The Norwegian Human Milk Study HUMIS Variations in levels of chlorinated pesticides, PCBs and PBDEs in Norwegian breast milk

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Introduction

Organochlorine pesticides (OCPs), polychlorinated, –dibenzo-pdioxins (PCDDs), -dibenzofurans (PCDFs), -biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) are among the persistent organic pollutants (POPs) that have been found to accumulate in human breast milk[1]. Because nursing children are exposed to these chemicals through the contaminated breast milk, health authorities worldwide are concerned for the infants' intake and therefore human milk monitoring programs are performed in many countries[2]. While restrictions and bans resulted in a decline of organochlorines (OCs) in human milk during the last decades, an increasing trend has been found for PBDEs[1]. The main goals of "The Norwegian Human Milk Study, HUMIS" are: to elucidate the human exposure in Norway to POPs, to identify dietary habits and other lifestyle factors that are associated with high levels of POPs in human milk, and to study the impact of exposure to the these contaminants on child health.[3]. This study reports preliminary results of recent levels of POPs in human milk in 4 different counties in Norway.

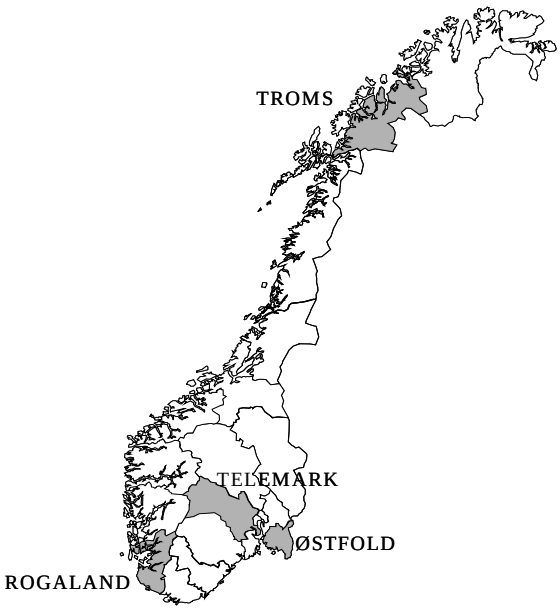

Figure 1. Map of Norway indicating the four counties studied

Materials and methods

Sampling and collection: HUMIS aims to recruit and collect milk samples from 6000 mothers, of which 800 milk samples have been collected and stored so far**.** In the first study phase 400 samples will be analysed for POPs. The mothers in this study collected up to 200 ml of breast milk in specially cleaned containers during a period of time between 2 and 12 weeks after delivery. Instructions were given how to avoid contamination and to keep the milk frozen in between the collecting days. Details of the mothers, such as age, health status, occupation, dietary habits and socio-economic factors were obtained. Both mothers giving birth to their first child (primipara) and mothers giving birth to their second or more child (multipara) participated in this study. The average age of the mothers was 29 years, with a range of 20-38. Preliminary data of POPs in 46 breast milk samples from 4 different Norwegian counties (Rogaland, Telemark, Troms and Østfold) are presented in this study (Figure 1).

Determination of OCP, PCBs and PBDEs: Concentrations of hexachlorobenzene (HCB), beta-hexachlorocyclohexane (β-HCH), p,p'-DDE, the sum of 10 indicator PCBs, IUPAC # : 28, 52, 74, 99, 101, 138, 153, 170, 180, 194, 8 mono-*ortho* PCBs # : 105, 114, 118, 123, 156, 157, 167, 189, and 6 PBDEs # :28, 47, 100, 99, 154 and 153 were measured at the Norwegian School of Veterinary Science. The extraction and lipid clean-up were done according to methods described earlier[4]. Prior to extraction the internal standards PCBs 29 and 112 and BDE 77, 119 and 181 were added. The lipid concentration of the milk was determined gravimetrically. Details for determination of OCPs and PCBs on a GC-ECD were described earlier[5].

The mono-*ortho* PCBs were determined on a GC-MS (Agilent 6890 Series, Agilent Technologies Avondale, PA, USA), connected to a DB-5MS column, 60m x 0.25 mm x 0.25 um (J&W Scientific). Carrier gas: He 1 ml/min constant flow. Temperature program as described earlier, slightly modified[5]. MSD specifications: negative chemical ionisation (NCI) in the SIM mode, $T_{ion\ source}$ 150 °C, $T_{quadrupole}$ 106 °C.

