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Kompletterande mätningar av syntetiska myskföreningar i bröstmjölk från förstföderskor i Uppsala, 1996-2003

Utfört av
Livsmedelsverket

Programområde
Hälsorelaterad miljöövervakning
Kontrakt nr 215 0313

Sakrapport till Naturvårdsverkets Miljöövervakning:

Kompletterande mätningar av syntetiska myskföreningar i bröstmjölk från förstföderskor i Uppsala, 1996-2003.

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Utförare:	**Livsmedelsverket**
Programområde:	**Hälsorelaterad miljöövervakning**
Delprogram:	**Exponering via livsmedel**
Undersökningar/uppdrag:	**Analys av syntetiska myskföreningar i bröstmjölk:** **1. Polycykliska myskföreningar (HHCB, AHTN, ADBI, ATII och AHDI)** **2. Nitromyskföreningar (MX, MK)**

Sanna Lignell, Marie Aune, Per Ola Darnerud och Anders Glynn
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Report to the Swedish Environmental Protection Agency, 2004-03-10

Sanna Lignell, Marie Aune, Per Ola Darnerud and Anders Glynn (the Swedish National Food Administration).

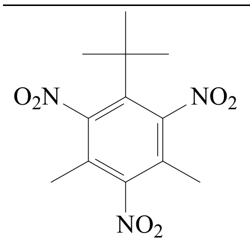
Synthetic musk compounds in breastmilk from primiparae women in Uppsala County, Sweden, 1996-2003.

Introduction

Synthetic musks have a widespread use as a substitute for natural musks in fragrances, and can be found in a number of consumer products such as laundry detergents, cleaning agents and cosmetic products (soaps, perfumes etc.). The most frequently used synthetic musks belong to the nitro musks (e.g. musk xylene (MX) and musk ketone (MK)) and to the polycyclic musks (e.g. HHCB, AHTN, ADBI, ATII, AHDI) (Figure 1).

Because of their high lipofility and chemical stability, musk compounds can be expected to accumulate in the human body, and it is important to investigate their occurrence in human milk.

NITRO MUSKS | **POLYCYCLIC MUSKS**



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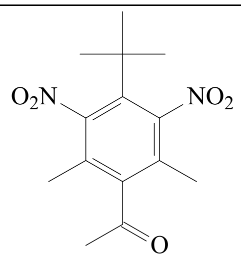

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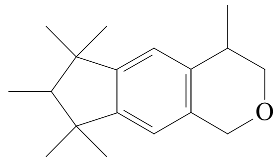

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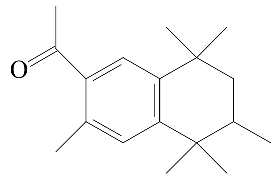

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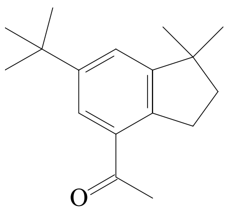
continuation **POLYCYCLIC MUSKS**



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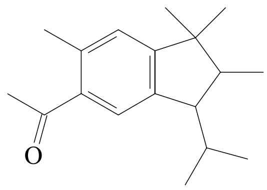

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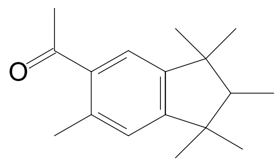

AHDI (Phantolide®)
CAS No. 15323-35-0

Figure 1. Chemical structures, names and CAS numbers of the nitro and polycyclic musk compounds analysed in breast milk from Swedish women.

For risk assessment purposes, the Swedish NFA (National Food Administration) has made recurrent measurements of levels of selected persistent organic pollutants (POP) (e.g. polychlorinated biphenyls (PCBs), dioxins, persistent pesticides and flame retardants) in human breast milk. The ambition is to follow changes in the levels of these environmental contaminants in human breast milk and to establish a time trend. The aim is also to evaluate possible health risks for the mother and in particular for the breastfed infant. The first sampling of breast milk performed by the NFA occurred in Uppsala County in 1996-1999. About 300 primiparae participated in this large study that to some parts was financially supported by the Swedish EPA (Environmental Protection Agency). The second sampling was performed in Uppsala in 2000-2001 (n=31) and the third sampling in Uppsala in 2002-2003 (n=31). A new sampling (2004-2005) is in progress. The investigations in 2000-2001, 2002-2003 and 2004-2005 have partly been financed by the Swedish EPA.

