DOI 10.1002/mnfr.200600008 Mol. Nutr. Food Res. 2006, *50*, 922 – 933

Dioxins, polychlorinated biphenyls and other organohalogen compounds in human milk

Levels, correlations, trends and exposure through breastfeeding

Peter Fürst

Chemisches Landes- und Staatliches Veterinäruntersuchungsamt, Münster, Germany

Polychlorinated dibenzo-*p*-dioxins (PCDD) and dibenzofurans (PCDF), together simplified termed "dioxins", polychlorinated biphenyls (PCB), polybrominated diphenylethers (PBDE) and organochlorine pesticides constitute lipophilic, persistent organic pollutants that bioaccumulate in the food chain and consequently can be found in humans at considerable concentrations. During the past 30 years our institute analyzed far more than 2000 individual human milk samples for organochlorine pesticides and PCB and over 1000 specimens for PCDD/PCDF. The results of these analyses provide an overview and reliable basis as to contamination of human milk with these compounds, their correlations among each other, the temporal trend of exposure through breastfeeding and the predominant parameters that influence the maternal body burden. It was found that the levels of most persistent organohalogen compounds in human milk decreased significantly over the past three decades and equally did their exposure through breastfeeding. Exceptions are PBDE, which are still extensively used as flame-retardants. PBDE levels in milk samples collected in the early 2000s are approximately 60% higher compared to specimens sampled 10 years before. Moreover, in contrast to PCB, PBDE show no significant correlation with PCDD/PCDF in human milk, which might be interpreted as an indication for another mode of human exposure.

Keywords: Dioxin-like and non dioxin-like polychlorinated biphenyls / Exposure / Human milk / Organochlorine pesticides / Polybrominated diphenylethers

Received: January 27, 2006; revised: April 2, 2006; accepted: April 2, 2006

1 Introduction

In the 1970s, findings of DDT and other organochlorine pesticides in human samples raised public and scientific concern about the safety of breastfeeding. It was in this context that the Government of the State North Rhine-Westphalia (NRW) in western Germany offered all nursing women to have their breast milk tested for organochlorine pesticides in the Chemical Control Laboratory, the predecessor institute of the Chemical and Veterinary Control Laboratory (CVUA), in Münster/NRW. During the past 30 years this offer was extended to other emerging compounds, such as polychlorinated biphenyls (PCB), polychlorinated dibenzo-*p*-dioxins (PCDD) and dibenzofurans (PCDF), heavy metals, radioactivity and brominated flame-retardants. To gain knowledge about the parameters that mainly affect the body burden with persistent organic pollutants, all mothers were asked to fill in a comprehensive questionnaire especially regarding personal, dietary and occupational information. Detailed statistical evaluations of these investigations based on several hundred analyses for dioxins, indicator PCB and predominant organochlorine pesticides were already reported earlier [1 – 3]. Those evaluations showed that personal data, such as age of the mother, length of breastfeeding period(s), number of breast-fed children, year of sample collection and specific consumption habits, predominantly influence the body burden with dioxins, indicator PCB and organochlorine pesticides. The age dependence of the PCDD/PCDF and PCB human body burden as well as their temporal trend was also demonstrated by analyses of human blood [4 – 6]. In contrast, the area of living

Correspondence: Dr. Peter Fürst, Chemisches Landes- und Staatliches Veterinäruntersuchungsamt, Joseph-König-Strasse 40, D-48147 Münster, Germany
E-mail: pfuerst@cvua.nrw.de
Fax: +49-251-9821-250

Abbreviations: **I-TEQ**, international TEQ; **PBDE**, polybrominated diphenylethers; **PCB**, polychlorinated biphenyls; **PCDD**, polychlorinated dibenzo-*p*-dioxins; **PCDF**, polychlorinated dibenzofurans; **TEF**, toxic equivalent factors; **TEQ**; toxic equivalents; **WHO**, World Health Organization

of the mother, whether urban or rural, generally did not have a significant influence on the PCDD/PCDF and indicator PCB levels in human milk [2]. This can be explained by the fact that dietary intake, being usually of widespread origin, constitutes the main route of exposure to these compounds, generally making up more than 90% of total intake [7, 8].

While in the 1980s and 1990s the analysis of PCB in human samples was mainly limited to the "indicator" congeners 28, 52, 101, 138, 153 and 180, in the past few years the determination of the PCB congeners 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, and 189 gained increasing importance. These congeners may adopt a similar spatial structure as the dioxins and after binding to the Ah receptor exhibit toxic effects comparable to PCDD/PCDF. As a consequence, toxic equivalent factors (TEF) were attributed to these congeners, termed dioxin-like PCB and they were considered for the derivation of a tolerable dioxin intake set up by the World Health Organization (WHO), Scientific Committee on Food (SCF) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (see [9], http://europa.eu.int/comm/food/fs/sc/scf/out90_en.pdf and Joint FAO/WHO Expert Committee on Food Additives, Fifty-seventh meeting, Rome, 5 – 14 June 2001). PCB 126 is of particular importance. Although being found in human samples only in picogram per gram (pg/g) fat amounts, due to its TEF value of 0.1, this congener significantly contributes to total PCDD/PCDF and PCB levels, expressed in toxic equivalents (TEQ).

Polybrominated diphenylethers (PBDE) belong to another group of compounds that gained increasing public and scientific concern during the past 10 years. Investigations demonstrated a doubling of PBDE levels in archived Swedish human milk samples every 5 years for the period 1972 – 1997 [10]. Similar increasing trends were also reported to be observed in countries, such as Norway, Faroer Islands and Japan [11 – 13]. Moreover, it was shown that the PBDE levels in human milk and blood of the US population often exceed the respective levels in European human samples by two orders of magnitude [14, 15]. Occupational exposure was demonstrated for several professions, mostly when in contact with plastic materials, *e.g.* as production workers, electronics dismantlers or intensive users of specific computer equipment [16, 17]. Moreover, indoor environments, such as household dust seem to be a significant source of human exposure to PBDE, at least in the United States, as shown by analyses of dust samples collected with vacuum cleaners (Wu, N., Webster, T., Hermann, T., Paepke, O. *et al*., Dioxin 2005, Abstract CD ROM ID: 1743). Thus, human PBDE exposure seems to be more complex and obviously arises by other means than human exposure to dioxins, PCB and organochlorine pesticides.

2 Materials and methods

2.1 Collection of human milk samples

All milk samples were sent in by nursing mothers residing in North Rhine-Westphalia/western Germany. Before sample collection, all mothers received detailed written information concerning sampling, storage and shipment. Moreover, all women were asked to fill in a comprehensive questionnaire. The information from these questionnaires together with the analytical results built the basis for the statistical evaluation of those parameters that essentially affect the maternal body burden.

