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RESEARCH ARTICLE

Sex, BMI and age in addition to dietary intakes influence blood concentrations and congener profiles of dioxins and PCBs

Helle K. Knutsen[1], Helen E. Kvalem[1], Margaretha Haugen[1], Helle M. Meltzer[1], Anne L. Brantsæter[1], Jan Alexander[1], Olaf Päpke[2], Veronica H. Liane[1], Georg Becher[1] and Cathrine Thomsen[1]

[1] Norwegian Institute of Public Health, Oslo, Norway
[2] Eurofins – Ergo Forschung GmbH, Neuläder Kamp, Hamburg, Germany

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Scope: The aim of this study was to i) characterize dietary polychlorinated biphenyls (PCBs) and dioxin exposure in consumers of fish from the PCB contaminated Lake Mjøsa in Norway ii) examine the influence of demographic factors on blood concentrations and congener composition of dioxins and PCBs, iii) characterize dietary sources and possible exposures above tolerable intake.

Methods and results: Blood samples were analysed for dioxin-like (dl) compounds (PCDD/Fs and dl-PCBs) and non-dl-PCBs (ndl-PCBs). Dietary exposures were calculated using food frequency questionnaires (n=64). Men had higher median intake of dl-compounds than women (1.2 and 0.85 pg TEQ/kg bw/day), but similar blood concentrations (23.3 and 25.8, pg TEQ/g lipid weight (lw)). For non-dl-PCBs, intakes (6.5 and 4.5 ng/kg bw/day) and blood concentrations (381 and 224 ng/g lw) were higher in men than in women. Blood concentrations correlated with dietary intakes in men only. Increasing BMI and age elevated blood concentrations mainly in women. Men and women had different blood congener profiles, with a higher share of PCB-126 in women, despite similar dietary congener profiles. Eleven participants exceeded the tolerable intake for dl-compounds. Fish from Lake Mjøsa was the main dietary source.

Conclusion: The higher influence of BMI and age for women than for men may have implications for risk assessment.

Keywords:
Blood / Diet / Dioxin / Polychlorinated biphenyls / Sex

1 Introduction

Diet is the major non-occupational source of exposure to dioxin-like (dl) compounds (PCDD/Fs (polychlorinated dibenzo-*p*-dioxins/furans) and dioxin-like polychlorinated biphenyls (dl-PCBs)) and non-dioxin-like PCBs (ndl-PCBs), contributing approximately 90% of the total exposure [1]. These substances are lipophilic and accumulate in the food

Correspondence: Dr. Helle K. Knutsen, Division of Environmental Medicine, Department of Food Safety and Nutrition (MIME), PO Box 4404 Nydalen N-0403 Oslo, Norway
E-mail: helle.knutsen@fhi.no
Fax: +47-21-07-66-86

Abbreviations: 2,3,7,8-TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; **CB,** chlorinated biphenyl; **dl-compounds,** dl-PCBs and PCDDs/PCDFs; **dl-PCBs,** dioxin-like polychlorinated biphenyls; **lw,** lipid weight; **mo-PCBs,** mono-ortho substituted PCBs; **ndl-PCBs,** non-dioxin-like PCBs; **no-PCBs,** non-ortho substituted PCBs; **PBDE,** polybrominated diphenyl ether; **PCBs,** polychlorinated biphenyls; **PCDDs/PCDFs,** polychlorinated dibenzo-p-dioxins/furans; **sum 6-PCB,** sum of CB-28, 52, 101, 138, 153 and 180; **sum 4-PCB,** sum of CB-101, 138, 153 and 180; **TEQ,** 2,3,7,8-TCDD toxic equivalents; **TWI,** tolerable weekly intake

chain. Although the dietary intake of dl-compounds is the major determinant of the blood concentration and congener profile in the general population, this relationship might be modified by demographic factors such as age, body fat mass, body weight, number of childbirths (parity), breastfeeding, sex and smoking status. How these variables influence blood concentrations together with dietary intake is not well characterized.

It is generally accepted that the dl-compounds exert the toxic effects (*i.e.* neuro-developmental, immune, hormonal and metabolic effects) by activation of the Ah-receptor, while the ndl-PCBs have different mechanism of action. Due to their similar physicochemical properties, the dl-compounds and the ndl-PCBs are found in the same food items, and ndl-PCBs affect similar end-points as the dl-compounds. Hence, it has been difficult to separate the effects resulting from dl-PCBs from those caused by ndl-PCBs. There is currently no internationally agreed tolerable intake of ndl-PCBs. On a weight basis, the ndl-PCBs constitute approximately 90% of the total PCB content in food. Six marker PCBs have often been determined to represent the complete mixture of all ndl-PCBs: CB-28, 52, 101, 138, 153 and 180 (sum 6-PCB). This approach has also recently been suggested for food and feed control purposes in the European Union.

Exposure assessments in both Europe and the USA show that the dietary intake and levels of dl-compounds and ndl-PCBs in humans are declining [2–6]. However, the intake of dl-compounds among certain subgroups may still be high and exceed the tolerable weekly intake (TWI) of 14 pg 2,3,7,8-TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) toxic equivalents (TEQ)/kg body weight/week set by the EU scientific committee on food [7].

In the present study, we investigated exposure to ndl-PCBs and dl-compounds in a group of hobby anglers and their families living by the largest lake in Norway, Lake Mjøsa. This lake is contaminated with both PCBs and polybrominated diphenyl ethers (PBDEs), which are structurally related to PCBs. Elevated PBDE-exposure in the same study group has been reported [8]. Lake Mjøsa is rich in fishing resources, is well-known for large trout and there is a strong tradition for both recreational and commercial fishing among people living around the lake. The contamination with PCBs has led to food consumption advisories from the Norwegian Food Control Authorities. The advice to the general population is to avoid eating Lake Mjøsa trout above 1 kg more often than once a month on average, and pregnant and lactating women should not eat trout above 1 kg and children and women of fertile age should not eat large trout from this lake regularly. Since the present study group has high consumption of food contaminated above background concentrations, they represent a population at risk of higher exposure than the TWI for dioxins and PCBs.

