Residues of Chlorinated Hydrocarbons in Marine Organisms in Relation to Size and Ecological Parameters
I. PCB, DDT, DDE, and DDD in Fishes and Molluscs from the English Channel

by W. ERNST, H. GOERKE, G. EDER, and R. G. SCHAEFER
Institut für Meeresforschung
285 Bremerhaven, FRG

Introduction

In order to provide more data for the characterization of organochlorine residue levels in marine food chains we analysed animals of three areas: English Channel (I), central North Sea (II), and eastern North Sea (III). Animal tissues were investigated for finding possible correlations between residue levels and body size or ecological parameters.

This paper deals with residues of PCBs, DDT, DDE, and DDD in five species from the English Channel. The organisms, which are listed in Table 1, were caught either by dredging or bottom trawling on Nov. 26/27, 1971 during the 158th cruise of FFS "Anton Dohrn". The compounds were determined by gas chromatography in two independent working groups, identifications were performed by the GC-MS technique.

TABLE 1

Stations of investigated species

Species	Station	Depth
Yellow gurnard, *Trigla lucerna* (Pisces)	50°26'N 00°03'E	56 m
Plaice, *Pleuronectes platessa* (Pisces)	50°26'N 00°03'E 50°00'N 03°40'W	56 m 71 m
Brill, *Scophthalmus rhombus* (Pisces)	50°00'N 03°40'W	71 m
Squid, *Loligo forbesi* (Cephalopoda)	50°26'N 00°03'E	56 m
Queen scallop, *Chlamys opercularis* (Bivalvia)	50°21'N 00°02'W	56 m

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Experimental

All species except the gurnards (Trigla lucerna) were wrapped in aluminium foil, placed in polyethylene bags and deep-frozen (-20°C) aboard. Trigla lucerna specimens were dissected aboard and the tissues packed and stored as described above for whole specimens. Samples were kept at -20°C until analyzed.

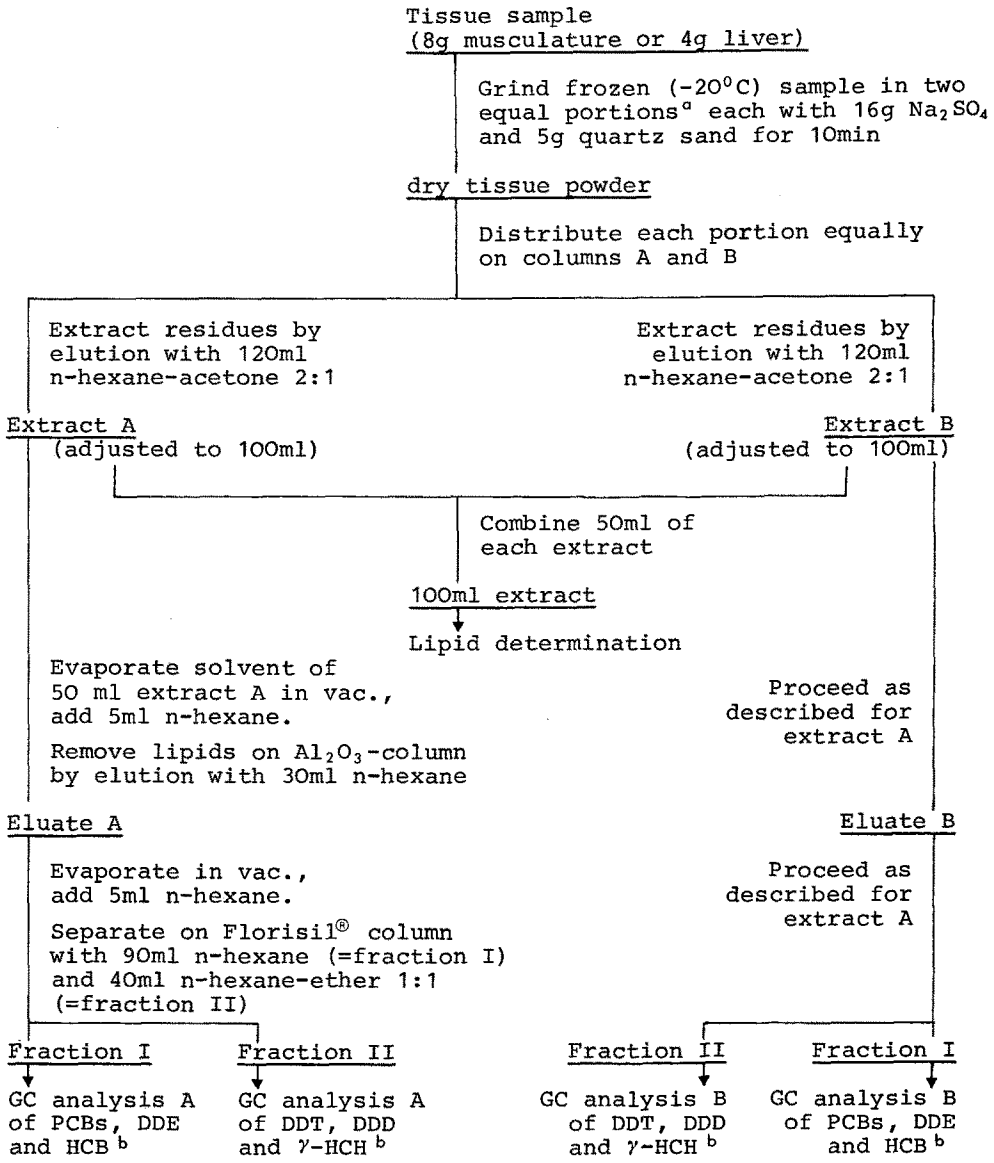
Tissue samples were prepared from organisms or tissues immediately after thawing and deep-frozen again for grinding. According to the expected residue concentrations these samples had different weights: musculature - 8 g, liver and digestive gland - 4 g, visceral adipose tissue - 1 g. The investigation of the visceral adipose tissue seemed to be advantageous for two reasons: 1. Analytical data were obtained more precisely than in tissues with very low residue concentrations. 2. The lipid content in this tissue is fairly constant as compared with other tissues in our study. Gurnards and flatfish were analyzed individually (Table 3); numbers of molluscs were sufficient to allow pooling of specimens of different size groups (Table 4). Tissues of different specimens contributed to the final samples with identical weights. In the case of Chlamys opercularis the digestive gland as a whole was taken.