Analyses of PBDEs were performed on a GC-MS (Agilent 6890 Series) mounted with a SPB-5, 60m x 0.25 mm x 0.25 µm. (Supelco Inc. Bellefonte, PA, USA) and equipped with a mass spectrometer (MS) quadrupol detector (Agilent 5973 network Mass Selective Detector). The split-less-time was 1 min. $T_{injector}$ 250 °C. Carrier gas: He 1 ml/min constant flow, 50 psi. Oven temperature program: 90 °C (2 min); 90-190 °C (25 °C min^{-1}); 190 °C (1 min); 190-250 °C (5 °C min^{-1}); 250 °C (1 min); 250-320 °C (2.5 °C min^{-1}); 320 °C (10 min). MSD specifications: NCI in the SIM mode at the m/z ratios 79 and 81. Ionization gas CH_4. $T_{ion\ source}$ 250 °C, $T_{quadrupole}$ 106 °C. Recoveries and CV of individual BDEs in spiked milk samples varied from 92-121% and 0.05-0.08 %, respectively. The laboratory's accredited analytical quality was approved in several international intercalibration tests.

Results and discussion

OCPs:

Low levels of OCPs were determined in human milk from the 4 different counties (Table 1). Levels of HCB seemed to be somewhat higher in Troms and Rogaland compared to Telemark and Østfold. The concentrations of HCB are in the same range as found in Sweden in 1997[1] and about one fourth of the corresponding levels in Russia 2000[6]. Withdrawal of HCB as a fungicide in 1980 resulted in a clear decline of this contaminant in human milk in Sweden[1]. Concentrations of β-HCH and p,p'-DDE were highest in Troms and showed the widest range in Rogaland. In agricultural districts such as Rogaland, p,p'-DDT was used as pesticide before its usage was restricted in 1970, and banned in 1988. Compared to Sweden and Canada, the β-HCH and p,p'-DDE levels presented in this study are in the same range[1,7], but much lower compared to breast milk from northern Russia where levels of 190 and 670 ng/g milk fat β-HCH and p,p'-DDE were measured in 2000[6].

PCBs and mono-ortho PCBs:

Low levels of sum 18 PCBs (10 indicator PCBs plus 8 mono-*ortho* PCBs) were found in the 4 counties (Table 1). As for the OCPs, highest levels and widest range of sum 18 PCBs were found in Rogaland. Up to now, statistical calculations were not performed because some of the counties still are represented with too few samples. The concentrations of sum 18 PCB levels in this study were comparable to findings in Sweden and Finland but slightly lower than results for The Netherlands[2].

Table 1. Median concentrations and ranges of HCB, β-HCH, p,p'-DDE, sum 18 PCBs, sum OCs and sum PBDEs (ng/g milk fat), and sum WHO-TEQs (pg/g milk fat) in human milk from 4 different counties in Norway sampled in 2003.

		Rogaland	**Telemark**	**Troms**	**Østfold**
		n=25	***n=2***	***n=5***	***n=14***
HCB	median	13.71	9.71	14.69	10.54
	range	(8.7-23.6)	(6.4-13)	(10.8-35.2)	(9-14.6)
β-HCH	median	6.7	3.92	4.46	5.48
	range	(3.4-22.3)	(2.1-5.8)	(3.2-7.7)	(3.2-10.1)
pp-DDE	median	45.7	24.20	59.46	47.12
	range	(12.9-184)	(15.4-33)	(23.9-90.6)	(17.2-101)
*Sum 18 PCBs**	median	135.1	73.0	132.4	110.4
	range	(43.3-266)	(45.7-100)	(65.1-183)	(65-188)
Sum OCs		301	166	327	252
	range	(168-615)	(103-229)	(151-444)	(142-417)
*Sum m-o PCB-TEQ***	median	4.37	2.04	3.86	3.25
	range	(1.1-10.1)	(1.5-2.6)	(1.9-5.5)	(1.8-6.3)
		n=17	***n= 4***	***n=8***	***n=3***
Sum PBDEs***	median	2.47	1.66	2.52	1.95
	range	(1.00-10.79)	(1.06-2.55)	(1.41-10.56)	(1.73-3.76)

*Sum 18 PCBs : sum of PCBs # 28, 52, 74, 99, 101, 138, 153, 170, 180, 194, and the mono-*ortho* PCBs # 105, 114, 118, 123, 156, 157, 167, 189
** Sum m-o TEQ: sum TEQ of PCBs # 105, 114, 118, 123, 156, 157, 167, 189
***Sum PBDEs: sum of PBDEs # 28, 47, 99, 100, 153, 154

WHO-TEQ for mono-ortho PCBs and infant exposure:
The concentrations of sum mono-*ortho* PCBs as WHO_{1998}[8] toxic equivalents (WHO-TEQs) are shown in Table 1. PCB 156, 118 and 157 were the major mono-*ortho* congeners, contributing about 60%, 21% and 15% to the sum mono-*ortho* PCBs respectively (Figure 2). The concentrations of TEQs were much lower in the two samples collected in central Telemark compared to the other counties. The reason for these differences is unknown but will be examined further when more samples become available. The present levels of sum mono-*ortho* PCBs are comparable to those found in Sweden in 1997[1]. Another recent study in Norwegian human milk showed that mono-*ortho* PCBs contributed 30% to the total TEQs for PCDDs/PCDFs, non-*ortho* and mono-*ortho*