The purpose of this study was to (i) characterize the exposure in Norwegians who are high consumers of fish from a contaminated lake by determining dietary intakes and blood concentrations of ndl-PCBs and dl-compounds, (ii) examine to what extent demographic factors influence concentration and congener composition of dl-compounds and ndl-PCBs in blood and (iii) characterize dietary sources and possible exposures above the tolerable intake of dl-compounds.

2 Materials and methods

2.1 Study subjects and sampling

This study population comprises high consumers of inland fish from Lake Mjøsa, which is in the south-eastern part of Norway. The recruitment process has been described in detail previously [8]. The participants provided blood samples and filled in comprehensive questionnaires regarding demographic information and dietary habits. The dietary questions covered both the regular diet and intake of fish from Lake Mjøsa. Of 66 individuals (41 men and 25 women), one was excluded because of low age (10 years), and one because the food questionnaires were incomplete. Of the remaining 64 participants, one had insufficient amount of blood for the determination of dl-compounds. The mean age among men and women were 58 (range 31–88) and 57 (range 37–79) years, respectively. The mean body weight of the participants was 86 kg for men (range 60–129) and 74 kg for women (range 57–79). The mean BMI was 27.0 for men (range 18.7–40.3) and 26.8 (range 21.7–37.3) for women. There were 15 current smokers in the group, of which three were women. The data collection and blood sampling was performed from October 2004 to May 2005 as described before [8]. Informed consent was obtained from all the participants, and the project was approved by the Regional Committee for Medical Research Ethics (id: S-04142).

2.2 Determination of ndl-PCBs in serum

Ndl-PCBs were determined in samples ($n = 64$) as described by Thomsen *et al.* [9]. Briefly, to serum samples ^{13}C-labeled internal standards were added and extracted by means of solid-phase extraction, and the lipids removed using sulphuric acid-silica columns. Both procedures were performed on an automated solid phase extractor. The extracts were analyzed using GC coupled to quadruple MS, operated in the electron-capture, negative ionization mode. In this detection mode, the responses of CB-28 and 52 were too low to be quantified (LOQ ~10 ng/g lipid weight (lw)). The LOQ for CB-101, 138, 153 and 180 was ~3 pg/g serum (~0.6 ng/g lw). The sum of CB-101, 138, 153 and 180 is denoted as sum 4-PCB hereafter. Ten procedural blanks were included in the total sample set, in which CB-101, 138 and 153 were found in all. The blank contribution of CB-138 and 153 was negligible (below 3.5% of the mean

concentration observed in the samples), while the measured CB-101 concentrations have been corrected by subtraction of the median blank content.

The analytical quality of determinations of ndl-PCBs was assured and proven by participation in the third round of the AMAP (Arctic Monitoring and Assessment Programme) ''Ring Test for Persistent Organic Pollutants in Human Serum'' in 2005. A *Z*-score <1 was obtained for CB-101, 138, 153 and 180.

Lipids (triglycerides, cholesterol and phospholipids) were determined enzymatically at the Haukeland University Hospital (Bergen, Norway) and the total lipid content was calculated according to the method described by Grimvall *et al.* [10].

2.3 Determination of PCDD/F and dl-PCBs in whole blood

Dl-compounds were determined in samples from the 63 participants who had delivered a sufficient amount of whole blood (~25 mL). All 17 of the 2,3,7,8-substituted PCDDs/PCDFs and the 12 dl-PCBs (non-ortho substituted PCBs (no-PCBs) CB-77, 81, 126, 169 and mono-ortho substituted PCBs (mo-PCBs) CB-105, 114, 118, 123, 156, 157, 167, 189) were determined as described in detail previously [11]. Briefly, ^{13}C-labeled internal standards were added and the samples liquid/liquid extracted by means of *n*-hexane and *n*-hexane/2-propanol. The lipid content was determined gravimetrically by weighing the dried fat residue. Subsequently, cleanup was done on a multicolumn system and the analyses performed using GC-HRMS. Two isotope masses were measured for each component and quantification was performed using the isotope dilution technique. The analytical method was successfully tested in various national and international quality control studies and proficiency tests such as Interlaboratory Comparison on Dioxins in Food organized by the Norwegian Institute of Public Health. Compounds not detected have been assigned concentration zero (lower bound approach). Concentrations of dl-compounds were calculated in toxic equivalents (TEQs) using the 2,3,7,8-TCDD toxic equivalence factors established by World Health Organization in 2005 [12].

2.4 Calculation of intake of dioxins and PCBs

An extensive database comprising concentrations of dioxins and PCBs in Norwegian foods has recently been established [13]. A separate database, with concentrations in different fish species from Lake Mjøsa, was created (see the online Supporting Information Table S1). Dietary exposure was assessed using a validated 12-page semi-quantitative food frequency questionnaire covering consumption over the last 12 months [13, 14]. In addition, questions about consumption of fish from Lake Mjøsa were asked [8]. Food frequencies were converted into food amounts (g/day) by multiplying with standard, gender-specific portion sizes. Calculation of dioxin and PCB intakes was based on lower bound mean concentrations in food using the 2,3,7,8-TCDD toxic equivalence factors established by World Health Organization in 2005 [12].

