# Combined microwave assisted extraction and gel permeation chromatography for the determination of organochlorine compounds in fatty tissue of marine mammals

**Kerstin Hummert, Walter Vetter, and Bernd Luckas**
Friedrich-Schiller Universität Jena, Institut für Ernährung und Umwelt, Dornburger Str. 25, 07743 Jena, Germany

## 1. Introduction

Microwave assisted extraction for the quantitative determination of organochlorine compounds (e. g. PCBs, PCDDs, HCHs, DDT and its metabolites, HCB) in soil, seeds, food, and water yielded at least similar recoveries as Soxhlet extraction[1-6].
Recently, we have presented a fast and effective sample preparation procedure for the determination of organochlorine compounds in fatty tissue of marine mammals by application of microwave extraction[7]. The organochlorines were extracted with 10 ml n-hexane and the required heating of the non-polar extraction solvent was achieved using discs of the microwave transformer Weflon®[8]. After extraction of 0.5 - 1.0 g of fatty tissue, aliquotes corresponding to 0.1 g fat were directly purified on silica gel deactivated with 30% water. The complete sample clean-up required only 70 ml of hexane and 3 g of silica. This micro method showed high recoveries and excellent reproducibility[7]. Though the microwave technique allowed to extract 1-2 g of blubber, the second clean-up step on silica gel was limited on 0.1 g extracted fat in samples.
Now we present a new method for the separation of higher amounts of extracted fat after microwave extraction by gel permeation chromatography (GPC) using bio beads S-X3. To simplify the sample clean-up, the microwave extraction medium n-hexane/Weflon® of the micro method was substituted by ethyl acetate/cyclohexane (1:1, v:v) which was also used as solvent in GPC. Therefore, no change of the solvent was necessary and after adjustment of the volume of the microwave extract to exactly 10 ml, the solution was directly injected into the GPC.
Due to the high dielectric constant of ethyl acetate, addition of the microwave transformer Weflon® was not necessary as it was in the case of n-hexane. The non-polar n-hexane has a particularly low dielectric constant and cannot be heated directly by microwave energy.
The efficiency of the combination microwave extraction/GPC was compared to the micro method using n-hexane/Weflon®.

## 2. Experimental

### Chemicals

Standard solutions of organochlorine compounds (10 ng/µl each) were obtained from Promochem, Wesel, Germany. Purities were claimed to be greater than 99 %. Silica gel 60, extra pure for column chromatography (particle size 0.063-0.200 mm), was purchased from Merck (Darmstadt, Germany). Ethyl acetate (Chromasolv quality) and cyclohexane (Pesta) were from Riedel-de Haen, Seelze, Germany. Isooctane (Rotipuran) was from Roth (Karlsruhe, Germany) and n-hexane (zur Rückstandsanalyse) was from Promochem (Wesel, Germany).
Discs of Weflon® (2.5 cm diameter, 0.3 cm thickness), applied as microwave transformer, were obtained from MLS, Leutkirch, Germany. Weflon® is an inert material on the basis of a carbon containing teflon derivate, usable up to 350°C.

Microwave conditions

The microwave extraction was performed in a MLS 1200 mega apparatus (MLS, Leutkirch, Germany). Microwave energy is produced by a 1200 W magnetron. The MLS 1200 mega system allows multistep programming of both microwave increments (0-1000 W, programmable in steps of 10 W and time) and cooling phases at room temperature. A detailed description of the system was recently presented[7]. 8 ml of ethyl acetate/cyclohexane (1:1, v:v) were added to approx. 1.5 g blubber and extracted by application of several heating and cooling phases (see results and discussion).

Gel permeations chromatography conditions

To separate extracted fat from organochlorine compounds an Autoprep 1002 (ABC, Analytical Biochemistry Laboratories, Columbia, USA) gel permeation chromatography system was used under standard conditions[9]. The volume of the ethyl acetate/cyclohexane extract containing approx. 1.5 g blubber was justified to 10 ml. The solution was automatically introduced in the 5 ml loop of the system. The dump and collect times were optimized using δ-HCH and HCB which are among the first and last eluting organochlorine compounds on bio beads S-X3[9].

