

Chlorobornanes in Water, Sediment, and Fish from Toxaphene Treated and Untreated Lakes in Western Canada

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Concentrations of toxaphene (CHB) and 2-*exo*,3-*endo*,5-*exo*,6-*endo*,8,9,10-heptachlorobornane (B7-1001; Hp-Sed), a major toxaphene component known to be present in the sediment of toxaphene treated lakes, were determined in water, sediment, and fish from 13 lakes to examine the relationship between chlorobornane levels in fish and environmental variables. The lakes were situated in either the boreal plain, montane, or subalpine ecozones in western Canada. Five of the lakes had been treated with toxaphene between 1958 and 1962. Mean concentration of CHB (all chlorobornane congeners) in fish from the lakes ranged from 1.9 to 303 $\mu\text{g}/\text{kg}$ with the maximum concentration occurring in lake trout from Bow Lake, an oligotrophic glacial fed lake with no record of toxaphene usage. CHB and in particular B7-1001 levels, were higher in water, sediment, and fish from treated relative to matched untreated lakes selected as "control" sites. CHB concentrations in sediment were not related to the levels measured in fish; however, levels of atmospheric CHB_{Atm} (CHB_{Atm} = CHB - [B7-1001]) in fish, from both treated and untreated lakes combined, were inversely related to indicators of lake productivity ($p < 0.05$, dissolved phosphorus $r = -0.64$, percent organic content of lake sediment $r = -0.84$, and mid-summer water temperature $r = -0.68$). These relationships suggest that the process and pathway of CHB_{Atm} accumulation in fish is mediated by limnological and perhaps physiological factors in addition to exposure levels. B7-1001 concentrations in fish were not related to indicators

of lake productivity or the characteristics of fish from the lakes (weight, age, lipid content), but were related to B7-1001 concentration in sediments ($r = 0.74$, $p < 0.05$).

Introduction

Toxaphene, a complex mixture of chlorobornanes (CHBs), was used primarily as an insecticide in agricultural crops (1), although it was also widely used in the 1950s and 1960s by sport fish managers to remove undesirable fish species from lakes (2–5). Manufacture of toxaphene was banned in the United States in 1982, although the use of existing stocks was permitted until 1986. Use was restricted in Canada in the 1970s (1). However, it has been shown to persist for decades in lakes where it was directly applied (5). Within aquatic systems, toxaphene biomagnifies through the food chain (6–8), and lakes with the longest food chains have been shown to have the highest concentrations of toxaphene in fish predators (9).

The objective of our study was to determine CHB concentrations in water, sediment, and fish from both toxaphene treated and untreated lakes. For sediment and fish, CHB or 21 congeners plus 2-*exo*,3-*endo*,5-*exo*,6-*endo*,8,9,10-heptachlorobornane, or B7-1001 [according to new systematic nomenclature (10)] were quantified. B7-1001 is the major heptachlorobornane known to be present in sediments of lakes treated with toxaphene (5, 11). We defined atmospherically derived toxaphene CHB_{Atm} as CHB - (B7-1001). In this paper, we test the hypotheses that CHB_{Atm} and B7-1001 concentrations in fish were (a) higher in water, sediment, and fish from lakes treated with toxaphene relative to untreated lakes and were related to (b) limnological aspects of lakes (temperature, dissolved phosphorus, elevation, and sediment organic content) or (c) fish characteristics (weight, age, lipid content). The samples for this study were collected in 1992–1993 from eight untreated lakes and from five lakes treated once with toxaphene between 1958 and 1962.

The 13 lakes of this study are situated either in boreal plain, montane, or subalpine ecozones. Eleven of the 13 lakes are in Alberta, and the other two are in British Columbia near the Alberta/British Columbia boundary (Figure 1). The lakes ranged in size from 24 ha (Peanut and Moab Lakes) to 2066 ha (Maligne), in maximum depth from 12 to 96 m, and in elevation from 534 to 1945 m above sea level (Table 1). Mid-summer water temperatures measured August 9–13, 1994, for the 2–5 m depth zone ranged from 9.5 to 20.9 °C. One or two fish species were present in most of the lakes, limiting the potential for biomagnification of CHBs through the food chain (9). Rainbow trout (*Oncorhynchus mykiss*) were collected from more than half of the lakes (eight) while brook trout (*Salvelinus fontinalis*), lake trout (*S. namaycush*), longnose suckers (*Catostomus catostomus*), and mountain whitefish (*Prosopium williamsoni*) were taken from the other lakes.

