ENVIRONMENTAL ANALYSIS

Microwave-Assisted Extraction and Accelerated Solvent Extraction with Ethyl Acetate–Cyclohexane before Determination of Organochlorines in Fish Tissue by Gas Chromatography with Electron-Capture Detection

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Focused open-vessel microwave-assisted extraction (FOV–MAE), closed-vessel microwave-assisted extraction (CV–MAE), and accelerated solvent extraction (ASE) were used for extraction before determination of organochlorine compounds (polychlorinated biphenyls, DDT, toxaphene, chlordane, hexachlorobenzene, hexachlorocyclohexanes, and dieldrin) in cod liver and fish fillets. Wet samples were extracted without the time-consuming step of lyophilization or other sample-drying procedures. Extractions were performed with the solvent mixture ethyl acetate–cyclohexane (1 + 1, v/v), which allowed direct use of gel-permeation chromatography without solvent exchange. For FOV–MAE, the solvent mixture removed water from the sample matrix via azeotropic distillation. The status of water removal was controlled during extraction by measuring the temperature of the distillate. After water removal, the temperature of the distillate increased and the solvent mixture became less polar. Only the pure extraction solvent allowed quantitative extraction of the organochlorine compounds. For CV–MAE, water could not be separated during the extraction. For this reason, the extraction procedure for wet fish tissue required 2 extraction steps: the first for manual removal of coextracted water, and the second for quantitative extraction of the organochlorine compounds with the pure solvent. Therefore, CV–MAE is less convenient for samples with high water content. For ASE, water in the sample was bound with Na_2SO_4. The reproducibility for each technique was very good (relative standard deviation was typically <10%); the slightly varying levels were attributed to deviations during sample cleanup and the generally low levels.

Polychlorinated biphenyls (PCBs), hexachlorocyclohexanes (HCHs), hexachlorobenzene (HCB), DDT, compounds of technical toxaphene (CTTs), dieldrin, and chlordane are ubiquitous anthropogenic chemicals. Because of their lipophilic nature and persistence, they are accumulated in environmental samples, and efficient analytical methods are required for the determination of these compounds.

After homogenization, extraction is the first step in the sample preparation procedure for the determination of organochlorine compounds. In modern analytical laboratories, classic Soxhlet extraction is being replaced more and more by faster, less solvent- and time-consuming techniques (1). Microwave-assisted extraction (MAE; 2–6) and accelerated solvent extraction (ASE; 7, 8) have been suggested as extraction methods for the determination of organochlorines in soil, sediment (2, 6), and adipose tissue (3).

The most common MAE technique is closed-vessel MAE (CV–MAE) under pressure and high temperature (1, 2, 9). An alternative to CV–MAE is focused open-vessel MAE (FOV–MAE), which operates at atmospheric pressure and with refluxing of the solvent (5, 9, 10). ASE® is a registered trademark of Dionex Corp. of the general method of pressurized liquid extraction. It is an automated extraction technique, which uses hot solvents and high pressure for extraction (7, 8).

The solvent mixture we used for both ASE and MAE was ethyl acetate–cyclohexane (1 + 1, v/v; 4). Nonpolar solvents like cyclohexane or *n*-hexane are best suited for the extraction of nonpolar organochlorines, but they cannot be heated directly by microwave energy (3, 9). Addition of ethyl acetate allows direct heating of the solvent and also supports its penetration into the pores of a wet sample matrix. Another advantage, after volume adjustment of the extract, is direct performance of gel-permeation chromatography (GPC) with Bio-Beads SX-3 (ABC, Analytical Biochemistry Colombia, Colombia, MO), because GPC can be performed with the same solvent mixture (4, 11). The combination of MAE and GPC avoids exchange of solvent, which is a time-consuming source of error. The composition of the azeotrope is 54:46 (12), which means that evaporation does not change the solvent composition significantly.

CV–MAE with ethyl acetate–cyclohexane (1 + 1, v/v) proved to be efficient for the determination of organochlorine compounds in seal blubber and cod livers, and high recoveries were obtained (4). FOV–MAE in combination with efficient

Received December 27, 1999. Accepted by JS March 1, 2000.
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sample cleanup was used earlier to extract the low organochlorine levels in eggs of penguins and skuas from the Antarctic (5), because it allowed the extraction of sample amounts of up to 50 g. It is also well suited for the extraction of lipids as a prelude to the determination of the fatty acid profile (13).

In this study, the solvent mixture ethyl acetate–cyclohexane (1 + 1, v/v) was applied to the ASE of cod livers (30% water) and the MAE of fresh fish fillets (73% water) without drying. The advantages of each technique are discussed.

Experimental

Sample Origin

Canned cod livers (origin not specified) were purchased from a food store in Jena, Germany, in 1998. Cod livers were homogenized before extraction in an Ultra-Turrax T25 (Janke & Kunkel, Staufen, Germany). Mackerels (*Scomber scrombrus*) were taken from the German North Sea in 1997, and herring (*Clupea harengus*) were taken from the central Baltic Sea in 1995. After collection, the complete fish were packed in aluminum foil and kept at –20°C until analysis. For analysis, fresh fish fillet was separated from 3 fishes, homogenized, and blended with Na_2SO_4 (1 + 1, w/w).

Chemicals and Organochlorine Standards

The following single standard solutions of organochlorines (each at 10 ng/μL) were obtained from Promochem (Wesel, Germany) or Dr. Ehrenstorfer (Augsburg, Germany): α-, β-, γ-, and δ-HCH, HCB, *p,p′*-DDT, *p,p′*-DDD, *p,p′*-DDE; PCBs 28, 52, 101, 118, 138, 149, 153, 163, 170, and 180; dieldrin; oxychlordane; *cis*- and *trans*-chlordane; and *cis*- and *trans*-nonachlor. CTTs quantitated in this study were 2-*exo*-3-*endo*-5-*exo*-9,9,10,10-heptachlorobornane (B7-1453), 2-*endo*-3-*exo*-5-*endo*-6-*exo*-8,8,9,10-octachlorobornane (B8-1412), 2-*endo*-3-*exo*-5-*endo*-6-*exo*-8,8,10,10-octachlorobornane (B8-1413, P-26), 2-*endo*-3-*exo*-5-*endo*-6-*exo*-8,9,10,10-octachlorobornane (B8-1414, P-40), 2-*exo*-3-*endo*-5-*exo*-8,9,9,10,10-octachlorobornane (B8-1945, P-41), 2-*exo*-5,5,8,9,9,10,10-octachlorobornane (B8-2229, P-44), 2,2,5,5,8,9,9,10,10-nonachlorobornane (B9-1025, P-62), and 2-*endo*-3-*exo*-5-*endo*-6-*exo*-8,8,9,10,10-nonachlorobornane (B9-1679, P-50). Hereafter, Andrews and Vetter (AV)-codes (14) are used and Parlar numbers (15) are in parentheses. B8-1412 was quantitated with the electron-capture detection (ECD) response factor of B8-1413 (P-26). A solution containing B7-1453 was calibrated by gas chromatography with flame-ionization detection (16). The other CTT standards were from Dr. Ehrenstorfer.