2.5 Statistical analysis

Statistical analyses were carried out using SPSS (version 17.0, SPSS, Chicago, IL, USA). Correlations were calculated using Spearman's rank, since the distributions of dietary intakes and serum concentrations were skewed. Dietary intake and blood concentration in men and women were compared using the Mann–Whitney rank-sum test. The relative mean congener contribution to total TEQ (% TEQ) of dl-compounds in diet and blood was normally distributed and was compared using *t*-test. Regression analyses were performed to identify factors that influenced the concentration and congener composition of dl-compounds and four of the ndl-PCBs (sum 4-PCB) in blood. The dietary intake, dietary congener composition, age, parity, breastfeeding, education level, sex and smoking status were included as covariates. For the variable age, the starting value was set at 30 years, while BMI was set with 20 as starting value. The models were built individually by stepwise exclusion of non-statistically significant covariates ($p<0.05$). Further, the models were checked for interactions, and interaction terms were included if statistically significant ($p<0.05$). Prior to the final regression analyses, the data were checked for outliers with high influence by using Cooks distance >1 as cut-off point. This excluded two participants from two analyses (CB-105 and 180).

3 Results

3.1 Dietary intake and blood concentrations

The median blood concentration of total TEQ in the study group was 22.3 pg TEQ/g lw (range 3.03–92.5), and the median sum of CB-101, 138, 153 and 180 (sum 4-PCB) in blood was 292 ng/g lw (range 73.2–1258) (Table 1). The calculated median total TEQ intake was 1.0 pg TEQ/kg bw/day (range 0.25–3.4) and the calculated median intake of sum 6-PCB was 5.3 ng/kg/day (range 0.98–27). The distribution of dietary intake and blood concentrations of dl-compounds and ndl-PCBs were skewed with a long tail toward higher concentrations. Dietary intakes and blood concentrations of the individual congeners can be found among the online Supporting Information (Supporting InformationTable S2).

The mean and median dietary intakes of dl-compounds were lower in women than in men (Table 1). These differences were statistically significant for PCDF and sum of

Table 1. Dietary intakes, blood concentrations and Spearman's correlations of dl-compounds and ndl-PCBs in the Lake Mjøsa study group, year 2003–2004

			Dietary intake[a)]				Blood concentration[b)]				Correlation		
		N	Min	Med.	Mean	Max	Min	Med.	Mean	Max	*r*	*p*	(95% CI)
PCDD	M	40	0.05	0.17	0.19	0.47	0.98	6.45	8.58	26.5	**0.37**	0.019	(0.07, 0.61)
	W	23	0.05	0.13*	0.13	0.23	3.56	6.21	7.64	16.9	0.05	0.812	(−0.37, 0.45)
	A	63	0.05	0.15	0.17	0.47	0.97	8.25	8.22	25.1	**0.30**	0.016	(0.06, 0.51)
PCDF	M	40	0.05	0.19	0.23	0.73	1.17	3.91	4.63	13.9	**0.50**	0.001	(0.22, 0.70)
	W	23	0.05	0.15**	0.15	0.29	1.46	3.49	3.75	9.28	0.05	0.830	(−0.37, 0.45)
	A	63	0.05	0.16	0.20	0.73	1.17	3.87	4.31	13.9	**0.39**	0.002	(0.16, 0.58)
PCDD/F	M	40	0.10	0.35	0.42	1.2	2.78	12.8	13.2	39.0	**0.40**	0.011	(0.10, 0.63)
	W	23	0.10	0.28**	0.29	0.52	5.02	10.2	11.4	26.1	0.05	0.812	(−0.37, 0.45)
	A	63	0.10	0.31	0.37	1.2	2.78	12.4	12.5	39.0	**0.32**	0.011	(0.08, 0.53)
no-PCB	M	40	0.20	0.76	0.93	2.4	0.00	9.01	11.1	46.3	**0.35**	0.029	(0.04, 0.60)
	W	23	0.15	0.54*	0.61	1.5	2.24	11.1	11.7	31.4	−0.04	0.865	(−0.44, 0.38)
	A	63	0.15	0.69	0.81	2.4	0.00	9.31	11.3	46.3	0.21	0.098	(−0.04, 0.44)
mo-PCB	M	40	0.01	0.05	0.06	0.19	0.24	1.72	2.16	7.28	0.31	0.051	(0.00, 0.57)
	W	23	0.01	0.03*	0.04	0.13	0.38	1.77	1.69	3.88	0.12	0.574	(−0.31, 0.51)
	A	63	0.01	0.04	0.05	0.19	0.24	1.73	1.98	7.28	**0.29**	0.002	(0.05, 0.50)
dl-PCB	M	40	0.21	0.81	0.99	2.6	0.25	10.6	13.3	53.5	**0.34**	0.032	(0.03, 0.59)
	W	23	0.15	0.57*	0.64	1.7	2.78	12.8	13.4	33.8	0.01	0.975	(−0.40, 0.42)
	A	63	0.15	0.73	0.86	2.6	0.25	11.3	13.3	53.5	0.23	0.075	(0.02, 0.45)
Sum TEQ	M	40	0.31	1.20	1.4	3.4	3.03	22.1	26.5	92.5	**0.37**	0.017	(0.07, 0.61)
	W	23	0.25	0.85*	0.93	2.1	7.86	25.1	24.8	52.5	−0.02	0.911	(−0.43, 0.40)
	A	63	0.25	1.00	1.2	3.4	3.03	22.3	25.8	92.5	**0.27**	0.032	(0.02, 0.49)
ndl-PCB[c)]	M	40	0.98	6.5	8.6	27	99.5	381	397	1258	**0.42**	0.007	(0.12, 0.65)
	W	24	1.2	4.5**	5.2	16	73.2	224**	224	421	−0.06	0.765	(−0.35, 0.35)
	A	64	0.98	5.3	7.3	27	73.2	292	332	1258	**0.44**	<0.001	(0.22, 0.62)

Abbreviations: M: men; W: women; A: all; *r*: regression coefficient, in bold if $p<0.05$; *p*: level of significance for correlation.
a) dl-compounds: pg TEQ/kg bw/day, ndl-PCB: ng/kg bw/day.
b) dl-compounds: pg TEQ/g lipids, ndl-PCB: ng/g lipids.
c) Sum 4-PCB in blood (CB 101, 138, 153, 180), Sum 6-PCB in diet; *$p<0.08$ and **$p<0.05$ denote differences between men and women compared.

