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POLYCHLORINATED BIPHENYLS (PCB),
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IN NORWAY

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SUMMARY

The Norwegian Institute for Air Research has collected 32 samples of human milk from three regions in Norway for assessing the residue levels of polychlorinated biphenyls (PCB), dibenzo-p-dioxins (PCDD) and dibenzofurans (PCDF). This study was part of a larger Scandinavian investigation. The overall study design of the Scandinavian investigation was to collect at least ten individual (non-pooled) samples of human milk from each of 6 regions in Norway and Sweden: a coastal area, an inland area, two industrialized areas, one with known dioxin sources, a large city and a city with a refuse incinerator plant. The mothers were chosen according to a set of selection criteria. Additional information on lifestyle and possible previous exposure was obtained using questionnaires.

The Norwegian samples were collected in the winter of 1985/1986 from Tromsø (the coastal area), Elverum-Løten-Hamar (the inland area), and Skien-Porsgrunn (the industrialized area where there is a known substantial dioxin source). The 32 Norwegian samples were analyzed either at the Department of Organic Chemistry, University of Umeå, Sweden, (28 samples), or at the Norwegian Institute for Air Research (4 samples), for PCDD and PCDF, and at the Department of Pharmacology and Toxicology, the Norwegian College of Veterinary Medicine for PCB.

No significant differences in the concentrations of PCBs were found between the three geographic areas. No regional differences were found in the levels of PCDDs and PCDFs based on the determination of TCDD equivalents. However, an indication of regional differences was found for some PCDD and PCDF congeners, with relation to known sources of these compounds, and possibly to traffic or urban environment. The values of the TCDD equivalents in Norway are close to the average value of data reported from studies in other countries.

SAMMENDRAG

32 morsmelkprøver ble samlet inn fra 3 områder i Norge for å bestemme konsentrasjonene av polyklorerte bifenyler (PCB), dibenzo-p-dioksiner (PCDD) og dibenzofuraner (PCDF). Undersøkelsen inngikk som en del av et større skandinavisk program som hadde som mål å samle inn minst 10 enkeltprøver (ikke blandete prøver) fra hver av 6 utvalgte områder i Sverige og Norge. Det ble valgt et kystområde, et område i innlandet, to industriområder (derav en med kjente dioksinkilder), en storby og en by med et søppelforbrenningsanlegg.

Mødrene ble utvalgt etter bl.a. alder (18-30 år), at de var førstegangsfødende og hadde bodd forholdsvis lenge på det nåværende bostedet (5 år). Dessuten ble det samlet inn opplysninger angående livsstil og mulig eksponering for de ovennevnte komponenter med hjelp av spørreskjemaer.

Prøvene fra Norge ble samlet inn i løpet av vinteren 1985/86 fra Tromsø-området (kystområdet), fra innlandet rundt Elverum-Løten-Hamar og fra Skien-Porsgrunn (industriområdet med en betydelig og kjent PCDF/PCDD-kilde). 28 prøver ble analysert for PCDD og PCDF ved Organisk institutt, Umeå Universitet (Prof. Ch. Rappe) og 4 prøver ved Norsk institutt for luftforskning. Norges Veterinærhøyskole i Oslo (Institutt for farmakologi og toksikologi) foretok PCB-analysene.

Ingen signifikante forskjeller i PCB-nivået ble funnet mellom de 3 norske områder. De beregnede TCDD-toksisitetsekvivalentene var ikke signifikant forskjellige for de 3 områdene. Indikasjoner på mulige konsentrasjonsforskjeller ble imidlertid funnet for enkelte PCDD- og PCDF-kongener som har sin opprinnelse fra kjente industrikilder eller kan knyttes til trafikk eller urbane miljøer. Nivået av TCDD ekvivalentene tilsvarer omtrent gjennomsnittet av resultater rapportert fra flere andre land.

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POLYCHLORINATED BIPHENYLS (PCB), DIBENZO-P-DIOXINS (PCDD) AND
DIBENZOFURANS (PCDF) IN HUMAN MILK FROM
THREE GEOGRAPHIC AREAS IN NORWAY

1 INTRODUCTION

In the history of the industrial society, our environment has been receiving an increasing number of various chemical compounds. Many are in continuous use in the world to-day, including pesticides, industrial products and by-products. In many cases their impact on the environment has not been studied and their toxic properties are unknown.

During the last 20 years, special attention has been given to a class of compounds, the persistent chlorinated hydrocarbons. Due to their chemical properties, these compounds degrade only very slowly in the environment and therefore may accumulate in living organisms. For instance, low concentrations of the pesticide DDT and the industrial chemicals polychlorinated biphenyls, PCBs, have been found widespread in the environment, and residues are also found in humans. For these reasons, most industrialized countries have restricted or banned the use of this type of compounds.

One way to study if humans have accumulated such chemicals has been to monitor human milk. The idea behind most human milk monitoring programs has been to elucidate the infant burden of those chemicals from nursing. However, studies of chemical contamination of human milk have also been used for assessing levels of environmental pollution by fat soluble substances in different areas within and between countries. In Norway, monitoring of human milk for assessing levels of DDT, PCBs and related compounds have been done periodically since 1970 at the Norwegian College of Veterinary Medicine (Bjerk, 1972; Brevik and Bjerk, 1978; Skaare, 1981; Skaare et al., 1988). These studies have demonstrated that the concentration of DDT has decreased significantly

following the restriction and ban of the DDT use. Thus, our knowledge about some environmental contaminants of the chlorinated hydrocarbon type is fairly good.

Less, however, is known about a family of compounds named polychlorinated dibenzodioxins, PCDDs, and dibenzofurans, PCDFs, which constitute 210 different chemical structures with quite different toxicological properties. Some sources of these compounds have been identified - they are undesired byproducts of different chemical reactions and are also formed by high-temperature thermal processes. PCDDs and PCDFs have generally a very high acute toxicity in laboratory animals and chronic toxic effects could be detected down to daily dosages of 1-10 ng/kg body weight. These substances are present in biological samples in quantities which have not been possible to detect until the beginning of the 1980s. Even these low quantities may have the potential for causing toxic effects. Therefore there was a need to determine to what extent Norwegians are exposed to these substances.

The State Pollution Control Authority and the Royal Norwegian Council for Scientific and Industrial Research asked the Norwegian Institute for Air Research (NILU) in 1985 to organize an investigation of the concentration levels of PCDD, PCDF and PCB in human milk in Norway. NILU was responsible for the practical details of the project. The National Institute of Public Health was responsible for medical aspects of the study. The Department of Pharmacology and Toxicology at the Norwegian College of Veterinary Medicine performed the PCB analysis. Chemical analysis of PCDDs and PCDFs were carried out either at the Department of Organic Chemistry, University of Umeå, Sweden, (28 samples), or at the Norwegian Institute for Air Research (4 samples). The study was later incorporated into a larger Scandinavian study.

2 GOAL OF THE INVESTIGATION

The project was designed to assess the following:

- To determine the approximate mean levels and range of PCDDs and PCDFs in Norwegian human milk in a biological defined group.
- To identify possible sources of PCDD and PCDF contamination of human milk.
- To determine if PCDD and PCDF pollution is a local or a general problem.
- To make a follow-up of earlier Norwegian investigations on PCBs and other organochlorines.

However, the number of samples is quite limited and the investigation will probably give only an indication of the general pollution load of the population. Unless large local variations exist with regard to PCDD and PCDF pollution, the possible sources of the human exposure will probably not be revealed. Nevertheless, the selection of sampling areas was done with the hope to meet the abovelisted goals.

3 GEOGRAPHIC LOCATION

Sweden and Norway have co-ordinated their studies. Each country collected samples from three different types of regions. Norway contributed with two presumed background areas, one by the coast and one inland, and with one industrialized area with a known dioxin source. Sweden contributed with measurements from a town with a large refuse incinerator, a major city, and an industrialized area with unknown dioxin emissions.

In Norway the three chosen study locations were:

- The coastal area consisted of the city of Tromsø and its surroundings. It was considered to be a region relatively free from air pollution. Fish is an important part of the diet.

