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Exposure of persistent organic pollutants (POPs) in relation to high fish consumption, part 3

Exponering för persistenta organiska miljöföroreningar hos högkonsumenter av fisk, del 3

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Aim

The overall aim for the health related environmental monitoring (HÄMI) is to assess hazardous pollutants and their exposures over time, especially for vulnerable and risk groups of the population, in order to initiate actions in limiting such exposures. In the current study the specific aim has been to assess the exposure of individual PCBs, HCB, oxychlordane, *p,p*-DDE and PBDE # 47 in 141 women with documented fish intake from three different regions in Sweden.

The study (contract 215 0311) is part of a joint HÄMI project in which other studies (HÄMI contracts 215 0105, 215 0307, 215 0309, 215 0310) assess the exposure for the fluorinated compounds PFOS and PFOA, the mercury exposure and the composition of fatty acids in the blood of the women. A questionnaire concerning fish consumption and information on other foods was used for assessment of the individual intake.

Abstract

Individual levels of 33 PCBs (#28, #52, #47/48, #74, #66, #101, #99/113, #110, #118, #114/122, #105, #153, #138, #128/167, #156, #157, # 178, #182/187, #183, #174, #177, #172/192, #180/193, #170/190, #189, #202, # 194, #199, #196/203, #195, # 208, #207, #206), HCB, oxychlordane, *p,p'*-DDE and PBDE #47 were determined in 141 plasma samples (0.5-3 ml) from Swedish females with documented high consumption of fish. The analytical method used includes solid-phase extraction (SPE) and high resolution mass spectrometry (GC-HRMS EI SIM).

Concentrations reported are given in pg/ml plasma and in ng/g plasma lipid (ppb). Lipid contents were determined enzymatically. For the sum of the 33 PCBs the average level, on lipid basis, was 340 ppb (range 1979-61 ppb), for PCB 153 the average was 81 ppb (range 460-13 ppb), HCB 41 ppb (range 143-12 ppb), oxychlordane 2.3 ppb (range 27-0.06), p,p-DDE 131 ppb (range 1114-7.3 ppb) and PBDE #47 had an average level of 2.5 ppb (range 25-0.4 ppb). A reference sample confirms the method performance to have a SD of 13% for the total PCB.

Background

The POPs included in this study are well-known to bioaccumulate in the aquatic food-chain, and fish has relatively high levels of these compounds compared to other foods. In the literature several studies including fish consumers show that consumption of fish affects the body burden of these pollutants. The risk for women is associated with a potential elevated perinatal exposure in connection with pregnancy and breast feeding, as well as with the endocrine disrupting estogenisity of the compounds and their metabolites. For PCB and DDE there is an increase of the bodyburden with age which entails age matching in comparing levels of exposure. For HCB and PBDE similar age related levels are not found.

PCBs

The use and production of PCBs have been banned for decades, but PCBs are still an environmental problem in the Baltic. The concentrations in food have declined since the reports by Sören Jensen and others in the 1970s and 80s. However, Bignert (2002) recently reported that in fish from some parts of the Baltic Sea, the decline has not continued during the 90s. This has been confirmed in other studies on fish in the Baltic Sea (Atuma et al., 1996, and Kiviranta et al., 2003).

DDTs

Declining levels of sumDDT has been reported in the Kattegatt and Skagerak in 1980-2000, (Bignert 2002). In biological samples the metabolite DDE is the most abundant of the group of DDTs. Levels of *p,p*-DDE are despite decreasing levels, still the highest among the organochlorine compounds.

HCB

Hexachlorobenzene, HCB, was withdrawn from the Swedish market in 1980 because of its carcinogenic effects and its persistence. Bignert (2002) reports decreasing concentrations of HCB in herring, cod, guillemot's eggs and dab in the Baltic proper since 1988.

Chlordane/Oxychlordane

Chlordane is a pesticide mixture of 140 different components with persistent and toxicological properties. Its use has been banned in Sweden since 1971, but it is found in fish, bird and seal from the Baltic Sea. When chlordane is metabolized oxychlordane is one possible metabolite in biological samples.

PBDEs

The increase in levels between the 70's and 2000' for the lower brominated BDEs in human milk and biota seems to level out according to recent studies (de Wit, 2002). PBDE #47 is the major PBDE congener in human tissue.