PCDD/F ($p<0.05$). However, the lower intakes were not reflected in lower blood concentrations in women. Women appeared to have higher blood content of no-PCBs as both mean and median concentrations were higher in women than in men, but no statistically significant differences in blood concentrations of sums of dl-compounds were observed between men and women.

For the ndl-PCBs (sum 4-PCB), the blood concentration was higher ($p=0.01$) in men (median 381 ng/g lw) than in women (median 224 ng/g lw), and the calculated dietary exposure to sum 6-PCB was also higher ($p<0.05$) for men (median 6.5 ng/kg bw/day) than for women (median 4.5 ng/kg bw/day). Men had wider ranges than women both for blood concentrations and dietary intakes (Table 1).

We found clear sex-specific differences in the bivariate correlations between dietary exposure and blood concentrations (Table 1). For women alone, dietary intake did not correlate with blood concentration for any group of compounds, whereas for men there were statistical significant correlations for all sums of congeners except for mo-PCBs. For women and men together as one group, the correlations were lower than for men and statistically significant for total TEQ, PCDDs, PCDFs, PCDD/Fs, mo-PCBs and ndl-PCBs but not for no-PCBs and dl-PCBs as a whole group.

To explore the possible impact of factors other than dietary intake on the blood concentrations of sums of dl-compounds and ndl-PCBs, we performed regression analyses (Table 2). The calculated dietary intake was a statistically significant predictor for PCDDs, PCDFs and sum 4-PCB, but not for the dl-PCBs. Age or age^2 were significant predictors for sum 4-PCB and PCDF and interacted with BMI and/or sex for all the dl-compounds. BMI appeared to be inversely associated with serum concentration of all sums of the congeners, except of sum 4-PCB, but as a consequence of the interactions; increasing BMI was positively associated with dioxins and dl-PCBs in blood. For PCDD, PCDF, total TEQ and sum 4-PCB, sex was a statistically significant covariate in the regression analysis. Women had lower concentration of these compounds than men when other covariates were taken into consideration. The effects of BMI and age were different for women and men (Table 2). Sex was coded 0 for men and 1 for women, and consequently, the interactions between sex and BMI and/or age in Table 2 only apply to women.

Table 2. Regression analysis of dl-compounds and sum PCB-4 in blood from participants in the Lake Mjøsa study 2003–2004, showing adjusted R^2, the constant and beta of the covariates with level of statistical significance

	R^2 adjust	Constant	Dietary intake	Age[a)]	[a)]Age^2	BMI[b)]	Age · BMI	Age^2 · BMI	♀	Age · ♀	Age^2 · ♀	BMI · ♀	Age · BMI · ♀	Age^2 · BMI · ♀
PCDD	58%	8.7	10.7*	−0.11		−1.2*	0.05**		−9.5*	0.34*		1.5*	−0.05*	
PCDF	60%	5.0	5.7**		−7.7E04*	−0.5**		2.3E−04**	−5.1*		0.002c	0.7*		−2.6−04**
PCDD/F	60%	14.1	7.9*	−0.20		−1.9*	0.07**		−15.1*	0.52*		2.2*	−0.08*	
no-PCB	47%	6.0	2.4		−2.5E−04	−0.9		4.6E−04*						
mo-PCB	47%	1.1	4.6	0.02		−0.2*	0.01*							
dl-PCB	48%	7.0	2.5	0.05		−1.4*	0.06*							
Total TEQ	58%	29.9	3.3	−0.39		−4.5*	0.18**		−30.2*	1.05*		4.7*	−0.16*	
Sum 4-PCB	49%	44.9	12.0*	8.74**					−120.7*					

a) 30 years set as baseline value for all, for age^2 this is age$^2-30^2$.
b) 20 kg/m2 set as baseline value for all, * $p \leq 0.05$, ** $p \leq 0.001$.

3.2 Factors affecting congener composition in blood

There were explicit and significant sex-specific differences in congener profile in blood (Fig. 1). The mean percentage contribution from no-CB-126 to total TEQ was 26% in men and 37% in women. The fraction of the no-CB-169 was higher in men than in women, *i.e.* 11 *versus* 7%. There were also small but significant differences in the relative contribution of 1,2,3,7,8-PeCDF, 2,3,4,7,8-PeCDF, CB-123, 156, 157, 167 and 189 between men and women ($p < 0.05$).

We investigated whether men and women had different congener composition in their diets. The relative contribution of dl-compounds to total TEQ intake was calculated for each individual. As shown in Fig. 1A, the mean congener composition of dl-compounds was quite similar in the diet among men and women, with no statistically significant differences and with no-CB-126 being the main contributor to total TEQ intake.

