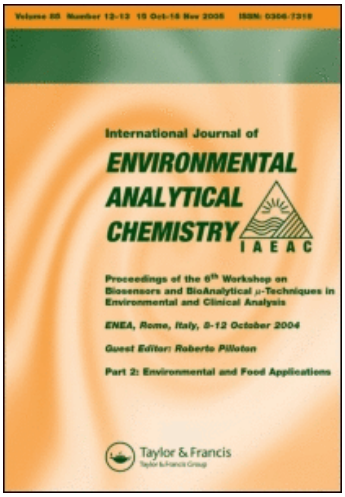

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Determination of Organochlorine Levels in Antarctic Skua and Penguin Eggs by Application of Combined Focused Open-Vessel Microwave-Assisted Extraction, Gel-Permeation Chromatography, Adsorption Chromatography, and GC/ECD

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DETERMINATION OF ORGANOCHLORINE LEVELS IN ANTARCTIC SKUA AND PENGUIN EGGS BY APPLICATION OF COMBINED FOCUSED OPEN-VESSEL MICROWAVE-ASSISTED EXTRACTION, GEL-PERMEATION CHROMATOGRAPHY, ADSORPTION CHROMATOGRAPHY, AND GC/ECD

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Levels of organochlorines (PCBs, DDT, toxaphene, chlordane, hexachlorobenzene (HCB), hexachlorocyclohexanes (HCHs), dieldrin, Q1) were determined in eggs of both penguins (Adelie *Pygoscelis adeliae*, Chinstrap *Pygoscelis antarctica*, Gentoo *Pygoscelis papua*) and skuas (South Polar Skua *Catharacta maccormicki*, Brown Skua *Catharacta antarctica lonnbergi*, Mixed Pair Skua *Catharacta maccormicki* × *lonnbergi*) from the Antarctic.

Focused open-vessel microwave-assisted extraction (FOV-MAE) was performed for the extraction of entire, partly lyophilised eggs (approx. 50 g). After gel-permeation chromatography (GPC) and adsorption chromatography on deactivated silica gel, the quantitation was performed by GC/ECD on two capillary columns of different polarity. Compounds of technical toxaphene (CTTs) were determined after separation of the PCBs. The sample clean-up method was validated with certified reference material SRM 1588.

In general, skua eggs revealed higher organochlorine levels than penguin eggs. Main contaminants in skua eggs were p,p′-DDE, PCB 153, and PCB 180 with levels about 10 – 350 μg/kg wet weight without shell (ww). Eggs of penguins were topped by levels of hexachlorobenzene (HCB) and p,p′-DDE (2 – 22 μg/kg ww), respectively. In skua eggs, the most abundant CTTs were B9–1679 (Parlar #50) > B8–1413 (Parlar #26) > B9–1025 (Parlar #62) > B8–1412, the levels were about 1-20 μg/kg ww. In penguin eggs, however, the order was B8–1413 (Parlar #26) > B9–1679 (Parlar #50) > B8–1412 > B9–1025 (Parlar #62), and the levels ranged from 0.02 – 0.8 μg/kg ww. A so far

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unknown heptachloro compound labelled Q1 caused an abundant peak in some samples. Levels of Q1 (2 – 126 µg/kg ww in skua eggs and 0.3 – 1.2 µg/kg ww without shell in penguin eggs) were estimated relative to the ECD response factor of trans-nonachlor.

Keywords: Focused open-vessel microwave-assisted extraction; organochlorines; eggs of penguin; eggs of skua; Antarctic

INTRODUCTION

PCBs and chlorinated pesticides (DDT, HCB, HCHs, dieldrin, chlordane, toxaphene) are typical anthropogenic chemicals. Due to their lipophilicity and persistence they are accumulated in environmental samples of agricultural and industrialised areas. By atmospheric long-range transport these xenobiotics are transported to remote areas like the Antarctic [1–2], where they have never been used.

In the late 1960s and 1970s studies dealed with the organochlorine contamination of eggs of penguins [3–5], others focused on different tissues of different Antarctic birds [6–7]. In studies analysing both skuas and penguins much higher levels of organochlorines were found in skua samples [5; 8–9]. The main topic of most studies was the determination of DDT and its metabolites. Levels of p,p′-DDE were about 6-4740 µg/kg lipid [3–5; 9], and of p,p′-DDT 5 – 400 µg/kg lipid in penguin eggs [4–5]. In skua eggs levels of p,p′-DDE of 1090 µg/kg lipid [9], 8000 – 28000 µg/kg lipid, and of p,p′-DDT from 700 – 2000 µg/kg lipid [5] were found. Data about single PCB congeners and chlordane are scarce, but Σ PCB residues are reported to be 2750 µg/kg lipid basis in skua eggs and 100 µg/kg lipid basis in Adelie penguin eggs [9]. Compounds of technical toxaphene (CTTs) have been detected in eggs of Antarctic birds [10], but their levels have not been determined quantitatively, yet.

To get more information about the present pollution status of the Antarctic, levels of organochlorines were determined in 9 penguin eggs and 10 skua eggs. According to earlier studies [3–5; 8–10], low levels were expected in eggs of Antarctic birds. Consequently, an efficient sample clean-up method was required. For this reason, focused open-vessel microwave-assisted extraction (FOV-MAE) was applied for the extraction of entire, partly lyophilised eggs.

Microwave-assisted extraction (MAE) proved to be a fast and effective sample preparation method for the determination of organochlorines in soil and sediment [11–12], as well as in adipose tissue [13]. Recently, MAE in closed-vessels was combined with gel-permeation chromatography (GPC) for the sample clean-up of fatty matrices [14]. In this work, we used the same method except MAE in focused open-vessels, which allowed the extraction of entire, partly lyophilised eggs.

EXPERIMENTAL

Samples

Samples were available of 10 skua eggs, namely 5 eggs of South Polar Skua *Catharacta maccormicki*, 3 eggs of Brown Skua *Catharacta antarctica lonnbergi*, and 2 eggs of Mixed Pair Skua *Catharacta maccormicki* × *lonnbergi*. Mixed Pair Skuas are pairs of usually a female Brown Skua and a male South Polar Skua [16]. Skuas are migratory birds, the South Polar Skuas spend the winter in the northern hemisphere, whereas the Brown Skuas stay in the Southern Ocean [16]. These dark gull-like seabirds are predatory seabirds [16]. In the investigation area, the South Polar Skua feeds extensively on fish, while the Brown Skua mainly lives on penguin chicks and eggs, and station garbage [42]. Skuas lay clutches of two eggs [16]. Eggs 66a and 66b are the first and second laid egg of the same female.