Earlier (in 1999-2000 and 2002), 44 randomly selected breast milk samples from the monitoring of environmental contaminants have been analysed for synthetic musk compounds. The results presented in Eriksson et al. (2003) indicated that the levels of HHCB, AHTN and MX decreased from 1998 to 2001. The investigation also pointed to the possibility of a relationship between perfume exposure from cosmetics and detergents, and musk levels in breast milk.

During 2003, 80 additional breast milk samples have been analysed for synthetic musk compounds. In this report results from the earlier (2002) and the latest (2003) analyses of nitro musks (MX, MK) and polycyclic musks (HHCB, AHTN, ADBI, ATII, AHDI) are presented. Results from the analysis in 1999-2000 have been excluded since the later analyses are of better quality. The total number of samples analysed in 2002 and 2003 is 101. The possible time trend and the possible relationship with use of perfumed products are investigated as well as relationships between musk levels and lifestyle factors such as age and body mass index (BMI).

The analyses of musk compounds in breast milk and the compilation of data was financed by the Swedish EPA (Environmental Protection Agency).

Material and methods

Recruitment of mothers and sampling

From January 1996 to May 1999 pregnant women (n=953) from the general population in Uppsala County were recruited as controls in a case-control study of risk factors for early miscarriages. All primiparas (women having their first baby) recruited from early fall 1996 and onwards (n=365) were in late pregnancy asked to participate in a breast milk study aimed at the analysis of environmental contaminants. Of these 365 primiparae women, 188 agreed to donate breast milk for chemical analysis. In early pregnancy, another 25 primiparas from Östhammar, located at the coast of the Baltic Sea in Uppsala County, were asked to participate in the breast milk study, and 16 of these women agreed to donate milk. The women answered extensive questionnaires about lifestyle, medical history, and dietary habits. In addition to the women recruited in 1996-1999, primiparas were also recruited in Uppsala County from April 2000 to March 2001 (n=31) and from March 2002 to February 2003 (n=31). Besides questionnaires about lifestyle etc the women recruited in 2000-2001 and 2002-2003 answered questions about their use of perfumed products (perfume, deodorant, skin lotion, laundry detergents and washing detergents) before and during pregnancy.

The routine for milk sampling was identical for all the participating women. The mothers got instructions on how to collect the milk at home, and they also got a breast milk pump and sampling bottles. The milk was collected during the third week after delivery (day 14-21 post partum) and the mothers were instructed to sample milk both in the beginning and at the end of the breast-feeding sessions. The goal was to sample a total of 500 ml from each mother during 7 days of sampling. The breast milk was kept cold, preferably in a freezer (-20°C), in hexane-washed glass bottles and the newly sampled milk was poured on top of the frozen milk.

Chemical analysis

Total 101 breast milk samples from the monitoring of environmental contaminants in 1996-1999, 2000-2001 and 2002-2003 were randomly selected for musk analysis. The only inclusion criteria was that the amount of breast milk had to be sufficient for the analysis. Data on age, body mass index, weight reduction from delivery to sampling and sampling year for the women selected for the study are compiled in Table 1.

Table 1. Data on age, BMI (body mass index), weight reduction and sampling year for the women participating in the study.

	n	mean	S.D.	median	min	max
Age at the sampling occasion (years)	101	29.2	3.8	29.6	21.0	37.4
BMI (kg/m^2) before pregnancy	101	22.8	3.4	22.2	16.2	37.7
Weight reduction from delivery to sampling (kg)	99	11.2	3.5	11.0	3.0	23.0

	n
Sampling year 1996	11
Sampling year 1997	15
Sampling year 1998	16
Sampling year 1999	15
Sampling year 2000	17
Sampling year 2001	6
Sampling year 2002	19
Sampling year 2003	2

The samples were analysed in 2002 and 2003 at the Institute of Chemical Technology, Department of Food Analysis, Prague, Czech Republic under the direction of professor Jana Hajšlová (Setkova L and Hajšlová J 2003). HHCB, AHTN, ADBI, ATII, MX and MK were analysed in all samples. AHDI was included in the analytical method in 2003 and was therefore only analysed in 80 samples.

