0***	$p < 0.001$
Affected fetuses/total fetuses	1/41	14/71	57/61	88/89	73/73	89/90	79/79	
Severity[d]	0.01 ± 0.01	0.18 ± 0.08	0.83 ± 0.09**	1.09 ± 0.06**	1.54 ± 0.06**	1.80 ± 0.07**	1.92 ± 0.06**	$p < 0.001$

[a] 10 ml corn oil/kg, gd 10.
[b] Difference in maternal body weight between gd 10 and 18 minus gravid uterus weight.
[c] Mean ± SEM.
[d] Per litter.
* $p < 0.05$ vs controls.
** $p < 0.01$ vs controls.
*** $p < 0.001$ vs controls.

TABLE 4

DEVELOPMENTAL TOXICITY OF 4PeBDF

	Dose (μg/kg[a])										
	0	25	50	100	200	400	800	1600	2400	4000	ANOVA
Numbers of litters	16	11	10	12	9	11	12	12	11	11	
Maternal effects											
Weight gain (g)[b]	2.0 ± 0.2[c]	2.5 ± 0.3	2.7 ± 0.5	2.5 ± 0.2	1.6 ± 0.3	2.0 ± 0.2	1.8 ± 0.5	1.9 ± 0.3	1.8 ± 0.2	2.3 ± 0.4	NS
Liver/body weight (×100)	6.9 ± 0.1	7.4 ± 0.1**	7.1 ± 0.2	7.4 ± 0.1**	7.6 ± 0.2*	7.9 ± 0.1***	8.4 ± 0.2***	8.5 ± 0.2***	8.3 ± 0.1***	8.7 ± 0.1***	$p < 0.001$
Fetal effects											
Weight[d]	1.16 ± 0.04	1.15 ± 0.01	1.12 ± 0.02	1.11 ± 0.04	1.12 ± 0.04	1.13 ± 0.02	1.14 ± 0.04	1.14 ± 0.02	1.11 ± 0.02	1.20 ± 0.02	NS
No. live[d]	8.8 ± 0.6	8.6 ± 0.4	9.0 ± 0.6	9.0 ± 0.5	8.1 ± 1.0	9.5 ± 0.3	9.0 ± 0.5	9.2 ± 0.5	9.1 ± 0.4	7.7 ± 0.3	NS
% Mortality[d]	7.2 ± 3.8	8.5 ± 2.9	10.0 ± 4.0	5.8 ± 1.8	5.8 ± 3.7	2.6 ± 1.3	7.4 ± 3.1	6.5 ± 3.5	10.0 ± 2.3	12.5 ± 2.9	NS
Cleft palate											
No. litters affected	0/16	0/11	0/10	0/12	0/9	0/11	0/12	1/12	4/11	11/11	
Incidence[d]	0	0	0	0	0	0	0	0.83 ± 0.83	13.28 ± 8.08*	93.02 ± 5.70***	$p < 0.001$
Affected fetuses/total fetuses	0/141	0/95	0/90	0/108	0/73	0/105	0/108	1/110	13/100	79/85	
Hydronephrosis											
No. litters affected	4/16	2/11	5/10	6/12	4/9	11/11	12/12	12/12	11/11	11/11	
Incidence[d]	3.8 ± 1.3	2.9 ± 1.5	10.6 ± 4.0	6.5 ± 1.7	16.7 ± 6.9	43.6 ± 6.9***	88.2 ± 3.2***	100.0***	99.0 ± 1.0***	100.0***	$p < 0.001$
Affected fetuses/total fetuses	6/157	3/95	10/89	7/110	11/78	46/105	97/111	112/112	99/100	85/85	
Severity[d]	0.02 ± 0.01	0.01 ± 0.01	0.06 ± 0.02	0.03 ± 0.01	0.16 ± 0.08	0.27 ± 0.05***	1.12 ± 0.11***	1.90 ± 0.06***	1.78 ± 0.11***	2.38 ± 0.08***	$p < 0.001$

[a] 10 ml corn oil/kg, gd 10.
[b] Difference in maternal body weight between gd 10 and 18 minus gravid uterus weight.
[c] Mean ± SEM.
[d] Per litter.
* $p < 0.05$ *vs controls.*
** $p < 0.01$ vs controls.
*** $p < 0.001$ vs controls.

fects on embryo/fetal mortality, body weight, or number of live fetuses.

As with the other three brominated compounds, cleft palate and hydronephrosis occurred at doses below those at which toxicity occurred. Cleft palate incidence jumped from 13 to 93% when the dose was raised from 2400 to 4000 μg/kg. The incidence of hydronephrosis was at first significantly increased *above control values at 400 μg 4PeBDF/kg*, but the severity score was low (0.3). By a dose of 1600 μg/kg, the hydronephrotic incidence was 100% and the severity was moderate.

DISCUSSION

The four brominated compounds used in this study, TBDD, TBDF, 1PeBDF, and 4PeBDF, all induce a pattern of structural malformations identical to that observed previously for TCDD and related chemicals including brominated naphthalenes (Miller and Birnbaum, 1986), chlorinated dibenzofurans (Weber *et al.*, 1984; Birnbaum *et al.*, 1987a), polychlorinated biphenyls (Marks *et al.*, 1981; Watanabe and Sugahara, 1981; Birnbaum *et al.*, 1987b), and azoxybenzenes (Hassoun *et al.*, 1984). The only structural abnormalities induced by these chemicals are cleft palate and hydronephrosis, and these teratа occur at doses below those at which maternal and fetal toxicity occurs (Birnbaum *et al.*, 1989). The increase in relative liver weight which occurs at low doses is due to an increase in absolute liver weight and represents an adaptive response (Poland and Knutson, 1982). Mason *et al.* (1987) have demonstrated that TBDD induces the same class of drug-metabolizing enzymes as TCDD. TBDF also appears to be active as an enzyme inducer (Zacharewski *et al.*, 1988).

Hydronephrosis has been shown to be a more sensitive indicator of TCDD-like teratogenesis than cleft palate (Birnbaum *et al.*, 1989; Couture *et al.*, 1990). However, in the studies reported here, the dose response was so steep that probit analysis was not appropriate. The ED50 values, however, can be estimated from the dose–response curves. Thus, for hydronephrotic incidence the approximate ED_{50} values in μg/kg are: TBDD, 9; TBDF, 12; 1PeBDF, 340; 4PeBDF, 437. The ED_{50} for TCDD-induced hydronephrosis has recently been estimated to be 4 μg/kg. Thus, TBDD appears to be almost half as teratogenic as TCDD in the induction of hydronephrosis. One interesting observation is the apparent "saturation" of the severity of hydronephrosis. Even at doses at which there was maternal and/or fetal toxicity, such as at the higher doses of TBDD and TBDF, the mean hydronephrotic severity score was only moderate. Similar observations have been reported previously with hexabromonaphthalene (Miller and Birnbaum, 1986) and with TCDD (Couture *et al.*, 1990). It is possible that fetuses with more severely affected kidneys are less likely to survive.