Furthermore, samples of 9 penguin eggs were analysed, namely 2 eggs of Adelie *Pygoscelis adeliae*, 3 eggs of Chinstrap *Pygoscelis antarctica*, and 2 eggs of Gentoo *Pygoscelis papua*. Two penguin eggs could not be specified, probably they were Gentoo eggs. The three *Pygoscelis* species are medium sized penguin species, they are black and white animals which lack bright colours on the head and body. They are not to be mixed up with the well known yellow-necked Emperor and King penguins. Penguins dont leave the Antarctic region and feed mainly on fish and krill *(Euphausia superba)* [16].

All 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; see **Figure 1**).

After collecting, the samples were weighed, the shells were removed and weighed (for wet weight of the samples without shell see **Tables I and II**) and the eggs were partly lyophilised (water content approx. 30%). The samples were packed in aluminium foil and kept at −20°C until analysis.

Chemicals and organochlorine standards

The following single standard solutions of organochlorines (10 ng/μL each) were obtained from Promochem, Wesel (Germany) or Dr. Ehrenstorfer, Augsburg (Germany): α-, β-, γ- and δ-hexachlorocyclohexane (HCH), hexachlorobenzene (HCB), p,p′-DDT, p,p′-DDD, p,p′-DDE, PCB 18, 28, 31, 44, 52, 99, 101, 146, 149, 118, 153, 138, 163, 180, 170, 194, dieldrin, oxy-, cis- and trans-chlordane, cis- and trans-nonachlor. CTTs quantified 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*,

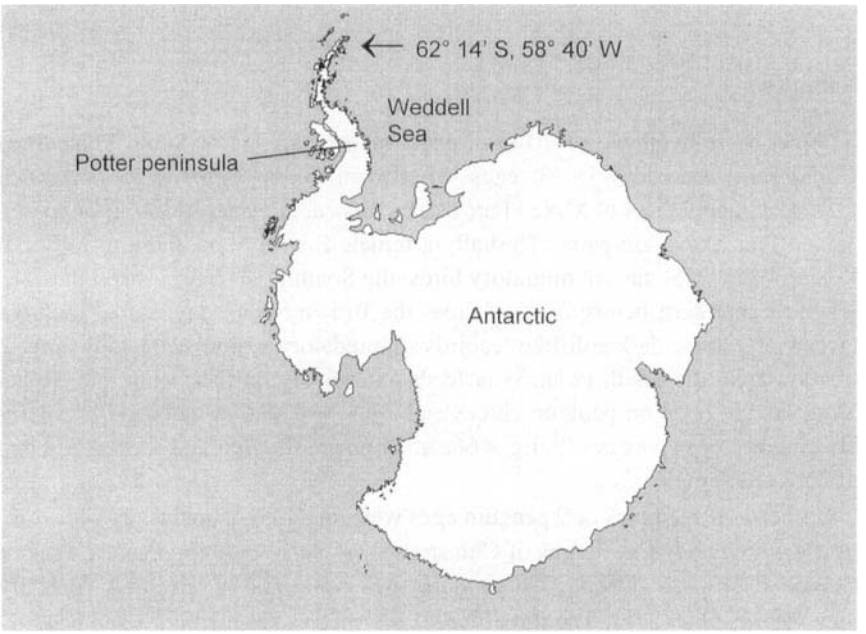

FIGURE 1 Map of the Antarctic with the sampling site

8, 8, 9, 10-octachlorobornane (B8–1412), 2-*endo*, 3-*exo*, 5-*endo*, 6-*exo*, 8, 8, 10, 10-octachlorobornane (B8–1413, Parlar #26), 2-*endo*, 3-*exo*, 5-*endo*, 6-*exo*, 8, 9, 10, 10-octachlorobornane (B8–1414, Parlar #40), 2-*exo*, 3-*endo*, 5-*exo*, 8, 9, 9, 10, 10-octachlorobornane (B8–1945, Parlar #41), 2-*exo*, 5, 5, 8, 9, 9, 10, 10-octachlorobornane (B8–2229, Parlar #44), 2, 2, 5, 5, 8, 9, 9, 10, 10-nonachlorobornane (B9–1025, Parlar #62), 2-*endo*, 3-*exo*, 5-*endo*, 6-*exo*, 8, 8, 9, 10, 10-nonachlorobornane (B9–1679, Parlar #50) which have been detected in tissue of Antarctic penguins [17]. Hereafter, AV-codes [18] are used and Parlar numbers [19] in parenthesis. B8–1412 was quantified with the ECD-response factor of B8–1413 (Parlar #26). A solution containing B7–1453 was calibrated by GC/FID [20]. The other CTT standards were from Dr. Ehrenstorfer.

Single standard solutions were combined to quantitation standards of 10, 20, 50, 100, 250, and 500 pg/μL, respectively. The response factors of the 100 pg/μL standard solutions were identical with those of certified standard solutions (N0813 and NC 378, Promochem). The first quantitation standard contained HCHs, HCB, p,p′-DDT, p,p′-DDD, p,p′-DDE, and PCBs, the second quantitation standard contained the chlordanes, and the third quantitation standard contained the eight CTTs. PCB 163 and dieldrin were used as single standards, PCB 99 and PCB 146 were only available as qualitative standards.