The milk samples were extracted with a mixture of hexane and diethylether. The extracts were filtrated and cleaned-up using gel permeation chromatography (GPC). Identification and quantification of the analytes were performed by capillary gas chromatography coupled to a mass selective detector operated in a selected ion monitoring mode (HRGC/LRMS/EI-SIM). Isotopically labeled AHTN-D_3 and musk xylene-D_{15} were used as internal standards to compensate matrix effects and improve overall precision of the measurements. Special precautions were taken to prevent secondary (intralaboratory) contamination that may occur from perfumes, deodorants, detergents etc. The relative standard deviation (RSD) of the whole analytical procedure was 6.5-22.4%, and the recoveries obtained by analysis of spiked samples was 95.1-106.1%. Limits of detection (LODs) and limits of quantification (LOQs) are shown in table 2. (Setkova L and Hajšlová J 2003)

Table 2. Limits of detection (LODs) and limits of quantitation (LOQs) of the analytical method used for determination of musk compounds in breast milk samples (µg/kg milk fat).

Analyse year	parameter	HHCB	AHTN	ADBI	ATII	AHDI	MX	MK
2002	LOD	3.0	3.0	1.5	1.5	*	4.5	4.5
	LOQ	6.0	6.0	3.0	3.0	*	9.0	9.0
2003	LOD	0.5	1.0	0.5	1.0	1.0	2.0	1.5
	LOQ	2.0	3.0	2.0	3.0	3.0	6.0	5.0

*not analysed in 2002

Results

Musk levels in human milk

Table 3 shows the results from the analysis of musk compounds in the breast milk samples. Values below LOQ were set to half the LOQ in the calculations of mean, standard deviation (S.D.) and median.

Table 3. Levels of HHCB, AHTN, ADBI, ATII, AHDI, MX and MK in breast milk (µg/kg milk fat) sampled from 1996 to 2003. Levels <LOQ (limit of quantification) have been calculated as ½LOQ.

Musk compound	n	mean	S.D.	median	min	max	No of samples <LOQ
HHCB	101	77.7	54.9	63.9	2.8	268	0
AHTN	101	13.3	12.0	10.4	1.5 (<3.0)	53.0	26
ADBI	101	2.0	2.0	1.0 (<2.0)	1.0 (<2.0)	11.0	75
ATII	101	2.4	2.1	1.5 (<3.0)	1.5 (<3.0)	12.6	77
AHDI	80	1.9	1.0	1.5 (<3.0)	1.5 (<3.0)	6.5	70
MX	101	15.1	16.5	9.5	3.0 (<6.0)	83.9	31
MK	101	4.2	3.8	2.5	2.5 (<5.0)	24.4	83

Lifestyle factors

Simple methods of regression analysis were used to evaluate possible relationships between levels of the musks HHCB, AHTN and MX in breast milk and age, body mass index and weight reduction from delivery to sampling. The evaluation was performed with both non-transformed and n-log transformed musk concentration values. ADBI, ATII, AHDI and MK were not evaluated statistically because of the large frequency of values below LOQ.

In the earlier investigation (Eriksson et al. 2003), age, body mass index and weight reduction from delivery to sampling showed no significant relationships with musk levels in milk. In the present study, body mass index showed a significant negative relationship with the n-log transformed level of AHTN ($p=0.039$). Since no significant relationship was observed with non-transformed AHTN levels, the relationship is not considered to be very certain. Figure 2 shows the level of AHTN in relation to age, body mass index and weight reduction of the mother.

