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Organochlorine Pesticides and Polychlorinated Biphenyl Congeners in Ooligan Grease: A Traditional Food Fat of British Columbia First Nations

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Levels of persistent organic pollutants including dichlorodiphenyltrichloroethane, hexachlorobenzene, hexachlorohexanes, dieldrin, chlordane, mirex, and polychlorinated biphenyls (PCB) were measured in ooligan fish (*Thaleichthys pacificus*) prepared in the way usually consumed and in ooligan grease collected from five communities (Nass River, Kitimaat, Bella Coola, Kingcome Inlet, and Knights Inlet) in the coastal area of British Columbia, Canada. Levels of chlorinated pesticides and PCB increased from the north to the south, with the lowest from Nass River and highest from Knights Inlet. Average levels of persistent organic pollutants (110 ng/g lipid of total chlorinated pesticide and 30 ng/g lipid of PCB) were lower than those reported in fish from the Great Lakes and similar to those from the Arctic and were below the regulation limits established by Health Canada. Therefore, consumption of ooligan grease presents minimal health risk from organochlorine exposure.

INTRODUCTION

Ooligan fish (*Thaleichthys pacificus*) is widely consumed among Indigenous Peoples in the coastal area of British Columbia, Canada (Macnair, 1971). Boiling, baking, grilling, smoking, drying, and salting are typical preparation methods. Extracted fat, known as ooligan grease, is widely used as a condiment as well as a native medicine for skin rashes or for various internal ailments (Garfield and Wingate, 1966; Edwards, 1978; McGregor, 1981). Cultural and nutritional values of ooligan grease have been documented by Kuhnlein *et al.* (1982).

T. pacificus grows to a maximum length of 30 cm and returns in early spring to fresh water rivers for spawning after 2–3 years at sea. The fish is commonly harvested along the Stikine, Nass, Skeena, Kitimaat, Bella Coola, Kingcome, Klinaklini, and Fraser rivers (Macnair, 1971). Persistent organic pollutants such as organochlorine pesticides and polychlorinated biphenyls (PCB) have been produced in large quantities in the last 40–60 years. Although uses of these chemicals have been banned or restricted in most industrialized countries, a significant amount of organic pollutants produced in the past still cycle in the environment because of their persistent nature (Tanabe, 1988; Barrie *et al.*, 1992). Significant levels of pollutants such as dichlorodiphenyltrichloroethane (DDT), hexachlorobenzene, hexachlorohexanes (HCH), dieldrin, chlordane, mirex, and PCB have been found in Arctic and Subarctic freshwater

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ecosystems (Lockhart *et al.*, 1992). Long range atmospheric and oceanic transport is believed to be the major source (Barrie *et al.*, 1992, Murray and Shearer, 1994). Since persistent organic pollutants have been shown to bioaccumulate and biomagnify through the food chain (Norstrom and Muir, 1994), there is increasing concern among First Nations communities about levels of contaminants in the highly prized ooligan fish and grease.

In this report, we present levels of persistent organic pollutants in the edible portion of fish prepared as usually consumed, as well as data from ooligan grease collected from different communities of coastal British Columbia. The potential for human exposure to these chemical compounds is assessed.

MATERIALS AND METHODS

Sampling

A total of 18 ooligan grease samples were collected from independent First Nation family preparations during March–April, 1994. The collection time coincided with the season of ooligan spawning and traditional grease preparation. Samples were collected from five primary areas of First Nations on the coast of British Columbia, Canada.[2] These areas, from North to South were Nass River (five samples), Kitimaat (five samples), Nuxalk Nation at Bella Coola (four samples), Knights Inlet (three samples), and Kingcome Inlet (one sample). Five fish samples (either raw or prepared) were also collected; Nass River (five dried fish), Bella Coola (five smoked fish), Knights Inlet (five raw fish), and Rivers Inlet (five dried fish). Ooligan grease samples were preserved in sealed cans and fish samples were frozen for shipment to the laboratory.

While the total number of grease samples is small, grease preparation in Bella Coola in 1982 represented the harvest and preparation of an approximate average of 6,300 kg of fish, yielding 380 liters of prepared grease (Kuhnlein *et al.*, 1982). Thus, these 18 grease samples likely represent the processing of more than 11,000 kg of fish by many of the traditional west coast families still following this practice.

