ENVIRONMENTAL RESEARCH PLAN
OF THE MINISTRY FOR THE ENVIRONMENT,
NATURE CONSERVATION AND NUCLEAR SAFETY

Action Programme "Environment and Health"

UFOPLAN Ref. No. 202 61 218/03

Residues of flame retardants in breast milk from Germany with specific regard to polybrominated diphenyl ethers (PBDEs)

Final Report

by
Bärbel Vieth, Thomas Rüdiger, Barbara Ostermann, Hans Mielke

Federal Institute for Risk Assessment
President: Prof. Dr. Dr. Andreas Hensel

Responsible investigators:
Dr. Bärbel Vieth and Dr. Thomas Rüdiger
Federal Institute for Risk Assessment

Commissioned by the Federal Environmental Agency

Berlin, May 2005

Report Cover Sheet

1. Report No.	2.	3.

4. Report Title
Residues of flame retardants in breast milk from Germany with specific regard to polybrominated diphenylethers (PBDE)

5. Author(s), Family Name(s), First Name(s)
Vieth, Bärbel, Rüdiger, Thomas, Ostermann, Barbara, Mielke, Hans

6. Performing Organisation (Name, Adress)
Federal Institute for Risk Assessment
Thielallee 88-92
D-14195 Berlin

7. Sponsoring Agency (Name, Address)
The Federal Environmental Agency (UBA)
P.O. box 1406
D-06813 Dessau

8. Report Date
14.05.2005

9. Publication Date

10. UFOPLAN-Ref. No.
2002 61 218/03

11. No. of Pages
92

12. No. of References
122

13. No. of Tables, Diagrams
18

14. No. of Figures
13

15. Supplementary Notes

16. Abstract

This study presents the observations on PBDE-levels in breast milk as well as possible influencing factors. The study-design enabled the specific analysis of the impacts of eating habits and the duration of breast-feeding. Further possible factors were pinpointed and examined using a questionnaire. The daily PBDE-intake of a fully breast-fed infant was estimated by a worst-case scenario.

In the period from November 2001 to March 2004, a total of 128 milk samples were taken from 89 nursing mothers (total collective) across Germany within 1 – 2 weeks and in some cases again appr. 12 weeks after child delivery. 41 women were on a mixed diet (cohort 1) and 32 were vegetarians or vegans (cohort 2). 16 mothers did not meet the criteria for participation. The 9 congeners BDE 28 (Tri-BDE) , 47, 66 (Tetra-BDE), 99, 100 (Penta-BDE), 153, 154 (Hexa-BDE), 183 (Hepta-BDE) and BDE 209 (Deca-BDE) were analysied. This study is one the most extensive examinations for PBDE in breast milk worldwide.

From the total collective, the mean value of the total PBDE (sum of 9 congeners) was calculated at 2,49 ng/g milk fat. In comparison with other european countries, the body burden in Germany falls among the lowest. The succession of the congeners BDE 47>153>99>100 are found to be identical in most european countries, which indicates similarity of exposure sources. Measurements in North America with mean values of 22 to 73 ng/g milk fat are 10 to 30 times higher that those in Germany. The different succession of the main congeners BDE 47>99>100>153 suggests that the exposure sources may be differ from those in Europe.

The decabromocongener BDE 209 was quantified in breast milk samples from Europe for the first time. These results confirm, that despite its low bioavailability the BDE 209 is absorbed and is present in human milk samples reflecting the low European background body burden.

For the first time, evidence was found, that both partial or total refrainment from the consumption of animal products and breast-feeding of several infants lead to significantly lower PBDE-levels. Accordingly, the average value of 1,65 ng/g fat from the breast milk samples of the vegetarian mothers was significantly lower than the average of 2,47 ng/g fat in samples of the mothers on a mixed diet. The number of mothers who were breast-feeding the 2nd or 3rd child was higher among the vegetarians than among those on a mixed diet, the observed differences in body burden between both cohorts were therefore attributed to nutrition as well as to the number of nursing periods. This was modelled by the multiple lineal regression.

A reduction in the PBDE-level after a 3-month breast-feeding period was not observed. It is possible that this observation period was too short. Age, body-mass-index, display screen exposure (computer and television) as also tobacco smoke were not proven to be influencing factors.

The PBDE-intake of a 4-month-old infant through breast-milk is 10.000 times lower than the lowest NOAEL derived from animal experiments and which has exhibited no adverse effects during observations. This very great margin of safety gives grounds, based on the present level of knowledge, for the assurance that breast-fed infants in Germany are not exposed to health risk. Subsequently, the 4 to 6 months breastfeeding period as recommended by the commission for nursing behaviour (National Stillkommission) can be unrestrictedly maintained in regard to the PBDE-intake.

17. Keywords
Brominated flame retardants, PBDE, breast milk, observation study, questionnaire, influencing factors, nutrition behavior, breast-feeding, breast-feeding periods, exposure of infants, breast-feeding recommendations

18. Price	19.	20.

Table of Contents **Page**

1 Introduction 5

2 Review of literature 7

2.1 Toxicology 7

2.1.1 Pentabromodiphenyl ether (PeBDE) 7

2.1.2 Octabromodiphenyl ether (OBDE) 9

2.1.3 Decabromodiphenyl ether (DBDE) 11

2.1.4 Endocrine effects 13

2.1.5 Neurotoxic effects 13

2.1.6 Risks involved in exposure of the newborn infant through breast milk 14

2.1.7 Regulatory measures 14

2.2 PBDE in human specimens 15

2.2.1 Data on PBDE in breast milk and blood 15

2.2.2 Prenatal exposure to PBDEs 21

2.2.3 Routes of exposure 23

3 Objectives of this study 26

4 Material and methods 28

4.1 Structure of the study, collection of data and samples 28

4.1.1 Study design 28

4.1.2 Questionnaire 29

4.1.3 Recruitment of test persons 30

4.1.4 Collection and storage of samples 31

4.2 Analysis 31

4.2.1 Placing of order for analysis 31

4.2.2 Preparation of sampling vessels / storage / dispatch of samples 31

4.2.3 Sample preparation and quantification 32
4.2.4 Minimization of blank values 33
4.2.5 Quality control / quality assurance 33
4.3 Statistical methods 35
4.3.1 Descriptive statistics 35
4.3.2 Testing of hypotheses 36
4.3.3 Explorative data analyses 36
5 Results 37
5.1 Characterization of the study populations 37
5.2 PBDE levels in the total cohort and the study cohort 39
5.3 Testing for normal distribution 41
5.4 Influence of the diet on PBDE levels in breast milk 43
5.4.1 Testing of study hypothesis I: Differences between omnivores and vegetarians 43
5.5 Influence of breastfeeding on PBDE levels 45
5.5.1 Testing of study hypothesis II: Changes in PBDE levels during the lactation period 45
5.5.2 Influence of the number of breastfed children on PBDE levels in breast milk 47
5.5.3 Influence of the duration of the lactation period on PBDE levels in extended breastfeeders 49
5.6 Joint evaluation of dietary habits and number of breastfed children 51
5.7 Testing of other potential confounders 53
5.8 Estimate of PBDE intake levels of the breastfed infant 56
5.9 Results of PBDE detection in human blood and comparison with the breast milk data 58
6 Discussion and assessment 60
6.1 PBDE levels in breast milk from Germany, and international comparison 60

6.2 The decabrominated congener (BDE-209) in breast milk 62
6.3 The influence of dietary habits on PBDE levels 63
6.4 The influence of breastfeeding on PBDE levels 66
6.5 Common model for the influencing factors of dietary habits and number of breastfed children 69
6.6 Assessment of PBDE intake of the breastfed infant 70
7 Summary 72
8 Conclusions and outlook 75
9 Acknowledgement 76
10 References 77
11 Lists 87
11.1 Abbreviations used 87
11.2 List of Tables 89
11.3 List of Figures 91

1 Introduction

Breastfeeding is regarded as the best choice of infant feeding and has been uncontested also as its most natural form. While breastfeeding mothers can control contamination of their breast milk by means of their direct behaviour with regard to several contaminants (e.g. pharmaceuticals or stimulants such as nicotine, alcohol and caffeine), the uptake of substances from the environment, mainly through accumulation in the food chain, can hardly be avoided by them. Therefore, reasons for monitoring breast milk contamination with persistent and lipophilic environmental contaminants also include the aspect of preventive health care.

In Germany, the levels of persistent organochlorine compounds detected in breast milk, such as DDT, PCBs and polychlorinated dibenzodioxins and dibenzofurans, have decreased by ca. 60 - 90 % in the last 15 - 30 years (Vieth, 2000, 2001, 2002). In contrast to these continuously decreasing trends, which could also be observed on an international level, strongly increasing levels (doubling every five years) of flame retardants belonging to the group of polybrominated diphenyl ethers (PBDEs) were detected in the 1972 - 1997 period in breast milk in a retrospective Swedish study published in 1999, which gave rise to great concern (Meironyte et al., 1999). So far, only few data on PBDEs in breast milk had been available in Germany so that the exposure situation could not be evaluated.

The focussed substance class of polybrominated diphenyl ethers (PBDEs) is used as an additive flame retardant preferably in plastic materials, such as polyurethane foam for upholstered furniture and motor vehicle seats, and in polymers in electronic equipment (computers, video recorders etc.), with quantities of up to 40 % of the mass percentage being added to the polymers. Given the above-mentioned uses, an exposure of consumers has been suspected.

Technical use has been made of three commercial products: Pentabromodiphenyl ether (PeBDE) with a world market demand of ca. 8,500 t/a, octabromodiphenyl ether (OBDE) with a worldwide quantity used of ca. 3,800 t/a, and the main product in quantitative terms, decabromodiphenyl ether (DBDE), accounting for 55,000 t/a. The technical products are mixtures of a number of single compounds with different degrees of bromination. The name of the technical product characterizes the mean degree of bromination of the single compounds contained.

In chemical terms, the brominated diphenyl ethers are characterized by a ether-bridged diphenyl structure whose congeners differ with regard to the number and position of bromine substituents on the phenyl ring. Analogous to the PCB nomenclature by

Ballschmiter, the 209 possible BDE congeners are designated by numbers, such as BDE-47, BDE-100 etc. (Ballschmiter, 1980). They are similar to dioxins both with regard to their chemical structure and to their toxicological profile.

Meanwhile, these compounds have become ubiquitous in the environment. They can be detected in the air, in the soil, in water and in the sediment as well as in aquatic biota, fish, meat, milk and eggs. Increasing levels of PBDE residues have been found over decades in sediments, fish, marine mammals and birds. Also in the aquatic food chain, a continuous increase in accumulated PBDE concentrations through the trophic levels has been observed (de Wit, 2002). Being persistent and lipophilic compounds with a potential of bioaccumulation, PBDEs comply with essential criteria of persistent organic pollutants (POPs). These have been defined in the Stockholm POPs Convention. Release into the environment of such chemicals and exposure to them is to be minimized or prevented.

In the framework of chemical assessment, the European Union (EU) has developed Risk Assessment Reports (RAR) for the three technical products used. In these reports, the data currently available were considered as insufficient for a comprehensive assessment of this class of compounds. Due to the confirmed potential of bioaccumulation and the indications found of influences on the neurological development, the research requirements formulated include, among other data, those on levels of PBDEs in breast milk and on the exposure of the breastfed infant (EU Risk Assessment Report Pentabromodiphenyl ether, 2000). Due to the great number of findings on PBDE in environmental and human specimens on the one hand and the lacking data on PBDE levels in breast milk in Germany on the other, a request to support measures for risk assessment had also been filed by the Bundesrat to the Federal Government (Bundesrats-Drucksache 97/01).

