PEAK PATTERNS OF CHLOROSTYRENES IN FISH AND FISH OILS FROM THE NORTH ATLANTIC USING NEW SYNTHETIC CONGENERS

Mehmet Coelhan, Iris Reil, and Harun Parlar

Lehrstuhl für Chemisch-Technische Analyse und Chemische Lebensmitteltechnologie, Technische Universität München, D-85350 Freising-Weihenstephan, Germany, Phone: +49-(0)8161-713284, Fax: +49-(0)8161-4418, Email: parlar@weihenstephan.de

Introduction

Octachlorostyrene (OCS, **11**, see Table 1) was first found in the environment in birds from the river Rhine and the Netherlands coastal area and later in fish and herons[1,2]. In the marine environment, OCS has been detected together with hexa- and heptachlorostyrene as a local pollutant in the Frierfjorden, Norway[3], and also in fish, water, and sediments from the North Sea and adjacent estuaries[4]. In the earlier 1980s, OCS and related compounds were found in considerable amounts in different parts of the Great Lakes[5].

The local source of Norwegian polychlorinated styrenes (PCS) is magnesium production, and these substances have been identified in samples of water, sediment, fish, and bird's eggs as well as blood from workers in the factory and humans with high consumption of fish[6]. As has been shown by Lahaniatis et al.[7], thermolysis of various chlorinated hydrocarbons, such as trichloroethylene, pentachlorobenzene, or pentachlorophenol, at temperatures between 600 and 800°C seems to be another source of octachlorostyrene (**11**). While toxicological relevance for humans is still questionable with OCS and investigations of toxicity of other PCS remain to be done, indications of a high bioaccumulation potential, a high toxicity for some aquatic organisms and promotor characteristics have been found[8,9].

Generally, penta- to octachlorostyrenes have been found in the aquatic environment. In most cases, only octachlorostyrene (**11**) was quantified, while the other chlorostyrenes were only occasionally detected[10]. The synthesis of hexa- and heptachlorostyrenes with fully chlorinated phenyl ring made the first standards of these groups available[11], while chlorostyrenes with a tetrachlorophenyl nucleus, especially **3**, **4**, and **6**, are still lacking as single substances. In spite of the great number of reports on residues of chlorostyrenes in the environment, no systematic study of residue composition has been executed. Indications for variations in peak patterns of PCS in fish from the river Elbe have been found with the residue composition depending on the sampling site[10], but no comparable data exist for samples from the Atlantic region. Therefore, we compared the peak patterns of chlorostyrenes in different fish species from different parts of the Atlantic and in several fish oils[12].

Experimental Section

Materials: Octachlorostyrene (**11**) was synthesized according to the method of Bieniek and Korte[19]. All other chlorostyrenes (see Table 1), except **3**, **4**, and **6**, were prepared according to the method of Kolsaker et al.[11]. For the quantification of **3**, **4**, and **6**, a new standard mixture was

prepared in the following way. 300 mg of $LiAlH_4$ in 20 ml diethyl ether was added dropwise during 2 hours to 1.14g (0.03 mol) of octachlorostyrene (**11**) in 30 ml of diethyl ether. The reaction was controlled by HRGC-MS analysis of samples taken every hour during the first 8 hours. After 48h, 20 ml of MeOH and 50 ml H_2O were added. The ether phase was dried over Na_2SO_4 and completely evaporated. The remaining oil was dissolved in a small volume of petroleum ether and purified by chromatography on a 1m silica gel column (70-230 mesh). After evaporation of the solvent, ca. 0.5g of a chlorostyrene mixture (see Figure 1a) remained. A final determination of the structure of the components **3**, **4**, and **6** was not possible. According to retention times in analogy to chlorinated biphenyls[13], it was assumed that **3** is the *p*-, **4** is *m*-, and **6** is the *o*-isomer of octachlorostyrene (**11**) monodechlorinated at the nucleus. All three components were quantified with **7** as external standard. All solvents were of purity grade for residue analysis. Na_2SO_4 was from Merck, Germany.