Lakes Treated with Toxaphene. Five of the lakes had been treated with toxaphene to remove native fish in preparation for rainbow trout and/or brook trout introductions. Peanut Lake was treated with 7.5 $\mu\text{g}/\text{L}$ toxaphene in September 1961, and Chatwin Lake was treated at 18.4 $\mu\text{g}/\text{L}$ in October 1962 (5). Records of the quantity of toxaphene used in the remaining three lakes were not available. In the

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FIGURE 1. Location of lakes in Alberta and British Columbia.

case of toxaphene-treated Annette Lake, longnose suckers have re-invaded and are the dominant fish species found in the lake. Fathead minnow (*Pimephales promelas*) and brook stickleback (*Culaea inconstans*) have been re-introduced into Peanut Lake, but the other three lakes have remained free of "coarse" fish species. We selected some untreated lakes to provide "control" sites for the treated lakes. The control and treated pairs were similar in fish species composition, lake elevation, turbidity, and often lake morphometry. The matched pairs of treated and untreated lakes are Moab-Cabin, Emerald-Maligne, and Annette-Beauvert, respectively.

Materials and Methods

Field Methods. Forty-liter grab samples of water were collected in March 1994 from a depth of 5 m near the center of two treated and three untreated lakes. Surface sediments were also collected from the top 3 cm by Ekman dredge or freeze-coring from each of the five toxaphene-treated and three untreated lakes. Samples were taken at the deepest site in each lake. Frozen sediments were thawed and homogenized by hand mixing. Percent loss-on-ignition (%LOI) was determined by weight difference before and after combustion of dried sediments at 550 °C for 1.5 h (12). Organic carbon content of the sediments was $0.4 \times \%LOI$ because carbon represents about 40% of organic matter (12/30, C:H:O, molecular weight 12:2:16).

A total of 91 fish were collected with monofilament gill nets, comprised of six panels 1.8 × 30 m in length with mesh sizes of (stretch measure) 25, 38, 51, 76, 102, and 125 mm. Fish were measured and weighed, and otoliths were taken for age determination. Whole fish were wrapped in acetone/hexane-rinsed aluminum foil, frozen on dry ice, and stored at -10 °C.

Water Extraction and Analysis. Low-level dissolved phosphorus analyses were completed at the Environment Canada nutrient laboratory in Saskatoon, Saskatchewan. Water samples were filtered through a Whatman GF/C filter and then subjected to acid hydrolysis in the presence of potassium persulfate. To each 50 mL of water was added 0.45 mL of 31% sulfuric acid and 0.21 g of potassium persulfate. The samples were autoclaved at 121 °C for 30 min to dissolve the persulfate. Samples are analyzed with a Technicon AutoAnalyzer II system consisting of a sampler, pump, manifold, colorimeter, and recorder. A calibration curve was prepared using standard concentrations, and the

TABLE 1. Location, Elevation, and Morphometry of Lakes, and Characteristics of Fish Analyzed for Toxaphene

lake	location		elevation m	area ha	max depth, m	fish assemblage, species	mid-summer temp (2–5 m), °C	dissolved phosphorus µg/L	sediment organic carbon, %	fish analyzed			
	latitude	longitude								mean weight (SD) N, g	mean lipid content (SD), %	mean age (SD), yr	species ^a
Annette	52 54	118 02	1019	29	23	LNSC, LKCH, BKTR	18.3	0.6	7.6	1198 (58.6)	5.2 (0.8)	7.7 (1.4)	LNSC
Emerald	51 26	116 33	1300	116	28	BKTR, RNTR	12.5	3.4	1.1	557 (246.5)	7.5 (2.1)	3.8 (1.5)	BKTR
Chatwin	54 15	110 51	534	71	16	RNTR	20.4	37.0	18.5	650 (415.4)	5.6 (0.9)	4.7 (1.2)	RNTR
Moab	52 39	117 57	1204	24	18	RNTR, LKTR, CISC, BLTR	17.4	1.0	2.8	581 (66.7)	14.5 (1.4)	2.0 (0.0)	RNTR
Peanut	54 01	114 21	660	24	14	RNTR, FTMN, BRST	20.9	9.0	15.9	376 (65.1)	3.0 (0.6)	4.1 (0.7)	RNTR
Beauvert	52 53	118 03	1021	32	25	LNSC, WHSC, MNWT, RNTR, BRTR	14.5	1.4	6.1	1340 (71.4)	7.8 (3.7)	9.8 (1.7)	LNSC
Bertha	49 01	113 56	1774	30	50	RNTR	12.2	1.8	— ^b	420 (65.3)	9.6 (2.0)	5.0 (1.2)	RNTR
Bow	51 40	116 27	1940	280	51	LKTR, MNWH	12.0	0.5	1.2	981 (200.9)	3.0 (1.3)	7.8 (1.1)	LKTR
Cabin	52 53	118 08	1219	32	21	RNTR, LKCH	17.6	4.0	6.6	2565 (1166.5)	7.1 (3.8)	8.8 (1.6)	LKTR
Hector	51 35	116 21	1752	590	87	LKTR, MNWH, CTTR	11.7	1.3	1.3	374 (55.4)	2.9 (0.6)	5.2 (1.1)	RNTR
Maligne	52 39	117 30	1671	2066	96	BKTR, RNTR	13.8	0.8	2.2	528 (77.3)	6.3 (1.3)	12.4 (1.5)	MITWT
Moat	52 43	118 19	1945	35	10	RNTR	—	—	—	741 (174.9)	7.4 (1.0)	4.6 (1.3)	BKTR
Sherbrooke	51 27	116 23	1800	35	12	RNTR, LKTR	9.5	0.8	1.0	479 (330.3)	6.6 (4.7)	6.0 (0.0)	RNTR
										253 (22.1)	5.8 (0.6)	5.8 (0.5)	RNTR

