Levels of Toxaphene Indicator Compounds (Chlorobornanes) in Fish

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1. Introduction

Toxaphene is probably the most complex pesticide and much more than one million tons of it have been used since 1946[1]. Toxaphene is a mixture consisting of polychlorinated monoterpenes, predominantly chlorobornanes. Minor constituents are chlorinated bornenes and camphenes. Owing to its widespread use and environmental stability, toxaphene residues have been identified in environmental matrices, including air, freshwater/sea water and soil. The highest concentrations of this fat-soluble pesticide have been detected in aquatic animals, particularly dolphins, whales and fish[2,3].
Toxaphene was found to have acute and chronical toxicity for aquatic and wildlife and may be a carcinogenic risk to humans[2]. An investigation into its environmental relevance according to ecotoxicological criteria led to a first ban of toxaphene in the Federal Republic of Germany in 1980 and in the USA in 1982[2]. In 1993 all toxaphene action levels are revoked by FDA[4], that means residues of toxaphene should not be present in any food items in the USA. The German maximum residue limit (MRL) is 0.1 mg polychloroterpenes per kg animal fat. Due to the fact that the laboratories could not determine toxaphene residues, the MRL for polychloroterpenes in Germany was established at 0.1mg/kg animal fat. Recently, a new analytical procedure based on the three indicator compounds **1 - 3**:

Indicator compound **1**: 2-exo,3-endo,5-exo,6-endo,8b, 8c,10a,10b-octachlorobornane
Indicator compound **2**: 2-exo,3-endo,5-exo,6-endo,8b, 8c,9c,10a,10b-nonachlorobornane
Indicator compound **3**: 2,2,5,5,8b,8c,9c,10a,10b-nonachlorobornane

was developed and validated in a collaborative study[5]. Marine animals are probably the main source of human toxaphene intake. Since especially the chlorobornanes **1 - 3** are also accumulated in humans, they have been determined in remarkable concentrations in human milk samples from populations with special food habits[6]. Therefore fish has to be taken into consideration when MRLs are established. For this reason we determined the chlorobornanes **1 - 3** in various fish species from different fishing areas.

2. Analytical Procedures

Determination of toxaphene residues in environmental matrices and food is very difficult. Most problems arise from the complex composition of toxaphene and the substantial differences in peak patterns in environmental samples as compared with the technical product.
Earlier GC/ECD methods often used an extensive cleanup combined with acid or alkaline treatment (or other 'derivatization' steps). In the presence of further chlorinated compounds adequate results often could not be achieved. Recently, the method predominantly mentioned in conjunction with determinations of toxaphene has been based on the more selective ECNI-MS detection, using technical toxaphene as a standard. Nevertheless, this method often overestimates the true residue because of differences between the response factors of chlorobornanes in samples and standards[7]. Moreover, reproducibility is poor on account of the influence of certain instrument parameters and the complexity of technical toxaphene.
To develop a method allowing an unambiguous identification and simple quantitative characterisation of toxaphene residues, we identified those chlorobornanes, which were present in marine fish in high concentrations. These chlorobornanes **1 - 3** are proposed as indicator compounds for toxaphene residues[7].

3. Experimental

Marine fish samples were caught by a research vessel or were supplied by Staatliches Veterinäramt Bremerhaven, Germany. Pooled samples of about five fishes caught together were used in each case.
Fat extractions were performed using the AOAC method 970.52 L(e), which has been specifically developed for extraction of organochlorine pesticide multiresidues from fish[8]. Alternatively, samples were extracted with dichloromethane/methanol according to a modified Blight and Dyer method[9].
The cleanup of the fat extracts and the gas chromatographic determination of toxaphene indicator compounds with ECD followed the same procedure as described elsewhere[5]. During ECNI-MS measurements, signals at 340, 341, 342, 343, 375, 377, 411, 415, 447 and 449 amu were recorded. In the case of EI-MS detection, fragment ions m/z 159, 231, 305 and 339 were used for quantification.
The individual toxaphene congeners are available from Promochem (Wesel, Germany) or Ehrenstorfer (Augsburg, Germany).

