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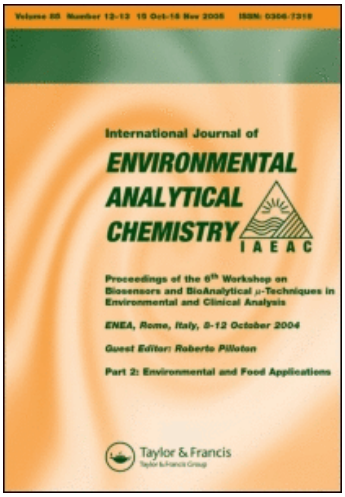

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Baseline Studies of the Global Pollution I. Occurrence of Organohalogens in Pristine European and Antarctic Aquatic Environments

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Baseline Studies of the Global Pollution

I. Occurrence of Organohalogens in Pristine European and Antarctic Aquatic Environments

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Pristine Alpian fresh water lakes with no run off were selected to monitor the atmospheric fall-out of C_6—C_{12} -organochlorine compounds. Off-shore marine areas were taken for monitoring the average marine pollution by these compounds.

In both cases fishes have been used as bioextractors. The analytical work-up combines solvent-partition, liquid chromatography and glass capillary gas chromatography with the electron capture detector. The identification is done by matching high-resolution retention indices of unknowns with those of reference compounds. The following compounds could be identified in the spawn of arctic chars (Salvelinus alpinus) caught in off-road Alpian lakes as well as in the liver of predatory antarctic cod (Dissostichus eleginoides) caught near South Georgia, as well as in Peru fish oil and crude sperm oil: hexachlorobenzene; α-, β-, γ-hexachlorocyclohexane; 4,4′-DDT; 4,4′-DDE; 4,4′-DDD; 2,4′-DDT; 2,4′-DDE; 2,4′-DDD; heptachloroepoxide; polychlorobiphenyls (PCB) and polychlorocamphenes.

Concentrations are given in nanogram/gram total lipid extract (ppb). First value Salvelinus (Alps), second value Dissostichus (Antarctic Ocean), α-HCH: 40/0,1; β-HCH: 4,1/0,1; γ-HCH: 17,2/0,1; HCB: 65/8; 4,4′-DDT: 59/4; 4,4′-DDE: 477/5; Σ DDT 646/11,4; Σ PCB: 1030/32; Σ PCC: 124/68.

KEY WORDS: Baseline study; Organohalogens; Alpian lakes; Antarctic Ocean; Glass capillary gas chromatography.

1. INTRODUCTION

A series of investigations has been started with the aim of a detailed qualitative and quantitative analysis of the global baseline occurrence mainly of organochlorine compounds brought into the environment deliberately, as pesticides, or involuntarily, as chlorinated solvents and plasticizers. All discussions of the fate of man-made chemicals in the

biosphere lead to the conclusion, that even so-called pristine environments should carry the strain of a defined baseline pollution, particularly of organic environmental chemicals. The mixing processes in the troposphere of either hemisphere will literally carry air-borne material around the world within 3–4 weeks. The sedimentation process and the washout by rain will eventually bring the particles or molecules to the ground. The surface and deep-sea currents of the oceans will be part of the global distribution system also. While the details of the mechanisms of global transport—particularly the role of particles and gaseous species—are still under study[1], the spreading of man-made chemicals can be easily detected[2, 3, 4, 5]. Numerous reports are available on the quantitative aspects of global pollution[2, 5, 6, 7]. Though the reports concentrate mainly on a few contaminants, the results do reflect the general trend.

We define as "pristine" such areas where no direct input of environmental chemicals occurs as a result of human activities. Such areas will be primarily found far from any settlement, e.g. in off-shore regions of the oceans, deserts, high mountainous areas, and the uninhabited or barely inhabited areas north and south of the respective polar circles.

The geophysical parameters of long-range transport, such as the air movements in the troposphere of both hemispheres and the surface and deep-sea currents in the oceans, define the level of indirect input of environmental chemicals. Maximum distance from any possible source, westerly winds and upwelling deep-sea water render the off-shore areas of the South Pacific west of South America one of the ultimates of a low-level input area under these aspects.

Should particulate transport in the troposphere show up as the main road of long distance transport for compounds such as the polychlorobiphenyls (PCB's), all factors governing the sedimentation process will be very effective in determining the levels of pristinity.

Besides the analytical challenge of this study—complex mixtures have to be analyzed and quantified in extremes as to sensitivity and specificity—we feel the question of the status quo of the "unpolluted" environment to be a wide open one. One might only question whether this unknown "status quo" is up to slow or fast changes. Furthermore, as long as the "status quo" is not known, any discussion about the ultimate effects of chemicals in the environment will be ill founded. However, knowing the "status quo", governmental or even interstate regulations might appear having only a local effect in terms of the environmental impact as long as the world production is not affected.

We have focussed our attention on a detailed qualitative analysis of the global pollution, because only such knowledge will reveal the full spectrum of compounds to be considered in the general ecological discussion.

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Furthermore, only single-component analysis, e.g., of the PCB's, can reveal whether particular physical properties or molecular structural parameters influence the spreading, if at all.

2. BIOEXTRACTION FOR LOW-LEVEL POLLUTION MEASUREMENTS

Baseline studies involve trace analyses of complex mixtures in the $1{:}10^{12}$ or $1{:}10^{15}$ range or even less. Though many xenobiotics can be detected directly either by selected ion GC-MS or electron-capture GC in the 0.1–10 picogram range, enrichment techniques still have to be applied, partly as a necessary step for the preseparation of complex samples. Considerable progress has been made in this field by adsorption techniques particularly for air and water analysis.[8,9,10] Though the operation of such techniques in off-road pristine environments is basically possible, we have used the well known effect of bioaccumulation [11,12] for baseline studies of aquatic environments.

The xenobiotics we are interested in are all to be considered hydrophobic. These compounds will be forced by the water to adhere to any surface less hydrophylic than water itself, as seen in the powerful enrichment by adsorption on aliphatic or aromatic hydrocarbon macromolecules. The cells of the micro-phytoplankton can be seen to act in the same way in aquatic environments. Instead of catching the micro-phytoplankton itself, we have made use of plankton-feeding species as bioextractors. Basically any propelled device carrying adsorption tubes through an aquatic environment would do the same, maybe even in a more defined way than bioextractors equilibrate with their environment.

