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Subchronic Toxicity of Baltic Herring Oil and its Fractions in the Rat II: Clinical Observations and Toxicological Parameters

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Abstract: This study aimed to increase the knowledge about the toxicity of fish-derived organohalogen pollutants in mammals. The strategy chosen was to separate organohalogen pollutants derived from Baltic herring (*Clupea harengus*) fillet, in order to obtain fractions with differing proportions of identified and unidentified halogenated pollutants, and to perform a subchronic toxicity study in rats, essentially according to the OECD guidelines, at three dose levels. Nordic Sea lodda (*Mallotus villosus*) oil, with low levels of persistent organohalogen pollutants, was used as an additional control diet. The toxicological examination showed that exposure to Baltic herring oil and its fractions at dose levels corresponding to a human intake in the range of 1.6 to 34.4 kg Baltic herring per week resulted in minimal effects. The spectrum of effects was similar to that, which is observed after low-level exposure to pollutants such as chlorinated dibenzo-*p*-dioxins and dibenzofurans (CDD/F) and chlorinated biphenyls, despite the fact that these contaminants contribute to a minor part of the extractable organically bound chlorine (EOCl). The study confirmed previous findings that induction of hepatic ethoxyresorufin deethylase (EROD) activity takes place at daily intake levels 0.15 ng fish-derived CDD/F-TEQs/kg body weight. The study also demonstrated that hepatic vitamin A reduction takes place at somewhat higher daily exposure levels, i.e. 0.16–0.30 ng fish-derived CDD/F-TEQs/kg body weight. Halogenated fatty acids, the major component of EOCl, could not be linked to any of the measured effects. From a risk management point of view, the study provides important new information of effect levels for Ah-receptor mediated responses following low level exposure to organohalogen compounds from a matrix relevant for human exposure.

The Baltic Sea is an important source of fish for commercial as well as sport/recreational fishermen, in the north of Europe. Due to intensive agricultural and industrial activities as well as long range atmospheric transport, the Baltic Sea has become one of the contaminated water bodies in Europe. Contaminants of major concern include chlorinated dibenzo-*p*-dioxins and dibenzofurans (CDD/F), chlorinated biphenyls (CB), 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT), DDT-metabolites and pesticides such as toxaphene and lindane as well as chlorinated paraffines (Jansson *et al.* 1993). The highest levels are found in fat predatory fish, as well as fish-eating birds and mammals. Fish consumption along the Scandinavian Baltic area has been positively correlated with CB and CDD/F levels in blood and human milk (Asplund *et al.* 1994; Vartiainen *et al.* 1997), suggesting that the general population acquires a substantial amount of these contaminants from fat fish species, such as herring and salmon (Darnerud *et al.* 2000) and that consumption of fatty fish from the Baltic is an important dietary source of persistent organic pollutants. Several studies have focused on the health of fishermen families on the east coast of Sweden, who eat comparatively large quantities of Baltic fish as compared to west coast fishermen families. In these studies it was demonstrated that fishermen from the east cost had higher serum levels of CBs and CDD/Fs compared with the west coast fishermen (Svensson *et al.* 1995a). High consumers of contaminated fish had an increased risk for stomach and skin cancer, although the total cancer risk was not changed (Svensson *et al.* 1995b). Fishermen wives had an increased body burden of CBs that was associated with the risk of low birth weight of their infants (Grimvall *et al.* 1997; Rylander *et al.* 1998).

In addition to the toxicologically well characterised organohalogen pollutants, Baltic fish also contains large amounts of less well characterised organohalogen compounds. During the 1990s it has been established that the majority (85–90%) of the halogenated compounds contributing to the extractable organically bound chlorine (EOCl) complex in fish are halogenated fatty acids (HFA) (Wesén 1995). The toxicity and persistence of halogenated fatty

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acids and other less well characterised compounds in fish, remain to be evaluated in mammals. A single study in rats fed diets containing brominated fatty acids for 35 days, demonstrated enlarged livers and showed elevated liver and heart fat content (Jones *et al.* 1983).

The present study was initiated due to concern about possible toxicological consequences associated with the exposure to these less well characterised organohalogen compounds via the consumption of Baltic fish. Different fractions of Baltic Sea herring (*Clupea harengus*) oil with differing proportions of identified and unidentified pollutants were isolated and chemically characterised. A subchronic toxicity study was performed with rats given oil or its fractions at different doses in the diet. Herring was chosen as the source of pollutants, because it is a common nutrient for humans, and therefore relevant from a general health perspective. This paper describes the subchronic toxicity study in detail, whereas levels of contaminants in the herring oil, its fractions, as well as in the resulting diets and liver tissues from exposed rats are described by Öberg *et al.* (2002).

Materials and Methods

Animals. Female Sprague-Dawley rats, ten weeks of age, obtained from Møllegård breeding centre Ltd., (Skensved, Denmark), were housed five per cage at constant environmental conditions (21–22° room temperature, 50–55% relative humidity, 12 hr light: 12 hr darkness schedule and a 100% fresh air exchange rate of 12–14 complete changes per hour). Rats were allowed to acclimatise five days prior to use. Pelleted food (R34, Lactamin, Sweden) and tap water were available *ad libitum* during the acclimatisation period. The test diets were supplied twice a week at the level of 20 g per day and animal. The experimental protocol, including animal housing and care during the study was approved by the Stockholm Northern Animal Ethic Committee (Stockholm, Sweden).

Feeding and diets. Herring oil was prepared from fish collected from the Baltic Sea outside Karlskrona (55°N/16°E) as described in detail by Öberg *et al.* (2002). The fish oil was fractionated into three fractions using a Wallenberg perforator (Jensen *et al.* 1992). In order to provide a nutritionally equivalent control group, cold pressed oil, with much lower levels of persistent organic pollutants, from Nordic Sea lodda (*Mallotus villosus)* fillet was also included in the study. The test diets (table 1) were prepared from R34 powder mixed with herring oil, its fractions or lodda oil (AnalyCen, Lidköping, Sweden). The fish consumption for Swedish population is estimated to be 0.21 kg fish/week (Becker 1994). This estimate was used in an attempt to relate the amount of fish ingested by the rats to the human fish consumption. To avoid adverse health effects associated with high fat consumption, the fat content in high dose diets did not exceed the 15% level. The fat concentrations in the diets were standardised at each dose level with soy oil to levels determined by the designed doses of the unfractionated oil. The diet containing lodda oil was prepared and tested only at the high dose level.

Chemical analysis of herring oil and its fractions. Fish oils and fractions were analysed for EOCl, HFA, hexachlorobenzene (HCB), hexachlorocyclohexane (ΣHCH, including α-, β- and γ-isomers), ΣDDT, including DDT and the metabolites DDE and DDD, eight chlorinated biphenyls (ΣCB, including CB28, 101, 105, 118, 138, 153, 156 and 180, numbered according to IUPAC), brominated diphenylethers ΣBDE, including BDEs 47, 99 and 100), and all 2,3,7,8-substituted CDD/Fs (ΣCDD/F). The concentrations of CDD/Fs were also summarised as toxic equivalents (CDD/F-TEQ) using the toxic equivalency factors (TEF) proposed by the World Health Organisation (van den Berg *et al.* 1998). In addition, all high dose diets were commercially analysed for vitamin A (AnalyCen AB, Lidköping, Sweden). The methods used for chemical analysis of fish oils and fractions, are fully reported by Öberg *et al.* (2002).