4. Results and Discussion

Typical ECD chromatograms of the lower polarity pesticide/PCB fraction and the more polar pesticide fraction are shown in Figures 1 and 2. A second chromatographic run on DB-1301 has shown that closely eluting or coeluting compounds could be excluded. Nearly all quantitative results were validated by ECNI-MS or EI-MS detection.
The results of the determination of toxaphene indicator components **1 - 3** are listed in the attached table.
High amounts of toxaphene indicator compound (based on wet weight) were determined especially in halibut, redfish, salmon, herring and mackerel. This is in agreement with the higher concentrations of other persistent organochlorines (PCBs, DDE, DDT) in fish species with a higt fat content. Lower differences between fish species are found when chlorobornane residue data are based on the fat content.

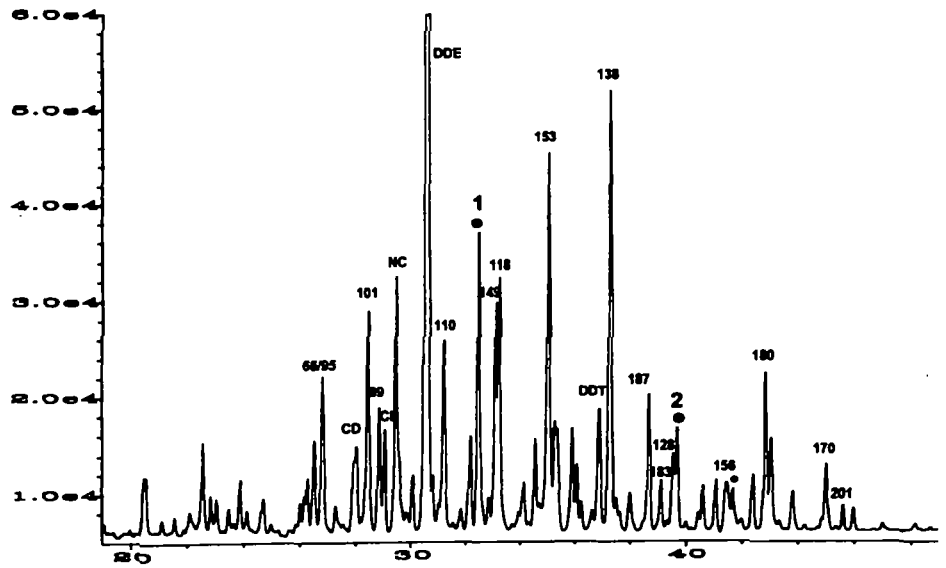

Figure 1: Gas chromatogram of the first silica gel fraction (hexane eluate) of a mixed marine fish sample recorded on DB-5 (numbers indicate PCB congeners, CD and NC indicate chlordane or nonachlor peaks; significant chlorobornanes are indicated by ●)

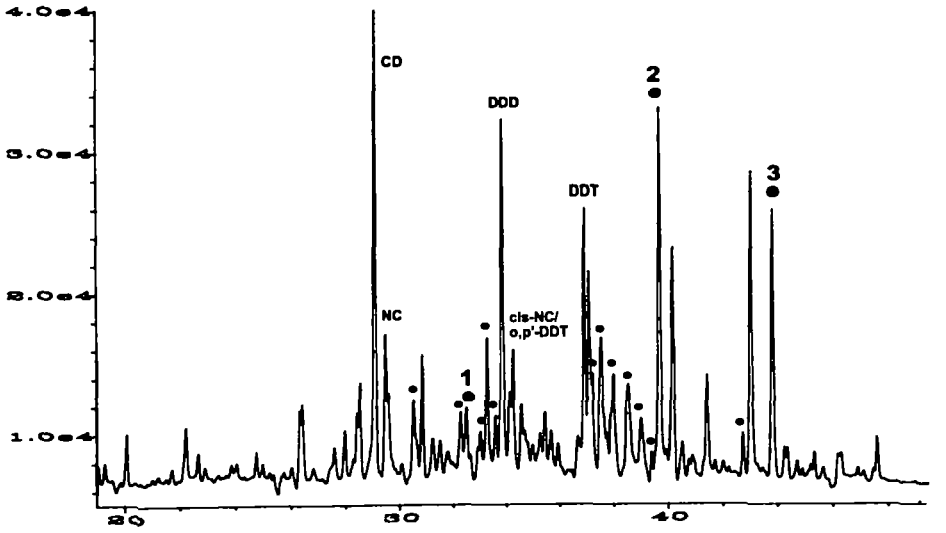

Figure 2: Gas chromatogram of the second silica gel fraction (hexane/toluene 65/35 eluate) of a mixed marine fish sample recorded on DB-5. (numbers indicate PCB congeners, CD and NC indicate chlordane or nonachlor peaks; significant chlorobornanes are indicated by ●)