^a LNSC, longnose sucker; LKCH, lake chub (*Couesius plumbeus*); BKTR, brook trout; RNTR, rainbow trout; LKTR, lake trout; MNWT, mountain whitefish; CISC, lake herring (*Coregonus artedii*); BLTR, bull trout (*Salvelinus confluentus*); FTMN, fathead minnow; BRST, brook stickleback; WHSC, white sucker (*Catostomus commersoni*); CTTR, cutthroat trout (*Oncorhynchus clarki*); ^b —, no data.

sample concentrations were determined by comparing the peak height of the sample against the calibration curve.

CHBs were extracted from water samples (unfiltered) into dichloromethane (DCM) using a Goulden large-volume liquid-liquid extractor (13, 14). The DCM extracts were shipped to the Freshwater Institute in Winnipeg, Canada, where they were stored at 4 °C until analysis. Extracts were dried over Na₂SO₄ (previously heated at 600 °C overnight), and the volume was reduced to approximately 1 mL using a rotary evaporator. The extract was exchanged into hexane and then separated into three fractions of increasing polarity on a Florisil column (8 g, 1.2% H₂O deactivated). The first fraction was eluted with hexane and contained PCBs, DDE, *trans*-nonachlor, chlorobenzenes, mirex, and a small proportion of CHBs, most notably T2 (2-*exo*,3-*endo*,5-*exo*,6-*endo*,8,8,10,10-octachlorobornane or B8-1413) (15, 16). Fraction 2 was eluted with hexane:DCM (85:15) and contained HCHs, chlordanes, and the remainder of the CHBs. The final fraction, containing dieldrin and heptachlor epoxide, was eluted with a 1:1 mixture of hexane:DCM. F1 and F2 were combined, spiked with ¹³C₈-Mirex and concentrated to 125 µL for mass spectral analysis.

Samples were analyzed using high-resolution gas chromatography-electron capture negative ion-high-resolution mass spectrometry (HRGC-ECNI-HRMS) in the selected ion mode on a Kratos Concept high-resolution mass spectrometer (EBE geometry) as described by Stern *et al.* (11). In brief, gas chromatographic separations were performed on a Hewlett-Packard 5890-II GC using a 60 m × 0.25 mm i.d. DB-5ms fused silica column (film thickness 0.24 µm) and pressure-programmed He carrier gas. Selected ion ECNIMS was performed at a resolving power of ~12000 (ion source temperature = 120 °C) using methane as the moderating gas and perfluorokerosene as the mass calibrant. Characteristic ions were monitored from the (M - Cl)⁻ isotopic cluster of the hepta- to nonachlorobornane homologue groups; C16 308.8962, 310.9323; C17 376.8573, 378.8543; C18 410.8183, 412.8154 were monitored. The ions used for quantification (in italics above), in the chlorine homologue classes were summed for a total toxaphene area, which was quantified against the area of a toxaphene standard (Radian Corporation). ¹³C₈-Mirex was monitored to correct for any variation in source performance of the mass spectrometer between runs. As no standard for B7-1001 was available, quantification was carried out using the relative response factor for total toxaphene. This response factor yielded similar results to the response factor for the heptachlorobornane congener, Parlar 32 (G. A. Stern, unpublished data).

CHBs were not detected in a 40-L blank sample of water from an artesian spring near Nokomis, Saskatchewan (51°30' N, 105°10' W). From ¹⁴C dating of dissolved inorganic carbon, it was established that this water was at the surface of the earth great than 40 000 years ago.