Single standard solutions were combined to obtain quantitation standards of 50 and 100 pg/μL. The response factors of the 100 pg/μL standard solutions were identical to those of certified standard solutions (N 0813 and NC 378, Promochem). Calibration curves (1–50 pg/μL for the CTTs and 10–500 pg/μL for the other organochlorines) were prepared to check the linearity of the area measurement. The solutions were distributed on the 2 capillary columns (*see* below), so that the calibration range was 5–250 pg (0.5–25 pg for CTTs).

Five quantitation standards were used, which contained (*1*) HCHs, *p,p′*-DDT, *p,p′*-DDD, *p,p′*-DDE, and dieldrin, (*2*) PCBs, (*3*) chlordane related compounds, (*4*) the 8 CTTs, and (*5*) HCB.

Certified reference material SRM 1588 (cod liver oil) was from Promochem. Ethyl acetate (residue analysis grade) was from Fluka (Neu-Ulm, Germany). Cyclohexane (Pestanal grade) was from Riedel-de Haen (Seelze, Germany). Isooctane (Rotipuran > 99.5% p.a.) was from Roth (Karlsruhe, Germany) and *n*-hexane (residue analysis grade) was from Promochem.

Silica Gel 60 (particle size 0.063–0.200 mm; Merck, Darmstadt, Germany) was activated for 16 h at 130°C. Anhydrous Na_2SO_4 (Merck) was dried for 16 h at 130°C before use.

Sample Lyophilization and Water Determination

Portions of 5 g homogenized mackerel fillet were placed on Petri dishes (10 cm id), frozen at –24°C, and then lyophilized for 24 h in a Beta 1–8k apparatus (Christ, Osterode, Germany) at –30°C and 0.37 mbar. The dishes were placed on plates and heated at 25°C. After lyophilization, the dry matrix was homogenized again in a mortar.

The water content was determined gravimetrically by weighing the samples before and after lyophilization. In addition, 10 g portions of the homogenized samples (cod liver and mackerel fillet) were placed on Petri dishes (10 cm id) and dried at 110°C until the weight was constant.

Instrumentation

FOV–MAE was performed with a Soxwave 100 (Prolabo, Paris, France) system. Energy was produced by a magnetron at 2450 MHz. The apparatus was equipped with a reflux column to recondense evaporated solvent. The system allowed multistep programming of microwave energy (maximum 300 W) and time of irradiation. For quantitative extraction, the glass connection piece between the quartz vessel and the reflux column was equipped with a 15 mL trap, which partly allowed the separation of solvent and water (5). For comparison, we also used a connection without a trap, which was the original piece delivered with the system (*see* below).

CV–MAE was performed with an MLS 1200 mega apparatus (MLS, Leutkirch, Germany). The system operated with closed vessels that could withstand pressures up to 80 bar and also allowed multistep programming of microwave energy (maximum 1000 W) and time of irradiation.

ASE was performed with a Dionex ASE 200 system (Dionex, Sunnyvale, CA).

Automated GPC was performed with 50 g Bio-Beads S-X3 in a 33 × 2.5 cm id column in combination with an Autoprep 1002 system (ABC, Analytical Biochemistry Columbia).

GC–ECD analyses were performed with a Hewlett-Packard (Waldbronn, Germany) 5890 gas chromatograph equipped with a splitter installed at the exit of the split/splitless injector (splitless time, 1.5 min) that divides the samples onto 2 capillary columns, and 2 ^{63}Ni electron cap-

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ture detectors. The samples were injected automatically (HP 7673 autosampler). The capillary columns of CP-Sil 2 and CP-Sil 8/20% C_{18} (both 50 m × 0.25 mm id, 0.25 μm film thickness) were from Chrompack (Middelburg, The Netherlands).

Helium was used as carrier gas at a constant flow of 1.3 mL/min. Nitrogen was used as the makeup gas. The injector (splitless) and detector temperatures were 250 and 300°C, respectively. After injection at 60°C (1.5 min), the GC oven temperature was increased at 40°C/min to 150°C (5 min), then at 2°C/min to 230°C, and finally at 5°C/min to 270°C (15 min). The total run time was 71.75 min.

Sample Extraction and Cleanup

FOV–MAE.—The extraction was performed after addition of the internal standard, perdeuterated α-HCH (α-PDHCH; 17). A 5 g portion of lyophilized mackerel fillet, a 10 g portion of fresh fish fillet blended with 10 g Na_2SO_4, or a 2 g portion of cod liver was placed in a 250 mL quartz glass tube, and FOV–MAE was performed with 60 mL ethyl acetate–cyclohexane (1 + 1, v/v).

Although the system was designed to use a cartridge similar to that in the Soxhlet system (or more precisely, the Twisselmann system), the samples were weighed directly into the flask.

The reservoir (15 mL) above the faucet originally designed to evaporate the solvent was used as a trap to separate coextracted water. The procedure was recently described in detail (5, 13). The fish fillet in the present study contained 73% water, and 10 g fish fillet corresponded to 7.3 mL water in the wet sample. At the end of the extraction, ca 6 mL water was separated in the trap and drained off. The aquatic phase was extracted with 5 mL *n*-hexane. No organochlorines were detected in this extract.

The extract in the quartz glass tube was filtered through 10 g Na_2SO_4, which was sufficient to separate the remaining water.

For FOV–MAE of cod liver (30% water), the small amount of water separated in the trap was not drained off, but the contents of the trap were combined completely with the extract in the quartz glass tube and filtered through Na_2SO_4.

A parallel extraction was also performed by using the standard glass connection between the vessel and the reflux column, which has no trap. With this system, the recondensed distillate dropped back into the extraction vessel without separation of water. The same extraction program was applied.

The extracts were concentrated by rotary evaporation to ca 7 mL, filtered once more through ca 2 g Na_2SO_4, and adjusted to 10 mL (fish fillet) or 20 mL (cod liver).

Aliquots of these solutions were analyzed by GPC and used for gravimetric determination of the lipid content. The values for extractable lipids and fatty acid composition agreed with those obtained after liquid–liquid extraction (13).

CV–MAE.—CV–MAE was performed by application of a microwave program consisting of 7 extraction cycles (3, 4). A 1.5 g portion of fresh mackerel fillet was blended with 1.5 g Na_2SO_4 and extracted with 10 mL ethyl acetate–cyclohexane (1 + 1, v/v) in an 80 mL quartz flask. During extraction, the aqueous and organic phases separated. After extraction, the coextracted water, which was at the bottom of the vessel, was separated manually by use of a Pasteur pipet. A 10 mL portion of fresh solvent was added, and the samples were extracted once again. The first and second extracts were combined. For comparison, 1.4 g lyophilized mackerel fillet (10 mL solvent) and 1.0 g cod liver (8 mL solvent) were extracted in 1 step (i.e., without separation of water).

