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Influence of Altitude and Age in the Accumulation of Organochlorine Compounds in Fish from High Mountain Lakes

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The analysis of hexachlorobenzene, hexachlorocyclohexanes, polychlorobiphenyls, and DDTs in muscle of fish from high mountain lakes shows that a proportion of their concentration variance depends on fish age and lake altitude. Interestingly, the magnitude of this share corresponds linearly with the log-transformed vapor pressure (V_p) of the organochlorine compounds (OC). Thus, the distributions of OC with $V_p < 10^{-2.5}$ Pa are mostly determined by these two variables. Altitude gradients mainly respond to temperature differences, involving concentration increases of 25−150 times between 8.7 and −2.3 °C. The age effect encompasses concentration increments of 2.4−7.8 for average lake differences between 2 and 13 yr. However, both effects are independent since no correlation between fish age and lake altitude is observed. Fish liver concentrations exhibit the same pattern, but the correlations are only significant for age, suggesting that the temperature trend is more related to long-term accumulation than episodic intake. The temperature effect is independent from compound origin. In addition, the sites situated at highest altitude, those most distant from possible ground pollution sources, are the most polluted. The results can be explained by condensation effects such as those described for the latitudinal trends that support the global distillation theory. However, in the high altitude lakes a temperature-dependent amplification mechanism, probably related to low metabolism and respiration at low temperatures, enhances OC accumulation in fish beyond the increases predicted from theoretical condensation and solubilization enthalpies. The observed temperature dependence suggests that a general remobilization of OC accumulated in high mountain areas could take place as a consequence of the general warming of these areas anticipated in the climatic change studies.

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Introduction

High mountain lakes offer "natural experiments" of exposure to long-range transported contamination in real environments since biota living in these ecosystems constitute stable communities that develop under chronic atmospheric pollution inputs. Thus, pollutants concentrated in the organisms from these lakes can be uniquely related to atmospheric deposition in these areas. In this respect, fish, as food chain top predators, are the most suitable marker organisms for the assessment of the contamination by the chemically stable organochlorine compounds (OC) in these ecosystems.

OC concentrations in fish muscle have been related to cold condensation effects due to local average air temperature (*1*). These preliminary observations were in agreement with the global distillation theory involving condensation effects for explaining the transfer of pollutants from low and medium latitudes to high latitudes (*2*−*4*). However, the concentrations of OC in fish also depend on other factors such as fish biology (e.g., age, sex) that must be adequately mastered for a full understanding of the environmental significance of the accumulation patterns in these organisms.

In this respect, a study of fish in 11 lakes located in high mountain sites of Norway (4), the Alps (2), the Tatra Mountains (1), the Pyrenees (1), Scotland (1), and the Rila Mountains (2) (Table 1, Figure 1) has been undertaken. Hexachlorobenzene (HCB), hexachlorocyclohexanes (HCH), DDTs, and polychlorobiphenyls (PCB) have been analyzed in muscle of 99 fish. OC composition in liver has been analyzed in 77 of these fish specimens (Table 2). Age was measured by examination of otoliths and scales. Sex and conditioning factors were also determined in all specimens. The resulting data set provides a description of the main factors, either biological or environmental, involved in the accumulation of OC in these organisms. In addition, the present data set has also been evaluated together with muscle data obtained from a previous study (*1*) resulting in a data set of 24 European high altitude lakes (Table 1, Figure 1) that contains OC concentrations from 217 muscle fish samples. This extended data set provides a comprehensive description of the main environmental and biological factors determining the accumulation of OC in fish from high mountain lakes.

Materials and Methods

Study Area and Sample Collection. Fish sampling followed standard test fishing procedures with multifilament gillnets. All fish were measured and dissected, and their sex was determined on site. Muscle fillets and livers were wrapped in a precleaned aluminum foil and stored at −20 °C until analysis. Otoliths and scales were kept for age determination, which was performed at NIVA.

Chemicals. For residue analysis, *n*-hexane, dichloromethane, isooctane, methanol, 95−97% concentrated sulfuric acid, acetone, and anhydrous sodium sulfate for analysis were from Merck (Darmstadt, Germany). Aluminum foil was rinsed with acetone and dried at ambient temperature prior to use. Cellulose extraction cartridges of 20 mm i.d. and 80 mm long were from Whatman (England). Neutral aluminum oxide type 507C was from Fluka AG (Switzerland).

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TABLE 1. Description of the High Mountain Lakes and Fish Included in the Study

	lake name	latitude (N)	longitude (E)	altitude[a] (m)	temp[b] (°C)	no. of fish analyzed	species	age[c] (yr)	conditioning factor[d] (cg cm^{-3})	sex[e]	lipid in muscle[f] (%)
1	Øvre Neådalsvatn	62.77778	8.98237	728	3.25	31	brown trout	4.7	1.3	1.6	2.9
2	Fallbekktjørna	62.74996	9.03719	1043	1.36	6	brown trout	11	0.89	1.7	0.62
3	Nedre Neadalsvatn	62.41243	7.98756	566	4.22	4	brown trout	4.75	1.1	1.75	2.1
4	Øvre Heimdalsvatnet	61.41877	8.89696	1088	1.09	4	brown trout	3.75	0.85	1.5	0.94
5	Stavsvatn	59.63500	8.11000	1053	0.6	10	brown trout	6.3	1.2	–[g]	1.9
6	Lochnagar	56.95914	−3.23128	785	3.7	9	brown trout	5.8	1.1	1.0	1.3
7	Maan	54.98861	−8.11667	436	8.7	5	brown trout	–	1.5	–	1.7
8	Zielony Staw Gąsienicowy	49.22890	20.00100	1671	1.9	5	brook trout	–	–	–	1.6
9	Vel'ké Hincovo	49.17970	20.06060	1946	0.5	5	brown trout	13	1.0	1.0	1.05
10	Gossenkoellesee	47.22528	11.01390	2413	−0.33	22	brown trout	4.55	1.0	1.6	1.9
11	Rotfelssee	47.22647	11.00796	2485	−0.74	5	arctic charr	7.6	0.76	1.6	1.2
12	Oberer Plenderlessee	47.19878	11.03815	2344	0.09	6	arctic charr	–	–	–	–
13	Mittlerer Plenderlessee	47.20474	11.04155	2317	0.24	4	arctic charr	–	1.2	–	2.3
14	Schwarzsee	46.96173	10.94621	2799	−2.31	3	arctic charr	4.4	1.0	–	4.4
15	Milchsee	46.72574	11.07237	2540	−0.65	6	arctic charr	12	1.2	–	4.05
16	Lungo	46.73333	11.08333	2384	0.24	6	arctic charr	10.5	1.4	–	1.2
17	Jörisee	46.77777	9.975	2519	0.32	9	brown trout	11	0.97	–	1.9
18	Paione inferiore	46.16889	8.19083	2002	3.0	5	rainbow trout	4.0	1.6	–	0.90
19	Aubé	42.74549	1.33801	2091	3.96	15	arctic charr	3.7	1.1	–	3.7
20	Redon	42.64208	0.77951	2240	3.18	39	brown trout	8.1	1.05	1.4	2.5
21	Okoto	42.19964	23.30584	2440	−0.09	5	brook trout	3.8	1.5	1.6	4.1
22	Bliznaka	42.20122	23.31497	2243	1.09	5	brown trout	3.0	1.0	1.8	2.5
23	Cimera	40.26666	−4.61388	2140	4.95	5	brook trout	5.0	1.5	–	1.0
24	Escura	40.35472	−7.63500	1680	8.05	3	rainbow trout	2.0	1.65	–	1.6