PCBs[9]. Assumed that this is still the case for 2003, the total TEQ could be calculated to 14.6 pg TEQ/g milk fat in Rogaland. The estimated daily intake of a 5-kg infant receiving 800 ml of a 3% fat breast milk per day would be 70 pg TEQ/kg body weight (bw)/day, or 490 pg TEQ/kg bw/week. In spite of the continued decrease of levels of POPs in Norwegian breast milk, this exceeds the tolerable life-long weekly intake (TWI) established by the EU (14 pg/kg b.w.) by a factor of about 35. However, the TWI has been established for lifetime exposures and cannot be applied to the short time period of breast-feeding.

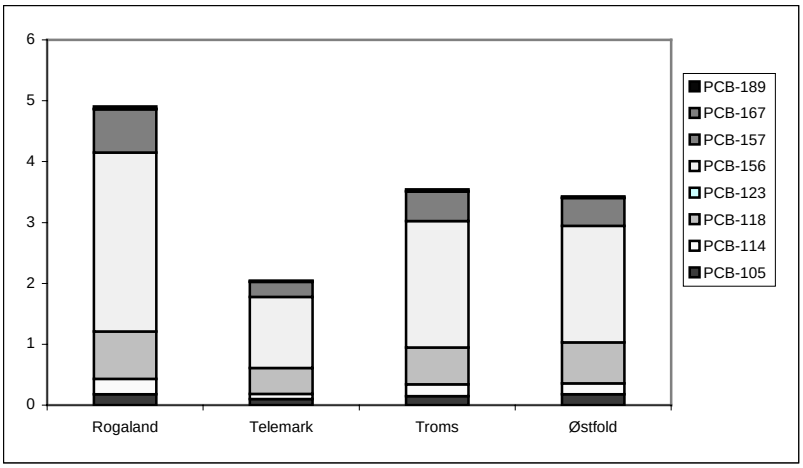

Figure 2. Mean concentrations of mono-*ortho* PCBs in WHO-TEQs (pg/g milk fat) for the different counties studied.

PBDEs:
The median and range of the sum of the six PBDEs in the breast milk from the different counties are shown in Table 1. BDE 47 was the dominating congener in all but five samples, in which BDE 153 was the most abundant compound. As for all the other POPs the lowest median concentration was observed in the samples from Telemark. The median sum PBDE were the same in Rogaland and Troms, as was also the case with the sum of the 18 PCBs. Analyses of more samples are in progress in order to reveal whether the PBDE levels in the counties differ significantly. The mean sum PBDE concentration of the 32 samples was 2.96 ng/g milk fat. As can be seen from the frequency distribution (Figure 3), two samples had a concentration above three times the mean. A wide individual variation in human PBDE levels has been reported previously[10,11,12,13]. Future statistical evaluations based on personal data from the questionnaire and the PBDE levels in a larger set of samples might clarify reasons for the elevated levels observed.

The mean sum PBDE level in these samples is in close agreement with the level reported in Norwegian breast milk pools from 2001, which was 3.04 ng/g milk fat (mean of sum of seven PBDEs)[11]. The increase in human PBDE levels observed during the last decades in Norway[11,14] thus seems to have levelled off.

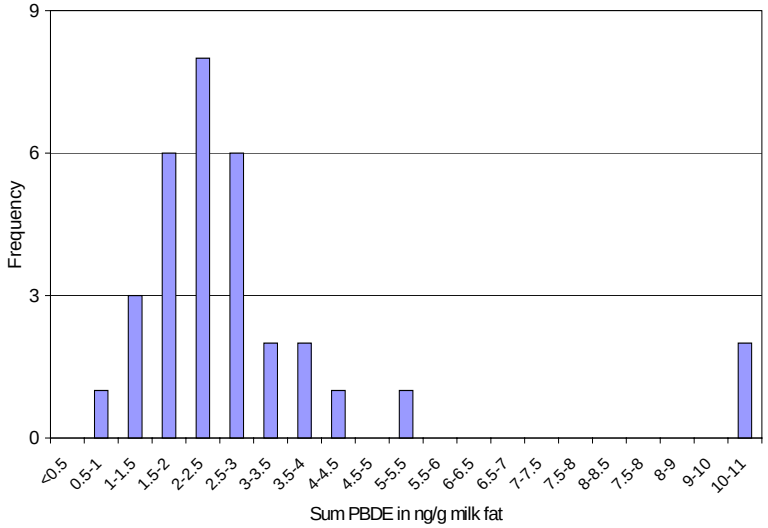

Figure 3. The frequency distribution of the sum of six PBDEs in ng/g milk fat in the 32 breast milk samples.

Acknowledgements

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