The extracts were concentrated by rotary evaporation to ca 7 mL, filtered through ca 2 g Na_2SO_4, and adjusted to 10 mL. The extracts were analyzed directly by GPC, and 1 mL was used for gravimetric determination of the lipid content.

ASE.—The ASE conditions for the ethyl acetate–cyclohexane (1 + 1, v/v) solvent were optimized by determination of the content of extractable lipids, which was compared with the content of extractable lipids obtained with the solvent *n*-hexane (18). Agreement in the content of extractable lipids was achieved under the following conditions: temperature, 125°C; pressure, 10 MPa; heat up, 6 min; 2 static cycles of 10 min; flush volume, 60%; purge, 1 MPa with nitrogen for 120 s.

A 5 g portion of cod liver was blended with 20 g Na_2SO_4 (1 + 4, w/w) and transferred to a 33 mL steel extraction cell. The remaining volume of the cell was filled with sea sand, and the extraction was performed with ASE by using the solvent ethyl acetate–cyclohexane (1 + 1, v/v). The extracts (ca 30 mL) were filtered through ca 10 g Na_2SO_4 and adjusted to 50 mL. The extracts were analyzed by GPC, and aliquots of these solutions were used for gravimetric determination of extractable lipids.

GPC.—After volume adjustment, the extracts were filtered through a 0.45 μm membrane filter. The sample was automatically introduced into the 5 mL sample loop of the system. GPC was used to separate the lipid fraction from the organochlorines. GPC was also performed with ethyl acetate–cyclohexane (1 + 1, v/v) as the solvent (4, 11). The dump and collection times were optimized with *trans*-chlordane and HCB, which are among the first- and last-eluted organochlorine compounds (11). The flow rate was set at 4.6 mL/min.

Adsorption chromatography with deactivated silica gel.—The GPC eluate was concentrated by rotary evaporation to ca 2 mL. A 2 mL portion of isooctane was added, and the solvent was evaporated in a nitrogen stream to ca 2 mL. The addition of isooctane and the evaporation were repeated twice for quantitative removal of the ethyl acetate.

Adsorption chromatography on deactivated silica was performed according to the method of Steinwandter and Schlüter (19), which was slightly modified (20). Activated silica gel was deactivated with 30% water (w/w) by shaking for 30 min. A 3 g portion of deactivated silica gel was slurry-packed in a glass column (1.0 cm id) and covered with Na_2SO_4. The isooctane extract of the sample was placed on the silica gel column, and the column was eluted with 60 mL *n*-hexane. The eluate was concentrated by rotary evaporation and by a nitrogen stream to 2 mL (cod liver), 1 mL (fish fillet, FOV–MAE), or 0.5 mL (fish fillet, CV–MAE) in a calibrated flask. Aliquots

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were analyzed by GC–ECD for the determination of PCBs and organochlorine pesticides, and for PCB/CTT group separation.

PCB/CTT group separation.—CTTs were quantitated after preseparation of the PCBs. A 1 mL (cod liver), 0.5 mL (fish fillet, FOV–MAE), or 0.2 mL (fish fillet, CV–MAE) aliquot of the solution, after adsorption chromatography with deactivated silica gel, was fractionated on a 30 × 1 cm id glass column filled with 8 g activated silica gel (21). PCBs were eluted with 48 mL *n*-hexane (PCB fraction), and CTTs and chlordane were eluted with 50 mL of a more polar solvent, which was more easily removed after the separation of a second fraction (CTT fraction; 21, 22). Instead of *n*-hexane–toluene (65 + 35, v/v), *n*-hexane–ethyl acetate (90 + 10, v/v) was used for elution of the CTT fraction (23). The eluates were condensed by rotary evaporation to ca 2 mL in calibrated flasks and evaporated in a nitrogen stream to 0.5 mL (cod liver) or 0.25 mL (fish fillet, FOV–MAE), or they were carefully evaporated to dryness in a nitrogen stream and dissolved in 100 µL *n*-hexane (fish fillet, CV–MAE). Aliquots were analyzed by GC–ECD for the quantitation of CTTs and chlordane.

Quality Control

α-PDHCH was used as the recovery standard to check losses of volatile organochlorines like α-HCH, lindane, and HCB during the sample concentration steps (17, 24).

The completeness of FOV–MAE, CV–MAE, and ASE was checked by repeating the extraction procedures with fresh solvent. In those extracts, no organochlorines were detected.

Two GC capillary columns of different polarity were used to check for peak interferences in the ECD chromatograms. For quantitation, it was required that a value determined with the first column be confirmed with the second column. In the case of deviations (coelution), the lower value was regarded as the correct one. Organochlorine levels were not extrapolated to 100% recovery; they represent the real measured levels.

The sample values were calculated with the standard having a concentration that was nearest to the sample concentration. Samples that revealed levels higher than the calibrated range were diluted before GC/ECD analysis.

The complete sample cleanup was validated with certified cod liver oil (SRM 1588; 5) by using CV–MAE ($n = 2$) as well as FOV–MAE ($n = 2$). Irrespective of the MAE system, all DDT and PCB levels were within the range certified by Schantz et al. (25).

Some samples were spiked, and after subtraction of the amount used for spiking, the results for the samples and the unspiked samples were the same.

Results and Discussion

Optimization of Extraction Programs

Microwave energy induces rotation of polar molecules, and the resulting molecular friction produces heat (9). Expressed in simple terms, it can be established that the higher the dielectric constant of a molecule, the more rotation is induced and the more effective is the heating by microwave energy (9, 26). Because microwave heating depends on the number and kinds of dipoles in the sample material, the water content of the sample exerts great influence on the heating by microwaves.

The present MAE systems (Soxwave 100 and MLS 1200 mega) had no temperature-pressure-controlling systems. Therefore, microwave extraction programs had to be adapted individually to each matrix, depending on the water content. Furthermore, apparent differences in the 2 MAE systems (vial size, sample amount, solvent amount) had to be considered.

Our goal was quantitative extraction within 30–40 min. No attempts to further reduce the extraction time were made. It may be possible to reduce this time, but for reasons of quality assurance, 30–40 min was selected to ensure quantitative extraction of a "worst-case" sample of the same type.