Experimental design and daily consumption of pollutants. Groups of ten rats (start body weight 189±6 g) were fed diets supplemented with Baltic herring oil and its fractions or lodda oil for up to 90 days (table 1). Additional groups of five animals were fed the low dose herring oil diet for 0, 6, and 39 weeks. Food consumption per cage was measured twice a week and was used to estimate the average food consumption per rat. Body weight was measured once a week. During the study the animals were observed regularly for clinical signs. The daily consumption of pollutants was calculated with respect to the results of the chemical analysis of fish oils and fractions, fish fat content of the diets, and the estimated daily food intake.

Toxicological parameters.

Postmortem analysis. The rats were anaesthetised with pentobarbital sodium (Mebumal® vet, ACO, Sweden), administered intraperitoneally at a dose of 150 mg/kg body weight and were bled to death via the abdominal aorta. The blood was collected, centrifuged and serum was frozen at −70° until analyses. Blood samples for haematological analyses were taken using EDTA as anticoagulant and stored cold until analyses. The necropsies were performed immediately after sacrifice. The order of sacrifice was randomised. During the necropsies a thorough macroscopical examination was performed, liver, spleen, thymus, lungs and kidneys were weighed, and specimens and samples for various studies were collected. Portions of liver, lungs and kidneys were frozen and stored at −70° for residue analysis and biochemical determinations.

Bone length and density. The left tibia was used for bone length and density measurement as described by Lind *et al.* (1999).

Histopathological examinations. The following tissues were fixed in buffered, neutral formaline solution and were processed for routine histology: adrenal gland, brain and cerebellum, colon, eye with optic nerve, harderian gland, heart, ileum, kidneys, liver, lungs, mammary gland, ovaries, parathyroids, pituitary gland, skeletal muscle, stomach, thymus, thyroid, urinary bladder, uterus. Samples from observed macroscopical lesions were also taken. After paraffin embedding, 4 µm thick sections were cut and stained with haematoxylin and eosin. Selected sections were stained with oil red, Prussian blue and Grocott-silver stain. Cells in the adenohypophysis were identified with immunoperoxidase techniques.

Haematology. Haemoglobin, haematocrit, erythrocyte count, platelet count and total leukocyte count were determined in an automated haematological analyser, the Sysmex F-800 (Toa Medical Electronics Co., Kobe, Japan). The erythrocyte indices mean corpuscular volume (MCV), and mean corpuscular haemoglobin concentration (MCHC) were calculated from the obtained results. Differential leukocyte counts were made manually on blood smears stained with May-Grunewald Giemsa stain using a microscope.

Clinical chemistry and biochemistry. The following serum components were determined: alanine aminotransferase (ALAT), albumin, alkaline phosphatase (ALP), aspartate aminotransferase (ASAT), bile acids, total bilirubin, calcium, cholesterol, creatinine, free fatty acids, glucose, γ-glutamate transpeptidase (γ-GT), glutamate dehydrogenase (GLDH), lactate dehydrogenase (LDH), inorganic phosphate, total serum protein, triglycerides, urea, and uric acid using a Cobas Mira (Roche, Basel, Switzerland) and reagents supplied by the manufacturer. The electrolytes, chloride, potassium and sodium were analysed on the same equipment using the ISE unit of

Table 1.

Composition of the test diets.

Type of diet	Dose level	Corresponding human fish intake[a] kg/week	Fat content of test diet[b] %	Soy oil content of test diet %	Fish oil content of test diet %
Control	Low	0	3.95	0.45	0
	Medium	0	5.75	2.25	0
	High	0	13.63	10.13	0
Lodda oil	High	34.4	13.63	0	10.13
Herring oil	Low	1.6	3.95	0	0.45
	Medium	8.2	5.75	0	2.25
	High	34.4	13.63	0	10.13
F 1	Low	1.6	3.95	0.07	0.38
	Medium	8.2	5.75	0.37	1.88
	High	34.4	13.63	1.67	8.46
F 2	Low	1.6	3.95	0.42	0.03
	Medium	8.2	5.75	2.09	0.16
	High	34.4	13.63	9.41	0.72
F 3	Low	1.6	3.95	0.42	0.03
	Medium	8.2	5.75	2.11	0.14
	High	34.4	13.63	9.48	0.65

[a] Equivalent to 8, 40 or 160 times the estimated weekly intake of fish in Sweden, calculated, assuming that 9 g of wet-weight fish equals 1 g of fish oil, mean body weight for adults 65 kg, mean body weight for rats 0.25 kg, mean daily food consumption for rats 20 g.
[b] The fat concentration in the R34 diet is standardised to 3.5 % (mainly fish fat).

the instrument. Total T4 (TT4) was determined with the Amerlite system (Amersham, UK) according to the protocol of the supplier with slight modifications. The TT4 assay buffer was diluted 5 times with demineralised water, the standard curve for TT4 ranged from 0 to 120 nmol TT4/l. Ascorbic acid and thiobarbituric acid reactive materials (TBARS) were determined in frozen liver by a method according to Yagi (1982).

Hepatic enzyme activities and lipid peroxidation. Uridine diphosphate-glucuronosyl transferase (UDPGT) and glutathione-S-transferase (GST) activities were determined using the method described by Burchell & Weatherill (1981) and Habig *et al.* (1974), respectively. *O*-dealkylation of 7-ethoxyresorufin (EROD) and 7-pentoxyresorufin (PROD) were determined in the hepatic S9-fraction by the method of Lubet *et al.* (1985). The lipid peroxidation level in liver was determined colorimetrically by the method of Uchiyama & Michara (1978), using malon dialdehyde as the standard. Liver protein concentrations were determined by the method of Lowry *et al.* (1951), using bovine serum albumin as the standard.

Vitamin A analysis. Frozen samples of liver, kidneys and lungs were analysed for vitamin A levels by HPLC (Håkansson *et al.* 1987), modified as described by Chu *et al.* (1995).

Chemical analysis of rat liver. Liver lipid fractions were extracted and analysed for HCB, ΣHCH, ΣDDT, ΣCB, ΣBDE and ΣCDD/F as described elsewhere (Öberg *et al.* 2002). The amount and concentration of organohalogens in liver were calculated with respect to liver weight and hepatic fat content.

Statistical analysis. All group data are reported as the arithmetic mean and standard deviation for groups of 5 or 10 animals. A significance level of $P<0.05$ was chosen. Statistical analyses were performed using SPSS Statistical Software (SPSS Inc. Chicago, USA). Values were analysed using the Kruskal-Wallis One Way ANOVA (analysis of variance). In cases of statistically significant differences between the control and treated groups or between the different dose groups, each group was tested against the control and low dose groups, respectively, using the Mann-Withney Rank Sum Test. Linear regression analysis was applied to achieve an overwiew of the relationship between CDD/F-TEQ-intake and toxicological variables, using PlotIT 3.20 (Scientific Programming Enterprises, MI, USA).