[a] Meters above sea level. [b] Annual average air temperatures. Meteorological data were supplied by the Department of Geology and Geophysics from the University of Edinburgh, U.K. Lake site air temperatures were calculated from WMO data and corrected with daily altitudinal lapse rates. [c] Average age of the fish analyzed in each lake. [d] Average CF of the fish analyzed in each lake. [e] Male = 1; female = 2. Average value of the fish analyzed in each lake. [f] Average value of the fish analyzed in each lake. [g] Not determined.

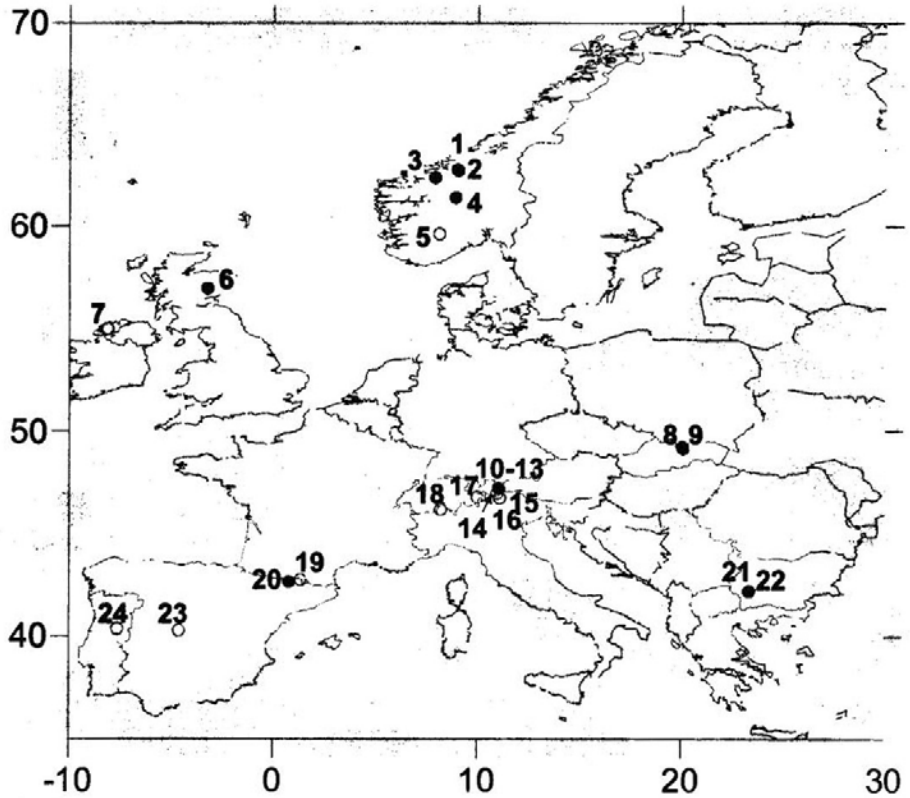

FIGURE 1. Map showing the high altitude lakes included in the study. Solid circles (●) indicate lakes in which fish were sampled and analyzed. Open circles (○) indicate other lakes from which muscle fish data were also available (*1*) and are included in the present data set. Numbers refer to the names in Table 1.

The purity of the solvents was checked by gas chromatography-electron capture detection (GC-ECD), and no peaks were detected. Sodium sulfate, aluminum oxide, and cartridges were precleaned by Soxhlet extraction with dichlo-

romethane:methanol (2:1, v/v) for 24 h before use. Sodium sulfate and aluminum oxide were activated overnight at 400 and 120 °C, respectively.

γ-Hexachlorocyclohexane (γ-HCH) and tetrabromobenzene (TBB) standards were from Aldrich-Chemie (Steinheim, Germany). α-HCH and PCBs (Nos. 28, 52, 101, 118, 138, 153, 180, and 209) were from Promochem (Wesel, Germany), and 4,4′-DDE, and 4,4′-DDT were from Dr. Ehrenstorfer (Augsburg, Germany).

Organochlorine Compound Analysis. Muscle and liver tissues were extracted and analyzed for OCs using the method described elsewhere (*5, 6*). Briefly, the samples were ground with activated sodium sulfate until a fine powder was obtained. This mixture was introduced into cellulose cartridges and Soxhlet-extracted with *n*-hexane:dichloromethane (4:1) for 18 h. Lipid content was determined gravimetrically using 20% of the extract. TBB and PCB 209 standards were added to the rest of the extract. Muscle extracts were subsequently cleaned up with sulfuric acid (5 times). All *n*-hexane solutions were combined and concentrated by vacuum rotary evaporation (20 °C, 20 Torr) to small volumes (ca. 500 μL), further concentrated to near dryness under a gentle nitrogen flow, and redissolved in 50 μL of isooctane. Liver extracts were cleaned up by elution through an aluminum oxide chromatographic column. Two fractions were collected. The first involved elution with 16.5 mL of *n*-hexane:dichloromethane (19:1, v:v) and 3 mL of *n*-hexane: dichloromethane (1:2, v:v) and provided HCB, PCBs, and DDTs. The second was obtained by elution with 13 mL of *n*-hexane:dichloromethane (1:2, v:v) to obtain the HCH. The two fractions were concentrated to 50 μL in isooctane as described above.