2.2 Analytical methodologies

2.2.1 Preliminary remarks

Analytical methodologies using combined high-resolution capillary GC/high-resolution MS (HRGC/HRMS) are nowadays state of the art for the determination of dioxins, PCB and PBDE. The advantages of this technology are increased sensitivity and specificity and the ability to use isotope labeled compounds as ideal internal standards. A reliable simultaneous determination of all three compound classes requires a spiking with isotope-labeled internal standards over a concentration range of four-to-five orders of magnitude because the respective levels in human milk differ tremendously. This is especially true for the indicator PCB 138, 153 and 180 each of which is normally present in human milk at levels of 50 – 100 ng/g fat, while the most toxic dioxin congener 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) only amounts to an average of about 1 pg/g fat at present. Thus, an adequate spiking with indicator PCB demands much higher levels than for dioxins. Unfortunately, it was shown that certain commercially available $^{13}C_{12}$-labeled PCB standards contain $^{13}C_{12}$-labeled polychlorinated dibenzofurans as impurities [18]. Although being found at approximately 0.2% only, due to the high spiking level this amount was sufficient to affect the simultaneous PCDD/PCDF determination significantly. The following apparently false reductions for native congeners were observed: 2,3,7,8-TCDF >90%, 2,3,4,7,8-PeCDF 75%, 1,2,3,6,7,8-HxCDF 25%, and 1,2,3,4,6,7,8-HpCDF 70% [18]. Therefore, the analyses of mono-ortho and indicator PCB on the one hand and non-ortho PCB and PCDD/PCDF on the other hand are performed as separate determinations. No disturbances caused by isotope-labeled standards were found during the analysis of PCB and PBDE, thus making a simultaneous determination possible after splitting of the final extract.

The analytical procedures were tested successfully in various national and international quality control studies and proficiency tests.

2.2.2 Extraction of human milk samples

The extraction of lipophilic persistent halogenated pollutants along with fat starting with 100 – 50 mL milk was performed after addition of potassium oxalate by liquid-liquid partitioning with ethanol, diethyl ether and pentane. The combined organic extracts were washed with water and dried over sodium sulfate. After careful solvent evaporation, gravimetric lipid determination was performed.

2.2.3 Determination of PCDD/PCDF and non-ortho PCB

An aliquot of 2 g fat was spiked with all 17 $^{13}C_{12}$-labeled PCDD/PCDF that possess a 2,3,7,8 chlorine substitution each at a concentration of 25 pg/sample and 3 $^{13}C_{12}$-labeled non-ortho PCB congeners (PCB 77, 126, 169) each at a concentration of 100 pg/sample. Fat and non-acid stable compounds were removed on a silica gel column coated with sulfuric acid. The hexane fraction containing PCDD/PCDF and PCB was carefully concentrated by rotary evaporation at 40°C under reduced pressure and subsequently applied to a Florisil column (3% water) to separate PCB (eluted with hexane) from PCDD/PCDF (eluted with toluene). While the PCB fraction was further cleaned up on a Chromosorb WHP/Charcoal SP-1 column, the PCDD/PCDF eluate was applied to a Carbopack C/Celite 545 column to remove any remaining non-planar compounds. The resulting two final extracts were separately analyzed by HRGC/HRMS on a Micromass AutoSpec system at a resolution of R = 10 000. Each batch of six samples was accompanied by a laboratory blank and/or a quality control pool.

2.2.4 Determination of mono-ortho and indicator PCB as well as PBDE

An aliquot of 0.25 g fat was spiked with a mixture of eight $^{13}C_{12}$-labeled mono-ortho PCB congeners (PCB 105, 114, 118, 123, 156, 157, 167, 189) each at a concentration of 500 pg/sample, six $^{13}C_{12}$-labeled indicator PCB congeners at a concentration of 500 pg/sample (PCB 28, 52, 101) and 5000 pg/sample (PCB 138, 153, 180), respectively, and six $^{13}C_{12}$-labeled PBDE congeners (PBDE 28, 47, 99, 153, 154 and 183) each at a concentration of 100 pg/sample. Fat and non-acid stable compounds were removed on a silica gel column coated with sulfuric acid. The hexane eluate was carefully concentrated by rotary evaporation at 40°C under reduced pressure and subsequently by a gentle stream of nitrogen to a final volume of approximately 100 µL. This extract was split and separately analyzed by HRGC/HRMS for mono-ortho/di-ortho PCB and PBDE, respectively. Each batch of six samples was accompanied by a laboratory blank and/or a quality control pool. Comprehensive precautions regarding laboratory equipment and conditions, such as use of solvents, reagents and glass ware, especially for the determination of PBDE are described in detail by Päpke *et al*. [19].

2.2.5 Determination of organochlorine pesticides

An aliquot of 0.5 g fat was dissolved in cyclohexane/ethyl acetate 1 : 1 and applied to a gel permeation chromatograph (GPC) to smoothly separate the partly acid labile organochlorine pesticides from milk fat. GPC separation was performed on BioBeads S-X3 with cyclohexane/ethyl acetate 1+1 as solvent. The resulting extract was further cleaned up on a silica gel mini column (1.5% water) subsequently using hexane, hexane/toluene (65+35) and toluene as eluents with increasing polarity. GC separation and detection of the final extracts was performed on two capillary columns of different polarity (DB-5, OV-1701) with electron capture detection (ECD). Epsilon-HCH was used as internal standard for all three fractions. This procedure was adapted from the so called "S-19 method" originally published by Specht and Tillkes [20, 21], which not only in Germany serves as the "gold standard" for pesticide analysis.

2.2.6 Quantification

Quantification of all compounds, whether determined by MS or by ECD, was based on multipoint calibration curves representing the respective concentrations in the samples. The long-term stability of the analytical procedures was checked by simultaneous analyses of quality control samples with strict requirement for the interpretation of their results that were evaluated by using quality control charts (Shewart plots).

3 Results and discussion

3.1 Dioxins

Out of 210 PCDD/PCDF, normally only those 17 congeners are found in human samples that possess a 2,3,7,8-chlorine substitution. Except specific circumstances, the levels of other congeners, if present, are generally only found near the LOD. On a concentration basis, the levels of the individual PCDD increase with increasing number of chlorine atoms. Regarding PCDF, 2,3,4,7,8-P_5CDF normally shows the highest concentrations followed by 1,2,3,4,7,8-H_6CDF, 1,2,3,6,7,8-H_6CDF and 2,3,4,6,7,8-H_6CDF. Although the total concentrations of PCDD/PCDF in human milk differ between mothers from different countries, the pattern of the 17 congeners is very similar in all samples. To facilitate risk assessment, regulatory control and comparison of data for these mixtures, it is common practice to express the results in 2,3,7,8-TCDD TEQ as the sum of the products of each congener concentration and its attributed TEQ. Based on increased knowledge about their toxic potency, the TEF