Chemicals and Standards

GC/MS grade solvents from Fisher Scientific (Montreal, QC) were used. ^{13}C-labeled PCB IUPAC Nos. 3, 77, 202, and 209, $^{13}C_{12}$-*p,p′*-DDT, and $^{13}C_6$-*g*-lindane were purchased from CIL (Andover, MA). Internal standard d_{12}-chrysene and chlorinated pesticides were purchased from Ultra Scientific (North Kingstown, RI). PCB standard solutions CLB-1-A, B, C, and D were obtained from the National Research Council of Canada (Halifax, NS).

Sample Preparation

Fish samples were slightly thawed and muscle of five fish collected from each site was cut into pieces, pooled (each fish contributed less than 20 g of tissue), and homogenized with an Osterizer blender. A modification of the extraction method described by Norstrom and Won (1985) was used for organochlorine extraction. Two

[2] See map in our companion paper in this issue.

grams (wet weight) of homogenate was spiked with an aliquot of surrogate internal standard solution containing ^{13}C-labeled PCB IUPAC Nos. 3, 77, 202, and 209, $^{13}C_{12}$-*p,p′*-DDT, and $^{13}C_6$-*g*-lindane. The sample was ground with 20 g of anhydrous sodium sulfate in a mortar until a free flowing powder was obtained. The ground fish sample was packed into a glass column (2.5 cm i.d. × 30 cm) and immersed with 75 ml of solvent (1:1 methylene chloride/hexane) for 45 min. The solvent was eluted from the column at a flow rate of 3–5 ml/min and further extracted with 200 ml of solvent. The extract was concentrated to 2 ml on a rotary evaporator, transferred, and further evaporated under a gentle stream of dry nitrogen to 1 ml. The fish extract or 2 g of ooligan grease was passed through an 0.45-μm teflon filter (SPE Ltd, Concord ON) and made up to 5 ml with solvent (1:1 methylene chloride/hexane). Each sample was applied onto a SX-3 Biobeads gel permeation column (3 cm i.d. × 70 cm; solvent: 1:1 methylene chloride/hexane; flow rate: 5 ml/min) connected to a Beckmann gold HPLC system (Beckmann, Fullerton CA). The first 175 ml containing higher molecular weight lipids were discarded. The next 115 ml containing the PCB and chlorinated pesticides were collected and concentrated to 100 μl; this was then made up to 1 ml with hexane and applied onto a florisil (Supelco, ON) column (1 cm. i.d. × 30 cm) for further purification and fractionation. Three fractions were collected: the first fraction (36 ml hexane) contained PCB congeners, hexachlorobenzene, *p,p′*-DDE, heptachlor, mirex, and photomirex; the second fraction (36 ml of 15% methylene chloride/hexane) contained hexachlorocyclohexanes, chlordanes, and *p,p′*-DDT; and the third fraction (55 ml 1:1 methylene chloride/hexane) contained heptachlor epoxide and dieldrin. The three fractions were concentrated to 50 μl, spiked with the volumetric internal standard d_{12}-chrysene and prepared for GC analysis with 100 μl with isooctane.

Analytical Method

Characterization of the three fractions was conducted using a Varian Saturn III GC-Ion trap mass spectrometer. A DB-5MS (J&W Scientific) (30 m × 0.25 mm i.d. and 0.25 μm film thickness) capillary column was used. Samples were loaded onto a Varian 8200CX autosampler and 1-μl injections were made using the sandwich injection technique. The SPI injector was used with the following conditions: initial temperature 110°C for 1 min then raised to 280°C at 150°C/min and held for 50 min. Initial column temperature was 80°C and held for 0.1 min, raised to 150°C at 10°C/min, raised to 265°C at 3°C/min, raised to 300°C at 15°C/min, and held for 5 min. Ion trap temperature was kept at 270°C with the electron multiplier set at 100 V over the 10^5 tuning voltage and transfer line was kept at 260°C. Perfluorotributylamine was used to tune the ion trap on a daily basis.