Mini silica gel column chromatography conditions

Silica gel was dried for 16 h at 130°C and deactivated with 30% water by subsequent shaking for 30 min. 3 g deactivated silica gel were placed in a glass column (1.0 mm internal diameter) which contained at both ends glass wool.
The GPC eluate was condensed in a rotavapor to approx. 2 ml. 2 ml of isooctane were added and the volume of the solvent was again reduced to approx. 1.5 ml in a nitrogen flow. After this procedure ethyl acetate and cyclohexane were quantitatively removed.
The isooctane extract of the sample was eluted on the silica gel column with 60 ml n-hexane. The whole procedure is according to the method of Steinwandter and Schlüter[10] which was slightly modified[11]. Extracts were reduced at first by rotary evaporation and finally carefully blown down with nitrogen. The final solutions were very clean and no further treatment e.g. with sulfuric acid was necessary (see Figure 1). Aliquotes were subjected to GC/ECD.

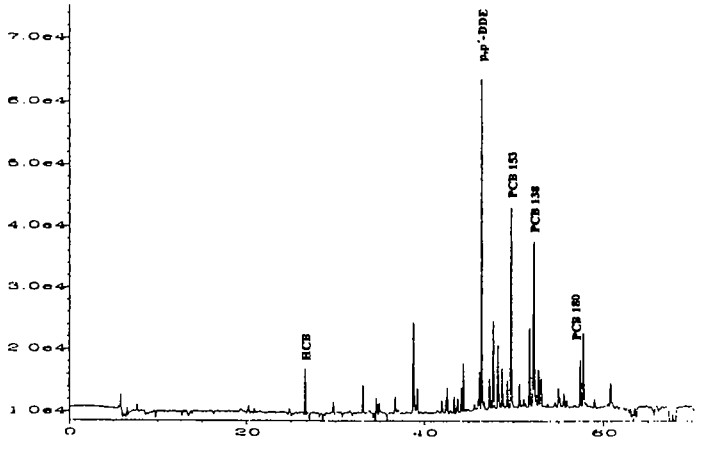


Figure 1: GC/ECD chromatogramm of grey seal blubber after microwave extraction with ethyl acetate/cyclohexane, GPC and silica chromatography clean-up

GC/ECD conditions

The GC separation was performed on an HP 5890 (Hewlett Packard) gas chromatograph equipped with both two capillary columns and $^{63}Ni$ electron capture detectors (ECD). The injector and detector temperatures were set at 220°C (splitless) and 300°C. Nitrogen was used as both make-up gas and carrier gas at a head pressure of 0.9 bar. CP-Sil 8/C18 20% and CP-Sil 19 capillary column (both: length 50 m; internal diameter 0.25 mm; film thickness 0.25 µm) were used (Chrompack, Middelburg, The Netherlands). After injection at 75°C (1.5 min) the GC oven was ramped at 15°C/min to 180°C (4 min), then ramped at 2°C/min to 240°C (10 min), and finally the oven was programmed at 20°C/min to 280°C (20 min). The total run time was 74.50 min.

3. Results and Discussion

Since no certified reference material was available for this study, blubber of a grey seal, stranded on Rügen (Baltic Sea, Germany) was used as a reference sample. The levels in the reference sample were determined in molten fat (i. e. 100% extractable fat) to ensure quantitative determination of the organochlorine contaminants[7]. 0.1 g molten fat of the reference sample, diluted in 2 ml n-hexane, were purified by matrix separation on deactivated silica gel (see above) and organochlorine levels were determined by GC/ECD.
The first row in Table 1 lists the levels of organochlorine compounds (PCB 153, PCB 138, PCB 180, p,p´-DDE and HCB) in molton blubber of the reference sample.
The second row in Table 1 lists recoveries determined with our optimized micro method including microwave assisted extraction with n-hexane/Weflon®[7]. Finally, Table 1 lists recoveries of combined microwave assisted extraction with ethyl acetate/cyclohexane followed by GPC (third row). Note that the microwave experiments were carried out with naturally contaminated blubber.