Tissue parts having had direct contact to aluminium foil were excluded from the final samples. Instruments and glass-ware were rinsed with redistilled acetone. Generally, from each sample two parallel extracts were prepared, which were treated separately as shown in Fig. 1 (exceptions are indicated in Tables 3 and 4). This procedure allowed the detection of any serious accidental contamination during the analyses. A prerequisite of the latter statement is the equipartition of the residues in the tissue powder on the two extraction columns. This was tested by measuring the equipartition of added DDT-^{14}C and naturally contained lipids.

The whole procedure for working up the tissue samples has been described in detail elsewhere (ERNST et al. 1974) and is summarized in Fig. 1. Two gas chromatographs were used under the conditions stated in Table 2. Calibration was done daily using 5 different concentrations of either PCB (Clophen® A60) + DDE or DDT + DDD. PCB values were calculated from the three major peaks and then averaged.

FIGURE 1

Procedure for two parallel residue analyses of a tissue sample



[a] Limited size of the mortar mill requires grinding in two portions.

[b] HCB and γ-HCH are not determined in this paper.

TABLE 2

Conditions of gas chromatographic analysis

	Beckman GC 5	Varian 2740
Column	1.8m, all glass, 2 mm i.d., 5 % SE 30 on Chromosorb W 80/100 mesh	1.8m, all glass, 2 mm i. d., 1.5 % SP-2250 (OV-17) +1.95 % SP-2401 (QF-1) on Supelcoport 100/120 mesh
Column temperature	200°C (DDD+DDT) 220°C (DDE+PCB)	190°C
Inlet temperature	10°C above column temperature	225°C
Detector	Beckman ECD	^{3}H-ECD
Detector temperature	300°C	225°C
Carrier-gas	helium, 40 ml/min	nitrogen, 30 ml/min

Reagents. Alumina, neutral (Woelm, Eschwege, Germany), heated 3 h at 850°C, deactivated with 5 % H_2O. Florisil® 60 - 100 mesh (Floridin Co., Pittsburgh, USA) heated 2 h at 650°C, addition of 0.4 - 0.6 % water (vapor state) after 24 h, ready for use after additional 24 h, storage in well closed glass stoppered flasks. Clophen® A60 (Bayer AG, Leverkusen, Germany). Other reference substances (Riedel de Haën, Seelze, Hannover, Germany). Na_2SO_4 anhydrous GR, heated 2 h at 650°C; quartz sand; acetone GR, distilled over 65 cm Widmer column; n-hexane for synthesis, distilled over 50 cm packed column; ether GR, threefold distilled (Merck, Darmstadt, Germany).