Before chromatographic analysis, an internal standard of tetrachloronaphthalene (TCN) and octachloronaphthalene (OCN) was added to correct for instrument variability. Samples were analyzed by GC-ECD (Hewlett-Packard 5890 series II) with a 50 m × 0.25 mm i.d. DB-5 capillary column (J&W Scientific, Folsom, CA) coated with 5% phenyl/95% methylpolysiloxane (film thickness 0.25 μm). The GC operated in splitless mode, and the oven temperature program started at 90 °C (held for 1 min), ramped to 120 °C at 10 °C min^{-1}, and then to ramped 310 °C at 4 °C min^{-1} (holding time 15 min). Injector and detector temperatures were 270 and 310 °C, respectively. Stringent precautions were observed for maintenance of the injector under clean conditions avoiding adsorptions that could deviate the system from linearity and increase the limits of detection and quantification. Helium and nitrogen were used as carrier (0.33 mL min^{-1}) and makeup (60 mL min^{-1}) gases, respectively.

Some samples were examined by negative ion chemical ionization mass spectrometry coupled to gas chromatography (GC–MS-NICI) for structural confirmation of the analyzed compounds. A GC system from Agilent Technologies 6890A coupled to an MS detector 5973N was used. The system was equipped with a HP-5MS column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) and run under the same oven temperature program as described above. Helium was used as the carrier gas (1 mL min^{-1}), and ammonia was chosen as the ionization gas (1.6×10^{-4} Pa). Transfer line and quadrupole temperatures were 280 and 150 °C, respectively. The selected ion program is reported elsewhere (*7*). Procedural blanks were analyzed for every set of six samples. The recovery of the surrogate standards (TBB and PCB 209) was calculated for each sample. Identification and quantification of all studied compounds were performed by injection of external standards at different concentrations. Relative responses to TCN and OCN were used in order to correct for instrumental variabilities, and this value was also corrected by the recovery of the surrogate standards.

Results

Fish Population Characteristics. All lakes are of natural origin, oligotrophic, situated above the regional tree line, and far from local pollution sources. Their hydrology only depends on atmospheric precipitation. The water chemistry of some of these lakes is reported elsewhere (*8*). Brown trout (*Salmo trutta*) is the most common fish species sampled, being present in 13 of the lakes (Table 1). Arctic charr (*Salvelinus alpinus*) is present in seven lakes, and brook trout (*Salvelinus fontinalis*) and rainbow trout (*Oncorhynchus mykiss*) are present in two lakes each. No piscivorous fish were present in any of the lakes included in the study.

The ages of the individual fish specimens range between 1 and 21 yr. Average ages of fish collected in each lake range between 2 (Escura) and 13 yr (Velke Hinçovo) (Table 1). The conditioning factors were generally low (e.g., the average lake values were between 0.85 and 1.3 and between 0.76 and 1.4 for brown trout and arctic charr, respectively) (Table 1). Brook trout and rainbow trout exhibited somewhat higher values, 1.5 and 1.6–1.65, respectively. Pooled representation of the conditioning factors versus age shows a significant linear correlation ($p < 0.05$; $n = 105$; $r^2 = 0.118$) indicating lower conditioning at higher age. A higher number of female than male fish were collected, although the overall figure is close to 50% each (Table 1). No relationship between lake location or altitude and age, sex, or conditioning factor is observed (Figure 2A–F and Table 1).

Lake-Averaged Concentrations. Mean OC concentrations and standard deviations for the lakes in which both fish muscle and liver have been analyzed are shown in Table 2. The mean lake values represent the average tissue concentrations of all specimens analyzed individually. Muscle concentrations are in the range of those in previously reported low and high altitude freshwater systems (*9–15*). Liver values are in the same range as results reported in Arctic systems (*12, 16, 17*). Most fish show higher OC concentrations in liver than in muscle, except in the Bulgarian lakes (Okoto and Bliznaka) and in Rotfelssee and Nedre Neådalsvatn for HCHs.

Total DDTs (4,4′-DDE + 4,4′-DDT) show the highest range of variation between lakes for both muscle (0.25–65 ng g^{-1} ww) and liver (0.78–64 ng g^{-1} ww) (Table 2). The concentrations of HCB and HCHs are more similar. In the first case, they range between 0.14 and 1.0 ng g^{-1} and between 0.13 and 3.0 ng g^{-1} in muscle and liver, respectively. In the second, they range between 0.1 and 1.6 ng g^{-1} in muscle and between 0.01 and 3.3 ng g^{-1} in liver. Lake-averaged concentrations of total PCB range between 0.68 and 17 ng g^{-1} in muscle and between 2.4 and 25 ng g^{-1} in liver (Table 2). The inter-lake range of variation is larger for the more chlorinated congeners. Thus, lake average concentrations of PCB 153 range between 0.16 and 5.1 ng g^{-1} and between 0.23 and 7.9 ng g^{-1} in muscle and liver, respectively, and for PCB 52 they range between 0.08 and 0.5 ng g^{-1} and between 0.33 and 2.1 ng g^{-1} in muscle and liver, respectively.