Extraction programs were optimized for power for CV–MAE by irradiating the sample at soft conditions (10 W) for 10 s. After irradiation, the vessels were opened, and the temperature of the solvent was promptly measured. In the next step, the irradiation time was set for 30 s at 10 W, on the basis of our experience with previous samples (3). Then the irradiation power was subsequently raised (from 20 to 300 W in 20 W steps) until the solvent inside the vessels was boiling (approximately 70°C) after the vessels were opened. This was achieved at a power of 300 W. Note that the temperature during extraction exceeds the boiling point because of the increase in pressure (4). Table 1 lists the optimized CV–MAE conditions for different matrixes. The higher the water content of the samples, the lower the microwave power required for extraction.

For FOV–MAE, conditions were optimized by starting at low power, followed by subsequent increases in power until

Table 1. Optimized extraction programs[a] for CV–MAE for different matrixes, depending on water content

Matrix	Water content, %	Extractable lipids, %	Power, W	Reference
Seal blubber	0	>90	1000	3, 4
Cod liver	30	50	500	4
Fish fillet (herring, mackerel; mixed with Na_2SO_4)	73	3.7–6.3	300	
Lyophilized mackerel fillet	0	12.3–13.3	300	

[a] Each extraction program consisted of 7 extraction cycles. One cycle included 30 s of irradiation, followed by 5 min of cooling without irradiation. Total extraction time was 38.5 min (3).

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Table 2. Optimized extraction programs for FOV–MAE for different matrixes, depending on water content

Matrix	Water content, %	Extractable lipids, %	Step 1, W (min)	Step 2, W (min)	Step 3, W (min)	Total extraction time, min
Seal blubber	<1	>90	75 (30)	—	—	30
Cod liver	30	50	30 (5)[a]	45 (5)	75 (30)	40
Partly lyophilized eggs (5)	ca 30	0.9–7.7	30 (7)[a]	45 (8)	60 (20)	35
Fish fillet (herring, mackerel; mixed with Na_2SO_4)	73	3.7–6.3	45 (15)	60 (15)	75 (5)	35
Lyophilized mackerel fillet	0	12.3–13.3	45 (15)	60 (15)	75 (5)	35

[a] Program was not optimized; step 1 was not necessary (*see* text).

the solvent refluxed. The optimized extraction programs for different matrixes are listed in Table 2.

Fish fillet samples with high water content (73%) were blended with Na_2SO_4 before the extraction, and the program was started at 45 W. The power was increased stepwise as water was removed from the sample matrix (*see* below). Figure 1 shows the GC–ECD chromatogram of a sample of herring fillet. The sample cleanup resulted in chromatograms of good quality.

For seal blubber samples with low water content (<1%) and high lipid content (>90%), a slow increase in power was unnecessary.

Samples with medium water content (30%), which were not blended with Na_2SO_4 before the extraction (cod liver, partly lyophilized eggs), may have had inhomogeneous distribution of water, which causes localized heating (5). To avoid delayed boiling, the extraction was begun at the lowest power (30 W), which was increased carefully. These samples were the first we extracted with FOV–MAE. Because of our limited experience, the extraction was started carefully at lowest power (5). On the basis of our current experience, the optimized extraction programs for cod liver and partly lyophilized eggs with medium water content could be started at a medium power of 45 or 60 W.

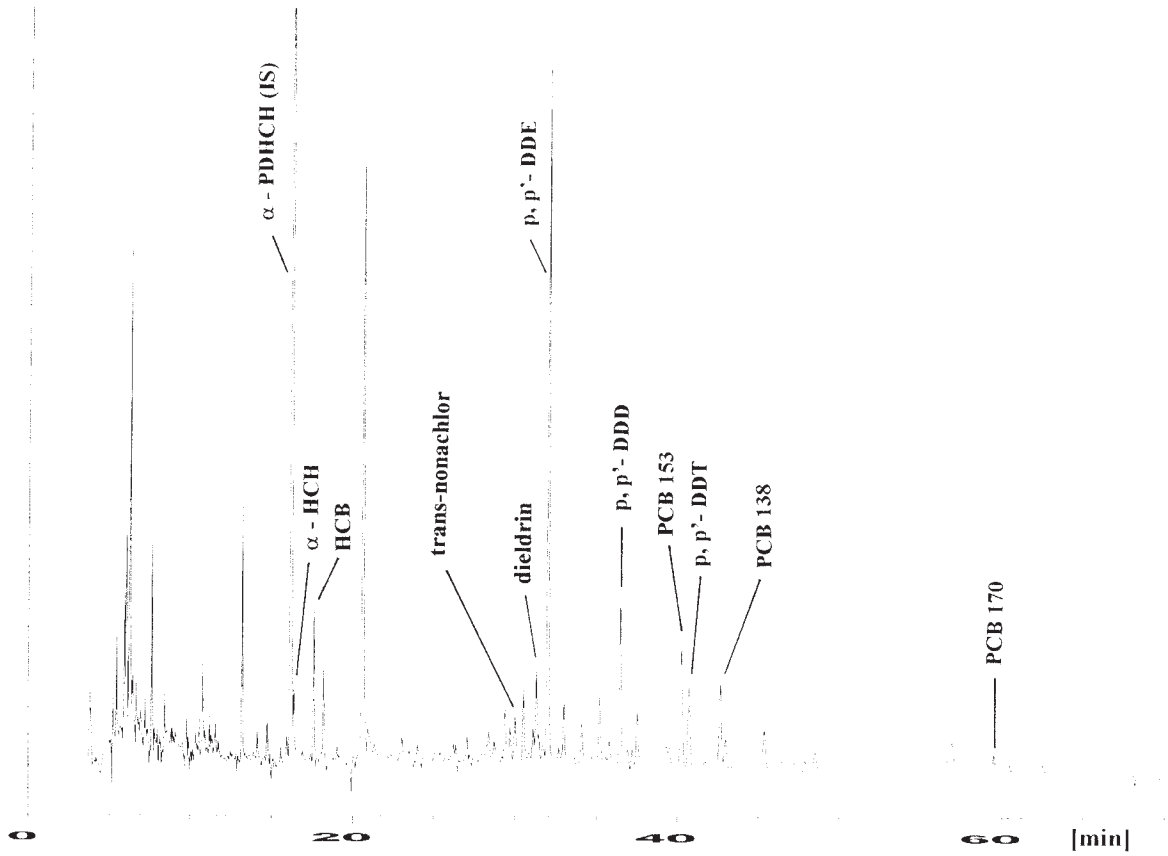

Figure 1. GC–ECD chromatogram (CP-Sil 2) obtained for fresh herring fillet after FOV–MAE, GPC, and cleanup on deactivated silica gel.

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With the FOV–MAE system and optimized extraction programs, different matrixes with an extractable lipid content from <1 to >90% and a water content from <1 to 73% can be extracted. It appears that water content, and not lipid content, is the main factor influencing the extraction conditions.