Probit analyses of the cleft palate data from these experiments as well as from recent studies in our laboratory with TCDD (Birnbaum *et al.*, 1989) demonstrate that the dose–response curves with a common slope provide a good fit (Fig. 2). Model fitting to a common slope is compatible with a common mechanism of action for these five chemicals, in agreement with previous studies for the chlorinated isomers (Birnbaum *et al.*, 1987b). Based on the probit analysis, the ED50 values (and 95% confidence intervals) in μg/kg are: TCDD, 15.3 (14.7–16.0); TBDD, 65.1 (60.5–70.0); TBDF, 153.5 (143.9–163.3); 1PeBDF, 4087.6 (3854.5–4348.2); 4PeBDF, 3024.3 (2859.4–3203.0). Since the slopes are assumed parallel, the potencies can be compared. If a toxic equivalency factor (TEF) of 1 is assigned to TCDD, then the following values can be assigned: TBDD, 0.235; TBDF, 0.100; 1PeBDF, 0.004; 4PeBDF, 0.005. Based on *in vitro* experiments, Mason *et al.* (1987) estimated that the activity for enzyme induction of TBDD relative to TCDD was approximately 0.2. They also noted that substitution of an additional bromine for a hydrogen atom, i.e., going from TBDD to 1,2,3,7,8-pentabro-

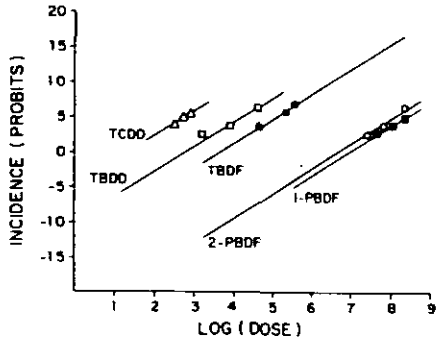

FIG. 2. Probit plot of cleft palate incidence. The induction of cleft palate by TCDD, TBDD, TBDF, 1PeBDF, and 4PeBDF was analyzed using a probit model fit to a common slope of 3.582 ± 0.177 (estimate ± standard error of the estimate).

Compound	Intercept
TCDD	−4.776 ± 0.484
TBDD	−9.956 ± 0.747
TBDF	−13.028 ± 0.925
1PeBDF	−24.783 ± 1.449
4PeBDF	−23.704 ± 1.412

modibenzo-*p*-dioxin, caused a 10- to 100-fold decrease in measures of toxicity *in vivo*. This is in good agreement with our comparisons between TBDF and both 1- and 4-PeBDF.

The differential effects of teratogenic potency between chlorination and bromination of the dibenzo-*p*-dioxins and dibenzofurans are of great interest. Using *in vitro* models, TCDF is nearly as toxic as TCDD, but *in vivo*, TCDF is much less toxic. This has been attributed to its being metabolized at a much greater rate than TCDD (Birnbaum, 1985). Likewise, 1PeCDF has *in vitro* properties similar to those of 4PeCDF, but is less toxic, as assessed by its teratogenicity (Birnbaum *et al.*, 1987a). Recent studies have indicated that this apparent discrepancy is also due to differential metabolism (Brewster and Birnbaum, 1987, 1988). In the case of the brominated isomers, TBDD and TBDF have similar teratogenic potencies, with TBDD being slightly more potent. This may be a reflection of the increased size of the bromine relative to the chlorine atom, making enzymatic attack on the C-4 position of the TBDF molecule more difficult. This position may be favored for metabolism (Burka and Overstreet, 1989). The similarity in TEF values for teratogenicity of 1- and 4PeBDF may also be due to the size of the bromine atom blocking enzymatic attack on the C-4 position of 1PeBDF. The reversal in relative potencies for TBDF and 4PeBDF vs TBDD compared to those of the chlorinated isomers may also be a reflection of the size of the bromine vs the chlorine atom. The effects of this class of chemicals all appear to involve initial binding of the ligand to a cytosolic protein, known as the Ah receptor (Poland and Knutson, 1982). The ligand must meet certain geometric requirements of size and shape. The presence of a fifth bromine atom would decrease the goodness of fit, resulting in lower binding affinity and lessened toxic effects.

In addition to steric considerations playing a role in decreased toxicity, changes in metabolism of the brominated isomers vs the chlorinated ones could play a role. The carbon–bromine bond has lower energy than the carbon–chlorine bond (Sovocool *et al.*, 1989). This could lead to greater rates of debromination than of dechlorination. Thus, the decrease in teratogenic effects of TBDD vs TCDD, while in part due to a slight decrease in binding to the Ah receptor (Mason *et al.*, 1987), may also reflect the loss of bromine from the parent compound, leading to a 2,3,7- or 2,3,8-tribromodibenzo-*p*-dioxin, a molecule which would be less toxic than TBDD. In addition, the relative weakness of the bromine–carbon bond appears to be reflected in the shorter whole-body half-life of TBDD in the rat, recently estimated to be approximately 17 days (Kedderis *et al.*, 1990) compared to 31 days for TCDD (Rose *et al.*, 1976).

In conclusion, brominated isomers and congeners of TCDD—TBDD, TBDF, 1PeBDF, and 4PeBDF—produce cleft palate and hydronephrosis in mice at doses below that at which maternal and/or fetal toxicity are evident. In general, these brominated isomers are slightly less potent than the chlorinated ones. However, TBDF is more toxic than TCDF, which may be due to a decrease in its rate of metabolism resulting in greater persistence of the toxic parent chemical. Studies are currently in progress to test this hypothesis.

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TOXICOLOGY AND APPLIED PHARMACOLOGY **107**, 153–163 (1991)

Aluminum Distribution into Brain and Liver of Rats and Rabbits following Intravenous Aluminum Lactate or Citrate: A Microdialysis Study[1,2]

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Aluminum Distribution into Brain and Liver of Rats and Rabbits following Intravenous Aluminum Lactate or Citrate: A Microdialysis Study. YOKEL, R. A., LIDUMS, V., MCNAMARA, P. J., AND UNGERSTEDT, U. (1991). *Toxicol. Appl. Pharmacol.* **107**, 153–163. Microdialysis probes were utilized to follow the appearance and disappearance of dialyzable aluminum (Al) in rat and rabbit brain and liver extracellular fluid compared to blood after iv Al lactate or Al citrate injection. Dialyzable Al was assumed to be the fraction not protein bound or self-associated into complexes > the molecular weight cutoff of the dialysis membrane. Aluminum concentrations peaked in brain frontal cortex and ventral hippocampus and in the liver in the first 20-min dialysis sample, indicating rapid Al penetration into the extracellular space of these organs. *In vitro* recovery experiments conducted with microdialysis probes at room temperature revealed an average dialysis efficiency of about 10% for both Al lactate and citrate. At 37°C Al recovery increased for both Al lactate and citrate. *In vivo* Al recovery from rabbit blood averaged 5.15% for Al lactate and 3.25% for Al citrate. These observations are consistent with results from recovery studies of other substances showing an increased recovery with increased temperature but an overestimate of recovery by *in vitro* methods. Tissue/blood Al ratios (TBR; representing dialyzable extracellular tissue Al ÷ dialyzable blood plasma Al) for liver were ≈1, suggesting unhindered diffusion of Al between blood and liver. In contrast, brain TBR were <1, demonstrating a partial blood–brain barrier to Al. The brain TBR for Al lactate was >TBR for Al citrate, suggesting that Al citrate did not preferentially penetrate the blood–brain barrier. Higher TBR were seen in the rabbit than the rat, perhaps contributing to the greater susceptibility of the rabbit to Al-induced neurobehavioral toxicity. Metals can be repetitively sampled in the extracellular space using microdialysis, enabling metal toxicokinetic determinations in these compartments. © 1991 Academic Press, Inc.

