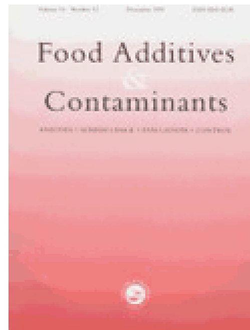

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Consumption of farmed salmon did not increase the levels of persistent organic pollutants (POP) in patients with metabolic disorders.

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Consumption of farmed salmon did not increase the levels of persistent organic pollutants (POP) in patients with metabolic disorders.

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Abstract

Objectives: Advice regarding the health benefits of fish consumption has been complicated by reports that certain fish species like salmon are burdened with levels of organochlorine contaminants (OCs) that may be potentially harmful in man. The aim of this study was to determine changes in the levels of persistent organic pollutants (POP) in human plasma and adipose tissue and to examine associations between POP levels and dietary fat intake during consumption of farmed salmon.

Methods: Twelve patients with non-alcoholic fatty liver disease consumed 380g farmed Atlantic salmon fillets for 15 weeks in an intervention study to examine the health benefits of farmed salmon. Concentrations of POP: hexachlorobenzene (HCB), *bis*-2, 2-(4-chlorophenyl) -1,1-trichloroethylene (*p,p'*-DDE), polychlorinated biphenyls (indicator (id-PCBs), mono-*ortho* PCBs (mo-PCBs), polybrominated diphenylethers (PBDEs), were measured in the salmon fillets, and in plasma and abdominal fat biopsies from the patients before and after intervention. Toxicity equivalent quantities (TEQs) for mo-PCBs were calculated.

Results: The concentrations of POPs in the salmon fillets were comparable to those typically found in farmed Atlantic salmon. 15 weeks consumption of these fillets was reflected in a significantly increased level of n-3 fatty acids in plasma. At the start of the study mean concentrations (ng/g lipid weight (lw)) of HCB, *p,p'*-DDE, sum id-PCBs, sum mo-PCBs, and sum PBDEs in plasma were 20±9, 115±136, 161±74, 24±12, and 3±2 and in abdominal fat 21±10, 191±300, 268±135, 40±20, and 4±4 (ng/g lw) respectively. After 15 weeks of salmon consumption the concentrations of POPs in samples of human plasma and abdominal fat had not increased but tended to decrease. The levels of CB156, CB167, sum mo-PCBs and sum $TEQs_{WHO2005}$ of mo-PCBs in fat biopsies decreased significantly ($p < 0.05$). After adjustment for age plasma total n-3 fatty acids correlated positively and oleic acid (18:1 n-9) correlated negatively with sum id-PCBs. Linoleic acid (18:2 n-6), on the other hand correlated positively with *p,p'*-DDE, sum-PBDEs and sum mo-PCBs.

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Conclusion: 15 weeks consumption of fatty fish did not affect the steady state levels of POPs in the study subjects. Significant associations were found between different plasma long chain fatty acids and different POP classes indicating that the latter have different food products as their main source for human exposure.

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Keywords: Atlantic salmon, organic pollutants, fat biopsies, dietary fat, correlation

Funding sources:

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Ethical approval.

The clinical study protocol has been approved by the The Regional Ethics Committee of Western Norway and all the study subjects did sign a written informed consent.

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Introduction

The health benefits of eating fish are well documented and seafoods rich in PUFAs have attracted special attention in this respect (Horrocks & Yeo, 1999; Mori et al, 1999). Fish and fish oil have beneficial effects in relation to a range of metabolic disorders, such as heart diseases, hypertension, inflammatory diseases, various cancer forms and possibly also diabetes II (Gebauer et al, 2006; Hu et al, 2001). Fish oil will further increase the bleeding time, reduce platelet aggregation, modulate inflammatory response and decrease slightly the blood pressure. Epidemiological evidence further suggests that populations consuming relatively large amounts of fish have lower risk of cardiovascular diseases and reduced mortality than those consuming little or no fish (Connor, 2000). The extent to which all these beneficial effects are attributable to the consumption of fish per se or can be achieved by fish oil as such is still a matter of debate. Furthermore, it is not clarified to what extent fish or fish oil may improve various disorders related to dyslipidemia and impaired inflammatory response such as fatty liver, ulcerative colitis and endothelial dysfunction among others.

Wild caught fish is still the most important source of seafood to humans, but farmed fish is getting increasingly important. However, advice regarding fish consumption has been complicated by reports that some fish species, such as farmed salmon, are burdened with potentially harmful levels of persistent organic pollutants (POPs), e.g.polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/PCDFs), dioxin-like polychlorinated biphenyls (dl-PCBs), non-dioxin-like PCBs (ndl-PCBs), brominated flame retardants (BFRs) like polybrominated diphenyl ethers (PBDEs), and organochlorine pesticides (OCPs) like hexachlorobenzene (HCB) and *bis*-2, 2-(4-chlorophenyl)-1,1-trichloroethylene (*p,p'*-DDE) (Hites et al, 2004a; Hites et al, 2004b; Mozaffarian & Rimm, 2006).

Due to their lipophilic properties organochlorines (OCs) bioaccumulate and biomagnify in the food chain. For humans the main exposure route of OCs is through the diet (Ahlborg et al, 1992; Liem et al, 2000). In addition to dietary exposure, humans might be

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exposed to particularly PBDEs through direct contact with dust, furniture and electric equipment (Darnerud, 2003; Sjodin et al, 2000). The total levels of OCPs, PCBs and PBDEs are higher in fish and other seafood, especially fatty fish and liver, than in most other foods, and fatty fish species such as salmon is thus an important source for POPs. In humans POPs are easily absorbed from the diet and distributed rapidly to fat rich organs and tissues.

Since 2004 several scientific papers as well as articles in national and international lay press have indicated that consumption of farmed Atlantic salmon may pose health risks that detract from the beneficial effects of fish consumption. Studies by Hites and co-authors (Hites et al, 2004a) and Foran and co-authors (Foran et al, 2005) conclude that farmed salmon contains higher concentrations of POPs than wild salmon, and that the former should not be eaten more than a few times per year due to potentially increased cancer risk. The risk assessment methods used in these papers were developed by both the United States Environmental Protection Agency (U.S. EPA) and the International Agency for Research on Cancer (IARC), but deviates in several aspects from methods used by international bodies like WHO/JECFA and EFSA. Thus, this contradicts current advice to increase fish consumption, and the European Food Safety Authority (EFSA) has concluded that there is no difference in wild vs. farmed fish with respect to consumer safety and stated that fish consumption, especially of fatty fish (1-2 servings a week) benefits health {SACN/COT (Scientific Advisory Committee on Nutrition/Committee on Toxicity, 2004 1220 /id}(EFSA, 2005). In a recent comprehensive assessment of fish and other seafood in the Norwegian diet the Norwegian scientific committee for food safety support the current Norwegian recommendations regarding consumption of fish, i.e. to eat a variety of fish including 2 meals of fatty fish per week (Skåre et al, 2006)

Currently, more than half the salmon sold globally is farm-raised in Northern Europe, Chile, Canada, and the United States (FAO, 2008; Hites et al, 2004).