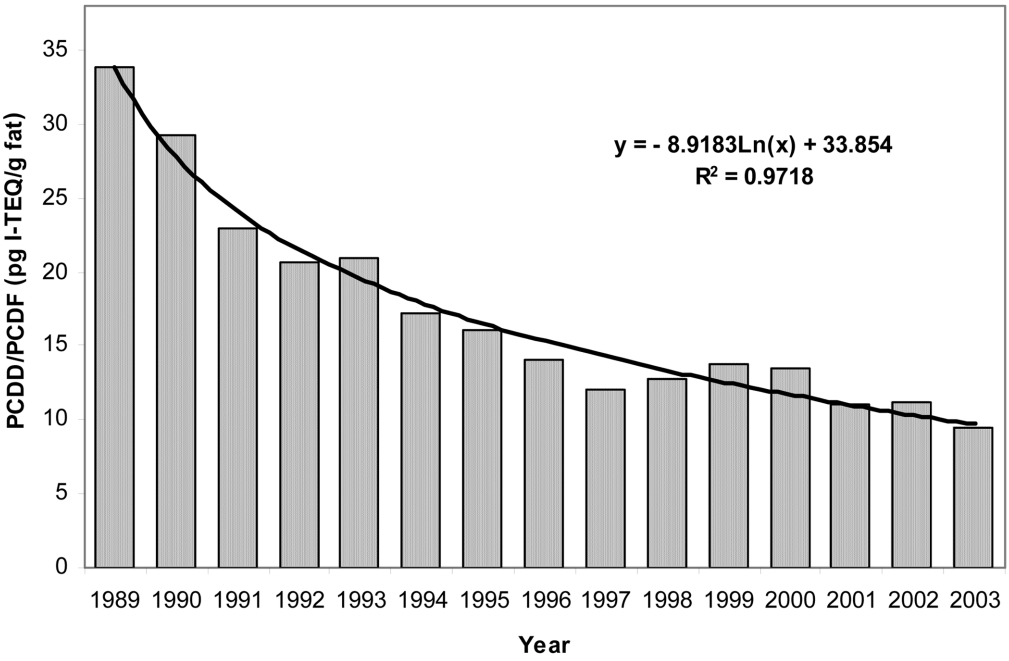

Figure 1. Median PCDD/PCDF levels (pg I-TEQ/g fat) in more than 1000 individual human milk samples from western Germany collected between 1989 and 2003.

values for individual PCDD/PCDF congeners were revised several times during the past three decades. Nowadays, the TEF values proposed by the World Health Organization [9] are mostly used. The resulting values are termed WHO-TEQ. Prior data are mostly expressed as international TEQ (I-TEQ) values. This calculation is based on the TEF model elaborated by a NATO/CCMS expert group in 1988 (NATO/CCMS, 1988: Pilot study on international information exchange on dioxins and related compounds. International toxicity equivalent factor (ITEF). Method of risk assessment for complex mixtures of dioxins and related compounds. Report No. 176). In contrast to the WHO model, the I-TEQ scheme only attributes TEF to PCDD/PCDF but not to dioxin-like PCB. Moreover, some congeners were attributed higher and others lower TEF values. Thus, detailed information of the TEF model that was applied for the conversion of analytical results into TEQ values is mandatory for a reliable comparison of results. By rule of thumb it can be said, that the WHO-PCDD/PCDF-TEQ values of human milk are approximately 15% higher than the corresponding levels expressed as I-TEQ.

Figure 1 shows the decrease of the median PCDD/PCDF levels in more than 1000 individual human milk samples from western Germany collected between 1989 and 2003. Since the WHO-TEF model was not proposed before 1998, all concentrations are expressed as picograms I-TEQ/g fat for better comparison. The results illustrate the significant decrease of PCDD/PCDF in the course of time. While the median concentration of human milk samples collected in 1989 was found to be 33.9 pg I-TEQ/g fat, the corresponding levels in 2003 only amounted to 9.8 pg I-TEQ/g fat or 11.5 pg WHO-PCDD/PCDF-TEQ/g fat, respectively. These data are consistent with results from other German regions (http://www.nlga.niedersachsen.de/master/C11137451_N11139066_L20_D0_I5800417.html) and with German human milk samples analyzed in the frame of the 3rd WHO Human Milk Field Study conducted in 2000/2002 [22].

Figure 1 indicates that the strong decrease observed in the 1990s has considerably slowed down and that the PCDD/PCDF levels in human milk are more or less leveling out. As can be seen, the median levels in 1998 and1999 were slightly higher than the levels in 1997. The same tendency was found in human milk and blood samples from other areas in Germany at that time [23]. During the same time a strong increase of dioxin levels in cow's milk samples occurred, which has been caused by highly contaminated Brazilian citrus pulp pellets as a component of feed for ruminants [24]. It was therefore assumed that the slightly higher body burden in 1998 and 1999 was associated with the consumption of food contaminated with dioxins from Brazilian citrus pulp [23].

In any case, the decreasing levels have a beneficial effect on the exposure through breastfeeding. Figure 2 shows the decline of the median daily PCDD/PCDF intake for nursed infants in the course of time. The calculations are based on

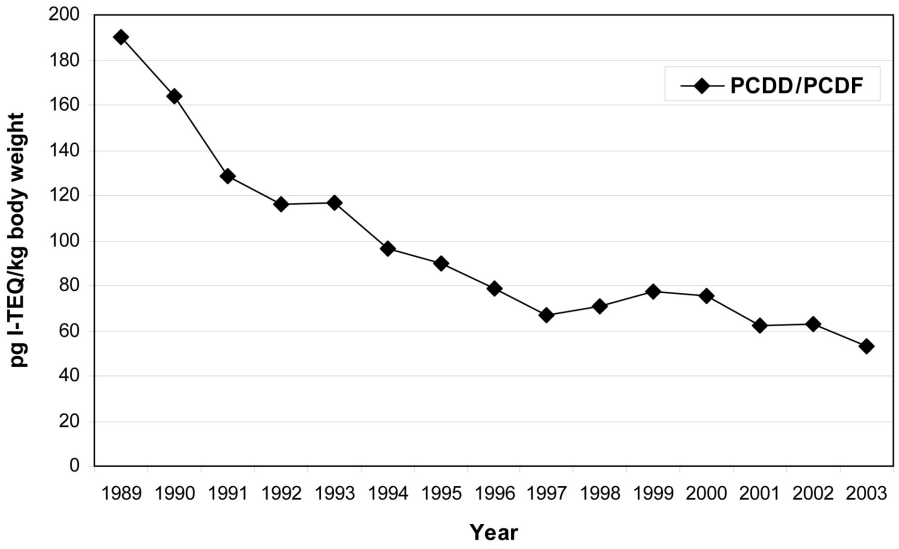

Figure 2. Temporal trend in daily PCDD/PCDF intake (pg I-TEQ/kg body weight, median) for exclusively breast-fed infants from western Germany between 1989 and 2003.

the assumption of a daily intake of 800 mL milk with a fat content of 3.5% for an exclusively breast-fed baby weighing 5 kg. During the period between 1989 and 2003, the median daily PCDD/PCDF intake decreased from 190 to 55 pg I-TEQ/kg body weight. Expressed as WHO-TEQ, the PCDD/PCDF intake for nursed infants in 2003 amounts to 64 pg WHO-TEQ/kg body weight and day that is approximately two orders of magnitude higher than the respective dietary intake for adults.