Tab. 1. Chlorostyrenes and their analytical parameters

Chlorostyrene (No.)	UV λ_{max} (nm)/ (log ε) in ethanol*	IR ($\nu_{olefine}$ C=C)*	EIMS $M^{+\bullet}$ and $(M-Cl)^+$	HRGC t_R (min)	Relative response factors EIMS-SIM $[M-HCl]^{+\bullet}$ or $[M-HCl_2]^+$
2,3,4,5,6-Pentachloro- (**1**)	221 (4.64)	–	274, 239	16.05	0.38
α,2,3,4,5,6-Hexachloro- (**2**)	216 (4.80)	1634	308, 273	19.55	0.66
α,β,β,2,3,5,6-Heptachloro- (**3**)	–	–	342, 307	20.13	
α,β,β,2,4,5,6-Heptachloro- (**4**)	–	–	342, 307	20.35	
(Z)-β,2,3,4,5,6-Hexachloro- (**5**)	213 (4.65)	1623	308, 273	22.34	0.48
α,β,β,2,3,4,5-Heptachloro- (**6**)	–	–	342, 307	22.58	
(E)-β,2,3,4,5,6-Hexachloro- (**7**)	227 (4.55)	1614	308, 273	24.00	0.42
(Z)-α,β,2,3,4,5,6-Heptachloro- (**8**)	216 (4.81)	1616	342, 307	24.32	0.79
β,β,2,3,4,5,6-Heptachloro- (**9**)	217 (4.70)	1616	342, 307	25.16	0.92
(E)-α,β,2,3,4,5,6-Heptachloro- (**10**)	216 (4.81)	1652	342, 307	27.30	0.95
Octachloro- (**11**)			376, 341	28.02	1.00

* from reference 11

Samples: Cod liver oils and fish oils were obtained from Germany, England, the USA, and Iceland. Fish (pool samples of 5-10 specimen per species) from different fishing areas was provided by the National Research Institute for Fisheries, Hamburg, Germany. The samples were kept below -18°C until use.

Clean-up of the fish oils: 2 g of the oil were dissolved in 10 ml sulfuric acid at 50°C for 30 min. 10 ml n-hexane was added and the mixture was centrifuged for 10 min (2000 U/min). The n-hexane phase was separated and reduced to 0.5 ml by an N_2-stream at room temperature. For the elimination of interfering substances and the rest of the oil (ca. 0.5%), a simple column chromatography was used. Mini columns were prepared with 1g silica gel 60 (70-230 mesh, activated at 140°C for 24h and then deactivated with 1.5% water) and ca. 0.5 cm anhydrous Na_2SO_4. The chlorostyrenes were in the first 3.6 ml of the eluent. This fraction was totally dried by an N_2-stream

and redissolved in 1 ml cyclohexane containing 40 pg/µl of pentachloronitrobenzene as injection standard.

Clean-up of the fish samples: 15-30 g fish was minced with 60-120 g anhydrous Na_2SO_4 to fine powder and Soxhlet extracted with n-hexane/acetone (1:1). After evaporation of the organic solvent, the extract was purified and transferred to cyclohexane in the same way as in the case of the fish samples.

HRGC-EIMS: All routine analyses were carried out with an HP 5890 Series III GC that was coupled to a Finnigan MAT 8200 MS. The column used was a DB5, 30m, i.d. 0.25 mm, film thickness 0.25 µm, and the GC conditions used were as follows: carrier gas He, flow rate 1.15 ml/min; temperature program: 50°C (1 min) → 150°C (70°C/min) (5 min) → 195°C (2°C/min) → 260°C (10°C/min) (15 min); splitless (1.5 min)/split injection, injection block 260°C, transfer line 230°C, ion source temperature 240°C. The emission current was 1 mA. The EI-SIM measurements were performed at 70 eV. The ions for identification and quantification were 238, 240, 271, 272, 273, 274, 342, and 344.