Results

Daily consumption of EOCl and identified compounds. The daily consumption of EOCl and HFA, estimated from the results of the chemical analysis of fish oils and fractions

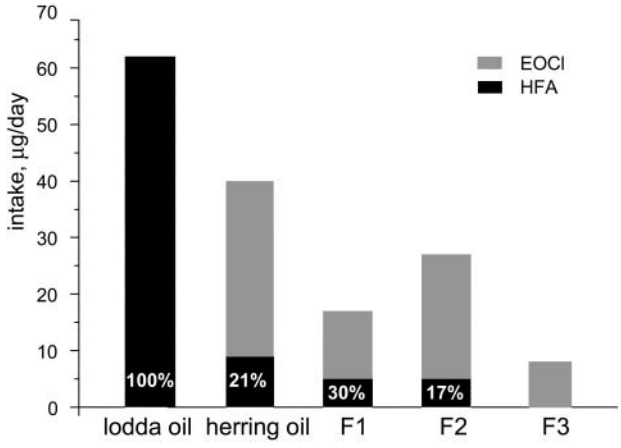

Fig. 1. Daily consumption of extractable organically bound chlorine (EOCl) and halogenated fatty acids (HFA) for female Sprague-Dawley rats fed high dose diets containing lodda oil, herring oil or its fractions for 13 weeks. No HFA was detected in the F3 diet. The daily consumption of EOCl and HFA was estimated from the concentrations in lodda oil, herring oil or its fractions, the fish fat content of the diets and the estimated daily food consumption.

(fully reported by Öberg *et al.* 2002) and the average food intake per rat in the different dietary groups, differed both qualitatively and quantitatively (fig. 1). Rats given the lodda oil diet had the highest estimated EOCl-intake of all dietary groups as almost the entire EOCl intake in this group consisted of HFA (fig. 1). The corresponding high dose group fed the herring oil diet had a slightly lower EOCl exposure, of which 21% consisted of HFA. The consumption of EOCl in rats fed the herring oil fractions at the high dose levels varied between 8 and 27 μg/day. In the high dose F1 and F2 dietary groups 30 and 17% of the EOCl, respectively, consisted of HFA, whereas the HFA intake in the F3 dietary group was below the detection limit. As seen in table 2, the rats fed the high dose of the herring oil diet consumed the highest amounts of all specified pesticides and persistent organohalogen pollutants, with the exception of the ΣDDT-consumption, which was highest in rats fed the high dose of the F2 diet. Intake levels of pollutants, in rats fed the F2 diet, were generally lower but still comparable with the herring oil group. Rats fed F3 diet consumed between 18 and 44% of the HCB, ΣCB, ΣBDE and ΣCDD/F, as compared to the herring oil group, whereas consumption of ΣHCH and ΣDDT were even lower (0.7 and 7%, respectively). Rats fed F1 diet consumed between 1 and 8% of most of the pollutants in comparison to the herring oil group, whereas the consumption of ΣCDD/F was 30%. Rats fed lodda oil diet consumed 23 and 16% of HCB and ΣHCH, respectively, and 3 and 2% of ΣDDT and ΣCB, respectively, as compared to rats fed the high dose herring oil diet.

The dietary vitamin A concentration varied between 7400 and 8300 IU/kg diet for all diets, with the exception of the F1 diet, which contained 5200 IU vitamin A /kg diet (data not shown).

Toxicological parameters.

Clinical observations, food consumption and body weights. No clinical signs of toxicity were observed and no animals died during the study. Food consumption was lower in rats fed diets at high-dose levels and in rats fed medium dose of F1 diet as compared to the corresponding low dose groups (table 3). Food intake was also lower in rats fed the high dose of the F1 diet as compared to the corresponding controls, given soya oil diet. Animals in all dietary groups gained weight (table 3). However, the body weight gain in rats fed high-dose F1, F2 and F3 diets was about 25% lower compared to the corresponding control groups.

Tissue weights. The relative liver weight was increased in rats fed herring oil and F1 diets at the high dose level compared with the corresponding low dose groups (table 3). As compared with corresponding control groups, the relative liver weight was significantly increased in rats fed diets containing lodda oil, herring oil or its fractions at the high dose level. Thymus weight was decreased in rats fed F1 and F3 diets in high dose groups compared with the corresponding controls and increased in rats fed the medium dose of F2 diet compared with the corresponding low dose group.

Table 2.

Daily consumption of identified contaminants and CDD/F-TEQ for female Sprague-Dawley rats fed diets containing lodda oil, herring oil or its fractions for 13 weeks[a,b].

Type of diet	Dose level	HCB[c] (ng/day)	ΣHCH[d] (ng/day)	ΣDDT[e] (ng/day)	ΣCB[f] (ng/day)	ΣBDE[g] (ng/day)	ΣCDD/F[h] (pg/day)	CDD/F-TEQ (pg/day)
Lodda oil	High	16	17	90	31	n.m.	n.m.	n.m.
Herring oil	Low	4	5	146	95	22	22	8
	Medium	17	26	712	463	108	105	38
	High	69	105	2910	1863	434	431	157
F 1	Low	0.1	0.1	2	7	1	7	1
	Medium	0.7	0.7	8	34	6	34	4
	High	3	3	33	148	27	132	17
F 2	Low	2	5	147	74	20	13	4
	Medium	13	25	792	400	109	72	20
	High	51	96	3027	1552	422	275	75
F3	Low	0.6	0	10	22	4	9	2
	Medium	3	0.2	49	108	20	45	10
	High	13	0.7	204	452	84	189	41

n.m.=not measured.

[a] The daily consumption of the contaminants was estimated from the contaminant concentrations in herring oil and its fractions (Öberg *et al.* 2002), the fish fat content of the diets and the estimated daily food consumption.

[b] CDD/F-TEQ are calculated according to WHO-TEF (van den Berg *et al.* 1998).

[c] Hexachlorobenzene.

[d] Hexachlorcyclohexanes, (including α-, β-, and γ-HCH).

[e] 1,1,1-Trichloro-2,2-bis(4-chlorophenyl)ethane (DDT), including DDE and DDD.

[f] Chlorinated biphenyls, including CBs 28, 101, 105, 118, 138, 153, 156, and 180 as numbered by IUPAC.

[g] Brominated diphenylethers including BDEs 47, 99 and 100.

[h] Chlorinated dibenzo-*p*-dioxins/furans, including all 2,3,7,8-substituted congeners.

There were no significant alterations of kidney, heart, spleen or lung weights (data not shown).

Bone length and density. Tibias were shorter in rats fed F1 (high dose), F2 (low dose) and F3 (low and high dose) diets as compared to the corresponding controls (table 3). A decrease in tibia density was observed in rats fed herring oil or F1 low dose diets as compared to the corresponding controls.

Histopathological examinations. Few macroscopical changes were noticed at necropsy. The most common finding consisted of white, elevated subpleural areas characteristic of alveolar histiocytosis, observed in the lungs of rats from all of the groups. The pituitary gland of one of the rats from the control group was moderately enlarged, showing a redish colour. Numerous rats belonging to the experimental groups and control rats, showed histological hepatic changes (table 4). The lesions observed consisted of foci of hepatocytes showing slightly enlarged, clear, vacuolated cytoplasm and lipidosis (fatty change). Not uncommonly, the portal triads also displayed moderate leukocytic infiltrations. Increased mitoses, both among the hepatocytes and the sinusoidal lining cells, were observed in some of the livers. Mononuclear leukocytes showing condensed cytoplasm and pyknosis were also found intravascularly. Fatty change appeared most frequently in the form of microvesicular lipidosis localised in the periportal areas. It was most prevalent in rats fed herring oil high dose diet, followed by rats fed high dose control diet. Rats fed low dose of F1 and F3 diets showed the lowest prevalence of hepatic lipidosis. In general, the hepatic changes were mild. The animals fed F2 and F3 diets exhibited discrete degenerative, necrotic and inflammatory lesions in the hepatocytes, Kupffer cells and bile duct epithelium, whereas rats fed herring oil diet during 39 weeks suffered from the most severe lesions (data not shown). Alveolar histiocytosis, occasionally accompanied by slight infiltrations of mononuclear leukocytes, was observed in rats from most of the groups. Alveolar histiocytosis generally was mild, consisting of a few elevated plaques located subpleurally in the more distal parts of the lung. In occasional rats, moderate alveolar histiocytosis showing numerous elevated plaques was found. Groups fed F1 high dose diet, were free from these pulmonary changes. Mineral casts (struvite) in tubular lumina, most commonly localised at the level of the corticomedular zone of the kidneys, was frequently observed, except for rats fed the low dose of herring oil diet and the high dose of F1 diet. The enlarged pituitary from one of the rats from the control group presented a benign tumour diagnosed as a pituitary adenoma.