Fish and Sediment Extraction Analysis. Extraction and analysis of fish and sediment were carried out by Axys Analytical, Sidney, British Columbia. Frozen whole fish samples were homogenized using a solvent-rinsed meat grinder and then refrozen until needed. Prior to extraction, the samples were thawed and further mixed to ensure homogeneous subsampling. Samples (about 10 g wet weight) were spiked with an internal standard (¹³C]PCB-180, [¹³C₁₂]2,2',3,4,4',5,5'-heptachlorobiphenyl, 80 ng), ground with Na₂SO₄ to a free flowing powder, packed into a glass chromatographic column containing DCM:hexane (1:1), and extracted by elution with 300 mL of additional solvent. The extract volume was reduced to 5 mL using a rotary evaporator and passed through a calibrated gel permeation column (Bio-Beads SX-3) to separate lipids. The 130–300 mL fraction was reduced down to 2 mL, and the toxaphene separated into two fractions on a Florisil column (8 g, 1.8% water

deactivated). The first fraction was eluted with 20 mL of hexane then by 25 mL of hexane:DCM (85:15) and was comprised of CHBs, PCBs, and most of the nonpolar chlorinated pesticides. The second fraction was eluted with 50 mL of hexane:DCM (1:1) and contained the organochlorine compounds.

About 5 g dry weight of sediment from each lake was ground with anhydrous sodium sulfate. The sediments were extracted by the same column elution method used for fish except the lipid separation step was omitted. Sulfur was removed from the sediment extracts by treatment with activated copper prior to Florisil chromatography.

Analysis was carried out as described by Fowler *et al.* (17). Briefly, samples were analyzed using GC-ECNIMS (nominal resolution) in the selected ion mode on a Finnigan Isco 50B mass spectrometer equipped with a Varian 3400 GC and a 60 m × 0.25 mm i.d. DB5 column (0.1 µm film thickness). Methane was used as the moderating gas. The two most intense isotopic peaks of the (M - Cl)⁻ ion clusters for the hepta- to decachlorobornanes were monitored along with characteristic ions for chlordane, nonachlor, and ¹³C-labeled PCBs. The latter were used as quantification internal and recovery standards. CHB quantitation was based on the total response of 21 prominent peaks present in the ECNIMS selected ion chromatograms of the technical standard and were well separated from all major components of technical chlordane, including the *cis* and *trans* isomers of chlordane and nonachlor. B7-1001 was quantified separately using the relative response factor from 2,2,5-*endo*,6-*exo*,8,9,10-heptachlorobornane (Parlar 32).

Detection limits were calculated on a sample-specific basis and were reported for each sample using instrument detection limits plus three times the standard deviation of the lowest concentration analysis (18). For sediments, the sample detection limits ranged from 0.06 to 0.7 µg/kg for CHB_{Atm} and B7-1001. For fish, the sample detection limits ranged from 0.02 to 0.2 µg/kg for CHB_{Atm} and B7-1001. A method blank of 0.1–0.2 g of cod liver oil with an expected "in-house" result of 5000 ± 1600 µg/L CHB_{Atm} was run with each batch of eight samples. Mean recovery was 4776 µg/L (SD = 804, range 3400–6000, N = 13). To evaluate analytical precision, homogenized tissue from 10 fish were re-submitted to Axys for analysis as "blind" duplicates. For these samples, the percent difference (PD = difference/mean × 100) ranged from 0% to 32% with a mean of 14%.

Results

CHB water concentrations in two treated lakes, Emerald and Annette, were 0.218 and 0.373 ng/L, respectively, and ranged from 0.062 to 0.108 ng/L in three untreated lakes (Table 2). B7-1001 corresponded to ~7% of CHB in untreated lakes and ranged from 12 to 22% in two treated lakes.

CHB concentrations in the surface sediment from the five treated lakes ranged from 2.6 to 110 µg/kg and were at or below the detection limit (0.3 µg/kg) in the untreated control lakes (Table 2). B7-1001 was the major CHB congener present in the treated lakes contributing from 81 to 99% of CHB.

CHB levels in fish ranged from 3.3 to 82.0 µg/kg in treated lakes and from 1.9 to 303 µg/kg in untreated lakes (Table 2). Mean CHB concentrations were found to be 15 times higher (range 2–22 times) in fish from treated lakes relative to matched untreated lakes selected as control sites (*p* < 0.05 in all cases, Student's *t* test for means between treated and untreated lakes). However, the highest CHB concentrations were recorded in fish taken from untreated Bow Lake. The mean maximum value recorded, 303 µg/kg, was from large lake trout (mean weight 2565 g, N = 5).

CHB Relationships among Water, Sediment, and Fish. CHB levels in lake water were not related to those in fish (*r*