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POPs exhibit a broad range of toxic and biological effects including cancer (Porta, 2001; Porta, 2006; Safi, 2002). No human data are available for quantitative risk assessment of PCDD/PCDFs, PCBs and PBDEs. Data from animal studies have therefore been used. For the PCDD/PCDFs and the dl-PCBs a tolerable intake has been established (EC SCF 2001, WHO, 2001). A threshold approach was used to derive at a tolerable intake of 14 pg TEQs TCDD/kg body wt. per week based on the effects on the developing male reproductive system resulting from the maternal body burden, which appear to be the effect triggered at the lowest dose. In addition to effects on the developing reproductive system several toxicological effects of dioxins and PCBs have been observed in the thyroid gland, liver, immune system, as well as on behaviour and the development of cancer, but it is not possible to distinguish between the effects resulting from dioxins and dl- PCBs and the effects from ndl-PCBs. Dioxins have been classified as carcinogenic to humans (IARC 1997). For PCDD/PCDFs and dl-PCBs the site specificities for the highest increased incidences of neoplasms are liver, lung and oral mucosa. In the liver, an increased incidence of hepatocellular adenoma and carcinoma is found (Huang et al, 2006; Salonen et al, 1995; Zhang et al, 2001). For the ndl-PCBs there are no reliable health based guidance values for human risk assessment (EFSA 2005).

There is limited toxicological information about the most toxic PBDEs. PBDEs in fish may be a risk factor for thyroid hormone imbalances (Darnerud, 2003) and for neurotoxic effects (Eriksson et al, 2001), and it is of further relevance that the important role of thyroid hormones in cardiac function can be disrupted by PCDDs/PCDFs and dl-PCBs, PBDEs, and their metabolites. The PCDDs/PCDFs, dl-PCBs, and ndl-PCBs have also been implicated in the atherosclerotic and cardiomyopathic processes.

Studies on fish consumption, with known levels of contaminants, and the resulting accumulation in human plasma and tissues are rare.

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As part of a dietary study to examine health effects of a large consumption of salmon in patients with metabolic disorders, it was of interest to assess if it was possible to detect any accumulation of POPs. The purpose of the present study was therefore to examine levels of POPs in human plasma and in human adipose tissue after a weekly consumption of 380 g farmed Atlantic salmon (2 meals per week) for up to 15 weeks.

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Methods

The 15 week intervention trial was conducted at Haukeland University Hospital, Bergen, Norway during 2005.

Study design.

A total of 12 patients with non-alcoholic fatty liver disease (NAFDL) were included in this study. NAFLD is associated with insulin resistance, obesity, hypertension, hypertriglyceridaemia and diabetes mellitus and is thus related to the metabolic syndrome. The reason why these patients were included was that they all participated in a larger intervention study to investigate effects of a high consumption of salmon.

The patients were all obese and they all agreed to undergo fat biopsies. Results from the larger clinical study will be published elsewhere.

The Regional Ethics Committee approved the study protocol and all included subjects gave their written informed consent.

Study diet

The participants consumed two servings of 190 g salmon each week (totally 380 g) for 15 weeks. The Atlantic salmon was produced by EWOS innovation, Dirdal, Norway. The fodder contained 30 % fat, with South-American fish oil as the lipid source. Study meals were prepared in the canteen at Haukeland University Hospital. The fillets were cooked and vacuum packed, ready for consumption at home. Durability of the fillets was 4 weeks when stored at 4°C. The salmon fillet used in the study contained 13.9% fat, corresponding to a weekly consumption of 52 g salmon fat for each study subject.

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Dietary restrictions

Four weeks before intervention with salmon and during the study all subjects had to adhere to a diet very low in omega 3 products. Participants in the intervention trial were told to avoid omega-3 products, including salmon, trout, herring and mackerel, products of these species and nutritional supplements, but were allowed to eat white fish products.

Dietary evaluation

A validated food frequency questionnaire (FFQ) was filled in at the start and at the end of the study (Brantsæter et al, 2007a). It is a semi-quantitative questionnaire designed to capture dietary habits and intake of dietary supplements during the last year. The FFQ includes questions about intake of 255 food items, among them 10 questions about cold cuts and spreads made of fish/shellfish, 16 questions about fish/shellfish eaten for dinner, and four questions about cod liver oil/cod liver oil capsules/fish oil capsules.

The questionnaires were optically read. Consumption frequencies were converted into food amounts (g/day) by the use of standard Norwegian portion sizes. FoodCalc (Lauritsen, 2005) and the Norwegian food composition table (Rimestad et al, 2001) were used for calculating nutrients from food. A database containing details of the declared content of supplements was developed for the calculation of nutrients from dietary supplements (Brantsæter et al, 2007b).

Analytical procedures

POPs were analysed in salmon fillet, in plasma and fat biopsies from the subjects participating in the study.

The salmon fillet was kept at - 20°C until analyzed. It consisted of epaxial muscle that was skinned, homogenized, and pooled (n=7) before being analyzed for POPs.

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Patient blood samples were drawn in a fasting condition on day 0 of the intervention and at the end of the study. The EDTA plasma was prepared within 1 h and kept at -70C until analyzed.

Fat biopsies

After collecting blood samples, a fat sample (approximately 0.1 g) was obtained by needle biopsy from the abdominal subcutaneous area under local anaesthesia. The tissue was frozen in liquid nitrogen, kept frozen at –70 $^{\circ}$C until analyzed.

Chemical analyses

Concentrations of hexachlorobenzene (HCB); *bis*-2, 2-(4-chlorophenyl)-1,1-trichloroethylene (*p,p'*-DDE); indicator (id) polychlorinated biphenyls (PCBs) (id-PCBs), IUPAC nos.: CB138, 153, 170, 180 and 194, mono-*ortho* PCBs (mo-PCBs): CB105, 114, 118, 123, 156, 157, 167 and 189, polybrominated diphenylethers (PBDEs): BDE28, 47, 99 and 100 in pooled samples of salmon and individual samples of human plasma and biopsies were detected and measured at the Norwegian School of Veterinary Science, Oslo, Norway.

