Determination of organochlorines (HCHs, DDT, PCBs, Toxaphene) in blubber of South African Seals (*Arctocephalus pusillus pusillus*)

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Introduction

Seals are top predators of the marine food chain and accumulate high levels of lipophilic and persistent organochlorines in their adipose tissue (blubber). Consequently, determination of organochlorine levels in seal blubber has been subject of many studies from all over the world including the remoted area of the Antarctic [1]. On the other hand, data on organochlorine residues in African seals are scarce.
In this presentation, organochlorine levels were determined in the blubber of 12 South African Fur Seals (*Arctocephalus pusillus pusillus*). The sample clean-up included microwave-assisted extraction (MAE) in combination with gel-permeation-chromatography (GPC), adsorption chromatography on deactivated silica and quantitation with GC/ECD [2][3]. Compounds of technical toxaphene (CTTs) were determined after separation from the PCBs [4].

Material and Methods

Samples

With permission of the Ministry of Fisheries and Marine Resources of the Republic of Namibia, blubber of 12 Samples of South African or Cape Fur Seals (*Arctocephalus pusillus pusillus*) were collected in April 1997 at Cape Cross, Namibia (see Figure 1). The South African Seal is indigenous to the waters of South Africa and South West Africa. This species prefers inshore waters and does not make wide migrations [5]. Ages were estimated by experts with a *a security of ± 1 year.*

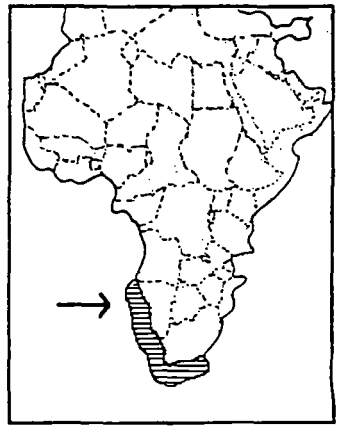

Figure 1: sampling site of the seals

Chemicals and organochlorine standards

Standard solutions of organochlorine compounds (10 ng/µL each) were from Promochem, Wesel, Germany or Dr. Ehrenstorfer, Augsburg, Germany. Silica gel 60 (particle size 0.063-0.200 mm) extra pure for column chromatography was from Merck, Darmstadt, Germany. Ethyl acetate (for residue analysis) was from Fluka, Neu-Ulm, Germany. Cyclohexane (Pestanal) was from Riedel-de Haen, Seelze, Germany. Isooctane (Rotipuran > 99.5% p.a.) was from Roth, Karlsruhe, Germany and n-hexane (for residue analysis) was from Promochem, Wesel, Germany.

The organochlorines were determined as Σ DDT (= sum of p,p'-DDT, p,p'-DDD, p,p'- DDE), Σ PCB (= sum of PCB 101, PCB 149, PCB 118, PCB 153, PCB 138, PCB 163, PCB 180), Σ HCH (= sum of α-, β- and γ-HCH) and Σ CTTs (= sum of B8-1413, B9-1679).

Microwave conditions

Microwave-assisted extraction was performed in an MLS 1200 mega apparatus (MLS, Leutkirch, Germany). It allows multistep programming of microwave energy and time of irradiation (0-1000 W, programmable in steps of 10 W). The whole procedure was recently presented in detail [2]. In brief, 8 mL of ethyl acetate/cyclohexane (1:1, v:v) were added to 1-2 g blubber and extracted by application of a microwave program with seven extraction cycles. Each cycle consisted of 30 s microwave extraction at 1000 W followed by 5 min without irradiation for cooling of the extracts. The extracts were adjusted to a volume of 10 mL and directly subjected to GPC.

Gel-permeation chromatography conditions

Gel-permeation chromatographic separation of extracted fat from organochlorines was obtained with *bio beads S-X3* in combination with an Autoprep 1002 (ABC, Analytical Biochemistry Columbia, USA) system. Ethyl acetate and cyclohexane (1:1, v:v) were used as the solvent. The dump and collection times were optimized using trans-chlordane and HCB which are among the first and last eluted organochlorine compounds [6].

Adsorption chromatography on deactivated silica

The GPC eluate was condensed in a rotavapor to approx. 2 mL, 2 mL of isooctane were added, and the solvent was evaporated in a nitrogen flow to approx. 2 mL. Addition of isooctane and evaporation was repeated twice. After this, the more volatile ethyl acetate was quantitatively removed. Adsorption chromatography on silica was performed with the method of Steinwandter and Schlüter [7] which was slightly modified [8]. The isooctane extract of the sample was eluted with 60 mL n-hexane from 3 g silica gel (deactivated with 30% water, w:w). The eluate was concentrated in a rotavapor, then carefully blown down in a nitrogen flow. The concentrated solutions were divided in two parts (1:1). One part was subjected to GC/ECD for the determination of HCHs, DDT, and PCBs. The second part was chromatographed on silica for the determination of the CTTs.

