

**The Human Milk Study, HUMIS.  
Presentation of a Birth Cohort study which aims to collect  
milk samples from 6000 mothers,  
for the assessment of persistent organic pollutants  
(POPS),  
relating it to exposure factors and health outcomes.**

Merete Eggesbø<sup>1</sup>, Hein Stigum<sup>1</sup>, Anucha Polder<sup>2</sup>, Gunilla Lindstrøm<sup>3</sup>, Georg Becher<sup>1</sup>,  
Janneche Utne Skaare<sup>2</sup>, Per Magnus<sup>1</sup>

<sup>1</sup>Norwegian Institute of Public Health, Oslo

<sup>2</sup>The Norwegian School of Veterinary Science, Oslo

<sup>3</sup>Ørebro University, Ørebro

## **Introduction**

Although PCB has been forbidden for more than 20 years now, and its levels in human milk is declining, it remains among the chemicals in human milk causing most concern with regard to its possible detrimental effects on the fetus and the breastfed child<sup>1</sup>. Due to our industry, amongst others, the Norwegian population has been rather heavily exposed to PCB<sup>2</sup>.

Furthermore, new environmental toxicants are steadily entering the scene, such as the Brominated flame retardants. The level of Brominated flame retardants in human milk has shown an exponential increase during the last ten years,<sup>3</sup> and this group of chemicals, are causing increasingly more concern. Studies from Sweden has shown that the levels differ greatly between individuals,<sup>4</sup> however, for reasons yet unknown. In Norway, the highest levels of Brominated flame retardants ever measured in the world was reported from fish in Mjøsa<sup>5</sup>.

Surprisingly few attempts has been made to identify dietary habits or other life style factors that are associated with the levels of these toxicants in human milk. Such knowledge is needed in order for accurate prophylactic measures to be taken by the population and of special importance to women before and during child bearing age, in order to keep the levels in human milk as low as possible. Furthermore, there is great need for more knowledge of the effects of these toxicants on child health. The need for more research in this field, especially the need for prospective exposure data and the need for interdisciplinary approaches has been specifically targeted<sup>1</sup>.

Therefore a research initiative was taken in Norway to establish a prospective birth cohort which aims to recruit 6000 mother/child pairs, in whom human milk samples are collected in infancy and information on health outcomes are collected throughout the child's first seven years of

life. The aim of this presentation is to describe this project in more detail and to give some preliminary results.

### **The main aims of the project**

The aim of this project is three-fold.

1. To assess the levels of given toxicants in human milk and to estimate the proportion of children that are at risk of adverse effects due to intrauterine exposure to these toxicants.
2. To identify dietary habits and other lifestyle factors that are associated with high toxicant levels in human milk.
3. To study the impact of exposure to the these toxicants on child health
  - a) Among 400 children on the outcomes  
-birthweight, allergic diseases and tendency for infections first year of life.
  - b) Among 6000 children  
-Nested case-control studies on specific health outcomes

### **Methods and Materials**

This project is a cooperation project between Epidemiological and toxicological research milieus at the Norwegian institute of Public Health, the Norwegian Veterinary Institute, the Norwegian School of Veterinary Science, as well as the Örebro University in Sweden.

In a population-based birth-cohort, mother and child pairs are consecutively recruited by health visitors. In general a health visitor calls upon all mothers in Norway 1-2 weeks after having given birth. At this visit a written consent is signed by the mothers that are willing to participate and subsequently posted by their health visitor. Participating mothers are then sent containers for milk and a questionnaire within 2 weeks after their consent has been registered at The Norwegian Institute of Public Health. Two dl of human milk is required from the mothers. The mothers are told that the preferred sample method is to collect 25 ml every morning on eight separate days before the child is 2 months of age. The mothers are encouraged to do the milking by hand avoiding any electrical or other types of pumping equipment. However, the mothers are free to decide how to sample and we also accept pumped milk from other times of the day and milk collected in a longer or shorter period of time. All details on how and when the milk was sampled are registered by a sampling scheme. The containers have been thoroughly washed and the absence of toxicants in its empty and washed state checked. When the container is filled it is posted by the mothers, and stored at -20 degrees in a Biobank of the Norwegian Institute of Public Health upon arrival.