To explore the sex-specific differences further, we performed a regression analysis of congener profiles of dl-compounds (Table 3) and ndl-PCBs (Table 4) in blood. The regression analysis showed that sex, age, BMI, smoking status, dietary congener profile and parity influenced the congener profile in blood, but the influence of these covariates differed between the congeners (Tables 3 and 4). The constants in Table 3 can be interpreted as the adjusted congener percentage in the blood samples. In descending order, 2,3,4,7,8-PeCDF, 1,2,3,7,8-PeCDD, CB-126, 2,3,7,8-TCDD and 1,2,3,6,7,8-HxCDD were the major contributors to the total TEQ (Table 3). The sex variable had a significant impact on the relative TEQ composition for ten congeners, including the four most abundant congeners. For nine of the ten congeners, the relative TEQ contribution was lower in women than in men, but women had increased proportions of CB-126 and the ndl-CB-138 in blood. Parity or breastfeeding affected only three congeners (CB-169, 105 and 123). Smoking reduced the TEQ share of 1,2,3,4,6,7,8-HpCDD; the beta for being a current smoker *versus* a non-smoker or previous smoker was 1.294 for this congener. The share of CB-169, 105, 114, 156 and 157 was increased for current smokers. Increasing age reduced the relative TEQ contribution mainly from CB-126 and 169, and also from CB-114, 156 and 167. On the other hand, the effect of age was opposite for the PCDD/Fs, as increasing age lead to a decreased relative contribution to the total TEQ. The exception was for 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD where age was not statistically significant. BMI was a significant covariate for the TEQ contribution of five dl-PCBs, of which four were inversely and one positively associated with BMI. The congener composition in the total diet, measured as the calculated TEQ proportion of each specific congener in the diet, was a significant covariate for four of the dl-compounds. For the TEQ share of 2,3,4,6,7,8-HxCDF, OCDF, CB 81 and CB-118, no statistically significant covariates were found. For the first three mentioned compounds, this might be explained by the fact that the

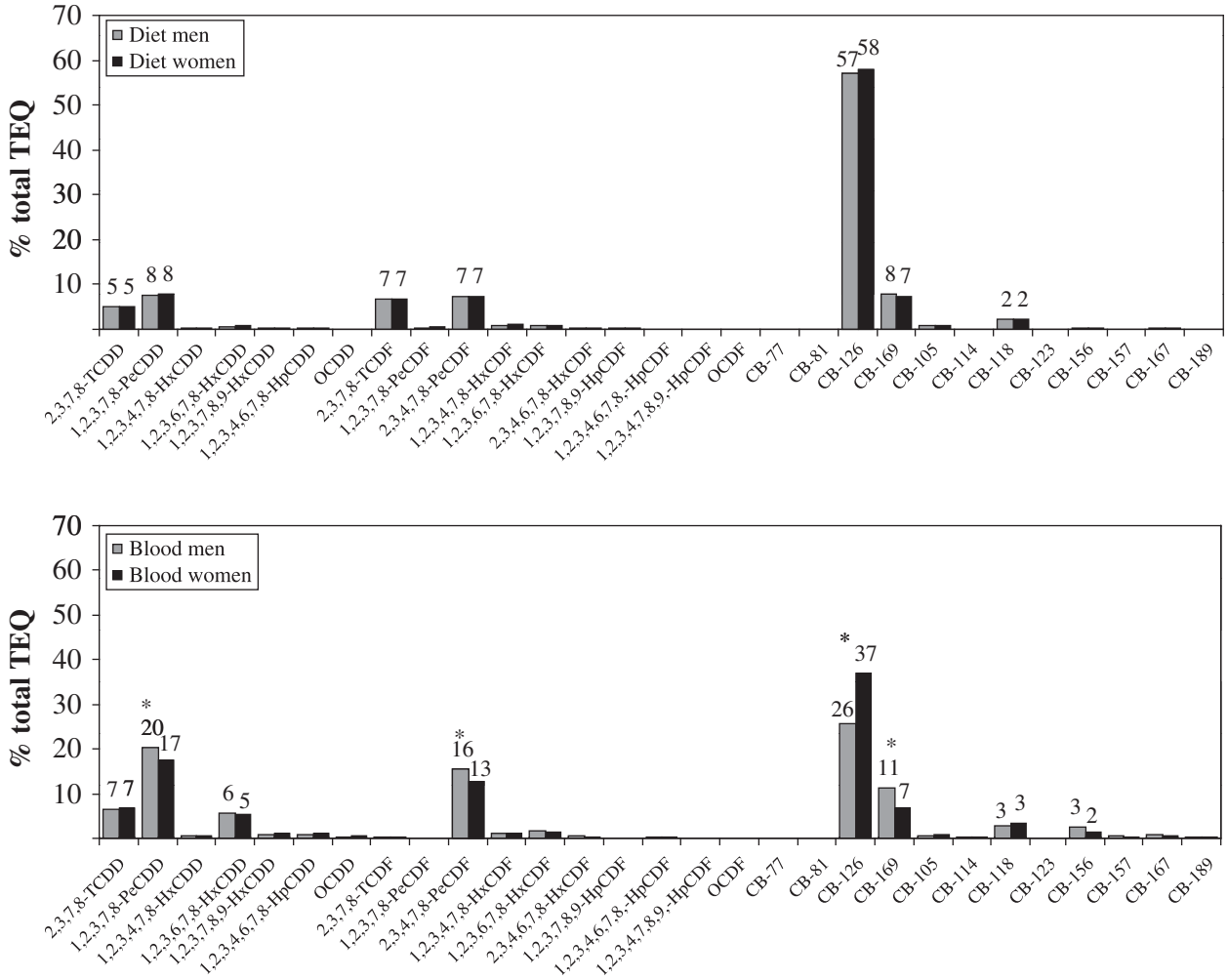


Figure 1. Relative mean congener composition (% TEQ) of dl-compounds in diet (A) and blood (B) in men and women in the Lake Mjøsa study group. Numbers above bars show proportion (%) of congeners contributing more than 1% to the total TEQ. Significant differences between men and women ($p < 0.05$) are indicated by * above bars for congeners contributing more than 1%.

concentrations were above the LOQ in only 1–2 participants (Supporting Information Table S2).

For the ndl-PCBs, CB-138, 153 and 180 were all major contributors to the congeners in blood, CB-153 being the most abundant (Supporting Information Table S2). The regression analysis on blood composition of ndl-PCBs showed that increasing age was associated with an increased fraction of CB-138 and 180 but a reduced contribution of CB-101 to the sum 4-PCB (Table 4). The share of CB-138 was distinctly larger for women. For the proportion of CB-153 to sum 4-PCB, no statistical significant covariates were found.