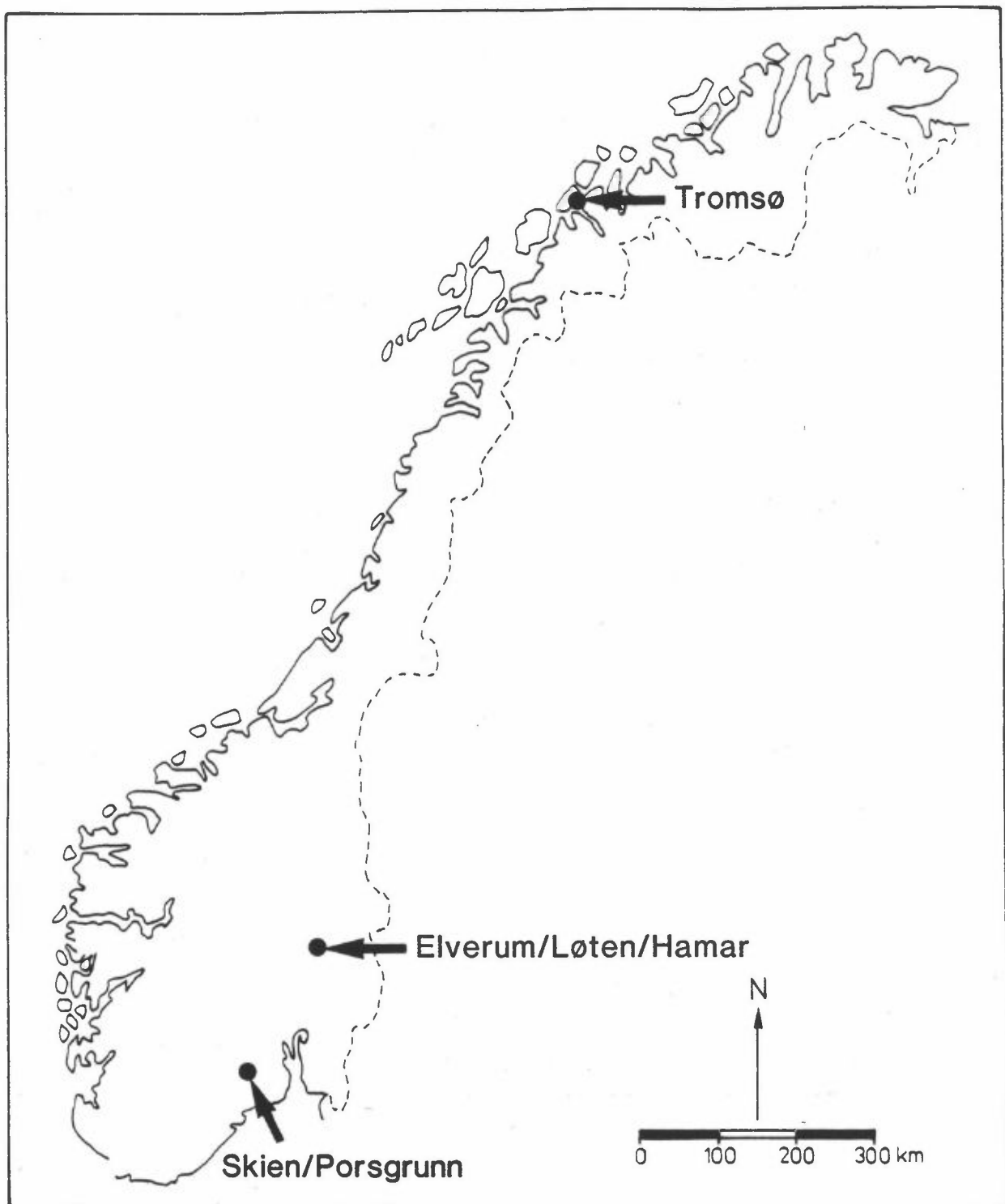


Figure 1: The three geographic areas where samples of milk were collected.

- The inland region included the area in and around Elverum, Løten and Hamar (referred to in the remainder of this report as Hamar). This area can also be considered a background area free from industrial emissions, it is exposed to pollution from wood burning in the winter (a typical situation in inland Norway). The population was assumed to eat less fish than the population of Tromsø.
- The industrial region included both the cities of Skien and Porsgrunn (referred to as Skien). Their inhabitants are exposed to air pollution from several industrial sources such as magnesium production emitting organic chlorine compounds and PCDD/PCDF (about 300 g/year of 2,3,7,8-TCDD equivalents to the waste water stream and about 6 g/year to the atmosphere).

4 MATERIALS AND METHODS

4.1 SELECTION CRITERIA FOR PARTICIPATING MOTHERS

The following is a list of the selection criteria for enrolling a mother into the study. These criteria were developed in co-operation with the other Scandinavian countries:

- only primiparae
 - only single births
 - mothers in good physical health
 - mothers whose psychological well-being was good
 - mothers having no problems with lactation
 - mothers between the ages of 18 and 30 years
 - mothers having lived in the same geographic area for at least 5 years.
-

4.2 SAMPLING PROCEDURE

A total of 350 to 500 ml were collected from each mother during the winter 1985/1986. The samples were collected daily at home during a one week period at any time of day, using a furnished breast pump which had been tested and found free from contamination, and a pre-washed collection bottle that held 500 ml. Electric pumps were not used. If necessary, the use of Oxytocin spray was allowed to facilitate let-down.

Each sub-sample collected was frozen. The next sample was then poured over the previously frozen sample and frozen again. After sampling was completed, the bottle was transported in a frozen condition to a central collecting point in the area. From there it was further transported frozen to NILU for analysis. A 50 ml aliquot of each sample was sent to the Norwegian College of Veterinary Medicine for the PCB analysis. Most of the PCDD and PCDF analyses were done at the University of Umeå, Sweden.

The National Institute of Public Health was in charge of the medical aspects of the study.

Sample collection was co-ordinated through the local health services. A local co-ordinator in each district (one of the participating community nurses) was responsible for organizing the local nurses, and disseminating information to them, and back to the central co-ordinator at NILU. Nurses in each geographic area visited the homes of the participating mothers where they explained the study. The mothers were also asked to fill out a questionnaire at this time.

Prior to sample collection, a meeting was held in each geographical location with all the participating nurses and the local co-ordinators, the central co-ordinator and the physician responsible for medical aspects of the study. This information meeting had three functions: 1) to present the objectives of the study and the procedures that need to be followed, 2) to respond to any questions the local personnel might have and 3) to discuss in detail the importance of information transfer to the mothers themselves.

It was of great importance that the mothers should not be worried or in any way psychologically burdened. In such a study they must not fear that they will not have enough milk for both the study and their child, or that there is anything wrong with their milk. Since the samples were not pooled (with the exception of two), each individual had the right to know their own values. However, these values were not given out automatically, only upon request. A brief report summarizing the findings of the three Norwegian regions was later given to each of the participating mothers.

Breast pumps were tested chemically and found to be free of contamination. The collection bottles were thoroughly cleaned and finally rinsed with acetone before they were delivered to the mothers.

Each mother was given careful directions as to the care of the pump. The pump should be rinsed with water and if desired boiled. However, soap was not allowed.

The breasts and hands were to be kept as clean as possible, though using as little soap as possible. When necessary to use soap, the breasts and hands should be thoroughly rinsed. If it was found necessary to use ointment on the nipples because of soreness, this was to be done outside of sampling time and the ointment should be removed prior to sampling.

4.3 ADDITIONAL INFORMATION OBTAINED VIA THE QUESTIONNAIRE

Each mother supplied the following information through a written questionnaire:

Personal information

- name, address, telephone number
- birth date, health status

Smoking habits

- current and previous smoking habits in addition to exposure to passive smoking

Occupation

- current and previous occupations held

Residence

- current and previous places of residence

Travel

- time spent and geographic location of vacation travelling outside the country

Dietary habits

- type of food eaten
- amount of fish eaten
- information on weight loss (> 10 kg) during the lifetime

Pregnancy and birth

- general information on the pregnancy
- general information on the delivery
- information on weight gain and loss during pregnancy and delivery

Miscellaneous

- exposure to a major fire or explosion
- use of fireplace, wood, coal or paraffin stove for heating.

4.4 CHEMICAL ANALYSIS AND TOXIC TCDD-EQUIVALENT CALCULATION

The PCDD/PCDF content of 28 human breast milk samples was determined by the group of Prof. C. Rappe, Department of Organic Chemistry, University of Umeå, Sweden. Details of the analytical procedure employed are given in Appendix I. Four samples were analysed at the Norwegian Institute for Air Research according to the working procedure FOG 1/86 (Manø et al., 1986). The results of all individual analyses are given in Appendix II.

PCB-analysis was carried out at the Department of Pharmacology and Toxicology at The Norwegian College of Veterinary Medicine, Oslo, by

the group of Dr Janneche Utne Skåre. Two independent techniques were used, the US Food & Drug Administration method (Sawyer, 1978) and an isomer specific method proposed by Codex (see Appendix III).

In order to assess comparable results of a sample related to dioxin potency, 2,3,7,8-TCDD equivalents were calculated as a weighted sum of the PCDD and PCDF using the Nordic TCDD-equivalent model, with coefficients given in Table 1 (WHO 1988a).

Table 1: Weights for the Nordic TCDD-equivalent model.

Compound	Weight factor
2,3,7,8-tetraCDD	1.000
1,2,3,7,8-pentaCDD	0.500
2,3,7,8-substituted hexaCDDs	0.100
1,2,3,4,6,7,8-heptaCDD	0.010
octaCDD	0.001
2,3,7,8-tetraCDF	0.100
1,2,3,7,8-pentaCDF	0.010
2,3,4,7,8-pentaCDF	0.500
2,3,7,8-substituted hexaCDFs	0.100
2,3,7,8-substituted heptaCDFs	0.010
octaCDF	0.001

4.5 STATISTICAL ANALYSIS

The statistical analysis had two goals: (1) to assess regional differences between levels of the compounds, and (2) to examine the data for any possible connections between lifestyle and chemical composition. Regional differences were assessed by multiple analysis of variance. In order to explore the effect of lifestyle (the subject of a separate report), it was necessary to reduce the number of variables in the investigation. The variables were divided into three groups: 1) PCBs, 2) PCDDs and PCDFs, and 3) the variables from the questionnaire. Each group was treated separately to investigate the structure of correlations within it. This resulted in the construction of new variables within the groups as weighted sums of the original variables. The weighted sums then replaced the original variables in the further

analysis. Using this approach, the risk of detecting false connections due to the linear dependencies within each group was substantially reduced.

Multiple analysis of variance and multiple regression analysis were used to investigate possible connections between the three groups of variables (Bartonova et al., 1988). Correlation coefficients between the chemical compounds are listed in Appendix IV.

5 RESULTS

Differences found between various groups of subjects, even if statistically significant, are small. Generally, the PCDD/PCDF concentrations are similar to those found in samples from Sweden, Denmark and North America, though lower than those measured in West Germany (WHO, 1988).

5.1 CONCENTRATIONS OF PCBs, DIOXINS AND DIBENZOFURANS IN HUMAN MILK FROM THE THREE AREAS, AND TIME TRENDS OF PCBs

The PCB results were calculated both on a fresh and a fat weight basis. The median, mean and the standard deviation of PCB residue levels calculated on a fat weight basis, in human milk from the 3 sampling areas are given in Table 2. The concentrations of p,p'-DDE and HCB are also given in the tables. As can be seen, no significant differences in the levels of PCBs, p,p'-DDE and HCB were found between the geographic areas concerned. Corresponding data on a fresh weight basis as well as individual values are given in Appendix III.

