Organochlorine patterns related to the fatty acid composition in cod livers and seal blubber

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Introduction

The determination of organochlorine residues is an important task in environmental chemistry. Organochlorines are high lipophilic substances which accumulate in the lipid phase. Consequently, seal blubber (lipid content >80%) and cod livers (lipid content approx. 40-50%) accumulate particularly high levels of organochlorines. The bioconcentration of these compounds is not only correlated to the lipid content but to the lipid composition [1]. Seafood lipids are known to contain high quantities of polyunsaturated fatty acids (PUFA) which are thought to be protective against certain diseases [2-4]. Polyunsaturated fatty acids, in particular 20:5(ω-3) (eicosapentaenoic acid, EPA) and 22:6(ω-3) (docosahexaenoic acid, DHA), are essential for the growth and development of animals in maricultures [5].
Minimization of clean-up methods in order to reduce the sample weights and the amount of solvents and chemicals is an important task in environmental chemistry. However, repeated sample preparations resulted in higher standard deviations for organochlorines if low sample amounts were used. The reason may be inhomogeneity of the lipid phase. The knowledge of the composition of the lipid phase is therefore an important information to understand the variations of organochlorines levels in a biomatrix.
A method was developed which allows the analysis of the lipid composition as well as the determination of organochlorine levels. Lipids were extracted from seal blubber and cod liver by application of microwave-assisted extraction (MAE) [6]. After sample clean-up, GC/FID analysis of fatty acids and GC/ECD analysis of organochlorines was performed for cod liver and seal blubber. With this method, the organochlorine pattern in the sample can be compared with the pattern of the fatty acids.

Material and Methods

Samples

The blubber of the Baikal seal (*Phoca sibirica*) was from an animal caught in 1995 on the east coast of Lake Baikal. Cod liver sample of this investigation was from canned cod liver purchased in March 1998. No details on the origin of the sample were reported.

Lipid phase extraction and sample clean-up for organochlorines

MAE was performed using the focused open-vessel system Soxwave 100 (Prolabo, France). Details on the extraction procedure and the sample clean-up of organochlorines (GPC, adsorption chromatography on silica) were recently described in detail [6].

Preparation of fatty acid methyl esters:
Between 100 and 200 mg of extracted lipids from seal blubber or cod livers were placed in a 10 mL screw cap vial. The samples were dissolved in 2 mL of isooctane and 0.3 mL of 30% $NaOCH_3$ were added. The closed vial was shaken for 1 to 2 min. Then, the solution was incubated for 30 min at 70 °C. After cooling to ambient temperature, 5 mL of 4% $KHSO_4$ were added and shaken. The sample was reposed for 30 min, and then 1 mL of isooctane was added. The clear upper (isooctane) layer contained the resulting methyl esters. An aliquote of this layer was injected into the GC to determine the fatty acid composition of both samples.

GC-conditions
The methylated fatty acids were determined with an HP 5890 gas chromatograph (Hewlett-Packard) equipped with a flame ionization detector (GC/FID). Injector and detector temperatures were both 260°C. Carrier gas was helium (1 bar). Detector ignition gasses were hydrogen (1.0 bar) and air (2.1 bar). A Stabilwax column (Restek) of 30 m length, 0.32 mm i. d., and 0.5 µm film thickness was installed in the GC.
Organochlorines were determined with GC/ECD. The conditions were reported elsewere in detail [7].

Results and discussion

MAE proved to be a suitable method for lipid extraction. The method showed good reproducibility. Figure 1 and 2 show GC/ECD and GC/FID chromatograms of cod livers and Figure 3 and 4 show the respective chromatograms in seal blubber. 10 or 12 fatty acids were identified by injection of standard solutions, and several further abundant FAMEs were detected in the FID chromatograms. Due to the low lipid amounts required (approx. 100 mg), this method is well suited for the determination of the lipid profile in environmental samples in parallel to the organochlorine content.
Therefore, a good technique for the testing of the homogeneity of the lipid phase and its influence on the organochlorine level is obtained. Further research will be aimed to investigate this topic.