Further questionnaires are sent to the families when the child is 6, 12 and 24 months and 7 years of age. A Microsoft Access database keeps track of all participating mothers and incoming as well as outgoing material and questionnaires.

The recruitment started in 2003 and is planned to continue until 6000 mother/child pairs have been recruited, provided sufficient funding. The recruitment takes place in six counties in Norway, which represent northern, southern, western, and eastern parts of Norway including both coastal and inland areas (Rogaland, Telemark, Troms, Østfold, Oppland and Akershus).

The first questionnaire provides information about maternal age, smoking habits, parity, place of residence, nationality, temporary residency abroad, maternal education and other socioeconomic factors. Furthermore, detailed information is obtained concerning the mothers dietary habits during the last year as well as her life time consumption of some specific food items known to be of special interest due to their high levels of toxicants (see also abstract: "Does consumption of different categories of seafood affect birthweight?" for more details concerning how information on dietary habits is obtained). Information is also obtained concerning how technological their lifestyle is and consumer patterns, amongst others. Information on breastfeeding patterns, the duration of exclusive as well as partial breastfeeding is obtained from the first questionnaires, as well as at 6, 12 and 24 months of age.

Health outcomes obtained in the first questionnaire are: birth weight, gestational length, pregnancy complications, congenital disorders or malformations, un-descended testis, any early infections, colicky behavior, eczema or food allergies. Child health is also mapped in subsequent questionnaires with special focus on the number and types of infections and allergic diseases, as well as unspecific disorders.

Information on psychomotoric development is obtained through the validated "Ages and stages" questionnaires and selected questions taken from ITQ at 6 and 12 and 24 months of age, while the content of the 7 years of age questionnaire is not yet decided upon.

Approximately one third of the mothers are also participants in an even larger cohort, namely "Mother and Child Cohort Study" and information that has been collected through that study will also be available on these mothers. This includes thorough information on diet collected prior to birth, occupation and information on a large number of other covariates and health outcomes as well as cord blood samples and blood samples of the mothers.

In order to compare characteristics of participating and not-participating women, information on the general population of birth-giving mothers during the period of 2000-2003 has been obtained

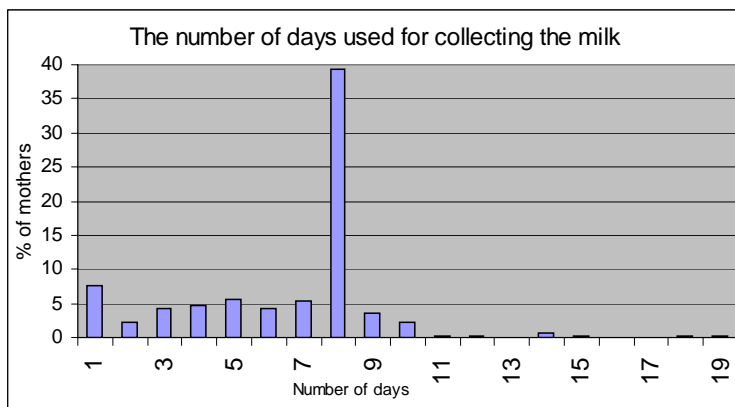
through the Norwegian Medical Birth Registry, which is a national registry containing information on all children born in Norway since 1967<sup>6</sup>.

T-tests were used for comparison of means and linear regression was used for the adjusted analysis. The regression model was tested for homogeneity of variances and possible departure from linearity. Calculations and statistical analysis were performed using the statistical data program - SPSS for Windows (v.11.0).