Concentrations in Individual Fish Specimens. Comparison of the log-transformed muscle and liver concentrations in the specimens where both measurements were available ($n = 77$) shows significant correlations for nearly all OC (Table 3). Higher concentrations in liver correspond to higher concentrations in muscle. Only HCB, PCB 28, PCB 52, and 4,4′-DDT do not exhibit significant correlation. These four compounds are those exhibiting lowest concentrations both in muscle and liver, 0.25–1.1 and 0.66–1.2 ng g^{-1}, respectively (Table 2). HCH concentrations are also similar to those of this OC group (0.54 and 0.93 ng g^{-1} in muscle and

TABLE 2. Comparison of Lake-Averaged Concentrations (±SD) in Fish Muscle and Liver in High Mountain Lakes[a]

lakes		*n*	HCB	PCB 52	PCB 153	PCB 180	total PCB	total HCHs	4,4′-DDE	4,4′-DDT	total DDTs
Øvre Neådalsvatn	muscle	14	0.58[b] ± 0.21[c]	0.13 ± 0.06	0.38 ± 0.16	0.19 ± 0.12	1.50 ± 0.57	0.28 ± 0.12	0.52 ± 0.32	0.22 ± 0.18	0.74 ± 0.31
	liver	13	0.64 ± 0.40	1.0 ± 0.88	0.97 ± 0.53	0.42 ± 0.21	5.6 ± 3.0	0.95 ± 1.5	0.69 ± 0.61	0.09 ± 0.15	0.78 ± 0.67
Fallbekktjørna	muscle	6	0.32 ± 0.25	0.09 ± 0.11	0.88 ± 0.52	0.37 ± 0.27	2.5 ± 1.3	0.10 ± 0.13	1.7 ± 1.2	0.19 ± 0.10	1.9 ± 1.2
	liver	6	3.0 ± 1.5	2.1 ± 1.8	6.4 ± 3.8	3.5 ± 3.0	25 ± 10	0.34 ± 0.37	18 ± 10	1.8 ± 0.52	20 ± 10
Nedre Neådalsvatn	muscle	4	0.32 ± 0.13	0.08 ± 0.05	0.16 ± 0.04	0.08 ± 0.01	0.68 ± 0.09	0.22 ± 0.08	0.20 ± 0.08	0.05 ± 0.02	0.25 ± 0.10
	liver	2	0.45 ± 0.40	0.65 ± 0.18	0.23 ± 0.11	0.01 ± 0.001	2.4 ± 0.81	0.01 ± 0.001	0.48 ± 0.03	0.55 ± 0.07	1.0 ± 0.1
Ve'ké Hincovo	muscle	5	0.30 ± 0.11	0.18 ± 0.08	5.07 ± 1.0	4.77 ± 1.8	17 ± 3.5	0.91 ± 0.44	33 ± 12	2.6 ± 0.97	36 ± 13
	liver	3	0.69 ± 0.02	0.81 ± 0.66	7.9 ± 0.65	5.9 ± 0.49	22 ± 4.1	3.2 ± 1.6	61 ± 11	2.8 ± 0.83	64 ± 10
Gossenköllesee	muscle	17	0.33 ± 0.09	0.16 ± 0.05	2.18 ± 0.79	1.9 ± 0.61	7.8 ± 2.5	0.27 ± 0.05	2.2 ± 0.85	0.43 ± 0.16	2.7 ± 0.95
	liver	16	0.47 ± 0.36	1.5 ± 1.1	5.8 ± 4.5	0.84 ± 0.46	19 ± 11	0.61 ± 0.42	3.6 ± 3.4	1.3 ± 0.51	4.0 ± 3.2
Rotfelssee	muscle	5	0.14 ± 0.05	0.37 ± 0.19	1.19 ± 0.40	1.2 ± 0.43	5.2 ± 1.2	0.33 ± 0.11	1.2 ± 0.47	0.39 ± 0.18	1.6 ± 0.65
	liver	5	0.47 ± 0.21	0.38 ± 0.10	4.8 ± 0.60	2.2 ± 0.54	17 ± 2.5	0.06 ± 0.03	5.6 ± 1.3	2.5 ± 0.42	8.2 ± 1.4
Redon	muscle	29	0.60 ± 0.36	0.30 ± 0.35	2.38 ± 1.65	1.5 ± 1.05	8.2 ± 4.8	1.6 ± 0.90	18 ± 13	1.2 ± 0.59	19 ± 13
	liver	24	0.67 ± 0.37	0.92 ± 0.85	3.4 ± 3.6	2.2 ± 2.5	12 ± 11	2.85 ± 2.5	18 ± 25	0.7 ± 1.2	18 ± 26
Okoto	muscle	5	1.00 ± 0.86	0.44 ± 0.23	1.91 ± 1.1	1.4 ± 0.72	8.1 ± 4.1	0.56 ± 0.19	62 ± 60	3.6 ± 1.9	65 ± 61
	liver	4	0.25 ± 0.27	0.33 ± 0.15	0.97 ± 0.25	0.44 ± 0.20	4.3 ± 1.4	0.17 ± 0.06	13 ± 4.2	0.64 ± 0.24	14 ± 4.3
Bliznaka	muscle	5	0.15 ± 0.15	0.48 ± 0.46	1.45 ± 1.4	0.86 ± 0.79	6.3 ± 5.0	0.57 ± 0.13	39 ± 66	1.2 ± 0.95	40 ± 66
	liver	4	0.13 ± 0.20	0.53 ± 0.37	0.99 ± 0.41	0.56 ± 0.40	5.1 ± 2.3	0.13 ± 0.06	7.1 ± 2.5	0.66 ± 0.29	7.8 ± 2.7
General Lake Distribution											
muscle											
min			0.14	0.08	0.16	0.08	0.68	0.10	0.20	0.05	0.25
max			1.00	0.48	5.1	4.8	17	1.6	62	3.6	65
max/min			7.2	6.0	33	60	25	16	310	67	260
mean ± SD			0.42 ± 0.28	0.25 ± 0.15	1.7 ± 1.5	1.4 ± 1.4	6.3 ± 4.8	0.54 ± 0.47	18 ± 22	1.1 ± 1.2	19 ± 24
liver											
min			0.13	0.33	0.23	0.01	2.4	0.01	0.48	0.09	0.78
max			3.0	2.1	7.9	5.9	25	3.3	61	2.8	64
max/min			22	6.4	34	760	10	240	130	30	81
mean ± SD			0.75 ± 0.85	0.92 ± 0.58	3.5 ± 2.8	1.8 ± 1.9	12 ± 8.5	0.93 ± 1.2	14 ± 19	1.2 ± 0.95	15 ± 19

[a] Units in ng g^{-1} wet weight. [b] Mean. [c] Standard deviation.