Our earlier studies

In a study of fish consumers around the lake Vättern in the Middle of Sweden (Rapport 74 Vätternvårdsförbundet, 2003) we find levels of PCB to be twice as high (1343 ng/g lipid, range 467 – 3465 ng/g) in Vätternfish consumers compared to a control group (654 ng/g lipid, range 122 – 1900 ng/g). For PCB #153 average was 354 ng/g for Vätternfish consumers and 172 ng/g for the controls. No differences were found for HCB (41 respectively 40 ng/g). For DDE and PBDE #47 the levels measured in blood of the Vätternfish consumers were 772 respectively 449 ng/g and 3.9 respectively 2.9 ng/g.

Material and methods

Study design and sampling

Swedish females (19-56 years, mean 40) with a high dietary intake of fish (consume fish several times a week) from three areas in Sweden were recruted:

1. North East Sweden (Västernorrlands- Västerbottens- och Gävleborgs län)
2. Mid Sweden (Västmanlands-, Värmlands- och Örebro län)
3. South West Sweden (Västra Götalands- och Hallands län)

All women gave blood in October 2001, and completed a dietary questionnaire of their fish intake during one year. Whole blood (2 x 5 ml; Venoject II® 5 ml, EDTA (K2): 9.8 mg, VP-050SDK, Terumo® Corporation, Leuven, Belgium) and serum (2 x 4 ml;

Venoject II® 4 ml, Gel+Clot Act., VP054SAS, Terumo® Corporation, Leuven, Belgium) was collected from 146 females for the joint project. POP levels in serum were analysed in this study. The questionnaire included information on the consumption of the following fish: frozen fish, fish dishes, mackerel (*Scomber scombrus*), tinned herring (*Clupea harengus*), tinned tuna (*Thynnus thynnus*), other tinned fish, salty herring (*Clupea harengus*), Baltic herring (*Clupea harengus*), smoked Baltic herring (*Clupea harengus*), smoked herring (*Clupea harengus*), Baltic salmon (S*almo salar*), other salmon (S*almo salar*), eel (*Anguilla anguilla*), pike (*Esox lucius*), pike-perch, burbot (*Lota maculosa*), perch (*Perca fluviatilis*), fresh halibut (*Hippoglossus hippoglossus*), tuna (Thynnus thynnus), swordfish (*Xiphias gladius*), angler (*Lophius piscatorius*), other fresh sea fish, liver from cod (*Gadus morhua*) and burbot (*Lota maculosa*), and shellfish (*Crustacea*). Information if the fish was bought or caught, and if pike, pike-perch and perch where caught from the Baltic Sea or a local lake was contained in the questionnaire. Consumption of sausage, pork, beef, game, dishes with minced meat, kidney and liver, chicken, other birds, and egg quantity were also recorded in the questionnaire

Chemical analyses

All analyses were done at the Dioxinlaboratory at MTM Research Centre, Örebro University. In a pilot study prior to the main study the analytical procedure was developed and tested in order to facilitate determination of lipid contents and the analytes in the limited sample volumes available (0.5-3 ml). Solid phase extraction clean-up and high resolution mass spectrometry met the demands for detection of most of the congeners (MDL 0.1-1 ng/g lipid). With MS (R=1000) a MDL of 1-5 ng/g lipid could be achieved. In order to be able to analyze as many congeners as possible we therefore chose HRMS for detection.

Determination of lipid content

The lipid content of the serum was determined at Örebro University Hospital by an enzymatic determination of triglycerides, cholesterol and phospholipids in a subsample of approximately 200 µl of serum. The total fat content was calculated by summarizing

triglycerides, cholesterol and phospholipids according to a method proposed by Grimvall et al., 1997. This enzymatic determination of the lipid content had good correlation with gravimetric determination.

Standards and reagents

Internal standards for PCBs, ^{13}C-labeled PCB #28, #52, #70, #101, #105, #118, #138, #153, #156, #170, #180, #194, #202, #206, and #209, were purchased from Wellington Laboratories. Internal standards for PBDEs, ^{13}C-labeled BDE #77 was from CIL, and #139 was purchased from Wellington Laboratories (Guelph, Ontario, Canada). A ^{13}C-labeled PCB recovery standard was used, containing PCB #81, #114, and #178. A quantification standard (IUPAC Nordic Standard, 99,9%) for PCBs included PCB #28, #52, #47, #74, #66, #101, #99, #110, #118, #114, #122, #105, #153, #141 #138, #128, #167, #156, #157, #187, #183, #180, #170, #189, #194, #206, #209, and for PBDEs congener #28, #47, #66, #85, #99, #100, #138, #153, #154, #183. For determination of pesticides Pesticides-Mix 13 from Labor Dr. Ehrenstorfer-Schäfers, Germany, was used. Organic solvents used were of pesticide grade and purchased from Riedel de Haën (methanol, n-hexane, dichloromethane, and 2-propanol). Formic acid was purchased from Merck, and water used for blank samples and column washing was water, gradient HPLC grade from Scharlau.