TABLE I Levels of organochlorines in skua eggs; Upper line: [µg/kg wet weight without shell], lower line: [µg/kg lipid basis]

	Brown Skua				*South Polar Skua*				*Mixed Pair Skua*	
	39a	*62b*	*37b*	*46x*	*10b*	*75a*	*66a*[4]	*66b*[4]	*28*	*13b*
ww [g]	106	87	111	93	85	84	82	82	108	82
fat [%]	4.1	5.0	3.0	7.0	5.8	6.1	7.0	6.2	1.3	5. 6
HCB	31	133	8	20	13	4	6	4	1.5	10
	759	2664	267	282	221	72	83	72	118	172
Σ HCH[1]	< 0.7	< 0.7	< 0.7	10	4	0.7	1	< 0.7	0.4	0.3
	< 17	< 14	< 23	137	61	12	20	< 11	27	6
PCB 101	1	det[2]	1	3	2	0.2	3	2	0.3	1
	27		21	39	35	3	43	37	20	18
PCB 149	1	< 1	1	3	1	0. 1	2	2	0.2	1
	14	< 20	26	37	25	2	35	28	18	14
PCB 118	28	25	4	37	8	1.2	12	10	0.7	3
	678	502	142	534	145	19	167	160	51	58
PCB 153	121	82	17	147	31	6	65	60	1.5	13
	2957	1639	559	2102	534	104	925	974	117	239
PCB 138	29	28	7	46	11	2	21	18	0.8	5
	702	556	241	651	182	30	305	292	64	85
PCB 163	1	1	2	3	2	0.3	5	4	0.3	1
	32	26	72	43	31	5	66	60	20	26
PCB 180	206	81	16	152	34	9	103	90	2	19
	5022	1625	535	2171	588	140	1475	1451	124	331
PCB 170	33	19	4	25	8	1	16	17	0.4	4
	796	382	139	350	136	16	223	282	31	63
PCB 194	53	16	3	17	6	2	23	20	0.4	4
	1292	315	111	238	102	25	333	319	30	63
Σ PCB[1]	472	252	55	431	103	21	250	223	6	50
	11520	5046	1845	6163	1778	346	3572	3603	474	897
p,p′-DDT	7	14	1	8	14	1	6	4	0.2	2
	176	289	24	118	233	15	82	59	18	38
p,p′-DDE	128	250	33	354	116	9	59	54	2	25
	3127	5006	1109	5057	2004	155	845	866	178	446
Σ DDT[1]	135	265	34	362	130	10	65	57	3	27
	3304	5295	1133	5175	2237	170	927	925	197	485
dieldrin	4	10	3	8	6	< 0.1	5	6	0.5	3
	95	193	94	115	100	< 2	77	92	41	49

	Brown Skua			*South Polar Skua*					*Mixed Pair Skua*	
	39a	*62b*	*37b*	*46x*	*10b*	*75a*	*66a*[4]	*66b*[4]	*28*	*13b*
oxychlordane	14	56	4	35	8	0.8	3	2	int[3]	4
	353	1119	118	500	130	14	43	32		66
cis-chlordane	< 0.2	1	< 0.2	< 0.2	0.7	0.1	0.9	0.9	< 0.2	0.5
	< 5	26	< 7	< 3	12	1	13	14	< 15	9
trans-	4	8	2	9	12	0.6	12	11	0.2	2
nonachlor	100	168	49	132	200	9	172	175	15	39
cis-	3	4	2	6	8	0.7	10	6	0.5	2
nonachlor	65	73	56	86	138	12	149	92	37	31
[1]Σ chlordane	21	69	7	50	28	2	26	19	1	8
	519	1386	223	718	479	36	377	313	52	144
Q1	126	109	29	26	31	2	16	12	11	73
	3073	2180	967	371	534	33	229	194	846	1304
B7–1453	< 0.2	1	0.2	< 0.2	< 0.2	int	< 0.2	< 0.2	< 0.2	0.2
	< 5	17	6	< 3	< 3		< 3	< 3	< 15	3
B8–1413	4	12	2	14	12	0.8	6	6	0.4	3
(#26)	88	242	65	205	198	13	82	91	27	56
B8–1412	2	7	2	6	6	0.4	6	4	0.2	2
	54	149	51	89	102	7	79	63	18	35
B8–1414	< 0.4	1	det	1	1	det	2	1	< 0.4	0.3
(#40)	< 10	14		16	19		29	23	< 31	5
B8–1945	< 0.3	1	0.2	1	1	det	2	1	< 0.3	0.3
(#41)	< 7	14	7	15	15		27	22	< 23	5
B8–2229	2	int	0.8	int	3	< 0.2	5	4	< 0.2	0.5
(#44)	49		27		52	< 3	68	64	< 15	10
B9–1679	6	16	3	26	19	2	17	13	0.4	4
(#50)	140	326	91	367	326	26	238	204	31	76
B9–1025	2	6	0.8	12	10	2	14	7	< 0.2	2
(#62)	50	122	25	166	169	28	204	116	< 15	39
Σ CTT[1]	16	44	8	60	51	5	51	36	1	13
	381	883	271	858	882	74	727	583	76	229

1: Σ HCH (= sum of β- and γ-HCH), Σ DDT (= sum of p,p′-DDT and p,p′- DDE), Σ PCB (= sum of all quantitatively determined PCBs listed in this Table), Σ CTT (= sum of all quantitatively determined CTTs listed in this Table), Σ chlordane (= sum of all quantitatively determined chlordane related compounds listed in this Table).
2: det detected qualitatively due to agreement of the retention times on the two capillary columns, quantitative determination was not possible because of base line noise.
3: int peak interfered.
4: egg 66a and 66b were the first and second laid egg of the same animal.

TABLE II Levels of organochlorines in penguin eggs; Upper line: [µg/kg wet weight without shell], lower line: [µg/kg lipid basis]