Aluminum (Al) is toxic to the brain, skeletal system, and blood-forming elements of the bone marrow (Boegman and Bates, 1984; Ott *et al.*, 1982; McGonigle and Parsons, 1985). Recently, adverse effects of Al on the liver, kidney, and spleen have been noted (Stein *et al.*, 1987). A causal relationship between Al and Alzheimer's disease has been suggested (Martyn *et al.*, 1989); however, this is highly controversial. As Al is not an essential element for any mammalian species, its presence in the body can only be regarded as undesirable. Aluminum has been shown to enter the human body by injection (during dialysis, intravenous feeding, and immunization), by oral absorption from Al-containing antacids/phosphate binders, and by inhalation (Yokel, 1988).

The metabolic fate of Al in humans and animals has been reviewed (Ganrot, 1986). It accumulates in organs to a variable extent,

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Immunologic and endocrine effects of the flame-retardant pentabromodiphenyl ether (DE-71) in C57BL/6J mice

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Abstract

Polybrominated diphenyl ethers are manufactured for use as flame retardants in commercial plastics and textiles in Europe and North America. These studies investigated the acute and subchronic immunotoxicity and endocrine effects of a commercial pentabromodiphenyl ether mixture, DE-71, in female C57BL/6 mice. Mice were orally exposed to acute single doses of DE-71 of 0, 0.8, 4.0, 20, 100, or 500 mg/kg, or to subchronic daily doses totaling 0, 250, 500, or 1000 mg/kg over a 14 day period. Immunotoxicity was assessed by measuring the plaque-forming cell response to sheep erythrocytes (SRBC) and natural killer cell (NKC) activity (basal and poly I:C stimulated) to YAC-1 target cells. Liver cytochrome P450 content and activities (ethoxyresorufin-*o*-deethylase (EROD) and pentoxyresorufin-*o*-deethylase (PROD)) as well as corticosterone (CS) and thyroxine (T4) concentrations were also measured. PROD activity was induced 3–5-fold in mice exposed acutely or subchronically to DE-71 at doses >250 mg/kg. EROD activity and total microsomal cytochrome P450 content were significantly induced only in mice treated subchronically with DE-71; maximum induction of EROD was 3.3-fold. Total serum T4 concentrations were significantly lower in mice treated acutely with DE-71 at all doses except the 100 mg/kg dose. Total and free T4 concentrations were dose-dependently decreased in DE-71-treated mice following subchronic exposure. Plasma CS levels were elevated following subchronic exposure to DE-71. The elevation

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of CS was correlated with order of capture at necropsy, suggesting an interactive effect of DE-71 and stress. In regard to immunotoxicity, significant suppression of the anti-SRBC response was seen only in mice exposed subchronically to 1000 mg DE-71/kg, an exposure that also resulted in decreased thymus weight. NKC activity was not altered by exposure to DE-71.

Key words: Antibody response; Corticosterone; Immunotoxicity; Natural killer cell; Pentabromodiphenyl ether; Thyroxine

1. Introduction

Brominated diphenyl ethers (BDE) are currently manufactured for use as flame retardant additives to various plastics, rubber, paints and resins (U.S. Environmental Protection Agency, 1991). This use has led to the appearance of residues of BDE, including pentabromodiphenyl ether (PBDE), in a variety of environments, both aquatic and terrestrial (U.S. Environmental Protection Agency, 1991). Pyrolysis of BDE can yield brominated dibenzofurans and dibenzodioxins of potential concern (Buser, 1986). Since there is potential for BDE to cause adverse environmental and human health effects, the U.S. Environmental Protection Agency has recommended that toxicity studies be conducted for commercially important BDE congeners and mixtures.

Structurally-related chlorinated diphenyl ethers (CDE) have been shown to cause liver enzyme induction as well as immune suppression in laboratory mice (Howie et al., 1990). In these studies, the potency of specific CDE congeners to induce hepatic microsomal ethoxyresorufin-*o*-deethylase (EROD) activity correlated with their humoral immunotoxicity as measured by the antibody response to SRBC. Von-Meyerinck et al. (1990) have described a commercial PBDE mixture, Bromkal-70, as a mixed-type inducer, stimulating both EROD and benzphetamine-*N*-demethylase cytochrome P450 activities. The present studies examined the potency of another commercial PBDE mixture, DE-71, to induce mixed-function oxygenase activities and to alter anti-SRBC responses in C57BL/6 mice exposed to a range of acute and subchronic doses of DE-71. Induction of hepatic cytochrome P450 1A1 and 1B1 subfamilies was evaluated by EROD and pentoxyresorufin-*o*-deethylase (PROD) activities, respectively.

Endocrine effects of BDE include hyperplasia of the thyroid gland in rats (U.S. Environmental Protection Agency, 1991). Thyroid hormones are endogenous iodinated diphenyl ethers bearing structural resemblance to PBDE and interference with normal thyroid hormone homeostasis has been proposed for structurally similar polychlorinated biphenyls (PCBs) (Brouwer, 1991). Halogenated compounds with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-like toxicity are also known to affect the thyroid in rats, causing

reduced thyroxin (T4) and triiodothyronine levels in addition to thyroid hyperplasia (Collins et al., 1977; Allen-Rowlands et al., 1981; Pazdernik and Rozman, 1985). The mechanisms of reduction of thyroid hormone levels by TCDD-like compounds has been shown to be due to induced hepatic UDP-glucuronyltransferase activity (Barter and Klaassen, 1992) as well as to effects at the level of the anterior pituitary and the thyroid (Gorski et al., 1988). Because natural killer cell (NKC) activity can be modulated by thyroid status (Kinoshita et al., 1991), NKC activity was examined in relation to circulating total and free T4 levels in DE-71-treated mice. Glucocorticoid (GC) hormone levels are also known to be modulated following exposure to various halogenated aromatic hydrocarbons including TCDD, presumably through a variety of mechanisms including altered metabolism (Gorski et al., 1988) and liver clearance through cytochrome P450 enzyme induction or suppression. Since GC hormones are also immunomodulatory, serum GC levels were monitored as possible indirect mediators of immunotoxicity of DE-71.