Removal of Water from the Sample Matrix

For FOV–MAE, the solvent removed water from the sample matrix because water formed a ternary azeotrope with ethyl acetate–cyclohexane. After refluxing, solvent and water were removed in 2 layers in the trap of the FOV–MAE system. After the trap was filled, the upper solvent layer redrained into the glass tube with the sample, and after the extraction, the water phase was removed. The status of the water removal was controlled by measuring the temperature of the azeotrope. After removal of the water, the temperature of the distillate increased to the boiling point of the binary azeotrope of ethyl acetate–cyclohexane (72.8°C).

When the glass connection is used without the water trap (*see* above), separation of water during the extraction is not possible. With this system, the organochlorine levels were only about 70% (52–89%) of those for the method that allowed separation of water. Consequently, only the pure, less polar extraction solvent enabled quantitative extraction of the organochlorines.

For this reason, CV–MAE of the fish fillet with high moisture content required 2 extraction steps: (*1*) extraction followed by manual removal of the water, and (*2*) repeated extraction for quantitative recovery of the organochlorines with the pure solvent. For fresh mackerel fillet, both extracts were analyzed separately. We found that the first and second steps extracted about 2/3 and about 1/3, respectively, of the organochlorines in the sample. No organochlorines were found in a third extraction. This distribution agrees well with the recovery obtained by FOV–MAE without the trap (*see* above). However, the 2-step method with CV–MAE is less convenient and more time consuming for samples with high water content.

A 10 g portion of fish fillet contained 7.3 mL water (*see* above). Anhydrous Na_2SO_4 is able to bind water as the decahydrate ($Na_2SO_4 \cdot 10H_2O$). Usually a 4-fold excess of Na_2SO_4 is recommended to bind water before the extraction of fish and meat (27). We used only (1 + 1, w/w). Note that we used the Soxwave system without a cartridge because of the large sample weight. A high surplus of Na_2SO_4 would have complicated filtration of the extract without loss of the analytes.

After the extraction of the fish fillet, 6–6.5 mL water was separated in the trap; this means the remaining 0.8–1.3 mL was bound to Na_2SO_4 or dissolved in the solvent. The remaining low amounts of water (in 60 mL solvent) did not change the polarity of the solvent significantly. Furthermore, because the extraction solvent became less polar during heating, this may have had a compensatory effect as well. Similar results were found during the extraction of 2 g cod liver with a content of 30% or 0.6 mL water, which was also performed quantitatively.

Under microwave irradiation, Na_2SO_4 was not able to bind with enough water. This was confirmed by the fact that the extraction using the glass connection without the water trap was not complete (*see* above): If Na_2SO_4 had bound the water during microwave extraction, the extraction of organochlorines without the water trap would have been quantitative as would the extraction in closed vessels without the 2-step method.

It appears that the main effect of blending the samples with Na_2SO_4 in MAE is not the binding of water, but an enlarged sample surface, which allows better penetration of the solvent into the pores of the sample matrix.

For ASE, water must be bound with Na_2SO_4, according to the Dionex application note (18). A 5 g portion of cod liver contained about 30% or 1.5 g water. Cod liver samples (5 g) were blended with 20 g Na_2SO_4 (18). Outside the ASE vessel, this amount of Na_2SO_4 should be more than suitable for quantitative binding of 1.5 g water. Nevertheless, the extract was cloudy after the extraction. After filtration through Na_2SO_4, the extracts were clear.

Extraction Efficiency for Organochlorines in Cod Liver

Commercially available cod livers were analyzed to check the reproducibility of FOV–MAE, CV–MAE, and ASE, and to compare the respective results obtained with the methods. To obtain the same concentration of organochlorines in the extracts for all methods, the sample weights were selected on the basis of the volume of solvent required for the extraction (FOV–MAE, 60 mL; CV–MAE, 8–10 mL; ASE, approximately 30 mL). After extraction and rotary evaporation, the volume was adjusted exactly. Despite the different sample weights and solvent volumes used with the different methods, the concentrations of the solutions were kept identical (weight and final volume of the extract for FOV–MAE: 2 g in 20 mL; CV–MAE: 1 g in 10 mL; and ASE: 5 g in 50 mL).

All samples were analyzed with the 3 extraction systems within 1 month. The levels obtained with ASE were slightly higher than those obtained with the MAE techniques, except for the levels of chlordane and CTTs (Table 3). However, repeated extraction with MAE or ASE resulted in no further organochlorine extraction, and the higher levels for ASE may be partly due to slightly inhomogeneous sample material. The reproducibility for each technique was very good, and most relative standard deviations (RSDs) were <5%. Nevertheless, there was a tendency toward smaller RSDs for CV–MAE, ASE, and FOV–MAE, in order of decreasing values. In general, the recovery of the internal standard α-PDHCH was >75%.

Composition of Organochlorines in Cod Liver

The main contaminant in cod liver was *p,p′*-DDE, followed by PCB 153. HCB levels were similar to those for PCB 52 and *p,p′*-DDT, and in the same order of magnitude as the levels of total HCHs. The ratios of *p,p′*-DDE:*p,p′*-DDD:*p,p′*-DDT were 1.0:0.36:0.05 for ASE, 1.0:0.37:0.06 for FOV–MAE, and 1.0:0.36:0.06 for CV–MAE. The good agreement of the ratios suggests that significant deg-

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Table 3. Organochlorine levels found in cod liver

Technique	Weight, g	Extractable lipids, %	Mean recovery of α-PDHCH, %	Organochlorine, μg/kg wet wt					
				Total DDT[a]	Total PCBs[b]	Total HCH[c]	HCB	B8-1413 (P-26)[d,e]	Total chlordane[d,f]
ASE (*n* = 10)	5	45.5 ± 0.7[d]	ND[g]	595 ± 18	810 ± 20	17 ± 3	27 ± 1	1.4 ± 0.4	72 ± 4
FOV–MAE (*n* = 7)	2	44.0 ± 0.9[h]	76	548 ± 15	761 ± 21	15 ± 1	25 ± 1	1.6 ± 0.2	71 ± 2
CV–MAE (*n* = 5)	1	43.4[i]	76	497 ± 32	704 ± 33	15 ± 1	23 ± 1	1.8 ± 0.2	69 ± 6

[a] Total DDT = sum of *p,p′*-DDT, *p,p′*-DDD, and *p,p′*-DDE.
[b] Total PCBs = sum of PCBs 52, 101, 149, 118, 153, 138, 163, 180, and 170.
[c] Total HCH = sum of α-, β-, and γ-HCH.
[d] *n* = 4.
[e] The other CTTs were below the detection limits: <0.4 μg/kg [B7-1453, B8-1412, B8-1414 (P-40), and B8-1945 (P-41)]; <0.6 μg/kg [B8-2229 (P-44)]; <2 μg/kg [B9-1679 (P-50)]; and <3 μg/kg [B9-1025 (P-62)].
[f] Total chlordane = sum of oxychlordane, *cis*- and *trans*-chlordane, and *cis*- and *trans*-nonachlor.
[g] ND = not determined.
[h] *n* = 5.
[i] *n* = 2.

radation of *p,p′*-DDT to more stable metabolites did not occur. *trans*-Nonachlor was the main chlordane-related compound in cod liver, followed by *cis*-nonachlor, *cis*-chlordane, oxychlordane, and *trans*-chlordane. B8-1413 (P-26) was the only CTT detected in cod liver; the other CTTs were below the detection limits (*see* footnote *e*, Table 3).