	Chinstrap			*Adelie*		*Gentoo*		*?*	
	1	*2*	*3*	*2*	*4*	*2*	*B*	*C*	*A*
ww [g]	88	85	89	87	68	92	94	69	118
fat [%]	4.1	1.7	2.3	0.9	-	7.7	2.4	3.2	6.8
HCB	9	5	4	12	15	9	8	22	11
	217	272	177	1370		121	343	678	161
Σ HCH[1]	< 0.2	< 0.2	0.9	< 0.2	0.3	0.1	0.3	< 0.2	< 0.2
	< 4	< 12	39	< 22		1	11	< 6	< 2
PCB 118	0.3	0.3	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	7	20	< 4	< 11		< 1	< 4	< 3	< 1
PCB 153	0.7	0.2	0.2	0.2	0.3	0.2	0.1	0.2	0.7
	16	12	10	18		2	5	7	10
PCB 138	0.2	0.1	0.1	0.1	<0.1	0.1	< 0.1	< 0.1	0.2
	5	7	4	7		1	<4	<3	3
PCB 163	0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.1
	2	< 6	< 4	< 11		< 1	< 4	< 3	1
PCB 180	0.3	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.5
	7	< 6	< 4	< 11		< 1	< 4	< 3	8
PCB 194	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.1
	< 2	< 6	< 4	< 11		< 1	< 4	< 3	2
Σ PCB[1]	1.5	0.7	0.3	0.2	0.3	0.3	0.1	0.2	1.6
	37	39	14	24		4	5	7	24
p.p'-DDT	1	0.5	0.4	0.1	0.4	0.2	< 0.1	0.2	0.4
	27	26	15	11		3	< 4	8	6
p.p'-DDE	13	4	4	3	5	2	2	4	5
	323	238	156	377		30	82	118	79
Σ DDT[1]	14	5	4	3	6	3	2	4	6
	350	265	171	388		33	82	125	86
dieldrin	0.7	0.4	0.2	0.8	*[4]	0.7	0.3	0.3	*
	17	22	10	89		9	13	9	
oxychlordane	0.5	0.3	0.2	0.2	< 0.1	0.7	0.4	0.7	1
	12	18	10	22		9	15	20	14
trans-nonachlor	0.4	0.3	0.2	0.4	0.4	1	< 0.1	< 0.1	1
	11	18	7	41		15	< 4	< 3	17
cis-nonachlor	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.3	0.5	< 0.1	< 0.1
	< 2	< 6	< 4	< 11		4	20	< 3	< 1
Σ chlordane	0.9	0.6	0.4	0.6	0.4	2.1	0.8	0.7	2.2
	23	35	17	63		28	35	20	32
Q1	1.2	0.6	0.3	0.3	< 0.1	1.2	0.3	0.3	0.7
	29	35	13	33		16	13	9	10

	Chinstrap			*Adelie*		*Gentoo*		*?*	
	1	*2*	*3*	*2*	*4*	*2*	*B*	*C*	*A*
B7–1453	0.02 0.5	0.01 0.6	0.01 0.4	det[2]	< 0.02	int[3]	0.01 0.4	< 0.02 < 0.6	< 0.02 < 0.3
B8–1413 (#26)	0.45 11	0.22 13	0.12 5	0.14 16	0.10	0.42 6	0.16 7	0.10 3	1 16
B8–1412	0.37 9	0.17 10	0.10 4	0.09 10	0.10	0.19 3	0.15 6	0.06 2	0.45 7
B8–1414 (#40)	0.08 2	0.04 2	det	det	< 0.02	0.02 0.3	det	< 0.02 < 0.6	< 0.02 < 0.3
B8–1945 (#41)	0.03 0.7	0.01 0.6	det	det	< 0.02	0.01 0.1	det	< 0.02 < 0.6	< 0.02 < 0.3
B8–2229 (#44)	0.17 4	0.02 1	0.03 1	0.01 1	0.02	det	0.04 2	< 0.02 < 0.6	< 0.02 < 0.3
B9–1679 (#50)	0.41 10	0.19 11	0.09 4	0.10 11	0.07	0.36 5	0.14 6	0.05 2	0.87 13
B9–1025 (#62)	0.25 6	0.08 5	0.07 3	0.07 8	0.06	0.20 3	0.08 3	< 0.02 < 0.6	0.21 3
Σ CTT[1]	1.8 43	0.7 44	0.4 18	0.4 46	0.4	1.2 16	0.6 24	0.2 7	3 38

1: Σ HCH (= sum of β- and γ-HCH), Σ DDT (= sum of p,p'-DDT and p,p'- DDE), Σ PCB (= sum of all quantitatively determined PCBs listed in this Table), Σ CTT (= sum of all quantitatively determined CTTs listed in this Table), Σ chlordane (= sum of all quantitatively determined chlordane related compounds listed in this Table).
2: det detected qualitatively due to agreement of the retention times on the two capillary columns, quantitative determination was not possible because of base line noise.
3: int peak interfered.
4: * sample was cleaned with sulfuric acid, dieldrin could not be determined.

Certified reference material SRM 1588 (cod liver oil) was from Promochem. Silica gel 60 (particle size 0.063–0.200 mm) was from Merck, Darmstadt (Germany). Ethyl acetate (for 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 (for residue analysis grade) was from Promochem.

Instruments

FOV-MAE was performed with a Soxwave 100 (Prolabo, France) system. Energy is produced by a magnetron at 2450 MHz. The system which operates at ambient pressure is equipped with a reflux column to avoid solvent losses during extraction. It allows multistep programming of microwave energy (max. 300 W) and time of irradiation. The glass connection of quartz vessel and reflux column

is equipped with a 20 mL water trap, which partly allows the separation of solvent and water.

Automated gel-permeation chromatography (GPC) was carried out with an Autoprep 1002 (ABC, Analytical Biochemistry Columbia, USA) with 50 g *bio beads S-X3* in a 33 cm × 2.5 cm i. d. column.

GC/ECD analyses were performed with an HP 5890 (Hewlett-Packard) gas chromatograph equipped with a splitter after the split/splitless injector (splitless time 1.5 min), that divides the samples onto two capillary columns and two ^{63}Ni electron capture detectors (ECDs). The samples were injected automatically (HP 7673 autosampler). The capillary columns CP-Sil 2 and CP-Sil 8/20% C_{18} (both: length 50 m, 0.25 mm i. d., 0.25 µm film thickness) were from Chrompack (Middelburg, The Netherlands).

Helium was used as carrier gas at a column head pressure of 1.2 bar, nitrogen was used as make-up gas. The injector (splitless) and detector temperatures were 250°C and 300°C, respectively. The GC oven program was the following: after injection at 60°C (1.5 min) the temperature 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.

Sample clean-up

Focused open-vessel microwave-assisted extraction (FOV-MAE)

The entire, partly lyophilised eggs (20–50 g) were separated from the aluminium foil by scraping off the main part with a spatula. Sample remainders were extracted with ethyl acetate/cyclohexane (1:1, v:v) in an ultrasonic bath and added to the main part.

The sample extracts were placed in quartz glass tubes (250 mL) and FOV-MAE was performed five times in the case of skua eggs and twice in the case of penguin eggs with 80 mL ethyl acetate/cyclohexane (1:1, v:v), respectively. At the beginning, the refluxing solvent filled the water trap (20 mL), in which solvent and coextracted water departed into two layers. After the water trap was filled, the refluxing solvent redrained into the tube with the sample. After FOV-MAE, the content of the water trap was drained off. The aquatic phase (approx. 15 mL per egg) was separated and extracted three times with 5 mL cyclohexane, respectively. The organic layer (15 mL cyclohexane extract) and 15 mL ethyl acetate were added to the combined FOV-MAE extracts. The combined extracts were filtered through 20 g Na_2SO_4 to separate water. Then, the extracts were concentrated in a rotavapor to approx. 40 mL (skua eggs) or 5 mL (penguin eggs), filtered once more through 2 g Na_2SO_4, and adjusted to

50 mL (skua eggs) or 20 mL (penguin eggs). The lipid content was determined gravimetrically by use of aliquots of these solutions.