2. Materials and methods

2.1. Animals

Adult (8-week-old) female C57BL/6J mice (The Jackson Laboratory, Bar Harbor, ME) from a colony sero-negative for murine hepatitis virus, were housed following National Institute of Health guidelines for the care and use of laboratory animals. Mice were housed six to a cage and maintained on a 12 h light cycle in a room with a laminar flow unit. Mice were provided with food (TEKLAD Rodent Chow, Madison, WI) and water ad libitum.

2.2. Experimental design

Mice (6/group) were dosed once by gavage with 0, 0.8, 4, 20, 100, or 500 mg/kg body weight DE-71 (Great Lakes Chemical Co., West Lafayette, IN) for the acute study. For the subchronic study, oral doses were 0, 18, 36, or 72 mg/kg per day for 14 days (6–8 mice/group), for total doses of 0, 250, 500, or 1000 mg/kg. Mice in the subchronic study were not given injections of SRBC when measurements of NKC activity or other endocrine or P450 endpoints were made. Mice treated acutely with DE-71 were all injected with SRBC. Peanut oil was used as the vehicle for DE-71. Mice were weighed daily to the nearest 0.1 g.

At the end of each experiment, mice were selected randomly from each cage, stratified across treatment groups and were killed by CO_2 asphyxiation. Spleen, thymus, liver and body weights were measured. Following either acute or subchronic exposure to DE-71, spleen cells were assayed for antibody production to SRBC, serum for total T4 and livers for microsomal

cytochrome P450 enzyme content and specific activities. Statistical analyses were performed using Student's *t*-test with significant differences accepted at the $P < 0.05$ level. Multiple linear regression was used to analyze the interactive effects of DE-71 and stress in the CS data. Statview 512K software for the Macintosh computer was used for statistical analyses.

2.3. Plaque-forming cell (PFC) assay for the antibody response to SRBC

Mice were sensitized by i.p. injection with 2.5×10^8 (Allen-Rowlands et al., 1981) SRBC 2 days after a single DE-71 exposure, or after 9 days of the 14 day chronic exposure regimen. Mice were killed 5 days after immunization in each study. Spleens were processed into single cell suspensions and the number of anti-SRBC plaques was determined using a modification (Deyo and Kerkvliet, 1990) of the method of Cunningham and Szenberg (Cunningham and Szenberg, 1968). Hanks' balanced salt solution with 2.5% fetal bovine serum (Hyclone, Logan, UT) with 10 mM HEPES buffer (pH = 7.4) was used as the medium throughout the assay.

2.4. Natural killer cell assay

Splenic NKC cytotoxicity was measured in a 4 h chromium release assay using the murine target cell line YAC-1 as previously described (Kerkvliet et al., 1982) except that Ultraculture® serum-free medium (Biowhittaker Labs, Walkersville, MD) was used throughout the assay. Thymocytes (Thy) from a control mouse were used as inactive effector cells to control for re-uptake of chromium. Poly(Inosinic:Cytidylic acid) (Poly I:C) (Sigma Chemical Co., St. Louis, MO) was added (25 μg/well) 2 h prior to addition of YAC-1 cells to stimulate NKC activity. Supernatants (100 μl) were harvested after 4 h incubation and counted on a gamma counter. Effector:target ratios of 100, 50, 25 and 12.5:1 were tested. Comparisons from the 100:1 ratio were reported as specific lysis calculated from the counts/min of: (Test-Thy/MR-SR) where SR was spontaneous release of chromium from YAC-1 cells incubated with media only and MR was YAC-1 cells incubated with 0.5% sodium dodecyl sulfate.

2.5. Hormone measurements

Total and free T4 and CS were measured in duplicate using commercial radioimmunoassay (RIA) kits (Diagnostic Products Corp. Los Angeles, CA). Lowest detectable limits for total T4, free T4 and CS were: 1.0 μg/dl, 1.0 pg/ml and 10 ng/ml, respectively. Mice were allowed to acclimate to the laboratory overnight before termination to lessen stress prior to necropsy.

2.6. Hepatic microsomal cytochrome P450 activities

Microsomes were prepared from livers that had been flash frozen in liquid nitrogen. Microsomes were prepared in phosphate buffer (0.1 M KH_2PO_4, 0.15 M KCl, 1 mM EDTA, 1 mM dithiothreitol, pH = 7.4) and stored at −70°C until assayed. Total protein was determined using a bicinconic acid kit (Pierce, Rockford, IL). Cytochrome P450 content was determined on a scanning spectrophotometer using the methods of Estabrook et al. (1972). Substrates and standards for the EROD and PROD enzyme assays were obtained from Molecular Probes (Eugene, OR). Enzyme activities of EROD and PROD were analyzed on a fluorometer using the methods of Prough et al. (1978).

3. Results

As shown in Table 1, the PFC response to SRBC was not significantly altered by a single acute dose of DE-71 as high as 500 mg/kg. Following subchronic exposure, the PFC response was modestly (63% of control, $P < 0.02$) suppressed only in mice that received a total dose of 1000 mg/kg DE-71. Neither resting nor (poly I:C) induced NKC activities were affected in mice treated subchronically with DE-71.

As shown in Fig. 1, serum T4 concentrations were decreased in a non-dose-dependent manner following acute exposure to DE-71. This effect was apparent at the lowest dose of DE-71 tested (0.8 mg/kg). Subchronic exposure to DE-71 produced a similar suppression of total serum T4 levels (Fig.

Table 1
Plaque-forming cell (PFC) number and natural killer cell (NKC) activity in C57BL/6 mice treated with a single dose or 14 daily doses of DE-71[a]

DE-71 (mg/kg)	PFC/10^6cells	NKC activity	
		Basal	Induced[b]
Acute			
0	1257 ± 126	n.d.[c]	n.d.
500	999 ± 89	n.d.	n.d.
Subchronic[d]			
0	1460 ± 108	24.77 ± 0.74	41.62 ± 1.48
250	1284 ± 195	26.73 ± 2.15	44.48 ± 2.72
500	1269 ± 219	30.77 ± 3.41	48.79 ± 2.86
1000	981 ± 146*	29.81 ± 3.15	43.77 ± 3.98

The data are representative of 2 experimental trials.
[a]Data are presented as mean ± S.E.M., $n = 6$/dose.
[b]Induced with Polyinosinic:cytidylic acid (Poly I:C).
[c] n.d., not done
[d]Total dose is shown for subchronic treatment.
*Significant difference from control $P < 0.02$.