The research project "Residues of flame retardants in breast milk from Germany with specific regard to polybrominated diphenyl ethers (PBDEs)" was commissioned by the Federal Environmental Agency with the aim to obtain well-founded data on PBDE levels in breast milk, to assess the exposure of the breastfed infant and to verify possible routes of exposure and influencing parameters, such as the diet.

Below, the results are presented of the analysis of 128 breast milk samples collected from 89 mothers. Thus, the present report represents one of the most comprehensive studies ever performed so far on PBDE levels in breast milk. For the first time, also results of samples collected from vegetarians are presented, and evidence is provided also for the significant influence of different dietary habits and the number of breastfed children on individual PBDE levels. This was only possible owing to the target-oriented study design.

2 Review of literature

2.1 Toxicology

PBDEs have a similar structure like PCBs, PBBs and PCDDs/PCDFs, and they also show similar properties with regard to toxicity. A fact worth mentioning is their structural similarity with the thyroid hormone, thyroxine (T4).

The majority of toxicological studies was performed using commercial PBDE mixtures,, which vary considerably with regard to their content of congeners and isomers, so that it is difficult to make statements about congener-specific effects. The major part of information is based on animal studies; data on direct effects in humans are rare and mainly available for the technical DBDE.

A comprehensive evaluation of the toxicological properties of the three technical products, PeBDE, OBDE and DBDE was carried out by the European Union in the context of the Risk Assessment Reports. These were completed recently and the Reports presented. (EU Risk Assessment Report Pentabromodiphenyl ether, 2000; EU Risk Assessment Report Octabromodiphenyl ether, 2002; EU Risk Assessment Report Decabromodiphenyl ether, 2003).

The information on toxicology summarized in this review of literature is mainly based on the Risk Assessment Reports by the European Union. References quoted in these documents are not stated separately in the following. Instead, reference is made to the Reports. More studies and up-to-date data have been added quoting the corresponding references.

2.1.1 Pentabromodiphenyl ether (PeBDE)

The technical compound, pentabromodiphenyl ether is a mixture of 24 - 38 % tetra-BDE, 50 - 60 % penta-BDE and 4 - 8 % hexa-BDE. The predominant congeners are BDE-47, BDE-99 and BDE-153.

Toxicokinetics

From animal experiments, it is understood that PeBDEs are absorbed after oral administration. Knowledge is scarce about other routes of exposure, however, due to the structural similarity with PBBs and PCBs it has been assumed that also PeBDEs may enter the body via other possible routes of exposure. Studies in rats have revealed that a major part of a single oral dose of PeBDE is excreted with the faeces in an unmetabolized

state within a period of 72 hours and that major quantities distribute in the skin and fatty tissue. Due to the poor solubility in water and the high molecular weight of PeBDEs and their metabolites, excretion takes place mainly via the biliary route, with the faeces, but also with breast milk (Darnerud, 1998; Meironyte, 1998).

With regard to the half-life of PeBDEs, the metabilization rate is assumed to be low. In the fatty tissue of rats, a half-life of PeBDE isomers of $t_{1/2}$ = 25 – 47 days was determined (von Meyerinck, 1990). It was assumed that in human fatty tissue, the substance persists for significantly longer periods (Sarver, 1997). Such assumption has been confirmed by up-to-date data on the elimination half-life times of the predominant congeners of commercial PeBDE in adult humans. Thus, the average elimination half-lives determined from the daily intake through foods were: for the tetrabrominated congener, BDE-47, 1.8 years, for the pentabrominated congeners, BDE-99 and BDE-100, 2.9 and 1.6 years, respectively, and for the hexabrominated congeners, BDE-153 and BDE-154, 6.5 and 3.3 years, respectively. Elimination half-lives as referred to human fatty tissue are markedly longer. The periods reported were: for BDE-47, 3.0 years, for BDE-99, 5.4 years, for BDE-100, 2.9 years, for BDE-153, 11.7 years and for BDE-154, 5.8 years, with these periods in general being longer in females than in males (Geyer, 2004). Markedly shorter elimination half-lives were determined for BDE-153 (680 days) and BDE-154 (270 days) by means of PBDE detection in the blood of exposed workers (Jakobsson, 2003).

Acute toxicity

Studies in rats performed with commercial PeBDE revealed a low acute toxicity. After oral administration, an induction of several hepatic enzymes was observed in addition to diarrhoea, tremor and reduced activity (Darnerud, 2001). Low acute toxicity was also found with regard to exposure by inhalation of PeBDE. In addition, only minor irritation of the eyes and the skin could be observed after single administration.

Effects associated with chronic exposure

Information on systemic effects after repeated exposure to PeBDE originates from studies in rats and mice. The liver represents an important target organ. In addition to hepatomegaly associated with histopathological changes, and induction of several hepatic enzymes, also disturbances of the cholesterol and porphyrin synthesis were observed. For chronic liver toxicity as the most sensitive endpoint, a NOAEL of 0.45 mg/kg b.w./d was determined by means of animal experiments.

With regard to the thyroid gland, a reduction of T4 levels associated with weight increase of the thyroid was observed in rats and mice, which can be explained by hepatic enzyme induction with consecutive increase of T4 conjugation and excretion, among other factors.

A decrease of CD4 and CD8 thymocytes in mice, but not in rats, could be demonstrated by exposure to the commercial product, Bromkal 70 (mainly BDE-47, BDE-99 and BDE-100). Relevance with regard to humans has remained unclear so far.

As far as repeated dermal exposure to PeBDE is concerned, only one study has been available so far demonstrating the occurrence of erythema and oedema as well as chloric acne reactions on rabbit ears.

With regard to humans, only one case report is available describing the development of chloric acne reactions in the face and on the back of a 31-year-old male who had spent several hours a day in front of the TV set and playing on the computer. Due to the poor evidence provided by this single case report it is difficult to demonstrate a relationship between exposure to PeBDE used in electronic equipment and the development of skin irritations.

Mutagenicity, carcinogenicity, reproductive and developmental toxicity, neurotoxicity

It could be demonstrated in numerous studies performed in bacteria, fungi and mammalian cells that PeBDEs are no cell mutagens. No data on carcinogenicity are available so far.

Fertility studies with regard to PeBDE are not available so far. No histopathological changes of the gonads or sexual organs could be demonstrated in a 90-day study in rats with oral administration of the product, DE-71, using quantities of up to 100 mg/kg b.w./d. No negative effects on the fetus could be demonstrated in a developmental study in rats exposed to the technical product, Saytex 115, at doses of up to 200 mg/kg b.w./d.

Abnormalities with regard to learning abilities and activity compared with the control group were demonstrated by a study examining mice for behavioural disturbances (Eriksson, 1998). These differences depended on the dose administered. Relevance for human health has not yet been elucidated.

2.1.2 Octabromodiphenyl ether (OBDE)

The technical product, octabromodiphenyl ether consists of a mixture of 10 - 12 % hexa-BDE, 43 - 44 % hepta-BDE, 31 - 35 % octa-BDE, 10 – 11 % nona-BDE and < 1 % deca-BDE, with the heptabrominated congener, BDE-183 being the predominant congener.

Toxicokinetics

Data obtained from animal studies have shown that after oral and inhalational exposure to commercial OBDE, the parent compounds and the metabolites accumulate in the liver and in the fatty tissue, and after inhalation, also in the lung tissue. So far, no exact statements can be made with regard to the degree of absorption, excretion and metabolization. After oral administration, OBDEs will induce numerous hepatic enzymes and the metabolism of foreign substances. No data are available on dermal absorption.

No human data are available so far on absorption, metabolism and excretion of OBDE. The hexa-, hepta-, octa- and nonabrominated congeners contained in commercial OBDE are absorbed in the human body, they distribute in the blood and fatty tissue and accumulate in the human fatty tissue due to their highly lipophilic character. BDE-183, a predominant congener of commercial OBDE, is detected in breast milk (Table 1). Stanley could detect OBDE in human fat (Stanley, 1991). Octabrominated congeners, but also nona-BDE and the heptabrominated congener, BDE-183 were detected in the blood of exposed workers in the electronics industry in a Swedish study (Sjödin, 1999). The elimination half-lives determined for the nona-BDEs detected in the blood of these exposed workers were between 17 and 85 days, for octa-BDEs, between 62 and 84 days, and for the hepta-BDE-183, 110 days, i.e. they were markedly shorter that the periods determined for PeBDEs (Sjödin 1999; Hagmar, 2000).

Acute toxicity and effects associated with chronic exposure

Due to the data currently available it has to be assumed that OBDEs exhibit only low acute toxicity in animals, no irritations of the skin or eyes were observed in animal studies. No data are available on sensitization of the respiratory tract or skin in humans.

As far as chronic exposure is concerned, only studies in rats with repeated oral and inhalational exposure to commercial OBDEs are available. A main target organ is the liver. Observations included an increase in liver weight, hepatomegaly, histopathological changes of liver cells, an induction of several hepatic enzymes and an increased incidence of disseminated hyperplastic nodules as well as changes in the porphyrin metabolism. After oral administration in rats, changes of the thyroid hormonal balance with a dose-dependent reduction of T4 and T3 in the serum and hyperplasia and histopathological changes of the thyroid were presented. Furthermore, a disturbance in the thyroid hormone metabolism associated with reduced T4 levels and elevated TSH levels in the serum were presented.

After oral administration, a dose-dependent increase in bromine levels in the liver and after inhalational administration also in the lungs were observed. Accumulation of OBDE in the fatty tissue and in the lungs was more pronounced than that in the hepatic tissue.

Mutagenicity, carcinogenicity, reproductive and developmental toxicity

No indications of mutagenicity were found in studies performed in salmonellas and mammalian cells. Based on the fact that no mutagenic properties were observed for PeBDE and DBDE, this can be assumed to also apply to OBDEs. At present, definitive statements on carcinogenicity cannot be made, no corresponding animal studies are available.

Fertility studies are also not available. Information on possible effects of OBDEs with regard to fertility has been obtained from subacute or subchronic studies in rats with oral or inhalational administration of commercial OBDEs. An increase in the weight of testes was observed. On inhalational exposure, neither negative effects with regard to the weight of testes or epididymis were observed nor any histopathological changes could be confirmed. With regard to the female reproductive organs, an absence of the corpus luteum was demonstrated in a 90-day inhalation study.

Effects regarding reproductive toxicity were observed in animal studies in rats in the context of two studies (decrease of the maternal weight and low fetal weights). In rabbits, exposure to OBDEs resulted in a minor loss of fetal weight. The lowest NOAEL considered has been 2 mg/kg b.w./d.

2.1.3 Decabromodiphenyl ether (DBDE)

The technical product, decabromodiphenyl ether consists mainly of the congener, BDE-209 (97 %) and contains less than 3 % nona-BDE.

Toxicokinetics

Only few data are available on toxicokinetics as referred to the human body. DBDE is absorbed by the body and distributes in the blood and fatty tissue. Thus, BDE-209, the predominant congener of the technical product, was detected in the blood of exposed workers in the electronics industry (Table 2). The elimination half-life times of BDE-209 determined in the blood of exposed workers were 6.8 and 14 days, respectively, and thus, are particularly short compared with those of the lower brominated congeners (Sjödin, 1999; Sjödin, 2000; Hagmar, 2000; Geyer, 2004). Obviously, elimination half-life times become shorter with increasing degree of bromination.

Animal studies have revealed that due to the high molecular weight, DBDE is absorbed through the gastrointestinal tract to a minor degree only (6 - 9.5 %), and a major part is excreted with the faeces. Due to the low oral absorption in rats, a low accumulation potential can be assumed. No data are available on bioaccumulation of DBDE in human fat. However, the fact that DBDE is obviously accumulated in the body fat has been confirmed by a study performed in the USA, which detected BDE-209 in breast milk (Schecter, 2003, Table 1). So far, no data have been available on BDE-209 in breast milk from Europe, where contamination has been considerably lower.