Acknowledgement
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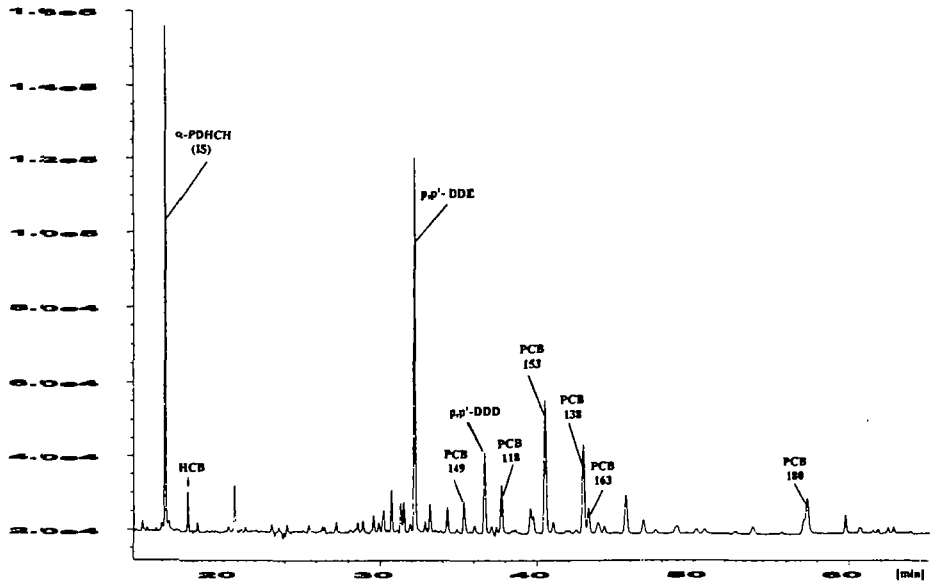

Fig.1- GC/ECD chromatogram of the organochlorine fraction of canned livers *on CP-Sil 2.*
GC oven program: 60°C (1.5 min), 40°C/min to 180°C (2 min), 2°C/min to 230°C (25 min), 10°C/min to 270°C (15 min).

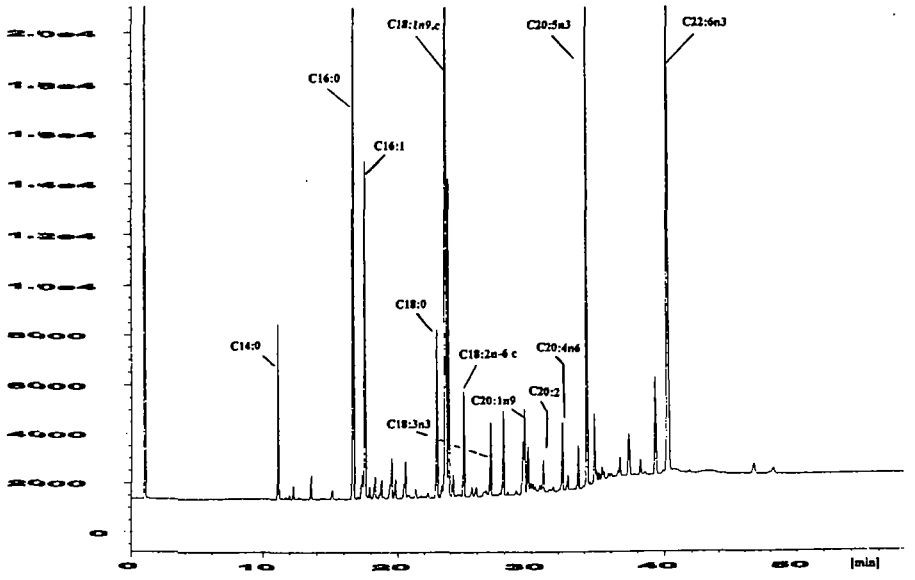

Fig.2- GC/FID chromatogram of the lipid fraction of canned cod livers on Stabilwax.
GC oven program: 120°C (3 min), 10°C/min to 170°C(6 min), 3°C/min to 230 °C, 20°C/min to 240°C (25min).