TABLE 2. Chlorobornanes (CHB) in Water, Sediment (Dry Weight), and Fish (Wet Weight) from Lakes Treated with Toxaphene and in Untreated Lakes

lake	treated with toxaphene, year	water ^a			sediment ^b			fish								
		CHB, ng/L	CHB _{Atm} , ng/L	B7-1001, ng/L	CHB, µg/kg	CHB _{Atm} , µg/kg	B7-1001, µg/kg	CHB, µg/kg	CHB _{Atm} , µg/kg		B7-1001, µg/kg					
													mean	(SD)	mean	(SD)
Rainbow Trout																
Moab	1958	—	—	—	86.4	3.4	83	73.1	25.2	(6.4)	47.9	(25.2)				
Emerald	1959	0.218	0.192	0.026	2.6	0.5	2.1	82	74.7	(49.4)	7.3	(7.5)				
Peanut	1961	—	—	—	110	<0.2	110	3.3	0.03	(0.06)	3.3	(1.8)				
Chatwin	1962	—	—	—	52	<0.2	52	42.6	1.3	(0.12)	41.3	(1.5)				
Cabin	no	0.092	0.085	0.007	<0.2	<0.2	<0.2	3.3	2.9	(1.9)	0.4	(0.16)				
Bertha	no	—	—	—	—	—	—	12.4	11.4	(2.7)	1	(0.4)				
Sherbrooke	no	—	—	—	—	—	—	12.2	11.94	(3.18)	0.3	(0.18)				
Moat	no	—	—	—	—	—	—	29.7	27.9	(21.5)	1.8	(1.4)				
Brook Trout																
Emerald	1959	0.218	0.192	0.026	2.6	0.5	2.1	59.3	57.1	(33.8)	2.2	(1.5)				
Maligne	no	0.062	0.058	0.004	—	—	—	32.2	31.4	(6.5)	0.8	(0.27)				
Longnose Sucker																
Annette	1959	0.373	0.292	0.081	73.1	5.1	68	38.8	13.7	(4.3)	25.1	(9.2)				
Beauvert	no	—	—	—	<0.3	<0.3	<0.3	1.9	1.7	(0.6)	0.2	(0.09)				
Mountain Whitefish																
Hector	no	—	—	—	—	—	—	75.7	73	(14.4)	2.7	(1.56)				
Lake Trout																
Bow (av 981 g)	no	0.108	0.101	0.007	0.2	0.17	0.03	161	155	(72.9)	5.76	(3.61)				
Bow (2565 g)	no	0.108	0.101	0.007	0.2	0.17	0.03	303	294	(235)	8.48	(5.91)				

^a Single large volume samples from each lake. Results are repeated opposite each fish species. ^b All results for CHB in sediment are by low-resolution ECNIMS except for results from Bow Lake where high-resolution ECNIMS was used. ^c —, no data.

TABLE 3. Correlation Coefficients (*r* for All Lakes Combined) between CHB in Fish and CHB in Water and Sediment and between CHB and Limnological Conditions and Fish Characteristics^a

fish	CHB (N)	CHB _{Atm} (N)	B7-1001 (N)
water			
CHB	0.14 (7)	— ^b	—
CHB _{Atm}	—	0.00 (7)	—
B7-1001	—	—	0.65 (7)
sediment			
CHB	0.06 (10) ^a	—	—
CHB _{Atm}	—	0.36 (10)	—
B7-1001	—	—	0.74* (10) ^a
elevation	0.39 (15)	0.77* (15)	-0.35 (15)
mid-summer temperature	-0.33 (14)	-0.68* (14)	0.43 (14)
dissolved phosphorus	-0.33 (14)	-0.64* (14)	0.15 (14)
sediment organic carbon	-0.58* (13)	-0.84* (13)	0.18 (13)
fish			
mean weight	0.36 (15)	0.32 (15)	0.21 (15)
mean lipid content	-0.01 (15)	-0.12 (15)	-0.03 (15)
mean age	0.21 (15)	0.60* (15)	-0.28 (15)

^a All log-log relationships. An asterisk (*) indicates significant at <0.05. ^b —, values less than detection in Table 2 = 0.1 µg/kg.

= 0.14, *N* = 7, *p* > 0.10, log-log relationship, Table 3). Bioaccumulation factors (concentration in fish wet wt/concentration in water) from water to fish ranged from 104 to 376 × 10³ in the treated lakes and from 36 to 2805 × 10³ in the untreated lakes. In lake sediments, CHB concentrations were not related to those in fish for treated lakes (*r* = -0.51, *N* = 6, *p* > 0.10, log-log relationship) or for all lakes combined (*r* = 0.06, *N* = 10, *p* > 0.10, Table 3). The relationship between CHB concentrations in fish and organic carbon content of lake sediment was significant (*r* = -0.58, *N* = 13, *p* > 0.05). Sediment organic carbon content ranged from 1.0 to 18.5%. The bioaccumulation factor from sediment to fish (BSAF = CHB lipid weight fish/CHB sediment organic carbon content) for treated lakes ranged from 0.1 to 6.2.