After intravenous administration, DBDE is metabolized by the liver. A metabolic debromination of BDE-209 to BDE-153 could be observed in a study in rainbow trout exposed to technical DBDE via the oral route (Kierkegaard, 1995). Viberg could demonstrate an uptake of DBDE into brain tissue of neonatal mice who had been postnatally exposed to a single dose via the oral route. The toxicological significance of such findings has still remained unclear (Viberg, 2001). An accumulation of DBDE has so far been observed to take place to a minor extent in fatty tissue and in the liver. At low concentrations, DBDE does not induce the metabolism of foreign substances, however, effects at higher concentrations cannot be excluded. The degree of bromination has been assumed to play an important role since PeBDEs cause a stronger enzyme induction than OBDEs, and no induction has been observed for DBDEs. For DBDE, neither data on dermal nor on pulmonary absorption are available. Based on the similarities with PCBs, a maximal dermal absorption of 1 % has been assumed.

Acute toxicity and effects associated with chronic exposure

Animal studies have shown a low acute toxicity on exposure via the oral, dermal and inhalational routes. Irritations of the skin and eyes did not occur. Furthermore, there were no indications of a development of chloric acne. No animal studies are available with regard to skin sensitization. Neither sensitization nor irritation of the skin were observed in a relatively large study in humans.

Generally, animal studies could demonstrate only minor systemic toxicity after chronic exposure. The liver, kidneys and thyroid were identified as the main target organs that generally underwent a minor increase in size. Lesions such as elevated incidence of thrombosis, liver degeneration, splenic fibrosis, hyperplasia of mandibular lymph nodes and hyperplasia of thyroidal C-cells were observed in a study in rats (NTP, 1986). A study in workers exposed to PBDE, including DBDE, for a period of six weeks demonstrated an increased prevalence of hypothyreosis associated with reduced T4 levels in the serum (Bahn, 1980).

Mutagenicity, carcinogenicity, reproductive and developmental toxicity

DBDE does not induce mutagenic effects neither in vivo nor in vitro. As an indication of carcinogenicity, an increased occurrence of neoplastic nodules of the liver was observed after exposure to DBDE both in mice and rats. In addition, a higher incidence of thyroid tumours was demonstrated in mice (NTP, 1986).

No effects indicating reproductive or developmental toxicity of DBDE could be found in animal studies. Thus, neither effects on fertility nor developmental disorders nor changes of the reproductive organs were found in rats and mice.

2.1.4 Endocrine effects

The different metabolites of PBDEs have a pronounced structural similarity with the thyroid hormones (T3 and T4) and show a very high affinity to the thyroid hormone distributor protein, transthyretin (Meerts, 1998; Meerts, 2000). In addition, they are also able to bind to thyroid hormone receptors, though with a lower affinity (Marsh, 1998). All commercial PBDEs interfere with the thyroid balance, with DBDE exhibiting the lowest potential compared with the other PBDEs (Hooper and McDonald, 2000). Clinical signs observed include those of a hypothyreosis associated with suppressed thyroid hormone levels in the plasma and thyroidal hyperplasia, as well as increased occurrence of thyroid carcinoma in mice (NTP, 1986; Hallgren and Darnerud, 1998; Hallgren, 2001). For DBDE products, a statistically significant increase of the incidence of thyroid hyperplasia was recorded in animals (NTP, 1986) and also demonstrated in humans (Bahn, 1980). It can be concluded that the thyroid hormone system is a sensitive target of PBDEs, which may also disrupt the development of the central nervous system during the pre- and postnatal stages.

2.1.5 Neurotoxic effects

A number of studies have demonstrated that commercial PBDEs may cause neurotoxic effects (Eriksson, 1998; Eriksson, 1999; Viberg, 2001). For the congerens, BDE-47 and BDE-99, which are the ones found most frequently in human tissue, effects observed in exposed newborn mice included a clear deviation of motor behaviour such as reduced total activity and impairment of locomotion. In addition, impaired learning abilities and memory disturbances were described. Disorders of both motor and cognitive development in the neonatally exposed mice showed clear effects also during adolescence (Eriksson, 1998).

There are several possible routes by which PBDEs may influence the development of the central nervous system:

Firstly, a decisive role is played by the interference with the thyroid hormone regulation resulting in disruption of the neuronal development both in rodents and humans (Morreale de Escobar, 2000; Porterfield, 2000; Morreale de Escobar, 2003) because the development of the brain is decisively controlled by these hormones above all during the fetal and neonatal stages. In addition, PBDEs may cause a disruption of several neurotransmitter systems (Eriksson, 1997; Viberg, 2000). Viberg described a significant relationship between behavioural neurological effects and changes in the cholinergic transmitter system after exposure to PBDE.

2.1.6 Risks involved in exposure of the newborn infant through breast milk

Due to the placental permeability to PBDEs, children become exposed to these compounds already during the prenatal stage and in addition postnatally through breastfeeding. Above all the lower brominated PBDE congeners (tetra to hexa) may lead to neoplasia, endocrine disruption and neurological developmental disorders. Deficient neuronal development caused by disturbances of the thyroid hormone regulation may result in behavioural disorders in children exposed for example through breast milk (Porterfield, 1994; Haddow, 1999). Thus, increasing incidences have been observed of hypothyreosis and disturbances of the neuronal development in children, which become manifest by learning and behavioural disorders (Chen, 1994; Huisman, 1995; Huisman, 1995; Koopman-Esseboom, 1996).

Due to the knowledge gained so far on toxicology, a risk assessment with regard to the feeding of breast milk to newborns, who are in a vulnerable stage, is particularly important because any interference with the above-described organ systems by PBDEs, which have a direct effect on the neuronal development, may cause extensive late sequelae. However, the data available do not permit any exact risk assessment so that further studies are required both with regard to exposure data and to toxicology.

2.1.7 Regulatory measures

PBDEs are not only present in biota but they also accumulate in human body fat, attack certain organ systems and are excreted in breast milk. Knowledge in the fields of ecological and human toxicology is scarce so far, and possible long-term consequences for humans and the environment cannot be reliably assessed at present.

Meanwhile, regulatory measures have been taken for precautionary reasons in order to minimize exposure to brominated flame retardants of the environment and humans including the breastfed infant. These apply to the products, penta- and octabromodiphenyl ether, both because of their higher toxic relevance compared with the decabrominated product, and because of their higher potential of bioaccumulation. Thus, the Seventh Regulation to Amend Legal Regulations on Chemicals (Siebte Verordnung zur Änderung chemikalienrechtlicher Verordnungen) stipulated a ban on the placing on the market and use of substances and products containing more than 0.1 % of pentabromodiphenyl ether or octabromodiphenyl ether, and was set into force on 15 August 2004 (Federal Gazette – Bundesgesetzblatt, 2003). The ban was based on a Directive of the European Union (European Union, Directive 2003/11/EC). In addition, the directive amending the WEEE Directive (Waste Electrical and Electronic Equipment) provides for the substitution of polybrominated flame retardants in electronic products from the beginning of 2008 (European Union, Directive 2003/108/EC).

2.2 PBDE in human specimens

2.2.1 Data on PBDE in breast milk and blood

PBDE have been detected in breast milk as well as in blood (including plasma or serum) and in human fatty tissue. Table 1 presents a synoptic view of current data on PBDEs in human specimens together with the corresponding references. If not quoted otherwise, the statements made below refer to the sources quoted in Table 1. The spectrum of PBDE congeners analyzed in the different studies was different in part. Although for this reason, the levels stated for total PBDEs as a sum of PBDEs analyzed are based on somewhat different data, such differences should not be of any significance because the predominant congeners were quantified in any case, and differences existed only in the quantification of minor components.

Temporal trends

PBDEs were detected in breast milk and human fatty tissue already 15 years ago (Krüger, 1988; Stanley et al., 1991). Krüger detected on average 2.64 ng/g fat in 25 breast milk samples from North Rhine-Westphalia. An exponential increase in PBDE levels with doubling every five years was reported in a retrospective Swedish breast milk study by Meironyte and Noren (1999, 2000) covering the period of 1972 – 1997. Since 1998, a reversion of trends has been associated with shifts in the pattern of congeners towards the high-brominated congeners, which has been attributed to the withdrawal of the

commercial PeBDE from the market in Sweden. Thus, in 2000, the average total PBDE level in breast milk from Sweden was 2.8 ng/g fat (Meironyte Guvenius, 2001). A similar trend in serum and breast milk was reported by Thomson et al., 2003, for Norway: The total PBDE levels increased from 0.5 ng/g fat to 4.1 ng/g fat between 1977 and 1998 and dropped to 3.0 ng/g fat until 2001. A doubling of BDE-47 levels in plasma within 5 years was also reported from the USA, with total PBDE levels increasing from ca. 2.5 ng/g fat in 1985 to 10.2 ng/g fat in 1990 and to a mean value of 66 ng/g fat in 2002 thus reaching considerably higher levels than in Europe (Sjödin et al., 2003). An increase of PBDE levels in breast milk on the Faeroe Islands from 1987 to 1998/99 from 1.5 to 7.2 ng/g fat, i.e. five times the first amount detected, was reported by Fängström et al., 2004. Also in blood samples from Germany, an increase of PBDE levels was observed between 1985 and 1999, which, however, was considerably less pronounced with a factor of 1.8 (Schröter-Kermani et al., 2000).

Data from Germany

So far, only a few studies on PBDE levels in human specimens from Germany have been published. In addition to the results by Krüger, more recent data on PBDE levels in blood or in breast milk have only been presented by Schröter-Kermani (2000), Fürst (2001), and Weber (2004). The data reported have been summarized in Table 1. Given mean total PBDE levels between 1.9 and 7.2 ng/g fat, the levels detected in Germany are in the range of the PBDE background exposure reported in different human matrices from other European countries. Reference has already been made to the temporal increase of PBDE levels in blood observed by Schröter-Kermani. The predominant congener has always been the tetrabrominated compound, BDE-47, followed by the congeners, BDE-153 and BDE-99. Their sum accounts for 70 – 80 % of the total PBDE levels detected.

International comparison of data / background levels

The mean PBDE levels detected in breast milk from Finland, Sweden, Norway, Italy, Belgium and the Netherlands have been in the range between 2.14 and 3.65 ng/g fat and thus are comparable as to the order of magnitude. Data from Great Britain and from the Faeroe Islands have shown that given average PBDE levels in breast milk of 6.6 and 7.2 ng/g fat, respectively, the background exposure is approximately twice as high in these countries, a fact suggesting possible additional exposure. In this context, Kalanzki et al. (2003) discussed the treatment of upholstered furniture, mattresses and other synthetic home textiles with flame retardants, which is legally prescribed in the UK and may contribute to elevated PBDE exposure of the consumer. Markedly elevated PCB levels in breast milk compared with data from other European countries were reported

from the Faeroe Islands. They are caused by the high quantities of fish and seal meat consumed by the local population. This could also be an approach to explaining the high PBDE levels found there.

Only few reports from Asia have become available so far. The lowest background exposure identified so far, i.e. an average total PBDE level of 0.5 ng/g fat in breast milk, was reported from Vietnam by Schecter (2004). Also PBDE levels in breast milk from Japan, namely a total PBDE level of 1.4 ng/g fat, have suggested a low exposure to exist in these Asian countries.

The total PBDE level in blood specimens from Australia was 11.0 ng/g fat and thus, markedly above those of European background exposure levels.