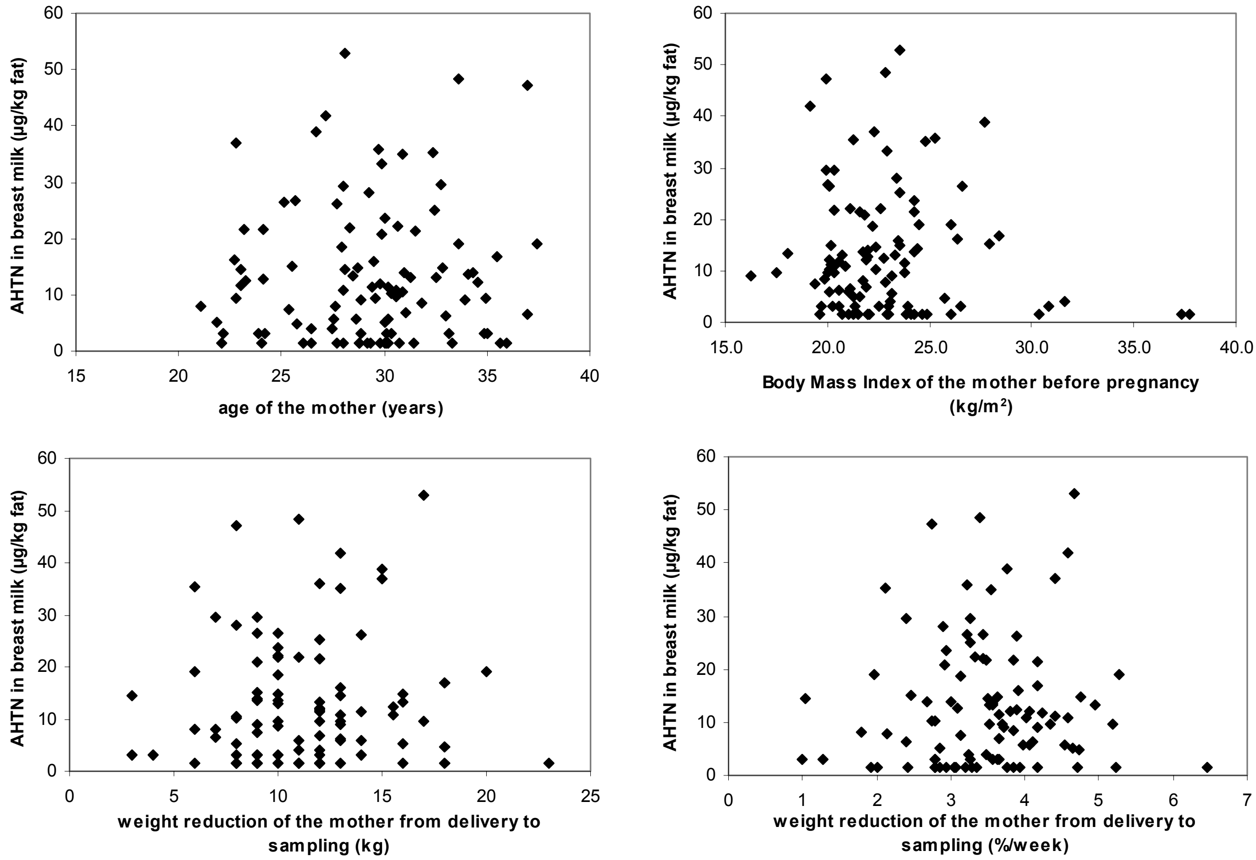

Figure 2. AHTN in breast milk (µg/kg milk fat) in relation to age, BMI (Body Mass Index) and weight reduction from delivery to sampling (kg and % per week) from primiparae women (n=101) from Uppsala County. Each symbol represents the AHTN level in one milk sample from one women.

<u>Time trend</u>

A possible time trend was investigated regarding HHCB, AHTN and MX. The musks ADBI, ATII, AHDI and MK were not evaluated because of the large frequency of values below LOQ.