Haematology. The haemoglobin level was decreased in rats fed the low dose of herring oil diet and increased in rats fed high dose diets containing F1 and F3 as compared to the corresponding controls (table 5). The haemoglobin level was increased in rats fed medium and high dose of the F1 diet and decreased in rats fed medium dose of the F2 diet compared to low dose groups. MCV was decreased in rats fed medium dose of the F2 and high dose of the F1 and F3

Table 3.

Weekly food consumption, body weight gain, relative tissue weights, tibia length and tibia bone density of female Sprague-Dawley rats fed diets containing lodda oil, herring oil or its fractions for 13 weeks[a].

Type of diet	Dose level	Weekly food consumption (g)	Body weight gain (g)	Relative liver weight (g/kg body wt)	Relative thymus weight (g/kg body wt)	Length of tibia (mm)	Density of tibia (g/cm^3)
Control	Low	129±9	125±18	31.0±4.3	1.2±0.4	39.3±0.9	0.84±0.08
	Medium	126±9	128±29	29.5±3.4	1.8±0.3	38.7±1.1	0.81±0.12
	High	118±9[c]	144±27	29.1±2.7	1.3±0.3	39.3±1.4	0.79±0.10
Lodda oil	High	152±9	139±22	32.4±2.9[b]	1.1±0.3	38.4±1.1	0.85±0.15
Herring oil	Low	134±6	140±20	30.7±2.0	1.3±0.2	39.1±0.8	0.76±0.10[b]
	Medium	131±7	131±19	30.4±1.8	1.3±0.4	39.2±0.9	0.74±0.05
	High	119±10[c]	140±20	32.5±1.7[b,c]	1.3±0.3	38.9±0.8	0.75±0.05
F 1	Low	135±5	135±22	30.5±3.1	1.3±0.4	39.4±1.1	0.76±0.06[b]
	Medium	126±9[c]	122±19	30.6±2.8	1.3±0.2	38.7±0.7	0.75±0.09
	High	109±6[b,c]	101±17[b,c]	35.4±3.1[b,c]	1.0±0.2[b]	37.8±0.6[b]	0.77±0.03
F 2	Low	125±6	110±21	31.6±4.0	1.0±0.2	37.8±0.9[b]	0.80±0.06
	Medium	126±9	116±20	30.1±3.0	1.4±0.3[c]	39.2±1.2	0.74±0.03
	High	107±12[c]	107±15[b]	31.9±2.5[b]	1.2±0.2	38.2±1.5	0.77±0.09
F 3	Low	123±6	101±8[b]	32.4±3.4	1.1±0.3	37.8±0.7[b]	0.90±0.10
	Medium	126±13	106±29	29.3±2.3	1.3±0.2	39.2±0.8	0.77±0.11
	High	113±3[c]	109±35[b]	33.2±5.6[b]	1.0±0.2[b]	37.9±0.9[b]	0.82±0.11

[a] Values represent the mean±S.D. of 10 animals except for the F 3 medium dose group in which values represent the mean±S.D. of 5 animals.
[b] Significantly different from the corresponding control group ($P<0.05$).
[c] Significantly different from the corresponding low dose group ($P<0.05$).

Table 4.
Incidence and severity of histological changes in liver, lungs and kidneys of female Sprague-Dawley rats fed diets containing herring oil or its fractions for 13 weeks[a].

		Hepatocytes				Kupffer cells		Portal triads					
Type of diet	Dose level	Steatosis/ lipidosis	Focal degeneration, necrosis, inflammation	Mitotic figures	Foci	Condensed nuclei	Proliferation	Inflammation	Bile duct epithelium	Condensed leukocytes	Alveolic histiocytosis	Nephrocalcinosis
Control	Low	2/3	3/3	3/3	0/3	1/3	0/3	1/3	0/3	0/3	1/3	1/3
	High	6/6	4/6	5/6	0/6	0/6	0/6	1/6	0/6	0/6	2/6	5/6
Lodda oil	High	5/6	3/6	1/6	1/6	0/6	0/6	0/6	0/6	0/6	4/6	4/6
Herring oil	Low	2/3	1/3	2/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3	0/3
	High	6/6	4/6	1/6	0/6	0/6	1/6	0/6	0/6	0/6	4/6	2/6
F 1	Low	0/3	3/3	1/3	2/3	0/3	0/3	0/3	0/3	0/3	1/3	1/3
	High	3/6	5/6	0/6	0/6	2/6	0/6	1/6	1/6	1/6	0/6	1/6
F 2	Low	1/3	3/3	2/3	0/3	1/3	0/3	0/3	2/3	0/3	1/3	1/3
	High	2/6	2/6	0/6	0/6	3/6	0/6	1/6	3/6	2/6	3/6	3/6
F 3	Low	0/3	3/3	1/3	1/3	3/3	2/3	0/3	3/3	1/3	1/3	1/3
	High	1/6	6/6	5/6	0/6	4/6	3/6	3/6	6/6	6/6	3/6	5/6

diets compared with corresponding controls and increased in rats fed high dose of the control diet compared with the corresponding low dose group. MCHC was higher in rats fed high dose of the F1 and F3 diets as compared to the corresponding controls. The total leukocyte counts were lower in rats fed F2 and F3 low dose diets as compared to the corresponding controls.

Clinical chemistry and biochemistry. ALP activity was increased in rats fed medium and high dose of the F1 diet as compared to the corresponding controls (table 6). ALAT activity was decreased in rats fed F1 diet at low dose level and F3 diet at medium and high dose levels compared with the corresponding controls. Serum albumin levels were increased in rats receiving F2 medium and high dose diet, and F3 high dose diet compared with the corresponding controls. Total serum protein levels were increased in animals fed high dose of diets containing herring oil, F1 and F2, compared to corresponding controls. Serum phosphorus levels were significantly decreased in rats fed F2 diet at the low dose level and F3 at the low and high dose level compared to corresponding controls. Serum cholesterol levels were increased in animals receiving medium dose of the F2 diet as compared to corresponding controls. There were no effects upon the following biochemical parameters when compared to corresponding control and low dose groups: ASAT, ascorbic acid, bile acids, total bilirubin, calcium, creatinine, free fatty acids, glucose, γ-GT, GLDH, LDH, inorganic phosphate, TBARS, triglycerides, urea, uric acid, urea nitrogen, chlorine, sodium, potassium, and TT4.