Obviously, the public concern about human milk analyses for contaminants decreased in parallel with the declining levels in the samples. During the past 2 years, only ten women requested an analysis, which is far too little to allow for evaluating the future trend of contamination.

3.2 Polychlorinated biphenyls

The non dioxin-like congeners 138, 153 and 180 represent the predominant PCB in human samples making up approximately 60% of total PCB. Consequently, the total PCB concentration in human milk samples can be obtained by multiplying the sum of the three congeners by 1.64. This was already shown by Schulte and Malisch in 1984 [25] and confirmed by the latest WHO coordinated exposure study on the levels of PCB and PCDD/PCDF in human milk [8].

Figure 3 shows the median concentrations for the three predominant PCB congeners in 2032 individual human samples from western Germany analyzed between 1984 and 2003. While the median levels in the 1980s were quite similar, the samples collected in the following years showed a clear downward trend presumably due to the strict European legal regulations concerning production, use, disposal and destruction of PCB. The human PCB body burden depends on the extent of use and composition of the technical products in the respective country, time of ban or severe restriction and the individual food consumption habits. As a result, the PCB levels in human milk samples from different regions differ worldwide considerably. The most recent risk assessment on non dioxin-like PCB performed by the European Food Safety Authority (EFSA) in 2005 gives a comprehensive overview on levels and relative contribution of individual PCB congeners to total PCB [8].

As for the dioxins, the decrease of PCB levels in human milk collected in western Germany led to a considerable decline of PCB exposure through breastfeeding. Figure 4 shows this decline. As already mentioned before, the total PCB levels were calculated by multiplying the sum of the three predominant indicator PCB 138, 153 and 180 by 1.64. The intake calculation was based on the same assumptions as for the dioxins. Although the median daily PCB exposure through breastfeeding decreased considerably from 7000 ng/kg body weight in 1984 to 1300 ng/kg body weight in 2003, the actual intake through breastfeeding is still one-to-two orders of magnitude higher than for adults.

Table 1 shows the levels of dioxin-like PCB in 175 human milk samples collected and analyzed between 2001 and 2003 in comparison with their corresponding PCDD/PCDF and total PCDD/PCDF/dioxin-like-PCB levels, respectively. All data are expressed as picograms WHO-TEQ/g milk fat. It can be clearly seen that the levels of PCDD/PCDF and dioxin-like PCB in human milk are similar and

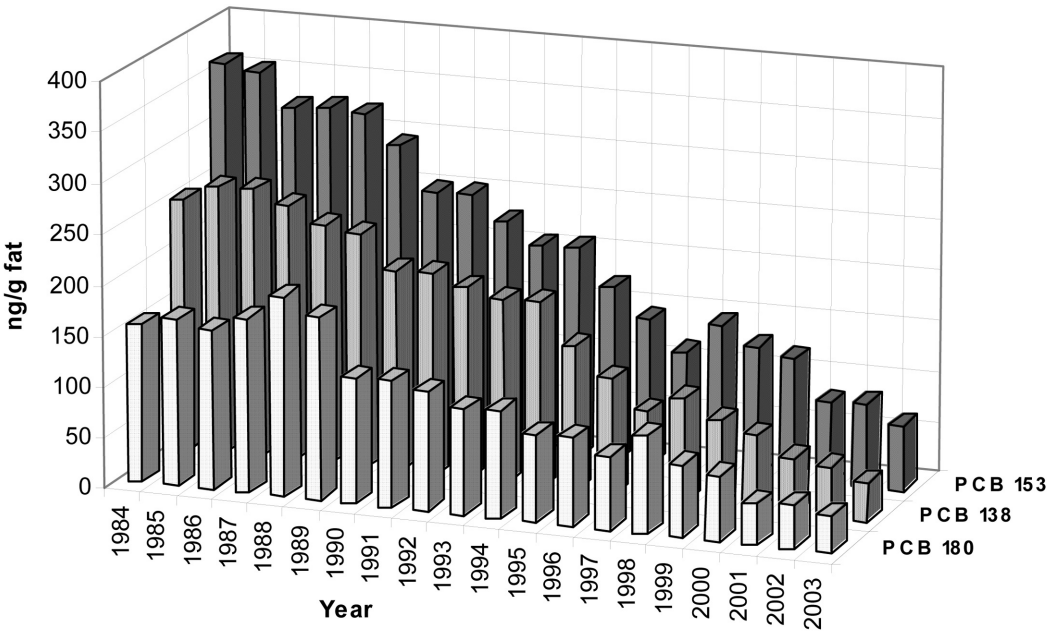

Figure 3. Temporal trend in predominant indicator PCB levels (ng/g fat, median) in human milk samples from western Germany collected between 1984 and 2003.

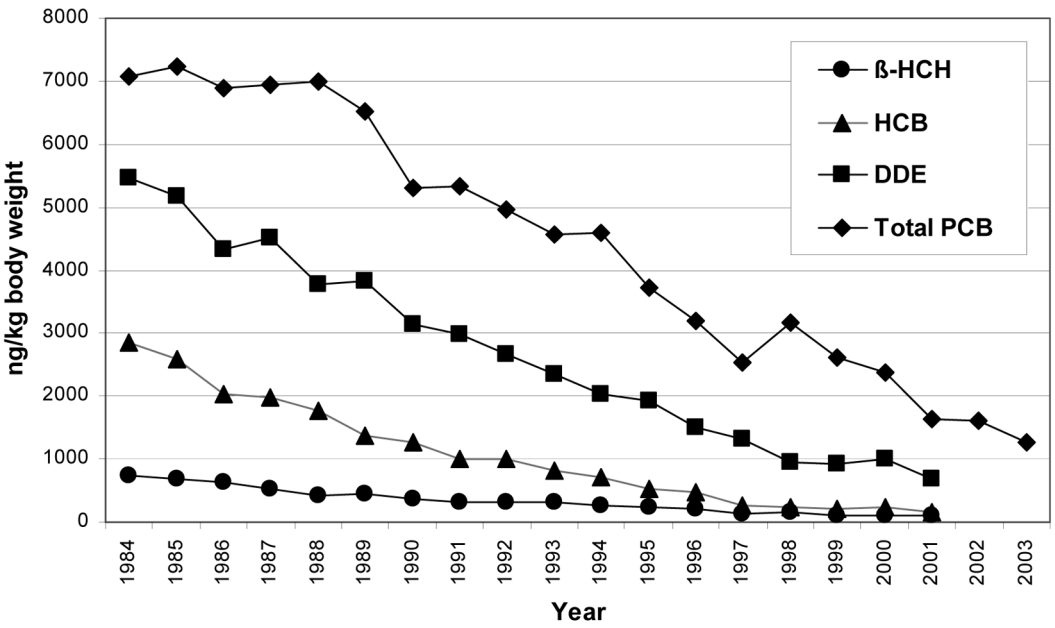

Figure 4. Temporal trend in daily intakes of organochlorine pesticides and total PCB (ng/kg body weight, median) for exclusively breast-fed infants from western Germany between 1984 and 2003.

the ratio between these two groups for women without a specific contamination is usually approximately 1:1 on a TEQ basis. Thus, the inclusion of the dioxin-like PCB into the TEQ scheme approximately doubles the WHO-TEQ concentrations in comparison to those derived from PCDD/PCDF only.