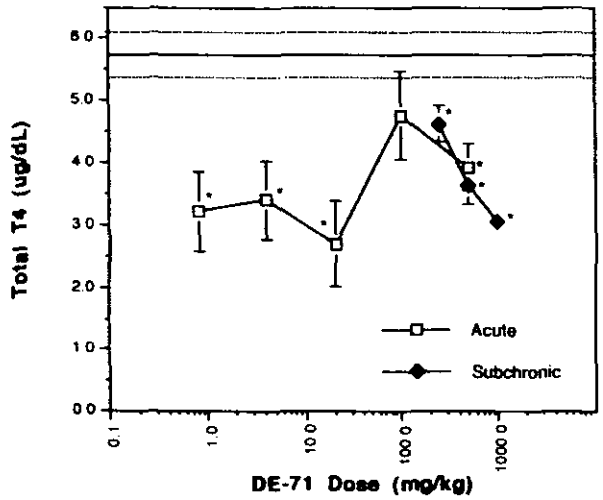

Fig. 1. Total T4 in female C57BL/6 mice (5–8/dose) treated by gavage with 0, 0.8, 4.0, 20, 100, or 500 mg/kg DE-71 in an acute exposure, or with 0, 250, 500, or 1000 mg/kg over a 14 day period (1 experimental iteration for each exposure regime). Data are presented as mean ± S.E.M. * indicates a significant difference from control (represented by horizontal lines for mean ± S.E.M. of 5.68 ± 0.32, n= 14.

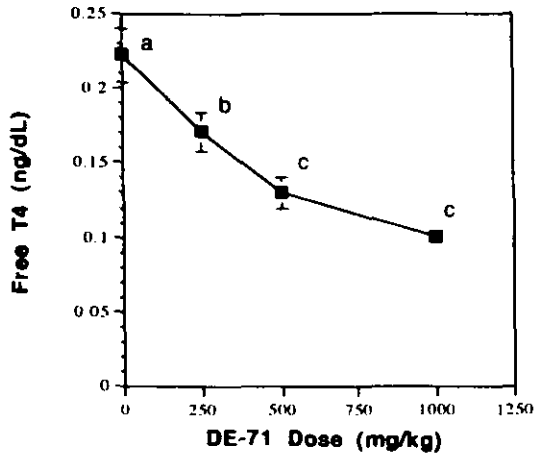

Fig. 2. Serum free T4 in female C57BL/6 mice (6/dose) treated by gavage with DE-71 (0, 18, 36, or 72 mg/kg per day) for 14 days (total dose is shown). Data are presented as mean ± S.E.M. The data are representative of 2 trials. Different letters indicate significant differences between treatment groups ($P < 0.05$).

1), with maximum suppression to 60% of control at the 1000 mg/kg dose. Free serum T4 was also suppressed by DE-71 and paralleled the response seen for total T4 (Fig. 2).

As shown in Fig. 3, corticosterone levels in the serum increased with increasing dosage and with order of kill. In addition, a highly significant ($P < 0.01$) statistical interaction of dose and order of kill, measured by multiple linear regression, was seen for the CS elevation.

Liver weight/body weight ratios were dose-dependently increased compared to controls following subchronic exposure (Table 2). Liver weight/body weight was also significantly increased in mice treated acutely with 500 mg/kg DE-71 compared to controls. Acute DE-71 treatments as high as 100 mg/kg had no effect on liver weight. Thymus weight was significantly decreased following subchronic treatment with 1000 mg DE-71/kg, while body and spleen weights were unchanged (Table 2).

Hepatic microsomes exhibited cytochrome P450 enzyme induction in terms of elevated PROD activity (2-fold) at the highest dose (500 mg/kg)

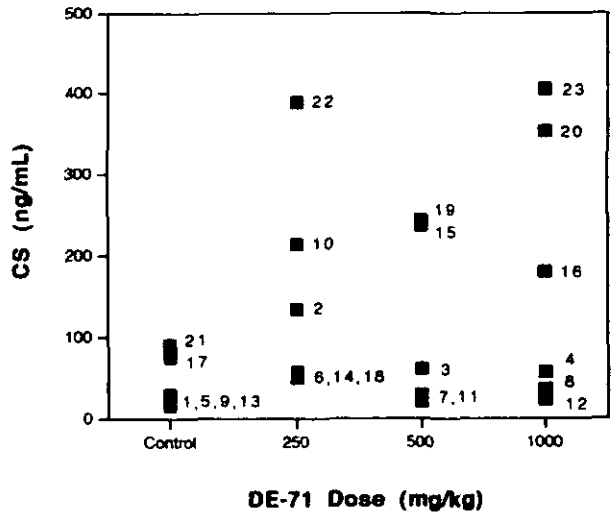

Fig. 3. Serum corticosterone (CS) levels in female C57BL/6 mice treated subchronically with DE-71 (0, 18, 36, or 72 mg/kg per day). Total dose is shown. The number by each data point indicates the order in which the animal was killed at time of necropsy. The data was collected from a single experiment. A significant interaction was seen between order of kill, DE-71 treatment and elevation of CS levels using multiple linear regression Y-Intercept = −79.742 β(order) = 44.903, β(DE-71 dose) = 0.111, R^2 = 0.478, S.E. = 95.381. Partial F-values were 14.067 for order, 4.456 for dose and 9.173 overall ($P < 0.01$).

Table 2
Liver, spleen, thymus and final body weights from female C57BL/6 mice treated with a single or 14 daily doses of DE-71[a]

DE-71 (mg/kg)	Final BW (g)	Liver/BW (mg/g)	Thymus/BW (mg/g)	Spleen/BW (mg/g)
Acute				
0	17.45 ± 0.14	51.19 ± 1.77	2.46 ± 0.24	4.30 ± 0.46
0.8	17.34 ± 0.48	53.89 ± 1.25	2.93 ± 0.36	4.26 ± 0.21
4.0	17.42 ± 0.32	52.81 ± 0.91	2.89 ± 0.21	4.13 ± 0.18
20	17.05 ± 0.39	48.88 ± 0.74	2.03 ± 0.31	3.55 ± 0.22
100	17.86 ± 0.18	50.70 ± 1.69	3.21 ± 0.30	4.36 ± 0.31
500	17.67 ± 0.37	60.51 ± 1.06[c]	2.91 ± 0.18	4.42 ± 0.24
Subchronic[b]				
0	17.30 ± 0.28	48.45 ± 1.25	3.14 ± 0.24	3.91 ± 0.17
250	17.22 ± 0.27	53.96 ± 0.62[c]	3.08 ± 0.19	3.89 ± 0.14
500	16.74 ± 0.35	58.19 ± 0.98[c]	3.25 ± 0.10	4.06 ± 0.16
1000	17.28 ± 0.35	62.13 ± 1.08[c]	2.62 ± 0.14[c]	3.56 ± 0.23

The data are representative of 2 experimental trials.
BW, Body weight.
[a]Data are presented as means ± S.E.M., $n = 6$–8/dose.
[b]Total dose is shown.
[c]Statistically different from control ($P < 0.05$).