No.	Species	Fishing Area	Fat (%)	Ind. 1 (µg/kg fat)	Ind. 2 (µg/kg fat)	Ind. 3 (µg/kg fat)	1-3 total (µg/kg fat)	Ind. 1 (µg/kg wet wt.)	Ind. 2 (µg/kg wet wt.)	Ind. 3 (µg/kg wet wt.)	1-3 total (µg/kg wet wt.)
1	Halibut	near Iceland	20,58	113	131	120	**364**	23	27	25	**75**
2	Halibut	near Iceland	6,90	119	210	171	**500**	8	15	12	**35**
3	Halibut	near Norway	12,00	50	78	61	**189**	6	9	7	**23**
4	Herring	central North Sea	5,69	142	265	206	**613**	8	15	12	**35**
5	Herring	near Norway	19,80	34	81	55	**170**	7	16	11	**34**
6	Herring	near Shetland I.	6,97	81	176	145	**402**	6	12	10	**28**
7	Herring	near Ireland	13,75	35	89	57	**181**	5	12	8	**25**
8	Herring	near Norway	18,90	25	43	34	**102**	5	8	6	**19**
9	Herring	Baltic Sea	10,20	54	70	40	**164**	6	7	4	**17**
10	Herring	Baltic Sea	7,91	68	84	53	**205**	5	7	4	**16**
11	Herring	Baltic Sea	5,03	98	109	51	**258**	5	5	3	**13**
12	Herring	central North Sea	9,10	41	58	31	**130**	4	5	3	**12**
13	Herring	near Ireland	10,40	25	48	37	**109**	3	5	4	**11**
14	Herring	Baltic Sea	8,30	44	52	36	**132**	4	4	3	**11**
15	Herring	central North Sea	5,12	50	80	53	**183**	3	4	3	**9**
16	Herring	Baltic Sea	4,29	67	78	38	**183**	3	3	2	**8**
17	Herring	near Ireland	7,56	21	45	21	**87**	2	3	2	**7**
18	Herring	central North Sea	8,20	19	31	9	**59**	2	3	1	**5**
19	Herring	North Sea (Skagerrak)	18,10	8	11	n.d.	**19**	2	2	n.d.	**4**
20	Herring	central North Sea	5,30	21	28	12	**61**	1	1	1	**3**
21	Herring	North Sea (Skagerrak)	18,00	n.d.	11	n.d.	**11**	n.d.	2	n.d.	**2**
22	Herring	North Sea (Skagerrak)	13,90	n.d.	7	n.d.	**7**	n.d.	1	n.d.	**1**
23	Herring	central North Sea	7,11	59	143	110	**312**	4	10	8	**22**
24	Herring	central North Sea	13,90	29	63	47	**139**	4	9	7	**19**
25	Herring	central North Sea	14,83	28	54	37	**119**	4	8	6	**18**
26	Herring	central North Sea	13,20	23	45	36	**103**	3	6	5	**14**
27	Herring	central North Sea	19,72	10	19	n.d.	**28**	2	4	n.d.	**6**
28	Herring	central North Sea	23,70	6	10	n.d.	**16**	2	2	n.d.	**4**
29	Redfish	near Iceland	4,5	153	216	337	**706**	7	10	15	**32**
30	Redfish	near Iceland	4,53	144	264	273	**681**	7	12	12	**31**
31	Redfish	North Atlantic	6,69	61	119	123	**304**	4	8	8	**20**
32	Redfish	near Norway	5,03	50	97	76	**223**	2	5	4	**11**
33	Redfish	North Atlantic	1,95	62	113	87	**262**	1	2	2	**5**
34	Salmon	near Iceland	23,70	27	38	27	**93**	7	9	7	**22**
35	Salmon	fjord in Norway	24,60	17	36	25	**78**	4	9	6	**19**
36	Salmon	fjord in Norway	18,30	19	32	22	**74**	3	6	4	**13**
37	Salmon	near Ireland	5,10	31	59	35	**125**	2	3	2	**6**
38	Salmon	near Chile	13,60	4	6	n.d.	**11**	1	1	n.d.	**1**
39	Makarel	nothern North Sea	21,44	23	30	26	**79**	5	6	6	**17**
40	Makarel	soutern North Sea	27,90	9	18	14	**41**	3	5	4	**11**
41	Makarel	nothern North Sea	10,60	16	32	34	**82**	2	3	4	**9**
42	Makarel	nothern North Sea	17,80	12	18	14	**44**	2	3	2	**8**
43	Makarel	nothern North Sea	10,58	17	28	26	**71**	2	3	3	**8**
44	Makarel	soutern North Sea	13,80	13	23	17	**53**	2	3	2	**7**
45	Makarel	central North Sea	19,10	n.d.	19	n.d.	**19**	n.d.	4	n.d.	**4**
46	Makarel	central North Sea	15,30	n.d.	15	n.d.	**15**	n.d.	2	n.d.	**2**
47	Eel	Baltic Sea	30,80	9	12	9	**30**	3	4	3	**9**
48	Eel	Baltic Sea	18,90	10	13	n.d.	**23**	2	3	n.d.	**4**
49	Eel	Poland	27,00	8	n.d.	n.d.	**8**	2	n.d.	n.d.	**2**
50	Pollock	near Iceland	0,28	126	180	87	**392**	0,4	0,5	0,2	**1,1**
51	Pollock	nothern North Sea	0,58	39	88	20	**147**	0,2	0,5	0,1	**0,9**
52	Pollock	North Sea	0,28	47	71	51	**169**	0,1	0,2	0,1	**0,5**
53	Alaska pollack	Pacific, Northwest	0,22	37	68	14	**119**	0,1	0,1	0,0	0,3
54	Alaska pollack	Artic Sea	0,16	27	79	16	**122**	0,0	0,1	0,0	0,2
55	Cod	near Iceland	0,23	145	253	102	**500**	0,3	0,6	0,2	1,2
56	Boniten	Gulf of Oman	0,12	20	123	52	**195**	0,0	0,1	0,1	0,2
57	Hake	Pacific, Southeast	0,71	4	9	6	**19**	0,0	0,1	0,0	0,1