The highest PBDE levels in human specimens have been reported from the USA and Canada. Since the test persons had not been subject to any recognizable occupational exposure, the levels reported should rather reflect the local background exposure. Given average levels of between 22 and 86 ng/g fat in human specimens, these values are by a factor of 10 to 100 higher than the PBDE levels detected in Europe. Schecter et al. (2003) reported maximum levels of > 400 ng/g fat in breast milk. Mazdai et al. (2003) detected as much as up to 580 ng/g fat (total PBDE level) in maternal serum. A percentage of 95 % of the world production of technical PeBDE has been used in the USA (www.bsef.com, 2003). This fact might contribute to the elevated internal exposure in North America in comparison to Europe.

The patterns of congeners identified in human specimens from the different countries are very similar. The predominant congener identified in almost all studies is the tetrabrominated congener, BDE-47. Other predominant congeners identified included BDE-99, BDE-153 and partly also BDE-100, with their order varying depending on the country of origin of the specimens. While in the specimens from most European and the two Asian countries, the order observed was BDE-153 > = BDE-99 > = BDE-100, Schecter et al. (2003) reported that in breast milk from the USA, the shares of BDE-99, BDE-100 and BDE-153 in the total PBDE level were 17, 8.5 and 6 %, respectively. Such congener pattern , which is obviously characteristic of specimens from North America, has been confirmed in other studies (Mazdai, 2003; Sjödin, 2003, 2004a; Ryan, 2004) and is in contrast to the congener patterns described in human specimens from the European countries. Also the breast milk samples from the Faeroe Islands exhibited a congener pattern that was strikingly different: The level of BDE-153 was twice as high as that of the otherwise mostly predominating BDE-47. It is unknown to what extent this fact is to be attributed to other sources of exposure, such as a very specific diet.

Table 1: Mean PBDE levels (congeners analyzed and total PBDE levels) in human specimens with background exposure – Current international data (in ng/g fat)

Country	Year	Matrix	N	BDE-28	BDE-47	BDE-66	BDE-85	BDE-99	BDE-100	BDE-153	BDE-154	BDE-183	BDE-209	Total PBDE	Reference
Data from Germany															
Germany	before 1988	BM [1]	25											2.64	Krüger, 1888
Germany	1985	B [2]	20		3.1									3.9	Schröter-Kermani, 2000
	1990		20		3.6									4.9	
	1995		20		3.7									5.6	
	1999		20		3.9									5.6	
Germany	1992	BM	1 P[9]	0.12	0.83	0.01	0.02	0.28	0.18	0.45	0.04	0.02		1.9	Fürst, 2001
	2000		7	0.15	0.85	0.03	0.05	0.3	0.2	0.7	0.04	0.05		2.4	
Germany	2002	BM	8		2.9		0.1	2.2	0.6	1.2	0.1	0.2		7.2	Weber, 2004
Data from Europe															
Belgium	2000-01	BM	14	0.09	1.69			0.35	0.17	0.43	0.12			2.85	Pirard, 2003
Faeroe Islands	1998-99	BM	10		1.7			1.0	1.0	3.6				7.2	Fängström, 2004
Finland	1994-98	BM	11	0.16	1.31			0.39		0.39				2.25	Strandman, 2000
Italy	1998-2000	BM	39	0.06	1.2	0.02	0.04	0.51	0.28	0.49	0.04	0.10		2.75	Ingelido, 2004
Netherlands	1998	BM	108	0.11	1.19	< 0.06	<0.08	0.37	0.31	0.95	<0.08	0.41		3.65	Baumann, 2003
Norway	2003	BM	38											2.96	Polder, 2004

Country	Year	Matrix	N	BDE-28	BDE-47	BDE-66	BDE-85	BDE-99	BDE-100	BDE-153	BDE-154	BDE-183	BDE-209	Total PBDE	Reference
Norway	1999	S [7]	10	0.24	1.5			0.31	0.35	0.59	0.35			3.34	Thomsen, 2002
Sweden	1997-2000	B, M[3] B, F[4]												8.1 5.6	Lindström, 2004
Sweden	2000-01	BM	15	0.06	1.15	0.02	0.04	0.21	0.14	0.32	0.02	0.01		2.14	Guvenius, 2003
UK	2001-03	BM	52		3			0.9	0.6	1.4	0.5			6.6	Kalantzki, 2003
Data from Asia															
Japan	2000	BM	13	0.09	0.53	0.02	0.01	0.15	0.17	0.34	0.03	0.04		1.4	Akutsu, 2003
Vietnam	2003	BM	2	0.03	0.13	0.01	< 0.01	0.08	0.05	0.09	0.01	0.02		0.48	Schecter, Quynh, 2004c
Data from Australia															
Australia	2003	B	10 P[9]		4.7			2.3		2.0	0.2			11.0	Harden, 2003
Data from North and Central America															
Canada	1994-99	MP [8]	10 P[9]	0.8	10.9		0.5	5.6	2.0	2.3	0.5	0.8		23.3	Ryan, 2004
Canada	2001-02	BM	98		12.9			3.3		1.3	0.2			22	Ryan, 2004a
USA	2001	MS[5]	12		28			5.7	4.2	2.9	0.3	0		37	Mazdai, 2003
USA	2002	BM	47	2.4	40.8	0.65	1.15	14.0	8.2	5.3	0.76	0.13	0.92 [6] (7/23)	73.5	Schecter, 2003
Mexico	2003	BM	7		1.7			0.6	0.8	0.8	0.2		0.3	4.4	Lopez, 2004

[1] BM = Breast milk, [2] B = Blood, [3] M = Male, [4] F = Female, [5] MS = Maternal serum, [6] Quantified in 7 out of 23 samples analyzed, [7] S=Serum, [8] MP= Maternal plasma, [9] P = Pooled sample

PBDE levels in persons exposed at the workplace

Sjödin et al. (1999, 2001), Thuresson (2002) and Jakobsson (2002) reported on elevated PBDE levels in the blood of exposed Swedish workers in different fields of the electronics industry compared with non-exposed control groups. The studies included occupational groups specifically exposed to PBDE due to their work, which was confirmed by indoor measurements of PBDEs at the workplaces. The levels detected are condensed in Table 2.

Table 2: PBDE levels in the blood of exposed workers (medians and ranges; Sjödin 1999, 2001, Thuresson, 2002, Jakobsson, 2002)

Occupational group	Year	BDE-47 (ng/g fat)	BDE-153 (ng/g fat)	BDE-183 (ng/g fat)	BDE-209 (ng/g fat)
Electronics dimantlers	1997	2.8 (<0.5-22.4)	4.5 (2.1-12.2)	8.0 (2.2-18.8)	4.8 (<0.9-9.5)
Workers in electronic scrap recycling	1998	2.4 (<0.3-12.9)	1.3 (0.8-2.5)	<0.3 (<0.3-1.2)	2.3 (<1.0-5.6)
Rubber workers	2000	0.6 (0.3-1.9)	0.8 (0.3-2.2)	<0.4 (<0.4-0.9)	27.8 (1.2-144)
Workers in cable insulation	2000	0.6 (<0.5-3.2)	1.4 (<0.6-3.3)	all <1.4	34.6 (6.7-278)
Computer clerks	1999	1.3 (<1.0-13.3)	2.6 (<1.3-5.8)	0.9 (0.2-4.6)	1.5 (<1.0-6.8)
Clerks / office	1997	1.4 (<0.5-4.8)	0.8 (0.5-3.3)	0.2 (<0.01-1.0)	<0.7 (<0.7-7.7)
Control group 1: Hospital cleaners	1997	1.5 (<0.5-16.2)	0.6 (0.4-4.9)	0.1 (0.02-0.3)	<0.7 (<0.7-3.7)
Control group 2: Abattoir workers	2000	1.3 (<0.5-6.2)	2.0 (1.2-3.8)	all <0.3	2.4 (0.9-9.3)

The pattern of congeners observed in the blood samples of the different groups reflects their specific occupational exposure. Thus, elevated BDE-209 levels were detected in the blood of rubber workers and workers in cable insulation, who had been exposed to the technical DBDE only, while levels of the lower brominated BDE-47, BDE-153 and BDE-183 remained unchanged. In addition, elevated levels of nona- and octa-BDEs were detected in the blood indicating a metabolic debromination of BDE-209. In contrast,

workers in the field of electronics recycling are exposed to the different technical products, PeBDE, OBDE and DBDE, which is reflected in elevated levels of all four congeners analyzed. Altogether, a high dust contamination is found at the workplaces of these occupational groups. Therefore, potential routes of exposure of these workers include increased inhalational or oral uptake of particle-bound PBDEs.

In contrast, no potential exposure could be found in computer technicians or clerks in spite of their intensive contacts with computers. The PBDE levels detected in the blood of these groups were not significantly elevated compared with workers not exposed to computers. Obviously, their occupational exposure to PBDEs is low.

Detection of BDE-209 in human specimens

In 1999, the commercial product, DBDE dominated the world market of brominated diphenyl ethers with ca. 55,000 t/a accounting for ca. 81 % (www.bsef.com, 2001). Nevertheless, it was not until 1999 that Sjödin could quantify BDE-209 in the blood of Swedish workers and the control group and thus provide evidence for its bioavailability after elevated exposure. In 2003, Schecter et al. reported on the detection of BDE-209 in 7 out of 23 American breast milk samples, with these reflecting a markedly higher background contamination compared with European samples. The mean level of BDE-209 detected was 0.9 ng/g fat, and thus, the concentration ranges were comparable to the corresponding levels detected in blood samples from Swedish workers. So far, no data had been available on BDE-209 in breast milk samples from Europe.

Elimination half-life times of PBDEs in humans have already been dealt with above in Chapters 2.1.1-2.1.3. They are specific of congeners and vary considerably, and in contrast to the dioxins and PCBs, they become shorter with increasing degree of halogenation. It cannot be assessed to what extent the different results are caused by the different data sets, which are based on intake data of the background exposure on the one hand, and on elevated PBDE levels in the blood of exposed workers, on the other. However, it can be stated that the elimination half-life times established for the different PBDE congeners, which are in the range between 7 days and 11.6 years (as referred to the fat content), are shorter than the values established for persistent organochlorine compounds.

2.2.2 Prenatal exposure to PBDEs

Infants become exposed to PBDE not only in the postnatal period through breastfeeding but also in the prenatal period, because PBDEs can cross the placenta. This has been

documented by data from Sweden, Japan, Canada, the Netherlands and the USA (Guvenius et al., 2003; Hirai et al., 2000; Ryan and van Ostdaam, 2004; Weiss et al., 2004; Mazdai, 2003), among others. In the context of these studies for an estimation of the prenatal exposure of the fetus and on the efficacy of the placental barrier, PBDE levels were analyzed in matrices such as placenta and cord blood, partly also maternal blood and breast milk in samples from one and the same person, so that also conclusions with regard to the comparability of blood and breast milk levels should be possible.

While data from the USA by Mazdai et al. (2003) detected comparable PBDE levels in fetal and maternal serum in 12 paired samples, other studies demonstrated the efficacy of the placental barrier, i.e. a lower PBDE exposure of the embryo. Guvenius et al. (2003) reported mean total PBDE levels in cord blood of 1.69 ng/g fat (N = 15), while the mean levels stated for maternal plasma and breast milk were 2.07 (N = 15) and 2.14 ng/g fat (N = 15), respectively. While the levels detected in blood and those in breast milk as referred to the fat content were comparable, the authors detected only 72 % of these in the cord blood. The data by Ryan (2004) and Weiss (2004) have confirmed that levels detected in cord blood amount to ca. 40 - 80 % (Ryan et al.: total PBDE = 8.6 – 17.5 ng/g fat; Weiss et al.: total PBDE = 8.5 ng/g fat) of the total PBDE levels detected in the maternal plasma (Ryan et al.: total PBDE = 21.6 – 25.1 ng/g fat; Weiss et al.: total PBDE = 10.7 ng/g fat; N= 78). The data reported in the Japanese study by Hirai et al. (2000) have suggested that the relative PBDE levels in cord blood could be even lower compared with the maternal blood. In this study, the average sum of BDE congeners detected in the cord blood (0.3 ng/g fat; N= 4) was lower by a factor of 3 than in the placenta and in maternal blood (0.97 and 1.04 ng/g fat, respectively; N = 4) and even lower by a factor of 5 than in the breast milk (1.5 ng/g fat; N= 4). When assessing the embryonal exposure, however, also the much lower fat content of the cord blood compared with the maternal blood has to be taken into account, which means an additional reduction in the exposure of the embryo.