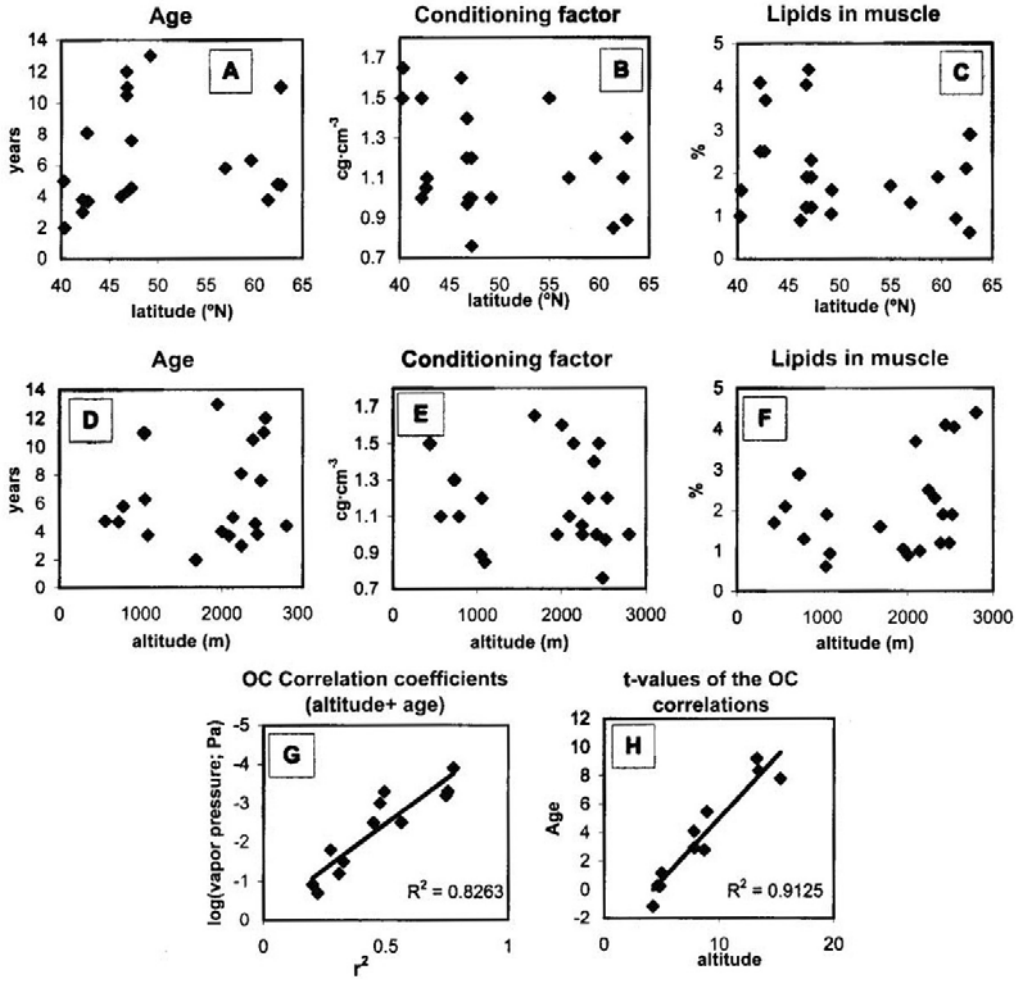

FIGURE 2. Upper row: distribution of lake average values of age (A, D), conditioning factor (B, E) and muscle lipid content (C, F) vs lake latitude (first row) and altitude (second row). Bottom row: (G) Comparison between the subcooled liquid vapor pressures of the organochlorine compounds and the correlation coefficients (r^2) of the linear multivariate regression analysis of log-transformed OC concentrations in muscle with altitude and age (Table 4). (H) Comparison of the *t* estimators for the altitude and age coefficients (coefficient value/standard error) of the linear multivariate regression analysis of the log-transformed OC concentrations in muscle. The individual values of the coefficients are reported in Table 4.

liver, respectively), but the correlation between both tissues is significant (Table 3). However, the range of variation of HCH concentrations (max/min ratios of 16 and 240 for muscle and liver, respectively, Table 2) is considerably larger than that of HCB and the volatile PCB (max/min ratios of 6−7 and 6−22 in muscle and liver, respectively, Table 2).

Explicative Factors. Redundancy analysis (RDA) can be used to determine whether some lake or fish features are significant in explaining the observed OC variance (*18*). The method consists of the extraction of the principal components of the log-transformed OC concentrations of the individual muscle fish (n = 99) with the constraint of being linear combinations of explicative factors such as altitude, age, lipid content, sex, length, weight, and conditioning factors. A forward selection of the factors is followed in order to discard redundant explanations. At each step, the significance of the explanatory variable under consideration is tested with a Monte Carlo method. According to this procedure, altitude is the most significant variable and accounted for 36% of the variance of the whole OC in fish muscle ($p < 0.001$). Then, successive selection (by order of significance) of age, lipid content, conditioning factor, and sex accounted respectively for 8.7% ($p < 0.001$), 9.4% ($p < 0.001$), 1.8% ($p < 0.001$), and 1.6% ($p < 0.01$) of the successively remaining variance after including the previous variable in the model of total variance. Length and weight did not contribute with any significant independent explanatory capacity.

Once the potential explanatory variables were identified, multiple regressions were used for assessment of their specific predictive capacity of the variance of each compound. To achieve robust models, only regression coefficients for variables with $p < 0.001$ were kept. Altitude was the most predictive variable except for HCB and PCB congener 28 (Table 4). Age was also important for the less volatile compounds. Interestingly, the percentage of variance explained decreased with increasing volatility, from about 78% for PCB 180 to 20% for HCH (Figure 2G). Regression coefficients and intercepts showed values within similar ranges with altitude coefficients increasing from high to low volatile compounds.