The median levels of HHCB, AHTN and MX in samples from different years were compared in a Kruskal-Wallis test (Table 4). Since the number of samples from 2001 was small (n=6), and they were all collected in the beginning of the year, the results from 2000 and 2001 were grouped together. Similarly, results from 2002 and 2003 (n=2) were put in one group. The results show that there are significant differences in levels of MX between samples from different years, but no significant differences in levels of HHCB and AHTN.

Non-parametric trend tests according to Cuzick (1985) were performed to evaluate possible time trends. The results show that there is a significant downward trend regarding AHTN ($p=0.024$) and MX ($p<0.0002$), but not regarding HHCB ($p=0.373$). As in the Kruskal-Wallis test, results from 2000 and 2001 as well as 2002 and 2003 were grouped together. Figure 3-5 shows the levels of HHCB, AHTN and MX respectively in relation to sampling year.

Table 4. Kruskal-Wallis test for comparison of medians. The levels of HHCB, AHTN and MX in samples from 1996 to 2002 have been compared.

Musk compound	**Year of sampling**	**n**	**Median level in breast milk (µg/kg fat)**	**p-value**
HHCB	1996	11	42.0	
	1997	15	63.9	
	1998	16	90.8	
	1999	15	124	
	2000-2001	23	49.1	
	2002-2003	21	55.8	0.183
AHTN	1996	11	14.0	
	1997	15	21.6	
	1998	16	12.4	
	1999	15	8.5	
	2000-2001	23	9.5	
	2002-2003	21	6.9	0.207
MX	1996	11	20.9	
	1997	15	13.2	
	1998	16	10.0	
	1999	15	8.0	
	2000-2001	23	8.9	
	2002-2003	21	3.0	0.005

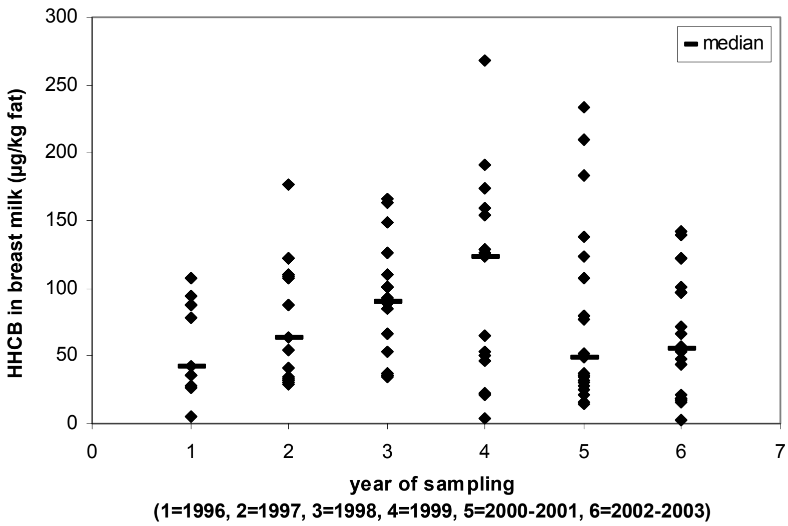

Figure 3. HHCB levels in breast milk samples (n=101) in relation to the year of sampling. Results from 2000 and 2001 as well as 2002 and 2003 have been grouped together. Each symbol corresponds to a single milk sample from one mother. No significant trend can be demonstrated with a non-parametric trend test.