Hepatic enzyme activities and lipid peroxidation. EROD-activity was elevated as compared to the corresponding controls in rats fed herring oil (all dose), F2 (low dose) and F3 (low and high doses) (table 7). As compared to the corresponding low dose groups EROD-activity was also elevated in rats fed medium and high dose of the herring oil diet, medium dose of the F1 diet and high dose of the F3 diet. PROD-activity was slightly depressed in rats fed F3 diet at low and high dose level compared with the corresponding controls. The PROD-activity was elevated in rats fed high dose of control and herring oil diets as compared with the corresponding low dose groups. Similar EROD- and PROD-activities were observed in rats fed the low dose of the herring oil diet for 39 weeks as compared to the animals fed the same diet for 6 and 13 weeks (data not shown). The UDP-GT activity was increased in rats fed the high dose of the F1 diet and the low and high dose of the F3 diet as compared to the corresponding controls (table 7). GST activity in liver was elevated in animals receiving F1 and F3 diets at high dose levels compared with the corresponding controls. Lipid peroxidation was higher in rats fed medium and high dose of the herring oil diet but lower in the medium dose F2 diet as compared to the corresponding controls. As compared to the corresponding low dose groups, elevated lipid peroxidation was observed in rats, fed the me-

Table 5.

Haematological parameters in plasma for female Sprague-Dawley rats fed diets containing lodda oil, herring oil or its fractions for 13 weeks[a].

Type of diet	Dose level	Hb (g/l)	MCV[d] (l)	MCHC[e] (g/l)	WBC[f] (*10^{12}/l)
Control	Low	133±5	55.1±1.7	334±7	6.7±7.0
	Medium	133±6	55.5±2.7	339±14	5.4±3.6
	High	129±3	59.2±2.9[c]	316±20	4. 3±1.0
Lodda oil	High	132±3	57.3±1.9	317±14	5.0±1.3
Herring oil	Low	127±5[b]	56.5±1.8	330±13	4.3±0.8
	Medium	129±4	55.2±1.7	334±6	5.4±0.1
	High	130±3	57.5±2.6	319±11	4.5±0.9
F 1	Low	132±8	55.5±1.1	335±4	7.2±6.7
	Medium	134±4[c]	54.5±1.9	349±9	4.2±1.5
	High	134±2[b,c]	54.7±1.4[b]	339 5[b]	3.8±1.2
F 2	Low	134±3	53.3±2.0	342±10	2.9±1.0[b]
	Medium	128±6[c]	52.0±0.6[b]	347±12	5.4±5.1
	High	128±14	56.1±6.3	338±9	3.5±1.2
F 3	Low	137±6	55.9±3.6	340±17	3.5±0.6[b]
	Medium	128±7	55.4±3.2	340±19	4.0±1.1
	High	135±4[b]	53.9±3.2[b]	338±10[b]	3.9±1.0

[a] Values represent the mean±S.D. of 10 animals except for the F 3 medium dose group in which values represent mean±S.D. of 5 animals.
[b] Significantly different from the corresponding control group ($P<0.05$).
[c] Significantly different from the corresponding low dose group ($P<0.05$).
[d] Mean corpuscular volume.
[e] Mean corpuscular haemoglobin concentration.
[f] White blood cell count.

dium and high doses of herring oil diet, and the high dose of the F1, F2 and F3 diets.

Vitamin A. Hepatic vitamin A was increased in rats fed the low dose of the herring oil and F1 diet, and decreased in rats fed the high dose of the F1, F2 and F3 diets, compared with the corresponding controls (table 7). Compared with the corresponding low dose groups, liver vitamin A was decreased in rats fed high dose of the herring oil, F1 and F2 diets and the medium dose of the F1 diet. In comparison

Table 6.

Clinical chemistry and biochemistry for female Sprague-Dawley rats fed diets containing lodda oil, herring oil or its fractions for 13 weeks[a].

Type of diet	Dose level	ALP (U/l)	ALAT (U/l)	Serum albumin (g/l)	Total serum protein (g/dl)	Serum phosphorus (mg/dl)	Serum cholesterol (mg/dl)
Control	Low	146±26	35±5	3.8±1.0	6.4±1.2	5.9±0.2	74±43
	Medium	131±39	39±3	3.6±0.3	5.9±0.4	5.9±0.6	77±8
	High	202±71	40±12	3.5±0.3	6.0±0.3	6.0±0.3	74±18
Lodda oil	High	342±100	33±6	3.8±0.4	6.4±0.2	5.6±0.4	61±9
Herring oil	Low	158±32	31±2	3.6±0.2	6.2±0.3	5.6±0.2	71±10
	Medium	166±38	31±6	3.7±0.4	6.2±0.5	5.7±0.4	61±15
	High	291±47	34±7	3.6±0.4	6.6±0.4[b]	5.9±0.4	62±11
F 1	Low	136±33	29±2[b]	3.7±0.3	6.3±0.5	5.7±0.2	75±8
	Medium	201±40[b]	27±5	3.9±0.3	6.5±0.4	5.6±0.5	75±12
	High	357±54[b]	35±11	4.0±0.5	6.9±0.5[b]	5.6±0.7	91±19
F 2	Low	170±46	35±7	3.5±0.4	6.0±0.6	5.2±0.6[b]	68±34
	Medium	146±61	35±12	4.0±0.3[b]	6.3±0.2	5.4±0.4	96±9[b]
	High	260±88	30±8	4.1±0.3[b]	6.4±0.3[b]	5.3±0.3	86±12
F 3	Low	167±36	29±5	3.3±1.6	6.7±0.7	5.0±0.6[b]	85±16
	Medium	138±24	25±6[b]	3.6±0.4	6.1±0.5	5.6±0.8	67±10
	High	196±48	22±4[b]	4.2±0.3[b]	6.7±0.6	5.0±0.3[b]	80±16

[a] Values represent the mean±S.D. of 10 animals exept for the F 3 medium dose group in which values represent mean±S.D. of 5 animals.
[b] Significantly different from the corresponding control group ($P<0.05$).

Table 7.

Hepatic enzyme activities, lipid peroxidation in liver and total amount of vitamin A in liver and kidneys of female Sprague-Dawley rats fed diets containing lodda oil, herring oil or its fractions for 13 weeks[a].

Type of diet	Dose level	EROD[e] (pmol/mg*min.)	PROD[f] (pmol/mg*min.)	UDPGT[g] (nmol/ mg*min.)	GST[h] (nmol/mg*min.)	Lipid peroxidation (abs/mg*10^{-3})	Hepatic vitamin A (mg)	Renal vitamin A (μg)
Control	Low	13±6	8±1	1.2 ±0.4	348±72	18±7	3.4±0.3	2.1±0.8
	Medium	27±7	12±3	1.0±0.4	312±50	24±7	3.5±0.8	1.7±0.4
	High	25±13	12±2[c]	1.4±0.3	297±64	31±16	3.3±0.7	2.2±0.5
Lodda oil	High	24±12	9±4	1.2±0.3	344±90	30±14	3.9±0.6	2.3±0.5
Herring oil	Low	23±3[b]	10±3	1.3±0.3	354±34	14±7	3.9±0.6[b]	2.0±0.3
	Medium	50±15[b,c]	11±4	1.2±0.4	333±55	102±27[b,c]	3.8±0.6	1.9±0.2[b]
	High	102±28[b,c]	13±2[c]	1.6±0.3	266±87	166±167[b,c]	3.0±0.4[c]	2.4±0.5[c]
F 1	Low	21±10	10±3	1.2±0.2	338±85	25±13	4.2±0.5[b]	2.1±0.5
	Medium	41±13[c]	13±4	1.2±0.2	355±96	34±45	3.2±0.4[c]	2.1±0.4[b]
	High	32±8	10±2	2.3±0.6[b]	459±109[b]	56±25[c]	2.4±0.3[b,c]	1.7±0.4[b,c]
F 2	Low	29±7[b]	9±3	1.3±0.4	424±110	12±5	3.1±0.4	1.7±0.3[b]
	Medium	32±1	12±3	1.0±0.3	384±120	10±7[b]	3.3±0.5	1.8±0.5
	High	45±21	12±4	1.5±0.4	373±76	43±44[c]	2.7±0.5[b,c]	2.1±0.5[c]
F 3	Low	31±6[b]	10±2[b,c]	1.7±0.5[b]	423±130	13±1	3.3±0.6	1.6±0.3
	Medium	25±9	11±4	1.1±0.2	423±108	15±4	3.2±0.7	2.1±0.2[b,c]
	High	49±4[b,c]	10±1[b]	2.4±1.2[b]	521±174[b]	20±7[c]	2.8±0.5[b]	1.8±0.5[b]