Extraction and clean up

During the whole sample preparation samples were protected from light because some of the PBDEs can be degraded by UV-light. The human biopsies (~0.1 g) were weighed, added internal standards CB29, 112 and 207, BDE77, 119, 181 and ^{13}C-BDE209 (Cambridge Isotope Laboratories, Inc., Andover, MA, USA), 10 mL of H_2O, and solvents. After homogenization, with an Ike Ultra-Turrax^{TM} (Janke & Kunkel, Gmbh & Co., KG, Germany) the lipids were extracted twice with cyclohexane and acetone (3:2) using an ultrasonic homogenizer (4710 Series, Cole Parmer Instruments Co., Chigaco, IL, USA). The plasma samples (~5 g) were weighed added the same internal standards, but were not homogenized

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before extraction. The extracts were centrifuged for 10 min at 3,000 rpm (Allegra 6R Beckman Coulter). The supernatants of the extractions were merged and concentrated to about 1 mL using a Zymark (evaporation system (TurboWap II, Zymark Corporation, Hopkinton, MA, USA) at 40^0C, and by a gentle flow of nitrogen (pressure 0.6 bar). The samples were then transferred quantitatively to a conical, calibrated glass tube, and the solvents were vaporized by a gentle flow of nitrogen (pressure 0.6 bar) until stable weight of the glass and lipid. The lipid determination was done gravimetrically. Thereafter, the extracted lipid was re-dissolved with one mL of cyclohexane. For cleanup (i.e., removal of lipids) the lipid were treated twice with ultra clean (purity 96%) concentrated H_2SO_4 (Scanpure, Chemscan AS, Elverum, Norway). The extracts were transferred to conical glass tubes and concentrated by a gentle flow of nitrogen to 0.5 mL final volume. The final extracts were transferred to amber GC-vials.

Determination of POPs

The separations and detections of the two organochlorine pesticides (OCPs) HCB, *p,p'*- DDE and id-PCBs were performed by high resolution GC (Agilent 6890 Series gas chromatography system; Agilent Technologies, PA, USA) equipped with an auto sampler (Agilent 7683 Series; Agilent Technologies), a dual column system with specifications SPB-5 and SPB-1701, both 60 m, 0.25 mm i.d. and 0.25 µm film thickness (Supelco, Bellefonte, PA, USA) coupled to two ^{63}Ni micro (µ) electron capture detectors (Agilent 6890 µ-ECD). Other details of the GC-ECD specifications have been described (Polder et al. 2008). The mo-PCBs and PBDEs were detected on a HRGC (Agilent 6890 Series), equipped with a SPB-5 column (60 m, 0.25 mm i.d., 0.25 µm film thickness; Supelco) and connected to a low resolution MS (LRMS) quadrupol detector (Agilent Technologies, Avondale, PA, USA), operating in selected ion monitoring (SIM) mode with negative chemical ionization (NCI). The carrier gas was Hydrogen (H_2) 5.0 pure (Yara Industrial AS, Oslo). Further details for

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detection of mo-PCBs and PBDEs were described by (Polder et al. 2008). The target ions used for the mo-PCBs were *m/z* 325.8 for CB123, -18, 114, 105; *m/z* 359.7 for CB156, 157, 167 and *m/z* 395.7 for CB189. The target ions for PBDEs were *m/z* 79/81. The lowest limits of detection (LODs) were as follows: HCB: 0.005 ng/g ww in plasma, 0.022 ng/g w/w in salmon, 0.1-5 ng/g ww in fat biopsies; *p,p'*-DDE: 0.006 ng/g ww in plasma, 0.042 ng/g w/w in salmon, 0.3-11 ng/g w/w in fat biopsies; id-PCBs: 0.002-0.01 ng/g ww in plasma, 0.02-0.1 ng/g w/w in salmon, 0.1-21 ng/g ww in fat biopsies; mo-PCBs: 0.001-0.002 ng/g ww in plasma and salmon, 0.04-3 ng/g in fat biopsies; PBDEs: 0.005-0.006 ng/g ww in plasma, 0.003-0.005 ng/g ww in salmon, 0.11-3.4 ng/g ww in fat biopsies. Levels of POPs, lower than LOD, were replaced by half-LOD values.

Analitical quality:

The laboratory is accredited by Norwegian Accreditation for testing the analysed chemicals in biological material according to the requirements of the NS-EN ISO/IEC 17025 (TEST 137). The results of recovery tests, performed on spiked whole blood of sheep ranged between 84-118% for OCs, 84-146% for mo-PCBs and 89-112% for PBDEs. Several blanks (solvents) and one blind (low fat milk) were included in every series. The laboratory's own reference sample of seal blubber was analysed for each of the analytical for testing reproducibility over time. Deviation in response of the GC system was controlled by analysing a standard for every 10th sample. The coefficient of variation for determinations in the laboratory's reference samples ranged between 7-24% for OCs, and 16-41% for the PBDEs. The analytical quality of the determinations of OCPs, PCBs and PBDEs was found satisfactory after participation in several relevant intercalibration tests: WHO, third round of coordinated exposure study on levels of PCBs, PCDD/PCDFs in breast milk (2000-2001); Quasimeme, Exercise 524, round 28, on PBDEs in human milk (2002) and AMAP ring-tests for PCBs and OCs in human serum (2001-2006).

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Calculations and statistical analysis

The estimated weekly intake (EWI) of the POPs, based on lipid weight, was calculated as follows: POPs concentrations in salmon fillet was multiplied with 380g consumed salmon per week, divided by the mean body weight of the patients (100kg) (Table 4).

Statistical analyses were carried out in SAS (Statistical Analysis System) version 9.1 (SAS Institute, Inc., Cary, NC), Prism 4.0 and SPSS 14.0 for Windows. All p values were two-sided and values < 0.05 were considered statistically significant. Concentrations are given as means with standard deviations (SD). Signed rank test were used to demonstrate significant differences. Relationships between pairs of measures were assessed using Pearson and Spearman correlation coefficients. Spearman partial correlations were also computed to assess whether any relationships found between two variables still existed after adjustment for patients' age.

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Results

Characteristics of the study population are given in Table 1. Three of the subjects included had hypertension and were treated with ACE-inhibitor and diuretics. One patient was treated with mesalazin for Crohns disease, 2 patients were treated with statins and 2 patients with metformin for diabetes mellitus. The patients increased their weight non-significantly from 98±15 kg before the study to 100±16 kg at the end of the study ($p = 0.4$).

Diet evaluation and fatty acid profile in plasma

The dietary questionnaire revealed that the estimated intake of n-3 increased significantly, otherwise no changes in energy and proportion of nutrients intake were observed during the intervention study (Table 2).

The fatty acid profile was measured in plasma samples to evaluate the subjects compliance with the study protocol. From Table 3 it can be seen that during the run-in period with dietary restrictions the plasma levels of n-3 fatty acids decreased significantly with more than 30% ($p<0,05$). During the study period most of the n-3 fatty acids consumed came from the study meals. Plasma n-3 levels increased as expected (Table 3). The same increase was observed for the ratio of n-3/n-6 fatty acid due to an increased content of EPA and DHA, but not of n-6.