**Determination of OCP, PCBs and mono-ortho PCBs.** Concentrations of hexachlorobenzene (HCB), beta-hexachlorocyclohexane ( $\beta$ -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<sup>7</sup>. 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<sup>8</sup>. 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  $\mu$ m (J&W Scientific). Carrier gas: He 1 ml/min constant flow. Temperature program as described earlier, slightly modified. MSD specifications: negative chemical ionisation (NCI) in the SIM mode,  $T_{\text{ion source}}$  150 °C,  $T_{\text{quadrupole}}$  106 °C.

## Results and discussion

**Preliminary characterization of the sample:** 800 milk samples have been collected and stored so far. Information from the first questionnaire is available for 667 of the mothers. The mothers had a median and mean age of 30 years (range 17-43 ) at the time of recruitment. The milk samples had been sampled through a median of 8 days (Figure 2). The milk had been sampled by hand in 66.6% the women. The first questionnaire was filled out when the child was median 6 weeks old.



In approximately one third of the mothers, this was the first child (Table 1). Comparing the characteristics of the participating women with the characteristics of the general population, shows that there is a selection of multipara women to the study. This is probably at least partly due the fact that the task of collecting human milk is perceived as easier for multipara women compared to primipara women. However, there is also a selection of non-smoking mothers, which may indicate that there is a selection of women from higher socioeconomic levels (Table 1).

Table 1. Characteristics of 660 mothers and new-borns recruited to the HUMIS project compared to the general population of birth giving mothers.

(The Norwegian Medical Birth Registry has provided information on the general population)

	Participants n=667		General population n=126182	
	mean	median	mean	median
Maternal age	30	30	29	29
Birth-weight (g)	3517	3590	3520	3570
Gestation length	274	278	279	279
Parity (Prior pregnancies)		%		%
0		34.9		43.8
1		39.7		35.6
2		18.3		15.3
3		3.4		3.9
≥4		1.1		1.4
Smoking habits				
No		81		68
Yes		6.6		17.4
Missing		12.4		14.6

## Preliminary results

### Dietary habits and health effects

Among the 645 mothers for whom information is yet available we have studied the association between consumption of different categories of seafood by the mother and the birth weight of the child, see separate short paper on this conference<sup>9</sup>.

**Dietary habits during pregnancy of products of special interest:** Specific food items such as pike, fish liver as dinner meals and fishliver/-eggs as patee ("Svolværpostei"), are among food items that have been identified as having a relative high content of environmental toxicants in Norway. Pregnant women are recommended not to eat these food items (Norwegian Food Control Authority). However, the degree to which this advice is commonly accepted and followed is not known. Thus we looked at the consumption of these food items: Fish liver patee was consumed during pregnancy in 12.1 % of the women, with a median yearly intake of 12 meals (ranging from 1-240, mean 28). Pike had been consumed as a dinner meal in 2.8% of the women, with a median yearly intake of 2 meals (range 1-5, mean 2.6 dinner meals pro year). Fish liver had been consumed during

the last year by 14.1% of the women, with a median yearly intake of 2 dinner meals (mean 3.8 range 1-52 ).

Thus, a rather high proportion of pregnant women are often consuming food items which they are advised against. The dietary intakes especially of fish liver meals among some of the women is high enough to cause concern. We do not know whether this is due to lack of knowledge of the dietary recommendations.

### **Association between risky dietary habits and level of POPS in human milk**

The association between consumption of fish liver patee, pike and fish liver on the one hand and the levels of POPS in the human milk samples on the other, were studied among the 47 mothers in whom the milk samples had been analyzed (For the result of the chemical analysis see separate paper <sup>10</sup>). Consumption of fish liver patee showed a significant association to the HCB levels in human milk in the bivariate analysis ( $p < .000$ ).