[a] Values represent the mean±S.D. of 10 animals except for the F 3 medium dose group in which values represent the mean±S.D. of 5 animals.
[b] Significantly different from the corresponding control group ($P<0.05$).
[c] Significantly different from the corresponding low dose group ($P<0.05$).
[e] *O*-Dealkylation of 7-ethoxyresorufin.
[f] *O*-Dealkylation of 7-pentoxyresorufin.
[g] Uridine diphosphate-glucuronosyl transferase.
[h] Glutathione-S-transferase.

to the corresponding controls, the renal vitamin A was higher in rats fed herring oil, F1 and F3 diets at middle dose and lower in rats fed high dose of the F1 and F3 diets, and low dose of the F2 diet. Compared with the corresponding low dose groups, the renal vitamin A content was increased in rats fed high doses of herring oil and F2 diets and medium dose of the F3 diet, but was decreased in rats fed the high dose of the F1 diet.

Residue levels in liver tissue. The hepatic fat content varied from 3.2% in rats fed F3 diet, to 8.1% in rats fed herring oil diet (table 8). Results from chemical analysis of residue levels in liver tissue are fully reported by Öberg *et al.* (2002). Briefly, for all dietary groups the most abundant pollutants were ΣDDT and ΣCB and rats fed herring oil diet had the highest liver levels of all contaminants except for ΣCDD/F, which was at its highest concentration in rats fed the F2

Table 8.

The hepatic fat content, and the concentrations of organohalogen pollutants and CDD/F-TEQ in liver lipids of female Sprague-Dawley rats fed high dose diets containing herring oil or its fractions for 13 weeks[a].

Type of diet	Hepatic fat content (%)	HCB[b] (ng/g lipid)	ΣHCH[c] (ng/g lipid)	ΣDDT[d] (ng/g lipid)	ΣCB[e] (ng/g lipid)	ΣBDE[f] (ng/g lipid)	ΣCDD/F[g] (ng/g lipid)	CDD/F-TEQ[h] (ng/g lipid)
Herring oil	8.1	55	54	2000	1500	269	6.4	2.7
F 1	4.4	7	9	45	130	33	2.8	0.7
F 2	5.5	39	44	1800	830	191	7.9	3.2
F 3	3.2	13	10	160	360	59	5	1.7

[a] Lipid content was measured gravimetrically.
[b] Hexachlorobenzene.
[c] Hexachlorcyclohexanes, (including α-, β- and γ-HCH).
[d] 1,1,1-Trichloro-2,2-bis(4-chlorophenyl)ethane (DDT), including DDE and DDD.
[e] Chlorinated biphenyls, including CBs 28, 101, 105, 118, 138, 153, 156, and 180 as numbered by IUPAC.
[f] Brominated diphenylethers, including BDEs 47, 99 and 100.
[g] Chlorinated dibenzo-*p*-dioxins/furans, including all 2,3,7,8-substituted congeners.
[h] CDD/F-TEQ are calculated according to WHO-TEF (van den Berg *et al.* 1998).

diet (table 8). Rats fed high F1, F2, and F3 diets had liver residue concentrations of HCB, ΣHCH, ΣCB, ΣBDE, and ΣDDT, which varied between between 2–17, 55–90 and 8–24%, respectively, as compared to the concentrations in rats fed herring oil. In contrast, liver concentrations of ΣCDD/Fs in rats fed high dose F1, F2 and F3 diets were 44, 123, and 78%, respectively, of that found in the herring oil group. There was a marked difference in the liver residue levels between ΣCDD/F and the other analysed contaminants. The ratios, between daily intake (table 2) and hepatic residue levels (table 8) were in the range 0.3–2.2 for all contaminants except for ΣCDD/F that showed ratios in the range 0.03–0.07, and ΣHCH that showed a ratio of 0.07 in the F3 dietary group. Data on bioaccumulation for up to 39 weeks are reported by Öberg *et al.* (2002).

Discussion

The present study aimed at improving the knowledge about the toxicity and persistence of fish-derived HFA and not yet identified EOCl components in mammals *in vivo.* The chosen strategy was to fractionate and separate organohalogen pollutants derived from Baltic herring fillet in order to obtain fish oil fractions with differing proportions of identified and unidentified pollutants and to perform a traditional subchronic toxicity study in female rats, essentially according to the OECD guidelines. Bearing in mind the well known highly bioactive CDD/Fs and CBs in Baltic fish, additional end-points serving as established markers for Ah-receptor mediated toxicity were included in this study.

The chosen dose levels of fish intake corresponded to 8, 40, or 160 times the estimated intake (=0.21 kg/week) in the Swedish population, based on measurement during extraction, that 9 g of wet-weight fish equals 1 g of fish oil and assuming a body weight for adults of 65 kg, a body weight for rats of 0.25 kg and a daily food consumption of 20 g table 1). On a fish oil basis, there was a continuos variation in doses from 0 to 10.13% fish oil in the test diets (table 1). The estimated intakes of EOCl and HFA corresponded to daily doses in the ranges 1.6–248 μg EOCl/kg body weight and up to 248 μg HFA/kg body weight, respectively. The estimated intakes of the different organohalogen contaminants corresponded to daily doses of 0.6–276 ng HCB/kg body weight, <0.16–420 ng ΣHCH/kg body weight, <7–12108 ng ΣDDT/kg body weight, 28–7452 ng ΣCBs/kg body weight, 4–1763 ng ΣBDE/kg body weight, 29–1724 pg ΣCDD/F/kg body weight, and 4–628 pg CDD/F-TEQ/kg, respectively (table 2).

Effects related to fish intake, in rats exposed to the herring oil or its fractions, were limited to decreased body weight gain and food intake, increased relative liver weight, increased hepatic EROD and UDP-GT activities and decreased hepatic vitamin A content. There were no effects on hepatic EROD activity or vitamin A levels in the group exposed to the lodda oil diet, when compared to the corresponding control group (table 7), suggesting that HFA do not induce typical dioxin-like responses. Since HFA is a group of compounds, there may be qualitative differences between the HFA from herring and lodda. There was no correlation between exposure to any specific compound, or compound group, and the observed fish-intake related effects on body weight gain, food consumption, liver changes or hepatic UDP-GT activity (data not shown). Observations in lungs, kidneys, and bone tissue, as well as hematology and clinical chemistry parameters could not be linked to any exposure parameter.