Plankton-feeding animals—whale sharks, fishes of the herring family, right whales—seem particularly appropriate for getting a good insight into the contamination of their aquatic environment. Within only two trophic levels an enrichment factor of up to 10^5 can be measured. The content of PCB rises from 0.1–3 ppt for seawater[4] to 0.1–5 ppm for liver, spawn or bodyfat of plankton-feeding fishes.[5,6,7] Predatory fishes often show even higher enrichment factors, but larger deviations are encountered due to a varying spectrum of food. Benthic fishes should give a good idea of the substances transported to the sea bottom by currents and the detritus.

Consequently fishes as members of the higher level of the aquatic food-web, that could be assigned to defined habitats and a known spectrum of food have been chosen as "bioextractors" being in equilibrium with their aquatic environment. Knowing growth rate and mean food intake, a bioaccumulation factor can be directly calculated in certain cases.[11,12]

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Metabolic changes of the xenobiotics are much less pronounced in fish than in e.g. birds or mammals.

Fish samples bring a major advantage to the analytical procedure. If only parts from the inside—liver, spawn, fillet—are investigated, the fishes can be considered "naturally contamination-free wrapped up." Therefore, the often decisive steps in low-level trace analysis—sampling, sample transport, sample storage—can be considered to be fully controlled and not the source of sample contamination. Wrapping up in aluminium foil and cooling to $-20°C$ has been used for transport and storage prior to the actual analysis.

Fish from both marine and fresh water sources have been collected, to see whether one can distinguish between water-air and air-transport mechanisms. Spawn rather than fillets has been used for the investigations, in order to ensure a close look at the body mobilization effect as well as the contamination of the reproduction system. Possibly the hatching of fish is not so much affected by a direct contamination from the outside aquatic environment, but rather more strongly by the infestation of the spawn by the fish itself, which implies that the fish has finally got its burden from the outside.

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3. KIND AND ORIGIN OF ENVIRONMENTAL SAMPLES

To gain an insight into the general baseline occurrence of organochlorines, the following environmental samples—considered to be typical for "unpolluted" and pristine areas—have been checked through:

3.1. Alpian lake fishes

Alpian lake fishes of the Salmon type, the Artic Char (*Salvelinus alpinus*), a plankton-feeding fish (24–28 cm, 45–65 g) caught in 1978 in two small off-road high-mountain lakes in the Wetterstein-mountains of the Tyrolian Alps, 110 km south-west of Munich: the Seebensee (area: $2 \cdot 10^5$ m^2; altitude: 1653 m) 47° 20′N 11°E, and the Drachensee (area: 10^5 m^2; altitude: 1880 m) 47° 30′N 11°E. The two small lakes, 5 km by air of the small winter resort Ehrwald near the Zugspitze, have been selected with the intention of excluding as far as possible any direct local input by farming or other human activities. The only source should be the direct and indirect Central European fall-out transported to the lakes, either by rain, snow or sedimentation. The rain fall within about 4 km^2 of mostly bare rocks in a medium altitude of 2000–2500 m feeds the lakes. Since the primary input of pollutants will be exclusively by air, compounds found in the fish could help to clarify the influence of physical parameters considered to be important in air-transport.

3.2. Antarctic marine fishes

Antarctic marine fishes of the antarctic cod family: Notothenia (*Notothenia rossi marmorata*) (60 cm, 2.1 kg) and (*Dissostichus eleginoides*) (135 cm, 19 kg) caught on the shelf of South Georgia (55° S, 40° W). Notothenia rossi marmorata feeds primarily on macrozooplankton, while Dissostichus eleginoides is a typical fish-feeding predatory species.[13] The fish have been caught south of the Antarctic convergence, a region mainly characterized by upwelling deep-sea water. Westerly winds and the westwind drift are the atmospheric and marine currents. The oil fields south of Tierra del Fuego (55°S, 70°W) have to be considered as the only major sources for xenobiotics in this antarctic region, except for xenobiotics from the agricultural activities in the southern part of South America. As the contaminants of the Notothenia rossi marmorata could not be clearly differentiated from the procedural blank, only the results of the predatory Dissostichus are presented.

Furthermore we have analyzed an industrial fish oil and crude sperm oil of sperm whales. Though the defined local origin of these oils can not be quoted, they give tentative information about the general areas from which they come.

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3.3. Peru fish oil

Peru fish oil is gained from the anchovy (*Engraulis ringens*) caught in the Peruvian upwelling water region. Engraulis is a small (10–15 cm) zooplankton-feeding fish caught mainly for industrial purposes.

The Pacific Ocean west of Peru and Chile can be considered to be one of the least polluted areas of both hemispheres as the input of xenobiotics, either by the westerly winds or the oceanic surface and deep-sea currents, will be small compared to other areas.

3.4 Sperm oil

Sperm oil is the waxy mass found in the head of the sperm whale (*Physeter macrocephalus*). Sperm whales feed mainly on molluscs (cuttle fishes), and on fish. The sample investigated probably comes from Japanese catches in the South Pacific or Antarctic Ocean. The feeding grounds of sperm whales can be considered in any case to be of a low global baseline pollution.

4. GENERAL ANALYTICAL ASPECTS

Effective measures for the exclusion of contamination during sample collection and sample preparation have been applied. All steps of the analysis have been checked for possible sources of contamination prior to the real run. Low-level pollution samples (0.01–10 ppb) could be analyzed only by drastically reducing the blank values caused by lab air, solvents, reagents and glassware.

Enrichment techniques and preseparations by liquid chromatography prior to high-resolution glass capillary gas chromatography with the electron-capture detector give highly detailed patterns of the environmental pollution.[14]

We have identified the compounds in multi-component chromatograms by comparing their "high resolution" retention indices with those of the reference standards.[15] As far as possible, technical products rather than pure active compounds have been used in the identification procedure. The internal ratios of compounds in technical products and their changes during their fate in the environment can lead to interesting conclusions about the dispersion, degradation or persistence of compounds under environmental conditions. A detailed analysis of these aspects for PCB's has been reported recently.[14]

So far we have concentrated on identifying the following groups: 1. DDT-originated compounds, 2. polychlorinated aromatics (benzenes, biphenyls, terphenyls, naphthalenes, toluenes, styrenes), 3. polychlorinated cyclodiene compounds, and 4, chlorinated C_2—C_{10} aliphatic or olefinic hydrocarbons.

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5. ANALYTICAL PROCEDURES

5.1. Solvents and materials

All solvents used were of pesticide analysis grade. If necessary they were distilled over sodium metal and potassium hydroxide. Concentrating 50 ml to 0.1 ml should show no interfering compounds. The adsorbents were cleaned by heating to 650°C for 2 h and they were cooled to room temperature under controlled conditions. Their water content has been controlled by adding defined amounts of water through the gas-phase. The equilibration time was one week.