Comparisons between PBDE levels in the maternal blood and in the breast milk can be drawn by means of the quoted data by Guvenius (2003) and by Hirai (2000). While Guvenius observed comparable levels in both matrices, the results obtained by Hirai indicated PBDE concentrations in the blood to be lower by ca. 1/3. Due to the limited number of samples, however, the evidence provided by these results is restricted.

2.2.3 Routes of exposure

Like for organochlorine compounds, oral uptake through foods of animal origin has been assumed to be a relevant main route of exposure to PBDEs of the general population, due to the properties of bioaccumulation and persistence. (Darnerud, 2001; Sjödin 2000a, 2000b). This assumption has been supported by the bioaccumulation of PBDEs through the several trophic levels of the aquatic food chain (Darnerud, 2001; de Wit, 2002). However, their persistence is lower compared with the organochlorine compounds. Nevertheless, direct evidence has been lacking so far with regard to food indeed representing a relevant route of exposure to PBDEs.

Up to the present, only a few studies have been available for an estimation of the daily dietary intake of PBDEs. They were almost exclusively conducted as market basket studies. In these studies, the daily intake was calculated on the basis of the PBDE levels detected in relevant foods in connection with the corresponding amounts consumed. For Sweden, Darnerud et al. (2001) calculated an average daily intake of 51 ng/d for an adult on the basis of market basket studies. Current data from Sweden have confirmed this order of magnitude stating a mean daily intake of 41 ng/d for women (0.58 ng/kg b.w. per day; median = 28 ng/d, 0.43 ng/kg b.w. per day) (Lind, 2002). In this study, different groups of foods were analyzed for BDE-47, BDE-99, BDE-100, BDE-153 and BDE-154. The dominating factor was fish consumption accounting for ca. 74 % of PBDE intake, while the consumption of meat and meat products and of milk and milk products accounted for less then 10 % each of total PBDE intake. Another study by Lind documented somewhat lower data (27 ng/day) for PBDE intake, with fish consumption accounting for ca. 48 %. The authors discussed a low fish consumption of the group of subjects tested in this study as a cause for the low intake levels. The relevance of fish consumption in Scandinavian dietary habits for PBDE uptake was also demonstrated by Sjödin et al. (2000a), who detected statistically significant differences of BDE-47 levels in the blood of consumers eating large amounts of fish and those eating small amounts of fish. For Canada, Ryan and Patry (2001) calculated the mean dietary intake of PBDEs to be 44 ng/d. The study included more than 40 commercial food groups of animal origin with a high fat content. In Canada, the consumption of meat and meat products accounting for 75 % of the PBDE intake is dominating, while the consumption of milk and milk products and that of fish account for no more than 6 and 3 %, respectively. In Spain, Bocio et al., 2003, calculated a mean PBDE intake of 97 ng/d (1.4 ng/kg b.w. and day) by means of market basket studies. The studies included a wide range of foods of both vegetal and animal origin which are typical of the dietary habits of the country. Fish consumption

accounted for ca. 31 % of PBDE intake, the consumption of vegetable fats and oils, for ca 25 %, and the consumption of meat and meat products, for ca. 20 %. In contrast, milk and milk products and eggs together accounted for no more than ca. 11 % of PBDE intake levels. A duplicate study from Great Britain has confirmed the order of magnitude of PBDE intake levels obtained by means of market basket studies. The highest PBDE intake levels have been reported from the USA recently, i.e. 2.0 ng/kg b.w. and day. This value is 1.5 – 3 times higher than the data obtained in Europe, however, it cannot explain the markedly higher PBDE levels detected in North American human specimens.

Table 3: Dietary intake levels of PBDE and percentage share of the different food groups – International data

Country	Total PBDE intake	Percentage accounted for by consumption of						Reference
		Fish	Meat	Milk products	Fat / oil	Eggs	Other	
Sweden	51 ng/d 0.7 ng/kg b.w./d							Darnerud, 2000
Sweden	27 ng/d 0.4 ng/kg b.w./d	48%	11%	26%	13%	2%		Lind, 2001
Sweden	41 ng/d 0.6 ng/kg b.w./d	74%	6%	8%	11%	1%		Lind, 2002
Canada	44 ng/d 0.6 ng/kg b.w./d	3%	77%	6%			15% [1]	Ryan, 2001
Spain	97 ng/d 1.4 ng/kg b.w./d	31%	20%	9%	25%	2%	12% [2]	Bocio, 2003
UK[3]	90.5 ng/d 1.3 ng/kg b.w./d							Wijesekera, 2002
USA	2.0 ng/kg b.w./d	10%	50%	30%				Schecter, 2004b

[1] Other not specified, [2] Other = Fruit, vegetables, cereals, [3] Duplicate study

The dietary intake levels reported from the European countries (between 41 and 97 ng/day) have suggested that exposure through the diet in these countries is of a comparable order of magnitude. In contrast, there are clear differences with regard to the shares of the individual food groups characterized by the specific national dietary habits. The dominating factor identified was either fish or meat consumption. Both categories taken together account for between 50 and 80 % of the daily dietary intake of PBDEs. If indeed, the diet represents a relevant route of exposure to PBDEs, lower PBDE body burdens should be expected to occur in populations refraining from the consumption of

fish and meat. It has not been examined so far whether a vegetarian diet would indeed result in lower PBDE body burdens.

Another issue discussed has been whether exposure to PBDE by inhalation and ingestion of dust could make a relevant contribution to the background body burden. Studies by Knoth have demonstrated that bound to household dust, PBDEs occur which probably originate from emissions from equipment such as PC, TV, matresses and synthetic upholstery material treated with flame retardants. The mean total PBDE level detected in dust from vacuum cleaners in German households is 1,800 ng/g dry matter with a considerable variability observed for all congeners (up to a factor of 100). In almost all samples, BDE-209 is by far the most predominant congener, which does not comply with the patterns of congeners detected in human specimens (Knoth et al. 2002, 2003). In contrast, Sjödin et al. (2004b) reported mean PBDE levels of ca. 3,750 ng/g house dust from the USA, while the levels detected by these authors in dust samples from German households (mean total PBDE ca. 100 ng/g dust) were signifcantly lower, also compared with the values detected by Knoth. BDE-209 is the predominant congener in all samples. However, it remains unclarified to what extent the dust fractions examined can indeed penetrate into the lungs with regard to their particle size. Nevertheless, evidence has been provided that higher body burdens result from inhalational uptake of PBDEs at least under conditions of high occupational exposure associated with dust exposure, as demonstrated by PBDE levels detected in the blood of exposed workers (Sjödin, 1999, 2001; Thuresson, 2002; Jakobsson, 2002).

From indoor air measurements in computer workrooms and in private homes, Wijesekera et al., 2002, calculated a theoretical inhalational PBDE uptake of 33 ng/day, on the basis of an assumed 100 % absorption. Taking into account also the dietary exposure to PBDE of 90.5 ng/d, which was also calculated in the above study, oral ingestion would account for 73 % of PBDE uptake and inhalation, for 27 %. However, the fact that PBDE levels in the blood of computer technicians were comparable to those of clerks would rather suggest that no significant contribution to the PBDE body burden is made by this route (see Table 2, Sjödin, 2001).

3 Objectives of this study

From Germany, only few data on PBDE levels in human specimens have been available so far. Due to the low number of samples examined, such data are insufficient to characterize the situation with regard to the PBDE background exposure in Germany. However, above all because of the toxic potential of PBDEs ingested through the breast milk by the still developing and vulnerable newborn infant, it is important to study in detail the current PBDE levels in breast milk samples from Germany and to estimate the exposure of the breastfed infant. In addition, the possible influence of factors such as age, diet and earlier lactation periods, as known from the organochlorine compounds, on PBDE levels in breast milk has been unclarified so far. Therefore, the study presented was to address the following terms of reference:

1. Based on the data on PBDEs in breast milk samples from Germany compiled in the context of this study, the current background exposure to these contaminants was to be characterized. For this purpose, the spectrum of congeners to be analyzed was to include also the predominant congener of the technical OBDE, the heptabromodiphenyl ether BDE-183, and the predominant congener of the technical DBDE, the decabromodiphenyl ether, BDE-209, in addition to the PBDE congeners quantified so far in human specimens (tri- to hexabromodiphenyl ethers, BDE-28, BDE-47, BDE-66, BDE-85, BDE-99, BDE-100, BDE-153 and BDE-154).

2. Comparative analyses of PBDE levels in breast milk samples from omnivorous women and from vegetarians were to elucidate to what extent the consumption of fat of animal origin contributes to the PBDE body burden, similar to that of dioxins. For this purpose, the study hypothesis I was formulated as follows: PBDE levels in breast milk from vegetarians are significantly lower than those in breast milk from omnivores.

3. In contrast to the levels of persistent organochlorine compounds, there has been no study so far that could demonstrate an influence of the duration of the lactation period on PBDE levels in breast milk. In the present study, the influence of the lactation on the PBDE levels in breast milk was to be examined by means of two defined sampling times. Study hypothesis II was formulated: PBDE levels in breast milk are significantly lower after a 3-month lactation period than at the beginning of the lactation period.

4. For a characterization of the cohorts and for the identification of possible external influencing factors or other parameters and other data (e.g. diet and consumption frequencies, age, body mass index, smoking habits, occupational exposure), the corresponding information was to be recorded for each woman in an accompanying questionnaire and their relevance tested with regard to PBDE levels in breast milk.

5. Based on blood and breast milk samples from the same test person, the intraindividual comparability of levels of PBDE congeners in both matrices (as referred to the fat content) was to be analyzed.

4 Material and methods

4.1 Structure of the study, collection of data and samples

The study described is an observation study. A structured study design was developed to address the terms of reference of the study in a target-oriented way. The corresponding test protocol has been enclosed as Annex 1. The coordination of the study was performed by the Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV) and its successor institute, the Federal Institute for Risk Assessment (BfR). Authorizations were obtained from the Ethics Committee of the Berlin Chamber of Physicians and of the Federal Commissioner for Data Protection (Annexes 3 and 4).

4.1.1 Study design

Breast milk samples were collected from 2 cohorts differentiated by their dietary habits. The first sampling was carried out in the 2nd week after child delivery, a second sample was requested three months later from those mothers who had exclusively breastfed their child up to that time. In addition, a blood sample was to be collected from some mothers at the time of the first sampling. Table 4 summarizes the schedule of the sampling plan.

Table 4: Schedule of the sampling plan

	1st sampling time 8+/-1 days post partum (later: 7th- 14th day p.p.)	2nd sampling time 12th week post partum
Cohort 1: Omnivorous women	ca. 40 mL breast milk; N = 40 ca. 20-40 mL blood; N open	ca. 40 mL breast milk; N = 20
Cohort 2: Vegetarian women	ca. 40 mL breast milk; N = 40	ca. 40 mL breast milk; N = 20

The number of samples that could be collected was limited due to the funds available and the costs of analyses. The estimation of case numbers was based on data available on PBDE levels in the blood (Schröter-Kermani, 2002). On the basis of the standard deviations of PBDE levels established in this publication it was calculated that by one-tailed testing of the study hypotheses on a significance level of p= 0.05 involving 40 test persons per group, demonstration of a significant difference of mean values of 27 % should be possible.