Extraction and clean-up

Extraction and sample clean up was performed after an adopted solid phase extraction (SPE) method (by Thomsen et al., 2001). In short, human plasma samples were extracted with solid phase extraction (SPE) and further cleaned using mini silica open columns. All glassware was rinsed with ethanol, n-hexane and dichloromethane. The frozen plasma sample was allowed to thaw in the refrigerator. Plasma, formic acid and 3 % 2-propanol were used in ratio 1:1:1. Plasma was added to the glass flasks. With every batch of samples a blank sample of water, gradient HPLC grade from Scharlau, was extracted. Equal amounts of formic acid were added and the samples where sonicated for 10 minutes. Internal standards were added. After 60 minutes 3 % 2-propanol was added and the samples were sonicated for 10 minutes. Prepacked 6 ml SPE columns (ISOLUTE,

200 mg, ENV+, from International Sorbent Technology, Mid Glamorgan, UK) were activated with 4 ml methanol, 4 ml dichloromethane, 8 ml dichloromethane/methanol (1:1 by volume), 6 ml methanol, and 6 ml water. 60 minutes after adding 3 % 2-propanol the samples were added on to the columns. The glass was washed with 6 ml 3 % 2-propanol and added onto the columns. After washing the columns with 8 ml 3 % 2-propanol and 8 ml 40 % methanol in water the column was put under vacuum suction together with application of nitrogen pressure on top until dryness. Elution was performed slowly (1 drop/sec) with 10 ml dichloromethane. All solutions including the sample solution were allowed to run through the column material without vacuum. The extracts were evaporated under a gentle stream of nitrogen and cleaned up on mini silica columns. Mini silica columns were plugged with glass wool and rinsed with ethanol, n-hexane, and dichloromethane, and packed with KOH-based silica and sulfuric acid silica. The columns were pre washed with 4 ml n-hexane, 4 ml dichloromethane and 2 ml n-hexane. The extracts were added on to the columns and eluted with 10 ml n-hexane. As a keeper 25 µl of tetradecane was added and the samples were evaporated to 25 µl under a gentle stream of nitrogen. The extracts were stored at -20 ºC until HRGC/HRMS analysis.

Mass spectrometry analysis

HRGC/HRMS analysis was performed on a Micromass Autospec Ultima operating at 10 000 resolution using EI ionization at 35 eV. All measurements were performed in the selective ion recording mode (SIR), monitoring the two most abundant ions of the molecular chlorine or bromine cluster. Quantification was performed using the internal standard method. Analyses were performed with a 60 m DB-5 (0.32 mm id, 25 µm) and a 30 m DB-5MS (0.25 mm id, 25 µm) column. Splitless injection was used to inject 1 µl of the final extract on the GC column. GC temperature programs were used to optimize the response (and minimize the degradation in both the injector and on the column) depending on column length and GC performance. The temperature program consisted of the following: The injector at 275 C, an initial oven temperature of 180 C held for 2 min, and heating to 250 C at a rate of 6 C/min, and then to 320 C at 3 C/min. Helium was used as a carrier gas at a flow rate of 1.5 ml/min.

Method performance and quality assurance

Method repeatability was controlled by extracting a reference sample (14 replicates) with every batch extracted. A blank sample (HPLC grade water) was also prepared with every batch extracted and analyzed. The detection levels were mainly in the range 0.1-1 ppb, depending on congener and sample amount. The MTM laboratory participates on a regular basis in international intercalibration studies. In studies organized by AMAP, QUASIMEME and the Norwegian Institute of Public Health the MTM laboratory shows good results.

Results and discussion

Totally 141 plasma samples were extracted and analyzed with above described methods. One sample had an insufficient volume for lipid determination and for this sample levels are given only on wet weight basis. In tables 1 and 2 results are presented of range (individual min and max), average and median levels for all POPs determined in 140 plasma samples. The % SDs for each congener, assessed from the reference sample (13 replicates) are included. For the sum of PCBs the % SD is 13%, which is very good bearing in mind the small sample volumes and number of congeners determined.

Table 1. Concentrations on lipid bases of individual PCB congener and sumPCBs lower (LB, nd= ½ DL) and upper bound (UB, nd=DL) in 140 plasma samples. Congener specific SD % calculated from 13 reference sample analysis.