CHB_{Atm} and B7-1001 in Fish. For treated and untreated lakes combined, CHB_{Atm} levels in fish were positively correlated with elevation (\log_{10} CHB_{Atm} = 4.46 \log_{10} elevation -

12.77, Figure 2, *r* = 0.77, *N* = 15, *p* < 0.01), were not correlated with CHB_{Atm} concentration in sediment (*r* = 0.36, *p* > 0.10, *N* = 10), but were correlated with mid-summer temperature of the epilimnion (\log_{10} CHB_{Atm} = 9.31 - 7.06 \log_{10} temperature, *r* = -0.68, *p* < 0.01, *N* = 14). Significant negative relationships were also observed with dissolved phosphorus in lake water (\log_{10} CHB_{Atm} = 1.44 - 1.24 \log_{10} phosphorus, *r* = -0.64, *N* = 14, *p* < 0.05), and the percent organic content of lake sediment (\log_{10} CHB_{Atm} = 2.04 - 1.95 \log_{10} percent organic content, *r* = -0.84, *N* = 13, *p* < 0.05). Organic carbon content of lake sediment was inversely related to lake elevation (*r* = -0.92, *N* = 11, *p* < 0.01).

For treated and untreated lakes combined, there was no significant relationship (*p* > 0.05) between CHB_{Atm} concentrations in fish and fish mean weight and lipid content (*r* = 0.32 and -0.12, respectively, *N* = 15, all values log transformed; Table 3). Lipid content of the fish ranged from 2.9% to 14.5% (*N* = 15). Mean CHB_{Atm} concentration in fish was

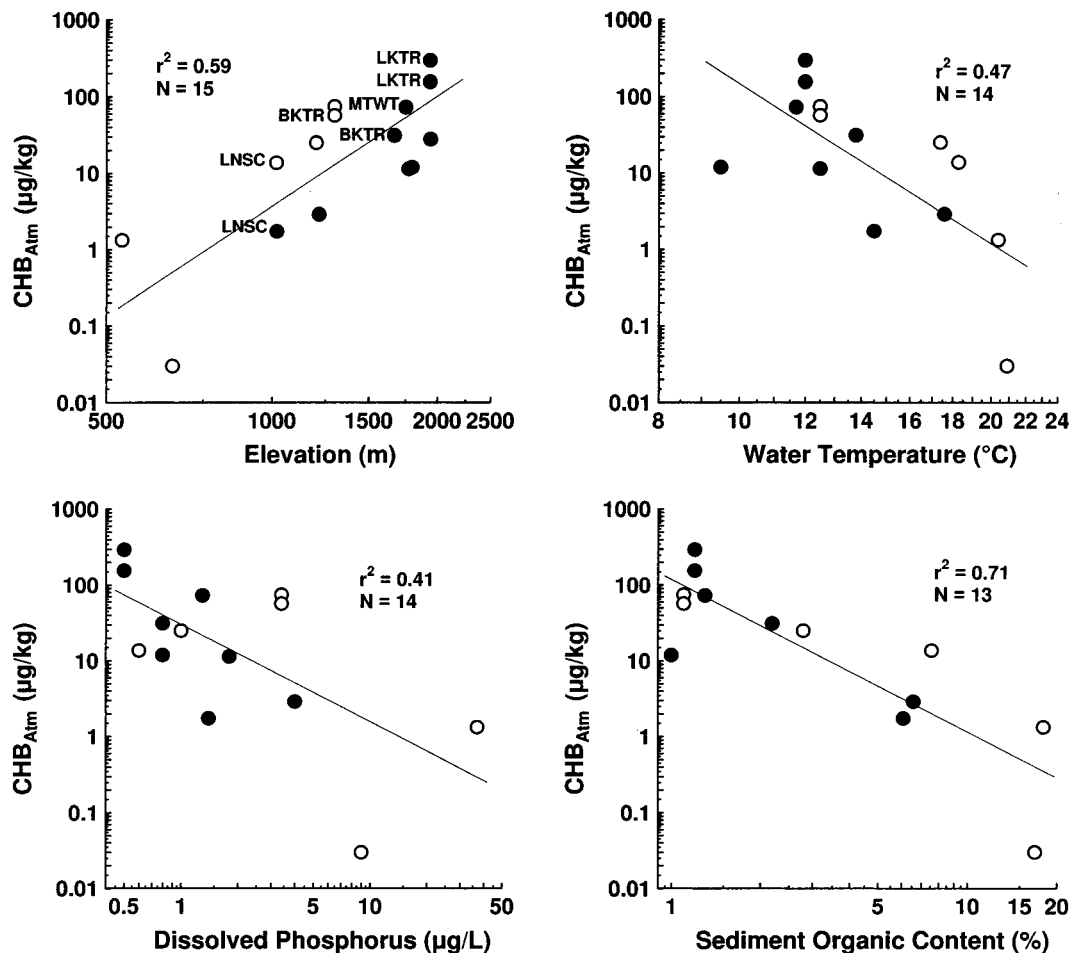


FIGURE 2. Relationship between CHB_{Atm} in rainbow trout and other fish species and lake elevation, mid-summer water temperature, dissolved phosphorus, and sediment organic content. All values were transformed to the \log_{10} (○, treated lakes; ●, untreated lakes; LKTR, lake trout; MTWT, mountain whitefish; BKTR, brook trout; LNSC, longnose sucker).

related to their mean age ($r = 0.60$, $p < 0.05$, all values log transformed).