The relative contribution of the various individual dioxin-like PCB to total PCDD/PCDF/dioxin-like-PCB-TEQ differs considerably. While, for example, the share of the non-ortho PCB 77 and 81 is below 0.01%, PCB 126 and 156 contribute on average approximately 25 and 13% to total WHO-TEQ, respectively, as depicted in Fig. 5.

Table 1. Dioxin-like PCB levels in 175 individual human milk samples collected between 2001 and 2003 in comparison to their corresponding PCDD/PCDF and total PCDD/PCDF/dioxin-like-PCB levels

Compound	Minimum	Maximum	Mean	Median	90th perc.	95th perc.
	pg WHO-TEQ/g milk fat					
Dioxin-like (dl) PCB	1.2	50.1	13.2	12.8	22.0	24.1
PCDD/PCDF	1.8	34.7	13.8	13.1	22.1	24.1
Total PCDD/PCDF/dl-PCB	3.0	78.7	27.0	26.3	42.5	48.8

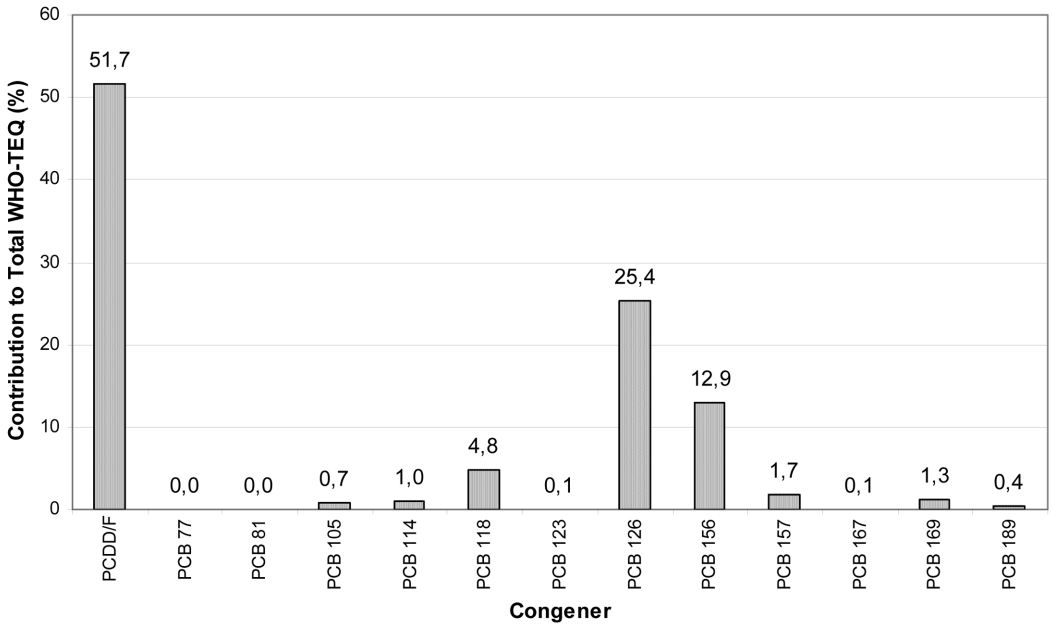

Figure 5. Contribution of PCDD/PCDF and individual dioxin-like-PCB congeners to total WHO-TEQ.

Figure 6 shows the correlation between the PCDD/PCDF and dioxin-like PCB levels, each expressed as picograms WHO-TEQ/g milk fat in 175 human milk samples collected between 2001 and 2003. The R^2 value of 0.78 indicates the good correlation between the two groups of contaminants in the analyzed human milk samples. Good correlations were also found for total PCB ((Σ PCB 138+153+180) × 1.64) and PCDD/PCDF-TEQ (Fig. 7) as well as for the sum of 6 indicator PCB and the sum of 12 dioxin-like PCB (Fig. 8), expressed as ng/g fat. The relatively high coefficients of correlation indicate a widely uniform exposure to PCDD/PCDF and PCB. It has to be stated, however, that all samples originate from a single area. These results cannot be used in general to derive dioxin levels from certain PCB levels and vice versa. Different consumption habits and exposure to diverse technical PCB mixtures with deviant composition used in the respective countries or regions may result in completely different levels and significantly weaker correlations. This was impressively demonstrated by the recent EFSA risk assessment on non-dioxin-like PCB [8].

3.3 Polybrominated diphenylethers

Out of 209 PBDE, PBDE 47, 99 and 153 are the predominant congeners that are generally found in samples from humans with background exposure. Table 2 compares PBDE levels in human milk samples from western Germany collected in 1992 and 2002. The results from 1992 originate from a manifold analysis of a human milk pool prepared with 1 kg milk fat from approximately 300 individual samples. This pool serves as a quality control pool that is analyzed in parallel with the routine samples since 1992 in order to check the long-term stability of the analytical procedures for the determination of organohalogen compounds. In contrast, the data from 2002 were taken from the analysis of 79 individual human milk samples. Therefore, some more detailed data on the distribution of the various congener levels could be given. The data show an increase of the levels between the two sampling periods of approximately 60%. However, it has to be considered that the results are only based on two sampling years. Unfortunately, no information is available about the concentrations

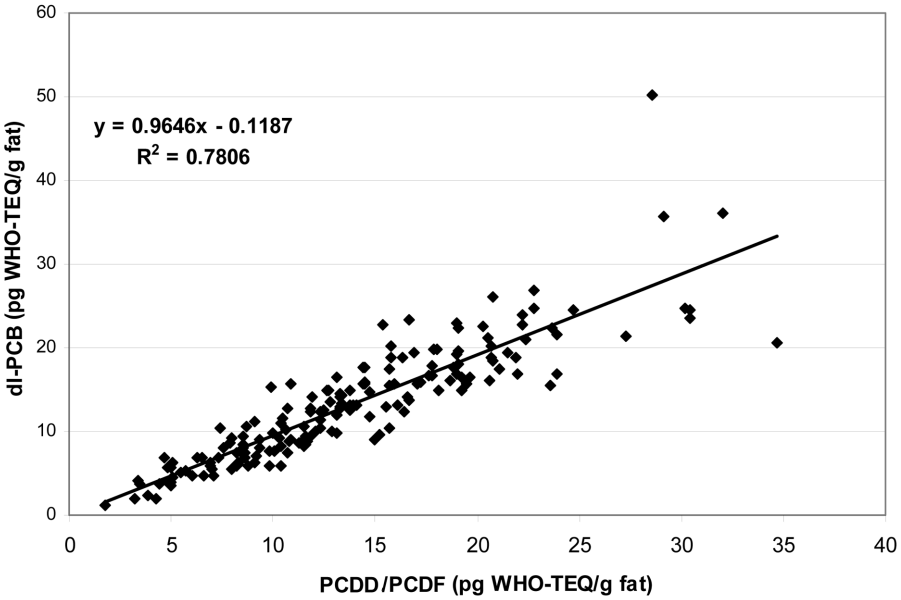

Figure 6. Correlation between WHO-TEQ (pg/g fat, median) in human milk derived from PCDD/PCDF and dioxin-like-PCB.

