SUBCHRONIC TOXICITY OF BALTIC HERRING OIL AND ITS FRACTIONS IN THE RAT

Natalia Stern[1], Mattias Öberg[1], Helena Casabona[1], Christina Trossvik[1], Ellu Manzoor[1], Niklas Johansson[1], Monica Lind[1], Kajsa Blomgren[1], Sören Jensen[2], Peter Haglund[3], Clas Wesén[4], Jan Örberg[5], Ricardo Feinstein[6], Anna Johansson[6], Ih Chu[7], Raymond Poon[7], Al Yagminas[7], Abraham Brouwer[8], Bernt Jones[9] and Helen Håkansson[1]

Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden[1] Department of Environmental Chemistry, Stockholm University, Stockholm, Sweden[2] Institute of Environmental Chemistry, Umeå University, Umeå, Sweden[3] Department of Technical Analytical Chemistry, Lund University, Lund, Sweden[4] Department of Environmental toxicology, Uppsala University, Uppsala, Sweden[5] Department of Pathology, The National Veterinary Institute, Uppsala, Sweden[6] Environmental Contaminants Section, Environmental Health Centre, Ottawa, Canada[7] Institute for Environmental Studies, Vrije University, Amsterdam, The Netherlands[8] Department of Clinical Chemistry, Swedish Agricultural University, Uppsala, Sweden[9]

Introduction

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 most contaminated water bodies in Europe. Existing levels of highly toxic organohalogen pollutants have been associated with reproductive injuries, immunosuppression, endocrine disturbanses and other toxicological problems in fish-eating birds and mammals. Compounds of major concern include polychlorinated dibenzo-*p*-dioxins and dibenzofurans (CDD/F), polychlorinated 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. Temporal trends, for these persistent organic pollutants in the Baltic Sea, show a general decline since the 1970s [1]. Yet, the overall intake of dioxin-like pollutants are of the same magnitude as the tolerable daily intake (TDI), proposed by the World Health Organization (WHO) and others [2,3]. In addition, various sub-groups in the human population eat much fish and therefore may exceed the TDI several times [4,5]. Fish consumption along the Scandinavian Baltic coastal area has been positively correlated with CB and CDD/F levels in blood and human milk [6,7]. Epidemiological data show correlations between high levels of fish-derived organohalogen pollutants and effects such as reduced birth weight and poor neurobehavioral development [8,9].

In addition to the toxicologically well characterised organohalogen pollutants, Baltic fish also contain a large amount of less well characterized halogenated organic compounds. During the 1990s it has been established that the majority (≈90 %) of the chlorinated compounds contributing to the extractable organically bound chlorine (EOCl) in fish are halogenated fatty acids (HFA) [10]. The possible toxicity and persistence of EOCl and HFA *in vivo* has not been evaluated. With the aim to quantify the contribution of the different fish-derivied organohalogens to the subchronic toxicity, the present study investigated the possible adverse health effects of different fractions of Baltic herring (*Clupea harengus)* oil, as well as the oil itself, in the rat. A major task in this study was therefore to separate the different types of organohalogen pollutants according to their chemical properties. The oil or its fractions were given at different doses in the diet for up to 39 weeks. Herring was chosen as the source of pollutants, because it is a general nutrient for humans, and therefore relevant from the human health perspective.

Methods and Materials

Baltic herring oil was extracted with isopropanol and fractionated with acetonitrile in a Wallenberg perforator to examine the contribution to toxicity and biological effects of different halogenated organic pollutants. Three fractions were derived. Nordic Sea lodda (*Mallotus villosus*) oil was used as a nutritionally equivalent control, essentially free from halogenated organic pollutants. Fish oils and the fractions were mixed into pelleted food and given to Sprague-Dawley female rats at three levels, corresponding to 8, 40 and 160 times the estimated human intake. Herring oil, its fractions, as well as liver tissues from exposed rats, were analyzed for: eight CBs, all 2,3,7,8-substituted CDD/Fs, hexachlorocyclohexanes (HCH), hexachlorobenzene (HCB), DDT and DDT-metabolites, as well as EOCl and HFA. A bio-assay (EROD) was used for measuring the dioxin-like enzyme induction activity.

The subchronic toxicity study was done essentially according to OECD guidelines. Bearing in mind the well known contribution of the highly bioactive CDD/Fs and CBs to the organohalogen contaminants in Baltic fish, additional end-points, serving as markers for Ah-receptor mediated toxicity, were included in the study (eg. vitamin A analysis). The toxicological examination included clinical observations, macroscopical and histopathological examinations, measurements of bone length and density, haematological analysis, clinical chemistry and biochemical examinations, measurements of hepatic enzyme activities and lipid peroxidation.

Results and Discussion

The fractionation procedure resulted in a substantial reduction of most of the pollutants in the triacylglycerol fraction (F1), and a pronounced enrichment of most of the pollutants into the two other fractions (F2 and F3). All contaminants were still present at some level in all fractions. The concentrations of organohalogens found in this study were representative for Baltic herring during the mid-1990s (Table 1). Rat liver tissue showed similar residue patterns as the diet, with the exception of CDD/Fs that had a higher liver retention than pesticides and CBs. The analysis of lodda oil confirmed the expected low levels of persistent organohalogen pollutants, but the total EOCl was similar and the HFA was higher compared to that of herring oil.