Table 3
Hepatic microsomal cytochrome P450 activities and content of female C57BL/6 mice treated with a single or 14 daily doses of DE-71[a]

DE-71 (mg/kg)	PROD (nmol/min/mg)	EROD (nmol/min/mg)	P450 (nmol/mg)
Acute			
0	0.046 ± 0.017	0.118 ± 0.010	0.664 ± 0.068
0.8	0.030 ± 0.004	0.134 ± 0.020	0.760 ± 0.064
4	0.029 ± 0.002	0.113 ± 0.023	0.660 ± 0.065
100	0.069 ± 0.011	0.109 ± 0.018	0.672 ± 0.128
500	0.136 ± 0.013[c]	0.147 ± 0.017	0.644 ± 0.076
Subchronic[b]			
0	0.104 ± 0.010	0.058 ± 0.005	0.619 ± 0.027
250	0.484 ± 0.037[c]	0.131 ± 0.011[c]	0.760 ± 0.020[c]
500	0.478 ± 0.058[c]	0.192 ± 0.025[c]	0.787 ± 0.033[c]
1000	0.389 ± 0.034[c]	0.192 ± 0.003[c]	0.882 ± 0.034[c]

The data represents one experimental trial.
[a]Data are presented as means ± S.E.M., $n = 6$–8/dose.
[b]Total dose is shown.
[c]Statistically different from control ($P < 0.05$).

following acute exposure (Table 3). Subchronic DE-71 exposure resulted in significant induction of PROD activity which was maximal with 250 mg DE-71/kg (4.7-fold), and an induction of EROD activity which was maximal at 500 mg DE-71/kg (3.3-fold) (Table 3). Hepatic microsomal P450 content was dose-dependently increased up to 40% above controls in mice treated subchronically with DE-71.

4. Discussion

Induction of hepatic microsomal EROD activity is generally correlated with humoral immunotoxicity to the anti-SRBC response from exposure to halogenated aromatic hydrocarbons (HAH) such as: dioxins (Vecchi et al., 1983), furans (Davis and Safe, 1988), biphenyls (Silkworth et al., 1984) and CDEs (Howie et al., 1990) in C57BL/6 mice. The correlation of these effects is largely explained by the Ah-receptor model (Poland and Glover, 1980). However, Howie et al. (1990) observed marked congener-specific differences in potencies for EROD induction and immunotoxicity in CDEs, including some congeners which did not fit the correlation seen with the majority of CDEs. The congeners which did not show classic Ah-receptor-dependent effects in their study included 2,3′,4,4′,5,5′-hexaCDE, a congener which exhibited low EROD-inducing potential and a lack of a dose-response in humoral immunotoxicity. Other mono-*ortho*-substituted CDE congeners tested were highly potent for both EROD induction and immunotoxicity. The lack of classic Ah-receptor-dependent effects is similar to the modest EROD induction and poorly correlated immunotoxicity seen in our study using DE-71. Based on these results it is clear that EROD activity per se does not parallel immune suppression when maximal EROD induction is relatively modest.

At equivalent doses, acute exposure to DE-71 did not yield as great an induction of P450 enzymes as did daily exposure for 14 days. This is likely due to the fact that acute exposure was given 8 days prior to necropsy, whereas subchronic exposure continued until the day prior to necropsy. Cytochrome P450 PROD and EROD activities were both significantly induced by subchronic exposure to DE-71, indicating that DE-71 induces both the IIB1 and IA1 forms of cytochrome P450 in mice. This is consistent with the results using Bromkal-70 in rats (Von Meyerinck et al., 1990) with the exception that, in our studies in mice, higher IIB1 type induction (using the PROD assay) and lower IA1 induction (using the EROD assay) was observed. The lack of highly pronounced induction of EROD activity in our study indicates either that rats are more readily induced by PBDE than mice, or that the DE-71 formulation of brominated diphenyl ethers is missing specific isomers that are present in the experiments using Bromkal-70 (Von

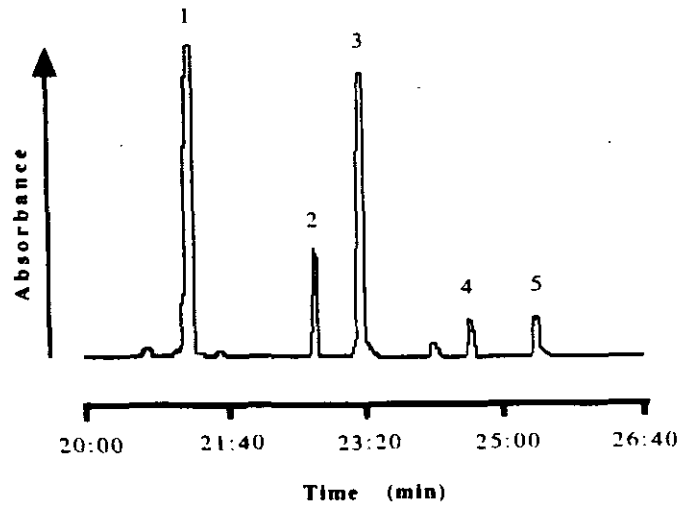

Fig. 4. Gas chromatogram of DE-71 showing five major peaks corresponding to 1, tetrabromodiphenyl ether (33%); 2 and 3, pentabromodiphenyl ethers (58%); 4 and 5, hexabromodiphenyl ethers (6%). Other minor peaks make up 3% of the mixture.

Meyerinck et al., 1990). While the gas chromatographic analysis of DE-71 (Fig. 4) appears to be very similar to Bromkal-70 (Von Meyerinck et al., 1990), the isomeric make-up of the formulation is not known.

It was apparent from our studies that the order in which mice were killed at the end of subchronic treatment correlated with CS levels, with higher CS levels seen in mice that remained in the cage the longest. This was likely due to stress caused by the repeated disturbance of the cage during captures. Interestingly, there was a significant interactive effect of DE-71 and the order of kill on elevated CS levels. These results suggest either an increase in CS production or a decreased clearance of CS by the liver in animals treated with DE-71 following acute stress. Further investigation into this phenomenon is warranted.

Excluding the 100 mg/kg acute dose, total T4 levels were significantly suppressed following all doses of DE-71 given, both acutely and subchronically. Interestingly, the degree of suppression of T4 was as great at low acute doses as it was at higher subchronic doses. Because of the different exposure protocols, it is difficult to directly compare mechanistic bases for the suppression. However, it suggests that the effect of DE-71 on T4 is saturable at very low doses and persists for at least 7 days after exposure is terminated.

Suppression of NKC activity has been reported in mice treated with T4

(Gupta et al., 1983; Stein-Streilein et al., 1987), or under conditions of hyper- and hypothyroidism in C57BL/6 mice (Kinoshita et al., 1991). The suppressed T4 levels seen in our study did not result in decreased NKC activity, suggesting that a depressed circulating T4 concentration alone is not sufficient to cause suppression of NKC activity. Interestingly, ^{125}I-induced hypothyroidism has also been shown to have no effect upon NKC activity (Sharma et al., 1982). Given the close structural similarity of some BDE congeners that may be present in DE-71 to that of T4, DE-71 could have either agonistic or antagonistic properties to T4 receptors. The suppression of T4 levels seen in DE-71 exposure is unlikely due to competition for binding to carrier proteins in the plasma since total and free T4 were affected in a similar dose-dependent manner. The cause of the lowered hormone concentration may instead be at the thyroid gland itself. This is supported by previous reports of bromine levels 12 times that of normal controls found in the thyroid gland of rats exposed to PBDE in the diet for 28 days (U.S. Environmental Protection Agency, 1988.) The potential for structure-specific binding to T4 receptors for chlorinated aromatic hydrocarbons has been reviewed (McKinney, 1989).