Some of the PCB compounds used as references were a gift from Bayer (Leverkusen, G.F.R.); the others were obtained from Aldrich-Europe (Beerse, Belgium), Analabs (North Haven, Conn., U.S.A.) and RFR Corp. (Hope, R.I., U.S.A.).

5.2. Sample extraction

Spawn or liver (10–25 g) together with up to 30 g Na_2SO_4 and 5 g quartz sand have been ground for 10 min in an agate mortar in a controlled atmosphere. The resulting dry powder has been extracted with 110 ml n-hexane + acetone (2 + 1), using a column technique.[16] Larger portions have been extracted in a soxhlet to avoid large amounts of solvent. An aliquot of the extract has been used for the determination of the extractable lipid content.

Oil samples (2–10 g) have been dissolved in 20 ml hexane.

5.3 Fat clean-up

The fat clean-up has been done first by solvent partition (dimethylformamide/n-hexane).[17,18] Some samples have been further cleaned by adsorption chromatography on 8 g alumina (de-activated by 5% H_2O), using 30 ml n-hexane as eluant. This clean-up step strongly reduces the β-hexachlorocyclohexane and partly the dieldrin.

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5.4. Pre-separation

The clean-up by adsorption chromatography on Florisil has been combined with a group preseparation. By modification of reported procedures, we found no discrimination of the PCB's, when carefully bringing the water content of Florisil to 1.25% and using n-hexane as eluant at a flow-rate of 5 ± 1 ml/min.

50 ml n-hexane will elute from 13 g Florisil the hexachlorobenzene, all the OCB's and 4,4 DDE together with part of 2,4'-DDE, traces of 2,4'- and 4,4'-DDT and a few front running polychlorocamphene components. Lowering the water content opens up the possibility of a loss of chlorinated PCB components. Increasing the amount of n-hexane or the water content of Florisil results in a less defined PCB-polychlorocamphene separation.

Next 50 ml hexane-diethylether (95 + 5) will elute the DDT-group and the more polar cyclodiene pesticides: endrin, dieldrin, heptachloroepoxide and the polychlorocamphenes. For an extensive fat clean-up, as is advisable for glass capillary gas chromatography, this hexane-diethylether eluate has to be run over a Florisil column (13 g, 1.25% H_2O) a second time.

5.5. Concentrating step

The eluates (50 ml) are concentrated by a rotary evaporator to 0.2–0.5 ml in a special glass vessel. 2–5 μl of this concentrate are used for G.C. analysis by splitless on-column injection. The overall enrichment factor ranges from 20 (10 g→0.5 ml) to 200 (40 g→0.2 ml).

5.6. Detection limit

The above moderate enrichment factors indicate that even samples for a baseline pollution study do not require extensive preconcentration. The basic problem is to rule out any contamination. One has to keep the procedural blank of PCB's and other contaminants close or below the detection limit of the analytical system. The cold column injection technique or the "solvent effect"[19] will allow up to 10 μl of sample to be injected on the glass capillary column without a decrease in separation efficiency over a long period. Most of the compounds of interest could easily be detected by the electron-capture detector at the 1 pg level. This brings the detection limit to 10 ppt per single component using a 5-μl injection of a 500-μl concentrate of a 10-g sample of extractable lipids or oil.

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5.7. Gas chromatography conditions

Instruments: Gas chromatograph Carlo Erba Fractovap 2101 AC
Detector: ECD Carlo Erba HT 25
Standing current 2.3–2.9 nA; Temperature 250°C ECD control module 251
Injector: Modified on-column injection port
Temperature: 250°C
Capillary column: Soda-lime (AR) glass, gas phase HCl etching; Carbowax 20 M deactivation; static coating; phase ratio 1000; liquid phase SE 30; internal diameter: 0.30 mm; length 35 m separation number (C_{12}–C_{13}): 48 carrier gas: hydrogen flow rate optimized
Temperature program: on-column injection at 40°C; 2 min at 40°C; 40° to 140°C temperature program 50°/min; 3 min at 140°C; 140° to 190° temperature program 1.6°/min; 190°C isothermnal.
Sample: 1–5 μl; solvent n-hexane.

6. RESULTS

6.1. Air-borne contaminants in Alpian lakes

Figure 1 compares the blank of the analytical procedure to the contamination of the spawn of arctic char (*Salvelinus alpinus*) sample. The entire analytical procedure from grinding of the sample to concentrating of the Florisil column eluate to 0.5 ml has been run through for Figure 1.1. The spawn extract—only cleaned by alumina chromatography but not preseparated by chromatography on Florisil using different eluants—is shown in Figure 1.2. Figure 2 presents the hexane eluate (Figure 2.1) and the hexane/5% diethylether eluate (Figure 2.3) from a Florisil column (1.25% water).