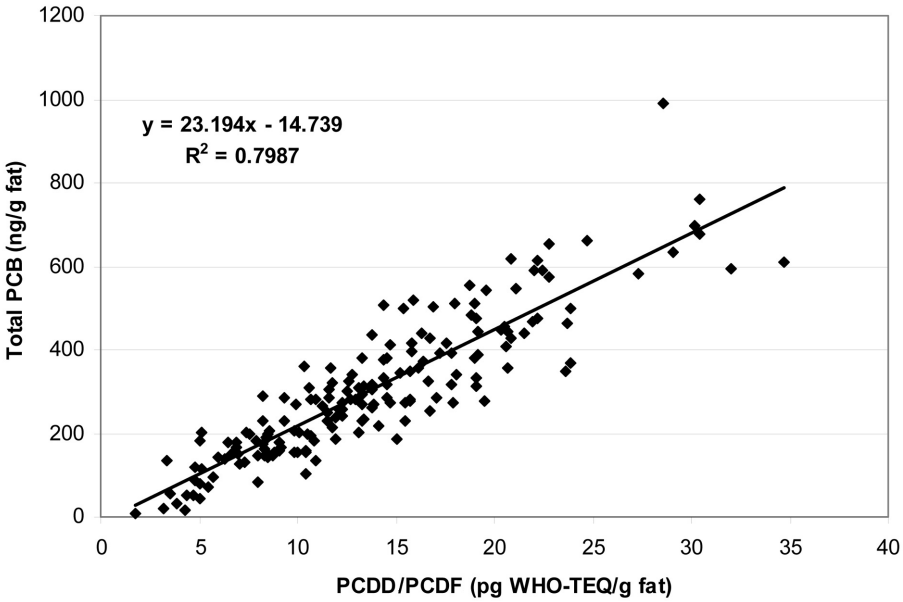

Figure 7. Correlation between PCDD/PCDF levels (pg WHO-TEQ/g fat) and total PCB levels (ng/g fat) in human milk samples from western Germany.

for the years between the two data points. Consequently, no conclusion can be drawn as to whether the levels are still increasing as in human milk samples from Norway and the Faroer Islands or are already decreasing as in human milk samples from Sweden [10].

The PBDE levels in the samples from 2002 are consistent with other analyses of human milk samples from Germany ([26, 27] and http://www.umweltbundesamt.org/fpdf-l/2921.pdf), Sweden [10], Norway [11], Finland [28], Italy [29] and Belgium [30]. Somewhat higher PBDE levels were reported for samples from the United Kingdom [31] and the Faroer Islands [12]. The highest PBDE levels and a different congener pattern were found so far in blood and human milk samples from the United States [14, 15, 32]. The Report "Residues of flame retardants in breast milk from Germany with specific regard to polybrominated diphenylethers (PBDE)" gives a comprehensive overview on the

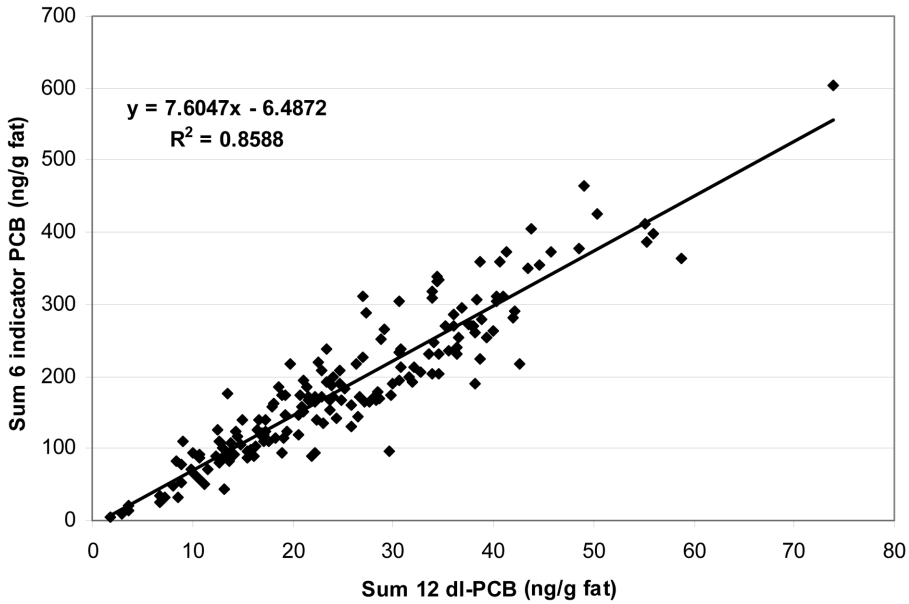

Figure 8. Correlation between the levels (ng/g fat) of the sum of six indicator PCB and the sum of 12 dioxin-like-PCB in human milk samples from western Germany.

Table 2. PBDE levels in human milk collected in 1992 and 2002

Congener	1992 (Pool; $n = 300$)	2002 (Individual samples; $n = 79$)					
		Minimum	Maximum	Mean	Median	90th perc.	95th perc.
	ng/g milk fat						
PBDE 28	0.127	0.025	1.44	0.129	0.100	0.188	0.234
PBDE 47	0.760	0.275	14.7	1.63	1.16	2.71	3.27
PBDE 66	0.011	0.003	0.151	0.018	0.013	0.028	0.042
PBDE 85	0.015	0.009	0.677	0.056	0.032	0.106	0.150
PBDE 99	0.236	0.085	4.79	0.747	0.394	1.62	2.47
PBDE 100	0.165	0.056	2.11	0.315	0.249	0.591	0.684
PBDE 153	0.454	0.065	2.76	0.740	0.677	1.14	1.30
PBDE 154	0.028	0.008	0.254	0.044	0.035	0.086	0.099
PBDE 183	0.069	0.010	0.351	0.067	0.053	0.139	0.174
Σ 8 PBDE	1.87	0.847	24.6	3.75	3.02	6.48	8.66

contamination of human milk with PBDE and possible factors that influence the burden (http://www.umweltbundesamt.org/fpdf-l/2921.pdf). For example, consumption of animal fat was found to be of special importance.