POP concentrations in salmon

The concentrations (ng/g wet weight (ww)) of HCB, *p,p'*-DDE, PBDEs, id-PCBs and mo-PCBs, and the concentrations (pg/g lw) of TEQs $_{WHO1998, WHO2005}$ of sum mo-PCBs in salmon fillet are presented in Table 4. The EWIs, based only on consumption of 380g salmon per week, for sums of HCB, *p,p'*-DDE, sum PBDEs, sum id-PCBs and sum mo-PCBs were 5, 14, 2, 27 and 10 ng/kg body weight, respectively. The EWI for TEQs$_{WHO1998}$ of sum mo-PCBs was 1.4 pg/kg body weight, while the corresponding EWI for TEQs$_{WHO2005}$ were 0.3 pg/kg body weight, respectively (Table 4).

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POP concentrations in plasma and abdominal fat

The concentrations (ng/g lw) of HCB, *p,p'*-DDE, PBDEs, id-PCBs and mo-PCBs in plasma and fat-biopsies are presented in Table 5 and 6. HCB and *p,p'*-DDE were detected in all samples. The id-PCBs and mo-PCBs were detected in >90% of the samples, but CB114 had a lower detection frequency in abdominal fat samples (>60%). PBDEs were found in low concentrations, approximately one order of magnitude lower than the other POPs. BDE47 was the major PBDE-congener, detected in 96% of the samples. The detection frequencies of BDE28, BDE99 and BDE100 were zero (0), 25 and 12% and 0, 54 and 30% in plasma and adipose tissue samples, respectively.

No significant difference was observed in the plasma levels comparing week 0 and week 15. A large inter-individual variation in POP concentrations was found. Significant reductions of the concentrations of CB156, CB167 and sum mo-PCBs were observed in the fat-biopsies ($p < 0.05$) after 15 weeks of salmon consumption. TEQs of CB156, CB167 and of sum mo-PCBs decreased similarly and with the same significant level ($p < 0.05$). The concentrations of all POP groups excluding HCB and sum PBDEs were significantly lower in plasma than in fat biopsies (Table 7). Within plasma there were positive correlations between all POP groups excluding PBDEs at week 15 (Table 8A). This was true also between plasma and fat biopsies (Fig.1 and Table 8B). Similar correlations were found also at week 0 and for POPs in the fat biopsies at week 0 and 15 (data not shown).

Correlations between POP concentrations, fatty acid levels and age

The data were further analysed for correlations between levels of id-PCBs in biopsies before the intervention, age and plasma levels of fatty acids. A significant correlation was found between age and id-PCBs in both biopsies ($r = 0.78$, $p < 0.005$) and plasma ($p < 0.001$) (Fig 2).

After adjustment for age correlations were found between plasma levels of specific fatty acids and the plasma levels of different POPs. Total n-3 fatty acids correlated positively

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and oleic acid (18:1 n-9) correlated negatively with sum id-PCBs (p= 0.027) respectively. Linoleic acid (18:2 n-6), on the other hand, did not correlate with these PCBs, but correlated positively with *p,p'*-DDE, sum PBDEs and sum mo-PCBs (p = 0.015, 0.0009 and 0.029, respectively) (Table 9).

No significant correlations were found between increase in plasma levels of n-3, EPA, DHA and reduction in indicator PCB levels in biopsies (n-3: -r = 0.62, p = 0.1, EPA: r= -0.62, p = 0.1, DHA: -0.51, p = 0.2, respectively.

No significant correlation was found between body weight and id-PCBs measured in biopsies (r = 0. 07, p = 0.84). (Data not shown).

Discussion

The main outcome of this study was that no accumulation of POPs could be detected in plasma or adipose tissue after consumption of fatty fish equivalent to two salmon meals per week (380g) for up to 15 weeks. The increase in plasma n-3 polyunsaturated fatty acid levels during the study period confirm intake of salmon during the intervention phase, as the plasma levels of EPA is used as a biomarker for the intake of fatty fish.

The small number of subjects included in the study implies that the results should be evaluated with caution. However, the results obtained are in line with previous studies and are also in accord with results from a larger cohort of patients (data to be published).

The levels of POPs in the salmon fillets consumed were comparable to those typically found in farmed salmon (Bethune et al, 2006; Knutsen et al, 2008; Hites et al, 2004b).

The estimated weekly intake (EWI) of $TEQs_{WHO1998}$ of sum mo-PCBs based on 380g salmon filet per week (1.4 pg/kg body weight/week) was lower than reported in a previous study (Bethune et al, 2006) and similar to the average exposure to $TEQs_{WHO1998}$ from all fish and seafood in Norway (Norwegian Scientific Committee for Food Safety; Skaare et al. 2007) In 2005 the WHO 1998 TEF values were revised, resulting in increased, decreased and

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unchanged TEF values for individual congeners of dioxins and dl-PCBs (non-*ortho* and mono-*ortho* PCBs) (van den Berg et al. 2006). In the present study both $TEFs_{WHO1998}$ and $TEFs_{WHO2005}$ were used in the calculation of EWIs. From Table 4 it can be seen that sum $TEQs_{WHO2005}$ for mono-*ortho*PCBs was reduced by approximately 80% compared to the corresponding $TEQs_{WHO1998}$.

Associations between fish consumption and blood levels of dioxin-related compounds and PCBs have been reported in a.o. Scandinavian (Asplund et al, 1994; Kiviranta et al. 2002), and Japanese populations (Arisawa et al, 2003). Many factors are known to influence net absorbance of PCBs and other POPs, namely compound properties (a.o. susceptibility to metabolism), the individual´s fat status, exposure history (age play a role) and diet. The plasma levels and pattern of POPs in the present study group were comparable with concentrations and congener profiles found previously in Norwegians and Europeans (Knutsen et al, 2008; Polder et al., 2008). The plasma concentrations of PCBs and DDE were, however, lower than what has been determined in a Norwegian population with high fish and fish-liver consumption (Furberg et al, 2002; Sandanger et al, 2006) and looking at a global scale considerably lower than in native Arctic and Russian populations known to have a relatively high exposure to POPs through their diet (Arisawa et al, 2003 and references therein).

The adipose tissue levels of indicator PCBs correlated with age of the patients, suggesting an accumulation of PCBs with age, consistent with the results of previous studies (Arisawa et al, 2003; Furberg et al, 2002; Papke, 1998; Sandanger et al, 2006). Similarly, Sjodin et al. found a significant effect of age on the levels of PCBs for subjects with a high dietary intake of fatty fish from the Baltic Sea (Sjodin et al, 2000). This may be explained by the fact that the PCBs accumulate in the human fat due to their lipophilic properties, and that the median half-lives for these congeners are long, in the order of 7–9 years and the body burden of these substances increases with age (Steenland et al, 2001). It is also reported that

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biological half-lives of POPs are longer in obese persons (Flesch-Janys et al, 1996; Juan et al. 2002).