In conclusion, the DE-71 mixture of BDE congeners appears to be modestly immunotoxic to the anti-SRBC response but not NKC activity in C57BL/6 mice at a subchronic dose totaling 1000 mg/kg DE-71. This same dose results in the weak, saturated induction of EROD and PROD liver enzyme activity, induction of total microsomal P450 content, suppression of circulating T4 and elevation of CS levels. Acute exposures to DE-71, while not immunotoxic, resulted in a modest induction of liver PROD activity following a high dose (500 mg/kg) and suppression of total serum T4 at much lower doses (0.8 mg/kg).

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INDUCTION OF XENOBIOTIC METABOLISM IN RATS BY BROMINATED DIPHENYL ETHERS ADMINISTERED FOR 90 DAYS

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SUMMARY

The administration of doses as low as 0.78 μmol/kg/day p.o. for 90 days of the commercial fire retardants pentabromodiphenyl ether and octabromodiphenyl ether to rats resulted in increased *O*-ethyl *O*-*p*-nitrophenyl phenylphosphonothioate (EPN) detoxification and *p*-nitroanisole demethylation. This dose of pentabromodiphenyl ether and higher doses of octabromodiphenyl ether also increased cytochrome P-450 and NADPH cytochrome *c* reductase. Measurements made at 30 and 60 days after the last dose showed that these indicators of induced xenobiotic metabolism return to control levels slowly. The results demonstrate that these inducers are not only potent but that their effects may be long-lasting.

INTRODUCTION

Induction of xenobiotic metabolism has been shown to occur following the administration of a variety of halogenated aromatic compounds including polychlorinated biphenyls [1—3], brominated biphenyls [4], chlorinated [5] and brominated benzenes [6], chlorinated dioxins [7] and chlorinated dibenzofurans [8]. More recently, commercial mixtures of brominated diphenyl ethers, which are used as fire retardants, have been shown to be potent inducers of xenobiotic metabolism [9].

In view of the accumulation of bromine in rat tissues following the administration of as little as 1.0 mg/kg/day of decabromobenzene for 2 years [10] and liver enlargement when it was fed at a level of 1% in the diet for 30 days [11], it was decided to try to determine if low levels of administration of brominated diphenyl ethers for long periods of time resulted in increases in xenobiotic metabolism and, if so, whether the induction might

Abbreviation: EPN, *O*-ethyl *O*-*p*-nitrophenyl phenylphosphonothioate.

be long-lasting because of the apparent retention of these compounds by the body.

METHODS

Pentabromodiphenyl ether (lot 345-146 A) and octabromodiphenyl ether (lot 476-109 F) were gifts of the Great Lakes Chemical Corp. (West Lafayette, IN). These are commercial mixtures whose compositions have previously been identified regarding relative contributions according to the degree of bromination [9]. These compounds were dissolved in corn oil and administered p.o. to male Sprague—Dawley rats (Laboratory Supply Co., Indianapolis, IN) weighing 200 to 250 g daily for 90 days. When the original doses of 6.25, 12.5 and 25 μmol/kg/day revealed extensive induction even at the lowest dose, the experiment was repeated at doses of 0.78, 1.56 and 3.13 μmol/kg/day.

1, 30 or 60 days after the last dose, the animals were anesthetized lightly with ether and portions of the liver were removed for fixation in buffered formalin, staining with hematoxylin and eosin, and histological evaluation. Another portion was used for measurement of EPN detoxification and *p*-nitroanisole demethylation according to the procedure of Kinoshita et al. [12]. The remaining liver was perfused in situ with cold isotonic saline and microsomal fractions prepared as previously described [13]. Measurements of NADPH cytochrome *c* reductase and cytochrome P-450 were made according to the procedure of Dallner [14]. Proteins were measured by the Lowry method [15].

Values were expressed as the mean ± S.E. for groups of 6 animals. Duncan's new multiple range-finding test was used to compare all treatments among themselves and with the control [16]. The level of significance selected was $P < 0.05$.

RESULTS

Initial 90-day studies revealed that the administration of 6.25, 12.5 or 25 μmol/kg/day of the brominated diphenyl ethers resulted in large increases in EPN detoxification, *p*-nitroanisole demethylation and cytochrome P-450 (Table I). It should be noted that pentabromodiphenyl ether did not cause large increases in NADPH cytochrome c reductase, and octabromodiphenyl ether did not cause significant increases even at the highest dose level.

When lower doses were tested, even the lowest dose of pentabromodiphenyl ether (0.78 μmol/kg/day) caused increases in EPN detoxification, *p*-nitroanisole demethylation, NADPH cytochrome *c* reductase and cytochrome P-450 (Table II). For EPN detoxification and *p*-nitroanisole demethylation there was a clear-cut dose response relationship. When the animals were allowed a 30-day recovery period following the last (90th) dose, elevations in these two measurements were still observable at all doses, although only

TABLE I

EFFECT OF PENTABROMODIPHENYL ETHER OR OCTABROMODIPHENYL ETHER FOR 90 DAYS ON EPN DETOXIFICATION, *p*-NITROANISOLE DEMETHYLATION, CYTOCHROME *c* REDUCTASE ACTIVITY AND CYTOCHROME P-450 CONTENT

Dose μmol/kg/day	EPN detoxification, μg *p*-nitrophenol/50 mg/30 min	*p*-Nitroanisole demethylation, μg *p*-nitrophenol/50 mg/30 min	Cytochrome *c* reductase, nmol cyto. *c* reduced/min/mg protein	Cytochrome P-450 nmol/mg protein
Pentabromodiphenyl ether				
0	9.6 ± 0.3[a]	7.6 ± 0.1[a]	88 ± 6[a]	0.60 ± 0.04[a]
6.25	17.7 ± 0.5[b]	25.6 ± 1.2[b]	117 ± 9[b]	1.02 ± 0.06[b]
12.5	21.5 ± 0.6[c]	34.6 ± 1.1[c]	120 ± 5[b]	1.16 ± 0.07[b]
25	26.5 ± 0.7[d]	50.1 ± 1.3[d]	138 ± 7[b]	1.14 ± 0.07[b]
Octabromodiphenyl ether				
0	9.7 ± 0.5[a]	10.0 ± 0.4[a]	171 ± 11[a]	1.04 ± 0.10[a]
6.25	21.3 ± 1.1[b]	20.9 ± 1.8[b]	197 ± 9[a]	1.56 ± 0.13[b]
12.5	26.9 ± 2.2[b]	27.3 ± 1.3[c]	196 ± 18[a]	1.27 ± 0.16[a,b]
25	37.1 ± 2.0[c]	38.4 ± 1.9[d]	208 ± 11[a]	1.46 ± 0.09[b]

[a–d] Values with same superscript are not significantly different from one another ($P > 0.05$).

with the highest dose was increased NADPH cytochrome *c* reductase activity present. Within this time period, cytochrome P-450 content had returned to control level. Even 60 days after the last dose slight elevations were noted in EPN detoxification and *p*-nitroanisole demethylation at the two higher (1.56 μmol/kg/day and 3.13 μmol/kg/day) levels.