Extraction Efficiency for Organochlorines in Fresh Fish Fillet

By extracting both lyophilized and fresh mackerel fillet with FOV–MAE and CV–MAE, it was shown that water in the sample had no influence on the results (Table 4). As expected, no water was separated in the trap during the extraction of lyophilized fish fillet.

There was no difference in the results obtained for FOV–MAE and CV–MAE of fresh and lyophilized mackerel fillet. In general, the recovery of the internal standard, α-PDHCH, was >70%. The smallest relative standard deviation, however, was obtained for the extraction of lyophilized mackerel fillet. This may be attributed to the homogenization of the sample matrix, which did not contain cell-bound water that might be distributed unevenly with an Ultra Turrax blender. The complicated extraction procedure with 2 extraction steps for CV–MAE, including several rinsing steps, may be one explanation for the higher RSD obtained with this method.

The sample cleanup resulted in relatively pure extracts of mackerel fillet (Figures 2–4). The GC–ECD chromatograms agreed well except the signal of *p,p′*-DDE, which was more abundant in lyophilized fish fillet. This is reflected in the slightly higher level of total DDT in lyophilized material (Table 4), whereas the levels of *p,p′*-DDT and *p,p′*-DDD were the same for all mackerel samples. No evidence was found for losses occurring during the extraction (*see* above); we did not find any explanation for this phenomenon.

Table 4. Organochlorine levels found in mackerel fillet

Technique	Weight, g	Extractable lipids, %[a]	Mean recovery of α-PDHCH, %	Organochlorine, μg/kg wet wt					
				Total DDT[b]	Total PCBs[c]	γ-HCH	Dieldrin	Total CTTs[d,e]	Total chlordane[e,f]
Lyophilized (*n* = 6)[g]	1.3	3.4 ± 0.1	73	13.9 ± 0.8	31 ± 2	1.3 ± 0.4	2.1 ± 0.1	2.6 ± 1.4	4.7 ± 0.9
FOV–MAE, fresh (*n* = 8)	10	3.6 ± 0.3	72	11.6 ± 1.3	37 ± 3	0.8 ± 0.2	2.7 ± 0.5	3.0 ± 0.8	5.2 ± 1.5
CV–MAE, fresh (*n* = 8)	1.6	3.7 ± 0.1	73	10.7 ± 1.7	34 ± 4	0.9 ± 0.1	2.6 ± 0.6	2.5 ± 1.0	4.6 ± 1.2

[a] *n* = 6.
[b] Total DDT = sum of *p,p′*-DDT, *p,p′*-DDD, and *p,p′*-DDE.
[c] Total PCBs = sum of PCBs 28, 52, 101, 149, 118, 153, 138, and 180.
[d] Total CTTs = sum of B7-1453, B8-1413 (P-26), B8-1412, B8-1414 (P-40), B8-1945 (P-41), B8-2229 (P-44), B9-1679 (P-50), and B9-1025 (P-62).
[e] *n* = 4.
[f] Total chlordane = sum of oxychlordane, *cis*- and *trans*-chlordane, and *cis*- and *trans*-nonachlor.
[g] Mean of *n* = 3 FOV–MAE and *n* = 3 CV–MAE.

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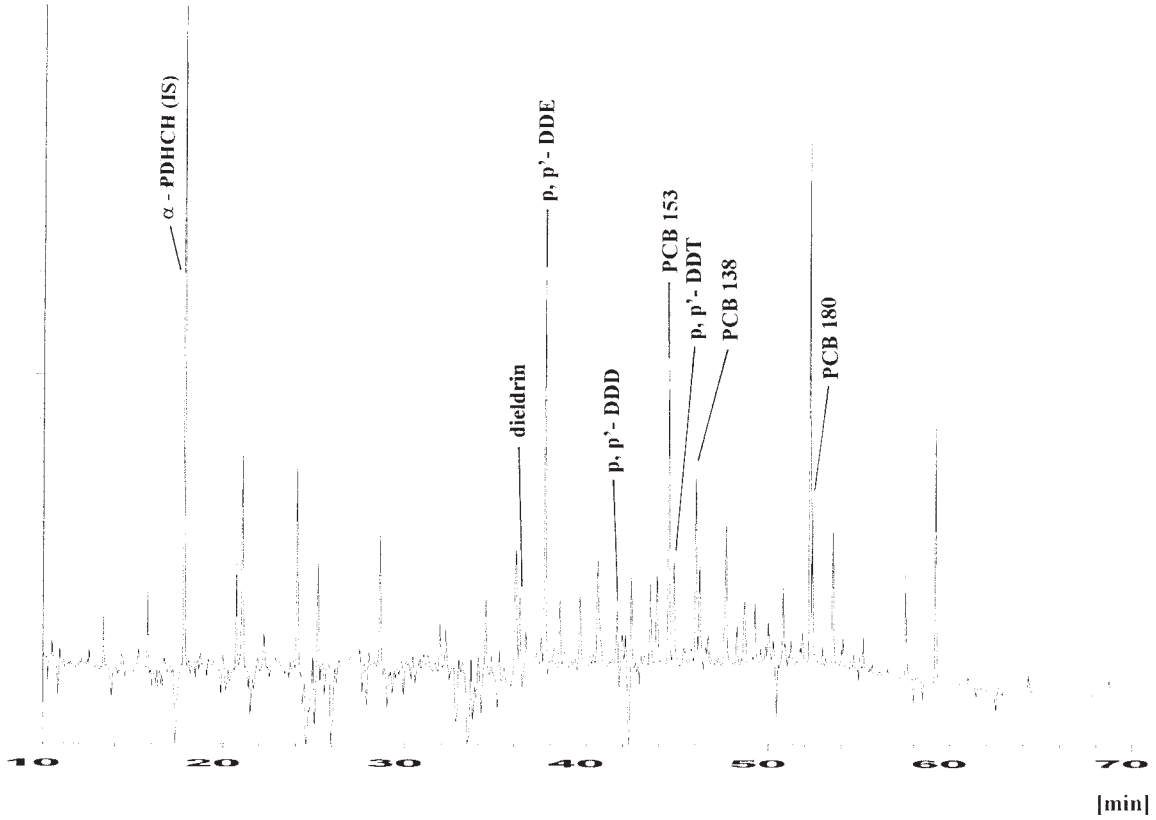

Figure 2. GC–ECD chromatogram (CP-Sil 2) obtained for lyophilized mackerel fillet after FOV–MAE, GPC, and cleanup on deactivated silica gel.