3.3 Dietary sources

In the present study, fish from Lake Mjøsa was the dominant dietary source of dl-compounds and ndl-PCBs, contributing approximately 43 and 54% of the mean total intake, respectively (Fig. 2). For all consumers, the mean total consumption of fish from the lake was 35 and 15 g/day for men and women, respectively. Trout contributed most to the intake of dl-compounds and ndl-PCBs. Mean trout consumption (consumers only) was 30 g/day (men, $n = 30$) and 15 g/day (women, $n = 22$). The most commonly eaten Lake Mjøsa fish was trout weighing between 0.8 and 3 kg [8]. The second main source was semi-oily and oily fish not originating from Lake Mjøsa, contributing 22 and 24% for dl-compounds and ndl-PCBs, respectively. Altogether, fish and other seafood, including fish from Lake Mjøsa, comprised 68 and 77% of the total calculated dietary exposure of dl-compounds and ndl-PCBs, respectively. Dairy products were the main non-fish source, contributing 12% to the total TEQ intake and 8% to the intake of ndl-PCBs. Only three of the participants consumed seagull eggs, but this still influenced the mean intake because of high contamination in seagull eggs [13].

Table 3. The beta of statistically significant covariates ($p \leq 0.05$) affecting the congener composition (% in each sample) of dl-compounds and ndl-PCBs in blood from 63 participants in the Lake Mjøsa study, 2003–2004

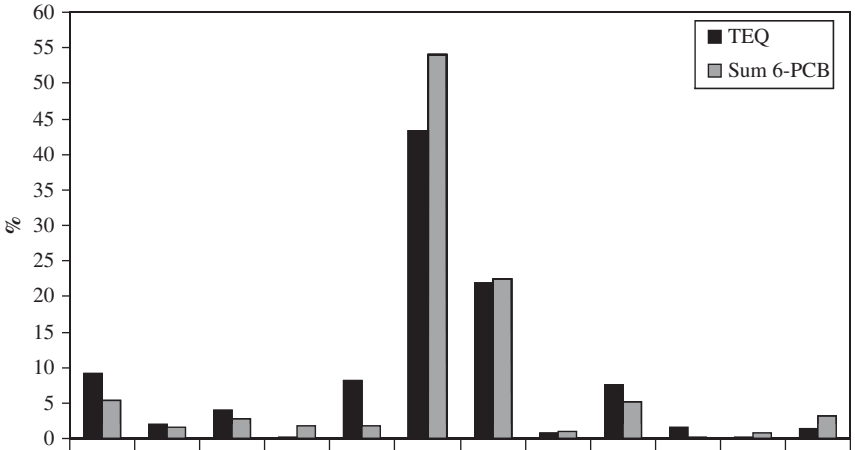
Congener[a)]	Adjusted R^2 (%)	Constant	Age[b)]	Age^2 [b)]	Woman	BMI[c)]	Smoker[d)]	% in diet	2 child births	Other 1	Other 2	N[e)]
2,3,7,8-TCDD	6.5	6.16								1.377[f)]		63
1,2,3,7,8-PeCDD	4.6	20.1			−2.642							63
1,2,3,4,7,8-HxCDD	17	1.13	−0.016									63
1,2,3,6,7,8-HxCDD	20	5.99	−0.058					1.633				63
1,2,3,7,8,9-HxCDD	17	1.54	−0.025									63
1,2,3,4,6,7,8-HpCDD	49	3.07	−0.228	0.00200			−1.294		0.545	0.410[g)]		63
OCDD	62	0.53	−0.022	0.00016				7.473		−0.824[h)]	0.006[i)]	63
2,3,7,8-TCDF	9.3	0.56	−0.008									63
1,2,3,7,8-PeCDF	3.5	0.11	−0.002									63
2,3,4,7,8-PeCDF	43	27.0	−1.123	0.00800	−8.054					0.184[j)]		63
1,2,3,4,7,8-HxCDF	54	3.48	−0.243	0.00200	−3.119				0.520	0.402[h)]	0.003[k)]	63
1,2,3,6,7,8-HxCDF	44	2.27	−0.209	0.00100								63
1,2,3,7,8,9-HxCDF	5.1	0.80	−0.011									63
1,2,3,4,6,7,8-HpCDF	41	1.29	−0.111	0.00100					0.532			63
1,2,3,4,7,8,9-HpCDF	44	0.13	−0.012	0.00008	−0.140					0.021[h)]		63
CB-77	3.9	0.02	−0.0002									63
CB-126	34	13.2	0.259		11.82	0.724						63
CB-169	61	2.22	0.848	0.00500	−4.439	−0.295	2.749			5.36[l)]		63
CB-105	44	0.58					0.107			0.12[m)]	2.153[n)]	61
CB-114	17	0.10	0.002				0.077					63
CB-123	1.6	0.04								0.009[o)]		63
CB-156	59	1.65	0.041		−0.863	−0.062	0.512					63
CB-157	61	0.24		0.00007	−0.185	−0.016	0.107	2.634				63
CB-167	32	0.50	0.008		−0.106							63
CB-189	47	0.31		0.00003	−0.111	−0.012						63

a) No significant covariates for 2,3,4,6,7,8-HxCDF, OCDF, CB-81 and CB-118.
b) 30 years set as baseline value, for age^2 this is age^2−30^2.
c) 20 kg/m^2 set as baseline value.
d) Current smoker.
e) Number of samples included in model, samples with too high leverage were excluded.
f) High school.
g) Age × smoke.
h) Congener % in diet × age.
i) Congener % in diet × age^2.
j) Age × sex.
k) Age^2 × sex.
l) Breastfeeding 0–1 month.
m) 1 child birth.
n) 1 child birth × smoke.
o) Breastfeeding 4 months or more.