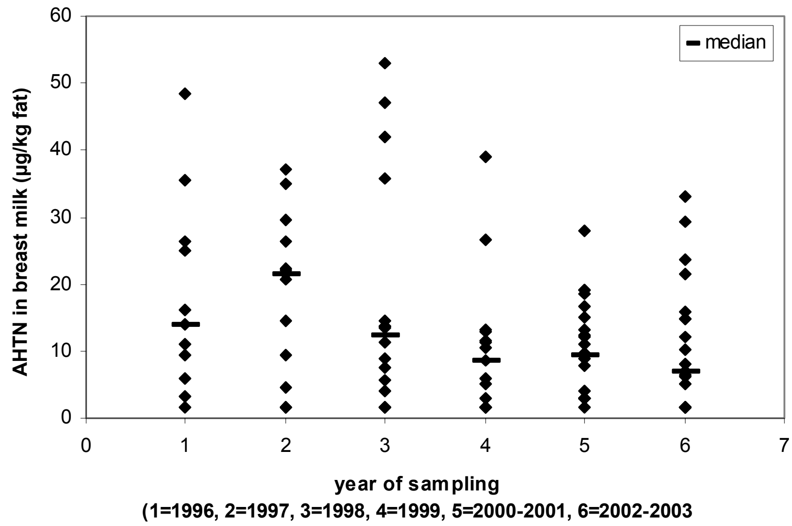

Figure 4. AHTN levels in breast milk samples (n=101) in relation to the year of sampling. Results from 2000 and 2001 as well as 2002 and 2003 have been grouped together. Each symbol corresponds to a single milk sample from one mother. A significant downward trend can be demonstrated in a non-parametric trend test (P=0.024).

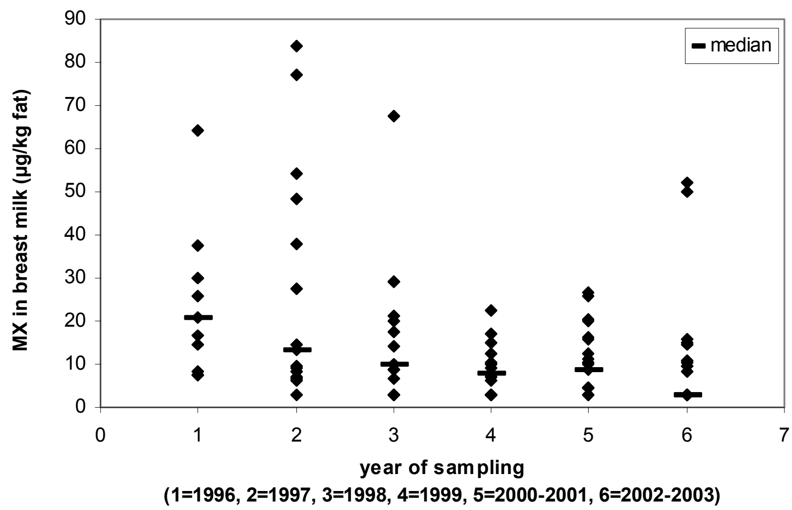

Figure 5. MX levels in breast milk samples (n=101) in relation to the year of sampling. Results from 2000 and 2001 as well as 2002 and 2003 have been grouped together. Each symbol corresponds to a single milk sample from one mother. A significant downward trend can be demonstrated in a non-parametric trend test (P<0.0002).

Use of perfumed products

Women who participated in the samplings in 2000-2001 and 2002-2003 (n=44) answered questions about use of perfumed cosmetics (perfume and deodorant) and detergents before and during pregnancy. In search for possible relationships between dermal perfume exposure and musk levels in breast milk the medians from “users” and “non-users” of perfumed products were compared by non-parametric Mann-Whitney U-tests. The results are shown in table 5. Women who answered incomplete to the questions have been excluded in the evaluations. ADBI, ATII, AHDI and MK were not evaluated because of the large frequency of values below LOQ.

Table 5. Results from non-parametric Mann-Whitney tests. Comparison of median levels of the musk compounds HHCB, AHTN and MX in breast milk from "users" and "non-users" of perfumed products.

Musk compound	User / non-user	n	Median concentration in milk (µg/kg milk fat)	P-value (user ≠ non-user)
Perfume – before pregnancy[1]				
HHCB	user	30	56.5	
	non-user	12	33.2	0.1223
AHTN	user	30	8.5	
	non-user	12	8.7	0.9556
MX	user	30	9.7	
	non-user	12	4.5	0.4194
Perfume – during pregnancy[1]				
HHCB	user	28	75.6	
	non-user	13	30.7	**0.0259**
AHTN	user	28	9.3	
	non-user	13	8.0	0.7794
MX	user	28	10.1	
	non-user	13	4.5	0.2684
Perfumed deodorant – before pregnancy[2]				
HHCB	user	34	51.0	
	non-user	9	43.6	0.9405
AHTN	user	34	9.3	
	non-user	9	3.0	0.1992
MX	user	34	6.5	
	non-user	9	4.5	0.7314
Perfumed deodorant – during pregnancy[2]				
HHCB	user	32	51.0	
	non-user	11	43.6	0.9445
AHTN	user	32	9.3	
	non-user	11	3.0	0.5403
MX	user	32	6.5	
	non-user	11	4.5	0.7279
Perfumed laundry detergent[3]				
HHCB	user	26	69.0	
	non-user	18	36.7	0.0517
AHTN	user	26	11.5	
	non-user	18	3.0	0.0593
MX	user	26	4.5	
	non-user	18	10.2	**0.0389**