Effects related to the CDD/F-TEQ intake. There was a significant correlation between CDD/F-TEQ intake and hepatic EROD activity in all dietary groups (fig. 2; $r^2=0.66$; $P<0.005$). A significant EROD induction as compared to corresponding controls was observed at the lowest TEQ-exposure level in F3 low dose group with daily CDD/F-TEQ-intake of 2 pg, corresponding to 8 pg CDD/F-TEQ/kg body weight. This effect on EROD was also seen in rats fed F2 (low dose), but may be an artefact of the low activity in the control group, since it was not dose dependent. The lowest exposure level that produced a significant and dose dependent induction in livers of rats was found at the medium dose of herring oil diet, corresponding to a daily intake of 152 pg CDD/F-TEQ/kg body weight. The corresponding no effect level (NOEL) is observed at 80 pg CDD/F-TEQ/kg body weight. This is in the same range as the lowest observed effect level (LOEL) of 100 pg/kg body weight per day for CYP1A1 induction by 2,3,7,8-tetrachloro dibenzo-*p*-dioxin (TCDD) in the rat reported elsewhere (Tritscher *et al.*1992). It has though to be noted that the

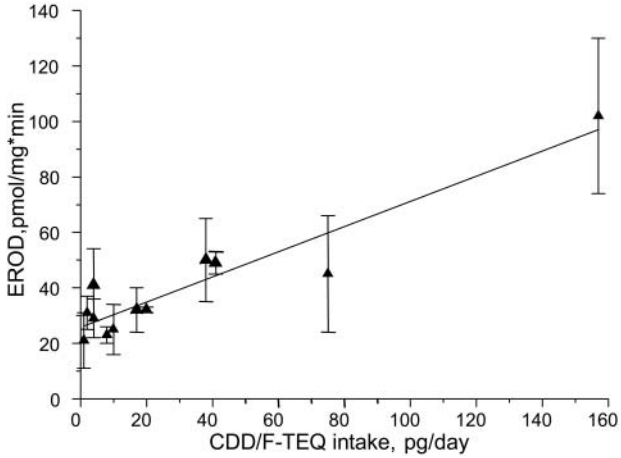

Fig. 2. Correlation between intake of chlorinated dibenzo-*p*-dioxin and furans summarized as toxic eqivalents (CDD/F-TEQ) and hepatic 7-ethoxyresorufin-*O*-deetylase (EROD) activity, for female Sprague-Dawley rats fed diets containing Baltic herring oil or its fractions for 13 weeks ($r^2=0.66$, $P<0.005$). Values represent the mean±S.D. of ten animals, except for one group in which values represent five animals. CDD/F-TEQ values are calculated using WHO-TEF values (van den Berg *et al.* 1998) and congener specific data from this study as reported by Öberg *et al.* (2002). The daily consumption was estimated from the CDD/F-concentrations in herring oil and its fractions, the fish fat content of the diets and the estimated daily food consumption.

total TEQ content in Baltic herring should be increased with at least a factor two, since dioxin-like CBs are not included (Bignert 2001). The EROD results of the present study are also in accordance with the EROD induction in Sprague-Dawley rats, which were fed diets supplemented with Lake Ontario or Pacific coho salmon for 13 weeks (Chu *et al.* 1984). The EROD activities in female rats fed the contaminated Lake Ontario diet varied from 0.002 to 0.093 nmole/mg/min. for low and high-dose groups, respectively, compared to 0.002 to 0.1 nmole/mg/min. in rats fed herring oil diets at the low and high dose levels, respectively, in the present study. It was not possible to compare EROD induction in the two studies on a TEQ exposure level, since no congener specific dioxin or CB analyses were performed by Chu *et al.* (1984). Although, exposure to HCB and ΣDDT were comparable in the two studies, there was a marked difference in CB exposure; the high dose group fed Lake Ontario salmon received at least a ten-fold higher exposure to CBs. These data suggest that dioxin contamination was low in the Lake Ontario salmon and/or that patterns of CB contamination of the fish are considerably different in the two studies i.e. on a relative scale Lake Ontario salmon seems to contain less planar and EROD-inducing CB congeners as compared to the present study. In the Canadian study, EROD-activities returned to background levels after a 13 weeks recovery period on a clean diet (Chu *et al.* 1984). It is known that induction of the hepatic UDP-GT activity requires considerably higher doses of TCDD as compared to EROD induction (Thunberg *et al.* 1980; Santostefano *et al.* 1998). The absence of correlation between the CDD/F-TEQ intake and hepatic UDP-GT activity in the present study is consistent with these data.

Comparisons between hepatic vitamin A levels (table 7) and CDD/F-TEQ exposure (table 2) can only be performed within dietary groups; comparisons between dietary groups are hampered for the following reasons. First, the vitamin A levels in the diets varied due to unintentional conditions during the commercial manufacture. For example, the low hepatic vitamin A content in the F1 high dose group, is most likely explained by the lower vitamin A content of the F1 high dose diet as compared to all other high dose diets (5200 IU/kg versus 7400–8000 IU/kg). In addition, diets that were enriched in triglycerides and lipids from Baltic herring (herring oil and F1) may have an enrichment of natural bioactive compounds such as vitamins, steroids, and lipids. The F2 and F3 diets, on the other hand, contained limited amounts of herring oil-derived lipids, and therefore these dietary groups were considered most appropriate for further investigation of the hepatic vitamin A dose response relationship, using vitamin A as a marker for Ah-receptor mediated toxicity. Even if hepatic vitamin A reduction in itself is unlikely to be a toxic effect, this parameter clearly serves as a marker for Ah-receptor mediated disruption of retinoid storage (Nilsson & Håkansson 2002). The hepatic vitamin A content in the F2 high dose group was significantly decreased both compared to the corresponding control group and the low dose group, while there was no effect in the medium dose group (table 7). Similarly, the hepatic vitamin A content in the F3 high dose group was significantly decreased compared to the corresponding control group, while there was no effect in the medium dose group (table 7). Thus the LOEL for hepatic vitamin A reduction in the present study was in the range 41–75 pg CDD/F-TEQs/day (table 2), i.e. 0.16–0.30 ng CDD/F-TEQs/kg body weight and day. (The corresponding NOEL was observed in the range 10–20 pg CDD/F-TEQs/day (table 2), i.e. 0.04–0.08 ng CDD/F-TEQs/kg body weight and day.) The previously reported LOEL of 14 ng/kg body weight and day for hepatic vitamin A reduction in a subchronic toxicity study in the rat (van Birgelen *et al.* 1995) is considerably higher than the effect level of CDD/F-TEQs in this study.

Effects related to intake of fish lipids. The decreased food intake, which was observed in all high dose groups as compared to animals fed corresponding low-dose diets (table 3) is likely to be attributed to the high lipid level in these diets. Rats given diets with a high fat concentration would have to eat less food to obtain a sufficient growth rate and therefore the decreased food consumption in the high-dose groups did not affect the body weight gain. There was no correlation between body weight gain and CDD/F-TEQ intake in the present study (data not shown). This finding is consistent with the lack of effect on body weight gain in female rats at total doses below 46.7 μg TEQ/kg body weight, when given as a mixture of four CDDs over a 20-week period (Viluksela *et al.* 1998). In the present study the highest total CDD/F-TEQ-dose in any herring oil dietary group was approximately 0.06 μg/kg body weight over the 13-week period. Our data are also consistent with the lack of effect on body weight gain in rats, which were exposed to organohalogen pollutants through a diet supplemented with Lake Ontario coho salmon for 13 weeks (Chu *et al.* 1984). In contrast, significantly lower growth rates as compared to control animals as well as to rats fed the herring and lodda oil diets was observed in animals fed diets supplemented with the fractions from the herring oil at the high dose levels (table 3). However, the decrease in growth did not correlate with any exposure parameter.