Gel-permeation chromatography

10 mL of the FOV-MAE extract were filtered through a 45 µm membrane filter and an aliquot of the internal standard α-PDHCH [21–22] was added. The sample was automatically introduced into the 5 mL sample loop of the system, the remaining solution went to waste. Gel-permeation chromatography was used to separate the lipid fraction from the organochlorines, it was performed with ethyl acetate/cyclohexane (1:1, v:v) as the solvent [23]. The dump and collection times were optimized using trans-chlordane and HCB, which are among the first and last eluted organochlorine compounds [23], the flow rate was set at 4.6 mL/min.

Adsorption chromatography with silica gel

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 to remove the more volatile ethyl acetate quantitatively.

Adsorption chromatography on deactivated silica was performed according to the method of Steinwandter and Schlüter [24], which was slightly modified [25]. Silica gel was dried for 16 h at 130°C and deactivated with 30 % water by shaking for 30 min. 3 g deactivated silica gel were placed in a glass column (1.0 cm i.d.) and covered with Na_2SO_4. The isooctane extract of the sample was placed on the silica gel column and eluted with 60 mL n-hexane. The eluate was concentrated by rotary evaporation and blowing down with nitrogen to 1 – 5 mL in calibrated flasks. Aliquots were subjected to GC/ECD and to PCB/CTT group separation.

Two extracts remained yellow after adsorption chromatography with silica gel. These sample extracts were treated for 24 h with 2 mL concentrated sulfuric acid and after this procedure the extracts were cleaned again by adsorption chromatography with silica gel. However, this effective clean-up step destroyed dieldrin and further acid instable compounds (see below).

To get an overview about the organochlorine levels in the sample, 100 µL of the FOV-MAE solution were submitted to silica gel clean-up after solvent exchange to isooctane as described above, without performance of GPC [13]. 3 g deactivated silica gel allows the separation of approx. 75 mg lipids from organochlorines. On the basis of 5 % lipid content in the eggs, 100 µL extract contained

approx. 25 mg lipids. The silica gel eluate was reduced again to 100 µL by rotary evaporation and blowing down in a nitrogen flow, and 1 µL was manually injected into the GC/ECD.

PCB/CTT group separation

CTTs were quantified after pre-separation of the PCBs. The solution after adsorption chromatography with silica gel was fractionated on a 30 cm × 1 cm i. d. glass column filled with 8 g silica gel, which was activated for 16 h at 130°C [15]. PCBs were quantitatively eluted with 48 mL n-hexane (PCB fraction), and CTTs and chlordane were eluted with 50 mL of a more polar solvent into a second fraction (CTT fraction) [15]. Instead of n-hexane/toluene (65:35, v:v) n-hexane/ethyl acetate (90:10, v:v) was used for the elution of the CTT fraction [26]. The eluates were condensed in a rotavapor to approx. 2 mL in calibrated flasks and blown down in a nitrogen flow to approx. 0.5 mL. Then the volume was adjusted with isooctane to 1.0 mL. Low contaminated samples were carefully blown down to dryness and dissolved with 100 µL n-hexane. Aliquots were subjected to GC/ECD for the quantitation of CTTs in penguin eggs and CTTs and chlordane in skua eggs.

Quality control

Perdeuterated α-HCH (α-PDHCH) was used as recovery standard to check losses of volatile organochlorines like α-HCH, lindane, and HCB during sample concentration steps [21–22]. For the clean-up of eggs, high recoveries of organochlorines were reached with the solvent ethyl acetate/cyclohexane (1:1, v:v) [27].

Two GC capillary columns of different polarity were used to check for peak interferences in the ECD chromatograms. For quantification, it was required that the value on one column was confirmed on the other column. In case of deviations (coelution) the lower value was regarded as the right one. Organochlorine levels were not extrapolated to 100% recovery, they represent the really measured levels.

Calibration curves of all compounds (10, 20, 50, 100, 250, 500 pg/µL, injection: 1 µL) were made to check the linearity of the measurement area. Note that the solutions were distributed on the two capillary columns, so that the calibration ranged from 5 – 250 pg. If there were deviations from linearity, the sample values obtained by calculation with the 100 pg/µL standard solutions were corrected. Samples that revealed levels higher than the calibrated range were diluted prior to GC/ECD analysis.

To control the quality of the PCB/CTT group separation, PCBs were randomly quantified in the PCB fraction of the PCB/CTT group separation and the values were compared with the PCB levels determined without group separation.

The complete sample clean-up was validated with certified cod liver oil (SRM 1588).

RESULTS AND DISCUSSION

Microwave-assisted extraction in open and closed vessels

Microwave-assisted extraction with ethyl acetate/cyclohexane (1:1, v:v) as the solvent allows a direct performance of GPC after volume adjustment of the MAE extract [14]. No solvent exchange is necessary, and therefore, this technique is fast and avoids losses of analytes. Established with a closed-vessel MAE system, the method proved to be efficient for the analysis of organochlorines in cod livers and seal blubber, and high recoveries were reached [14].

In the meantime the complete sample clean-up method was validated with certified cod liver oil SRM 1588 using closed-vessel MAE (n = 2) as well as focused open-vessel MAE (n = 2). Irrespective of the MAE system, all DDT and PCB levels (exception: PCB 138 level was lower due to the separation of PCB 163 on CP-Sil 2 [28]) were within the certified range [29]. The reproducibility of both methods was checked by analysing commercially available cod livers. A very good reproducibility and a good agreement of both MAE methods was found. Therefore, both MAE techniques are efficient and suitable for extraction in view of the quantitative determination of organochlorines.

For the extraction of entire skua and penguin eggs, FOV-MAE was the method of choice due to the ability of this system to apply high sample amounts of about 50 g. Furthermore, the application of entire eggs avoided problems related to homogenisation of the samples. FOV-MAE in combination with an efficient sample clean-up is well suited for the determination of organochlorines in low contaminated samples.