A total of 51 PCBs and 17 chlorinated pesticides was screened in the samples. Levels were measured using the internal standard method in conjunction with the corresponding external standards. ^{13}C PCBs, $^{13}C_{12}$-*p,p′*-DDT, and $^{13}C_6$-*g*-lindane surrogates were only used to evaluate the extraction and purification efficiency of the analytical method. Masses were scanned from m/z 150 to 550 amu and confirmation of identity was performed by comparing mass spectra of the identified compound in the sample with one of the external standards stored in the calibration file eluting in the retention time window. Linearity of the ion-trap-ms was verified by injecting levels of standards ranging from 10 to 1000 pg/μl. Any compound measured below this minimum quantitation limit and that had a matching spectra was given the mini-

mum detected limit of 1 pg/μl corresponding to 0.05 ng/g of fish tissue or ooligan grease. A single injection was sufficient to characterize each fraction for PCBs and chlorinated pesticides. The following PCB congeners were determined using the sum of the signal produced by two respective ions: dechlorinated (No. 15; m/z 222 and 224), trichlorinated (Nos. 18, 31; m/z 256 and 258), tetrachlorinated (Nos. 54, 52, 49, 44, 40, 60, 77; m/z 290 and 292), pentachlorinated (Nos. 103, 121, 101, 86, 87, 118, 114, 105; m/z 324 and 326), hexachlorinated (Nos. 154, 151, 143, 153, 141, 137, 138, 129, 159, 128, 156; m/z 360 and 362), heptachlorinated (Nos. 182/187, 183, 185, 171, 173, 180, 191, 170, 189; m/z 394 and 396), octachlorinated (Nos. 202, 201, 199, 196/203, 195, 194, 205; m/z 428 and 430), nonachlorinated (Nos. 208, 207, 206; m/z 464 and 466), decachlorinated (No. 209; m/z 498 and 500). The following chlorinated pesticides were also determined using the same algorithm: pentachlorobenzene (m/z 250 and 252), α-HCH, β-HCH, γ-HCH (m/z 217 and 219), hexachlorobenzene (m/z 284 and 286), heptachlor (m/z 272 and 274), heptachlor epoxide (m/z 353 and 355), *trans*-chlordane, *cis*-chlordane (m/z 373 and 375), *trans*-nonachlor, *cis*-nonachlor (m/z 407 and 409), *p,p′*-DDE (m/z 316 and 318), *p,p′*-DDD, *p,p′*-DDT (235 and 237), photomirex, mirex (m/z 272 and 274).

Quality Control Assurance

With each batch of samples standard reference materials measured were CLB-1 PCB solutions from the National Research Council of Canada and SRM 1588 organics in cod liver oil from the National Institute of Standards and Technology. Results were consistently within 1 SD of certified values. Our laboratory also participated in Environment Canada's Northern Contaminants QA/QC Program.

Statistical Methods

Differences in contaminant levels were tested by one-way analysis of variance (ANOVA) followed by Tukey rank tests (SYSTAT, Version 5.02, SYSTAT Inc., Evanston, IL, U.S.A.). A P value of <0.05 was considered significant in all statistical tests.

RESULTS AND DISCUSSION

The GC-ion-trap-MS methods employed for the determination of organochlorine pesticides and PCB proved to be reliable, linear within a range of 0.5–50 ng/g, repeatable, reproducible, and sensitive. The recoveries of selected PCB and chlorinated pesticide internal standards were uniformly greater than 95%. Levels of total chlorinated pesticide (the sum of pentachlorobenzene, α-HCH, β-HCH, γ-HCH, hexachlorobenzene, heptachlor, heptachlor epoxide, *trans*-chlordane, *cis*-chlordane, *trans*-nonachlor, *cis*-nonachlor, *p,p′*-DDE, *p,p′*-DDD, *p,p′*-DDT, photomirex, mirex) and total PCBs (the sum of 51 congeners) in ooligan grease collected from different sites are presented in Table 1. There was relatively little intrasite variation in the levels of persistent organic pollutants in the grease samples; the highest coefficient of variation was 20% at Kitimaat area. These results suggest that the intrinsic levels in the fish oil are quite uniform and/or the efficiencies of extraction methods adopted by different households at the same site were similar.