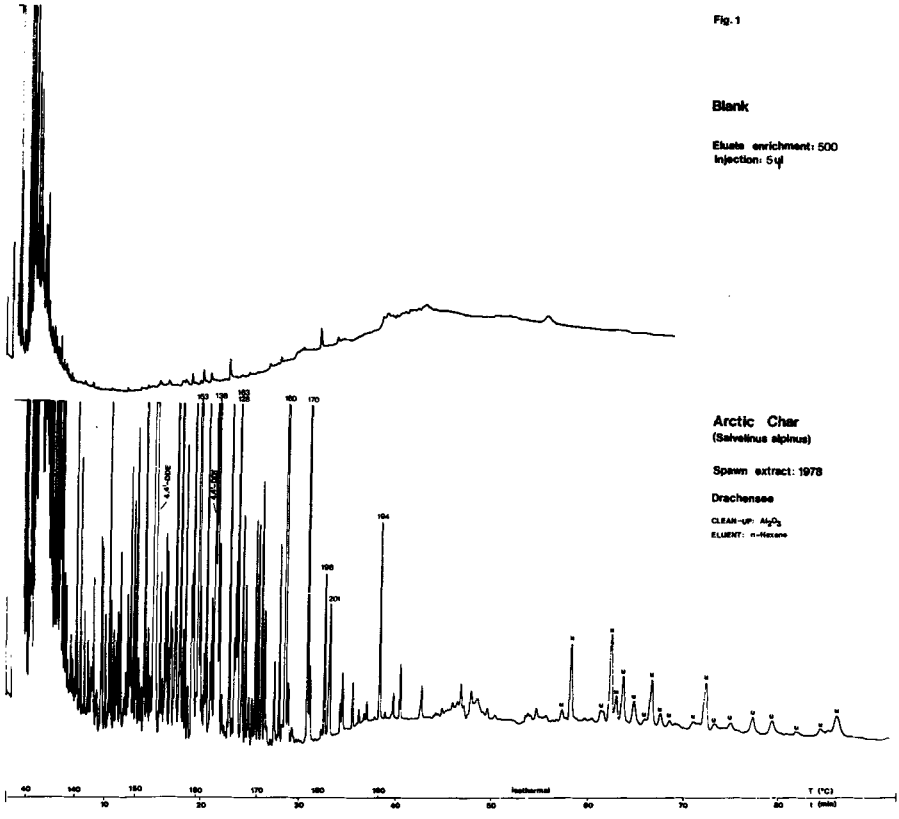

FIGURE 1 Figure 1.1. ECD-gas chromatogram of blank run of analytical procedure; Figure 1.2 Total contaminants in spawn of arctic char (Salvelinus alpinus) caught in Drachensee (1180 m), Tyrolian Alps. Aluminia (5% H_2O)/hexane eluate. Both chromatograms have been recorded at the same sensitivity settings.

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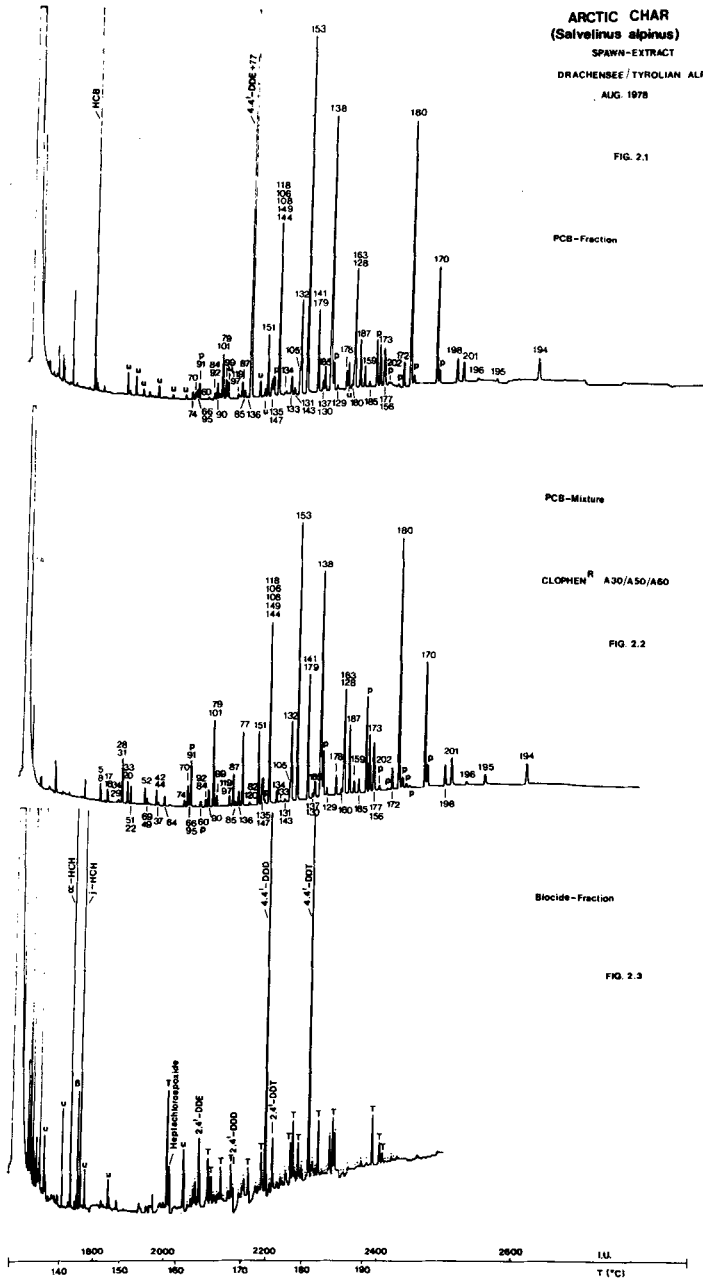

FIGURE 2 ECD-gas chromatogram of pre-separated contaminants in spawn of artic char (Salvelinus alpinus). Experimental data see text.

FIGURE 2.1 Florisil/n-hexane eluate. Figure 2.2 Mixture of technical polychlorobiphenyls, Clophen A 30, A 50, A 60 (1+1+2) as simulation standard; Figure 2.3. Florisil/n-hexane—diethylether (95+5) eluate.

T = polychloroterpene (PCC); dot = polychloroterpene; p = polychlorobiphenyl (PCB) of unknown structure; number = identified polychlorobiphenyl see Table 2; HCH = hexachlorocyclohexane; HCB = hexachlorobenzene; DDT = dichlorodiphenyltrichloroethane; DDD = dichlorodiphenyldichloroethane; DDE = dichlorodiphenyldichloroethene; u = unknown; not marked = unknown.

In the hexane eluate (Figure 2.1) the PCB's can be detected. To rationalize the identification of defined PCB's we worked out a systematic numbering. Arranging the PCB's in a sequence according to the IUPAC rules for systematic substituents numbering, gives each individual PCB component a defined position. The number of this position has been used as a symbol to identify the individual PCB's. The numbers 1–3 named for monochloro; 4–15 for dichloro; 16–39 for trichloro; 40–81 for tetrachloro; 82–127 for pentachloro; 128–169 for hexachloro; 170–193 for heptachloro; 194–205 for octachloro; 206–208 for nonachloro and 209 for decachloro biphenyl. Table 1 gives the structural assignments of the PCB components found in the environmental samples. The PCB components have been identified using retention index matching of standards and samples.[14,15] For components not available as reference compounds, we compared their calculated retention indices with those measured for the sample.[20,21]

None of the dichloro- and only minute amounts of trichloro-biphenyls can be detected, while several unknown compounds are found in this elution range. The general impression is of a nearly unchanged PCB pattern, since the PCB mixture found in the spawn of Salvelinus could be fairly well simulated by mixing Clophen® A 30/ A 50/ A 60 in the ratio of 1 + 1 + 2 (Figure 2.2). The sum of PCB compounds 141 and 179 and the so far unidentified PCB prior to 173 seem to be reduced in the fish sample as compared to the simulation mixture.