[1]women who reported a use of perfume once a week or more are defined as "users"
[2]women who reported a use of perfumed deodorant daily or at least 3 days a week are defined as "users"
[3]users of perfumed detergents are defined as "users"

Women who reported a use of perfume once a week or more are defined as "users" in the evaluation presented in table 5. HHCB-levels in milk from "users" of perfume during pregnancy were significant higher than in milk from "non-users" (Figure 6). However, the three women with the highest levels of HHCB in their milk exerted a high influence on the result. When these women are excluded, the significance disappears.

Women who reported a use of perfumed deodorant daily or at least 3 days a week are defined as "users" in the evaluation presented in table 5. No significant differences between "users" and "non-users" of perfumed deodorant could be detected.

Finally, users of perfumed detergents are defined as "users" and users of fragrance free detergents are defined as "non-users" in table 5. The median levels of HHCB and AHTN in milk from "users" of perfumed detergents were higher than in milk from "non-users" although the differences were not significant. MX-levels in milk from users of perfumed detergents were significant *lower* than in milk from non-users. However, the difference is not significant when the women with the two highest levels of MX in their breast milk are excluded.

Only 44 women answered questions about use of perfumed products, and the observed relationships are therefore uncertain. A few women with the highest levels of musks in their milk exerted an disproportionately high influence on the results of the statistical analysis.

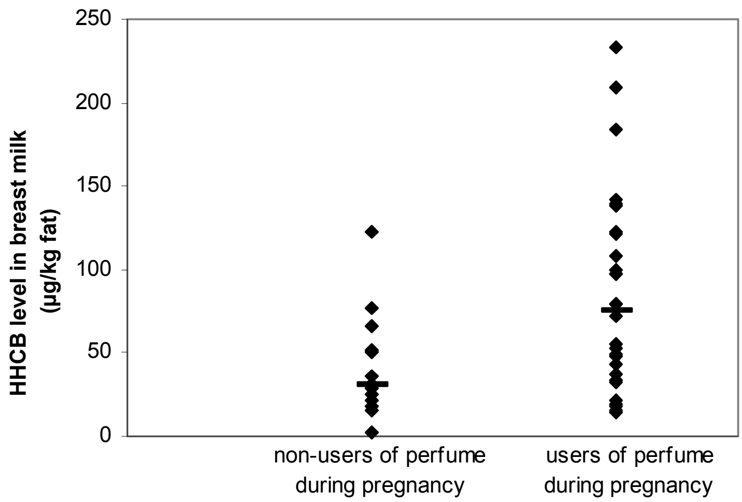

Figure 6. Comparison of HHCB levels in breast milk from non-users (n=13) and users (n=28) of perfume during pregnancy. Each symbol corresponds to a single milk sample from one mother. The HHCB-levels in milk from "users" were significant higher than in milk from "non-users" in a non-parametric Mann-Whitney tests (p=0.026). The lines indicate the median level of HHCB in milk from "non-users" and "users" of perfume respectively.

Discussion

The musk levels were somewhat lower in this study compared to the study presented in 2003 (Eriksson et al. 2003) (Table 6). In the earlier study, values from the analyses performed in 1999-2000 were included. At that point, the analytical method was not as well developed as in 2002-2003, and enough attention was not paid to the risk of secondary contamination. The results from the analyses in 1999-2000 were therefore excluded in this report.