Grease samples collected from Nass River had significantly lower concentrations

TABLE 1

CHLORINATED PESTICIDES AND PCB CONCENTRATIONS (ng/g LIPID) IN OOLIGAN GREASE SAMPLES FROM BRITISH COLUMBIA, CANADA (MEAN ± SD)

SITE [2]	N	∑CBZ	∑HCH	∑CHLOR	∑DDT	∑MIREX	DIELDRIN	∑PST[†]	∑PCB
Nass River	5	10 ± 2.8	14 ± 3.5	27 ± 2.1*	36 ± 2.4*	0.1 ± 0.04	8 ± 0.8	94 ± 3.7*	24 ± 2.4*
Kitimaat	5	20 ± 17.1	30 ± 8.1*	31 ± 2.9	50 ± 3.2	0	9 ± 0.6	140 ± 29	33 ± 2.7
Bella Coola	4	16 ± 4.0	10 ± 5.1	30 ± 5.4	50 ± 6.3	0	13 ± 4.7	130 ± 16	42 ± 4.3
Kingcome Inlet	1	11	15	49	88	0.1	12	175	57
Knights Inlet	3	11 ± 2.8	30 ± 15*	43 ± 2.5*	80 ± 8.8*	0.073 ± 0.0032	11 ± 2.6	170 ± 15*	48 ± 3.2*
MAXIMUM RESIDUE LIMIT		300	N/A	N/A	5000	100	200		2000

[†] ∑PST is the sum of ∑CBZ, ∑HCH, ∑CHLOR, ∑DDT, ∑MIREX, and DIELDRIN

* denotes significantly different ($p<0.05$) from other sites. ANOVA analysis for inter-site (all sites except for Kingcome Inlet) difference was significant for ∑HCH, ∑CHLOR, ∑DDT, ∑PST and ∑PCB.

and those from Knights Inlet had significantly higher concentrations of both chlorinated pesticide and PCB than the other sites (Table 1). There was an increasing trend of both chlorinated pesticide and PCB from north to south, suggesting more contamination closer to industrial areas. Among the different groups of chlorinated pesticides, only chlordane and DDT showed the north–south increasing trend. Muir *et al.* (1988) reported a similar north–south gradient for chlordane, DDT, and PCB; levels in arctic cod were two to five times lower than those in northwest Pacific fish. These results suggest that these three groups of contaminants may originate in the south and be transported to Northern Canada.

Figure 1 shows the levels of 17 chlorinated pesticide compounds measured in ooligan grease samples from different sites. All 17 chlorinated pesticide compounds except for pentachlorobenzene, heptachlor, and mirex were detected in ooligan grease samples. The relative abundance of the other 14 compounds was similar among the five different sites, suggesting lack of point source contamination of any particular contaminant. The most abundant chlorinated pesticide in grease samples was total DDT (36 to 51% of total chlorinated pesticide) followed by total chlordane (22 to 28% of total chlorinated pesticide). The relatively high levels of chlordane found in ooligan grease samples in comparison with other persistent organic pollutants, (e.g., DDT and PCB) are of interest, because this contaminant is believed to be a unique characteristic of marine environment in polar regions. The total chlordane/total PCB ratio in this study was about 1.0, which is similar to the ratio found in marine fish in the Arctic (Muir *et al.*, 1988) and Antarctica (Kawano *et al.*, 1986) and much higher than the ratios (0.02–0.2) found in salmon, red fish, and herring oil from the North Atlantic and in herring and char from the Baltic (Zitko, 1978; Jansson *et al.*, 1979; Miolanen *et al.*, 1982; Kramer *et al.*, 1984). The relatively high chlordane/PCB ratio in ooligan grease from the B.C. coast suggests that chlordane may originate from long-range atmospheric transport similar to that found in the polar regions.