The hexane eluate (Figure 2.1) further reveals the occurrence of hexachlorobenzene and 4,4′-DDE as major contaminants in the fish spawn. Other members of the DDT-group are found in the hexane/5% diethylether eluate (Figure 2.3), e.g. 4,4′-DDT and 4,4′-DDD, while 2,4′-DDT, 2,4′-DDE and 2,4′-DDD are rather weak. The ratio of 4,4′-DDT to 2,4′-DDT has changed to 6 : 1 as compared to about 4 : 1 in the technical mixture. We take this to be the result of the missing 1,4-disubstitution in one of the phenyl rings of 2,4′-DDT, which facilitates its degradation. This interpretation is backed by the fact that the metabolites 2,4′-DDE and 2,4′-DDD are never found to be enhanced to the same extent as are the 4,4′-derivatives (see Table 2).

The α- and the γ-isomer of the hexachlorocyclohexane group are found in the hexane-diethylether eluate (Figure 2.3). The α-isomer is the major component of the technical benzene hexachloride (HCH), composing up to 65% of the product. The technical HCH is used in many cases as an economical insecticide. The γ-isomer, Lindane, the major insecticide compound of the HCH-group is also widely used, particularly in indoor-applications and forestry pest-management. Probably as a result of the alumina clean-up step, only minor amounts of β-HCH could be detected.

Besides heptachloroepoxide and other as yet unidentified compounds,

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TABLE I

Major PCB-Components in Environmental Samples

PCB-Number[a]	Structure	PCB-Number[a]	Structure
	Trichloro-biphenyls		
28	2,4,4′	31	2,4′5
	Tetrachloro-biphenyls		
40	2,2′,3,3′	66	2,3′,4,4′
41	2,2′,3,4	69	2,3′,4,6
42	2,2′,3,4′	70	2,3′,4′,5
44	2,2′,3,5	74	2,4,4′,5
47	2,2′,4,4′	77	3,3′,4,4′
49	2,2′,4,5′	79	3,3′,4,5′
52	2,2′,5,5′		
	Pentachloro-biphenyls		
83	2,2′,3,3′5	101	2,2′,4,5,5′
84	2,2′,3,3′,6	102	2,2′,4,5,6′
85	2,2′,3,4,4′	105	2,3,3′,4,4′
87	2,2′,3,4,5′	106	2,3,3′,4,5
90	2,2′,3,4′,5	118	2,3′,4,4′,5
91	2,2′,3,4′,6	119	2,3′,4,4′,6
97	2,2′,3′,4,5	120	2,3′,4,5,5′
99	2,2′,4,4′,5		
	Hexachloro-biphenyls		
128	2,2′,3,3′,4,4′	143	2,2′,3,4,5,6′
129	2,2′,3,3′,4,5	144	2,2′,3,4,5′,6
130	2,2′,3,3′,4,5′	147	2,2′,3,4′,5,6
131	2,2′,3,3′,4,6	149	2,2′,3,4′,5′,6
132	2,2′,3,3′,4,6′	151	2,2′,3,5,5′6
133	2,2′,3,3′,5,5′	153	2,2′,4,4′,5,5′
134	2,2′,3,3′,5,6	156	2,3,3′,4,4′,5
135	2,2′,3,3′,5,6′	159	2,3,3′,4,5,5′
136	2,2′,3,3′,6,6′	160	2,3,3′,4,5,6
137	2,2′,3,4,4′,5	163	2,3,3′,4′,5,6
138	2,2′,3,4,4′,5′	165	2,3,3′,5,5′,6
141	2,2′,3,4,5,5′		
	Heptachloro-biphenyls		
170	2,2′,3,3′,4,4′,5	179	2,2′,3,3′,5,6,6′
172	2,2′,3,3′,4,5,5′	180	2,2′,3,4,4′,5,5′
173	2,2′,3,3′,4,5,6	185	2,2′,3,4,5,5′,6
177	2,2′,3,3′,4′,5,6	187	2,2′,3,4′,5,5′,6
178	2,2′,3,3′,5,5′,6		
	Octachloro-biphenyls		
194	2,2′,3,3′,4,4′,5,5′	198	2,2′,3,3′,4,5,5′6
195	2,2′,3,3′,4,4′,5,6	200	2,2′,3,3′,4,5′,6,6′
196	2,2′,3,3′,4,4′,5′,6	201	2,2′,3,3′,4′,5,5′,6
197	2,2′,3,3′,4,4′,6,6′	202	2,2′,3,3′,5,5′,6,6′

[a]Systematic numbering according to IUPAC rules of substituent numbering; 1 = 2-chlorobiphenyl; 209 = decachloro-biphenyl [23].

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TABLE II

Quantitative results of baseline pollution measurements via bioaccumulation. The values are given in nanogram per gram total extractable lipids (ppb)

Sample Region	Spawn[a] Salvelinus Mid-Europe ng/g	Liver[b] Dissostichus Antarctic ng/g	Peru fish oil South Pacific ng/g	Sperm oil Southern Hemisphere Ocean ng/g
α-HCH[(1)]	40.2	0.1	7.4	1.5
β-HCH	4.1	0.1	1.5	n.id.
γ-HCH	17.2	0.1	1.5	1.5
HCB[(2)]	65	7.5	3	4
Σ DDT[(3)]	646	11.5	73	270
4,4'-DDT[(4)]	59	3.9	10	19
2,4'-DDT	10	1.0	1	7
4,4'-DDD[(5)]	79	0.6	10	93
2,4'-DDD	6	0.2	2	40
4,4'-DDE[(6)]	477	5.2	44	97
2,4'-DDE	15	0.6	6	14
PCB[(7)]	1030	32	368	752
Heptachloroepoxide	5	0.02	n.id.	n.id.
Σ PCC[(8)] (Toxaphen)	124	68	20	72

n.id. = not identified.
[a]14% extractable lipids, pooled sample of 4 fishes.
[b]32% extractable lipids, 1 fish.
(1) Hexachlorocyclohexane; (2) Hexachlorobenzene; (3) Sum of the six identified compounds of the DDT-group; (4) Dichlorodiphenyltrichloroethane; (5) Dichlorodiphenyldichloroethane; (6) Dichlorodiphenyldichloroethene; (7) Polychlorobiphenyl; (8) Polychloroterpenes.

marked by an u (unknown), a vast amount of polychloroterpenes marked by T (major components) or a dot (minor components) has been identified by retention index matching and pattern analysis in the the hexane-diethylether eluate.

The occurrence of polychloroterpenes—Polychlorocamphene (USSR), Strobane, Toxaphene (U.S)—with a modified pattern as compared to Toxaphene in these Alpian fish can be taken as indicative of a long-range transport. The polychloroterpenes are used in Central-Europe only as rodenticides, if at all. They are however, heavily applied for pest management in e.g. cotton fields.