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 fish per week resulted in effects that could be described as minimal, even at the high dose level. Any effects noted in this study can not be conclusively linked to a specific contaminant, due to the complex nature of the mixture employed.

The identified pollutants such as CDD/Fs and CBs contribute to a minor part of the organically bound chlorine in the fish lipids. However, the results of the study have demonstrated that the spectrum of toxic effects was similar to that, which is observed after low-dose exposure to these pollutants (Table 2). Certain effects, such as EROD induction and hepatic vitamin A reduction occurred at lower CDD/F-intake levels than have previously been demonstrated, i.e at 0.012 and 0.23 ng CDD/F-TEQs/kg body weight and day, respectively, corresponding to weekly intakes of 1.6 and 34.4 kg Baltic herring/kg body weight. Interactions between contaminants, with regard to these effects, were additive, and consistent with the TEQ concept, i.e. the expected responses based on the chemically determined doses were obtained.

There were no evidence of any toxic effects at the 39 weeks exposure, except for the microscopic liver lesions, which were considered mild and eventually reversible. The group exposed to the lodda oil diet with high level of HFA and EOCl and low level of identified persistent organohalogen pollutants, did not shown any significant toxicological alterations.

In conclusion, the toxicological examination showed that the major effects were due to pollutants such as chlorinated biphenyls, dioxins and furans, despite the fact that they contribute to a minor part of the extractable organically bound chlorine. Halogenated fatty acids did not seem to cause any harmful effects. From a risk assessment point of view the present study provide important new information about low effect levels for endpoints associated with Ah-receptor activation following low level exposure to organohalogen pollutants from a matrix relevant for human exposure. 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.

Table 1. Concentrations of organohalogen pollutants, halogenated fatty acids and enzyme induction based dioxin equivalents (TEQ) measured in vitro, in herring oil, its fractions, and lodda oil.

Oil/fraction	EOCl (μg/g)	HCB (ng/g)	ΣHCH (ng/g)	ΣDDT (ng/g)	ΣCB (ng/g)	ΣCDD/F (pg/g)	HFA (μg/g)	EROD-TEQ (pg/g)
Herring oil	23	41	61	1 700	1 100	250	5 ±1	2 500
F 1	13	2	≤2	≤25	100	100	4 ±1	n.d.
F 2	241	460	870	28 000	13 600	2 500	43 ±11	27 300
F 3	78	120	≤7	1 900	4 300	1 800	n.d.	13 500
Lodda oil	28	9.8	7.8	40.9	14.3	n.m.	28 ±4	n.d.

n.d. = not detected
n.m. = not measured
values are based on pooled samples

Table 2. Daily intake of HFAs and CDD/F-TEQ and toxicological parameters

Type of diet	Control	Lodda oil	Herring oil	F1	F2	F3
Fish oil content of the diet, %	0	10.13	10.13	8.46	0.72	0.65
HFA intake, µg/day	n.m.	45	9	6	5	n.d.
CDD/F-TEQ intake, pg/day	n.m.	n.m.	220	25	129	58
Weekly food consumption	⇩		⇩	⬇ ⇩	⇩	⇩
Body weight gain				⬇ ⇩	⬇	⬇
Relative liver weight		⬆	⬆ ⇧	⬆ ⇧	⬆	⬆
EROD			⬆ ⇧			⬆ ⇧
Hepatic vitamin A			⇩	⬇ ⇩	⬇ ⇩	⬇

n.m. = not analyzed
n.d. = not detected
⬆⬇ Significantly different from the corresponding control group
⇧ ⇩ Significantly different from the corresponding low dose group

Acknowledgments
This study was performed with the support from the Swedish Environmental Protection Agency (Nr 30512)

References

1. Bignert A., Olsson M., Persson S., Jensen S., Zakrisson S., Lizén K., Eriksson U., Häggberg L., and Alsberg T. (1998) Environ. Poll. 99, 177.
2. Ahlborg U.G., Håkansson H., Waern F., and Hanberg A. (1988) Miljörapport 49 (in Swedish), nordisk ministerråd, Kopenhagen, Denmark
3. van Leeuwen F.X.R., and Younes M. (2000) Food Add. Contam. 17, 223.
4. Svensson B.G., Nilsson A, Jonsson E., Schutz A., Åkesson B., and Hagmar L. (1995) Scand. J. Work Environ. Health 21, 96.
5. Liem A., Furst P., and Rappe C. (2000) Food Add. Contam. 17, 241.
6. Vartiainen T., Jaakkola J.J.K., Saarikoski S., and Tuomisto J. (1998) Environ. Health Perspect. 106, 61
7. Asplund L., Svensson B.G., Nilsson A., Eriksson U., Jansson B., Jensen S., Widequist U., and Skerfving S. (1994) Arch. Environ. Health 49, 477.
8. Jacobsson J.L. and Jacobsson S.W. (1997) Neurotoxicol. 18, 415.
9. Rylander L., strömberg U., Dyremark E., Östman C., Nilsson-Ehle P., and Hagmar L. (1998) Am. J. Epidemiol. 147,493.
10. Wesén C., Mu H., Lund-Kvernheim A., and Larsson P. (1992) J. Chromatogr. 625, 257.