Apparatus. Mortar mill, type Pulverisette 2, with agate equipment (Fritsch, Idar-Oberstein, Germany). Glass columns for extraction 13x280 mm, solvent reservoir on top 50x100 mm. Al_2O_3 and Florisil® columns 5x480 mm and 10x 480 mm resp., solvent reservoirs on top 35x120 mm (see

ERNST et al. 1974). Column fillings: 8 g Al_2O_3 and 13 g Florisil® resp. Rotary vacuum evaporator (Büchi, Flavil, Switzerland). Gas chromatographs and conditions: see Table 1. Mass spectrometer: Varian MAT CH7 coupled with GC Varian Aerograph 2740, packed column.

Results and discussion

Analytical results are shown in Table 3 for gurnards and flatfishes, in Table 4 for scallops and squids. PCBs were evaluated on the basis of Clophen® A60, and in the DDT group only p,p'-isomers were measured. Residue concentrations are stated with two digits except when below 10 ppb; then one digit is given. Gas chromatographic data could be verified by applying the GC-MS technique. In order to obtain mass spectra the extracts had to be extremely concentrated. Both DDT compounds and PCB components were detected (SCHAEFER 1974).

Generally, residue concentrations increased with rising lipid content of the tissues in the order: musculature, liver/digestive gland, visceral adipose tissue. However, the residue amounts per lipid weight were highest in muscle tissues. This may be interpreted either by different metabolic activities in different tissues or by unknown mechanisms of fixation of the pollutants to different matrices.

For gurnard and squid correlations between residue concentration and animal weight were calculated by linear regression analysis. Scallops did not exhibit any correlation, and flatfishes were excluded from the calculation, because only a few animals of similar size were available. In liver and adipose tissue of gurnard positive correlations between residue concentration (wet weight basis) and animal weight seem to exist for some of the pollutants (Tables 5 and 6). Although the number of samples is small, this indicates an increase of residue levels with body size. For squid as an exception see below.

Strong correlations of organochlorine residues to size and / or age were found for DDT, DDD, DDE in Atlantic salmon (Salmo salar) by ANDERSON and FENDERSON (1970), for DDT in lake trout (Salvelinus namaycush) by YOUNGS et al. (1972) and REINERT (1970), for PCB in Cayuga Lake trout by BACHE et al. (1972), and in cod (Gadus morhua) by STENERSEN and KVALVAG (1972) and BJERK (1973). The variation of organochlorine residue levels with age in seals (Pagophilus groenlandicus) was investigated by ADDISON et al. (1973) using the blubber of the animals. The majority of the data reported in the

TABLE 3

Residue concentrations in intestinal adipose tissue (F), liver (L) and skeletal muscle (M) of fishes. Values are averaged from two analyses except when indicated by [a]. w: ppm - wet weight; l: ppm - lipid weight; nd: not detected, detection limit 0.0005 ppm; nm: not measured

Species	Wet weight (g)	Tissue and no. of animals		Lipid (%)	Residue concentration (µg/g)										PCB[c]/ΣDDT
					PCB		DDE		DDD		DDT		ΣDDT[c]		
					w	l	w	l	w	l	w	l	w	l	
Trigla lucerna[a]	174	F	1	73.9	3.3	4.5	0.53	0.71	0.083	0.11	0.54	0.73	1.2	1.6	2.9
	202	F	1	78.9	2.3	2.9	0.49	0.62	0.076	0.10	0.48	0.61	1.1	1.3	2.2
	249	F	1	78.4	2.2	2.8	0.41	0.52	0.057	0.073	0.39	0.49	0.85	1.1	2.6
	312	F	1	81.1	2.7	3.4	0.39	0.47	0.081	0.10	0.23	0.28	0.70	0.86	3.9
	320	F	1	76.5	4.9	6.4	0.46	0.60	0.14	0.18	0.59	0.78	1.2	1.6	4.1
	571	F	1	86.7	4.8	5.6	0.74	0.85	0.12	0.14	0.41	0.48	1.3	1.5	3.8
	808	F	1	87.7	5.7	6.5	1.2	1.4	0.10	0.12	0.26	0.29	1.6	1.8	3.6
Trigla lucerna[a]	14[b]	L	4	8.1	0.19	2.4	0.021	0.26	0.005	0.064	0.005	0.064	0.031	0.38	6.3
	66[b]	L	3	12.4	0.18	1.4	0.022	0.17	0.014	0.11	0.026	0.21	0.061	0.49	2.9
	103[b]	L	2	22.6	0.38	1.7	0.062	0.27	0.015	0.07	0.030	0.13	0.11	0.47	3.5
	174	L	1	25.9	0.96	3.7	0.14	0.54	0.047	0.18	0.10	0.38	0.29	1.1	3.3
	202	L	1	14.2	0.30	2.1	0.057	0.40	0.015	0.11	0.017	0.12	0.089	0.63	3.3
	249	L	1	21.7	0.34	1.6	0.047	0.21	0.021	0.10	0.091	0.42	0.16	0.73	2.1
	312	L	1	35.8	1.0	2.8	0.13	0.35	nm	nm	nm	nm	nm	nm	nm
	320	L	1	24.0	1.7	6.9	0.044	0.18	0.027	0.11	0.096	0.40	0.17	0.69	10
	571	L	1	35.4	0.78	2.2	0.067	0.19	0.039	0.11	0.11	0.31	0.22	0.61	3.6
	808	L	1	29.3	1.4	4.8	0.25	0.87	0.081	0.28	0.15	0.51	0.48	1.7	2.9