The relative contribution of age and altitude to the variance of each compound is shown by dividing the respective regression coefficients by their standard errors (*t* values). For all compounds the influence of altitude was larger than age (Figure 2H), as expected from the RDA, but interestingly the respective influence of both variables increased similarly at decreasing compound volatility (Figure

TABLE 3. Correlation Coefficients (r^2) and Enrichment Ratios of Log-Transformed Concentrations of Organochlorine Compounds in Muscle and Liver from Fish Specimens in Which Both Sample Tissues Were Analyzed

		muscle						liver							
	muscle vs liver[a]	age[b]		conditioning factor[b]		altitude[c]	temp[c]	age[a]		conditioning factor[a]		altitude[c]	temp[c]		
compd	r^2	r^2	21/1 yr old ratio[d]	r^2	1.5/0.70 ratio[e]	r^2	r^2	r^2	21/1 yr old ratio[f]	r^2	1.5/0.70 ratio[g]	r^2	r^2	log (K_{ow})[h]	vapor pressure (Pa)
α-HCH	0.113**	0.008	–[i]	0.071*	5.9	0.378	0.105	0.112**	5.4	0.001	–	0.028	0.001	3.9	$10^{-0.92}$
γ-HCH	0.129**	0.034	–	0.001	–	0.222	0.009	0.113**	7.7	0.001	–	0.035	0.000	3.9	$10^{-0.70}$
HCB	0.046	0.004	–	0.240**	12	0.016	0.075	0.066*	2.7	0.004	–	0.208	0.028	5.5	$10^{-1.2}$
PCB 28	0.014	0.002	–	0.027	–	0.553*	0.420	0.001	–	0.000	–	0.012	0.061	5.6	$10^{-1.5}$
PCB 52	0.004	0.008	–	0.035	–	0.694**	0.257	0.007	–	0.006	–	0.160	0.053	6	$10^{-1.8}$
4,4′-DDE	0.574**	0.184**	42	0.010	–	0.484*	0.180	0.291**	8.4	0.121**	0.069	0.331	0.268	5.7	$10^{-2.5}$
4,4′-DDT	0.040	0.069**	4.7	0.030	–	0.565*	0.275	0.002	–	0.171**	0.025	0.239	0.405	5.8	$10^{-3.3}$
PCB 101	0.078**	0.072**	4.9	0.000	–	0.523*	0.107	0.070*	1.8	0.127**	0.21	0.001	0.063	6.4	$10^{-2.5}$
PCB 118	0.255**	0.129**	5.7	0.000	–	0.645**	0.340	0.083**	2.1	0.065*	0.29	0.079	0.202	6.7	$10^{-3.0}$
PCB 153	0.406**	0.291**	15	0.004	–	0.640**	0.427	0.211**	3.4	0.265**	0.071	0.212	0.353	6.9	$10^{-3.3}$
PCB 138	0.208**	0.260**	14	0.000	–	0.695**	0.462*	0.146**	3.1	0.203**	0.076	0.213	0.411	6.8	$10^{-3.2}$
PCB 180	0.278**	0.207**	14	0.015	–	0.717**	0.512*	0.316**	7.0	0.209**	0.046	0.254	0.311	7.4	$10^{-3.9}$

[a] Concentrations of the individual specimens ($n = 77$). [b] Concentrations of the individual specimens($n = 99$). [c] Lake averaged concentrations ($n = 9$; Table 2). [d] Maximum increases for the largest age intervals represented by the fish included in Table 1 as predicted by the corresponding curve-fitting equations. [e] Maximum increases for the largest conditioning factor intervals represented by the fish included in Table 1 as predicted by the corresponding curve-fitting equations. [f] Maximum increases for the largest age intervals represented by the fish included in Table 2 as predicted by the corresponding curve-fitting equations. [g] Maximum increases for the largest conditioning factor intervals represented by the fish included in Table 2 as predicted by the corresponding curve-fitting equations. [h] Refs *29* and *30*. [i] Only values for the significant correlations are given. *, $p < 0.05$; **, $p < 0.01$.

TABLE 4. Multivariate and Correlation Analysis of the Log-Transformed Concentrations of Organochlorine Compounds in Fish Muscle

	multiple regression[a]				correlation analysis[c]			
compd	altitude coeff	age coeff	intercept	r^2	altitude (r^2)	temp (r^2)	Δ temp[d] (8.7 °C/−2.3 °C)	slopes (10^3 K)
α-HCH	0.0008	–[b]	2.9	0.201	0.205*	0.087 (0.087)[e]	–[f]	–
γ-HCH	0.0008	–	4.3	0.222	0.055	0.042 (0.044)	–	–
HCB	–	–	–	–	0.051	0.148 (0.141)	–	–
PCB 28	–	–	–	–	0.221*	0.054 (0.049)	–	–
PCB 52	0.0006	–	3.5	0.275	0.171*	0.001 (0.002)	–	–
4,4′-DDE	0.0016	0.1761	4.0	0.564	0.606**	0.373** (0.391)**	150	15.1
4,4′-DDT	0.0011	0.0666	3.6	0.494	0.569**	0.450** (0.449)**	68	12.7
PCB 101	0.001	0.0696	3.5	0.450	0.587**	0.154 (0.154)	–	–
PCB 118	0.0009	0.0827	3.7	0.477	0.347**	0.416** (0.403)**	25	9.7
PCB 153	0.001	0.1288	4.3	0.754	0.598**	0.611** (0.606)**	57	12.1
PCB 138	0.0011	0.1209	4.2	0.746	0.610**	0.583** (0.575)**	58	12.2
PCB 180	0.006	0.0514	1.5	0.776	0.599**	0.634** (0.630)**	75	13.0

[a] Calculated using the concentrations of the individual specimens as pg g^{-1} wet weight ($n = 99$). [b] Only the significant coefficients are shown. [c] Calculated using the average lake concentrations (muscle) ($n = 24$; Table 1). [d] Maximum increase for the largest temperature interval of the lakes included in Table 1 as predicted by the curve-fitting equation. [e] Calculated after normalization by muscle lipid content (Table 1). [f] Only significant values are given. *, $p < 0.05$. **, $p < 0.01$

2G). This correspondence did not result from altitude–age correlation in the samples (Figure 2D).