The number of blood samples was difficult to plan from the beginning, due to volumes required of 20 - 40 mL that could have affected willingness of the test persons.

The test persons had to comply with the following inclusion criteria: Written consent to participation, normal course of pregnancy and healthy child, primipara and normal BMI. In addition, the mothers were to be of German origin in order to represent the corresponding dietary habits. Mothers who were on a mixed diet, i.e. including foods of animal origin, were assigned to Cohort 1, representing the omnivores. Cohort 2 consisted of mothers who had been on a lacto-ovo vegetarian diet for at least 5 years, i.e. excluding meat / meat products and fish / fish products, but including milk, milk products and eggs, or of mothers on a vegan diet, i.e. also refraining from the consumption of milk, milk products and eggs. Exclusion criteria were multigravidity and a long stay abroad (>6 months) within the last 5 years.

The study design planned and exclusion criteria had to be changed during sample collection in order to be able to collect the numbers of samples required. The study design was extended as follows:

- Sampling, which had initially been restricted to the Berlin region for pragmatic reasons, was performed on a national level.
- The time of first sampling was extended from initially the 8^{th} +/-1^{st} day post partum to the 2^{nd} week post partum.
- Mothers nursing their second or third child were included. Conditions included an unchanged housing environment, unchanged dietary habits, an approximately constant BMI and an interval of at least 2 years from the end of the last lactation period.

Prerequisites for a comparability of PBDE levels in breast milk from women nursing their first child and those nursing their second or third child included the reaching of a steady state again during the non-lactation periods, and comparable living conditions prior to and between the deliveries with regard to factors possibly influencing the individual PBDE levels. These parameters were controlled. The period required to reach the steady state of two years was estimated on the basis of the elimination half-life times for BDE-183 and BDE-209 by Sjödin et al. (1999, 2001).

Therefore, it has been assumed that the above changes in the study design had no significant influence on the test targets of the study. No changes were made to the estimations of case numbers.

4.1.2 Questionnaire

For the characterization of the cohorts of test persons and the recording of potential sources of exposure and influencing factors or other parameters and data, the

corresponding data for each woman were recorded in an accompanying questionnaire. These included also personal data, which required an authorization by the Federal Commissioner for Data Protection (see Annex 3).

Data recorded included, among others, the mother's age, body mass index (height, weight), country of birth, information on previous pregnancies and lactation periods, housing environment, information on extended stays abroad, on education and occupation (computer workplace), smoking habits and frequencies of consumption for 4 categories of foods of animal origin (meat / meat products, fish, milk / milk products, eggs). The questionnaire has been enclosed as Annex 2.

4.1.3 Recruitment of test persons

In the Berlin region, nursing puerperant women in obstetric hospitals were directly addressed and asked for their participation in the study. However, the number of women recruited, particularly among vegetarians, gave rise to concerns that the study could fail.

Under the headline, "Research for healthy breastfeeding", pregnant vegetarian women were addressed by the BgVV Press and Public Relations Office and a BgVV leaflet (see Annex 5) and asked for their support and participation in the study. This leaflet was displayed and distributed in the cooperating hospitals, the competent social-medical services, by obstetric practitioners and midwives and in birth centres and also by means of posting at the Berlin Universities. Other locations used included ecological shops for organic food, health food shops and vegetarian restaurants, in order to address vegetarians in a more target-oriented way.

The internet with its special forums (such as the Veganis-Forum, Rohkost-Forum, Vegan.de, Vegetarisch fit etc.) was used to inform vegetarian women on a national level about our study. Calls for participation in the study were also published in special national print media, such as the organic food magazines, "Schrot & Korn" and "natürlich vegetarisch" (see Annex 5). The latter magazine is published by the Vegetarier-Bund Deutschlands e.V. (Vegetarian Federation of Germany), with whom cooperation had been agreed upon with regard to the study. We were able to win Mr. PD Dr. Hahn, University of Hannover, as a cooperating partner for this project. In parallel, cooperation was initiated with the "Arbeitsgemeinschaft Freier Stillgruppen" (team of free breastfeeding groups), who published a call for participation in the study in their specialist journal, "Stillzeit" (see Annex 5). Only owing to the great number of these activities, it was eventually possible to recruit the number of test persons / vegetarians required for our study.

4.1.4 Collection and storage of samples

Samples were collected in the period between November 2001 and March 2004.

Altogether, 128 milk samples (of these, 89 first samples and 39 second samples) and 7 blood samples were collected from 89 women. 73 mothers complied with the inclusion criteria, of these, 41 were omnivores, 31 were vegetarians and 1 a vegan also refraining from the consumption of milk, milk products and eggs. In addition, samples were available from 4 extended breastfeeders (lactation periods of 8 – 23 months).

For sampling, the mothers were given specially cleaned and aluminium-wrapped glass vessels and a written instruction for sample collection. Milk collection was performed after breastfeeding the child by manual expressing of the residual milk directly into the test vessel. Blood sampling was performed either in obstetricians' practices or by the physician participating in the project, using specially cleaned and heparinized glass tubes.

As a rule, the samples were stored during the sampling period in the mother's refrigerator (ca. 1-2 days at 4 °C), and after collection of a sufficient quantity, in the freezer (at -20 °C).

In the Berlin region, the samples were picked up personally from the participants. On the national level, the glass vessels specially cleaned at the BfR, and the freeze packs and styrofoam boxes required for return were sent to the mothers by mail. The frozen and appropriately packaged samples were transported from the mother to the BfR by express courier service not later than on the next day after sampling and stored at the institute at -80 °C.

4.2 Analysis

4.2.1 Placing of order for analysis

The order for analysis was placed with an independent laboratory following an international restricted call for tender. Altogether, 13 applications were received. After review of the application documents on the reliability of the analytical method used including the quantification of BDE-209, on the limits of determination achieved and on the costs, the order was placed with the company, ERGO Forschungsgesellschaft m.b.H., Hamburg.

4.2.2 Preparation of sampling vessels / storage / dispatch of samples

For sample collection, 50 and 100 mL glass vials with screw caps and teflon seals were cleaned with double-distilled water, ethanol and dichloromethane (for residue analysis)

and wrapped with aluminium foil to protect the samples against photolytic debromination. The samples were dispatched by courier service to the independent laboratory under dry ice.

4.2.3 Sample preparation and quantification

For the determination of polybrominated diphenyl ethers in the breast milk and blood samples, the isotope dilution method was used. Detailed working instructions are presented in the final reports by the company, ERGO (Annexes 6 and 7).

Extraction including fat determination

After addition of ethanol, dipotassium oxalate and diethyl ether and the corresponding 13C-labelled diphenyl ethers (one internal standard for each degree of bromination to be determined, see Table 5), 10 g of the sample were extracted with pentane by liquid-liquid partition.

Blood was extracted twice with hexane and twice with hexane/isopropanol by liquid-liquid partition, after addition of ethanol and water and the 13C-labelled PBDE standards.

Gravimetric fat determination was performed after drying of the extract.

Clean-up of the extract

After uptake of the residue in hexane, purification was performed by means of solid-phase extraction first on a combination column consisting of sulfuric-acid treated silica gel, silica gel and alumina from which elution was performed with hexane. A second solid-phase extraction was carried out on a potassium silicate / silica gel / alumina column from which elution was performed with a hexane / dichloromethane mixture. After transfer of the eluate to toluene, 13C-BDE-139 was added as a injection standard. In order to prevent photolytic debromination of the PBDEs during preparation and storage of the samples, the flasks were wrapped with aluminium foil or amber glass vials were used.

Quantification

The separation and quantification of PBDEs was performed by means of gas chromatography (GC) using high resolution mass spectrometry (HRMS/EI) and the isotope dilution method, i.e. using the internal standards added prior to extraction, on a VG AutoSpec or a MAT95 by Finnigan. The components were identified by comparison of the retention times with the corresponding internal standards and the evaluation of two mass traces. Table 5 summarizes the mass traces used for the detection of the native PBDEs (m/z) and the limits of determination achieved.

Table 5: ^{13}C-labelled internal standards used, mass traces (m/z) for detection and quantification and mean limits of determination of the breast milk method

Congener	Internal standards ^{13}C-UL PBDE	Mass traces (m/z) native congener	LOD [1](ng/g fat)
BDE-128	2,4,4'- triBDE	405.803 / 407.801	0.0075
BDE-47	2,2',4,4'- tetraBDE	483.713 / 485.711	0.056
BDE-66	2,2',4,4'- tetraBDE	483.713 / 485.711	0.0075
BDE-99	2,2',4,4',5- pentaBDE	403.787 / 405.785	0.038
BDE-100	2,2´4,4´,6- pentaBDE [2]	403.787 / 405.785	0.0075
BDE-153	2,2',4,4',5,5'- hexaBDE	481.698 / 483.696	0.011
BDE-154	2,2',4,4',5,6'- hexaBDE	481.698 / 483.696	0.0075
BDE-183	2,2',3,4,4',5,6'- heptaBDE	561.606 / 563.604	0.015
BDE-209	2,2',3,3',4,4',5,6,6'- decaBDE	797.336 / 799.333	0.075

[1] LOD = Limit of determination; [2] only used from 4th.preparation series

4.2.4 Minimization of blank values

To minimize secondary contamination of samples during their preparation, which can markedly increase blank values in PBDE analysis, the following measures were taken: No use of plastic containers, special cleaning of all glass vessels with solvent, previous testing of all adsorbents and solvents, minimization of the volumes of solvents used, volume reduction in water bath without using a rotary evaporator, quick opening and closing of vessels. In each preparation series, blank value samples were analyzed. Positive findings in samples were only recorded if the sample exceeded the blank value by a factor of 2 at least.

4.2.5 Quality control / quality assurance

To control the correctness of analytical results (trueness, recovery), two deuterated quality control pools (breast milk samples fortified at 2 defined concentration levels with the native PBDE congeners) were analyzed together with each preparation series. The mean recovery rates were between 80 and 105 % at both concentration levels for all congeners to be analyzed. The precision of the analytical method was established by two independent ways:

a) To determine the standard deviation within a series (N = 6) and to determine the day-to-day standard deviation by analysis in each preparation series (N = 21), the

independent laboratory used a laboratory-internal breast milk pool with PBDE levels corresponding approximately to the background exposure in Germany.

b) 10 control samples of a breast milk pool were encoded together with the breast milk samples by the BfR and dispatched to the independent laboratory. Neither the exact number of these control samples of the pool nor the code numbers assigned to them were known to the laboratory. After submission of all analytical results, the day-to-day standard deviation was also calculated for these BfR control samples.

The relative standard deviations obtained by this way are summarized in Table 6. In addition, duplicate analyses were carried out for 8 samples. Their results are shown in the corresponding diagrams of Annex 6.

As expected, the relative standard deviation within a series is lower than that from day to day (determined by means of the laboratory-internal breast milk pool), the mean values of both test series are comparable. In both of these test series, the relative standard deviation for the predominant congeners, BDE-47, BDE-99 and BDE-153 is below 10 %, while the day-to-day precision determined for these three congeners by means of the BfR control samples is only slightly higher, i.e. 7 – 14 %.

The established parameters, correctness and precision have demonstrated that the analytical method is well suitable for the determination of PBDE background levels in breast milk.