No significant differences were found between the geographic areas in the values of the 2,3,7,8-TCDD equivalents. However, concentrations of certain of the PCDFs were found to be higher (at the 5% significance level) in the Skien-Porsgrunn area. This concerns the concentrations of the 1,2,3,4,7,8-hexaCDF, the 1,2,3,6,7,8-hexaCDF and the 2,3,4,6,7,8-hexaCDF which were higher than the corresponding concentrations found in the regions surrounding Tromsø and Hamar. The mean

and median values of the analysed compounds are summarized in Tables 3a-b. Individual values of these congeners are given in Appendix II.

Table 2: Concentrations of PCBs, p,p'-DDE, HCB (ng/g fat weight) and percent of fat in human milk from the three geographic regions. The results are listed as mean, median and standard deviation values.

	Location		
	TROMSØ	HAMAR	SKIEN
% of fat			
median value	3.3	3.7	3.5
mean value	3.5	3.9	3.5
stand. dev.	.7	1.0	1.3
PCBs			
median value	474.5	515.5	511.5
mean value	561.7	507.1	533.4
stand. dev.	167.1	135.3	234.6
pp-DDE			
median value	594.0	475.5	342.5
mean value	625.1	518.0	390.4
stand. dev.	400.2	201.1	125.7
HCB			
median value	68.5	51.5	66.0
mean value	74.6	54.4	73.5
stand. dev.	20.5	11.4	32.1

The time trend of PCBs is shown in Table 4. Current concentrations of PCBs in Norwegian human milk are compared with the corresponding concentrations obtained in 1970, 1976, 1979, and 1982. The means and the standard deviations together with the number of samples analyzed are given. Basically the same analytical methods were used in all the surveys. The data show a marked reduction in the PCB levels since 1982. Further details are given in Appendix III.

Table 3a: Concentrations of PCDD congeners (pg/g fat weight) and of the TCDD-equivalent in human milk from the three geographic regions. The results are listed as mean, median and standard deviations.

	Location		
	TROMSØ	HAMAR	SKIEN
dioxin 2378-tetra			
median value	2.8	2.4	2.2
mean value	2.9	2.5	2.7
stand. dev.	.5	.5	1.2
dioxin 12378-penta			
median value	4.5	4.4	5.0
mean value	4.7	4.7	5.0
stand. dev.	1.1	1.4	1.8
dioxin 1234/678-hexa			
median value	19.8	15.8	19.6
mean value	19.2	18.8	20.3
stand. dev.	4.7	9.2	6.2
dioxin 123789-hexa			
median value	4.9	4.3	3.0
mean value	4.7	4.8	3.2
stand. dev.	.9	2.6	1.0
dioxin 1234678-hepta			
median value	38.6	35.8	33.3
mean value	36.0	40.3	36.6
stand. dev.	7.5	20.6	17.8
dioxin octa			
median value	138.0	160.5	142.5
mean value	154.6	149.9	156.0
stand. dev.	59.0	54.8	79.2
TCDD-equivalent			
median value	14.8	13.8	16.7
mean value	15.9	14.8	19.1
stand. dev.	3.7	4.9	7.5

Table 3b: Concentrations of PCDF congeners (pg/g fat weight) in human milk from the three geographic regions. The results are listed as mean, median and standard deviation values.

	Location		
	TROMSØ	HAMAR	SKIEN
furan 2378-tetra			
median value	4.0	4.2	4.7
mean value	4.3	4.1	4.9
stand. dev.	1.1	1.0	2.3
furan 12378-penta			
median value	.7	.7	1.1
mean value	.8	.8	1.3
stand. dev.	.3	.3	1.0
furan 23478-penta			
median value	11.0	10.1	14.1
mean value	12.9	11.4	17.7
stand. dev.	4.7	4.3	8.4
furan 123478-hexa			
median value	3.7	4.1	6.0
mean value	3.6	4.6	7.8
stand. dev.	.8	1.6	5.1
furan 123678-hexa			
median value	2.3	2.3	4.6
mean value	2.6	2.7	5.3
stand. dev.	.6	1.1	2.9
furan 123789-hexa			
median value	.7	.7	.7
mean value	.7	.7	.7
stand. dev.	.3	.0	.4
furan 234678-hexa			
median value	.9	.7	1.5
mean value	.9	1.0	1.7
stand. dev.	.4	.5	.7
furan hepta			
median value	6.0	5.0	5.8
mean value	6.2	5.5	5.6
stand. dev.	1.9	1.6	1.9
furan octa			
median value	1.2	1.2	1.2
mean value	1.1	1.2	2.5
stand. dev.	.3	.0	3.0

Table 4: Residues (ppb, $\mu\text{g/kg}$ fat weight) of PCBs in Norwegian human milk 1970a, 1976b, 1979c, 1982d, and 1986.

PCBs				
1970	1976	1979	1982	1986
367 \pm 167 (25)	960 \pm 360 ^e (14)	1220 \pm 400 (19)	1000 \pm 350 (20)	534 \pm 174 ^f (28)

Results are expressed as means \pm S.D., numbers analyzed are listed in parantheses.

a) Bjerk (1972)

b) Brevik and Bjerk (1978)

c) Skaare (1981)

d) Skaare, Sande & Tuveng (1987)

e) Significant difference ($P < 0.05$) between 1970 and 1976

f) Significant difference ($P < 0.05$) between 1982 and 1986.

5.2 IDENTIFICATION OF POSSIBLE SOURCES OF THE CONTAMINANTS

Using the information provided by the questionnaire, the values of PCDFs and PCDDs were statistically analyzed for effects of lifestyle and other confounding factors. This is the subjects of its own report (Bartonova et al., 1988). We present here only a short summary of the findings.

The effects of various personal characteristics were investigated; the effect of age of the mother at childbirth; her current and previous smoking habits; type of residence heating; mother's living in more densely populated areas or in Oslo; her travelling to Southern Europe; changes in her diet; type of fish the mother eats regularly; a history of a substantial weight reduction diet (more than 10 kg) as well as weight loss in the first week after delivery. Characteristics of the three geographic locations according to the respondent's personal parameters are summarized in Table 5a-b.

In most cases, no relation was detected between parameters of life-style and concentrations of the chemical compounds in the mother's milk. Neither amount nor type of fish eaten were found to significantly influence the values of dioxins. No significant effect was found related to smoking history, use of open fire heating, or exposition to a major explosion. However, several dependencies were found to be statistically significant.

Table 5a: Mean values of several personal characteristics for the three locations.

	Location		
	TROMSØ	HAMAR	SKIEN
Mother's age at delivery mean value (years)	24.9	24.1	23.7
Mean weight loss in the 1st week after delivery (kg)	9.4	10.2	8.5
Mean number of fish meals consumed per month	7.7	4.8	4.7

Table 5b: Characteristics of the three locations according to selected lifestyle parameters.

	Location		
	TROMSØ	HAMAR	SKIEN
Suffering from allergy			
no	12	9	9
yes		1	1
Observed major fire			
no	6	8	10
yes	6	2	
Positive smoking history			
no	2	4	8
yes	10	6	2
Travelled to South			
no	11	7	10
yes	1	3	
Mother's overweight			
no	3	8	9
yes	9	2	1
History of reduct. diet			
no	10	8	6
yes	2	2	4
Subst. diet change			
no	10	10	9
yes	2		1
Ever lived in Oslo			
no	10	8	8
yes	2	2	2
Use of open fire heating			
no	8	1	6
yes	4	9	4

Influence of urban environment: Analysis of variance revealed significantly higher levels of the tetra to hepta PCDDs and of the tetra, penta and hexa (with the exception of 1,2,3,7,8,9-hexa CDF) PCDFs in those respondents who during their lifetime had lived in Oslo. The concentrations of the compounds in the two groups are given in Table 6.

Table 6: Mean values of PCBs, PCDDs and PCDFs in milk according to history of living in Oslo of the mother.

	Ever lived in Oslo	
	no	yes
Number of respondents	26	6
% of fat	3.8	3.1
PCBs	516.9	597.2
pp-DDE	527.6	491.0
HCB	65.5	72.7
dioxin 2378-tetra	2.6	3.3
dioxin 12378-penta	4.3	6.2
dioxins 1234/678-hexa	18.2	24.9
dioxin 123789-hexa	4.0	5.5
dioxin 1234678-hepta	33.5	55.0
dioxin octa	147.5	180.0
furan 2378-tetra	4.2	5.4
furan 12378-penta	.8	1.5
furan 23478-penta	12.5	20.2
furan 123478-hexa	4.5	8.5
furan 123678-hexa	3.1	5.0
furan 123789-hexa	.7	.7
furan 234678-hexa	1.0	1.8
furan hepta	5.6	6.5
furan octa	1.6	1.2
TCDD-equivalent	15.2	22.5

Effect of travelling to Southern Europe: Only 4 subjects had travelled to Southern Europe within the last year before delivery. However, this group was different than the non-travelling subjects. In breast milk of the travelling group, significantly higher values of hexa dioxin isomers were found. Mean values of the concentrations found in both groups are given in Table 7.

Table 7: Mean values of concentrations of chemical compounds found in the milk of mothers according to their travelling to Southern Europe in the last year before delivery.