Due to bans and restrictions on use and output of dioxins, PCBs and OCPs there has been a distinct decline in environmental contamination. In humans, up to 60% reductions in levels of dioxins and PCBs have been reported during the last couple of decades both in Norway and in Europe (Becher et al, 2002; Papke, 1998; Hagmar et al, 2006; Polder et al., 2008). The age of the present study group ranged from 20 to 67 years, meaning that some of the study persons have lived through "rise and fall" of f.i. PCB concentrations in the environment and in food while others have been considerably less exposed. Thus, the large inter individual variation in POP concentrations could possibly be explained by the wide range in age of the study subjects. Differences in dietary habits and sex differences may also play a role.

Considering the measured levels of POPs in the fat biopsy on a fat mass basis, and assuming a body fat content in the study population in the range 20 – 30%, it can be estimated that the dietary intake, on the average, is less than 1% of the contaminant level in the body fat. In evaluating the outcome of the present study, one should take into account exposure to POPs from other sources than marine food, the age distribution of the study population and further consider the long half lives of POPs, factors that have been discussed above. Realizing that several half lives may be required for achieving a steady state level of POPs in the human body, it is not unreasonable that the dietary exposure to POPs in the present study population will not affect this steady state level in the short term. In addition the n-3 PUFA plasma concentrations of the patients were fairly high 4 weeks before the intervention indicating that this patient group probably had a relatively high intake of these PUFAs via fish consumption or diet supplements before the intervention. Thus, it should not be unexpected that this study, in which the subjects have consumed 380 g salmon weekly for 15 weeks, demonstrated non significant changes in POP plasma concentrations at the end of the study.

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Similar results in pooled plasma samples were also recently demonstrated in a group of coronary heart disease (CHD) patients (Bethune et al, 2006).

Examining the levels of POPs in biopsies from abdominal fat in the subjects (Table 6), significant reductions could be observed during the study period for CB156, CB167, sum mo-PCBs, and sum TEQs of mo-PCBs. No relationship was found between increase in n-3 levels in plasma and reduction of CB 156, CB167 or sum mo-PCBs levels in the fat biopsies. There is no obvious explanation for the reduction in circulating concentrations (particularly of mo-PCBs) in plasma and in fat biopsies observed in the study subjects. The mono-*ortho* substituted PCBs are less persistent than the id-PCBs and an increased metabolism, and/or excretion of these mono-ortho substituted PCBs is possible.

The question if high intake of fatty acids from fish would influence the toxicokinetics of these contaminants is however unknown. Besides any effects on the toxicokinetics of POPs, the accumulation and distribution of these contaminants may be influenced by changes in the diet during the study period decreasing the exposure to the study subjects of contaminants from other food sources. Seasonal variations in the exposure of organic contaminants from other sources should perhaps neither be excluded.

Much of the available literature addressing POP exposure and human health effects has relied upon blood (serum or plasma) for quantifying exposure. Adipose tissue has been suggested as the preferred matrix for human exposure since it represents cumulative internal exposure in comparison to blood that may represent more recent exposures. In the present study both plasma and adipose tissue samples were analyzed and significantly lower concentrations of all POP groups excluding HCB and PBDEs were found in plasma. Due to the lipophilicity of such compounds and the small amounts of lipids in blood this was not unexpected. There are, however, limited data with regard to distribution of dioxins and PCBs in human tissue. The liver and adipose tissues of both experimental animals and humans have been shown to sequester these compounds from blood in a dose dependent manner (World

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Health Organization (WHO), 1998). Dose-dependent distribution of these POPs has been reported to be due to the induction of binding proteins in the liver (Van den et al, 1994). Iida et al. (2007) have recently investigated the concentrations and distribution of PCDDs/PCDFs, dl-PCBs, and id-PCBs in blood, lung, liver, bile, pancreas, spleen, kidney and mesentery fat in human tissues from 20 donors. They demonstrated parallel levels of POPs in blood and tissues. Positive correlations were observed among the concentrations of dioxins in various tissues. They concluded that the congener levels in the lung, liver, pancreas, spleen and mesentery fat can be predicted from the blood levels. However, correlations between matrices for specific POPs have varied between studies and some authors have concluded that blood concentrations are poorly predictors of adipose tissue concentrations (Whitcomb et al, 2005). The findings of correlations between all POP groups excluding PBDEs in both plasma and adipose tissue as well as between the two matrices may point to different exposure routes for PBDEs compared to the other POPs. Human exposure routes to PBDEs have not been established but both diet and house dust are suspected routes of exposure while diet is the most important route for the other POPs (Harrad et al, 2006; Ohta et al, 2002). Thus, the present finding of positive correlations between all POP groups excluding PBDEs may suggest that plasma may be an acceptable proxy for quantifying POP exposure in human health studies when diet is the major source of POP exposure.

Fish and other seafood are natural sources of the marine n-3 fatty acids EPA and DHA, and the EPA concentration in plasma is considered a good biomarker for fish consumption. A positive correlation was found between total n-3 PUFAs and id-PCBs and sum PCBs (sum of id-PCBs and mo-PCBs) in plasma indicating that fish is a major source of PCBs. Unexpectedly, no correlation was found between the mo-PCBs and EPA which is in contrast to the finding of Arisawa et al. (2003) who found a positive association between plasma concentrations of EPA and TEQ concentrations of total dioxins, PCDDs/Fs and PCBs after having adjusted for age and other covariates.

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The other class of polyunsaturated fatty acids (PUFAs), the n-6 fatty acids, are present in a great variety of foods (vegetables. oils, cereals and meat are rich in n-6 PUFAs) and the precursor for this class of PUFAs is linoleic acid which is found in relatively high concentration in plant oil. Thus, linoleic acid may be used as a biomarker of the consumption of plant oil. Particularly soy oil is rich in linoleic acid, and due to extensive use of soy oil and soy beans in food and feed linoleic acid is the dominating polyunsaturated fatty acid in the Norwegian diet. Thus, the positive correlation between linoleic acid and the specific POP groups HCB, *p,p'*-DDE, sum PBDEs and sum mo-PCBs may suggest that other sources than fish, soy oil in particular, may be important sources for these POP groups.

Oleic acid is a n-9 monounsaturated fatty acid for which the main food sources are various meat products, milk and cheese in addition to olive oil. The negative association between oleic acid and id-PCBs, in contrast to the positive correlation observed with n-3 PUFAs, may indicate that these food groups at the expense of fish products will decrease the body burden of certain POPs.