Table 6: Results of quality control of PBDE determination in breast milk: Within-series precision and day-to-day precision (Päpke, 2004)

Congener	Within-series precision (N = 6)		Day-to-day precision (N = 16 - 21)		BfR control samples[1] (N = 10)	
	Mean value (ng/g fat)	RSD [2] (%)	Mean value (ng/g fat)	RSD [2] (%)	Mean value (ng/g fat)	RSD [2] (%)
BDE-28	0.036	14	0.032	10	0.02	32
BDE-47	0.73	1	0.69	6	0.57	14
BDE-66	0.009	13	0.0082	20	0.01	111 [3]
BDE-99	0.27	2	0.28	8	0.21	12
BDE-100	0.18	4	0.17	23	0.11	12
BDE-153	0.38	1	0.98	6	0.28	7
BDE-154	0.025	3	0.026	6	0.02	19
BDE-183	0.075	5	0.075	12	0.03	32
BDE-209	0.13	8	0.15	22	n.d. [4]	-

[1] corresponding to the day-to-day precision, [2] RSD = relative standard deviation, [3] concentration detected is near detection limit, [4] n.d. = not detectable

4.3 Statistical methods

4.3.1 Descriptive statistics

The cohort of test persons was characterized in terms of descriptive statistics with regard to age, height, weight and body mass index. The description includes the case number N, minimum and maximum, the mean and the standard deviation. The description was performed separately for the total cohort (all women recruited), the study cohort (all women complying with inclusion criteria), for the group of omnivorous women and that of vegetarian women also including the vegan. Data on smoking habits, number of breastfed children and country of birth were compared between the groups. The PBDE levels detected were characterized in terms of descriptive statistics both with regard to the single congeners and the total PBDE level (sum of single congeners), stating the number of samples N, the median, the maximum, the arithmetic mean, and the percentage share of single congeners in the total concentration (calculated from the mean values). Concentrations of single congeners that were below the limit of determination were included using half the value of the limit of determination.

4.3.2 Testing of hypotheses

Testing for normal distribution was performed both for the values measured and for their logarithmic values separately for both sampling times using the corresponding histograms and the Kolmogorov-Smirnov test of fit.

The study hypotheses were tested using the t test, always on the basis of the measured values in their logarithmic form. The significance level used was a = 5 %.

For the testing of study hypothesis I, the one-tailed t test for independent random samples was used. A comparison was made between the PBDE levels detected in breast milk samples from omnivores and in those from vegetarians collected at the first time of sampling.

For the testing of study hypothesis II, the mean values of the PBDE levels of the first and those of the second time of sampling were compared using the one-tailed t test for dependent samples. Only the samples from those mothers were included who had collected samples at both times of sampling.

The influence of the number of breastfed children on PBDE levels was tested using the one-tailed t test for independent random samples on the basis of the data of the first sampling time in their logarithmic form. The samples from primiparae and multiparae were assigned to two corresponding test groups.

The influence of the type of diet on the values measured was compared with that of the number of breastfed children by multiple linear regression. For this purpose, the data from the first time of sampling were used. This linear model of logarithmic values was converted into a mutiplicative model for the non-logarithmic data. The calculated factors of this multiplicative model permit a comparison between the influence of the type of diet and the influence of the number of breastfed children on the values measured.

4.3.3 Explorative data analyses

The testing for possible correlations between the PBDE levels and potential influencing factors such as age, BMI, hours of screen exposure and smoking habits was performed by means of scatter diagrams or box-whisker plots using the data in their logarithmic form.

For the metrically scaled parameters, age, BMI and hours of screen exposure, the Pearson correlation coefficient was calculated, and for the categorial variable of smoking habits, the Spearman correlation coefficient. As a basis, the logarithmic PBDE levels detected at the first time of sampling were used.

5 Results

Below, the term of total cohort is used to refer to the data and samples from all mothers, irrespective of the compliance with the inclusion and exclusion criteria described in Chapter 4.1. In contrast, the data and samples referred to as belonging to the study cohort include only those from mothers complying with the inclusion criteria. The data of the study cohort formed the basis for the testing of the study hypotheses. The study cohort was divided into Cohort 1, representing the omnivorous women, and Cohort 2, representing the vegetarians and the vegan.

5.1 Characterization of the study populations

The total cohort consisted of 89 mothers of which 73 complied with the inclusion criteria and were assigned to the study cohort. 16 test persons did not comply with the inclusion criteria: 15 of these had collected the first sample at a much later time, and one woman was nursing her fourth child already. The study cohort consisted of 41 omnivores (Cohort 1) and 31 vegetarians and 1 vegan, of which the latter was assigned to the vegetarians, if not specified otherwise (Cohort 2).

The different evaluation groups of the total cohort, the study cohort, the omnivores and the vegetarians were characterized with regard to the parameters of age, hight, weight at the first time of sampling, body mass index, hours spent working on the PC and watching TV, smoking habits, number of breastfed children and country of birth by descriptive statistics for the metric parameters and by frequency distribution for the non-metric data. The data have been condensed into Table 7.

There are no differences found between the total cohort on the one hand and the study cohort on the other, with regard to the parameters of age, height, weight and BMI. In addition, only minor differences exist between these two cohorts with regard to the smoking habits, the number of breastfed children and the countries of birth. Hence, the two cohorts are well comparable with regard to the distribution of all parameters mentioned. Therefore, possible differences in the PBDE levels between these two groups are rather coincidental and not to be attributed to the systemic influence of any of the parameters.

Also for the groups of omnivores and vegetarians, the descriptive statistic data for age, height, body weight and BMI show an equal distribution. In contrast, a comparison of hours of screen exposure, smoking habits, number of breastfed children and country of birth show marked differences between these thwo groups. For example, vegetarians

work on the PC and watch TV less often. Although the share of non-smokers is higher in the group of vegetarians (56 %) than in that of omnivores (46 %), this difference is not statistically significant. In addition, the vegetarians have breastfed more children, on average. Only 2 of the vegetarians originated from the new federal Länder, while 32 % (N = 13) of the omnivores were born in these East German Länder. These parameters will have to be taken into account when discussing possible differences in PBDE levels between the two cohorts.

Table 7: Characterization of the evaluation groups, total cohort, study cohort, omnivores, and vegetarians with regard to age, height, weight, body mass index (BMI), hours of screen exposure, smoking habits, number of breastfed children, country of birth

		Total cohort	Study cohort	Omnivores Cohort 1	Vegetarians Cohort 2
		N = 89	N = 73	N = 41	N = 32
Age (years)	Mean ± SD	31.9 ± 5.7	31.8 ± 5.6	31.9 ± 5.8	31.7± 5.3
	Min – Max	18 - 44	18 - 44	18 - 44	19 - 42
Height (cm)	Mean ± SD	167.9 ± 7.0	167.8 ± 7.1	167.8 ± 7.3	167.9 ± 6.9
	Min – Max	153 -184	153 - 182	153 - 182	156 - 182
Weight (kg) current[1]	Mean ± SD	64.9 ± 9.0	65.2 ± 8.8	65.3 ± 8.6	65.1 ± 9.2
	Min – Max	48 - 89	48 - 89	48 - 82	51 - 89
BMI (kg/m^2) current[1]	Mean ± SD	23 ± 2.9	23.1 ± 2.9	23.2 ± 2.8	23.1 ± 2.9
	Min – Max	18.1 - 32.3	18.1 - 32.3	18.1 - 29.0	18.6 - 32.3
Screen exposure per week[2]	Mean ± SD		32.4 ± 20.8	37.5 ± 21.5	25.8 ± 18.0
	Min – Max		0 - 80	4 - 80	0 - 65
Smoking habits	Non-smoker	51 (57%)	37 (51%)	19 (46%)	18 (56%)
	Former smoker	33 (37%)	31 (42%)	18 (44%)	13 (41%)
	Smoker	5 (6%)	5 (7%)	4 (10%)	1 (3%)
Number of breastfed children	1 child	56 (63%)	51 (70%)	31 (76%)	20 (63%)
	2 children	23 (26%	17 (23%)	8 (19%)	9 (28%)
	3 children	9 (10%)	5 (7%)	2 (5%)	3 (9%)
	4 children	1 (1%)			
Country of birth	Germany (West)	70 (78%)	56 (77%)	26 (63%)	30 (94%)
	Germany (East)	17 (19%)	15 (21%	13 (32%)	2 (6%)
	Poland	1 (1%)	1 (1%)	1 (2%)	
	Switzerland	1 (1%)	1 (1%)	1 (1%)	

[1] current = first time of sampling
[2] Hours of screen exposure per week as a sum of the hours spent on TV and on PC (private and work purposes)

Significant differences between the group of omnivores and that of vegetarians include, of course, their dietary habits. While the frequencies of consumption both of milk and milk

products and eggs do not show any difference, the difference between the frequencies of consumption of meat and meat products and fish and fish products is statistically significant. Table 8 provides a synoptic view. It has to be mentioned that on recommendation of their obstetricians, some of the vegetarians also consumed low amounts of fish for the limited period of their pregnancy.

Table 8: Comparison of consumption frequencies of foods of animal origin between omnivores (Cohort 1) and vegetarians (Cohort 2)

	Cohort	Frequency of replies (in %) Number of meals						Consumption frequency per month				Difference
		Almost never	Once per month	2-3 times per month	Once per week	2-3 times per week	Almost daily	Min	Max	MV	SD	P value
Fish	1	0.0	24.4	41.5	29.3	4.9	4.9	1	10	2.9	1.96	0.000
	2	80.6	3.2	16.1	0.0	0.0	0.0	0	2.5	0.4	0.94	
Meat	1	9.8	0.0	4.9	14.6	51.2	19.5	0	30	11.7	9.76	0.000
	2	100	0.0	0.0	0.0	0.0	0.0	0	0	0.00	0.00	
Milk	1	0.0	0.0	0.0	4.9	19.5	75.6	4	30	24.8	9.29	0.915
	2	9.7	0.0	0.0	0.0	6.5	83.9	0	30	25.8	9.92	
Eggs	1	2.4	2.4	12.2	56.1	22.0	4.9	0	30	6.2	6.14	0.767
	2	16.1	3.2	22.6	29.0	22.6	6.5	0	30	5.9	7.28	

5.2 PBDE levels in the total cohort and the study cohort

The PBDE levels detected in the total cohort (N = 89) and in the study cohort (N = 73) have been summarized in Table 9. Only the samples collected at the first time of sampling were taken into account, measured values below the limit of determination were included with half the limit of determination.

The mean total PBDE level for the total cohort is 2.49 ng/g fat. The maximum level detected of 17.8 ng/g fat was strikingly high. The sample exhibiting the strinkingly high contamination level originated from a mother who did not comply with the inclusion criteria. The questionnaire completed by this mother did not provide any information on a possible specific exposure. The levels detected in the study cohort were slightly lower, with a mean of 2.11 ng/g fat and a maximum level detected of 7.25 ng/g fat.

Attention is particularly drawn to the detection of the decabrominated congener, BDE-209, which was quantified in ca. 50 % of all samples, with mean levels detected of 0.21 ng/g fat and a maximum detected of 4.5 ng/g fat (total cohort).

The median as the more robust parameter is less prone to outliers, its comparison shows a very good correspondence for all congeners. The exclusion of the samples and women, respectively, who did not comply with the inclusion criteria did not change the PBDE concentrations (medians) of congeners. Also the percent composition with regard to the two cohorts is almost the same. Therefore, the statements made below are only based on the data of the study cohort.