In fish from treated lakes, B7-1001 represented from 65% to 99% of CHB (mean = 82%) with the exception of fish from Emerald Lake (Table 2). B7-1001 concentrations were higher in fish from treated lakes relative to matched untreated lakes by 3–180 times (mean = 106 times, $p < 0.05$ in all cases). The relationship between B7-1001 in fish and in sediment was significant ($r = 0.74$, $p < 0.05$; Table 3). B7-1001 in fish was not related to lake elevation, mid-summer water temperature, dissolved phosphorus in lake water, organic content of lake sediments, or to fish characteristics (weight, age, lipid content).

Discussion

CHBs were detected in water, sediment, and fish from all of the lakes surveyed in the boreal plain, montane, or subalpine ecozones in western Canada. Although toxaphene was used in limited quantities in the Canadian prairies for insect control until 1980, the presence of CHBs in this region is thought to be mainly due to long-range transport and deposition.

Atmospheric transport of toxaphene from areas of high use of this pesticide in the southern United States to higher latitudes has been demonstrated by residue analysis of air, water, and fish (19–21). To determine the potential source of atmospheric CHB_{Atm} , 5-day back trajectories at 700 mb (height of ≈ 1640 m) from Bow Lake (see Table 1) were computed every 6 h from January 1, 1990, to December 31, 1992 except for several days from February 18 to April 6 in 1991 and 1992. The trajectories were obtained from the

model developed by Olson *et al.* (22). The origin of the air masses for the other montane and subalpine lakes is expected to be similar to that found for Bow Lake.

Six-hour, 5-day back trajectories of the atmosphere for 3 years indicate that the air mass over Bow Lake originates from the Pacific and Arctic Oceans 60%, from the United States 21%, from Canada and Alaska 11%, and from Siberia 8% of the time. However, air mass movement from the United States (excluding Alaska) increased to 31% during the summer months (June–August) when organochlorine contaminant concentrations in the air are at a maximum (21). Of the 1 330 000 tons of toxaphene estimated to have been used globally from 1950 to 1993, the United States accounted for at least 20%, and Canada accounted for less than 0.01% (1). Thus, atmospheric transport from the United States could be an important source of persistent CHB to the study area.

B7-1001 was, in most part, associated with lakes treated with toxaphene (Table 2). A hexachlorobornane (Hxsed or B6-923) that is also prominent in sediments of toxaphene treated lakes (5, 11) was not determined in sediment and fish samples analyzed in this study. Although B7-1001 is present in the technical mixture in small amounts, anaerobic reductive dechlorination of other, less stable, CHB congeners present in the applied mixture is thought to be the most likely pathway leading to the predominant B7-1001 levels observed in treated lake sediment (5, 11). That B7-1001 is a major end product is not surprising, because like T2 (B8-1413; 11) and T12 (B9-1679), two CHBs known for their persistence in marine mammals, B7-1001 has the same

staggered 2-*exo*,3-*endo*,5-*exo*,6-*endo* ring structure (11, 15, 16). Relationships were not significant between B7-1001 in fish and limnological features of lakes or the condition of the fish (weight, age, lipid content). However, B7-1001 levels in fish were related to environmental concentrations of B7-1001 in sediment (Table 3) and may also be related to B7-1001 concentration in water. Additional data are required to evaluate a water–fish relationship. B7-1001 was more rapidly eliminated by salmonids than B8–1413 or B9–1679 and did not biomagnify in laboratory dietary accumulation studies (23). Because B7-1001 was also found in untreated lakes, analysis of only CHB_{Atm} would underestimate atmospheric contribution to lakes. However, the difference between CHB and CHB_{Atm} was small in water, sediment, and fish from untreated lakes.

The low CHB concentrations in surface sediment from Emerald Lake (2.6 µg/kg) relative to other treated lakes (mean = 80 µg/kg) may have been caused by deep burial of the applied toxaphene or loss through the outlet. Annual accumulation rates of sediment can be greater than 1 cm in lakes with inlets originating from glaciers (24). Active glaciers are present in the Emerald Lake drainage basin, and creeks flowing from glaciers to Emerald Lake are laden with fine clay particles or rock flour during summer. Consequently, Secchi depth in this lake was about 2 m. Clay that has a particle size of less than 50 µm from glaciers (24) may also act as a barrier to diffusion of chlorobornanes (25).