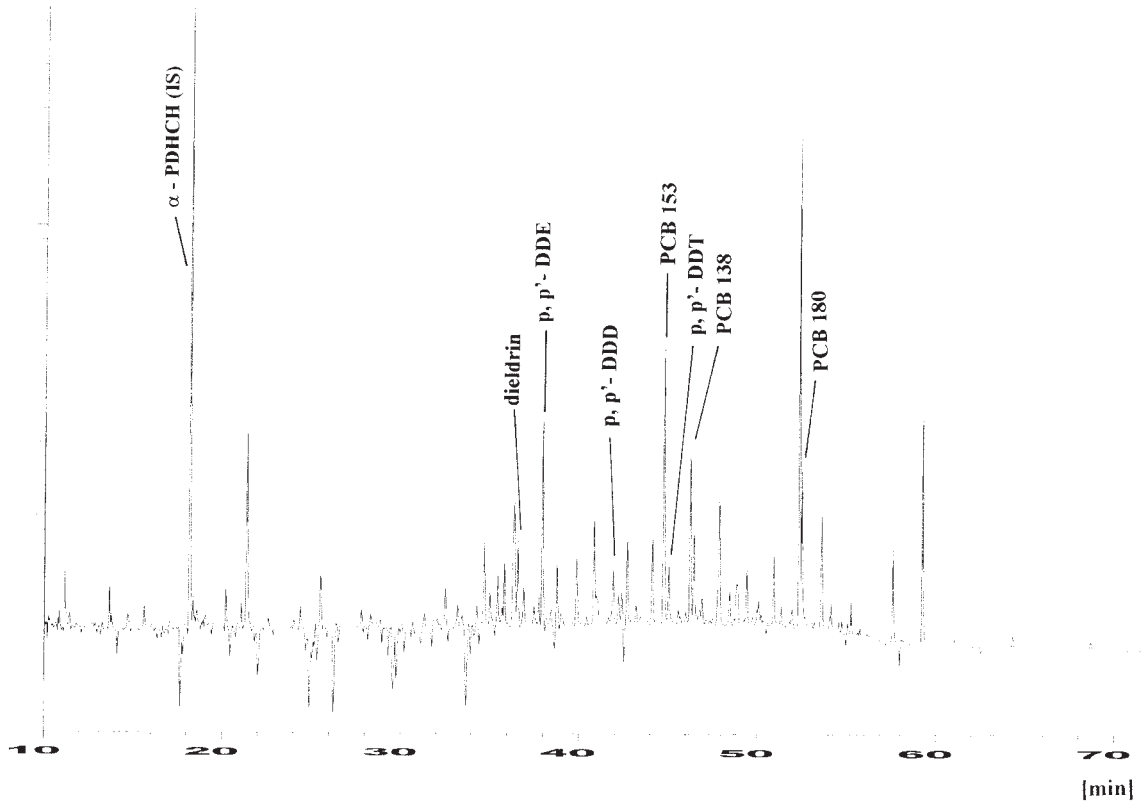

Figure 3. GC–ECD chromatogram (CP-Sil 2) obtained for fresh mackerel fillet after FOV–MAE, GPC, and cleanup on deactivated silica gel.

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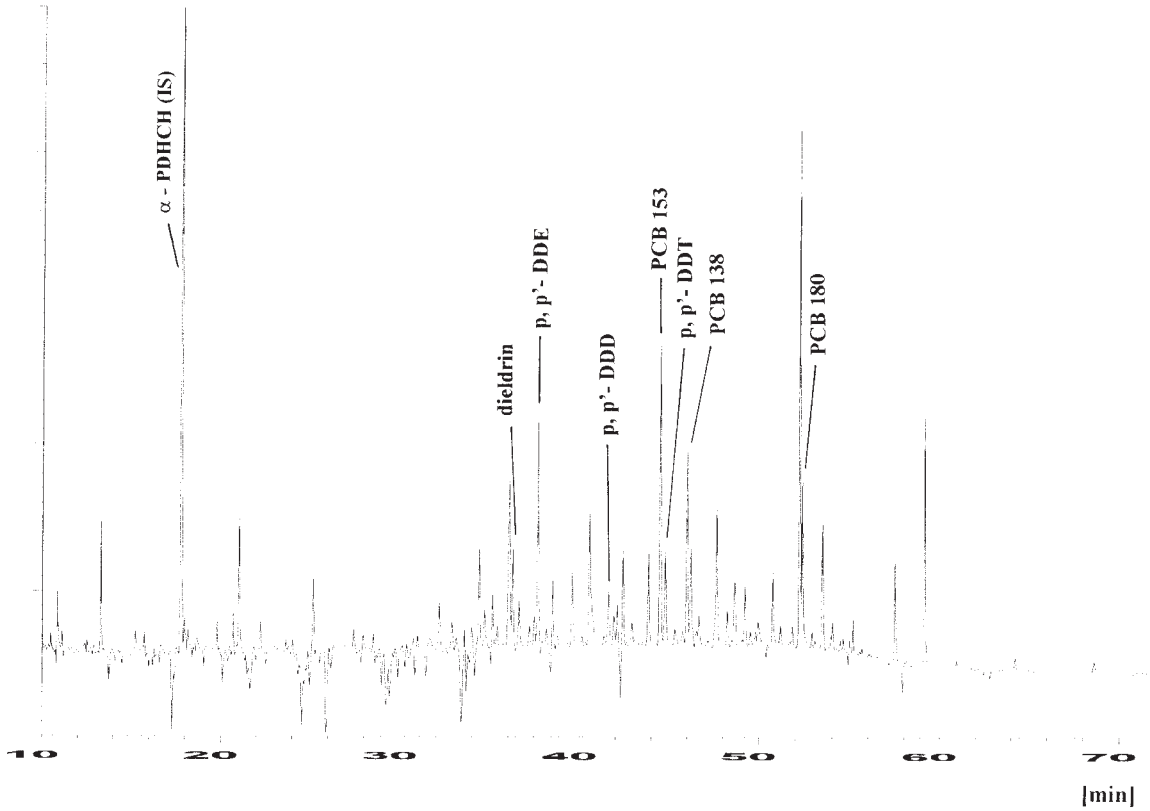

Figure 4. GC–ECD chromatogram (CP-Sil 2) obtained for fresh mackerel fillet after CV–MAE, GPC, and cleanup on deactivated silica gel.

Composition of Organochlorines in Mackerel Fillet

In the mackerel fillet analyzed, the levels of total PCBs, dieldrin, total CTTs, and total chlordane were the highest for FOV–MAE, but not the levels of total DDT, γ-HCH, and extractable lipids. The most abundant organochlorine contaminant in mackerel fillet was PCB 153, followed by *p,p′*-DDE and PCB 138. *cis*- and *trans*-chlordane were below the detection limit of 0.2 μg/kg, as was B7-1453 (<0.1 μg/kg). Levels of dieldrin and total CTTs were of the same order of magnitude.