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6.2. Contaminants in the Antarctic Ocean

For a first check-up of the baseline pollution of the southern hemisphere we have focussed on fishes from the Antarctic Ocean south of the antarctic convergence. Figure 3 represents the results from the liver extract of *Dissostichus eleginoides*, a predatory antarctic cod, caught near South Georgia. Size (135 cm) and weight (19 kg) promised a high degree of accumulation.

In the n-hexane eluate (Figure 3.1) hexachlorobenzene and 4,4′-DDE can be detected as major contaminants, together with several polychloroterpenes marked with T. The occurrence of minor amounts of 2,4′-DDE, 2,4′-DDT and 4,4′-DDT in this eluate proves elution of all PCB compounds to be complete. The PCB's are found only in very minute amounts (see compounds 153, 138, 180 and 170). Since the typical PCB-degradation pattern cannot be analyzed due to the minute amounts, the presence of the PCB's cannot unambiguously be attributed to environmental pollution. However, we feel a contamination during the clean-up procedure can be excluded.

The n-hexane-diethylether (5%) eluate (Figure 3.2) further reveals a broad spectrum of contaminants to be present in the antarctic region. Besides a group of so far unidentified compounds, marked by a u, we have identified: the α-, β- and γ-isomers of the hexachlorocyclohexane (HCH) group, heptachloroepoxide. The DDT-group is represented by the compounds: 4,4′-DDT, 4,4′-DDD, 2,4′-DDT, 2,4′-DDE and 2,4′-DDD. The 4,4′-DDE has been eluted in the hexane fraction (Figure 3.1). A surprisingly high amount of 4,4′-DDT can be detected. Since the metabolism of DDT to either 4,4′-DDE or 4,4′-DDD can be considered quite fast in terms of years, the strong 4,4′-DDT peak together with a correspondingly strong one for the 2,4′-DDT suggests a continuous and/or recent input.

Next to the DDT-group, the polychloroterpenes, marked by a T or a dot, represent the major part of the organochlorine compounds found in the hexane-diethylether eluate of the Dissostichus liver extract. The identification of the polychloroterpenes has been done by retention index matching and pattern analysis of reference and sample. The compounds close to retention index 2600 marked by u, though not identified yet, are most likely metabolites of the polychloroterpenes.[22] Figure 3.3 compares technical Toxaphene with the modified mixture as found in the antarctic fish sample (Figure 3.2). The world-wide occurrence of polychloroterpenes has been established recently.[23] Earlier reports on Toxaphene in air samples, taken in the Bermuda Islands and on cruises between Bermuda and the U.S. coast, did not prove this fact, though they suggested it

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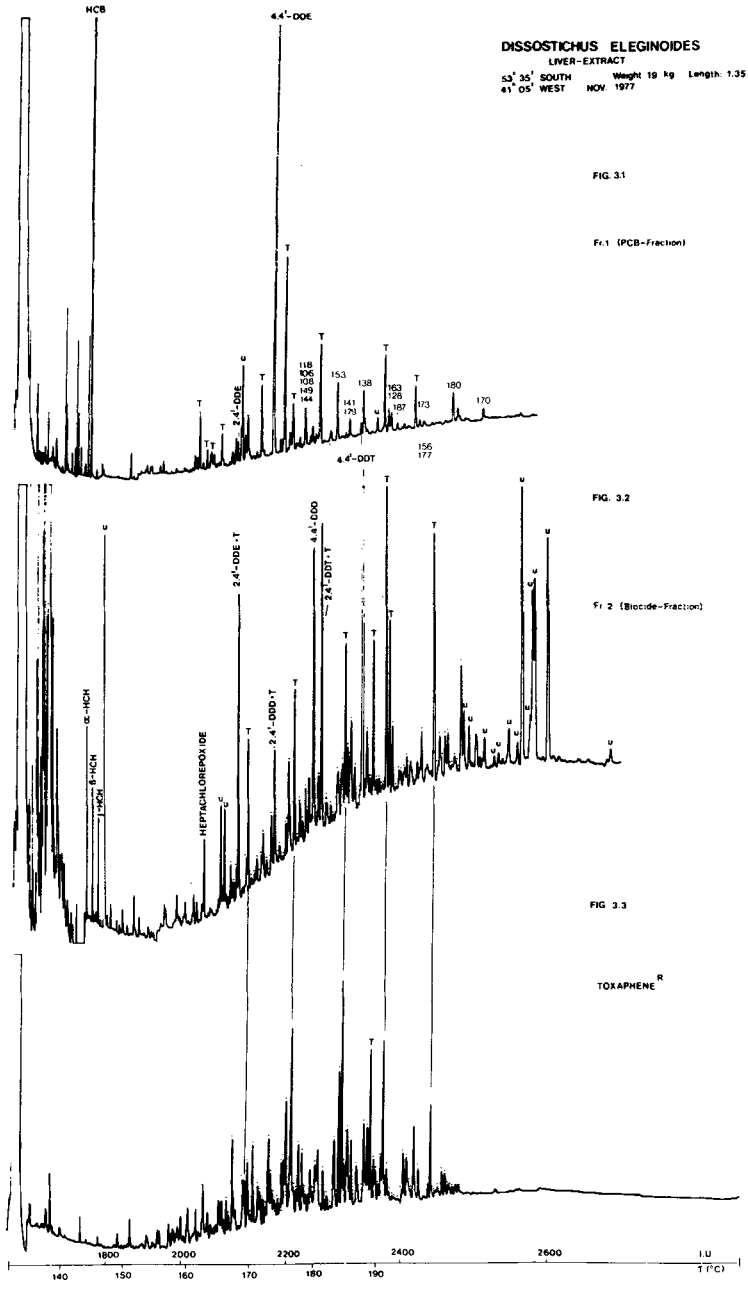

FIGURE 3 ECD-gas chromatogram of pre-separated contaminants in liver of antarctic cod of the Nothothenia family (Dissostichus eleginoides) caught near South Georgia, Antarctic Ocean. Experimental data see text.

Figure 3.1. Florisil/hexane eluate; Figure 3.2. Florisil/hexane—diethylether (95+5) eluate; Figure 3.3 Technical polychlorocamphene (Toxaphene®) for comparison. Abbreviations see Figure 2.

strongly.[24,25] As the polychloroterpenes are known to be potent mutagens their world-wide occurrence even in samples from pristine areas should cause some concern.

Liver of antarctic Notothenia fish has been investigated a decade ago.[26] No compound of the o,p'-DDT-group, no PCB's and no polychloroterpenes have been reported in that study.