	Travelling to South	
	no	yes
Number of respondents	28	4
% of fat	3.7	3.1
PCBs	520.2	650.3
pp-DDE	514.4	565.0
HCB	66.8	69.7
dioxin 2378-tetra	2.7	2.6
dioxin 12378-penta	4.8	5.7
dioxins 1234/678-hexa	18.3	27.3
dioxin 123789-hexa	4.0	6.1
dioxin 1234678-hepta	35.2	53.8
dioxin octa	152.8	159.0
furan 2378-tetra	4.5	3.8
furan 12378-penta	1.0	.7
furan 23478-penta	13.9	14.2
furan 123478-hexa	5.3	4.6
furan 123678-hexa	3.5	3.0
furan 123789-hexa	.7	.5
furan 234678-hexa	1.2	1.2
furan hepta	5.9	5.5
furan octa	1.6	1.1
TCDD-equivalent	16.4	18.0

Effect of age: Mothers enrolled in the study were between 18 and 30 years of age. Positive regression relations were found between levels of PCBs and 1,2,3,7,8,9-hexa CDF and age. Increased concentrations with age were found in breast milk.

6 DISCUSSION

Concentration levels. The mean levels of PCBs, p,p'-DDE, and HCB are among the lowest reported both in the Scandinavian countries and in the world (Jensen, 1983, 1987). The mean levels of the PCDD and PCDF compounds are not very different from the mean of values reported both in the Scandinavian countries and in the world (Lindström, 1988; Sundhedsstyrelsen, 1987; Rantanen, 1987; WHO, 1988).

The Nordic TCDD equivalent method (Nordisk Ministerråd, 1988) can be used to compare the dioxin levels. We can classify the samples from the countries that reported their values to the WHO into three groups (Figure 2): (1) low TCDD-equivalent values (below 10 pg/g on fat basis) comprising samples from certain areas in Vietnam, Thailand, New Zealand (2) moderate TCDD-equivalent levels (between 10 and 20 pg/g on fat basis) - samples from Canada, USA, Austria, Denmark, Norway and Yugoslavia (3) higher values of TCDD-equivalent, i.e. above 20 pg/g on fat basis, - British Columbia in Canada, certain areas in Vietnam, and in the Federal Republic of Germany. Based on this crude classification, we may conclude that levels in Norway are comparable with the levels reported from other industrialized countries, but lower than the levels reported for the FRG.

Geographic differences. The findings reported here revealing no geographical differences in PCB, p,p'-DDE and HCB concentrations agree well with earlier findings in Norway. Brevik and Bjerk (1987) reported geographical differences in HCB levels in their human milk investigation in 1976. However, no geographic differences in organochlorines contamination level were found in 1979, when 133 Norwegian human milk samples were analyzed from 7 cities located in different areas of the country and representing various degrees of industrialization (Skaare, 1981). Monitoring of seabirds (Ingebrigtsen et al., 1984), saltwater fish (Skaare et al., 1985), and birds of prey (Frøslie et al., 1986) in Norway has demonstrated that the concentration levels in such biological samples are approximately the same in different areas of the country. This supports the assumption that concentrations in human milk probably reflect more global distribution than local or regional sources.

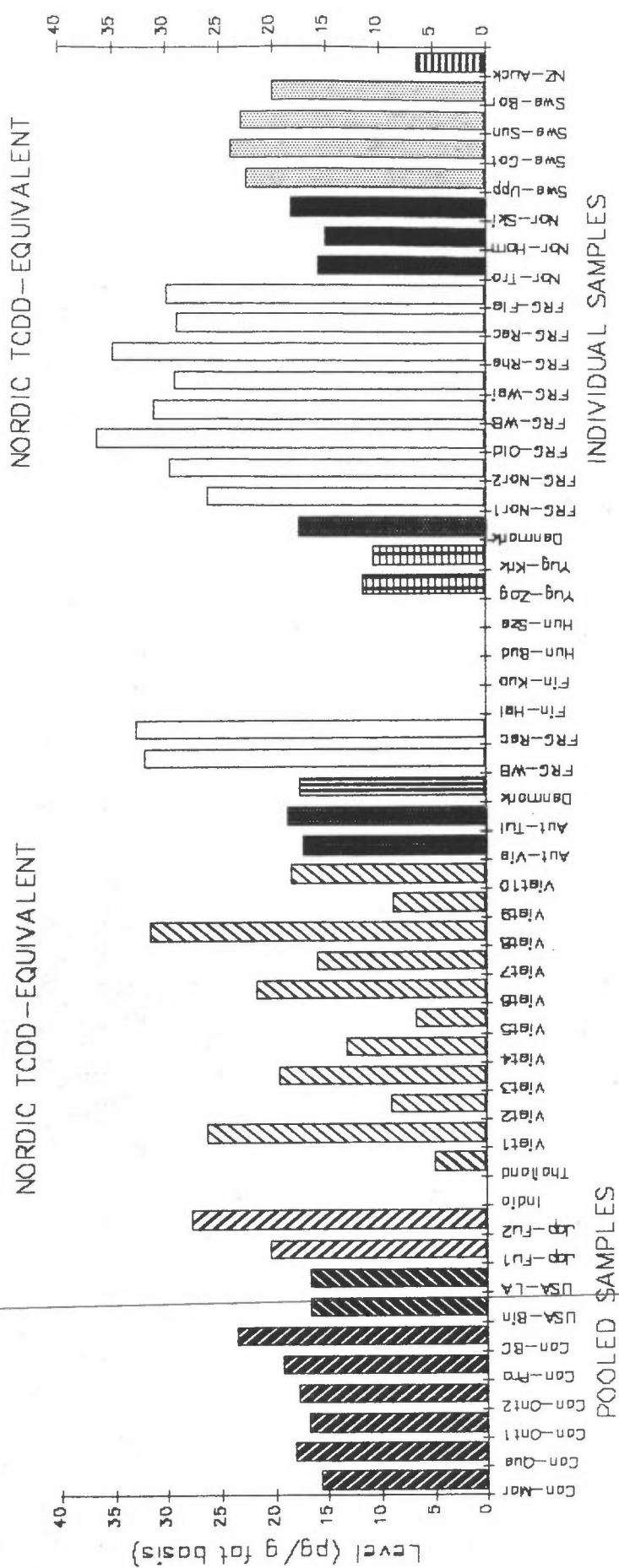


Figure 2: Mean values of the Nordic TCDD-equivalents for various locations (based on the WHO 1988b). For the 5 locations with zero (missing) values, it was not possible to determine the TCDD-equivalent due to the missing 2,3,7,8-TCDD concentration. For all locations, a missing component with weight equal to or less than 0.1 (see Table 1) was considered to be zero.

Small geographical differences were found in certain PCDD and PCDF congener levels, probably due to the known source of PCDFs in Porsgrunn. These compounds are of moderate biological significance. No other geographical differences were found, indicating that there is no major geographical trend throughout the country. However, this study does indicate the possibility that concentrations may be somewhat higher for persons staying for a longer period in Oslo. The results of the statistical analysis show that PCDD/PCDF concentrations there are significantly higher.

Factors affecting the levels of PCBs, p,p'-DDE, HCB, PCDDs and PCDFs in human milk and possible sources of organochlorine compounds. The findings of this study, relating the personal and environmental characteristics of the respondents to the concentration levels, have to be interpreted with great caution. They are based on a set of only 32 subjects, whose inclusion in the study was not based on their personal characteristics related to lifestyle or known exposure other than listed in 4.1.

Earlier surveys have reported correlations between PCB and DDT contamination levels in human with the mother's age, food and smoking habits, living conditions etc. For instance, in Sweden the main non-occupational source of these organochlorine compounds is presumably the diet, especially certain fish species (Jensen, 1983). This study confirmed the relation of PCB with age, but did not reveal any other differences in contamination levels related to the status of the mother and her environment. This may reflect small differences in exposure which may not be possible to detect with the design used in this study.

An overall description of sources, atmospheric transport and deposition of chlorinated hydrocarbons is given in Semb & Pacyna, 1988. No surveys have been carried out so far to reveal the relation between the sources of PCDDs and PCDFs, ambient concentration levels and personal burden. This study, does not allow any conclusions as to sources of dioxins and dibenzofurans. No indication was found that exposure to major fire increases the PCDD and PCDF concentrations in human milk. Smoking or a varying fish diet did not influence the concentrations in

breast milk significantly, indicating that the contributions from these sources to the present individuals, if any, are small.

Time trends in PCBs. In spite of the fact that the use of PCBs in Norway was restricted to closed systems since 1971, the mean levels in human milk were more than tripled from 1970 to 1979. A ban on the use of PCBs was imposed in 1979. The slight downward trend from 1979 to 1982, followed by a significant decrease (50%) during the period 1982-1986, demonstrates a positive effect of the restriction and the ban.

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APPENDIX I

Analytical Methodology for the Determination of PCDDs/PCDFs
in Human Milk from Norway

Analytical Methodology for the Determination of PCDDs/PCDFs
in Human Milk from Norway

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Totally 28 samples of human milk from Norway (see Appendix II) have been analysed during September and October 1987 at the Department of Organic Chemistry, University of Umeå. The analysis was done according to the following description:

* Extraction of the lipid fraction

In connection to the lipid extraction (DEE-hexane, Ref. 1) each milk sample (168.3-416.2 g) was fortified with 100 pg of the ^{13}C -congeners listed (Ref. 2) below.

2,3,7,8-TetraCDD
2,3,7,8-TetraCDF

1,2,3,7,8-PeCDD
1,2,3,7,8-PeCDF
2,3,4,7,8-PeCDF

1,2,3,6,7,8-HxCDD
1,2,3,4,7,8-HxCDD

1,2,3,4,6,7,8-HpCDD
1,2,3,4,6,7,8-HpCDF

OCDD

The lipid content ranges from 2.5-14.5 g (mean = 10.5 g) for the different samples corresponding to 1.5-7.8% (mean = 3.8%).