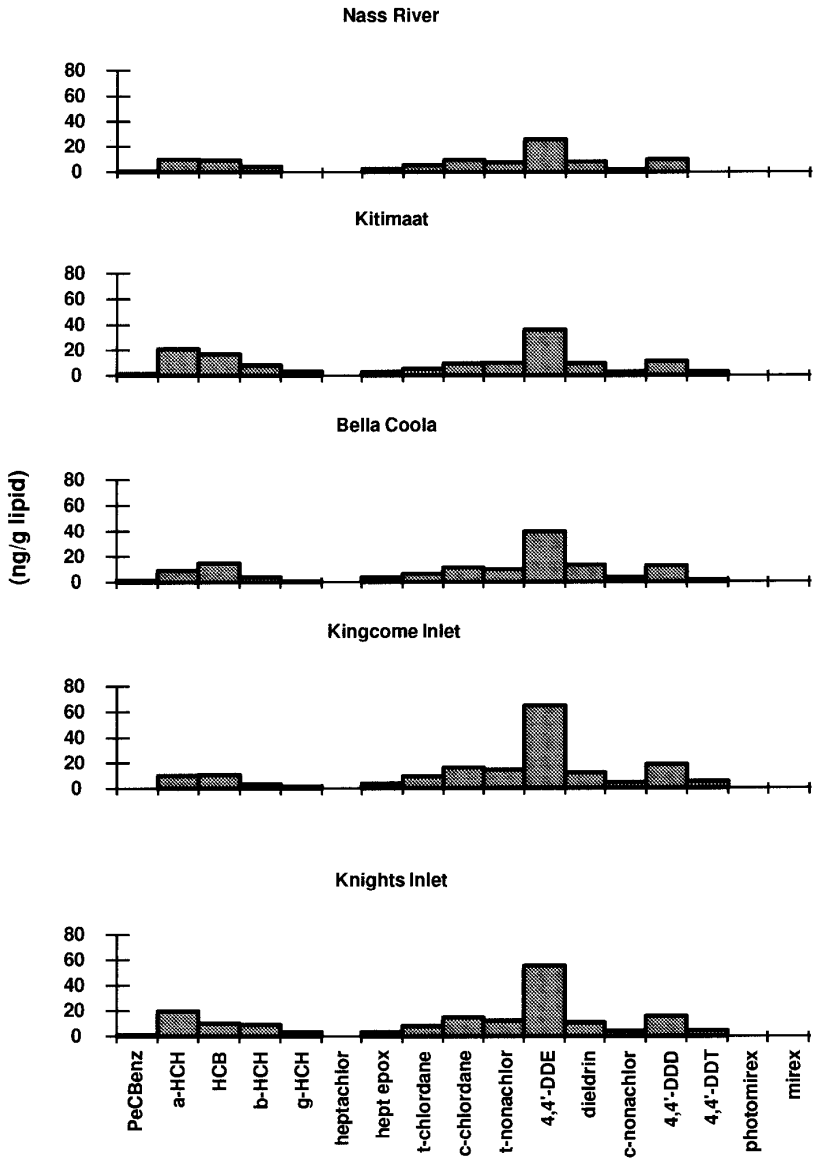

FIG. 1. Concentrations of chlorinated pesticides measured in ooligan grease samples.

The PCB congener profiles for ooligan grease collected from different sites were very similar (Fig. 2). Highest concentrations were found in congeners 153, 138, 118, 151, 118, 101, and 52. These patterns are similar to those of Aroclor standard mixtures (1242/1254/1260), suggesting little metabolism of these congeners by the fish. No geographical difference was detected in the proportion of PCB homologue either in the grease or fish samples (data not shown). Therefore, we pooled the data from the ooligan fish samples collected from different sites and presented the proportion of PCB homologue in Fig. 3. The predominant congeners were the penta- and hexa-

