LEVELS OF EXTRACTABLE ORGANICALLY BOUND CHLORINE AND IDENTIFIED ORGANOHALOGEN POLLUTANTS IN BALTIC SEA HERRING OIL, ITS FRACTIONS AND RAT LIVER.

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Introduction

Studies of halogenated organic pollutants in the environment and measurement of the extractable organically bound halogen (EOX) have shown that identified compounds constitute a small part of total EOX. In fish, between 0.1 and 16 % of extractable organically bound chlorine (EOCl) has been chemically identified as well-known contaminants and previous results suggest that a considerable portion of the remaining, EOCl consists of esterbound chlorinated compounds tentatively identified as chlorinated long-chain fatty acids (1). The toxicity and persistence of halogenated organic pollutants, such as PCBs and DDT are well documented, while the possible toxicity and persistence of the unidentified chlorinated organic compounds remain to be evaluated. By studying the properties of fractions of herring oil it would be possible to further investigate health effects of identified and unidentified chlorinated organic compounds in fish from the Baltic sea. This extended abstract describes congener specific levels of contaminants in herring oil, its fractions and in liver tissue from rats fed herring oil or fractions thereof for 13 weeks. Toxicological data from this study were briefly described earlier (2).

Materials and Methods

In May 1993, Baltic Herring (*Clupea harengus*) was collected from the coastal area of the Baltic Sea outside Karlskrona (N 55°, E 16°) in Sweden. The time of fishing and the size of herrings were chosen to get high levels of contaminants with low variance between individuals. After removal of skin, tail and intestine the fish were homogenised and extracted three times with fresh distilled isopropanol at 65° C.

The fractioning of herring oil was carried out in the Wallenberg perforator, where the oil was allocated with acetonitrile (3). Three fractions (F1, F2 and F3) were obtained. The F1 fraction was supposed to mostly contain triglycerids and free fatty acids, while the two ”fat-free” fractions (F2 and F3) were supposed to contain pollutants in different concentrations. Female Sprague-Dawley rats were fed pellet diets containing different doses of oil or fractions thereof for 13 weeks. In an additional study rats were given low dose of herring oil diet for 6 and 39 weeks.

Lipid extraction of rat livers was carried out by homogenising with acetone in a separatory funnel with glasfilter disk. The homogenate was then pressed under nitrogen atmosphere down to a lower funnel containing 50 ml of 0.1M phosphoric acid in 0.9% sodium chloride. This procedure was

repeated with two 20 ml portions of hexane. After shaking and discarding of the lower phase the organic phase was decanted to a 50 ml weighed Erlenmeir flask and the solvent was evaporated.

The liver lipid extract, fish oil and fractions were analysed for individual CBs and pesticides by using a gaschromatograph (GC) with an electron capture detector. The analysed substances were hexachlorobenzene (HCB), hexachlorocyclohexane (ΣHCH, including α-, β- and γ-), dichlorodiphenyltrichloroethane (ΣDDT, including DDT, DDE, and DDD), and eight chlorinated biphenyls (Σ8CB, including CB28, 101, 105, 118, 138, 153, 156, and 180). Individual PCDD/F congeners were analysed for congeners by the use of gas chromatography/mass spectrometry (GC/MS). The concentration of EOCl was determined with Neutron Activating Analysis (NAA).

Results and Discussion

The herring oil contained 23 μg EOCl /g (Table 1). Of the analysed EOCl nearly one tenth was identified. ΣDDT and Σ8CB were the major contributors and were found in concentrations that were 30 times higher than those of HCB and ΣHCH, and 5600 times higher than the concentration of ΣPCDD/F. About three fourth of the ΣDDT was identified as DDE (data not shown). This is a normal pattern in the Baltic ecosystem (4). The 8CBs were present in the following concentration order: CB28< CB156< CB105< CB180< CB118< CB101< CB138< CB153, ranging from 10 to 310 ng/g (data not shown). This order was similar in both oil and fractions. Among the PCDD/Fs the 2,3,4,7,8-PeCDF was the most abundant congener accounting for 44 % of the ΣPCDD/F content. 2,3,7,8-TCDF accounted for 18 % of the ΣPCDD/F content (data not shown).

The F1-fraction, which constituted 86 % of the fractionated oil, contained 13 μg EOCl /g and of this almost nothing was identified. In comparison to the other fractions, F1 had a higher proportion of ΣPCDD/F. The main congeners among the PCDD/Fs in F1 were OCDD and 2,3,4,7,8-PeCDF, accounting for 55 % and 16 % of the ΣPCDD/F content, respectively.

The highest concentrations of all identified pollutants, including EOCl, were found in fraction F2, which constituted 7 % of the fractionated oil. Of the 241 μg EOCl /g, about one fifth was identified. Almost all the recovered ΣHCH was found in this fraction. Among the PCDD/Fs 2,3,4,7,8-PeCDF was most abundant followed by OCDD and 2,3,7,8-TCDF, accounting for 33, 22 and 21 % of the ΣPCDD/F content, respectively.