Table 4. The beta of statistically significant covariates ($p \leq 0.05$) affecting the congener composition (%) of ndl-PCBs in blood from 64 participants in the Lake Mjøsa study, year 2003–2004

Congener	Adjusted R^2 (%)	Constant	[a)]Age	[a)]Age^2	Woman	N[b)]
CB-101	40	21.55	−1.982	0.01500		64
CB-138	28	12.23	1.307	−0.01100	2.083	64
CB-153[c)]						
CB-180	13	29.38	0.001			63

a) 30 years set as baseline value, for age^2.
b) Number of samples included in model, samples with too high leverage were excluded.
c) No statistically significant covariates.



	Dairy prod.	Eggs	Meat	Veg. fats	Other	Lake Mjøsa fish	Oily fish	Lean fish	Fish liver	Shell-fish	Fish oils	Sea-gull eggs
TEQ	0.11	0.03	0.05	0.00	0.10	0.54	0.27	0.01	0.09	0.02	0.00	0.02
Sum 6-PCB	0.42	0.13	0.22	0.14	0.14	4.24	1.76	0.07	0.40	0.02	0.06	0.24

Figure 2. The mean contribution (% daily intake) of food groups to intake of dl-compounds (TEQ) and ndl-PCBs (Sum 6-PCB) in the Lake Mjøsa study group ($n = 64$). Values in the table show mean intake from each food group (pg TEQ/kg bw/day for dl-compounds and ng/kg bw/day for sum 6-PCB). Dairy prod.: All milk-based products including butter and cheese; Veg. fats: margarine and all vegetable fats and oil; Other: bread, cereals, nuts, greens, sweets, dry foods, drinks; Oily fish: all fish with fat content above 2%; Lean fish: all fish with fat content of 2% or lower. Fish liver: Fish liver, roe and roe-liver pate.

Table 5. Regression analysis of total TEQ and sum 4-PCB in blood from participants in the Lake Mjøsa study using consumption of fish from Lake Mjøsa as independent variable.

	Adjusted R^2	Co-variate	Beta	p
Total TEQ	66%		14.30	
		Lake Mjøsa Fish (g/day)	0.172	<0.001
		Age[a)]	0.167	0.392
		BMI[b)]	−1.921	0.035
		Age*BMI	0.076	0.006
Sum 4-PCB	45%		144.04	
		Lake Mjøsa Fish (g/day)	0.911	0.196
		Age[a)]	6.777	<0.001
		Woman	−117.75	<0.001

a) 30 years set as baseline value, for age^2 this is age^2−30^2.
b) 20 kg/m^2 set as baseline value.

Since data on dioxins and PCBs in fish from lake Mjøsa is limited (Supporting Information Table S1), we additionally performed regression analysis of total TEQ in blood using self-reported total consumption of fish from Lake Mjøsa. A higher degree of the variance in blood total TEQ was explained with this model ($R^2 = 66\%$, Table 5) than with the model using estimated total TEQ intake ($R^2 = 58\%$, Table 2). For sum 4-PCB in blood, the regression analysis with self-reported local fish consumption ($R^2 = 45\%$, Table 5) was comparable with the regression with estimated intake of sum 6-PCB in diet ($R^2 = 49\%$), but the consumption of fish from the lake was not a significant determinant.

3.4 Exposure above tolerable intake

Eleven of the sixty-four participants had a calculated dietary intake of dl-compounds higher than the TWI (14 pg TEQ/kg body weight/wk) set by the EU Scientific Committee on

Food [7]. No tolerable intake for ndl-PCBs has been agreed upon by international risk assessment bodies; however, 10 ng sum 6-PCB/kg bw/day has been suggested as a temporary guideline [15, 16]. Fifteen of the participants in the present study (4 in addition to the 11 who had intake above the TWI for dl-compounds) had higher intake than 10 ng sum 6-PCB/kg bw/day. According to EFSA, such a long-term intake would correspond to about 250 ng/g lw in blood [17]. Since the blood concentrations of CB-28 and 52 were less than 2.5% of the concentrations of CB-138, 153 and 180, the difference between serum sum 6-PCB *versus* serum sum 4-PCB is considered to be negligible. Thirty-seven of the 64 participants had higher serum concentrations of sum 4-PCB than 250 ng/g lw.

4 Discussion

Despite higher intakes of dl-compounds and PCBs *per* kg body weight in men than in women, we found that men and women had equal concentrations of dl-compounds in blood. Women and men also had similar congener composition of their diets, but surprisingly, women had a much higher relative blood concentration of CB-126 than men, and the difference remained when the covariate BMI was taken into consideration. Bivariate analyses resulted in significant correlations between dietary dioxin and PCB intakes and blood concentrations in the whole group, but following stratification it remained significant only in men. To our knowledge, this is the first time sex differences in blood concentrations and composition of dioxins and PCBs have been observed when adjusted for dietary intake *per* kg bw.

We have, however, for the structurally related PBDEs, previously reported analogous sex-specific differences in correlations between dietary exposure and blood concentrations both in the Lake Mjøsa study and in the Norwegian Fish and Game study [8, 18].