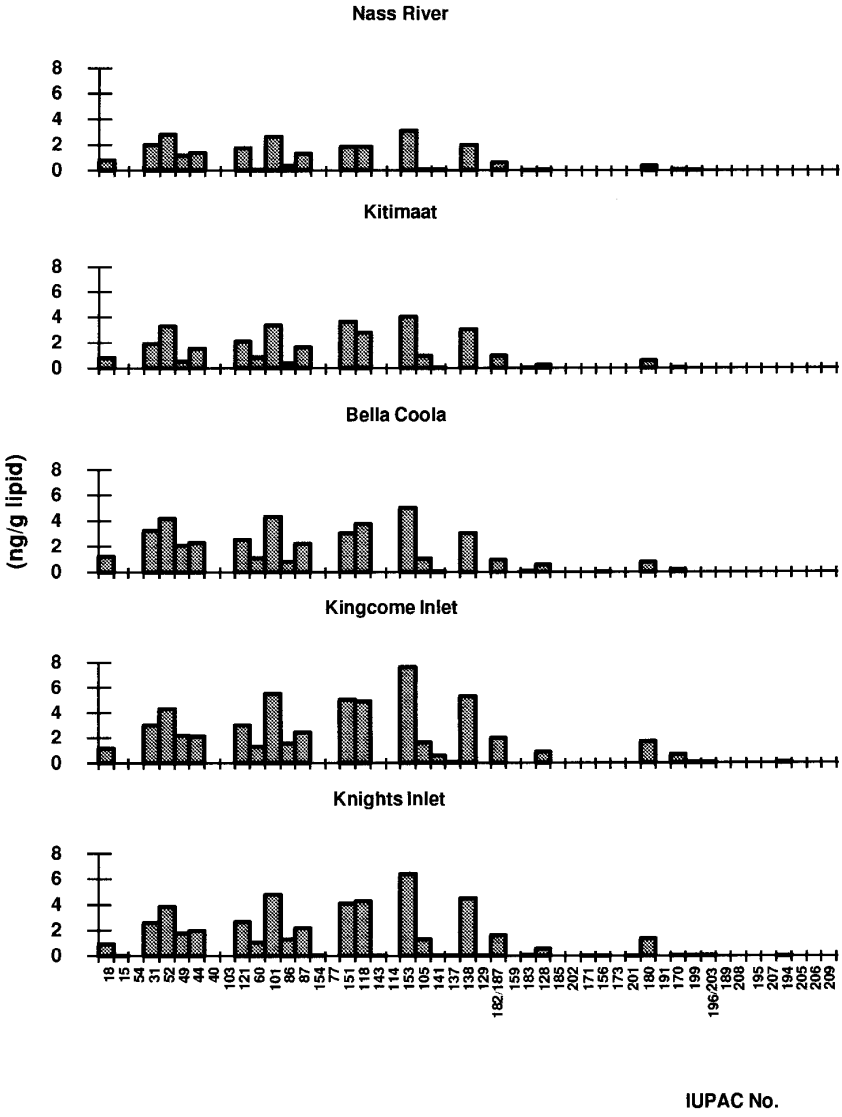

FIG. 2. Concentrations of PCB congeners in ooligan grease samples.

chlorinated congeners (33 and 32%), which are similar to the pattern reported for both marine and freshwater fish in the literature (Duinker and Hillebrand, 1983; Oliver and Niimi, 1988; Newsome and Andrews, 1993). However, unlike fish reported from the Great Lakes and North Sea, the proportion of tetrachlorinated congeners (21%) were higher than the heptachlorinated congeners (3%) (Fig. 3). Similar results were reported for arctic cod collected from the high arctic (Muir *et al.*, 1988). These results suggest that fish collected from less polluted areas may accumulate congeners with lower number of chlorines. However, variations between species may also be due to differences in proportions of homologues in ingested food (Subramanian *et al.*, 1983) and to the size of the fish (Muir *et al.*, 1988). Nevertheless, PCB mixtures appear to

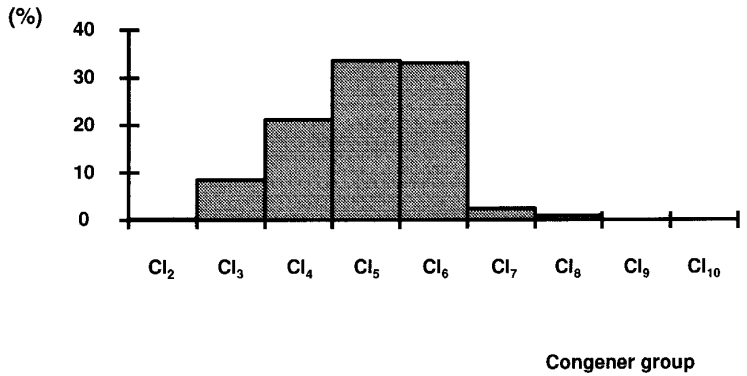

FIG. 3. Percentage of PCB homologues of total PCB in pooled ooligan fish samples from different sites in British Columbia, Canada.

be transported from the south to the north on a global scale, and they accumulated in fish with little metabolic alteration.