Table 2. HCB levels in human milk according to consumption of specific food items adjusted for maternal age. The unadjusted analysis shows number (N), mean HCB concentration in ng/g milk fat and p-value for differences between categories and the adjusted analysis shows estimated increase in HCB concentrations per meal/year (B) with confidence intervals (CI), based on linear regression.

		Bivariate (unadjusted)			Adjusted coefficients		
		HCB (ng/g fat-weight)					
		N	Mean	p-value	B	(95% CI interval)	p-value
All		46	13.5				
Fish liver/egg patee	No	41	12.6	<.000			
	Yes	5	21.1				
	+1meal/year				0.44	(.29-.59)	<.000
Fish liver dinner	No	39	13.0	0.11			
	Yes	7	16.2				
	+1meal/year				0.13	(-0.42-0.68)	0.64
Lean fish	<52 meals	23	13.4	0.94			
	≥52 meals/year	23	13.5				
	+1meal/year				-0.005	(-.03-.02)	0.67

In a linear regression model adjusting for maternal age and yearly consumption of lean fish and fish liver, the association was still highly significant ( $p < .000$ ) and the effect of each additional yearly meal was estimated to lead to an increase in the HCB levels in human milk of .44 ng/g fat-weight (CI.3-.6). However, these analysis are based on small numbers. Consumption of Svolvær patee did not show any association with the other POPS measured.

Consumption of fish liver showed a non-significant association with HCB in human milk, however, this association lost strength in the multivariate analysis adjusting for consumption of svolvær patee, lean fish and maternal age, and no association with the other POPs measured.

Consumption of pike showed a significant association with PCB 194 in human milk, in the bivariate analysis ( $p=0.046$ ), but this association was no longer significant when adjusting for maternal age in a linear regression model. Neither was consumption of pike associated with higher or lower levels of any of the other measured toxicants in the human milk samples.

Due to the small sample size there is a risk of type 2 error: e.g. not finding associations that are there, due to lack of strength. Furthermore, significant associations may have occurred due to random constellations and this may explain the association between svolvær patee and HCB levels. On the other hand the size of the effect estimate and the strength of the p-value indicates that there may be an actual association between frequent consumption of patee and HCB levels in the human milk. Other factors associated with consumption of Svolvær patee needs to be considered as well. This will be further clarified when the analysis of more human milk samples are completed.

## Conclusion

**This project will increase our knowledge concerning the exposure levels to toxicants among new-born's in Norway today. Furthermore, knowledge will be gained on the importance of dietary and other life style factors of the population, for the toxicant levels in human milk, which upon prophylactic measures may be based. Last, but not the least, we hope that this project will let us gain knowledge of the effects of these toxicants on human health.**

## References

1. Schutz D, Moy G.G., Kaferstein F.K. WHO/FSF/FOS/98.4. 2004.
2. Becher G, Haug Småstuen L, Nicolaysen T, Polder A, Skaare JU. Organohalogen Compounds 2002;56:325-8.
3. Meironyte D, Noren K, Bergman A. Environ.Health A 1999;58:329-41.
4. Lind Y, Darnerud PO, Atuma S. Environ.Res. 2003;93:186-94.
5. Fjeld E. Press release 2003  
[http://www.niva.no/nyheter/Pressemeldinger/pres\\_bromerte\\_flammehemmere\\_200303.htm](http://www.niva.no/nyheter/Pressemeldinger/pres_bromerte_flammehemmere_200303.htm)
6. Irgens LM. Acta Obstet.Gynecol.Scand. 2000;79:435-9.
7. Brevik EM. Bull Environ Contam Toxicol 1978, 19:281-286.



8. Polder A, Odland JO, Tkachev A, Føreid S, Savinova TN, Skaare. *JU Sci Total Environ* 2003, 306: 179-195.
9. Fristad, R.F., Eggesbø M., Stigum H., Magnus P. Submitted Dioxin 2004.
10. Polder A., Thomsen B., Becher G., Skaare JU:, Løken K., Eggesbø M. Submitted Dioxin 2004.