Only the blood concentrations of ndl-PCBs differed significantly between men and women. However, sex was important for blood concentrations of PCDDs, PCDFs and, as a consequence, for total TEQ in regression analyses. Sex interacted with age and BMI for PCDDs, PCDFs and total TEQ. It appears that BMI and age might affect uptake and/or elimination of PCDDs and PCDFs differently in men and women. Sex-based differences in drug metabolism are regarded as the primary cause of sex-dependent pharmacokinetics [19]. Interestingly, females have higher protein and mRNA expression of CYP3A4, which is a predominant catalyst of oxidative metabolism of exogenous and endogenous compounds in human liver [20, 21]. CYP3A4 is induced by pharmaceuticals and environmental pollutants such as PCBs *via* activation of nuclear receptors. Interestingly, in a study on adults from the Faroe Islands, a positive association between CYP3A4 activity and sum PCB and PCB-TEQ was found for men only, although women seemed to have slightly higher CYP3A4 activity than men. Men also had both higher dietary intake and higher blood concentration of PCBs [22]. On the other hand, and in line with our findings, PCB half-lives were 1.5–10 times longer in women than in men in former capacitor workers [23]. In contrast to what was found for PCDD/Fs and ndl-PCBs, neither sex nor dietary intakes were important covariates for the dl-PCBs in our study. The explained variance was also lower for the dl-PCBs (48%) and ndl-PCBs (49%) than for the PCDD/Fs (60%). Although it is recognized that childbirths and lactation reduce body burden of dioxins and PCBs, these were not significant factors in the present study. The effects of increasing age on blood concentration of dioxins and PCBs can be attributed to their long half-lives and a higher exposure in the past.

Interestingly, the sex-specific differences were evident for the relative proportions of the four major contributors to total TEQ in blood (1,2,3,7,8-PeCDD, 2,3,4,7,8-PeCDF, CB-126 and CB-169). Although females had higher relative content of CB-126, they had lower proportions of the other three major congeners than males. Regression analysis of absolute concentration of CB-126 and 169 in blood showed, however, that being female was a significant covariate for both, being negative for CB-169 and positive for CB-126. For the two other major congeners, sex was not a significant covariate (data not shown). The higher concentration of CB-126 explains how total TEQ can be similar in blood from men and women when the dietary intake of total TEQ in women is lower. In contrast to our finding, the prediction model for CB-126 in the University of Michigan Dioxin Exposure Study did not show any association with female sex, but in agreement with our observations, significant association between total TEQ and increasing age and BMI was seen [24].

Smoking was a factor affecting congener composition in blood, but not total TEQ. It has previously been reported that the half-life of dl-compounds in the body is affected by smoking [25]. Cigarette smoking induces CYP 450 enzymes, i.e. CYP1, and might thereby increase metabolism and excretion of dioxins and PCBs from the body. Only the relative 1,2,3,4,6,7,8-HpCDD contribution to total TEQ were decreased by current smoking. Smoking was a positive predictor for mainly CB-169, but also CB-105, 114, 156 and 157.

Exposure to dl-compounds and PCBs among regular consumers and high-fish consumers has also been investigated in the Norwegian Fish and Game study using the same food frequency questionnaire [13]. The ranges of both calculated dietary intakes and blood concentrations in the present study were within those in the Norwegian Fish and Game study part C. The total TEQ in blood (median 22.3 pg TEQ/g lw) was lower than that found in both the regular group (median 28.7 pg TEQ/g lw) and in the high consumption group (median 35.1 pg TEQ/g lw) in the Norwegian Fish and Game study, whereas the sum 4-PCB concentration was similar to the high consumers (median 292 ng/g lw in lake Mjøsa study *versus*

301 ng/g lw in the high consumption group). The median dietary TEQ intake and sum 6-PCB intake in the Lake Mjøsa study group was between those in the representative and high consumers in the Norwegian Fish and Game study part C. The exposure levels in the present study were expected to be higher, since a large proportion of the participants in the present study had a higher consumption of fish from Lake Mjøsa than the consumption advisories set by the Norwegian Food Control Authorities. In the present study group, the average male self-reported consumption of trout larger than 1 kg (consumers only) was in fact three to four times higher than the recommended maximal consumption [8].

Of the 64 participants, 37 had higher serum concentrations of sum 4-PCB than 250 ng/g lw, which in theory would be a result of a long-term median intake above 10 ng sum 6-PCB/kg bw/day [17], a guidance value for ndl-PCBs suggested previously [15, 16]. Since the current mean intake of ndl-PCBs in the present study was lower (7.3 ng/kg bw/day), this could imply that fish consumption and/or contamination of fish had been higher in the past. As previously discussed in [13], limitations in the food frequency questionnaire, including the participants' recall of food consumption, are other possible explanations. All dietary surveys based on recall rely on memory, which is subject to a variety of errors. Use of standardized portion sizes in our study may also create error, and finally, there are limitations in the database on dioxins and PCBs [13]. Particularly, there were relatively few analytical data on Lake Mjøsa fish, as shown in Supporting Information Table S1. This may also explain why total consumption of fish from Lake Mjøsa (g/day) resulted in higher proportion of explained variance of the blood concentrations than calculated dietary TEQ intakes, with the exception of sum 6-PCB (Table 5).

In conclusion, both the blood concentrations and dietary total TEQ intakes among high consumers of fish from Lake Mjøsa were within the range found among randomly selected and high fish consumers in the rest of Norway, despite PCB contamination of fish in Lake Mjøsa. Dietary intakes were significantly correlated with blood concentrations in men only. In addition to dietary intakes, BMI, age and sex were major determinants of blood concentration of dioxins and PCBs in regression analyses. For ndl-PCBs, both dietary intakes and blood concentrations were lower in women than in men. For dl-compounds, blood concentrations were equal in men and women despite higher dietary intakes in men. Similar dietary congener profiles of dl-compounds resulted in different blood congener profiles in men and women, and particularly, women had higher fraction of CB-126 than men. Age, sex and BMI were the most influential factors for the congener composition. Overall, the present results show that the relationship between dietary intake and blood concentrations of dioxins and PCBs is different in men and women, and that factors other than diet have stronger influence in women than in men. This may have implications for risk assessment of dioxins and PCBs.

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