Congener	Range ng/g lipid	Average ng/g lipid	Median ng/g lipid	Reference SD %
PCB#28	0.58-41	3.3	2.3	17
PCB#52	0.24-5.5	0.95	0.68	17
PCB#47/48	0.23-2.7	0.88	0.69	17
PCB#74	0.32-18	2.7	2.0	12
PCB#66	0.12-11	0.85	0.61	29
PCB#101	0.19-8.7	1.1	0.74	24

PCB#99/113	0.62-26	4.2	3.3	10
PCB#110	0.06-3.4	0.47	0.33	26
PCB#118	1.6-64	10	7.7	10
PCB#114	0.04-2.4	0.46	0.34	27
PCB#105	0.20-9.6	1.6	1.2	16
PCB#153	13-461	81	63	12
PCB#138	10-348	60	47	12
PCB#128	0.45-19	3.6	2.9	16
PCB#156	1.3-43	7.6	6.1	13
PCB#157	0.23-7.8	1.4	1.2	18
PCB#178	0.35-30	3.4	2.7	25
PCB#182/187	1.9-99	14	11	15
PCB#183	0.86-33	6.1	5.0	17
PCB#174	0.49-32	4.0	3.0	14
PCB#177	0.39-15	2.6	2.2	15
PCB#172/192	0.46-18	3.1	2.6	16
PCB#180/193	12-394	70	61	15
PCB#170/190	4.8-165	29	25	13
PCB#189	0.23-8.9	1.4	1.2	21
PCB#202	0.27-14	2.0	1.6	28
PCB#194	1.4-45	8.2	7.0	17
PCB#199	1.1-39	6.2	5.1	28
PCB#203/196	1.2-37	7.1	5.8	26
PCB#195	0.13-7.4	1.7	1.4	28
PCB#208	0.037-4.1	0.65	0.50	39
PCB#207	0.034-2.5	0.32	0.25	38
PCB#206	0.14-16	1.9	1.5	19
Sum PCBs LB	61-1980	340	268	13
Sum PCBs UB	123-1980	343	273	13

Table 2. Concentrations on lipid bases of HCB, oxychlordane, *p,p'*-DDE, and PBDE #47 in 140 plasma samples. Congener specific SD % calculated from 13 reference sample analysis are included.

	Ranges	Average	Median	Reference
Congener	ng/g lipid	ng/g lipid	ng/g lipid	SD %
HCB	11-143	41	30	33
Oxyklordan	0.06-27	2.3	1.6	49*
p,p-DDE	7.0-1114	131	94	16
BDE#47	0.39-25	2.4	1.4	22

* due to levels close to DL

In the Appendix (Bilaga SNV1) individual levels on lipid bases for all 140 samples are given. The complete data (including also the wet weight based data) from this study is available in the SNV Data Base. Correlations of total PCB, PCB #153, HCB, *p,p*-DDE, oxychlordane and PBDE # 47 with the levels of other pollutants (fluorinated an mercury) and with the information from the questionnaire can be found in the Final Report (contracts 215 0307, 215 0309, 215 0310, 215 0105, 215 0311) including parts 1, 2 and 3 of the HÄMI project.

References

Bignert A 2002. www.nrm.se/mg/mcom02.pdf. Comments concerning the National Swedish Contaminant Monitoring Programme in Marine Biota.

de Wit C. An overview of brominated flame retardants in the environment. *Chemosphere* 46, 583-624 (2002).

Grimvall E., Rylander L., Nilsson-Ehle P., Nilsson U., Strömberg U., Hagmar L., Östman C. *Arch. Environ. Contam. Toxicol*. 32, 329-336 (1997).

Kiviranta H., Vartiainen T., Parmanne R., Hallikainen A., and Koistinen J. *Chemosphere* 50, 1201-1216 (2003).

Atuma S.S., Linder C-E, Wicklund-Glynn A., Andersson Ö., and Larsson L. *Chemosphere* 33, 791-799 (1996).

R. Bjerselius et al., 2004. Report to the Swedish Environmental Protection Agency, 2004-01-23. Persistent organic pollutants (POPs) in fish from the Baltic Sea, Sweden, 2000-2002.

Thomsen, C., Lundanes, E. and Becher, G. A simplified method for determination of tetrabrombisphenol A and polybrominated diphenyl ethers in human plasma and serum. *J. Sep. Sci*., 24, 282-290 (2001).

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