Discussion

Age Dependence. OC in muscle versus age shows significant correlation coefficients for DDTs and PCB congeners 101, 118, 153, 138, and 180 (Table 3). In liver, the correlation is significant for the same compounds except 4,4′-DDT (Table 3). Liver concentrations of α-HCH, γ-HCH, and HCB are also age correlated. The differences of OC muscle concentration between 1 and 21 yr, the largest age span among individual fish, show increased ratios between 4.7 (4,4′-DDT) and 42 (4,4′-DDE) (Table 3). These intervals can be used to estimate the maximal concentration increase that could be attributed to aging as calculated from curve fitting (log(concn) vs age). PCB increases for the same age interval span between 4.9 and 15. In liver, application of the same approach gives rise to lower factors, between 1.8 and 8.4. Again, the increase for 4,4′-DDE is the largest. The lower increase ratio in liver as compared to muscle may reflect the lower long-term accumulation capacity of OC in the former than in the latter (*19*).

Among all compounds significantly correlated to age, the less volatile PCBs (vapor pressure between $10^{-3.2}$ and $10^{-3.9}$ Pa; Table 3) are those exhibiting higher correlation coefficients both in muscle and in liver. These compounds are also those with higher log(K_{ow}), 6.8–7.4. Both properties may be indicative of higher persistence in the lakes and in fish tissues. The age-dependent accumulation of HCH in liver but not in muscle may reflect the lower lipophilic character of these compounds (log(K_{ow}) = 3.9) in relation to the more chlorinated PCB as well as their lower chemical stability in fish metabolism. Thus, as OC are more lipophilic and metabolically inert their accumulation in fish muscle increases (*20–22*).

Altitude Dependence. The log-transformed lake-averaged concentrations in muscle ($n = 24$) show a strong dependence with altitude for all PCBs, DDTs, and α- HCH (Table 4, Figure 3). OC concentrations in liver also increase with lake altitude (Table 3). However, the increment is not strong enough to be statistically significant. The difference in correlation coefficients between these two tissues suggest that the observed altitudinal dependence reflects more the long-term OC accumulation patterns than episodic pollution events. In this respect, the significant altitudinal correlation for α-HCH but not for γ-HCH (Table 4) is also consistent with long-term effects, since atmospheric deposition of the former in high mountain areas reflects nearly steady-state pollution transfer, whereas the latter mostly corresponds to seasonal agricultural activities (*23*, *24*).

The altitudinal trends of the OC exhibiting significant correlations is not related to compound origin. DDTs were mainly introduced into the environment as an agricultural pesticide whereas PCBs are industrial products also used in urban applications. The observed altitudinal correlations are therefore consistent with the global distillation theory (*2*–*4*) involving condensation in cold zones, in this case high mountain areas instead of high latitude regions.

Temperature Dependence. Among other aspects, the altitudinal gradient reflects the changes in air temperature. The condensation effect in the high mountain areas can be evaluated by compilation of the mean annual air temperatures at each site (Table 1). Correlation of the muscle and liver concentration data with the reciprocal of absolute temperatures exhibit a general trend that is similar to the one observed for the altitudinal correlations (Tables 3 and 4, Figure 3). The significant correlations between OC concentrations in muscle and the reciprocal of temperature are observed for the less volatile compounds (vapor pressure < $10^{-2.5}$ Pa). In the extended data set ($n = 24$), all examined compounds from this group except PCB 101 give significant correlations (Table 4). Lower temperatures (higher altitude) involve higher OC concentrations. No significant correlations are observed for liver concentrations (also in the case of altitudes; Table 3). Normalization of the concentration values to lipid content provides about the same correlation coefficients and statistical significance.

The results of the reduced data set ($n = 9$, the one from which both muscle and liver OC were available) are also consistent with this general trend (Table 3). However, only the least volatile compounds of the series (vapor pressure lower than $10^{-3.1}$ Pa) exhibit significant correlation. However, also in this case, all compounds with vapor pressure < $10^{-2.5}$ Pa exhibit increasing concentrations with decreasing temperatures. This reduced data set encompasses lakes with annual average air temperatures ranging between −0.74 and 4.2 °C whereas the extended data set encompasses a larger span, between −2.3 and 8.7 °C. In the first case, the shorter temperature range and the lower number of lakes in the series probably indicates that only the compounds exhibiting the strongest temperature dependence (e.g., the least volatile) may provide a statistically significant trend within the background concentration variability of all fish.

The number of significantly correlated OCs in muscle is lower for temperature than for altitude both in the smaller and in the extended data-set (Tables 3 and 4). This difference probably reflects uncertainties in the estimation of the average annual air temperatures. Although the values used correspond to the best estimates obtained with meteorological modeling, no direct measurements at each site were generally available. However, use of maximum or minimum monthly averaged values at each site does not involve significant changes in correlation coefficients.

The concentration increases associated with the temperature range of the whole data set involve ratios between 25 and 150 (Table 4). These ratios are larger than those observed for the age effects discussed above. The difference is even larger when considering that the age ratios indicated in Table 3 refer to individual fish. Recalculation of these ratios to age averages for each lake (between 2 and 13 yr) show increase ratios between 2.4 and 7.8. These smaller ratios are only given for reference. In fact, they are not of significance for the temperature dependences since ages are not correlated to lake altitude or temperature (Figure 2D).

The temperature dependence of the accumulation of the less volatile OC in these high mountain lakes is consistent with previous findings related to the global distillation theory. Thus, areas of high accumulation of the more volatile OC such as HCB (vapor pressure $10^{-1.2}$ Pa) are located at 60 and 70° N, with mean air temperatures between −7 and −12 °C (*25*). The average air temperatures of the highest lakes included in this study range between −0.33 and −2.3 °C (Table 1), which is insufficient for trapping volatile compounds such as HCB but allow the retention of the less volatile OCs. In this respect, recent studies have shown that whereas the atmospheric deposition of OC is rather uniform throughout Europe, the ultimate pollutant composition retained in the high altitude lakes depends on local annual average air temperature (*23*).