6.3. Contaminants in marine oils of the Southern Hemisphere

Figure 4 compares the Florisil/n-hexane eluates of Peru fish oil and sperm oil. While the Peru fish oil is representative for the eastern part of the South Pacific, the sperm oil cannot be attributed unambiguously to a defined geographic region, though its origin most likely is the southern-hemisphere sperm-whale population.

Quite a few unknown compounds, marked with a u, and HCB are present. For the majority of PCB compounds the structure could unequivocally be assigned (Table 1). Though the pattern of the PCB's looks quite similar for both samples, a detailed inspection reveals marked differences. Whether these differences reflect the different metabolism of anchovy and sperm whale or a different input of the various PCB mixtures cannot be decided yet. As compared to other mammals, the metabolism of PCB's in the sperm acetic organ of the sperm whale is surprisingly much less pronounced.

The strong peak prior to 4,4'-DDE, marked as unknown (Figure 4.1), appears to be a metabolite of polychloroterpenes as this compound can be synthezised from technical Toxaphene by model degradation experiments with silver nitrate. Minor amounts of 2,4'-DDE, and a few polychloroterpenes marked by a T could be detected in the hexane eluate too. These compounds are in the front portion of the next group of substances, as shown in Figure 5.

Figure 5 presents for the sperm oil (Figure 5.1) and the Peru fish oil (Figure 5.2) the eluates containing the hexachlorocyclohexanes, the polychloroterpenes and the DDT-group except for 4,4'-DDE, which elutes in fraction 1, (Figure 4). The differences in intensities in Figure 5.1 and 5.2 reflect the differences in contamination of both samples.

Three hexachlorocyclohexane isomers—α, β, γ—are found in the Peru fish oil, while the β-isomer is missing in the sperm oil. This is due to alumina clean-up of this sample prior to the Florisil separation.

The whole DDT-group is rather strong in the sperm oil with high contents of even the 2,4'-isomers. The ratio of 4,4' to 2,4'-DDT can be taken as an indication of a recent input of technical DDT, as pointed out before. This ratio comes close to 11 for the Peru fish oil, compared to 4–5

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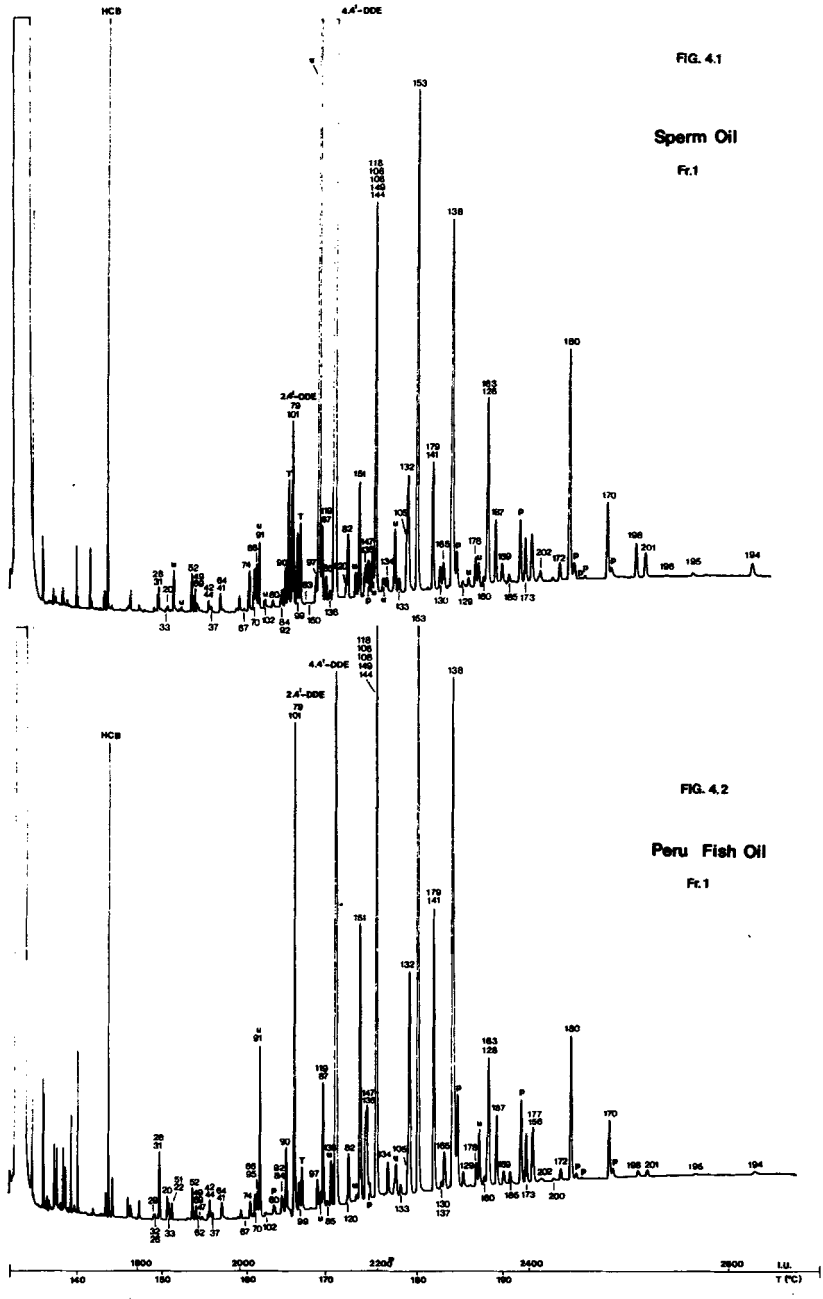

FIGURE 4 ECD-gas chromatogram of pre-separated contaminants in whale sperm oil and Peru fish oil. Experimental data see text.

Figure 4.1. Florisil/hexane eluate sperm oil; Figure 4.2. Florisil/hexane eluate Peru fish oil.

Abbreviations see Figure 2.