PCB/CTT group separation

Prior to the quantitation of CTTs, a PCB/CTT group separation was performed [4]. 8 g activated silica gel (16 h at 130°C) were applied. PCBs were quantitatively eluted with 48 mL n-hexane, while the CTTs were quantitatively collected in a second fraction with a more polar

solvent. The original method of Krock et al. [4] was slightly modified using n-hexane/ethyl acetate (90:10, v:v) for the elution of CTTs [9]. The eluates were concentrated in a rotavapor and blown down in a nitrogen flow. Aliquotes were subjected to GC/ECD.

GC/ECD conditions

The GC/ECD analyses were performed with an HP 5890 (Hewlett-Packard) gas chromatograph equipped with two capillary columns and two ^{63}Ni electron capture detectors (ECD). The injector (splitless) and detector temperatures were 250°C and 300°C. Helium was used as carrier gas at a column head pressure of 1.2 bar. Nitrogen was used as make-up gas. The capillary columns CP-Sil 2 and CP-Sil 8/20% C18 (both: length 50 m, 0.25 mm internal diameter, 0.25 µm film thickness) were from Chrompack, Middelburg, The Netherlands. After injection at 60°C (1.5 min) the GC oven was ramped at 40°C/min to 180°C (2 min), then ramped at 2°C/min to 230°C (25 min), and finally ramped at 10°C/min to 270°C (15 min). The total run time was 75.5 min.

Results and Discussion

MAE is an excellent method for the extraction of adipose tissues like seal blubber in view of the quantitative determination of organochlorines [3][10]. After volume adjustment the ethyl acetate/cyclohexane extracts after MAE can be directly injected into the GPC. Since this solvent mixture was also used for GPC, no solvent exchange after MAE was necessary. Figure 2 shows a typical chromatogram of a purifed organochlorine blubber extract of an African Fur seal. The chromatogram is dominated by the peak of p,p'-DDE.

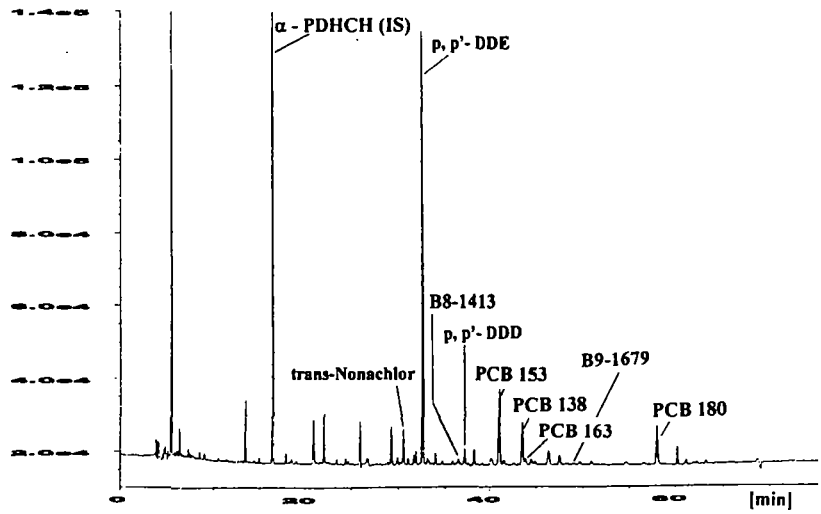

Figure 2: GC/ECD chromatogram of African seal blubber after microwave-assisted extraction, gel-permeation chromatography and silica clean-up

Levels of organochlorines and biological parameters of three samples are listed in Table 1. These three samples belong to those with the highest levels of the present African seal blubber samples.

Table 1: Levels of organochlorines in the blubber of two African Seals (µg/kg)

	Σ HCHs	Σ DDT	Σ PCBs	Σ CTTs
# 1 (m,5) *	13	422	104	49
# 2 (m,5)	25	1090	743	22
# 3 (f,4)	6	363	110	15

* (sex, age in years)

In all samples, the highest levels were found for Σ DDT. This is in agreement with findings in African fauna [12].
The levels belong to the lowest found in seals. Although the use of DDT, HCH, and other organochlorines was reported in Africa, the levels showed only concentrations similar to those of Antarctic Weddell Seals (*Leptonychotes wedellii*) which accumulated on average Σ DDT 105 µg/kg [1]. Seals from other regions like Norway (Σ DDT 1226 µg/kg), Iceland (Σ DDT 1546 µg/kg), North Sea, Germany (Σ DDT 3903 µg/kg) [1], or from the Black Sea (Σ DDT 70000 µg/kg) [11] revealed higher organochlorine residues.

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