Microwave heating depends on the number and kind of dipoles existing in the sample material [30]. Due to the high dielectric constant of water, the water content of the sample exerts great influence on the heating by microwaves. The present FOV-MAE system (Soxwave 100) works without heat controller, and in this case the microwave extraction program has to be adapted to each matrix depending on the water content.

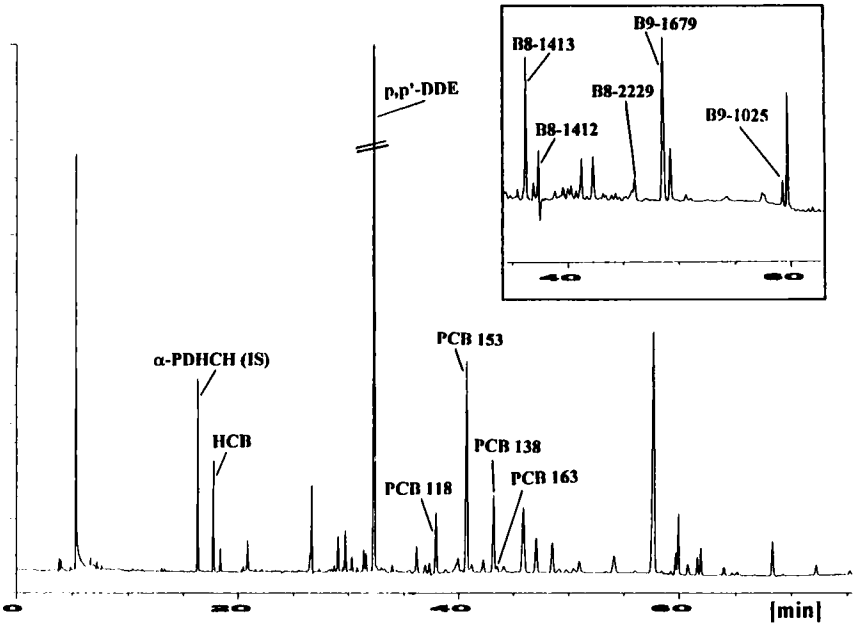

FIGURE 2 GC/ECD-chromatogram (CP-Sil 2) of a skua egg after microwave-assisted extraction, GPC and clean-up with deactivated silica gel; Upper chromatogram: GC/ECD-chromatogram (part) of the CTT fraction

The water content of the partly lyophilised eggs was approx. 30 % (estimated on the basis of the volume of 15 mL of coextracted water). The extraction program was developed with lyophilised eggs of hen, which had similar water content. Inhomogenic distribution of water in the sample matrix caused localised heating. To avoid delayed boiling, the extraction was started at low power, which was raised subsequently to maintain refluxing of the solvent. The optimised extraction program consisted of the following irradiation steps: 7 min at 30 W, then 8 min at 45 W, and finally 20 min at 60 W (total extraction time: 35 min).

FOV-MAE was repeatedly performed with 80 mL ethyl acetate/cyclohexane (1:1, v:v), respectively, and the organochlorine content was determined by GC/ECD in each extract. The extraction procedure was repeated with new solvent until the level of p,p′-DDE in the respective step was < 2 % of the p,p′-DDE level of the combined earlier extracts. This was obtained for skua eggs after five extractions with 80 mL ethyl acetate/cyclohexane (1:1, v:v), respectively. In a relatively high contaminated sample (skua 46x), within the two first extraction steps approx. 85 % of the organochlorines (p,p′-DDE, PCB 153, 138, 180) were extracted, in the third step approx. 8 %, in the fourth step approx. 5 % and in the fifth step < 2 %. Due to the lower levels in penguin eggs these samples were only extracted twice.

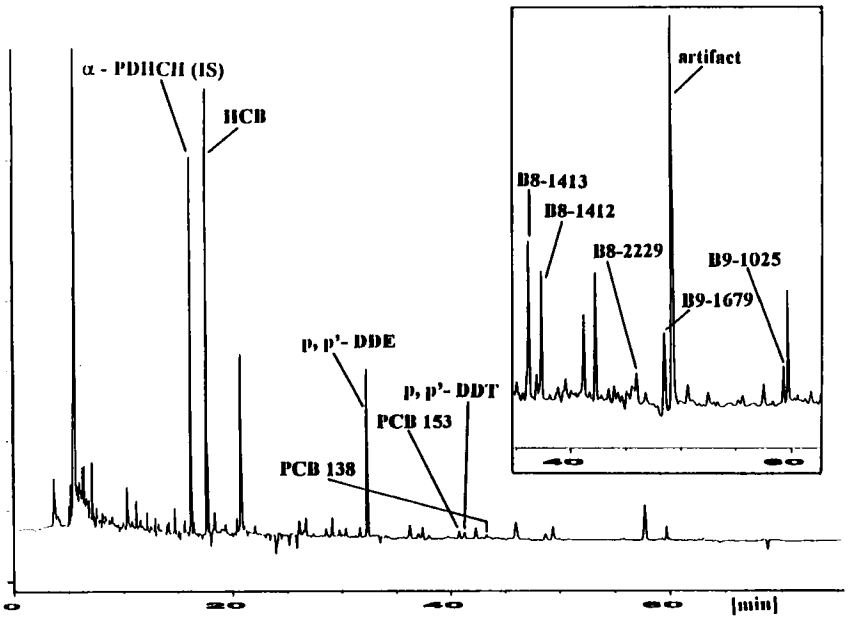

FIGURE 3 GC/ECD-chromatogram (CP-Sil 2) of a penguin egg after microwave-assisted extraction, GPC and clean-up with deactivated silica gel; Upper chromatogram: GC/ECD-chromatogram (part) of the CTT fraction

Organochlorine levels in skua and penguin eggs

The sample clean-up resulted in pure extracts (see **Figures 2 and 3**). The GC/ECD-chromatograms were dominated by the peak of p,p′-DDE or PCB 180 in skua eggs and by HCB in penguin eggs.

Depending on sample weight, the detection limits for penguin eggs were approx. 0.1 µg/kg wet weight without shell (ww) for all substances and for skua eggs approx. 1 µg/kg ww for PCB congeners, 0.7 µg/kg ww for p,p′-DDD, and 0.3 µg/kg ww for α-HCH and trans-chlordane. The detection limits of further compounds are listed in **Tables I and II**. Recoveries of the internal standard α-PDHCH were generally > 75%.