The levels of CTT congeners decreased in the order B9-1679 (P-50) > B8-1413 (P-26) > B9-1025 (P-62) > B8-1412 > B8-1414 (P-40) > B8-2229 (P-44) > B8-1945 (P-41). The different levels are attributed to deviations during sample cleanup and the generally low levels.

Composition of Organochlorines in Herring Fillet

The herring fillet analyzed was extracted only by FOV–MAE. The organochlorine levels found are listed in Table 5.

The RSDs (except for total chlordane) were comparable to the RSDs obtained for the FOV–MAE of mackerel fillet. The most abundant organochlorine contaminant in herring fillet

Table 5. Organochlorine levels found in herring fillet after FOV–MAE

				Organochlorine, μg/kg wet wt					
Parameter	Weight, g	Mean recovery of α-PDHCH, %	Fat, %[a]	Total DDT[b]	Total PCBs[c]	Dieldrin	Total HCH[d]	HCB	Total chlordane[e]
FOV–MAE, fresh (n = 6)	10	77	6.3	53	39	4.0	6.5	4.1	7.0
Range			6.2–6.3	50–59	38–42	3.4–4.4	5.9–7.0	3.8–4.3	6.2–9.0
Relative standard deviation, %				6.0	4.3	7.9	7.3	5.3	14.2

[a] n = 2.
[b] Total DDT = sum of *p,p′*-DDT, *p,p′*-DDD, and *p,p′*-DDE.
[c] Total PCBs = sum of PCBs 101, 149, 118, 153, 138, 163, 180, and 170.
[d] Total HCH = sum of α-, β-, and γ-HCH.
[e] Total chlordane = sum of oxychlordane, *cis*- and *trans*-chlordane, and *cis*- and *trans*-nonachlor.

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was *p,p′*-DDE, followed by *p,p′*-DDD, PCB 153, and PCB 138. *Trans*-nonachlor was the main chlordane-related compound determined in herring fillet, followed by *cis*-nonachlor. Levels of dieldrin and HCB, as well as levels of total HCH and total chlordane, were of the same order of magnitude. CTT levels were not determined.

Conclusions

The solvent ethyl acetate–cyclohexane (1 + 1, v/v) is well suited for ASE, FOV–MAE, and CV–MAE of organochlorines in tissue with a wide range of lipid and water content. The solvent mixture allows quantitative extraction of water-containing samples like cod liver (30% water) and fresh fish fillet (73% water) without drying, and the time-consuming step of lyophilization is avoided.

Acknowledgments

We thank F. Höfler and S. Pörschmann (Dionex, Idstein, Germany) for lending us the ASE 200 system.

References

(1) Lopez-Avila, V. (1999) *J. AOAC Int.* **82**, 217–222

(2) Lopez-Avila, V., Young, R., & Beckert, W.F. (1994) *Anal. Chem.* **66**, 1097–1106

(3) Hummert, K., Vetter, W., & Luckas, B. (1996) *Chromatographia* **42**, 300–304

(4) Vetter, W., Weichbrodt, M., Hummert, K., Glotz, D., & Luckas, B. (1998) *Chemosphere* **37**, 2439–2449

(5) Weichbrodt, M., Vetter, W., Scholz, E., Luckas, B., & Reinhardt, K. (1999) *Int. J. Environ. Anal. Chem.* **73**, 309–328

(6) Onuska, F.I., & Terry, K.A. (1993) *Chromatographia* **36**, 191–194

(7) Höfler, F., Ezzell, J., & Richter, B. (1995) *LaborPraxis* **3**, 62–67

(8) Richter, B.E., Jones, B.A., Ezzell, J.L., Porter, N., Avdalovic, N., & Pohl, C. (1996) *Anal. Chem.* **68**, 1033–1039

(9) Kingston, H.M., & Jassie, L.B. (Eds) (1988) *Introduction to Microwave Sample Preparation—Theory and Practice*, American Chemical Society, Library of Congress Cataloging-in-Publication Data, Washington, DC

(10) Vetter, W., Weichbrodt, M., Scholz, E., Luckas, B., & Oelschläger, H. (1999) *Mar. Pollut. Bull.* **38**, 830–836

(11) Specht, W., & Tillkes, M. (1985) *Fresenius Z. Anal. Chem.* **322**, 443–455

(12) Weast, R.C. (Ed.) (1976) *Handbook of Chemistry and Physics*, 56th Ed., CRC Press, Cleveland, OH

(13) Batista, A., Vetter, W., & Luckas, B. (2000) *Eur. Food Res. Technol.* (in press)

(14) Andrews, P., & Vetter, W. (1995) *Chemosphere* **31**, 3879–3886

(15) Parlar, H., Angerhöfer, D., Coelhan, M., & Kimmel, L. (1995) *Organohalogen Compd.* **26**, 357–362

(16) Krock, B., Vetter, W., & Luckas, B. (1996) *Chemosphere* **33**, 1005–1019

(17) Vetter, W., & Luckas, B. (1995) *J. High Resolut. Chromatogr.* **18**, 643–646

(18) *Application Note 321* (1996) Dionex Corp., Sunnyvale, CA

(19) Steinwandter, H., & Schlüter, H. (1978) *Dtsch. Lebensm. Rundsch.* **74**, 139–141

(20) Vetter, W., Natzeck, C., Luckas, B., Heidemann, G., Kiabi, B., & Karami, M. (1995) *Chemosphere* **30**, 1685–1696

(21) Krock, B., Vetter, W., & Luckas, B. (1997) *Chemosphere* **35**, 1519–1530

(22) Klobes, U. (1999) Doctoral Thesis, University of Jena, Jena, Germany

(23) Klobes, U., Vetter, W., Luckas, B., & Hottinger, G. (1998) *Organohalogen Compd.* **35**, 359–362

(24) Vetter, W., & Luckas, B. (1999) *Fresenius Environ. Bull.* **8**, 7–13

(25) Schantz, M.M., Parris, R.M., Kurz, J., Ballschmiter, K., & Wise, S.A. (1993) *Fresenius' J. Anal. Chem.* **346**, 766–778

(26) Kingston, H.M., & Haswell, S.J. (Eds) (1997) *Microwave-Enhanced Chemistry*, American Chemical Society, Library of Congress Cataloging-in-Publication Data, Washington, DC

(27) Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG, Verfahren L 00.00 38/2, Extraktion des Fettes, der Pestizide und PCB und Bestimmung des Fettgehaltes (1998) in *Lebensmittelrecht*, G. Klein, H.-J. Rabe, H. Weiss (Eds), Behr's Verlag, Hamburg, Germany

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