TABLE 3 (continued)

Trigla lucerna	14[b]	M	4	0.53	0.048	9.1	0.005	0.89	0.001	0.21	0.004	0.67	0.009	1.8	5.1
	66[b]	M	3	0.33	0.063	19	0.004	1.3	0.001	0.38	0.005	1.5	0.010	3.2	6.0
	103[b]	M	2	0.31	0.032	10	0.002	0.68	nd	nd	0.003	0.89	0.005	1.7	6.0
	174	M	1	0.25	0.046	18	0.004	1.5	0.001	0.50	0.006	2.2	0.011	4.2	4.3
	202	M	1	0.43	0.040	9.3	0.003	0.70	0.001	0.20	0.004	0.97	0.008	1.9	5.0
	249	M	1	0.52	0.057	11	0.004	0.66	0.001	0.23	0.006	1.1	0.010	2.0	2.0
	312	M	1	0.64	0.037	5.8	0.004	0.59	0.001	0.20	0.004	0.63	0.009	1.4	4.1
	320	M	1	0.43	0.048	11	0.002	0.55	0.002	0.53	0.008	1.8	0.012	2.9	3.9
	571	M	1	0.50	0.047	9.3	0.003	0.55	0.002	0.31	0.004	0.80	0.008	1.7	5.6
	808	M	1	1.3	0.12	9.5	0.012	0.90	0.003	0.24	0.010	0.72	0.025	1.9	5.1
Pleuronectes platessa	400	L	1	36.9	2.2	5.9	0.13	0.35	0.12	0.33	0.21	0.57	0.46	1.3	4.7
	427	L	1	16.0	1.1	7.1	0.083	0.52	0.048	0.30	0.029	0.18	0.16	1.0	7.1
	637	L	1	20.0	0.40	2.0	0.037	0.19	0.035	0.18	0.048	0.24	0.12	0.61	3.3
	400	M	1	0.42	0.039	9.2	0.002	0.43	0.001	0.27	0.004	1.1	0.007	1.8	5.2
	427	M	1	0.31	0.023	7.3	0.001	0.35	nd	nd	0.001	0.45	0.003	0.84	8.7
	637	M	1	0.25	0.014	5.6	0.001	0.21	nd	nd	0.002	0.73	0.003	1.0	5.6
Scophthalmus rhombus	1695	L	1	30.8	2.8	9.2	0.27	0.88	0.14	0.44	0.49	1.6	0.90	2.9	3.2
	1695	M	1	0.08	0.025	32	0.001	1.6	nd	nd	0.003	4.1	0.005	6.3	5.2

[a] Single analysis.
[b] Average wet weight.
[c] Calculated before rounding of values.

TABLE 4

Residue concentrations in digestive gland (DG), adductor muscle (AM) and mantle musculature (MM) of pooled molluscs. Values are averaged from two analyses except when indicated by [a]. w: ppm - wet weight; l: ppm - lipid weight; nd: not detected, detection limit 0.0005 ppm