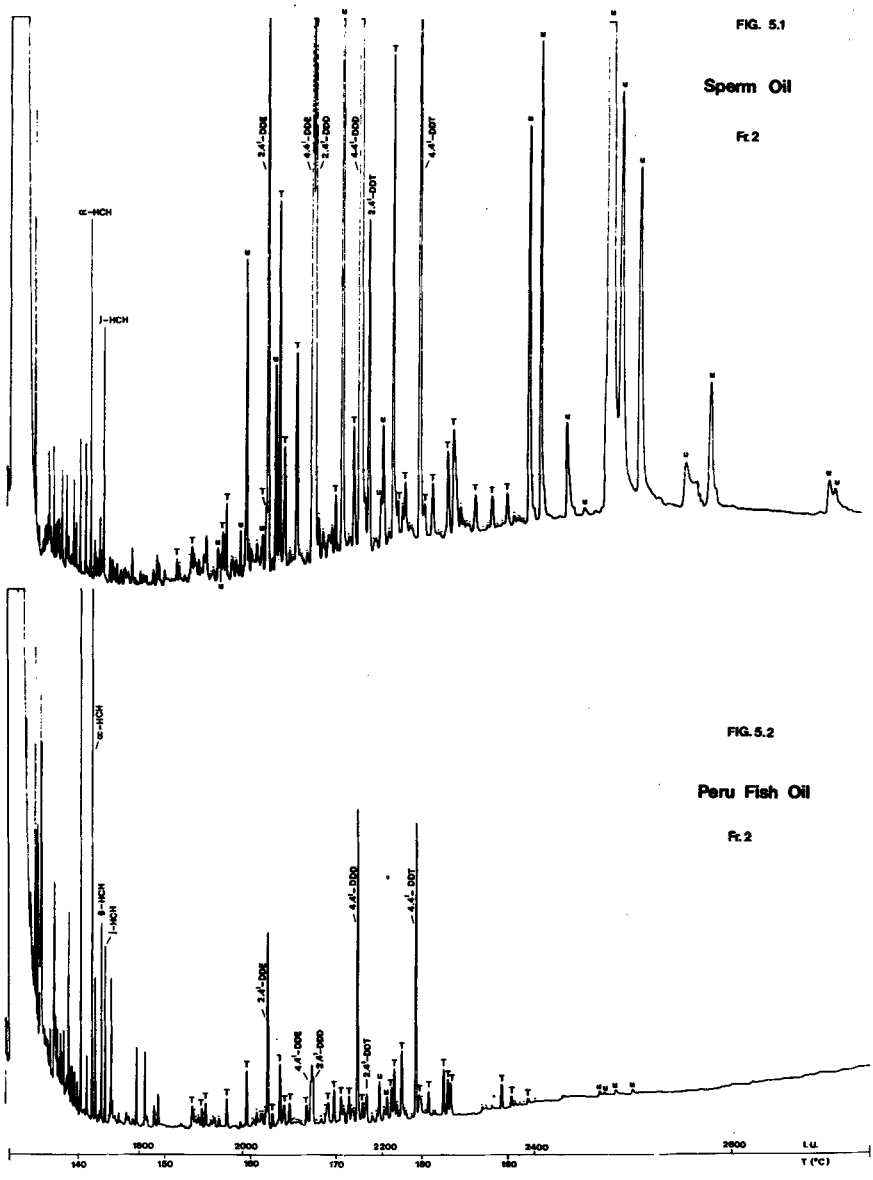

Figure 5 ECD-gas chromatogram of pre-separated contaminants in whale sperm oil and Peru fish oil. Experimental data see text.

Figure 5.1. Florisil/hexane-diethylether (95+5) eluate sperm oil; Figure 5.2. Florisil/hexane-diethylether (95+5) eluate Peru fish oil.

Abbreviations see Figure 2.

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for the technical DDT mixture. The DDT accumulated by the Peruvian anchovy therefore appears to be "old" in terms of time of occurrence in the biosphere. The decrease of 2,4′-DDT agrees with generally less pronounced occurrence of 2,4′-DDT metabolites as 2,4′-DDE or 2,4′-DDD in the fish oil.

Polychloroterpenes, marked by a T or a dot, occur in both samples. The pattern is distinctly different from that of the technical mixtures. So far we can only speculate about the identity of the high-boiling unknowns eluting after the PCB's (Figure 5.1). The possibility of long-chain esters or similar long-chain hydrocarbons as a result of an incomplete clean-up has been excluded by repeating the sample run using a flame ionisation detector. At highest sensitivity setting only minute peaks could be detected. Complex mixtures of xenobiotics eluting in that range would be the bromobiphenyls, the polychlorodibenzofurans, the polychlorodibenzodioxins, the polychloroterphenyls or polar metabolites of the polychloroterpenes.

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6.4. Quantitative analysis

Since our clean-up procedure is a standard method, we have based the results of the quantitative analysis on recovery rates as reported in the literature.[16,18] For the Florisil elution the recovery has been checked by optimizing the separation of PCB's and polychloroterpenes.

The quantitation procedure is straightforward in those cases where only one compound is involved which can be clearly separated from all interferences. The hexachlorobenzene, the hexachlorocyclohexane isomers, the dichlorodiphenylethanes and the cyclodiene pesticides except chlordane belong to this group. Problems arise when complex mixtures such as the polychlorobiphenyls, chlordane or polychloroterpenes have to be quantitated. Due to a partial metabolism of these mixtures, comparison of standard mixtures involves a systematic error that cannot easily be avoided.

While the polychlorobiphenyls can be chemically transformed into one single product, either decachlorobiphenyl[27] or biphenyl,[28] this is not possible for the polychloroterpenes. For the PCB quantitation we have compromised by making a "closest fit simulation" mixture and measuring areas or peak heights of several peaks. Summing up all peaks corrected by the specific ECD response factor would minimize the systematic error. This could be done best by a computer program. In the case of the polychloroterpenes, we have used technical Toxaphene as a standard and constructed a calibration curve by adding the peak heights of several major peaks of the standard. The same peaks have been summed up in

the sample. The result should be correct within 10–25%, or even better. Table 2 summarizes the results.

One could start to comment about the significance of the quantitative results. We feel that though the samples can be regarded as highly statistical the basis for a thorough interpretation is still too small. A few aspects however are clearly demonstrated by Figures 1–5 and the values of Table 2.

1. All samples of what one would call pristine environments reveal about the same complex qualitative pattern of pollution, though the quantitative extent can vary remarkably for single components.

2. The arctic fish sample gives by far the lowest contamination values, except for the polychloroterpenes and hexachlorobenzene,

3. The Central European fish sample, where only an input of the contaminants by air should be possible, shows consistently the highest values.

4. Though many contaminants have been identified, the structure of quite a few major unknowns remains to be elucidated, particularly in the region above 2500 and below 1800 retention index units.

It is too early to speculate about the parameters ruling the indirect input of contaminants, e.g, the primary sources and the extent of transport by water or air. We hope further sampling and further progress in analytical techniques will help to clarify the remaining questions. In particular type, extent and geographic areas of the main sources of the global baseline pollution will be of interest.

The results available so far suggest a *complex* contamination of the ecosphere with organochlorine compounds.

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