In summary, in the present intervention study consumption of fatty fish, equivalent to two salmon meals (servings) per week for 15 weeks, did not result in accumulation of POPs in plasma or adipose tissue and thus did not affect the steady state levels of POPs in the study subjects. Some of the POPs investigated even showed reduced levels in adipose tissue. The levels of POPs in the salmon fillets consumed were comparable to those typically found in farmed salmon. Plasma may be an acceptable proxy for quantifying POP exposure in human health studies when diet is the major source of POP exposure.

According to significant associations found between different long chain fatty acids and the different POP groups after adjusting for age it may be assumed that fatty fish is a major source of exposure only for the id-PCBs which are the most persistent PCBs. For the

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mo-PCBs other food sources than fatty fish may be more important. Unexpectedly, plant oil appear to be an important source for the PBDEs, *p,p'*-DDE and the mo-PCBs.

An expected increase in plasma n-3 (poly-unsaturated fatty acids) PUFA levels due to consumption of fatty fish was found. Thus, from a toxicological perspective there should be no risk associated with eating salmon in amounts equivalent to two meals per week.

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LEGEND TO FIGURES.

Fig 1. Correlation between mo-PCBs in plasma and fat biopsies before intervention in patients with non-alcoholic fatty liver disease. ($r = 0.63$, $p < 0.05$).

Fig 2. Correlation between age and id-PCBs in plasma before intervention in patients with non-alcoholic fatty liver disease. Regression line: Sum idPCBs = -42.87362 + 4.610354*AGE. $p < 0.001$.

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Table 1.
Characteristics of the study population

No of patients	12
Age (median and range)	44 (20-67)
Sex (male/female)	3/9
Steatosis hepatis	12
BMI (kg/m^2)	33±5.2
Diabetes mellitus type II	2
Crohns disease	1

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Table 2.
Estimated intake of fat (g/day) before and during a weekly consumption of 380 g farmed Atlantic salmon for 15 weeks.

Dietary fat intake	Before inclusion n= 8		During study period n = 10	
	Median (P25, P75)	Mean (SD)	Median (P25, P75)	Mean (SD)
Total fat intake	69.7 (61.6,91.0)	75.3 (17.7)	73.3 (57.0,85.8)	73.4 (20.1)
EPA/DHA	0.56 (0.34,0.78)	0.73 (0.69)	2.29 (2.26,2.36)*	2.30 (0.05)
Total n-3	1.9 (1.4,2.9)	2.2 (1.1)	3.76 (3.60,4.07)*	3.87 (0.38)
18:2 n-6	10.9 (9.4,14.6)	12.5 (4.6)	10.3 (7.3,14.9)	11.1 (4.2)
Ratio n-6/n-3	6.0 (4.7,7.3)	6.0 (1.6)	2.6 (2.0,3.6)*	2.8 (0.9)
Total monounsaturated	23.2 (21.1,33.3)	26.3 (7.6)	26.0 (20.1,33.0)	26.4 (7.1)
Total saturated	27.1 (23.0,28.6)	26.8 (4.0)	23.6 (19.6,29.6)	25.3 (8.9)

Values are given as gram daily intake of total fat or groups of fatty acids. Data are calculated based on a diet questionnaire as described in materials and methods. Significant differences were determined by a signed rank test. * $p<0.05$ between, before and after intervention.
EPA = Eicosapentaenoic acid, DHA = Docosahexaenoic acid

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Table 3. Plasma levels of specific fatty acids, groups of fatty acids and n-3/n-6 fatty acids ratio in the study subjects before inclusion, after 4 weeks run-in and at the end of the study.

	Week -4 % w/w	Week 0 % w/w	Week 15 % w/w	P value -4 and 0/ 0 and 15
EPA	1.26 ± 0.83	0.79 ± 0.33	1.26 ± 060	P <0.05 / =0.11
DHA	3.19 ± 1,18	2.17 ± 0.67	2.79 ± 0.92	P <0.05 / =0.26
Total n-3	5.88 ± 1,88	3.89 ± 1,15	6.17 ± 1,64	P <0.05 / <0.05
Total n-6	33.18 ± 3,39	32.85 ± 3,39	30.44 ± 2,24	P = 0.73 / =0.19
Ratio n-3/n-6	0.18 ± 0.05	0.12 ± 0.03	0.20 ± 0.05	P < 0.005/ <0.01
Oleic acid	20.03 ± 2,48	21.97 ± 2,76	22.82 ± 1,99	P < 0.01 / =0.64
Total saturated	33.25 ± 2,40	31.47 ± 2,49	31.09 ± 2,35	P = 0.16 / =0.95

Values are given as w/w % determined as described in materials and methods.
Significant differences were determined by a signed rank test, and are given as differences between week -4 and 0/week 0 and 15. (n = 8 – 11).
EPA = Eicosapentaenoic acid, DHA = Docosahexaenoic acid

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Table 4

Concentrations of OCs (ng/g wet weight (ww)), PBDEs and TEQs ($TEF_{WHO1998}$[1] and $TEF_{WHO2005}$[2]) (pg/g ww) in farmed Atlantic salmon. The estimated weekly intake (EWI) was based on consumption of 380g salmon/week and body weight of 100 kg.

POP	ng/g wet weight	EWI (ng/kg/week)
HCB	1.36	5.2
p,p'-DDE	3.55	14
BDE47	0.34	1.3
Sum PBDEs[3]	0.59	2.3
PCB153	2.77	11
Sum id-PCBs[4]	7.09	27
PCB118	1.61	6.1
PCB156	0.18	0.7
Sum mo-PCBs[5]	2.70	10
TEQsWHO1998	**pg/g**	**EWI (pg/kg/week)**
Sum mo-PCBs	0.37	1.42
TEQsWHO2005		
Sum mo-PCBs	0.08	0.31

[1] Ref: Van den Berg et al., 1998
[2] Ref: Van den Berg et al., 2006
[3] Sum PBDEs = BDE28, 47, 99,100
[4] Sum id-PCBs = CB138, 153, 170, 180 and 194
[5] Sum mo-PCBs = CB105, 114, 118, 123, 156, 157, 167 and 189

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Table 5.
Concentrations of OCs, PBDEs (ng/g lipid weight (lw)) and TEQs$_{WHO2005}$[1] of mo-PCBs (pg/g lw) in plasma before and after intervention