Species	Average wet weight[b] (g)	Tissue and no. of animals		Lipid (%)	Residue concentration (μg/g)										PCB[c]/ΣDDT
					PCB		DDE		DDD		DDT		ΣDDT[c]		
					w	l	w	l	w	l	w	l	w	l	
Chlamys opercularis[a]	1.9	DG	13	3.6	0.069	1.9	0.009	0.25	0.010	0.28	0.025	0.70	0.044	1.2	1.6
	4.5	DG	16	8.9	0.24	2.7	0.043	0.48	0.006	0.070	0.023	0.25	0.071	0.81	3.4
	7.5	DG	11	9.6	0.11	1.2	0.017	0.18	0.033	0.34	0.035	0.37	0.086	0.89	1.3
	10.4	DG	6	9.1	0.20	2.2	0.042	0.46	0.009	0.97	0.026	0.29	0.077	0.85	2.7
Chlamys opercularis	1.9	AM	13	0.13	0.022	17	0.001	0.53	nd	nd	0.006	4.3	0.007	5.0	3.4
	4.5	AM	16	0.34	0.030	9.0	0.001	0.42	nd	nd	0.001	0.30	0.003	0.84	11
	7.5	AM	11	0.17	0.048	29	0.002	1.4	0.001	0.78	0.003	1.7	0.007	3.9	7.4
	10.4	AM	6	0.16	0.035	22	0.002	1.1	nd	nd	0.001	0.81	0.003	2.1	11
Loligo forbesi	260	MM	4	1.2	0.18	15	0.012	0.99	0.012	1.0	0.035	2.9	0.059	4.9	3.1
	365	MM	4	1.0	0.17	17	0.012	1.1	0.002	0.23	0.014	1.4	0.029	2.7	6.0
	533	MM	4	1.1	0.10	9.1	0.005	0.47	0.001	0.13	0.007	0.66	0.014	1.3	7.3
	1030	MM	2	1.2	0.11	8.9	0.006	0.49	0.003	0.25	0.013	1.1	0.021	1.8	4.9
	1420	MM	1	0.91	0.083	9.0	0.004	0.47	0.001	0.09	0.007	0.76	0.012	1.3	6.8

[a] Single analysis.
[b] Soft parts in the case of Chlamys opercularis.
[c] Calculated before rounding of figures.

TABLE 5

Trigla lucerna. Coefficient r of correlation between residue concentration in liver and animal wet weight. N=9; P: Level of significance; ns: not significant

	Wet weight basis		Lipid weight basis	
	r	P	r	P
PCB [a]	0.65	ns	0.43	ns
DDE	0.73	<0.05	0.58	ns
DDD	0.87	<0.01	0.75	<0.02
DDT	0.85	<0.01	0.71	<0.05
ΣDDT	0.85	<0.01	0.75	<0.02

[a] calculated as Clophen® A60.

TABLE 6

Trigla lucerna. Coefficient r of correlation between residue concentration in visceral adipose tissue and animal wet weight. N=7; P: Level of significance; ns: not significant

	Wet weight basis		Lipid weight basis	
	r	P	r	P
PCB [a]	0.81	<0.05	0.69	ns
DDE	0.92	<0.01	0.88	<0.01
DDD	0.41	ns	0.28	ns
DDT	-0.53	ns	-0.59	ns
ΣDDT	0.70	ns	0.51	ns

[a] calculated as Clophen® A60.

literature is not readily comparable with our findings, because they refer to analyses of whole fish instead of tissues, but in some cases, when lipids were included in the calculations (REINERT 1970, ANDERSON and FENDERSON 1970, ADDISON et al. 1973), similar results were obtained in various species. Even though there are contradictory findings in the literature regarding the correlation of residue data with the lipid content of the organisms, the importance of lipids as the principal repository for organochlorines cannot be overlooked. Misinterpretations might be avoided, if the condition factors of the fish were considered, as discussed for instance by ANDERSON and FENDERSON (1970). In the case of DDT, REINERT (1970) found that in lake trout residue levels based on whole fish increased with animal size, but that residue concentrations based on lipid weight reached a steady state when the fish had grown to a certain size.

Regarding these findings, it is necessary to examine whether a rising lipid content of the growing animal is the only cause of a positive correlation between residue level and size. Since in some cases coefficients of correlation between residue concentration on lipid weight basis and body weight are significant within the 95 % confidence limits (Tables 5 and 6), the age of the animal is shown to be important.

In squid the residue concentrations in muscle (wet weight basis) tended to decline with increasing animal size, however, the correlation coefficients $\underline{r}$=-0.83 (PCBs) and $\underline{r}$=-0.65 (ΣDDT) are not significant within the 95 % confidence limits. At the moment and on the basis of the scarce material a detailed discussion is premature.

Concentrations of PCB were always higher than those of ΣDDT as indicated by PCB/ΣDDT ratios between 3 and 7 in most cases.

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