Figure 9 shows the correlation between the levels of PCDD/PCDF (pg WHO-TEQ/g fat) and the sum of the eight PBDE (ng/g fat) determined in the 79 human milk samples collected 2002 in western Germany. The graph clearly demonstrates that no significant interrelation exists between the levels of these two classes of organic persistent pollutants. This substantiates that differences in dietary exposure exist and other routes of exposure may have an additional effect on human PBDE body burden [33]. For samples collected in the United States, Wu *et al*. (Wu, N., Webster, T., Herrmann, T., Paepke, O. *et al*., Dioxin 2005, Abstract CD ROM ID: 1743) found an association between PBDE levels in human milk and house dust. However, they concluded that it is not clear if direct exposure to dust is important (*e.g.* via inhalation, ingestion or dermal routes), or if the levels in dust are a marker for some other routes of exposure. Further studies seem to be necessary to gain more detailed knowledge on the temporal trend of human PBDE burden and the relative contributions of the different exposure routes to human body burden.

Assuming the same intake conditions (milk intake, fat content, weight of baby) as for the other persistent organohalogen compounds mentioned, the median PBDE concentration in the human milk samples from 2002 would result in a

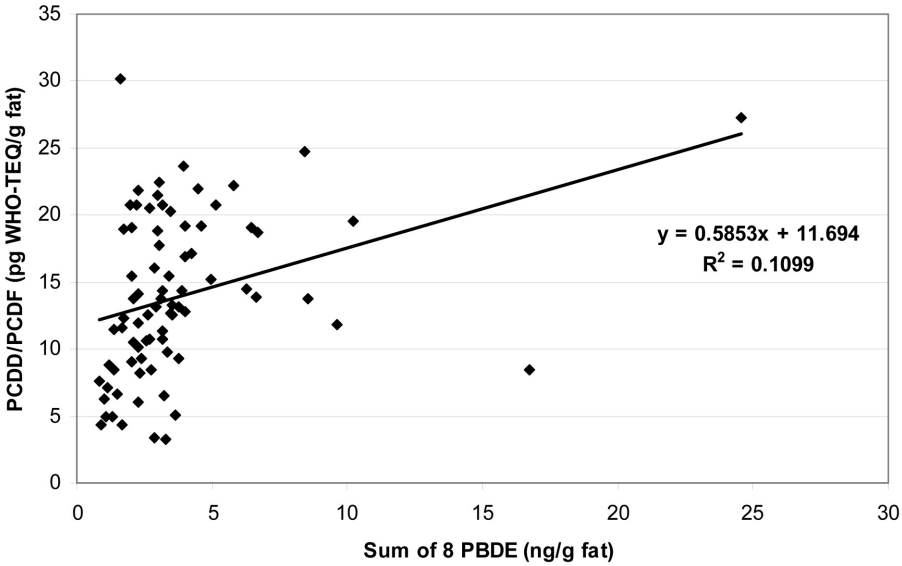

Figure 9. Correlation between the levels of PCDD/PCDF (pg WHO-TEQ/g fat) and the sum of eight PBDE (ng/g fat) in human milk samples from western Germany.

median PBDE intake of 17 ng/kg body weight and day for an exclusively breast-fed baby. This intake is approximately a factor of ten lower than the respective exposure for nursed babies in the United States where a median daily intake of 190 ng/kg body weight can be estimated based on the median PBDE concentrations of 34 ng/g fat in breast milk reported by Schecter *et al* [15].

3.4 Organochlorine pesticides

The use of DDT and other persistent organochlorine pesticides has been severely restricted and/or banned since the late 1970s in all Member States of the European Union. These regulations resulted in a significant decrease of the contamination of the food chain. However, due to the long half-lives of a number of organochlorine pesticides, these active substances or their metabolites which might be more stable than the respective parent compound, can still be found in human milk even in those countries that banned the production, use and trade some 30 years ago. Figure 10 shows the median levels for the persistent DDT metabolite p,p′-DDE, beta-hexachlorocyclohexane (β-HCH) and hexachlorobenzene (HCB) in more than 1900 individual human milk samples collected and analyzed between 1984 and 2001 in western Germany. The graph illustrates the clear downward trend during the past two decades for these three persistent organic pesticides. The highest pesticide levels are still found for p,p′-DDE. Actual samples from mothers with no known recent elevated exposure show almost exclusively the metabolite p,p′-DDE and virtually no parent compound p,p′-DDT.

Hexachlorobenzene has been used both as a fungicide and as an industrial chemical. While its intentional production has declined during the past two decades, HCB is still formed as a by-product during the manufacture of industrial chemicals, several pesticide formulations and during thermal processes, such as municipal or waste incineration. However, even this unintentional formation shows a declining tendency, which should have a further beneficial effect on the contamination of human milk with this compound.

Beta-hexachlorocyclohexane (β-HCH) shows a lesser insecticidal activity than gamma-HCH and is far more persistent in the environment and biota and thus has a high potential for bioaccumulation along the food chain. Therefore, technical HCH products containing less than 99% gamma-HCH (Lindane) were already banned in the European Union as from 1981. Despite this long ban, β-HCH can still be determined in human milk samples from western Germany although a declining tendency can be observed as shown for the median levels (Figure 10).

The temporal trend of the resulting intake estimations for nursed infants is depicted in Figure 4 together with the intake values for total PCB. All data are expressed as ng/kg body weight and day based on the above mentioned assumptions concerning the daily intake of breast milk. It is a striking fact that even 30 years after the ban on DDT in Germany, the median daily intake for an exclusively breast-fed baby still amounts to almost 700 ng/kg body weight and day. Since DDT is still used for vector control in some countries, it cannot be excluded that even in future a minor exposure to this substance may still occur due to imports of food or feed from the respective countries.

