Levels of organochlorines in penguin and skua eggs from the Antarctic - determination after application of focused open-vessel microwave-assisted extraction in combination with gel-permeation-chromatography

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Introduction

Levels of organochlorines were determined in eggs of penguin (Adelie *Pygoscelis adeliae*, Chinstrap *Pygoscelis antarctica*, Gentoo *Pygoscelis papua*) and skua (South Polar Skua *Catharacta maccormicki*, Brown Skua *Catharacta antarctica lonnbergi*, Mixed Pair Skua *Catharacta maccormicki x lonnbergi*) species. The results supplement earlier studies of the organochlorine levels in Antarctic birds [1-3] and provide additional data on the present pollution status of the Antarctic.
Microwave-assisted extraction (MAE) was used to extract entire, partly lyophilized eggs (20-50 g). MAE is a fast and effictive sample preparation method for the quantitative determination of organochlorines (e.g. PCBs, HCHs, DDT, HCB, toxaphene) in adipose tissue. Recently, MAE in closed-vessels was combined with gel-permeation chromatography (GPC) for the sample clean-up of 1-2 g of cod livers and seal blubber for the determination of organochlorines [4,5].
Focused open-vessel MAE (FOV-MAE) of the eggs was carried out with ethyl acetate/cyclohexane (1:1, v:v) as the solvent. Fat and matrix remainders were separated from the organochlorine fraction by GPC followed by adsorption chromatography on deactivated silica gel. Compounds of technical toxaphene (CTTs) were determined after separation from the PCBs [6]. The organochlorines were determined in the pure extracts by GC/ECD.

Material and Methods

Samples

The samples were collected in winter 1993/1994 on the Potter peninsula in the Antarctic near Base Cientifica Argentina JUBANY (62° 14` 18`` S, 58° 40` W).

Chemicals and organochlorine standards

Standard solutions of organochlorines (10 ng/µL each) were obtained from Promochem, Wesel (Germany) or Dr. Ehrenstorfer, Augsburg (Germany). Silica gel 60 (particle size 0.063-0.200 mm) 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 118, PCB 153, PCB 138, PCB 180), Σ HCH (= sum of α-, β- and γ-HCH) and Σ CTTs (= sum of B8-1413, B8-1412, B8-2229, B9-1679).

Microwave conditions

Microwave-assisted extraction was performed in a focused open-vessel microwave system Soxwave 100 (Prolabo, France). It allows multistep programming of microwave energy (max. 300 W) and time of irradiation. The system operates at ambient pressure and uses a reflux column to avoid solvent losses during extraction. The extraction program was developed with lyophilized eggs of hen and consisted of the following irradation steps: 7 min at 30 W, then 8 min at 45 W, and finally 20 min at 60 W (total extraction time: 35 min).

Entire, partly lyophilized penguin and skua eggs (20-50 g) were placed in glass tubes (250 mL) and extracted five times with 80 mL ethyl acetate/cyclohexane (1:1,v:v), respectively. The extracts of each sample were combined, concentrated in a rotavapor and adjusted to 20 mL (penguin eggs) or 50 mL (skua eggs). The colors of the extracts ranged from yellow to deep orange. 100 µL of the extract were submitted to the clean-up with deactivated silica gel (see below), the eluate was reduced again to 100 µL by rotary evaporation and blowing down in a nitrogen flow. This solution was injected into the GC to get an overview about the organochlorine residue levels in the sample.

Gel-permeation-chromatography conditions

An Autoprep 1002 (ABC, Analytical Biochemistry Columbia, USA) with 50 g *bio beads S-X3* was used with ethyl acetate/cyclohexane (v:v, 1:1) was the solvent [7]. The dump and collection times were optimized using trans-chlordane and HCB which are among the first and last eluted organochlorine compounds [7].

Mini silica gel chromatography conditions

To separate matrix remainders a sample clean-up was performed with 3 g deactivated silica gel (30 % water) according to the method of Steinwandter and Schlüter [8], which was slightly modified [9]. The GPC eluate was concentrated 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. The addition of isooctane and the evaporation was repeated twice for quantitative removal of the more volatile ethyl acetate. The isooctane extract of the sample was placed on the silica gel column which was eluted with 60 mL n-hexane. The eluates were concentrated in a rotavapor and by blowing down with nitrogen. Those extracts which remained yellow after this procedure were treated with 2 mL concentrated sulfuric acid to destroy coextracted colouring. Aliquotes were subjected to GC/ECD or subject to PCB/CTT group separation.