**** Cleanup and enrichment process of PCDDs/PCDFs**

The total lipid fraction was applied to a liquid chromatography system (Ref. 3) containing a basic silica column followed by a PX-21 carbon column and an acidic alox column completing the cleanup process. System blanks were treated in the same way.

***** Identification and Quantification of the PCDDs/PCDFs**

The thirteen 2,3,7,8-substituted congeners detected by HRGC-HRMS in the milk samples were identified on basis of the standard criteria (Ref. 4).

HRGC-data: Column DB-5, 60 m, id. 0.32 mm, splitless inj.

HRMS-data: VG 70-250E, EI, res. 6000, SIR monitoring; incl. lock mass (see Appendix I/2).

GC-MS-analysis: An internal standard (IS) consisting of the following $^{12}\text{C}_{12}$ -furan congeners was added to the sample prior to the injection: 2,4,6,7/2,3,4,6,8/1,3,4,6,7,8/1,2,3,4,6,8,9. The same amount of this IS was added to the $^{12}\text{C}_{12}$ -quantification standard (see Appendix I/3), holding a concentration of totally 100 pg of each isomer, which also contained the $^{13}\text{C}_{12}$ -congeners in the same concentration as in the samples (100 pg). One tenth of each sample and quantification standard was used in the analysis. Tetradecane was present in both samples and standards.

The quantification of the fifteen congeners reported on was done using equation (1) and the $^{13}\text{C}_{12}$ -recoveries were calculated by equation (2).

$$C_x = \frac{A_x \cdot C_{q\text{-mix}} \cdot X_{\text{tot}} \cdot A_{13\text{-C-q-mix}}}{A_{x\text{-q-mix}} \cdot V_g \cdot A_{13\text{-C-x}}} \quad \text{equation (1)}$$

$$\text{Recovery} = \frac{A_{13\text{-C-x}} \cdot A_{\text{IS-x}} \cdot 100}{A_{13\text{-C-q-mix}} \cdot A_{\text{IS-q-mix}}} \quad \text{equation (2)}$$

C_x	= amount of the PCDD/PCDF congener in ppt on lipid basis
A_x	= area of the analysed congener (M ion)
$q\text{-mix}$	= pg of the congener in the quantification standard
X_{tot}	= factor accounting for the congener in the total sample (1/10)
$A_{13\text{-C-q-mix}}$	= area of the corresponding congener in the standard
$A_{x\text{-q-mix}}$	= area of the congener in the standard
V_g	= total lipid fraction in g
$A_{13\text{-C-x}}$	= area of the ^{13}C -congener in the sample

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APPENDIX I/2

* SYSTEM:DBSQL Parameters for Selective Ion Recording [G# 1 KVE] (Sector)			
DAT Data filename	B:6421592	IRV Maximum volts	6000
REF Reference filename	PFK	IMR Maximum mass at IRV	2405
INS Instrument	1:70-250	DTM TLine 0:15:00 0:31:30 Mode EI-1S Gas	
ACH Customer account	UME	CHN MCasD SCasD DCasD	MCasD SCasD DCasD
ACV Accelerating volts	6000	A 303.9016	00 20
RES Instrument resolution	6000	B 305.8967	00 20
SMP #Samples	1	C 317.9389	00 20
INJ #Injections	1	D 318.8782	100 20
GRP #Groups	5	E 318.8782	00 20
CLS #Calibration scans	0	F 319.8965	00 20
CST Cal. scan time (s)	10	G 321.8936	00 20
CTL Cal. tolerance (ppm)	100	H 333.9338	00 20
CEX Cal. examination	N		
PEX Peak examination	N		
LRS Look span (peak widths)	2.0		
LST Look step (peak widths)	0.02		
FLO Fast Look on	Y		

* SYSTEM:DBSQL Parameters for Selective Ion Recording [G# 2 KVE] (Sector)			
DAT Data filename	B:6421592	IRV Maximum volts	6000
REF Reference filename	PFK	IMR Maximum mass at IRV	2405
INS Instrument	1:70-250	DTM TLine 0:31:40 0:36:00 Mode EI-1S Gas	
ACH Customer account	UME	CHN MCasD SCasD DCasD	MCasD SCasD DCasD
ACV Accelerating volts	6000	A 337.8627	00 20
RES Instrument resolution	6000	B 339.8537	00 20
SMP #Samples	1	C 342.9792	100 20
INJ #Injections	1	D 342.9792	00 20
GRP #Groups	5	E 351.9000	00 20
CLS #Calibration scans	0	F 353.8576	00 20
CST Cal. scan time (s)	10	G 355.8546	00 20
CTL Cal. tolerance (ppm)	100	H 367.8949	00 20
CEX Cal. examination	N		
PEX Peak examination	N		
LRS Look span (peak widths)	2.0		
LST Look step (peak widths)	0.02		
FLO Fast Look on	Y		

* SYSTEM:DBSQL Parameters for Selective Ion Recording [G# 3 KVE] (Sector)			
DAT Data filename	B:6421592	IRV Maximum volts	6000
REF Reference filename	PFK	IMR Maximum mass at IRV	2405
INS Instrument	1:70-250	DTM TLine 0:36:10 0:41:00 Mode EI-1S Gas	
ACH Customer account	UME	CHN MCasD SCasD DCasD	MCasD SCasD DCasD
ACV Accelerating volts	6000	A 371.8236	00 20
RES Instrument resolution	6000	B 373.8207	00 20
SMP #Samples	1	C 380.9760	100 20
INJ #Injections	1	D 380.9760	00 20
GRP #Groups	5	E 385.8610	00 20
CLS #Calibration scans	0	F 387.8156	00 20
CST Cal. scan time (s)	10	G 389.8156	00 20
CTL Cal. tolerance (ppm)	100	H 401.8559	00 20
CEX Cal. examination	N		
PEX Peak examination	N		
LRS Look span (peak widths)	2.0		
LST Look step (peak widths)	0.02		
FLO Fast Look on	Y		

* SYSTEM:DBSQL Parameters for Selective Ion Recording [G# 4 KVE] (Sector)			
DAT Data filename	B:6421592	IRV Maximum volts	6000
REF Reference filename	PFK	IMR Maximum mass at IRV	2405
INS Instrument	1:70-250	DTM TLine 0:41:10 0:48:00 Mode EI-1S Gas	
ACH Customer account	UME	CHN MCasD SCasD DCasD	MCasD SCasD DCasD
ACV Accelerating volts	6000	A 392.9760	100 20
RES Instrument resolution	6000	B 392.9760	00 20
SMP #Samples	1	C 405.7847	00 20
INJ #Injections	1	D 407.7818	00 20
GRP #Groups	5	E 419.8220	00 20
CLS #Calibration scans	0	F 421.7767	00 20
CST Cal. scan time (s)	10	G 423.7767	00 20
CTL Cal. tolerance (ppm)	100	H 435.8163	00 20
CEX Cal. examination	N		
PEX Peak examination	N		
LRS Look span (peak widths)	2.0		
LST Look step (peak widths)	0.02		
FLO Fast Look on	Y		

* SYSTEM:DBSQL Parameters for Selective Ion Recording [G# 5 KVE] (Sector)			
DAT Data filename	B:6421592	IRV Maximum volts	6000
REF Reference filename	PFK	IMR Maximum mass at IRV	2405
INS Instrument	1:70-250	DTM TLine 0:48:10 0:54:00 Mode EI-1S Gas	
ACH Customer account	UME	CHN MCasD SCasD DCasD	MCasD SCasD DCasD
ACV Accelerating volts	6000	A 441.7457	30 10
RES Instrument resolution	6000	B 443.7398	30 10
SMP #Samples	1	C 454.9728	100 10
INJ #Injections	1	D 454.9728	30 10
GRP #Groups	5	E 457.7377	30 10
CLS #Calibration scans	0	F 459.7348	30 10
CST Cal. scan time (s)	10	G 471.7758	30 10
CTL Cal. tolerance (ppm)	100		
CEX Cal. examination	N		
PEX Peak examination	N		
LRS Look span (peak widths)	2.0		
LST Look step (peak widths)	0.02		
FLO Fast Look on	Y		

TXT Samples 1: 1/10 NORWAY HUMAN MILK

'I'=sort masses 'H'=hardcopy RETURN=next ESC=prev CTRL/P=abort

'O'=go 'Q'=quit 'C'=create 'DEL'=delete 'O'=overwrite 'Z'=zero <group,sample>

APPENDIX I/3

 $^{12}\text{C}_{12}$ -quantification standardDioxins

2,3,7,8 tetra

1,2,3,7,8 penta

1,2,3,4,7,8 hexa

1,2,3,6,7,8 hexa

1,2,3,7,8,9 hexa

1,2,3,4,6,7,8 hepta

octa

Furans

2,3,7,8 tetra

1,2,3,7,8 penta

2,3,4,7,8 penta

1,2,3,4,7,8 hexa

1,2,3,6,7,8 hexa

1,2,3,7,8,9 hexa

2,3,4,6,7,8 hexa

1,2,3,4,6,7,8 hepta

1,2,3,4,7,8,9 hepta

octa

APPENDIX II

Individual PCDD/PCDF results
for all the human breast milk samples
analysed both at the Department of Organic Chemistry,
University of Umeå
and at the Norwegian Institute for Air Research