Octabromobenzene caused no induction of NADPH cytochrome *c* reductase and cytochrome P-450 at these lower doses (Table III). However, again the dose of 0.78 μmol/kg/day caused increases in both EPN detoxification and *p*-nitroanisole demethylation and larger increases were seen with increasing doses. After a 30-day recovery period only in the animals receiving the highest dose of 3.13 μmol/kg/day was there evidence of the maintenance of an induced state. These elevations were still observable 60 days after the last dose.

When the livers from the rats treated with the brominated diphenyl ethers in the low dose study were examined by light microscopy, no abnormalities were observed which could be related to the diphenyl ether administration.

DISCUSSION

Previous data had indicated that the octabrominated and pentabrominated diphenyl ethers used in this study were potent inducers of xenobiotic meta-

TABLE II

EFFECT OF PENTABROMODIPHENYL ETHER FOR 90 DAYS FOLLOWED BY RECOVERY PERIODS ON EPN DETOXIFICATION, *p*-NITROANISOLE DEMETHYLATION, NADPH CYTOCHROME *c* REDUCTASE AND CYTOCHROME P-450

Dose μmol/kg/day	EPN detoxification, μg *p*-nitrophenol/50 mg/30 min	*p*-Nitroanisole demethylation, μg *p*-nitrophenol/50 mg/30 min	NADPH cytochrome *c* reductase, nmol cyto. *c* reduced/min/mg protein	Cytochrome P-450, nmol/mg protein
Administration for 90 days				
0	5.8 ± 0.1[a]	5.5 ± 0.4[a]	165 ± 10[a]	1.05 ± 0.02[a]
0.78	8.1 ± 0.2[b]	9.7 ± 0.6[b]	215 ± 13[b]	1.41 ± 0.07[b]
1.56	8.9 ± 0.3[b]	12.2 ± 0.8[c]	194 ± 21[a,b]	1.26 ± 0.06[b]
3.13	11.4 ± 0.6[c]	16.9 ± 0.4[d]	209 ± 14[a,b]	1.25 ± 0.09[b]
Administration for 90 days plus a 30-day recovery period				
0	6.0 ± 0.2[a]	5.5 ± 0.4[a]	128 ± 9[a]	0.80 ± 0.07[a]
0.78	7.2 ± 0.2[b]	7.5 ± 0.3[b]	138 ± 1[a,b]	0.87 ± 0.04[a]
1.56	7.4 ± 0.5[b]	7.9 ± 0.4[b]	138 ± 8[a,b]	0.96 ± 0.18[a]
3.13	9.1 ± 0.2[c]	10.7 ± 0.9[c]	152 ± 6[b]	0.90 ± 0.13[a]
Administration for 90 days plus a 60-day recovery period				
0	6.3 ± 0.3[a]	3.6 ± 0.3[a]	155 ± 9[a]	0.82 ± 0.07[a]
0.78	6.4 ± 0.3[a]	3.8 ± 0.2[a]	162 ± 14[a]	0.85 ± 0.12[a]
1.56	7.7 ± 0.3[b]	4.6 ± 1.0[b]	148 ± 7[a]	0.85 ± 0.06[a]
3.13	7.5 ± 0.4[b]	5.6 ± 0.3[c]	172 ± 8[a]	0.82 ± 0.06[a]

[a–d] For each time period, values with same superscript are not significantly different from one another ($P > 0.05$).

bolism when administered at a dose of 100 μmol/kg/day for 14 days. They caused much larger increases than were seen with bis (*p*-bromodiphenyl) ether, the fully substituted decabromodiphenyl ether or the parent compound diphenyl ether [9]. In the present study it was important to determine if low levels over a prolonged period of time would cause such an induction and if this might be long-lasting because of the distinct possibility of retention of these materials.

It was demonstrated that doses of less than 1 μmol/kg/day, if given for 90 days, could cause increases in xenobiotic metabolism. As might be expected, at higher doses even larger increases were observed. Depending upon the dose, some changes were still observable 30 to 60 days after the last dose. Although measurements of tissue content were not made, it is probable that this extended period of induction is the result of the potency of these agents as inducers and accumulation in sites such as adipose tissue and liver. This would be similar to the explanation of Hart and Fouts [17] for the prolonged elevations in xenobiotic metabolism following the feeding of DDT.

TABLE III

EFFECT OF OCTABROMODIPHENYL ETHER FOR 90 DAYS FOLLOWED BY RECOVERY PERIODS ON EPN DETOXIFICATION, *p*-NITROANISOLE DEMETHYLATION, NADPH CYTOCHROME *c* REDUCTASE AND CYTOCHROME P-450

Dose μmol/kg/day	EPN detoxification, μg *p*-nitrophenol/50 mg/30 min	*p*-Nitroanisole demethylation, μg *p*-nitrophenol/50 mg/30 min	NADPH cytochrome *c* reductase, nmol cyto. *c* reduced/min/mg protein	Cytochrome P-450, nmol/mg protein
Administration for 90 days				
0	7.1 ± 0.3[a]	9.0 ± 0.4[a]	199 ± 14[a]	1.08 ± 0.09[a]
0.78	9.5 ± 0.4[b]	11.9 ± 0.6[b]	190 ± 8[a]	1.04 ± 0.05[a]
1.56	11.0 ± 0.6[b]	13.8 ± 0.4[c]	217 ± 13[a]	1.21 ± 0.06[a]
3.13	12.4 ± 0.5[c]	14.5 ± 0.8[c]	216 ± 22[a]	1.24 ± 0.08[a]
Administration for 90 days plus a 30-day recovery period				
0	10.6 ± 0.4[a,b]	12.0 ± 0.6[a]	104 ± 4[a,b]	0.88 ± 0.05[a]
0.78	9.9 ± 0.9[a]	13.4 ± 0.8[a,b]	86 ± 6[a]	0.82 ± 0.05[a]
1.56	10.9 ± 0.8[a,b]	13.7 ± 0.4[a,b]	109 ± 8[b]	0.89 ± 0.05[a]
3.13	12.4 ± 0.7[b]	14.3 ± 0.7[b]	103 ± 8[a,b]	0.88 ± 0.03[a]
Administration for 90 days plus a 60-day recovery period				
0	6.8 ± 0.3[a]	6.3 ± 0.3[a]	157 ± 6[a]	0.93 ± 0.02[a]
0.78	8.3 ± 0.4[a,b]	7.9 ± 0.3[b]	151 ± 5[a]	0.95 ± 0.06[a]
1.56	9.2 ± 0.9[b]	8.4 ± 0.3[b]	165 ± 6[a]	0.92 ± 0.02[a]
3.13	11.4 ± 0.7[c]	10.6 ± 0.7[c]	165 ± 7[a]	0.92 ± 0.04[a]

[a–c] For each time period, values with same superscript are not significantly different from one another ($P > 0.05$).

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