Toxicological response in rats fed Baltic Sea Herring oil and its fractions for 13 weeks

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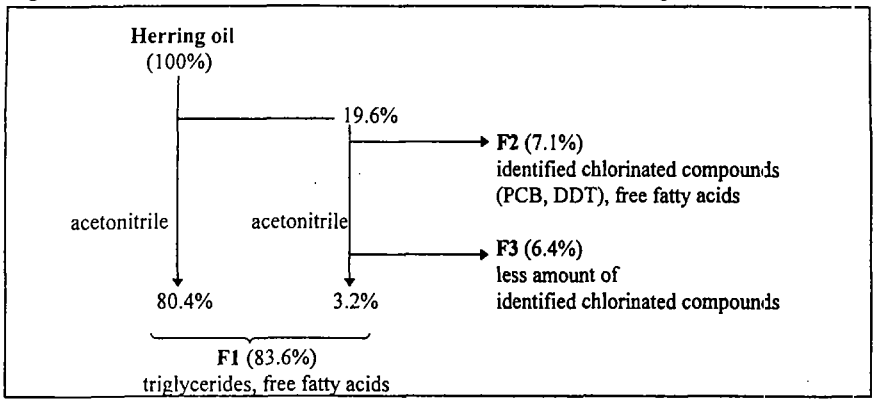
Introduction

Studies of known halogenated organic pollutants in the environment and measurment of the extractable organically bound halogen have shown that identified compounds constitute a small part. In fish, up to 10-15% of extractable organically bound chlorine (EOCl) has been chemically identified. Previous results shows that a considerable portion of the remaining, unidentified EOCl consist of esterbound chlorinated compounds tentatively identified as chlorinated long-chain fatty acids. The toxicity and persistence of known halogenated organic pollutants, such as PCBs and DDT are well documented, while the toxicity and persistence of the unidentified chlorinated organic compounds remain to be evaluated. The present study was initiated to investigate the possible health effects of chlorinated organic compounds in Baltic herring (*Clupea harengus*). Herring was chosen because it is a general food item for humans and therefore relevant from a general health perspective. By fractionating herring oil and mix the oil and its fractions into different diets, given to rats, the aim of the present study was to identify fractions that were responsible for toxicological responses. The fractionation was carried out in a Wallenberg perforator (Fig 1).

Figure 1. Fractionation scheme for separating contaminants in herring oil



Experimental design

The aim of the study was to cover the dose-interval from the NOEL to the real effect level. The dose levels should be comparable to the estimated human intake of 0.21kg fish/week. The low, medium and high dose diets were standardised to about 4, 6 and 14% fat with soy oil. Low, medium and high dose levels of the fish oil were designed to correspond 10, 50 and 225 times as compared to the estimated human fish intake. Groups of 10 SD rats were fed diets supplemented with Baltic Sea Herring fillet oil and its fractions for 13 weeks. Bioaccumulation is a condition to attain biological effects under the low-dose exposure and the additional groups of animals fed the low dose herring fillet diet were killed after 0, 6 and 39 weeks. The studied parameters and used methods are shown in Table 1. For addition to the parameters recommended by the OECD guidelines we have analysed parameters which could be altered by identified chlorinated compounds.

Results

No animals died during the study and no abnormal clinical signs were observed. The parameters that were altered in high-dose groups and expected effects of identified compounds are shown in Table 2. Food consumption was significantly lower in rats fed the high dose of the F1 diet compared to the corresponding controls. Animals in all dietary groups gained weight. However, the body weight gain was significantly decreased in rats fed high-dose F1, F2 and F3 diets as compared to corresponding control groups. Significant microscopically changes occurred in the livers of rats fed all kind of diets and these change were mild but dose-dependent. The lesions observed consisted of lipidosis (fatty change), small foci of degeneration, necrosis and inflammation in hepatic parenchyma and portal triads. Some of these changes, such as hepatic lipidosis, could represent unspecific hepatocyte responses, that can be elicited by various factors, including a high fat content in the diet. Relative liver weight was significantly increased in rats fed herring fillet and F1 diets at the high dose level compared with the corresponding low dose groups. Thymus weight was significantly decreased in rats fed F1 and F3 diets in high dose groups compared with the corresponding controls. The EROD activity was elevated in rats fed Baltic herring fillet diet and F3 diet at the high dose compared with the corresponding controls The PROD activity was weakly depressed in rats fed F3 diet at high dose level. GST and UDP-GT activity in liver was elevated in animals receiving F1 diet and F3 diets at high dose levels compared with the