DIOXINS AND FURANS IN HUMAN MILK FROM TROMSØ PPT ON FAT BASIS

Sample no.	1	2	3	4	5	6	7+8*	9	10	11	12		
Congeners	Umeå	Umeå	NILU	Umeå	Umeå	Umeå	Umeå	Umeå	Umeå	Umeå	NILU	Mean	Range
<u>DIOXINS</u>													
2,3,7,8-tetra	3.0	2.3	2.4	2.5	3.8	4.0	2.9	2.7	2.5	3.0	2.6	2.9	2.3-4.0
1,2,3,7,8-penta	5.4	3.7	5.5	3.7	6.6	6.5	5.1	3.4	3.6	4.0	4.0	4.7	3.4-6.6
1,2,3,4,7,8-hexa	20.2	12.6	4.1	19.5	21.2	20.6	21.4	17.1	14.7	15.4	1.7	19.0	12.6-30.7
1,2,3,6,7,8-hexa			26.6								14.4		
1,2,3,7,8,9-hexa	5.0	3.3	4.5	4.8	4.0	5.9	5.3	4.7	5.3	5.0	2.9	4.6	2.9-5.9
1,2,3,4,6,7,8-hepta	39.7	20.3	37.3	40.2	38.5	42.9	38.8	36.9	23.3	30.1	44.9	35.7	20.3-44.9
Octa	115	190	127	318	129	144	172	115	87	132	154	153	87-318
<u>FURANS</u>													
2,3,7,8-tetra	4.7	3.5	3.1	5.0	3.6	4.2	5.7	3.8	3.4	6.6	3.4	4.2	3.1-6.6
1,2,3,7,8-penta	1.1	0.4	0.5	0.6	1.3	1.1	1.0	0.8	0.6	0.7	0.7	0.8	0.4-1.3
2,3,4,7,8-penta	18.1	11.2	10.8	10.3	22.5	17.8	14.7	9.5	8.0	10.6	7.0	12.8	7.0-22.5
1,2,3,4,7,8-hexa	4.2	4.4	2.8	4.1	5.1	4.2	3.7	3.0	2.6	2.8	2.4	3.6	2.4-5.1
1,2,3,6,7,8-hexa	3.8	3.4	2.4	3.1	3.1	2.8	2.3	2.2	1.8	1.9	1.9	2.5	1.8-3.8
1,2,3,7,8,9-hexa	<1.5	<1.5	<0.1	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<0.1	n.d.	n.d.
2,3,4,6,7,8-hexa	0.3	0.9	0.7	0.9	1.5	1.4	1.4	0.8	0.5	0.6	0.9	0.9	0.3-1.5
1,2,3,4,6,7,8-hepta	6.7	4.4	6.4	9.1	5.0	5.5	6.2	5.9	3.5	5.6	10.4	6.2	3.5-10.4
Octa	<2.5	<2.5	0.8	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<0.5	<2.5	n.d.-0.8
Fat content %	2.8	3.0	5.0	3.0	3.5	2.4	3.0	3.2	7.8	2.8	2.5	3.5	2.4-7.8

UMEÅ - measured at the Department of Organic Chemistry, University of Umeå

NILU - measured at the Norwegian Institute for Air Research

* Samples no. 7 and 8 were pooled.

DIOXINS AND FURANS IN HUMAN MILK FROM HAMAR
PPT ON FAT BASIS

Sample no.	13	14	15	16	17	18	19	20	21	22		
Congeners	Umeå	Umeå	Umeå	Umeå	Umeå	Umeå	Umeå	Umeå	Umeå	Umeå	Mean	Range
<u>DIOXINS</u>												
2,3,7,8-tetra	2.0	2.4	2.6	2.9	2.4	1.7	3.6	2.0	2.5	2.6	2.5	1.7-3.6
1,2,3,7,8-penta	4.1	3.8	4.9	5.2	4.8	3.4	8.5	3.7	4.1	4.7	4.7	3.4-8.5
1,2,3,4,7,8-hexa	15.8	15.6	19.9	15.9	18.2	12.0	42.9	14.2	10.4	23.2	18.8	10.4-42.9
1,2,3,6,7,8-hexa												
1,2,3,7,8,9-hexa	4.2	3.1	4.8	4.5	5.2	3.2	11.0	3.2	2.1	7.2	4.9	2.1-11.0
1,2,3,4,6,7,8-hepta	32.1	24.9	59.7	38.9	51.1	17.3	86.0	36.6	21.4	35.1	40.3	17.3-86.0
Octa	157	102	164	180	237	97	188	107	65	202	150	65-237
<u>FURANS</u>												
2,3,7,8-tetra	2.9	5.0	5.1	5.4	4.1	2.7	4.3	3.7	5.1	2.9	4.1	2.7-5.4
1,2,3,7,8-penta	0.8	1.1	0.5	0.7	1.1	0.5	1.2	0.5	0.8	0.4	0.8	0.4-1.2
2,3,4,7,8-penta	9.9	10.5	12.7	9.8	10.0	8.5	23.4	8.9	10.6	10.2	11.4	8.5-23.4
1,2,3,4,7,8-hexa	3.2	4.9	3.9	4.1	5.9	3.3	8.6	4.1	4.0	4.1	4.6	3.2-8.6
1,2,3,6,7,8-hexa	2.5	2.4	2.3	2.1	4.9	1.7	4.7	2.2	2.3	2.1	2.7	1.7-4.9
1,2,3,7,8,9-hexa	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	n.d.	n.d.
2,3,4,6,7,8-hexa	0.7	0.9	1.1	0.6	1.5	0.5	2.2	0.8	0.7	0.7	1.0	0.5-2.2
1,2,3,4,6,7,8-hepta	4.6	7.7	3.9	5.4	8.0	3.5	7.0	4.7	3.9	6.7	5.5	3.5-8.0
Octa	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	n.d.	n.d.
Fat content %	4.4	2.4	3.2	4.5	3.8	4.3	2.7	5.2	3.3	5.1	3.9	2.4-5.2

UMEÅ - measured at the Department of Organic Chemistry, University of Umeå

NILU - measured at the Norwegian Institute for Air Research

DIOXINS AND FURANS IN HUMAN MILK FROM SKIEN/PORSGRUNN
PPT ON FAT BASIS

Sample no.	23	24	25	26	27	28	29	30	31	32		
Congeners	Umeå	Umeå	Umeå	NILU	Umeå	Umeå	NILU	Umeå	Umeå	Umeå	Mean	Range
<u>DIOXINS</u>												
2,3,7,8-tetra	2.5	5.2	3.3	2.0	2.5	2.0	4.2	2.0	1.6	1.8	2.7	1.6-5.2
1,2,3,7,8-penta	5.0	9.3	7.2	5.6	5.0	4.6	3.7	5.5	3.6	3.1	5.3	3.1-9.3
1,2,3,4,7,8-hexa	15.7	25.5	28.5	3.1	18.9	16.6	3.2	23.9	14.0	10.8	20.3	10.8-28.8
1,2,3,6,7,8-hexa				25.7			17.1					
1,2,3,7,8,9-hexa	3.1	3.9	4.8	1.6	2.9	2.8	1.8	4.0	4.1	2.8	3.2	1.6-4.8
1,2,3,4,6,7,8-hepta	25.9	76.0	55.9	26.5	38.9	16.2	35.2	31.4	39.5	20.5	36.6	16.2-76.0
Octa	103	356	142	183	149	112	194	83	143	95	156	83-356
<u>FURANS</u>												
2,3,7,8-tetra	3.7	9.8	5.5	5.2	5.3	2.1	2.6	4.3	7.0	3.1	4.9	2.1-9.8
1,2,3,7,8-penta	0.7	4.0	1.7	1.7	1.3	0.3	1.3	0.6	0.3	0.9	1.3	0.3-4.0
2,3,4,7,8-penta	13.2	33.2	31.7	21.4	15.5	12.6	12.2	15.1	12.2	9.9	17.7	9.9-33.2
1,2,3,4,7,8-hexa	5.5	21.1	10.5	6.6	8.5	4.1	5.6	4.4	5.3	6.4	7.8	4.1-21.1
1,2,3,6,7,8-hexa	4.4	13.0	5.4	6.0	5.5	3.6	4.8	3.9	3.3	3.3	5.3	3.3-13.0
1,2,3,7,8,9-hexa	<1.5	<1.5	<1.5	<0.1	<1.5	<1.5	0.2	<1.5	<1.5	<1.5	0.8	n.d.-0.8
2,3,4,6,7,8-hexa	2.2	3.0	2.9	1.5	1.5	1.1	1.3	1.4	1.8	0.7	1.7	0.7-3.0
1,2,3,4,6,7,8-hepta	4.2	9.1	6.4	6.5	2.8	4.2	7.6	3.7	6.7	5.3	5.7	2.8-9.1
Octa	<2.5	<2.5	<2.5	10.4	<2.5	<2.5	5.1	<2.5	<2.5	<2.5	2.5	n.d.-10.4
Fat content %	5.0	1.5	2.9	2.8	4.3	4.3	3.0	4.0	2.3	4.9	3.5	1.5-4.9

UMEÅ - measured at the Department of Organic Chemistry, University of Umeå

NILU - measured at the Norwegian Institute for Air Research

APPENDIX III

Analytical methods of PCB determination in human milk

Norwegian College of Veterinary Medicine/National Veterinary Institute
Janneche Utne Skåre

SFT-project no. 235/87

Materials and methods

Two parallels of each sample were analyzed. For details about the analytical procedures see Skaare (1981) and Skaare et al. (1987). The method includes extraction of fat and organochlorines (OC) from the sample using a mixture of hexane and acetone (ultrasonic extraction). Percent extracted fat is determined by calibration. The sample extract is cleaned up with acid, alkaline and/or gel permeation chromatography. Separation and quantification of the individual OC components is done by electron capture - gas chromatography (ec-glc). Both packed and capillary glc columns were used.

In addition to PCBs and HCB the following OC compounds were identified and quantified: alpha, beta, gamma - hexachlorocyclohexane (HCH), aldrin, dieldrin, endrin, heptachlor, p,p-DDT, p,p-DDE, p,p-TDE, by comparison of retention times and glc peak area and/or glc peak height respectively, in the sample and in the standards.