After the PCB/CTT group separation the sample extracts were concentrated down to 100 µL for the determination of CTTs. This volume of 100 µL corresponded with the extract of approx. 20 g of the partly lyophilised egg. This allowed it to quantify also CTTs in the ppt-range.

Levels of organochlorines based on wet weight without shell and lipid content in skua and penguin eggs are listed in **Tables I and II**, respectively.

Congener specific organochlorine levels in skua eggs

The lipid content in skua eggs (see **Table I**) ranged from 3.0 – 7.0 %, which is in agreement with findings of Risebrough et al. [5], who found a lipid content of 6 % in penguin and skua eggs. Egg skua 28a formed an exception, this sample revealed a particularly low lipid content and low levels of organochlorines. It is noteworthy that the content of this egg was addled. However, Gardner et al. [31] reported of no influence of putrefaction on the organochlorine levels. Low organochlorine levels were also measured in the other sample of Mixed Pair Skua (egg 13b) and egg 75a.

In the DDT group, p,p′-DDE was the dominant contaminant, followed by p,p′-DDT. p,p′-DDD was below the detection limit in all samples. Next to p,p′-DDE, the most abundant contaminants in skua eggs were PCB 153 and PCB 180 (10 – 350 μg/kg ww), which accounted for approx. 60 – 70% of all determined PCB congeners. PCB 180 was on the same level or higher concentrated than PCB 153. Both are among the most persistent PCB congeners as they have no vicinal hydrogens and are substituted at both para-positions [32–33]. Furthermore, PCB 153 is a major component in the most heavily applied technical PCB mixtures [34], and the PCB pattern of the most aquatic animals is dominated by this congener [35]. PCB 138 is another abundant congener in technical PCB mixtures, but due to the vicinal hydrogens it is not as persistent as PCB 153. PCBs 118, 170, and 194 were also abundant PCB congeners with relatively high levels (approx. 2 – 50 μg/kg ww). PCB 99 and PCB 146 were detected in all samples and the levels were estimated ranging from 1 – 12 μg/kg ww. Levels of p,p′-DDE, p,p′-DDT and PCBs were in the same order of magnitude as recently reported by Court [9], but Risebrough [5] found much higher levels in 1970.

In the group of the HCHs, β-HCH and γ-HCH were detected in some samples in low concentrations (mostly < 1 μg/kg), but α-HCH was severally below the detection limit. Strong variations were found for HCB levels, and in selected samples HCB was among the major contaminants.

CTT levels, ranging from 1 – 20 μg/kg ww, decreased in the order B9–1679 (Parlar #50) > B8–1413 (Parlar #26) > B9–1025 (Parlar #62) > B8–1412. These four congeners accounted for approx. 90 % and B9–1679 (Parlar #50) accounted for approx. 36 % of the sum of the eight CTTs (see **Figure 4**).

CTT and chlordane levels were comparable, but approx. one order of magnitude lower concentrated than PCBs. The most abundant chlordane related compounds in skua eggs were oxychlordane and trans-nonachlor. Dieldrin was determined in most of the samples, the levels were comparable with those of CTTs and chlordane (0.5 – 10 μg/kg ww).

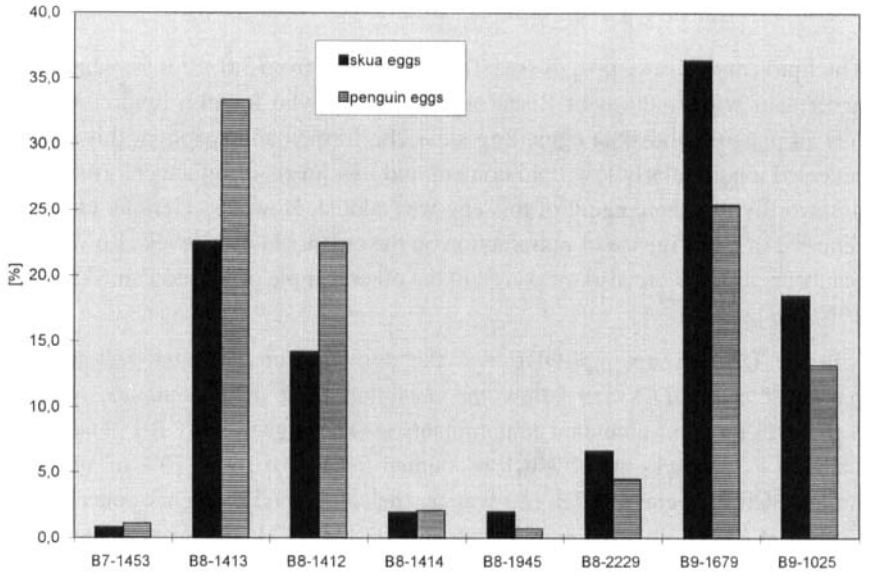

FIGURE 4 CTTs in skua and penguin eggs

In some skua eggs, a compound called Q1 was almost as abundant as p,p′-DDE (see **Table I**). Recently, this compound was identified by GC/MS as a heptachloro compound in the blubber of South African Seals [36]. As the retention time of Q1 was comparable with trans-nonachlor on both stationary phases, and due to the lack of standard solutions, levels of Q1 were calculated by using the GC/ECD response factor of trans-nonachlor. With this technique, Q1 levels were estimated to range from 2 – 126 μg/kg ww. Q1 levels were significantly higher in Brown Skua eggs than in South Polar Skua eggs (see **Table II**). Q1 levels were roughly comparable to HCB levels. Work is ongoing to elucidate the chemical structure of Q1.

Furthermore, it has to be mentioned that the second laid egg (skua 66 b) revealed comparable or a little bit lower levels of lipid and organochlorines than the first laid egg (skua 66a) of the same female.

Congener specific organochlorine levels in penguin eggs

The lipid content in penguin eggs ranged from 0.9 – 7.7 %. According to this, a sample with very high levels based on ww was not necessarily the one with very high levels based on the lipid content (e. g. egg penguin A). On the other hand, Risebrough [3] reported similar organochlorine levels and a fat content of 10 % in all three penguin species.