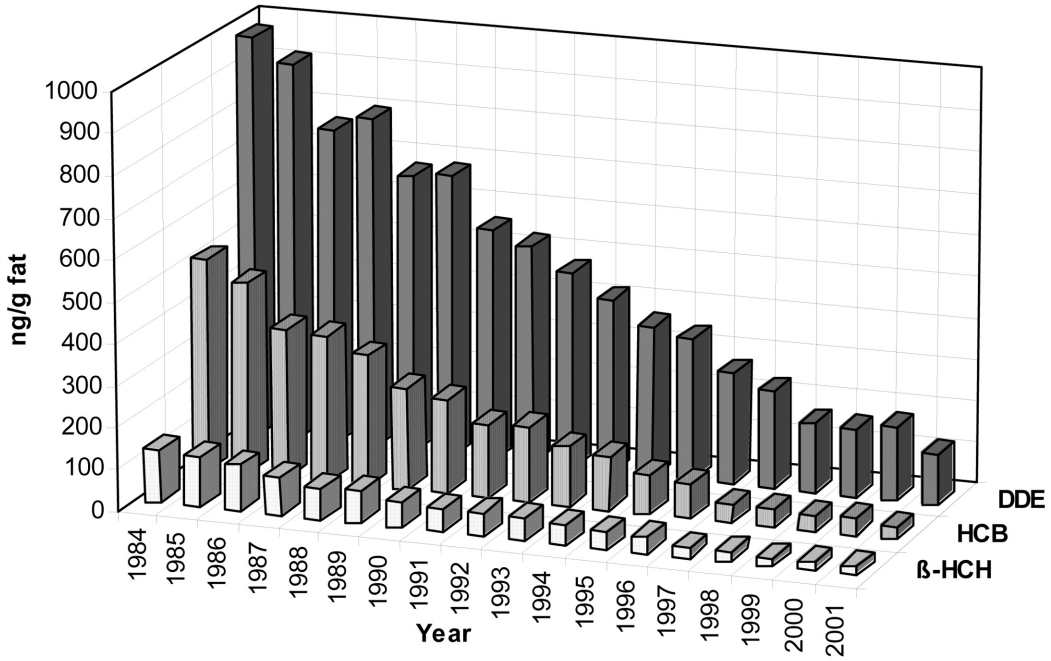

Figure 10. Temporal trend for prevalent organochlorine pesticides (ng/g fat, median) in human milk samples from western Germany collected between 1984 and 2001.

4 Concluding remarks

In conclusion, it can be said that the ban of persistent organochlorine pesticides and the numerous measures to restrict the use of certain hazardous compounds and to minimize their emission into the environment has had beneficial effects on the contamination of human milk and consequently the exposure through breastfeeding. For most of the persistent organohalogen compounds the actual levels in human milk are 70 · – 95% lower compared to samples from the 1980s. Exceptions are, however, the PBDE, which show higher levels nowadays than 10 years ago. Future analyses for these compounds will reflect how the legal regulations for PBDE will have affected levels in humans and exposure through breastfeeding.

The technical assistance of Ludger Wessel, Mathias Keitlinghaus, Ursula Möhlenkamp and Lothar Bathe is gratefully acknowledged.

5 References

[1] Fürst, P., Krüger, C., Meemken, H.-A., Groebel, W., *Chemosphere* 1989, *18,* 439 – 444.

[2] Fürst, P., Fürst, C., Wilmers, K., *Chemosphere* 1992, *25*, 1029 – 1038.

[3] Fürst, P., Fürst, C., Wilmers, K., *Environ. Health Perspect.* 1994, *102,* 187 – 193.

[4] Schrey, P., Wittsiepe, J., Ewers, U., Exner, M., Selenka, F., *Organohalogen Compounds* 1992, *9,* 261 – 267.

[5] Wittsiepe, J., Schrey, P., Ewers, U., Selenka, F., Wilhelm, M., *Chemosphere* 2000, *40,* 1103 – 1109.

[6] Päpke, O., *Environ. Health Perspect.* 1998, *106,* 723 – 731.

[7] Fürst, P., Beck, H., Theelen, R., *Toxic Substances J.* 1992, *12,* 133 – 150.

[8] European Food Safety Authority (EFSA), *EFSA J.* 2005, *284,* 1 – 137.

[9] van den Berg, M., Birnbaum, L., Bosveld, A. T. C., Brunstrom, B., *et al*., *Environ. Health Perspect.* 1998, *106,* 775 – 792.

[10] Noren, K., Meironyte, D., *Chemosphere* 2000, *40*, 1111 – 1123.

[11] Thomsen, C., Lundanes, E., Becher, G., *Environ. Sci. Technol.* 2002, *36*, 1414 – 1418.

[12] Fangstrom, B., Strid, A., Grandjean, P., Weihe, P., Bergman, A., *Environ. Health.* 2005, *4*, 12 – 20.

[13] Akutsu, K., Kitagawa, M., Nakaza, H., Makino, T., *et al*., *Chemosphere* 2003, *53*, 645 – 654.

[14] Päpke, O., Bathe, L., Bergman, A., Fürst, P., *et al*., *Organohalogen Compounds* 2001, *52*, 197 – 200.

[15] Schecter, A., Pavuk, M., Paepke, O., Ryan, J. J., *et al*., *Environ. Health Perspect.* 2003, *111*, 1723 – 1729.

[16] Sjödin, A., Hagmar, L., Klasson-Wehler, E., Kronhom-Diab, K., *et al*., *Environ. Health Perspect.* 1999, *107*, 643 – 648.

[17] Jakobsson, K., Thuresson, K., Rylander, L., Sjödin, A., *et al*., *Chemosphere* 2002, *46*, 709 – 716.

[18] Fürst, P., Bathe, L., Malisch, R., Winterhalter, H., *et al*., *Organohalogen Compounds* 1999, *40*, 109 – 113.

[19] Päpke, O., Herrmann, T., Fürst, P., *Talanta* 2004, *63*, 1203 – 1211.

[20] Specht, W., Tillkes, M., *Fresenius. Z. Anal. Chem*. 1980, *301*, 300 – 307.

[21] Specht, W., Tillkes, M., *Fresenius. Z. Anal. Chem.* 1985, *322*, 443 – 453.

[22] Malisch, R., van Leeuwen, F. X. R., *Organohalogen Compounds* 2003, *64*, 140 – 143.

[23] Vieth, B., Albrecht, M., Bruns-Weller, E., Fürst, P., *et al*., *Organohalogen Compounds* 2002, *57*, 65 – 68.

[24] Malisch, R., *Chemosphere* 2000, *1041 – 1053*.

[25] Schulte, E., Malisch, R., *Z. Anal. Chem.* 1984, *319*, 54 – 59.

[26] Vieth, B., Herrmann, T., Mielke, H., Ostermann, B., *et al.*, *Organohalogen Compounds* 2004, *66*, 2613 – 2618.

[27] Weber, H., Heseker, H., *Ernährungsumschau* 2004, *51*, 4 – 9.

[28] Strandman, T., Koistinen, J., Vartiainen, T., *Organohalogen Compounds* 2000, *47*, 61 – 65.

[29] Ingelido, A., Di Domenico, A., Ballard, T., De Felip, E., *et al*., *Organohalogen Compounds* 2004, *66*, 2689 – 2694.

[30] Pirard, C., De Pauw, E., Focant, J.-F., *Organohalogen Compounds* 2003, *61*, 263 – 266.

[31] Kalantzki, O., Alcock, R. E., Martin, F., Thomas, G., Jones, K., *Organohalogen Compounds* 2003, *61*, 9 – 12.

[32] Schecter, A., Päpke, O., Tung, K. C., Joseph, J., *et al*., *J. Occup. Environ. Med.* 2005, *47*, 199 – 211.

[33] Schecter, A., Päpke, O., Joseph, J. E., Tung, K. C., *J. Toxicol. Environ. Health A*. 2005, *68*, 501 – 513.