CHB levels were higher in fish from treated relative to untreated lakes regardless of species or their feeding habits (profundal and benthic littoral invertebrates for suckers; pelagic and littoral invertebrates for brook trout and rainbow trout). However, the absence of a direct relationship between CHB and CHB_{Atm} concentrations in fish and water or sediment suggests that the pathway for movement of this contaminant from the physical environment to the aquatic food web is complex. In Emerald Lake, for example, CHB levels were 32 times higher in rainbow trout than in sediment, but the opposite pattern was evident in Peanut Lake. There, CHB levels were 33 times higher in sediment as compared to fish. Duration of exposure, however, could account for these differences. While Peanut Lake was stocked with rainbow trout only 1 year prior to this study, trout from Emerald Lake ranged in age from 4 to 6 years. Our data indicate that high levels of CHB are maintained in the water column over contaminated sediments of treated lakes, but the processes and pathways of CHB accumulation in fish must be mediated by ecological or limnological factors in addition to exposure levels.

Concentrations of CHB_{Atm} in fish were not directly related to CHB_{Atm} in water and sediment. The significant negative relationship between CHB_{Atm} in fish and dissolved phosphorus in lake water, organic content in lake sediment, and mid-summer water temperature suggests that accumulation of CHB_{Atm} in fish is inversely related to indicators of lake productivity. Untreated oligotrophic lakes at high elevations such as Bow and Hector had the highest levels of CHBs in fish while treated eutrophic lakes at low elevation such as Peanut and Chatwin had lower levels of CHB. This suggests that loss of CHB by biodegradation, binding on organic particles followed by sedimentation, or transformation was more rapid in the more productive lakes. Chlorobornane metabolism or depuration also might be directly related to temperature-dependent physiological processes. Although temperature–depuration rate relationships for toxaphene have not been determined, depuration rates for toxaphene in fish are species dependent (26).

A study in southern Sweden on persistent organic pollutants in pike (*Esox lucius*) found that contaminant levels in fish were related to lake trophic class (27). There, PCB and DDE levels in pike also decreased as lake productivity

increased. In Oregon, levels of applied toxaphene in water, aquatic plants, invertebrates, fish, and sediments were less in a productive lake 3 years after application as compared with a less productive lake (4). Based on application concentration, the productive lake should have had two times higher levels of toxaphene. When the lakes we studied were grouped as either treated or untreated, lower concentrations of toxaphene in fish were generally associated with more productive or more eutrophic aquatic habitats relative to those that were oligotrophic.

Kidd *et al.* (9) have shown that chlorobornane concentrations in fish tissue are related to food chain length. In our study area, rainbow trout, brook trout, and mountain whitefish generally feed on chironomids, caddisflies, amphipods, *Daphnia*, and mayflies (28–32), suggesting that among lake differences in trophic position or food chain length to these salmonids would be small. Forage fish were present in only a few of the lakes we studied (Cabin, Peanut, and Bow). The presence of forage fish in Cabin and Peanut Lakes was not associated with elevated levels of chlorobornanes in rainbow trout. For the predacious lake trout from Bow Lake, however, their high CHB levels were likely because of the significant proportion of mountain whitefish in their diet. Our unpublished data show that the diet of small and large lake trout, the two size groups we assessed for toxaphene, included 13% and 56% mountain whitefish (*N* = 32 and 16 stomachs), respectively.

Lipid content was not related to, and therefore was not a principal determinant of, the among lake body burden of CHB. A similar conclusion was reached for lake trout, burbot (*Lota lota*), and lake whitefish (*Coregonus clupeaformis*) from southern Yukon lakes (33). However, within lake body burden of CHB in lake trout from Yukon lakes was related to muscle lipid content. Furthermore, trophic level lipid content was related to trophic level toxaphene concentration (34). For log transformed data, neither CHB_{Atm} or B7-1001 were related to mean weight of fish from our study area. Among lake differences in mean weight were also not related to CHB levels in fish from Yukon lakes. Moreover, Muir *et al.* (35) found that CHB levels in burbot taken from lakes and rivers located from 42° to 67° N in central North America were not related to their weight. The significant relationship between length of time of exposure (age) and CHB_{Atm} for our study area may indicate that, while many biochemical properties of CHB_{Atm} are not known, these congeners continue to accumulate in fish tissue over time. As with other organochlorine contaminants, CHB may become elevated in older fish, especially if their diet switches to fish.

In conclusion, CHB concentrations (CHB_{Atm} and the B7-1001 congener) in water, sediment, and fish in the early 1990s were greater in lakes that were treated with toxaphene between 1958 and 1962 as compared with untreated lakes that were selected as “control” sites. However, the highest concentrations of CHB_{Atm} were for fish from untreated, cold oligotrophic lakes situated at high elevations. The strong inverse relationship between CHB_{Atm} levels in fish and measures of lake productivity and thermal regime suggests that limnological aspects of lakes influence CHB_{Atm} concentration in fish. These limnological conditions may set limits for bioaccumulation of toxaphene through the food chain.

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