If disregarding some low concentrations of indicator compounds in herring caught in the Skagerrak (No. 19, 21, 22) a dependence on residue concentration from the fishing area could not be detected. Otherwise, an influence of fish length (as parameter for age) on toxaphene amount was found for herring samples 23 - 28. In this group of selected herring samples caught together in the northern part of the North Sea the mean length was 33.25, 31.75, 30.5, 29.5, 26.0, and 24.75cm, respectively. Based on wet weight, the total amounts of chlorobornanes **1** - **3** were rising with length from 3.8 to 22.2 µg/kg (see table). Most individual indicator concentrations increased in the same order.
Although the table does not contain data on PCBs and chlorinated pesticides, it should be mentioned that the total amount of chlorobornanes **1** - **3** often exceeded the total amount of PCBs 138, 153 and 180 or that of cis-chlordane and trans-nonachlor. This demonstrates the special importance and suitability of these toxaphene components for residue analysis.

5. Conclusion

Residues of toxaphene were detected in all fish samples analysed. For the sum of the indicator compounds **1** - **3**, levels up to 0.5, 0.58 and 0.7mg/kg fat were found in halibut, herring and redfish, respectively. The concentrations of indicator compounds measured were in the range well known for PCBs in fish.
Determination of the indicator chlorobornanes **1** - **3** by GC/ECD is simple, allows an easier confirmation and reproducible quantitation. Systematic errors resulting from the utilization of different technical standards or detectors are eliminated. Using this procedure, data from different laboratories are really comparable and time trends in residue situation are better detectable. From these reasons the establishment of maximum residue limits should be based on these indicator compounds.

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7. References

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