Table 6. Comparison of results from the earlier (Eriksson et al. 2003) and the present study of musk levels in breast milk. Note that the results from the investigation in 2003 are included in the present investigation. Results from analyses performed in 1999-2000 (included in the earlier report) were however excluded in the present report.

Study	n	HHCB		AHTN		MX	
		mean	min-max	mean	min-max	mean	min-max
Eriksson et al. 2003	44	125	14.5-370	23.8	<6-71.4	21.8	<9-107
present investigation	101	77.7	2.8-268	13.3	<3-53.0	15.1	<6-83.9

In the earlier investigation (Eriksson et al. 2003), age, body mass index and weight reduction from delivery to sampling showed no significant relationships with musk levels in milk. The results in the present expanded study were similar.

Because of their high lipofility, musk compounds can be expected to accumulate in the human body. Nevertheless, in contrast to other persistent lipophilic contaminants, e.g. PCBs (polychlorinated biphenyl), no association was demonstrated between musk levels in breast milk and age. Kokot-Helbling et al. (1995) reported that the half-life of musk xylene in human subjects is about 100 days, as compared to several years for PCBs. Faster elimination of musk compounds may explain the absence of a correlation between musk levels and the age. Another explanation may be the route of exposure. It has been suggested that percutaneous absorption after dermal application of cosmetics may be the most relevant route for musk exposure (Liebl et al., 2000; Rimkus, 1998), while oral uptake from food is the main source of human exposure to organochlorine compounds such as PCBs. The individual differences in exposure to musk compounds, e.g. use of perfumed products, may therefore mask any variation in musk levels in breast milk due to age.

The earlier investigation of musks in breast milk (Eriksson et al. 2003) indicated that the levels of HHCB, AHTN and MX have decreased from 1998 to 2001. The number of analysed samples was small (n=41), and it was concluded that in order to verify the trend there was a need for a more extensive investigation. More samples have now been analysed (n=101), and the time trend has been investigated again. Non-parametric trend tests according to Cuzick (1985) showed that there is a significant downward trend regarding AHTN ($p=0.024$) and MX ($p<0.0002$), but not regarding HHCB ($p=0.373$) from 1996 to 2002-2003. The observed trends are, however, uncertain, and if they are real the levels are decreasing slowly. Maybe the individual differences in exposure to musk compounds lead to a variation in musk levels in breast milk that makes it difficult to prove a time trend.

The earlier investigation of musks in breast milk (Eriksson et al. 2003) also pointed to the possibility that there is a relationship between perfume exposure from cosmetics and detergents and musk levels in breast milk. Only 23 women answered questions about use of perfumed products, and the relationships were therefore uncertain. The relationship has now been investigated again with data from total 44 women. As in the earlier investigation, HHCB-levels in breast milk from women using perfume during pregnancy (once a week or more) were significantly higher than in milk from women who reported less or no use. No relationship between use of perfumed deodorant and levels of HHCB, AHTN or MX in breast milk could be demonstrated. In the earlier study AHTN-levels in milk from users of perfumed deodorant was significantly higher than in milk from non-users. Finally, in the present study, MX-levels in milk from users of perfumed detergents were significantly *lower* than in milk from non-users. In the earlier study AHTN-levels were higher in milk from users of perfumed laundry detergents compared with non-users. The conclusion is that except for the relationship between HHCB-level and use of perfume, the relationships demonstrated in the earlier investigation could not be verified in this study. It has been suggested that percutaneous absorption may be the most relevant route of exposure to musk compounds (Liebl et al., 2000; Rimkus, 1998). Limited data may explain the absence of relationships in this study. A few women with the highest levels of musks in their milk exerted an disproportionately high influence on the results of the statistic analysis. Another explanation may be that it is difficult and uncertain to estimate the exposure to musk compounds using questionnaires. For example, the musk contents in the products that the women were using are unknown, as well as the applied amounts. The conclusions are that a relationship between dermal perfume exposure and musk levels in breast milk can not be excluded, and that it is difficult, if not impossible, to estimate the exposure to musks by questionnaires.

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