PCB/CTT group separation

For the determination of the CTTs, the final solution was fractionated on 8 g silica gel as described by Krock et al. [6]. PCBs were quantitatively eluted with 48 mL n-hexane, and CTTs were eluted with a more polar solvent in a second fraction [6]. Instead of n-hexane/toluene (65:35, v:v) n-hexane/ethyl acetate (90:10, v:v) was used for elution of the CTTs [10]. The eluates were condensed in a rotavapor and blown down in a nitrogen flow. Aliquotes were subjected to GC/ECD. CTTs were determined by quantification of B8-1413, B8-1412, B8-2229, B9-1679, which are the most abundant CTT congeners in penguin tissue [11].

GC/ECD conditions

GC/ECD analyses were performed on an HP 5890 (Hewlett-Packard) gas chromatograph equipped with two capillary columns and two ^{63}Ni ECDs. The injector (splitless) and detector temperatures were 250°C and 300°C. Helium was used as carrier gas at a head pressure of 1.2 bar. Nitrogen was used as make-up gas. The capillary columns CP-Sil 8/ 20% C18 and CP-Sil 2 (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

Closed vessel MAE in combination with GPC is a well suited method for the extraction of organochlorines from fatty tissue [4,5]. The same solvent (ethyl acetate/cyclohexane) was used for MAE and GPC, and GPC was performed directly after volume adjustment of the MAE extract. For the extraction of high sample amounts focused open-vessel microwave-assisted extraction (FOV-MAE) in combination with GPC was applied. This technique combines the benefits of closed vessel MAE with the facility to apply sample amounts of 50 g or more. Consequently, FOV-MAE proved to be an excellent method for the extraction in view of the quantitative determination of organochlorines in low contaminated samples like the eggs of Antarcic birds (ppb to ppt range) because it allows the use of high sample amounts (entire eggs). Furthermore, the application of entire eggs avoids problems related to homogenization of the samples. The sample clean-up resulted in pure extracts (see Figure 1).

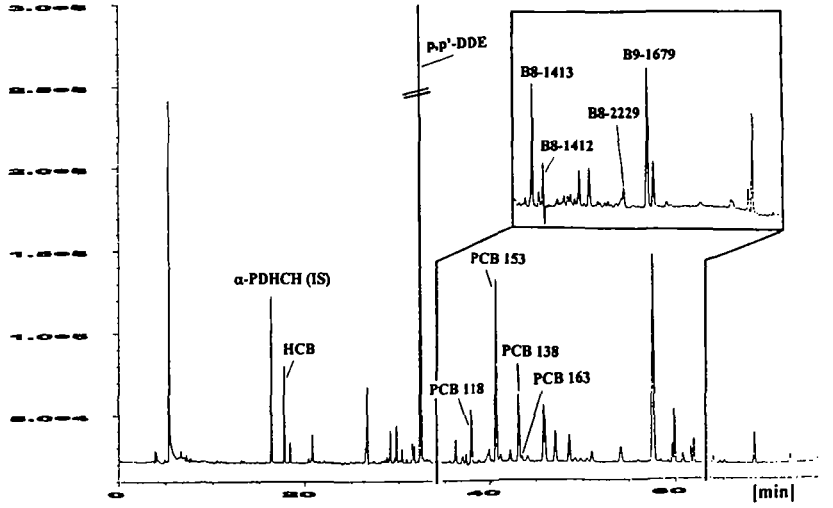

Figure 1: GC/ECD chromatogram of a skua egg after microwave-assisted extraction, GPC and clean-up on deactivated silica
Upper chromatogram: GC/ECD chromatogram (part) of the CTT fraction

Organochlorines are transfered from the dam to the eggs. The survey of organochlorine contamination in Antarctic penguins and skuas could be completed by analysis of these animals, but unfortunately they were not at our disposal. Levels of organochlorines in a penguin egg (*Chinstrap*) and a skua egg (*Catharacta maccormicki*) are listed in Table 1.

Table 1: Organochlorine levels in a penguin (*Chinstrap*) and a skua (*C. maccormicki*) egg (µg/kg wet weight without shell)

	Σ HCHs	HCB	Σ DDT	Σ PCBs	Σ CTTs
penguin egg	n. d.	8.0	13	1.9	1.0
skua egg	8.0	16	270	280	43

In general, skua eggs were significantly higher polluted with organochlorines than penguin eggs. On the other hand, the organochlorine levels were subject to strong variations in dependence of the respective skua and penguin species. This is due to differences in the diets of skuas and penguins but also within skua and penguin species [12,13]. Higher levels in skua eggs most likely originate from the fact that skuas are top predators of the Antarctic food chain which mainly feed on other birds and eggs. Furthermore, skuas are migratory birds that spend the Antarctic winter in the northern hemisphere which is higher polluted with organochlorines and the organochlorine levels of skuas are at least partly accumulated from prey caught in the northern hemisphere [2,3]. In contrast, the major prey of penguins, which don't leave the Antarctic [2,3], is krill (*Euphasus superba*).

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