Two methods were used for the PCB quantification:

Method 1, US-FDA-method, is based on the determination of individual glc-peak concentrations on a packed glc column, OV-101. The method was published by US FDA in 1978 (Sawyer, 1978). It involves the use of Aroclor 1260 as the PCB standard. This method was also used for PCB determination in human milk in the UNEP/WHO "Pilot project on assessment of human exposure to pollutants through biological monitoring". A detailed description of this method is included in the report from this project (Slorach and Vaz, 1983).

The concentrations of as many PCB peaks as possible are summed to give sum-PCB. In Norwegian human milk the most important PCB peaks are nos. 125, 146, 174, 280, and 332 ($R_{DDE} \times 100$). Thus, in this survey PCB

quantification by means of the US FDA method is the sum of the PCB concentrations of these peaks:

$$\text{Sum-PCB} = \text{PCB}^1 = \text{sum} (125 + 146 + 174 + 280 + 332)$$

Method 2, the Codex method, is an isomer specific method based on a suggestion from Codex 1986. The following 7 PCB compounds were recommended as indicator substances for quantification of PCBs in biological material. These were: 28 (2,4,4'-trichlorobiphenyl), 52 (2,2',5,5'-tetrachlorobiphenyl), 101 (2,2',4,5,5'-pentachlorobiphenyl), 118 (2,3',4,4',5-pentachlorobiphenyl), 138 (2,2',3,4,4',5'-hexachlorobiphenyl), 153 (2,2',4,4',5,5'-hexachlorobiphenyl), and 180 (2,2',3,4,4',5,5'-heptachlorobiphenyl).

See Ballschmitter and Zell (1980) for numbering system.

Of the 7 individual PCBs, only 4 were found in the Norwegian human milk samples. These were nos. 118, 153, 138, and 180. Thus, in this survey PCB quantification by the Codex method will be:

$$\text{Sum-PCB} = \text{PCBs}^2 = \text{sum} (118 + 153 + 138 + 180).$$

Analytical quality assurance, AQA-testing. Recoveries of pesticides, Aroclor 1260, the individual PCB compounds 118, 138, 153, and 180, and HCB were checked by fortification of cow's milk. The average recoveries varied from 96 to 105%.

The laboratory is periodically participating in interlaboratory AQA-tests. F.i. participation in an AQA-test organized by WHO/UNEP, with spiked cow milk samples provided from the National Food Administration in Sweden, demonstrated that our laboratory had good analytical quality (percentage deviation from spike concentration of beta-HCH, p,p'-DDE and PCBs were less than $\pm 20\%$).

Results and discussion

Methods: In this report are presented only the results from packed column - glc. For congener and isomer specific PCB determination, capillary column - glc must be used. The FDA-method, which is based on packed column - glc, cannot reveal small differences in the PCB composition in the samples since only few individual PCB congeners are separated. However, it is this method or comparable ones that have been used for PCB quantification in the human milk monitoring programs organized in our Department in 1970, 1976, 1979, 1982, and 1986. Thus, the results from all the surveys can be compared to study the trend in PCB contamination in Norwegian human milk.

The results from the FDA- and the Codex-method are compared in Table 5. As can be seen, the PCB levels calculated by the Codex-method are 55-60% of the corresponding levels calculated by the FDA-method. The four PCB isomers (118, 138, 156, and 180) overlap 4 of the 5 glc-peaks in Aroclor 1260 (125, 146, 174, and 280) which are used for the PCB quantification. The packed column glc-peaks of Aroclor 1260 may contain several PCB congeners/isomers. Thus, the concentration factor used for a glc-peak in Aroclor 1260 may correspond to several PCB compounds, which may explain the differences in the PCB levels calculated by the 2 methods. Capillary glc of the samples will reveal if the selected PCB isomers can be used or if additional or other PCB compounds should be chosen for PCB determination in Norwegian human milk.

Table 1: Residues (ppb, $\mu\text{g/kg}$ fat weight, $\mu\text{g/kg}$ wet weight in parentheses) of PCBs, p,p'-DDE and HCB in human milk from TROMSØ.

Sample no	% extr. fat	PCBs ¹	p,p'-DDE	HCB
1	3.1	786 (24)	771 (24)	108 (3)
2	3.5	580 (20)	802 (28)	69 (2)
4	3.4	479 (16)	208 (7)	76 (3)
5	3.9	866 (34)	717 (28)	104 (4)
6	2.8	709 (20)	1403 (39)	94 (3)
7	3.3	470 (16)	337 (11)	68 (2)
8	3.0	463 (14)	229 (7)	64 (2)
9	3.5	423 (15)	471 (17)	56 (2)
10	5.4	455 (24)	1066 (57)	53 (3)
11	3.0	386 (12)	247 (8)	54 (2)

Results are means of 2 parallels

1) PCBs: US-FDA-method

Table 2: Residues (ppb, $\mu\text{g/kg}$ fat weight, $\mu\text{g/kg}$ wet weight in parentheses) of PCBs, p,p'-DDE and HCB in human milk from HAMAR.

Sample no	% extr. fat	PCBs ¹	p,p'-DDE	HCB
13	2.6	666 (17)	548 (14)	70 (2)
15	3.8	614 (23)	477 (18)	68 (3)
21	3.7	480 (17)	303 (11)	50 (2)
16	4.7	623 (30)	773 (37)	40 (2)
18	4.4	306 (13)	226 (10)	45 (2)
17	3.6	359 (13)	430 (15)	55 (2)
20	5.2	397 (21)	413 (22)	46 (2)
22	5.2	551 (28)	866 (45)	53 (3)
19	2.8	671 (19)	670 (19)	71 (2)
14	2.8	404 (11)	474 (13)	46 (1)

Results are means of 2 parallels

1) PCBs: US-FDA-method

Table 3: Residues (ppb, $\mu\text{g/kg}$ fat weight, $\mu\text{g/kg}$ wet weight in parentheses) of PCBs, p,p'-DDE and HCB in human milk from SKIEN/PORSGRUNN.

Sample no	% extr. fat	PCBs ¹	p,p'-DDE	HCB
31	2.7	255 (7)	323 (9)	46 (2)
23	5.5	373 (21)	323 (18)	44 (2)
30	2.3	699 (16)	366 (8)	125 (3)
28	4.2	524 (22)	301 (13)	42 (2)
32	4.4	288 (13)	362 (16)	69 (3)
24	1.5	682 (11)	518 (8)	84 (1)
25	2.9	947 (27)	645 (19)	115 (3)
27	4.4	499 (22)	285 (13)	63 (3)

Results are means of 2 parallels

1) PCBs: US-FDA-method

Table 4: Residues (ppb, $\mu\text{g/kg}$ fat weight) of PCBs, p,p'-DDE, and HCB in Norwegian human milk.

Locality	% extr. fat	PCBs ¹	p,p'-DDE	HCB
Tromsø	3.5 ± 0.7	562 ± 167	625 ± 400	75 ± 20
Hamar	3.9 ± 1.0	507 ± 135	518 ± 201	54 ± 11
Porsgrunn/Skien	3.5 ± 1.3	533 ± 235	390 ± 126	74 ± 32

Results are expressed as means \pm S.D.

1) PCBs: US-FDA-method

Significant differences ($P < 0.05$) are indicated by different superscript.

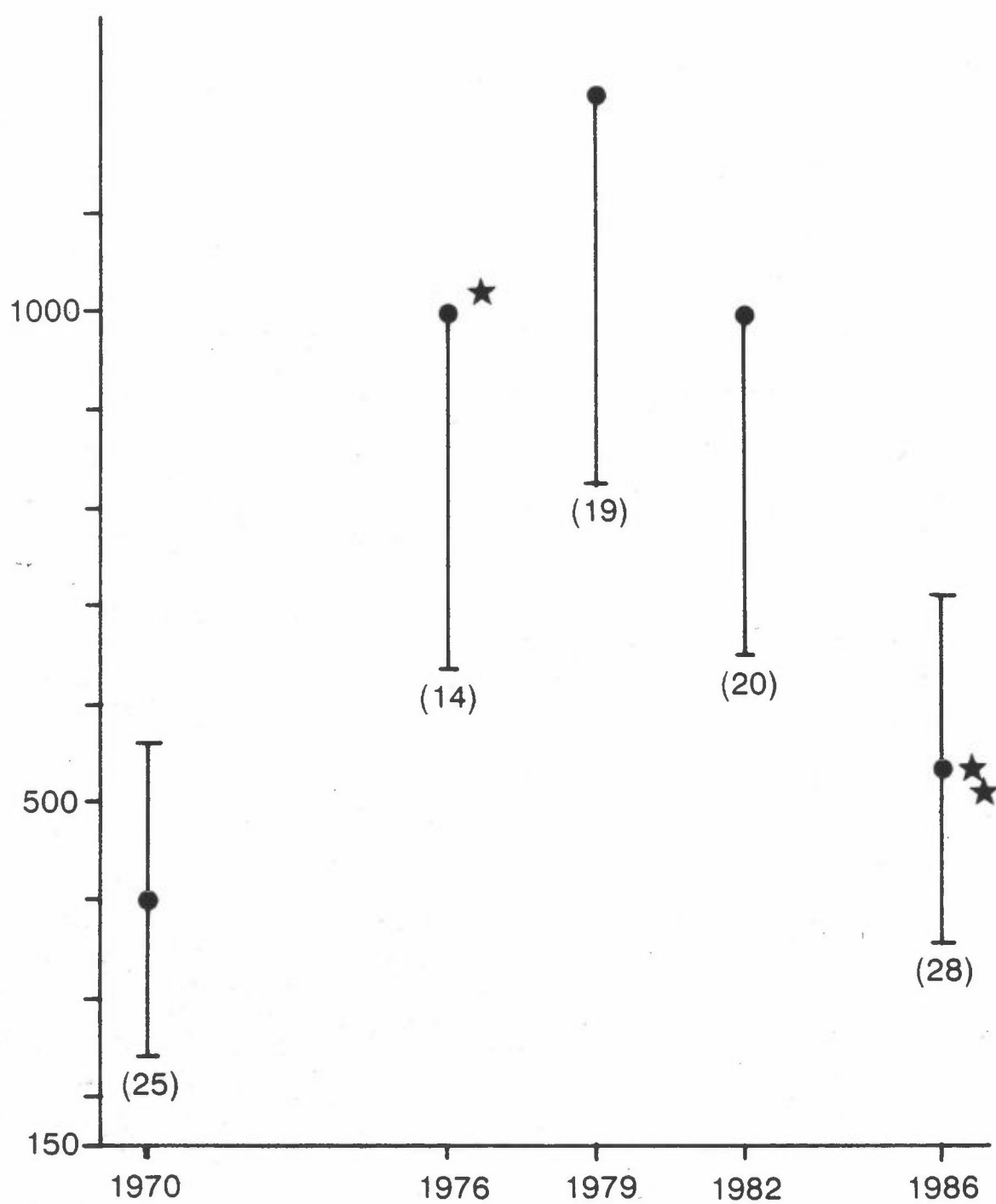
Table 5: Residues (ppb, $\mu\text{g/kg}$ fat weight) of PCBs in Norwegian human milk. Comparison of "US FDA"- and "Codex"-methods of PCB quantitation.