TABLE III Ratios in skua and penguin eggs

		$\Sigma DDT^1/\Sigma PCB^1$	$\Sigma PCB/\Sigma CTT^1$	$\Sigma CTT/\Sigma chlordane^1$	*Q1/trans-nonachlor*	*HCB/Q1*
Brown	39a	0.3	30	0.7	31	0.2
Skua	62b	1.0	5.7	0.6	13	1.2
	37b	0.6	6.8	1.2	20	0.3
South	46x	0.8	7.2	1.2	2.8	0.8
Polar	10b	1.3	2.0	1.8	2.7	0.4
Skua	75a	0.5	4.7	2.0	4.1	2.2
	66a	0.3	4.9	1.9	1.3	0.4
	66b	0.3	6.2	1.9	1.1	0.4
Mixed	28	0.4	6.2	1.5	59	0.1
Pair Skua	13b	0.5	3.9	1.6	34	0.1
Penguin	1	9.6	0.8	1.9	2.7	7.4
Chinstrap	2	6.7	0.9	1.2	2.1	7.7
	3	12.3	0.8	1.1	2.2	13.6
Penguin	2	15.9	0.5	0.7	0.7	41.1
Adelie	4	16.8	0.9	0.9	–	–
Penguin	2	9.1	0.2	0.6	1	7.8
Gentoo	B	19.6	0.2	0.7	4.1	27.5
Penguin	C	19.0	1.0	0.3	3.0	72.3
(?)	A	3.6	0.6	1.2	0.6	15.6

1: Σ DDT (= sum of p,p′-DDT and p,p′- DDE), Σ PCB (= sum of all quantitatively determined PCBs listed in **Tables I and II**), Σ CTT (= sum of all quantitatively determined CTTs listed in **Tables I and II**), Σ chlordane (= sum of all quantitatively determined chlordane related compounds listed in **Tables I and II**).

HCB and p,p′-DDE were the most abundant organochlorines in penguin eggs with levels about 2 – 20 μg/kg ww. High HCB levels were also typical of penguin tissue [37] and blood of Adelie penguins [38]. Levels of PCB congeners ranged from 0.1 – 0.7 μg/kg ww and decreased in the order PCB 153 > PCB 180 > PCB 138 > PCB 118. Comparing these results with the results of earlier studies, levels of p,p′-DDE [3–5; 9] and HCB [9] were in the same order of magnitude, but PCB levels were slightly higher (70 – 100 μg/kg lipid basis) [3; 9] than those determined in the present samples.

α-HCH, β-HCH, and γ-HCH were detected in four different samples, respectively. The levels were in the same order of magnitude than the PCB levels.

CTT levels have not yet been quantified in eggs of Antarctic birds, but Ballschmiter et al. detected toxaphene in eggs of gentoo penguins [10]. The present penguin eggs were very lowly contaminated with CTTs (0.02 – 0.8 μg/kg ww). CTT levels in penguin eggs decreased in the order B8–1413 (Parlar #26) > B9–1679 (Parlar #50) > B8–1412 > B9–1025 (Parlar #62), and B8–1413 accounted for approx. 33 % of the sum of the eight CTTs (see **Figure 4**). The four CTT congeners, which accounted for approx. 90 % of the sum of the eight CTTs, are reported to be the most abundant in penguin tissue as well [17].

CTT levels were comparable with chlordane levels, but much lower than PCB levels (one order of magnitude). The most abundant chlordane related compounds in penguin eggs were oxychlordane and trans-nonachlor. Dieldrin was abundant in the most of the samples. The levels ranged from 0.2 – 0.8 μg/kg ww and were in the same order of magnitude than those reported three decades ago [4]. They were a little bit higher than the CTT and chlordane levels. Levels of Q1 in penguin eggs ranged from 0.3 – 1.2 μg/kg ww.

Ratios of organochlorine contaminants and differences between skua and penguin eggs

The following differences in organochlorine levels in skua and penguin eggs could be established. The ratio Σ DDT / Σ PCB in penguin eggs was > 3.6, whereas in skua eggs ratios < 1.3 were found (see **Table III**). This difference is in agreement with findings of some earlier studies [3; 5], but not with Court, who found similar Σ DDT / Σ PCB ratios in skua and penguin eggs, respectively [9]. Note that Σ DDT / Σ PCB ratios are depending on the number of PCB congeners included in Σ PCB.

Skuas are migratory birds that spend the Antarctic winter in the northern hemisphere which is higher polluted with organochlorines than the remote area of the Antarctic, and the organochlorine levels of skuas are at least partly accumulated from prey caught in the northern hemisphere [4; 6]. This could be a reason for the high PCB levels in skua eggs, which were in the same order of magnitude as the p,p′-DDE levels.

The ratio Σ PCB / Σ CTT revealed differences between skua and penguin eggs as well (see **Table III**), and there was also a change in the abundance of CTT congeners (see **Figure 4**). No differences in the ratios Σ CTT / Σ chlordane between skua and penguin eggs were found.

Furthermore, there were differences in the ratios Q1 / trans-nonachlor between skuas and penguins. All penguin species and the South Polar Skua *Catharacta*

maccormicki revealed Q1 / trans-nonachlor ratios < 5, whereas eggs of Brown Skua *Catharacta antarctica lonnbergi* and Mixed Pair Skua *Catharacta maccormicki* × *lonnbergi* revealed ratios > 10 (see **Table III**).

In general, skua eggs revealed strongly varying levels from egg to egg (one order of magnitude and higher), the levels of the penguin eggs were comparable within the penguin species.

CONCLUSIONS

The sample clean-up including focused-open vessel microwave-assisted extraction enabled to determine organochlorines in the low µg/kg range in eggs of Antarctic skuas and penguins. The results confirm that DDT is still a major contaminant in the Antarctic [3-5; 9]. In contrast to seal blubber from the Antarctic [39; 40], toxaphene was not a major contaminant in Antarctic birds, although CTT and PCB levels were comparable in penguin eggs.

In general, skuas were significantly higher polluted with organochlorines than penguins [8; 9; 41]. Lower organochlorine levels in penguins were attributed to different feeding habits of skuas and penguins. Furthermore, skuas spend the winter outside the Antarctic. In addition, organochlorine levels in eggs of penguin species were relatively constant while the organochlorine levels in skua eggs were subject to strong variations, also in dependence of the respective species [42–43]. Therefore, penguins seem to be more suited as bioindicators for the assessment of the organochlorine pollution in the Antarctic.

Compared to residue levels of eggs of birds of other parts of the world, however, it can be noticed that skua and penguin eggs accumulate much lower levels of organochlorines [44–46].

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