corresponding controls. The total amount of vitamin A in the liver was significantly decreased in rats fed F1 and F2 at high dose level compared with the corresponding controls. The performed chemical analysis showed that the triglycerid-fraction F1 had the far lowest concentration of both EOCl and identified compounds. F2 had the highest concentrations of known chlorinated pollutants whereas F3 had concentrations of about 30-50% compared to F2 exept for sDDT and sHCH, which were almost absent in F3. The Herring fillet oil and F2 had the same proportions of the contaminants, but the concentrations in Herring fillet oil were only one tenth of those in F2 (Table 3). In the livers of the rats fed high doses for 13 weeks we found highest amounts of contaminants in those fed herring fillet oil followed by those fed F2 (Table 3). The TEQ were 2264 and 1557 pg/liver respectively. F1 and F3 fed rats only reached amounts of about 15-30% of the known contaminants compared to F2. sDDT was even more concentrated in the F2 fed rats. When the differences in fat amount of the livers were taken in account the picture was changed a bit and the concentrations more alike. The concentration (pg/g fat) of TEQ was 2743 and 3199 in livers from rats fed fillet oil and F2 respectively. F3 fed rats had higher concentrations of all analysed compounds as compared to F1 fed rats, but only about half the concentration of F2 fed rats. sDDT was even more concentrated in F2 fed rats.

Conclusions

Toxicological effects, noted in the study, are well in accordance with the effects expected to occur from the identified chlorinated compounds. However, it remains to be discussed if the concentrations of these compounds in the different fractions can account for all the observed alterations

Table 1. The studied parameters

Parameters	
Food consumption	2 times a week
Clinical observations	weekly
Body weight	weekly
Organ weights	liver, spleen, thymus, lungs, kidneys were weighed
Bone weight and density	measure of the bone volume, wet and dry weight (tibia)
Histopatology	formalin fixed tissues embedded in paraffin, sectioned and stained. The tissues examined were: stomach, ileum, colon, urinary bladder, liver, kidneys, adrenal gland, mammary gland, uterus, ovaries, thyroid, parathyroid, skeletal muscle, thymus, heart, lungs, eye and optic nerve, harderian gland, brain and cerebellum.
Hematology	Hematocrit, Hb, erythrocyte count, platelet count, total leukocyte count, MCV, MCH, and MCHC were analysed
Clinical chemistry and biochemistry	ALAT, albumin, ALP, ASAT, bile acids, total bilirubin, calcium, cholesterol, creatinine, free fatty acids, glucose, γ-GT, GLDH, LDH, inorganic phosphate, total serum protein, triglycerides, urea, and uric acid. electrolytes chloride, potassium and sodium were analysed
	Total T4 was determined in plasma
	Ascorbic acid and TBARS were determined in frozen liver.
Hepatic enzyme activities	UDP-glucuronosyl transferase (UDPGT) and glutation-S-transferase (GST) activity were determined
	O-dealkylation of 7-ethoxyresorufin (EROD) and 7-pentoxyresorufin (PROD) were determined in the hepatic S9 fraction
Lipid peroxidation	Lipid peroxidation level in liver was determined
Hepatic vitamin A	Frozen samples of liver, kidneys and lungs were analyzed for vitamin A levels

Table 2. Quantitative parameter alteration for female Sprague-Dawley rats fed diets differing in contents and types of fat for 13 weeks

Parameter	Herring oil	F1	F2	F3
Food consumption	no effect	↓	no effect	no effect
Body weight gain	no effect	↓	↓	↓
Relative thymus weight	no effect	↓	no effect	↓
EROD	↑	no effect	no effect	↑
PROD	no effect	no effect	no effect	↓
UDPGT	no effect	↑	no effect	↑
GST	no effect	↑	no effect	↑
Hepatic vitamin A	no effect	↓	↓	no effect

↑ - increase compared with the corresponding R34 controls
↓ - decrease compared with the corresponding R34 controls

Table 3. Concentrations of EOCl and identified compounds (HCB, HCH, 8CBDDT) (ng/g) in herring oil and its fractions and in livers (ng/g hepatic lipid) for female SD rats, fed high dose diets for 13 weeks

Parameter		Herring oil	F1	F2	F3
EOCl	μg/g oil	23	13	241	78
HCB	ng/g oil	41	2	460	120
	ng/g lipid	*55*	*7*	*39*	*13*
sHCH	ng/g oil	61	≤2	870	≤7
	ng/g lipid	*54*	*9*	*44*	*10*
sDDT	ng/g oil	1700	≤25	28000	1900
	ng/g lipid	*200*	*45*	*1800*	*160*
s8CB's	ng/g oil	1100	100	14000	4300
	ng/g lipid	*1500*	*130*	*830*	*360*
TEQ	pg/g oil	130	17	1163	554
	ng/g lipid	*2743*	*730*	*3199*	*1693*
Σ PCDDs/Fs	pg/g	91	13	680	395
	pg/g lipid	*2702*	*726*	*3169*	*1682*