Table 1: Levels of organochlorine compounds in grey seal blubber (µg/kg in pure molton fat), and recoveries (%) of organochlorine compounds and extractable fat (%) from naturally contaminated blubber (n=3)

| | PCB 153 | PCB 138 | PCB 180 | p,p´-DDE | HCB | % fat |
|---|---|---|---|---|---|---|
| µg/kg | 2810 | 2264 | 595 | 3186 | 331 | 100 |
| MW-Weflon® | 97.5 | 97.8 | 99.6 | 98.1 | 95.1 | 96.9 |
| MW-GPC | 107.5 | 98.0 | 96.4 | 96.2 | 84.8 | 96.8 |

In our first experiments using microwave extraction with ethyl acetate/cyclohexane, the parameters (heating time and microwave energy as well as cooling phases) had to be optimized. Best results were obtained by application of seven extraction cycles (EC). Each EC consisted of 30 s microwave extraction at 1000 W followed by a cooling phase of 5 min. Note that the instrumental conditions were identical to microwave assisted extraction using n-hexane/Weflon®[7]. However, using ethyl acetate/cyclohexane the microwave transformer Weflon® was not necessary.
After optimization of the microwave extraction parameters, the recoveries of PCB 153, PCB 138, PCB 180, p,p´-DDE generally exceeded 95%. The semivolative compounds (e. g. HCB) yielded lower recoveries which, however, also reached 85% for the complete sample clean-up procedure including microwave extraction, GPC and silica gel chromatography (see Table 1). Work is ongoing to optimize the recoveries for HCB.
Note that the micro method using microwave assisted extraction using n-hexane/Weflon® generally yielded recoveries exeeded 95 %[7].
In parallel to the determination of organochlorine compounds the amount of extractable fat was determined gravimetrically. The level of extractable fat by microwave assisted extraction was identical to the level determined by Soxhlet extraction[7].

## 4. Conclusions

Microwave assisted extraction coupled with gel permeation chromatography is an effective technique to determine the levels of organochlorine contaminants in fatty tissue. Compared to the micro method (microwave assisted extraction using n-hexane/Weflon® followed by silica gel chromatography) higher fat content can be removed and therefore, the combination of microwave extraction with ethyl acetate/cyclohexane and GPC with the same solvent is a suitable method for the quantitative determination of organochlorine compounds low contaminated sample material.

## 5. References

1) Lopez-Avila V., J. Benedicto, C. Charan, R. Young (1995): Determination of PCBs in soils/sediments by microwave-assisted extraction and GC/ECD or ELISA. Environ. Sci. Technol. 29, 2709-2712
2) Onuska F.I., K.A. Terry (1993): Extraction of pesticides from sediment using microwave technique. Chromatographia 36, 191-194
3) Lopez-Avila V., R. Young, W.F. Beckert (1994): Microwave-assisted extraction of organic compounds from standard reference soils and sediments. Anal. Chem. 66, 1097-1106
4) Onuska F.I., K.A. Terry (1995): Microwave extraction in analytical chemistry of pollutants: polychlorinated biphenyls. J. High Resol. Chromatogr. 18, 417-421
5) Schlabach M., A. Biseth, H. Gundersen, M. Oehme (1995): Microwave assisted extraction of solid samples for PCDD/PCDF-Analysis. Organohal. Compds. 23, 105-108
6) Lopez-Avila V., R. Young, N. Teplitsky (1996): Microwave-assisted extraction as an alternative to soxhlet, sonication, and supercritical fluid extraction. J. Am. Off. Anal. Chem. 79, 142-156
7) Hummert K., W. Vetter, B. Luckas (1996): Fast and effective sample preparation procedure for the determination of organochlorine compounds in fatty tissue of marine mammals by application of microwave extraction. Chromatographia 42, 300-304
8) Lautenschläger, W., T. Schweizer (1990): Mikrowellentechnik im Labor- und Prozeßbereich. LaborPraxis 14, 376-382
9) Specht W., M. Tillkes (1985): Gas-chromatographische Bestimmung von Rückständen an Pflanzenbehandlungsmitteln nach clean-up über Gel-Chromatographie und Mini Kieselgel-Säulen-Chromatographie.5.Mitteilung: Methode zur Aufarbeitung von Lebensmitteln und Futtermitteln pflanzlicher und tierischer Herkunft für die Multirückstandsbestimmung lipid- und wasserlöslicher Pflanzenbehandlungsmittel. Fresenius Z. Anal. Chem. 322, 443-455
10) Steinwandter H., H. Schlüter, (1978): Eine einfache Mikromethode zur Bestimmung von Chlorkohlenwasserstoff-Pestiziden. Dtsche. Lebensm. Rdschau. 74: 139-141
11) Vetter W., C. Natzeck, B. Luckas, G. Heidemann, B. Kiabi, M. Karami (1995): Chlorinated hydrocarbons in the blubber of a seal (*Phoca caspica*) from the Caspian Sea. Chemosphere 30, 1685-1696