Locality	PCBs ¹	PCBs ²	$\frac{\text{sum PCB}^1}{\text{sum PCB}^2} \%$
Tromsø	562 ± 167	342 ± 87	61.5 ± 4.0
Hamar	507 ± 135	274 ± 72	54.6 ± 7.7
Porsgrunn/Skien	533 ± 235	299 ± 155	55.1 ± 7.6

Results are expressed as means \pm S.D.

1) PCBs: US-FDA-method

2) PCBs: Codex-method



RESIDUES OF PCBs IN NORWEGIAN HUMAN MILK (PPB, $\mu\text{g/kg}$ fat weight)

* Significant difference ($P < 0.05$) between 1976 and 1970.

** Significant difference ($P < 0.05$) between 1986 and 1982.

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APPENDIX IV

Correlation coefficients between the chemical compounds

Correlations	PCBs	p,p'-DDE	HCB	fat %	2378-tetra CDD
PCBs	1.0000	.5079*	.7739**	-.3501	.5913**
p,p'-DDE	.5079*	1.0000	.2893	.0524	.4179
HCB	.7739**	.2893	1.0000	-.5210*	.4237
fat %	-.3501	.0524	-.5210*	1.0000	-.4125
2378-tetra CDD	.5913**	.4179	.4237	-.4125	1.0000
12378-penta CDD	.6940**	.3006	.5321*	-.4543	.6907**
1234/678-hexa CDD	.5744*	.2120	.4811*	-.3919	.4322
123789-hexa CDD	.2784	.3959	.1724	-.1750	.2762
1234678-hepta CDD	.4372	.1613	.3394	-.4562	.6152**
octa CDD	.0634	.0087	.0113	-.1148	.1560
2378-tetra CDF	.0889	-.1648	.1464	-.5758*	.4041
12378-penta CDF	.3809	.0727	.3702	-.5114*	.7208**
23478-penta CDF	.7043**	.1942	.6462**	-.5218*	.6389**
123478-hexa CDF	.3002	-.0106	.2800	-.4129	.5811**
123678-hexa CDF	.2931	-.0417	.3189	-.4362	.5454*
123789-hexa CDF	.0088	.0237	-.0130	.0908	-.0812
234678-hexa CDF	.3238	-.0456	.3281	-.3998	.4883*
1234678-hepta CDF	.0638	.0022	.1340	-.4811*	.3872
octa CDF	.0000	.0000	-.0000	-.0000	-.0086

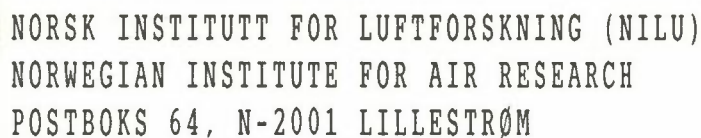
Correlations	123-penta CDD	1234/678-hexa CDD	123789-hexa CDD	1234678-hepta CDD	octa CDD
PCBs	.6940**	.5744*	.2784	.4372	.0634
p,p'-DDE	.3006	.2120	.3959	.1613	.0087
HCB	.5321*	.4811*	.1724	.3394	.0113
fat %	-.4543	-.3919	-.1750	-.4562	-.1148
2378-tetra CDD	.6907**	.4322	.2762	.6152**	.1560
12378-penta CDD	1.0000	.7686**	.4467	.7467**	.0725
1234/678-hexa CDD	.7686**	1.0000	.5916**	.6902**	.2919
123789-hexa CDD	.4467	.5916**	1.0000	.6158**	.2991
1234678-hepta CDD	.7467**	.6902**	.6158**	1.0000	.3540
octa CDD	.0725	.2919	.2991	.3540	1.0000
2378-tetra CDF	.4612*	.1618	.0702	.4848*	.0773
12378-penta CDF	.7031**	.3836	-.0265	.5369*	.0376
23478-penta CDF	.8843**	.6402**	.2043	.6041**	.0214
123478-hexa CDF	.7052**	.3800	.0309	.5835**	.0433
123678-hexa CDF	.6684**	.3920	-.0798	.5057*	.0983
123789-hexa CDF	-.0405	-.2805	.1045	-.0663	-.0033
234678-hexa CDF	.7230**	.5195*	.1527	.6113**	.1156
1234678-hepta CDF	.2209	.3164	.1618	.4744*	.5325*
octa CDF	.0322	.2429	-.3420	-.1391	.1869

Minimum pairwise N of cases: 28
 2-tailed Signif: * < .01 ** < .001

Correlations	2378-tetra CDF	12378- penta CDF	23478- penta CDF	123478- hexa CDF	123678- hexa CDF
PCBs	.0889	.3809	.7043**	.3002	.2931
p,p'-DDE	-.1648	.0727	.1942	-.0106	-.0417
HCB	.1464	.3702	.6462**	.2800	.3189
fat %	-.5758*	-.5114*	-.5218*	-.4129	-.4362
2378-tetra CDD	.4041	.7208**	.6389**	.5811**	.5454*
12378-penta CDD	.4612*	.7031**	.8843**	.7052**	.6684**
1234/678-hexa CDD	.1618	.3836	.6402**	.3800	.3920
123789-hexa CDD	.0702	-.0265	.2043	.0309	-.0798
1234678-hepta CDD	.4848*	.5369*	.6041**	.5835**	.5057*
octa CDD	.0773	.0376	.0214	.0433	.0983
2378-tetra CDF	1.0000	.6353**	.5297*	.6340**	.5708**
12378-penta CDF	.6353**	1.0000	.7882**	.8922**	.8803**
23478-penta CDF	.5297*	.7882**	1.0000	.7928**	.7559**
123478-hexa CDF	.6340**	.8922**	.7928**	1.0000	.9467**
123678-hexa CDF	.5708**	.8803**	.7559**	.9467**	1.0000
123789-hexa CDF	.1331	-.0607	.0262	.1334	.0385
234678-hexa CDF	.5071*	.6523**	.8016**	.7737**	.7456**
1234678-hepta CDF	.2888	.3873	.1943	.2877	.3118
octa CDF	.0174	.2368	.2007	.0957	.2625

Correlations	123789- hexa CDF	234678- hexa CDF	1234678- hepta CDF	octa CDF
PCBs	.0088	.3238	.0638	.0000
p,p'-DDE	.0237	-.0456	.0022	.0000
HCB	-.0130	.3281	.1340	-.0000
fat %	.0908	-.3998	-.4811*	-.0000
2378-tetra CDD	-.0812	.4883*	.3872	-.0086
12378-penta CDD	-.0405	.7230**	.2209	.0322
1234/678-hexa CDD	-.2805	.5195*	.3164	.2429
123789-hexa CDD	.1045	.1527	.1618	-.3420
1234678-hepta CDD	-.0663	.6113**	.4744*	-.1391
octa CDD	-.0033	.1156	.5325*	.1869
2378-tetra CDF	.1331	.5071*	.2888	.0174
12378-penta CDF	-.0607	.6523**	.3873	.2368
23478-penta CDF	.0262	.8016**	.1943	.2007
123478-hexa CDF	.1334	.7737**	.2877	.0957
123678-hexa CDF	.0385	.7456**	.3118	.2625
123789-hexa CDF	1.0000	.0420	-.4920*	-.4023
234678-hexa CDF	.0420	1.0000	.2442	.1023
1234678-hepta CDF	-.4920*	.2442	1.0000	.0848
octa CDF	-.4023	.1023	.0848	1.0000

Minimum pairwise N of cases: 28
 2-tailed Signif: * < .01 ** < .001



TITLE Polychlorinated biphenyls (PCB), dibenzodioxins (PCDD) and dibenzofurans (PCDF) in human milk from three geographic areas in Norway.
ABSTRACT (max. 300 characters, 7 lines) 32 samples of human breast milk taken in winter 1985/86 in three regions were analyzed for PCB, PCDD and PCDF. Study design and analytical procedures are described. No regional differences in PCB or toxic PCDD/PCDF equivalent were found. Several PCDFs were higher in one region. All detected concentrations rank between the medium levels found in other countries.

* Kategorier:	Åpen - kan bestilles fra NILU	A
	Må bestilles gjennom oppdragsgiver	B
	Kan ikke utleveres	C