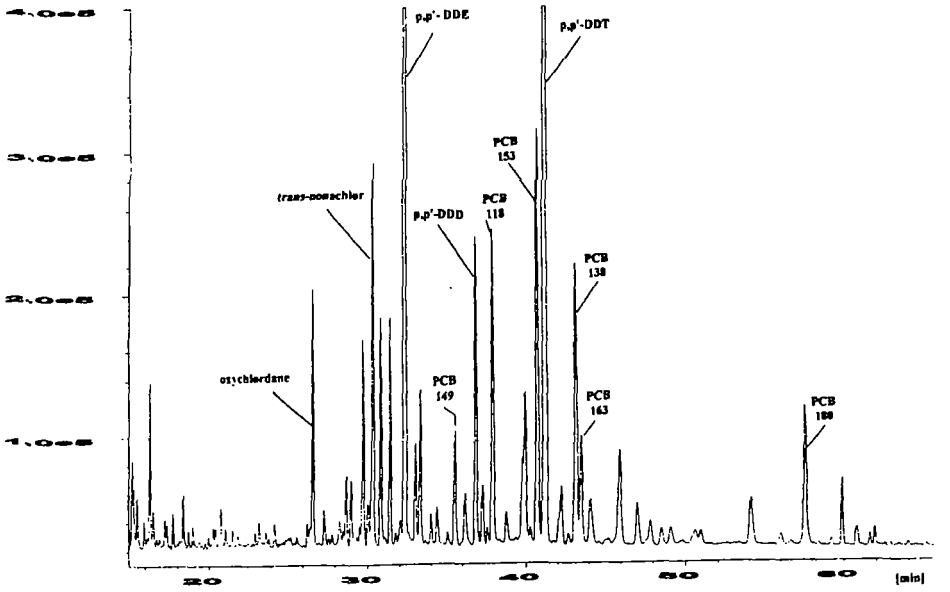

Fig.3- GC/ECD chromatogram of the organochlorine fraction of the blubber extract of a Baikal seal (*Phoca sibirica*) on CP-Sil 2.
GC oven program: 60°C (1.5 min), 40°C/min to 180°C (2 min), 2°C/min to 230°C (25 min), 10°C/min to 270°C (15 min).

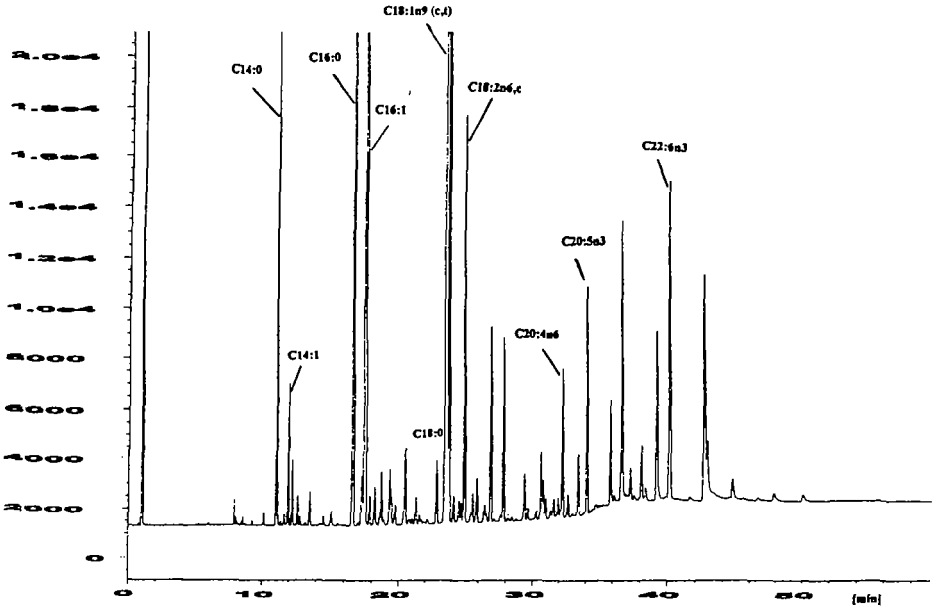

Fig.4- GC/FID chromatogram of the lipid fraction of a Baikal seal (*Phoca sibirica*) on Stabilwax.
GC oven program: 120°C (3 min), 10°C/min to 170°C(6 min), 3°C/min to 230 °C, 20°C/min to 240°C (25min).