The F3-fraction, which constituted 7 % of the fractionated oil, contained 78 μg EOCl /g and nearly one tenth of this was identified. The main PCDD/F congeners were OCDD, 2,3,4,7,8-PeCDF and 2,3,7,8-TCDF accounting for 41, 26 and 11 % of the ΣPCDD/F content, respectively.

TABLE 1. Concentrations of extractable organically bound chlorine (EOCl), identified organohalogen compounds and TEQ content in herring oil and its fractions.

Oil/fraction	EOCl µg/g	HCB ng/g	ΣHCH ng/g	ΣDDT ng/g	Σ8CB ng/g	ΣPCDDs/F pg/g	TEQ[a]
Herring oil	23	41	61	1700	1100	250	130
F 1	13	2	. 2	. 25	100	100	17
F 2	241	460	870	28000	13600	2500	1164
F 3	78	120	. 7	1900	4300	1800	554

[a] According to (5), but based on the analysed congeners.

Using the concept of toxic equivalency factors we calculated equivalent TCDD-concentrations (TEQ)(5). Among the chlorinated dioxins, furans and biphenyls analysed, the congener that contributed with the greatest part of total TEQ in herring oil was 2,3,4,7,8-PeCDF (42 %), followed by CB156 (16 %) and 1,2,3,7,8-PeCDD (14 %) (data not shown). These three congeners were also the most important in F1, F2 and F3. It has been shown that the dioxin-like CB congener with greatest impact on TEQ in Baltic herring is the non-*ortho*-CB 126 (6). However this congener was not analysed in the present study. Assuming the same congener proportions as found in (6), CB126 would contribute with 78 pg TEQ/g in the present herring oil.

Concentrations of all analysed organohalogen pollutants in livers from rats fed the different diets appeared in the following order: Oil> F2> F3> F1, with the exception of ΣPCDD/F (Table 2). The most abundant halogenated pollutants were Σ8CB and ΣDDT. Of the ΣDDT the major congener was DDE, but in rats fed F1-diet DDT was found to be present at a higher concentration (data not shown). The eight CBs were present in the following order: 28< 156< 101< 105< 180< 118< 138< 153 (data not shown). This is the same order as in the diets with exception for CB101. Among the PCDD/Fs in rats fed herring oil diet 2,3,4,7,8-PeCDF was the most abundant congener, accounting for 73 % of the ΣPCDD/F content (data not shown). 2,3,4,7,8-PeCDF was also the most abundant congener in the livers of rats fed the fraction diets. The level of OCDD was substantial, but lower in comparison with the oil and fractions. Comparing the levels of contaminants in oil and fractions in table 1 with the liver concentrations in table 2, the observant reader may question the comparatively high values in livers from rats fed oil and F1 diet. This is explained by the fact that these diets contained the major proportions of the herring oil. The liver values reflect the absolute intake of pollutants.

TABLE 2. Concentrations of identified contaminants in livers of female Sprague-Dawley rats fed high dose diets of herring oil and its fractions for 13 weeks, and the mean intake of TEQ per rat via the food.[a b]

Diet	HCB ng/g	ΣHCH ng/g	ΣDDT ng/g	Σ8CB ng/g	ΣPCDD/F ng/g	TEQ ng/week
Herring oil	55	54	2000	1500	6.4	1.42
F 1	7	9	45	130	2.8	0.13
F 2	39	44	1800	830	7.9	0.85
F 3	13	10	160	360	5.0	0.42

[a] Concentrations are ng/g lipid
[b] According to (4), but only with contribution from the analysed congeners.

In the time-course study, accumulation was observed for HCB, ΣDDT and Σ8CB was observed, whereas no accumulation was observed for ΣHCH (Table 3). Over the course of the last 33 weeks HCB, ΣDDT and Σ8CB increased by 40, 79 and 63 %, respectively.

In summary, this study showed that, when extracted from herring oil by acetonitrile in the Wallenberg perforator, ΣPCDD/F had a slightly different distribution between different fractions of the herring oil, compared to other organohalogen compounds. The analysis of herring oil and its fractions showed that a limited number of congeners contribute to the majority of the total TEQ amount, and that CBs contribute with a substantial part. The present study also showed that contaminants extracted from Baltic fish were taken up by the rats and distributed to the liver. Also, the retention pattern was similar in fish and rats. The toxicological investigation reported by Stern et al. (1998) concluded that the type of observed effects were well in accordance with the effects expected to occur from the identified compounds. However, it remains to be evaluated if the doses of separate pollutants in the fractionated herring oil could account for the observed alterations.

TABLE 3. Concentrations of identified contaminants in liver of female Sprague-Dawley rats fed low dose herring oil diet for 0, 6, 13, and 39 weeks.[a]

Exposure (weeks)	HCB	ΣHCH	ΣDDT	Σ8PCB
0	4	9	<17	39
6	5	17	106	80
13	6	11	120	80
39	7	12	190	130

[a] Concentrations are ng/g lipid

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