		Week 0		Week 15		Difference		
POP	N.	Median	Mean±SD	Median	Mean±SD	Median	Mean±SD	P[1]
Lipid %	12	0.67	0.68±0.2	0.64	0.63±0.1	-0.03	-0.05±0.1	0.380
HCB	12	19	20±9	20	20±7	0.5	-0.2±3	0.569
p,p'-DDE	12	68	115±136	64	117±150	0.2	2±28	0.677
BDE 47	12	1	2±2	1	2±2	0.03	-0.±0.3	0.910
Sum PBDEs	12	2	3±2	2	3±2	0.1	-0.2±0.6	0.677
PCB138	12	40	41±19	39	40±15	0.76	-0.78±7	0.677
PCB153	12	66	67±29	64	66±24	0.88	-0.63±11	0.622
PCB170	12	11	12±7	11	12±6	0.335	0.08±2.3	0.424
PCB180	12	35	36±18	39	37±16	2.1	1.1±6	0.064
PCB194	12	5	6±3	5	6±4	0.0	0.5±3	0.677
Sum id-PCBs	12	160	161±74	162	161±63	6	0.3±26	0.151
PCB105	12	2	2.3±1.2	2	2±1	0.0	-0.1±0.3	0.622
PCB114	12	0.45	0.5±0.3	0.5	0.5±0.2	0.01	0.004	0.424
PCB118	12	11	11±6	10	11±5	-0.03	-0.3±1.6	0.850
PCB156	12	5	6±4	6	6±3	0.04	-0.1±0.8	0.970
PCB157	12	1	2±0.9	1	2±0.8	0.0	-0.04±0.2	0.791
PCB167	12	2	2±1	2	2±1	0.0	-0.05±0.3	0.970
PCB189	12	0	0.5±0.3	0	0.5±0.3	0.0	-0.02±0.1	0.569
Sum mo-PCBs	12	26	24±12	24	24±11	-0.02	-0.6±3	0.970
TEQPCB105	12	0.07	0.1±0.04	0.07	0.06±0.03	0	-0.003±0.01	0.622
TEQPCB114	12	0.01	0.02±0.01	0.02	0.02±0.01	0	0±0.003	0.424
TEQPCB118	12	0.33	0.3±0.2	0.3	0.3±0.2	-0.001	-0.01±0.05	0.850
TEQPCB156	12	0.2	0.2±0.1	0.2	0.2±0.1	0.001	-0.004±0.02	0.970
TEQPCB157	12	0.04	0.05±0.03	0.04	0.05±0.02	0	-0.001±0.01	0.791
TEQPCB167	12	0.1	0.06±0.04	0.06	0.06±0.03	0	-0.002±0.01	0.970
TEQPCB189	12	0.01	0.02±0.01	0.01	0.02±0.01	0	-0.001±0.003	0.569
Sum TEQs[1] mo-PCBs	12	0.8	0.7±0.4	0.7	0.7±0.3	-0.001	-0.02±0.1	0.970

[1] Ref: Van den Berg et al., 2006
[2] P-value for difference: Signed Rank test.
For sum id-PCBs, sum mo-PCBs, sum TEQs of mo-PCBs: see Table 4

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Table 6.

Concentrations of OCs, PBDEs (ng/g lipid weight (lw)) and TEQs $_{WHO2005}$[1] of mo-PCBs (pg/g lw) in adipose tissue before and after intervention.

		Week 0		Week 15		Difference		
POP	N	Median	Mean±SD	Median	Mean±SD	Median	Mean±SD	P[1]
Lipid %	12	79	73±23	78	71±22	-3	-1±35	0.850
HCB	12	18	21±10	19	20±8	-0.7	-1±4	0.266
p,p'-DDE	12	109	191±30	117	185±214	-2	-6±29	0.677
BDE47	12	2.1	3±3	2	3±3	-0.6	-0.6±0.9	0.064
Sum PBDEs	12	2.5	4±4	2	4±3	-0.5	-0.54±2	0.223
CB138	12	61	65±29	63	60±23	-3	-5±19	0.266
CB153	12	96	100±46	100	91±35	-5	-9±32	0.380
CB180	12	62	65±39	64	59±27	-4	-7±20	0.266
CB170	12	24	26±15	25	24±11	-2	-2±7	0.204
CB194	12	11	11±7	11	11±5.3	-0.7	-0.8±3	0.266
Sum id-PCBs	12	258	268±135	277	245±99	-11	-23±80	0.339
CB105	12	4	4±2	3	3±2	-0.4	-0.5±0.9	0.110
CB114	12	0.6	0.7±0.6	0.8	0.6±0.5	-0.1	-0.08±0.5	0.569
CB118	12	16	16±8	13	15±8	-0.8	-2±3	0.176
CB156	12	12	13±7.1	11	11±6	-1.1	-1±2	0.042*
CB157	12	2.5	3±1.3	2.4	2±1	-0.2	-0.4±0.7	0.052
CB167	12	2.8	3±1.7	2.7	3±2	-0.33	-0.5±0.7	0.042*
CB189	12	1.1	1±0.7	0.75	0.9±0.7	-0.02	-0.2±0.4	0.339
Sum mo-PCBs	12	37	40±20	38	36±17	-3.4	-5±7	0.042*
TEQPCB105	12	0.1	0.1±0.1	0.08	0.1±0.1	-0.012	-0.01±0.03	0.109
TEQPCB114	12	0.02	0.02±0. 02	0.02	0.02±0.01	-0.002	0.002±0.01	0.569
TEQPCB118	12	0.5	0.5±0.3	0.4	0.5±0.2	-0.02	-0.05±0.1	0.176
TEQPCB156	12	0.4	0.4±0.2	0.3	0.4±0.2	-0.03	-0.04±0.07	0.042*
TEQPCB157	12	0.07	0.07±0.04	0.07	0.07±0.04	-0.006	-0.01±0.02	0.052
TEQPCB167	12	0.08	0.09±0.05	0.08	0.08±0.05	-0.01	-0.02±0.02	0.042*
TEQPCB189	12	0.03	0.03±0.02	0.02	0.03±0.02	-0.001	0.005±0.01	0.339
Sum TEQs[1] mo-PCBs	12	1	1±0.6	1	1±0.5	-0.1	-0.1±0.2	0.042*

[1] Ref: Van den Berg et al., 2006

[2] P-value for difference: Signed Rank test.