Experimental Pseudo-Enthalphies. Calculation of phase change pseudo-enthalpies from the slopes of the significant temperature-correlated OC 9700−15 100 ($\Delta H = SR \ln(10)$, S = slope of the regression straight line, $R = 8.314$ J K^{-1} mol^{-1}, $\ln(10) = 2.303$) give values of 180−290 kJ mol^{-1} (180−250 kJ mol^{-1} for the PCB). Assuming that gas-phase OC are incorporated into water and transferred from this compartment into fish following a water−octanol partition model, these experimental values *are significantly higher than* the summed theoretical condensation (*26*) (86−97 kJ mol^{-1}) and solubilization (*27*) (45−48 kJ mol^{-1}) enthalpies of these compounds. The higher experimental than theoretical values must reflect some additional temperature-dependent mechanism enhancing OC accumulation in fish. Higher preservation of OC at lower temperatures is unlikely since the relative content of the more labile species (e.g., 4,4′-DDT vs 4,4′-DDE) is not altitude correlated. Furthermore, as indicated above, variations in water properties or fish length, weight, or age are not in correspondence with elevation gradients.

As shown in previous studies (*1*), sediment inventories of low volatile OC in high altitude lakes have a temperature-dependent distribution that is compatible with the increase rates predicted from the theoretical condensation and solubilization enthalpies. The observed deviation in fish must respond to biological processes that amplify the retention effect of low temperatures. Assuming a model in which OC are essentially incorporated into fish by food consumption and exchange through gills but returned to lake waters mainly through gill exchange, variations in the water ventilation rates through the gills (*28*) may be at the cause of this additional increase. Thus, lower temperatures involve lower metabolic activity and therefore lower respiration rate. They also involve higher oxygen solubility and therefore the need of lower water circulation through the gills for the same oxygen amount. Both processes encompass lower gill capacity for OC exchange. In contrast, OC ingestion through food consumption is much more rapid and less temperature-dependent since it encompasses direct intake of OC already associated to organic constituents. The limited depuration through the gills may also be responsible for the progressive OC accumulation with age as fish are unable to eliminate part of the ingested OC through time.

The retention of OC by condensation effects and lower metabolic/respiration rate at low temperatures may therefore account for the higher concentrations of these compounds

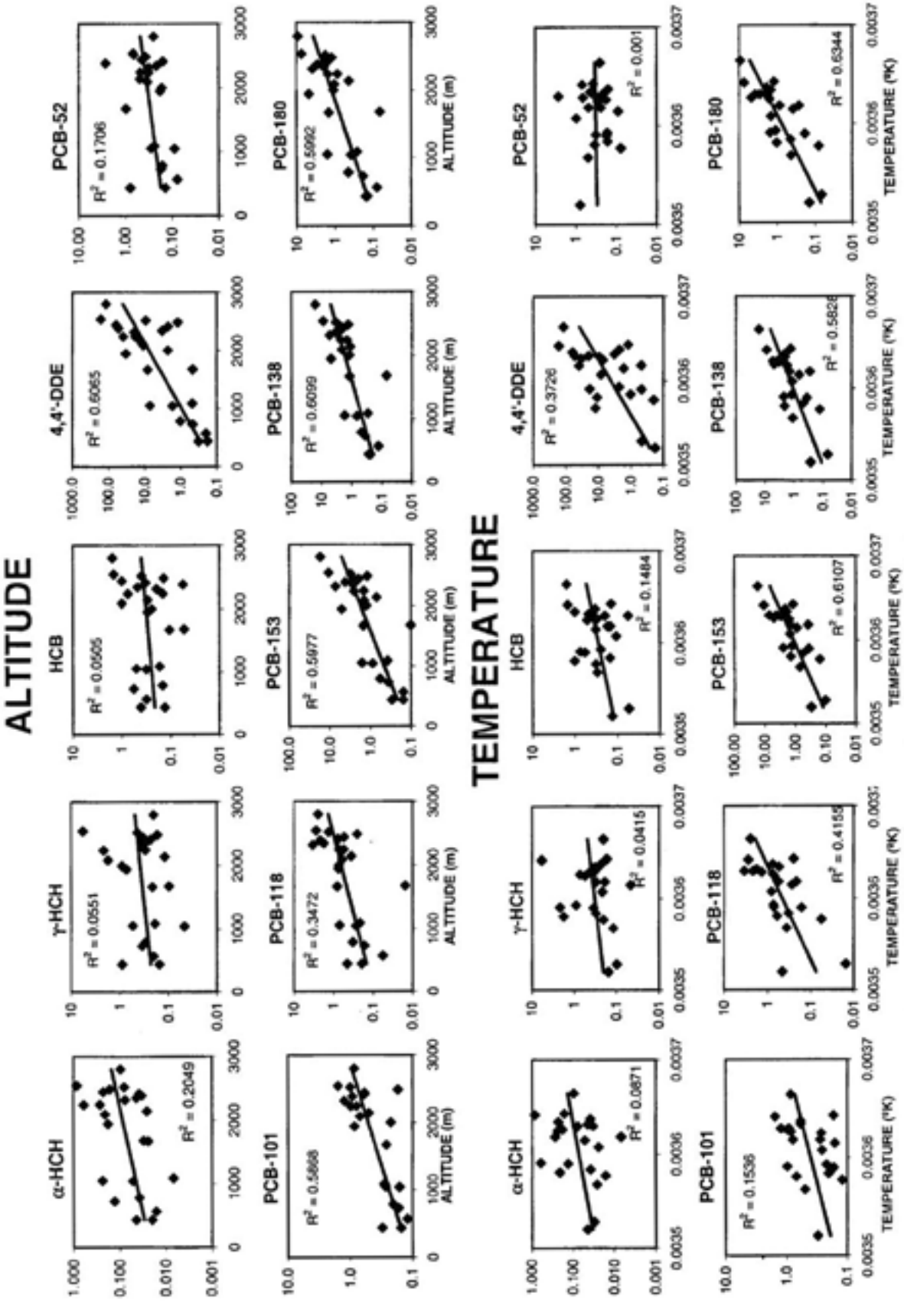

FIGURE 3. Examples of the distributions of lake-averaged ($n = 24$) muscle concentrations of selected organochlorine compounds vs lake altitude and reciprocal of temperature.

in fish at higher lake altitude and older age. These trends do not reflect any age−altitude correlation in the data set (Figure 2D) but the concurrence of temperature-related processes leading to this distribution. Both processes tend to retain the less volatile and more hydrophobic OC in fish muscle and, to a lesser extent, liver. The present results also illustrate that high mountain areas may be priority sites for the study of the forthcoming redistribution of OC as consequence of global warming.

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