Both chlorinated pesticide and PCB profiles of ooligan grease from British Columbia showed similarity to those found in marine fish from the Arctic. Levels of persistent organic pollutants in the pooled fish muscle samples are presented in Table 2. Both lipid and water contents were decreased by drying and smoking. Nevertheless, the same north–south gradient was observed in chlorinated pesticide and PCB concentrations (Nass River < Bella Coola < Knights River) when expressed as ng/g lipid.

Figure 4 compares levels of 17 persistent organic pollutants in fish collected from the three sites. Some differences of relative abundance are observed: α-HCH and γ-HCH were not found in the smoked sample from Bella Coola; HCB and γ-HCH were not found in the dried sample from Nass River; all of the 14 compounds were detected in the raw sample from Knights River. Because these particular organic pollutants are relatively more volatile, they may be lost during the preparation process.

TABLE 2

CHLORINATED PESTICIDES AND PCB CONCENTRATIONS IN OOLIGAN FISH SAMPLES (POOLED SAMPLE FROM FIVE FISH) FROM BRITISH COLUMBIA, CANADA

SITE	PREPARATION*	LIPID (%)	WATER (%)	∑CBZ	∑HCH	∑CHLOR	∑DDT	∑MIREX	DIELDRIN	∑PST†	∑PCB
Nass River	Dried	15.5	59.2	0.2	10.7	47.3	50.1	0.0	13.1	121	17
Bella Coola	Smoked	21.9	69.6	12.2	5.7	29.5	54.7	0.1	10.0	112	37
Knights Inlet	Raw	16.7	72.2	20.5	46.6	70.2	104.9	0.3	19.9	262	55
MAXIMUM RESIDUE LIMIT				300	N/A	N/A	5000	100	200		2000

* Pooled sample from 5 fish.

† ∑PST is the sum of ∑CBZ, ∑HCH, ∑CHLOR, ∑DDT, ∑MIREX, and DIELDRIN

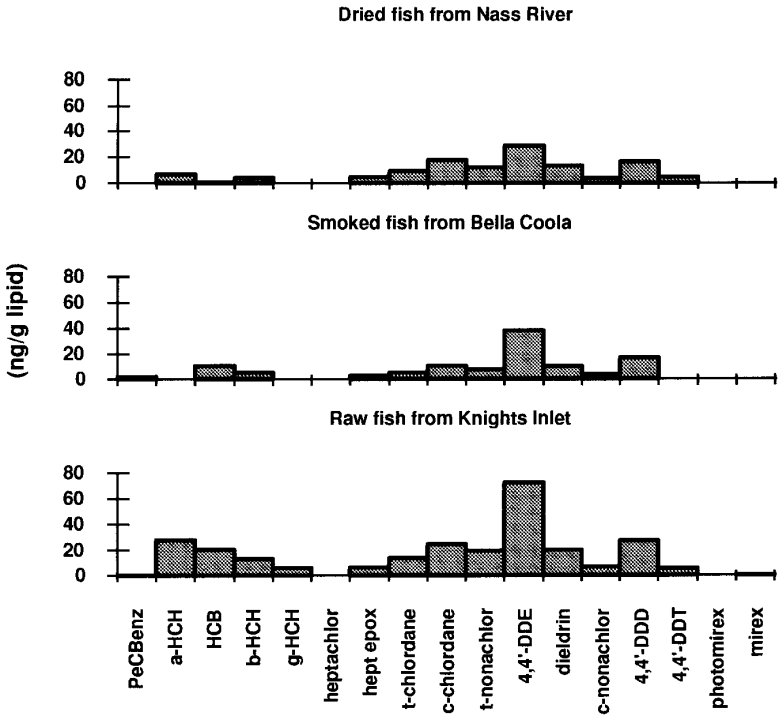

FIG. 4. Concentrations of chlorinated pesticides measured in ooligan fish samples collected from three sites in British Columbia, Canada.