Results and Discussion

In all 20 samples, at least some of the components could be detected. Generally concentrations in fish oils were lower than in fish. The highest total residues (>70 ng/g fat) in fish were found in three samples from the North Cape (Sample 12) and from the North Sea (Samples 13 and 20), and medium values (ca. 30-40 ng/g fat) in five samples from Spitsbergen (Sample 11), Southern Ireland (Sample 14), Eastern Greenland (Sample 16), Iceland (Sample 17), and the Northern Shetlands (Sample 10). Mean residue values for the single chlorostyrenes are shown in Figure 1. Main components were E-β,2,3,4,5,6-hexa- (**7**) and octachlorostyrene (**11**) with amounts of ca. 0.4 to 40 ng/g fat, which represent between 30 and 100% of the total residues. Next to them is α,2,3,4,5,6-hexachlorostyrene (**2**), which has been detected in at least 13 of the 20 samples, though in lower concentrations (ca. 0.1-9 ng/g fat). All other components were found in only 3-10 of the 20 samples in concentrations of ca. 0.2-13 ng/g fat. The concentrations of **7** and **11** show approximately the same trend ($R^2 = 0.866$), while no correlation of the concentration of **11** with that of other components can be seen. The peak patterns of all samples investigated are similar only as far as that **7** and **11** are dominating in fish samples, independent of the total amount of chlorostyrenes, while either **11** or **7** or **1** are the main components in fish oil samples. Because reduction by $LiAlH_4$ in diethyl ether, which with some restrictions may simulate the tendency of formation of lesser chlorinated hydrocarbons from **11** in the environment, gave mainly the heptachlorostyrenes **3**, **4**, and **8** instead of the hexachloro components **2** and **7** and because hexachlorostyrenes, e.g. 2 and 7, were not detected in activated sludge[14], probably all chlorostyrenes found in the fish samples are formed directly by technical processes, but not or only in minor amounts in the environment.

An evaluation of water pollution or distribution pathways can be made only with restrictions because of the small number of samples, of which only the fish samples can be considered, and the lack of biological information. Fish samples from the northern European coastal areas generally showed slightly higher concentrations (calculated in fresh weight) than those from the western parts of the North Atlantic. This may reflect input from industrial countries via coastal areas and streams (North Sea, Norway, Great Britain) as well as long or short range transport by the main oceanic streams (North Cape). To distinguish between these two factors more data are needed, especially from the eastern coast of the USA and Canada. Nevertheless, comparison with residues found *in*

the river Elbe, which were in the range of ca. 0 to 45 ng/g fresh weight[10], indicate an input of chlorostyrenes mainly via streams.

The results of this work show that only two of the single PCS, (E)-β,2,3,4,5,6-hexachlorostyrene (**7**) and octachlorostyrene (**11**), are found in higher amounts in marine fish and fish oil samples. Whether the chlorostyrene patterns found in fish and fish oils are typical only for these types of samples or may be found also in other marine samples, has to be decided in future on the basis of more data especially of water, sediment, and other biotic samples.

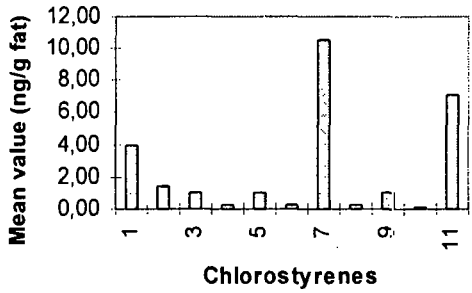

Figure 2: Mean residues of single chlorostyrenes (in ng/g fat) in fish and fish oil samples from the North Atlantic

Acknowledgement
This work was financially supported by the Fond der Chemischen Industrie.

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