For sum id-PCBs, sum mo-PCBs, sum TEQs of mo-PCBs: see Table 4

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Table 7.
Differences between concentrations of POPs in plasma samples and fat biopsies from the study subjects at start and end of the study. (n= 12)

		Plasma			Fat biopsy			Differences			
Parameter	Week	Median	Mean	St. dev	Median	Mean	St. dev	Median	Mean	St. dev	P-value *
HCB	0	18.726	19.822	9.126	18.029	21.433	9.721	-0.955	-1.610	3.707	0.2661
*p, pÕ*DDE	0	67.663	114.453	136.356	109.003	191.177	229.552	-41.341	-76.724	96.669	**0.0005**
Sum BDEs	0	2.467	3.194	2.083	2.549	4.181	4.077	-0.032	-0.987	2.204	0.2661
Sum id-PCBs	0	160.075	160.713	74.321	258.460	267.737	134.492	-99.467	-107.024	78.629	**0.0005**
Summ o-PCBs	0	26.322	24.231	12.483	37.410	40.193	20.403	-11.884	-15.962	10.245	**0.0005**
HCB	15	20.339	19.651	6.937	18.661	20.069	8.177	-0.216	-0.419	2.738	0.7910
*p, pÕ*DDE	15	63.621	116.818	149.963	117.238	184.842	214.206	-49.147	-68.025	66.664	**0.0005**
Sum BDEs	15	2.227	2.998	1.810	2.387	3.589	3.028	-0.528	-0.590	1.383	0.2661
Sum id-PCBs	15	162.025	160.982	62.524	276.948	244.964	99.285	-76.599	-83.981	49.088	**0.0005**
Summ o-PCBs	15	24.182	23.583	10.965	37.568	35.736	17.260	-11.538	-12.153	7.894	**0.0005**

* Signed Rank Test
For sum id-PCBs and summ o-PCBs: see Table 4

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Table 8A.
Correlation between different POPs in plasma samples from the study subjects at the end of the study. (n = 12) *

	HCB	*p,pÕ*DDE	Sum BDEs	Sum id-PCBs	Sum mo-PCBs
HCB	-	n.s.	n.s.	0.91608 <.0001	0.91608 <.0001
***p,pÕ*DDE**	n.s.	-	n.s.	0.72727 0.0074	0.72028 0.0082
Sum BDEs	n.s.	n.s.	-	n.s.	n.s.
Sum id PCBs	0.91608 <.0001	n.s.	-0.12587 0.6967	-	0.93706 <.0001
Sum moPCBs	0.91608 <.0001	0.72028 0.0082	n.s.	0.93706 <.0001	-

* Pearson and Spearman correlation coefficients. n.s = not significant

Table 8B.
Correlation between different POPs in plasma samples and fat biopsies from the study subjects at the end of the study. (n = 12) *

POPs in fat biopsies	POPs in plasma sample				
	HCB	*p,pÕ*DDE	Sum BDEs	Sum id PCBs	Sum mo PCBs
HCB	0.93706 <.0001	n.s	n.s	0.86713 0.0003	0.90210 <.0001
***p,pÕ*DDE**	0.76224 0.0040	0.95105 <.0001	n.s	0.81818 0.0011	0.85315 0.0004
Sum BDEs	n.s	n.s	0.81818 0.0011	n.s	n.s
Sum id PCBs	0.90909 <.0001	0.77622 0.0030	n.s	0.93706 <.0001	0.85315 0.0004
Sum mo PCBs	0.92308 <.0001	0.81818 0.0011	n.s	0.90210 <.0001	0.93706 <.0001

* Pearson and Spearman correlation coefficients. n.s = not significant
For sum id-PCBs and sum mo-PCBs: see Table 4

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Table 9.
Summary of correlations after adjustment for age between plasma fatty acids in study subjects before inclusion and the plasma levels of selected contaminants at start of the study. (n= 12) *

Plasma fatty acids	**POPs in plasma samples**			
	***p,p'*-DDE**	**Sum BDEs**	**Sum id-PCBs**	**Sum mo-PCBs**
Sum n-3	n.s.	n.s.	0.688 p = 0.027	n.s.
C 18:1 n-9	n.s.	n.s.	- 0.739 P = 0.015	n.s.
C 18:2 n-6	0.737 p = 0.015	0.874 p = 0.0009	n.s.	0.683 p = 0.029

- Spearman partial correlations coefficients adjusted for age. n.s = not significant

For sum id-PCBs and sum mo-PCBs: see Table 4

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Figure 2

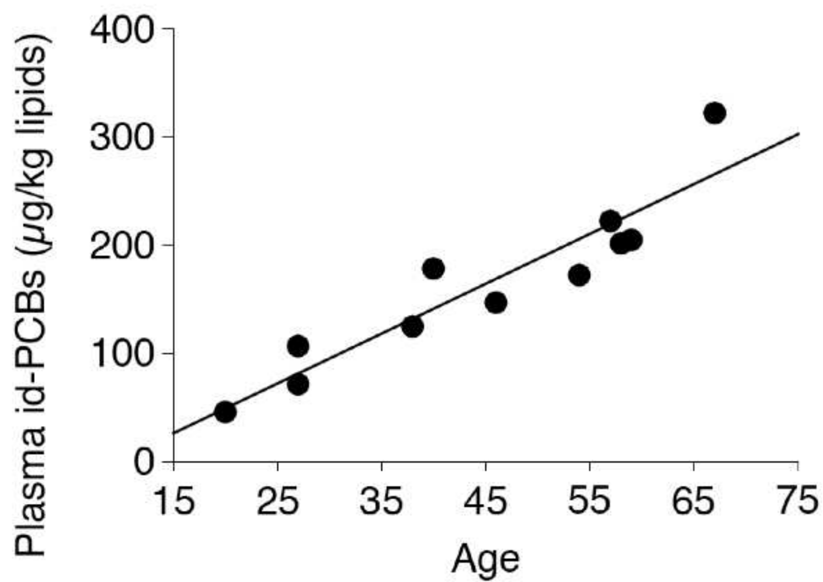

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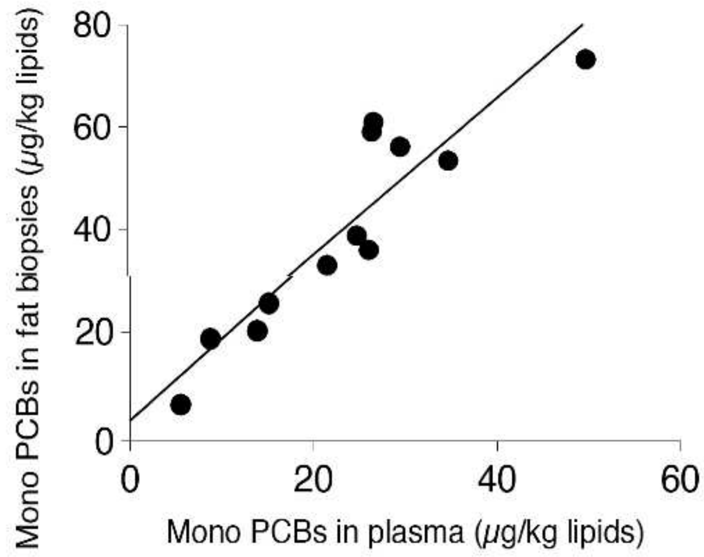
Figure 1



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