PCB profiles of ooligan grease and smoked fish collected from Bella Coola were compared (Fig. 5). For ease of comparison, concentrations of congeners were expressed as ratios of the concentrations of congener 153 (the congener with the highest concentration in both samples). The patterns are very similar, suggesting that the two food preparation methods did not affect the PCB congener profiles, i.e., no change in the concentrations of any particular congeners. These results suggest that food preparation may affect concentrations of the more volatile chlorinated pesticide but had little effect on the PCB congener profile.

The persistent organic pollutant levels in both grease and fish were generally low (Tables 1 and 2). Average total chlorinated pesticide and total PCB levels were 110 ng/g lipid and 31 ng/g lipid, respectively, in grease samples and 165 ng/g lipid and 36 ng/g lipid, respectively, in fish samples. No comparable data are available in the literature for persistent organic pollutant levels of ooligan fish. Our data are similar to those in fish of similar size and lipid content. For example, pacific herring collected in the Arctic contained 185 ng/g for chlorinated pesticide and 42 ng/g for PCB (Muir *et al.*, 1987). Our data are much lower than the values reported for smelt collected in the Great Lakes (1218 ng/g for chlorinated pesticide and 1926 ng/g for PCB) (Newsome and Andrews, 1993).

The persistent organic pollutant levels in ooligan fish were at least an order of magnitude lower than the maximum residual limit established by Health Canada or the action level established by the U.S. Food and Drug Administration (Table 2). We

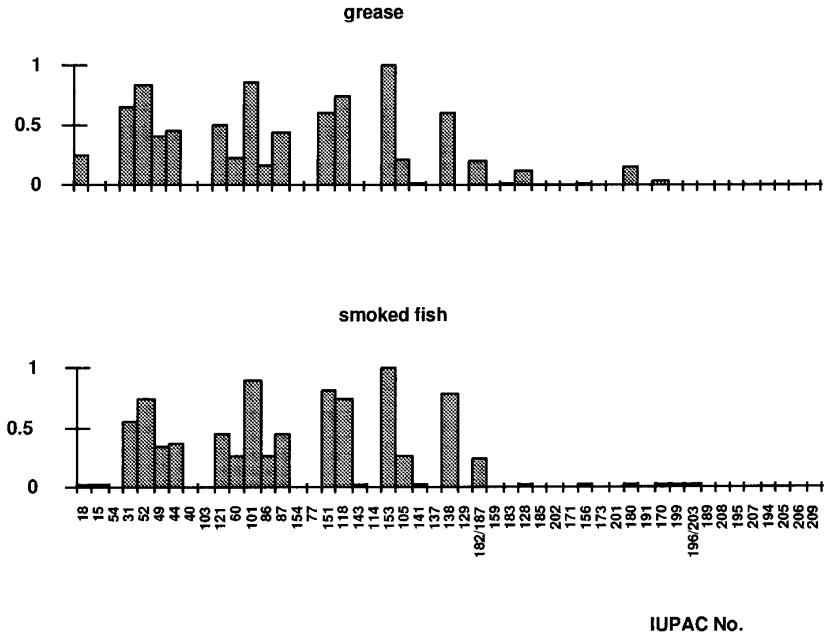

FIG. 5. Comparison of PCB congener profiles in grease and smoked fish samples collected from Bella Coola, British Columbia, Canada. Levels of all congeners are expressed as ratios of the level of the most abundant congener (congener 153) in both samples.

could not find any residual regulation for HCH and chlordane levels in fish; however, tolerable daily intake (TDI) levels for HCH and chlordane are 0.3 and 0.05 μg/kg body weight/day, respectively (Health Canada, personal communication). Therefore, an adult weighing 65 kg would have to consume 650 g of ooligan grease per day to exceed the TDI for HCH and 70 g of grease to exceed the TDI for chlordane. Since it is unlikely that First Nations Peoples would daily consume this quantity of grease on a year-round basis, the health risk of persistent organic pollutants exposure associated with consumption of the grease is minimal. In contrast, its consumption should be encouraged, in consideration of the important cultural and nutritional values.

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