Relative liver weights were increased to a similar extent in rats fed high dose herring oil diet, with a high content of identified chlorinated contaminants, and in rats fed the lodda oil diet, with a low content of these compounds (table 3). Thus, it is unlikely that the increase in relative liver weights is directly related to the identified organohalogen contaminants. In fact, there was no correlation between the intake of CDD/F-TEQs and the liver weights; neither for the absolute nor relative weights (data not shown). However, there was a correlation between the relative liver weights and the fish oil content of the experimental diets within the high dose groups ($r^2=0.14$; $P<0.005$), and for all dietary groups together ($r^2=0.09$; $P<0.005$). These correlations were also observed for absolute weights (data not

shown). Thus, the increased liver weights in the groups fed lodda oil, herring oil and F1 diets appear to be due to the fish lipid concentration. However, not in a clear linear manner. Some of the microscopic liver changes observed, such as hepatic lipidosis, could represent unspecific hepatocyte responses. These can be elicited by various factors, including a high fat content in the diet. In the groups with high lipid content, lipidosis was found twice as often in rats fed lodda and herring oil diets as compared to rats fed diets containing herring oil fractions. In addition, the morphology of the lesions suggests that the hepatic changes were not caused by the high dietary fat content itself; further supporting the assumption that fish lipids in the diet independent of the organohalogen pollutants are responsible for the observed liver changes. Since the 39 weeks exposure group exhibited more complex hepatic changes, the occurrence of reparative processes superimposed to the toxic injury cannot be excluded. Another possibility is progression to hyperplasia. It would be of interest to follow the course of the hepatic alterations for up to two years, to evaluate whether the outcome will be more chronic alterations, hyperplasia, neoplasia or complete restitution. Taken together, the histopathological findings are indicative of hepatotoxicity, albeit in a low grade.

Observations in lungs, kidneys, bone tissue, haematology and clinical chemistry. The frequency of alveolar histiocytosis in the present study was higher in groups fed diets containing lodda or herring oil (table 4). This lesion has also been noticed after hypophysectomy, treatment with anorectic drugs, feeding rats with diets high in lipids or deficient in fatty acids, choline, or pantothenic acid (Dungworth *et al.* 1992). Repeated pulmonary injury, but also disturbances in lipid metabolism are suspected to be involved in the genesis of the lesion. Since alveolar histiocytosis has been reported with varying frequency also in untreated rats, it need not necessarily be related to the experimental treatments applied in the present study. The opposite alternative, however, cannot be ruled out.

The observed changes in kidney histology were not related to any fish-derived components. Nephrocalcinosis, as it was observed in the present study (table 4) is rather common in rats. The frequency varies depending on numerous factors, such as the rat strain, sex and age, but the composition of the diet is of major importance. Female rats seem to develop nephrocalcinosis more easily than male rats and the lesions are more readily induced in young rats than in young adult or old rats. Thus, the rat-related factors in the present study probably have contributed to the unexpected frequency of nephrocalcinosis. These data are in agreement with previous studies (Chu *et al.* 1984; Villeneuve *et al.* 1981) when both contaminated and non-contaminated fish-supplemented diets induced focal calcification of tubules at the corticomedullary junctions, which was greater in female than in male animals.

Tibia bone length and density were significantly affected in some groups, however, the effects were not consistent with regard to dose within any of the groups (table 3). The decreased tibia length in rats given F1 or F3 diets is most likely not due to a specific effect on bone tissue, but could be a consequence of the reduced body weight gain in these rats. There were no correlations between tibia lenght or density and the TEQ-intake in the present study (data not shown), consistent with the observation that decreased tibia length and mechanical strength occur at weekly doses of 1.7 ìg TCDD/kg body weight but not at the 0.17 µg/kg body weight dose level, when given s.c. over a 20 week period to female Long-Evans and Han/Wistar rats (Viluksela *et al.* 2000), i.e. at doses well above the maximum, total weekly TEQ-dose of 0.004 µg/kg body weight in the present study. The only evident haematological differences among groups consuming fish diets and control groups were weak alterations in Hb, MCV, MCHC and WBC. There were also differences between the controls and different fish diets for some biochemical parameters as ALP and ALAT activities, serum albumin, cholesterol, protein and phosphorus levels. These findings do not represent any grave concern regarding the health of the rats since many of them probably represent adaptive responses to the diets and would probably diminish, as reported by others, if a recovery phase was part of the experimental design, wherein the control diet replaced the fish diets (Chu *et al.* 1984).

There were no treatment-related effects on serum TT4 levels in the present study (data not shown). The lack of effect is consistent with the estimated LOEL of 47 ng TCDD/kg body weight per day in the rat for plasma thyroid hormone reductions in a 13-weeks dietary exposure study (van Birgelen *et al.* 1994), as compared to the highest daily dose level in the present study, which was estimated to be about 0.6 ng CDD/F-TEQ/kg body weight.

To conclude, the toxicological examination showed that exposure to Baltic herring oil and its fractions at dose levels, corresponding to a human intake in the range of 1.6 to 34.4 kg Baltic herring fillet per week, resulted in effects that could be described as minimal, even at the high dose level. HFA, the major component of EOCl, did not cause any effects. The spectrum of toxic effects was similar to that, which is observed after low-level exposure to pollutants such as CDD/Fs and CBs, despite the fact that these contaminants contribute to a minor part of the EOCl.

From a risk management point of view the present study provide important information about effect levels for endpoints associated with Ah-receptor activation following low level exposure to organohalogen pollutants from a matrix relevant for human exposure. Increased EROD activity was seen in rats with a daily intake of 0.15 ng CDD/F-TEQ/kg body weight. A decrease of hepatic vitamin A that was dependent on TEQ intake was observed at 0.16–0.30 ng CDD/F-TEQ/kg body weight. There were no evidence that the severity of effects increased for any parameter as the exposure went on for up to 39 weeks, except for the microscopic liver lesions, which were considered mild and eventually reversible.

In order to protect the consumers from potential negative

health effects due to dioxin exposure via consumption of fish, the Council of the European Union has decided on maximum levels of CDD/Fs in foods with a limit of 4 pg CDD/F-TEQ/g fresh weight of fish muscle (Council Regulation 2001). However, a derogation for fatty fish from the Baltic region has been offered Sweden and Finland in the legislation, meaning that within these countries fatty fishes over the maximum levels could be caught and sold on the national markets. In Sweden, since many years, the strategy to protect consumers has been establishment of dietary recommendations on fatty fish from the Baltic Sea and certain other Swedish waters. According to these recommendations, girls and women in childbearing ages should not eat fatty fish from the Baltic Sea more than once per month, whereas the recommended limit for other consumers is once per week. The chemical analysis of the herring in this study showed a concentration of about 10 pg CDD/F-TEQ/g fish muscle. The European Union has proposed a tolerable daily intake of 2 pg TEQ/kg body weight. Our results supports a continued use of dietary recommendations on fatty fish from the Baltic Sea and that the focus on CDD/Fs and CBs may be appropriate, even if the limited effect panorama makes it difficult to draw any conclusions with regard to the possible health risks associated with fish intake due to other types of contaminants than the dioxin-like.

The present concern for reproductive and developmental consequences of organohalogen exposure have not been addressed in the present study; higher dose levels and/or other experimental designs are needed to adequately pick up additional end-points and or physiological consequences of relevance for the perinatal period. However, it need to be recognised that, enrichment of natural bioactive compounds such as vitamins, steroids, and lipids introduces confounding effects, which need to be controlled for, and thus may limit the possibilities to increase the dose levels.

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