Table 9: PBDE concentrations in breast milk samples from the total cohort and the study cohort (in ng/g fat; levels < LOD included as 50 % of LOD level; 1st time of sampling)

	Total cohort; N = 89					Study cohort; N = 73				
	MV	Median	Max	%[1]	N<LOD[2]	MV	Median.	Max	%[1]	N<LOD[2]
BDE-28	0.04	0.03	0.03	2%	17%	0.04	0.03	0.17	2%	15%
BDE-47	0.91	0.51	6.8	37%	2%	0.76	0.53	4.5	36%	1%
BDE-66	0.01	0.01	0.2	0%	30%	0.01	0.01	0.06	0%	30%
BDE-99	0.38	0.16	6.4	15%	3%	0.23	0.14	1.3	11%	3%
BDE-100	0.26	0.15	2.2	10%	0%	0.2	0.15	1.1	9%	0%
BDE-153	0.59	0.49	1.9	24%	0%	0.6	0.5	1.9	28%	0%
BDE-154	0.03	0.02	0.35	1%	7%	0.02	0.02	0.07	1%	4%
BDE-183	0.08	0.03	0.63	3%	17%	0.08	0.03	0.63	4%	19%
BDE-209	0.21	0.10	4.5	8%	51%	0.17	0.1	1	8%	53%
Total PBDE	2.49	1.72	17.8	100%		2.11	1.75	7.25	100%	

[1] Percentage share of the congener in total PBDE level
[2] Percentage share of the measued values below the limit of determination (LOD)

The predominant congener is the tetrabrominated congener BDE-47 (36 % of the total PBDE level), followed by the hexabrominated congener BDE 153 (28 %) and the pentabrominated congener BDE-99 (11 %). The sum of these three predominant congeners accounts for more than 75 % of the total body burden. In contrast, the tribrominated congener BDE-28, the tetrabrominated congener BDE-66, and the hexabrominated congener BDE-154 with shares of < 2 % each are to be considered as minor components.

The pattern of congeners, i.e. the shares of the single congeners in the total PBDE level is depicted in Fig. 1.

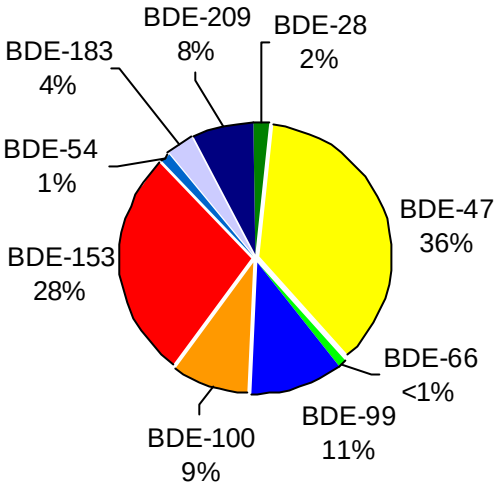

Fig. 1 Shares of single PBDE congeners in the total PBDE level in breast milk samples from Germany

5.3 Testing for normal distribution

The levels of environmental contaminants, such as dioxins, in human specimens follow a log-normal distribution. It was to be demonstrated whether this fact also applies to PBDE levels in breast milk. Another reason to test the distribution function of PBDE levels in breast milk was that it had to be decided whether the t test or parameter-free tests had to be used for the testing of the study hypotheses.

The samples of the study cohort formed the basis for the testing of the study hypotheses, because only these complied with the inclusion criteria. The data were tested for their distribution function separately by the first and second time of sampling.

The histograms of the total PBDE level of the study cohort with curves of the normal distribution shown have been depicted in Fig. 2 and Fig. 3. They demonstrate also the PBDE levels in breast milk to follow a log-normal distribution. This is confirmed by the Kolmogorov-Smirnov test of fit for both times of sampling, both for the whole study population and for the two cohorts of omnivores and vegetarians, and also for all single congeners and the total PBDE level.

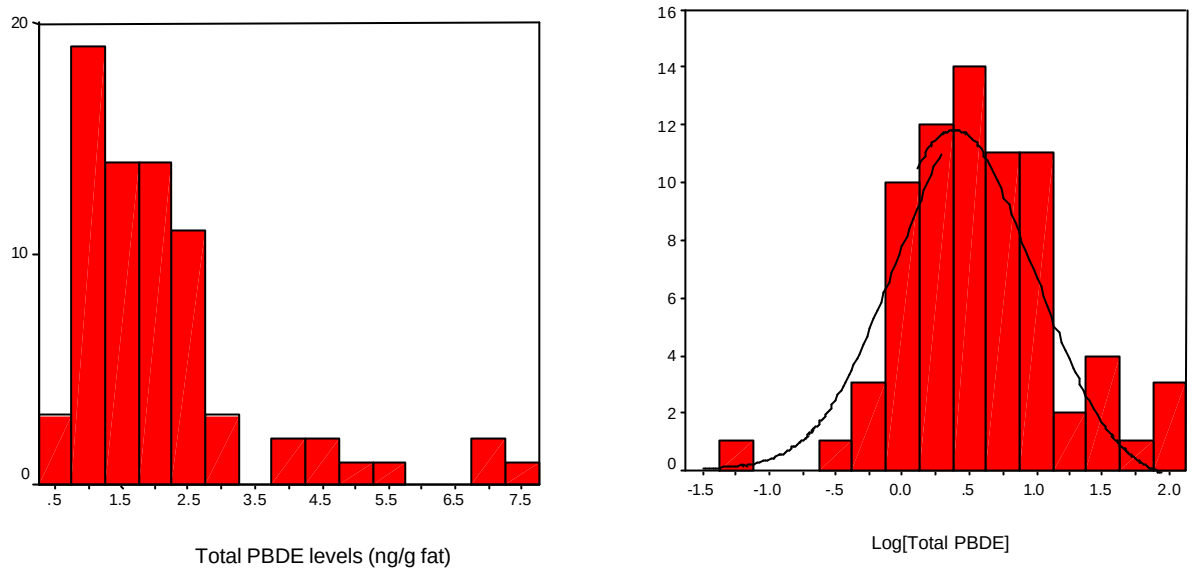

Fig. 2: Histograms for total PBDE levels and log [total PBDE] in breast milk from the first time of sampling including the curve of the normal distribution (study cohort; N = 73)

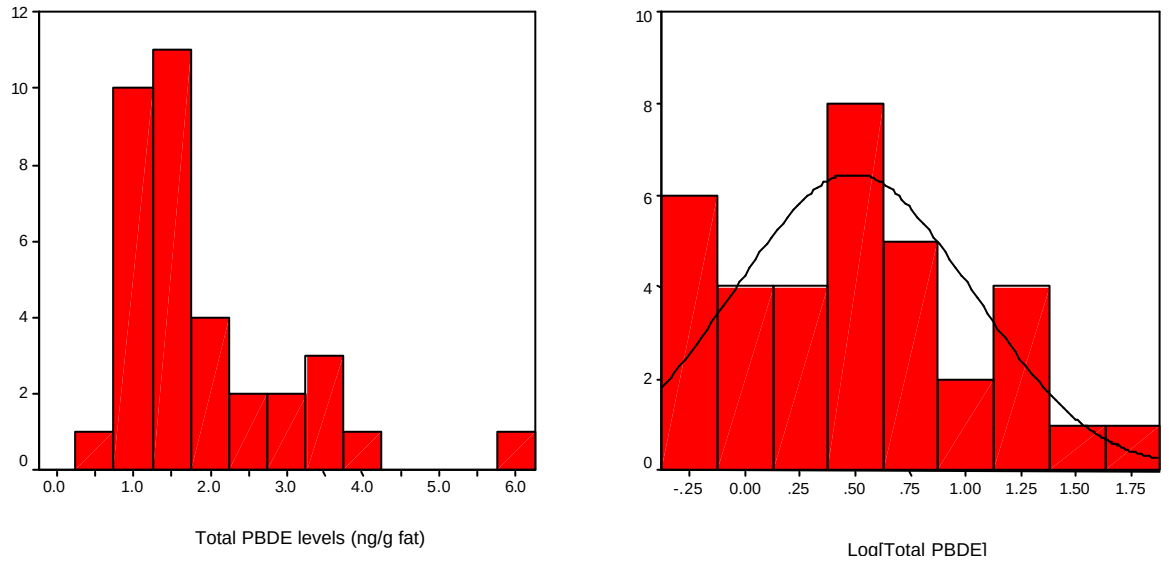

Fig. 3 Histograms for total PBDE levels and log [total PBDE] in breast milk from the second time of sampling including the curve of the normal distribution (study cohort; N = 35)

Given the confirmed log-normal distribution, the t tests on the basis of the logarithmic values were used for statistical testing of the study hypotheses I and II according to the study design.

5.4 Influence of the diet on PBDE levels in breast milk

5.4.1 Testing of study hypothesis I: Differences between omnivores and vegetarians

It was formulated in study hypothesis I that PBDE levels in breast milk samples from vegetarians (Cohort 2) are significantly lower than those in samples from omnivores (Cohort 1). For the statistical evaluation, the sample from the vegan woman was also included in Cohort 2.

The possible influence of the different dietary habits was visualized by means of the box-whisker plot by graphical comparison of the total PBDE levels in breast milk samples from omnivores, vegetarians and one vegan. The tendency becomes clear that partial or complete refraining from the consumption of foods of animal origin reduces the PBDE body burden and thus, PBDE levels in breast milk. The reservation must be made that a single sample provides only limited evidence.

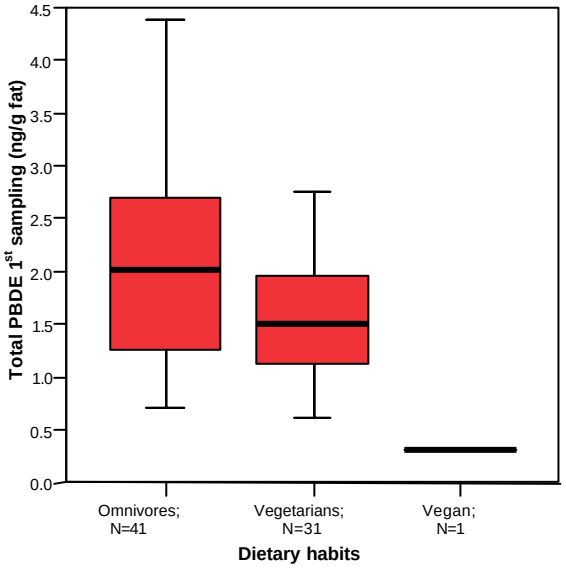

Fig. 4: Box-whisker plot for total PBDE levels in breast milk samples from omnivores, vegetarians and one vegan (1st time of sampling)

The one-tailed t test was used to test the significance (significance level of 0.5) of mean value differences between the two cohorts on the basis of the logarithmic values of the first time of sampling. All mothers included belonged to the study cohort, i.e. they complied with the inclusion criteria

The mean PBDE levels for both cohorts and the results of the statistical test have been summarized in Table 10.

Table 10: Comparison between the mean values of PBDE levels in breast milk from omnivores (Cohort 1) and those from vegetarians/vegan (Cohort 2), and results of the one-tailed t test (1st time of sampling)

	Cohort 1 N = 41 (ng/g fat)	Cohort 2 N = 32 (ng/g fat)	Difference of MV	t test p value
BDE-28	0.04	0.04	0 %	0.109
BDE-47	0.95	0.53	- 44 %	0.005*
BDE-66	0.013	0.0085	-34 %	0.003*
BDE-99	0.29	0.15	- 48 %	0.001*
BDE-100	0.23	0.16	- 30 %	0.001*
BDE-153	0.66	0.52	- 21 %	0.013*
BDE-154	0.03	0.02	- 33 %	0.000*
BDE-183	0.09	0.07	- 22 %	0.032*
BDE-209	0.17	0.16	- 6 %	0.375
Total PBDE	2.47	1.65	- 33 %	0.005*

* Difference is significant

The mean levels of all PBDE congeners and thus, also the total PBDE is lower in the samples from vegetarians than in those from omnivores. The mean levels of relevant congeners in Cohort 2 are by 21 – 48 % lower than those in Cohort 1.

The differences are statistically significant for the congeners, BDE-47, BDE-66, BDE-99, BDE-100, BDE-153, BDE-154, and BDE-183 and of course for the total PBDE level, while the congeners, BDE-66 and BDE-154 represent only minor components. No statistically significant difference in mean values between the two cohorts could be found for the congeners, BDE-28 and BDE-209. This has been illustrated by the box-whisker plots below for the congeners with statistically significant differences in mean values and quantitatively relevant levels.

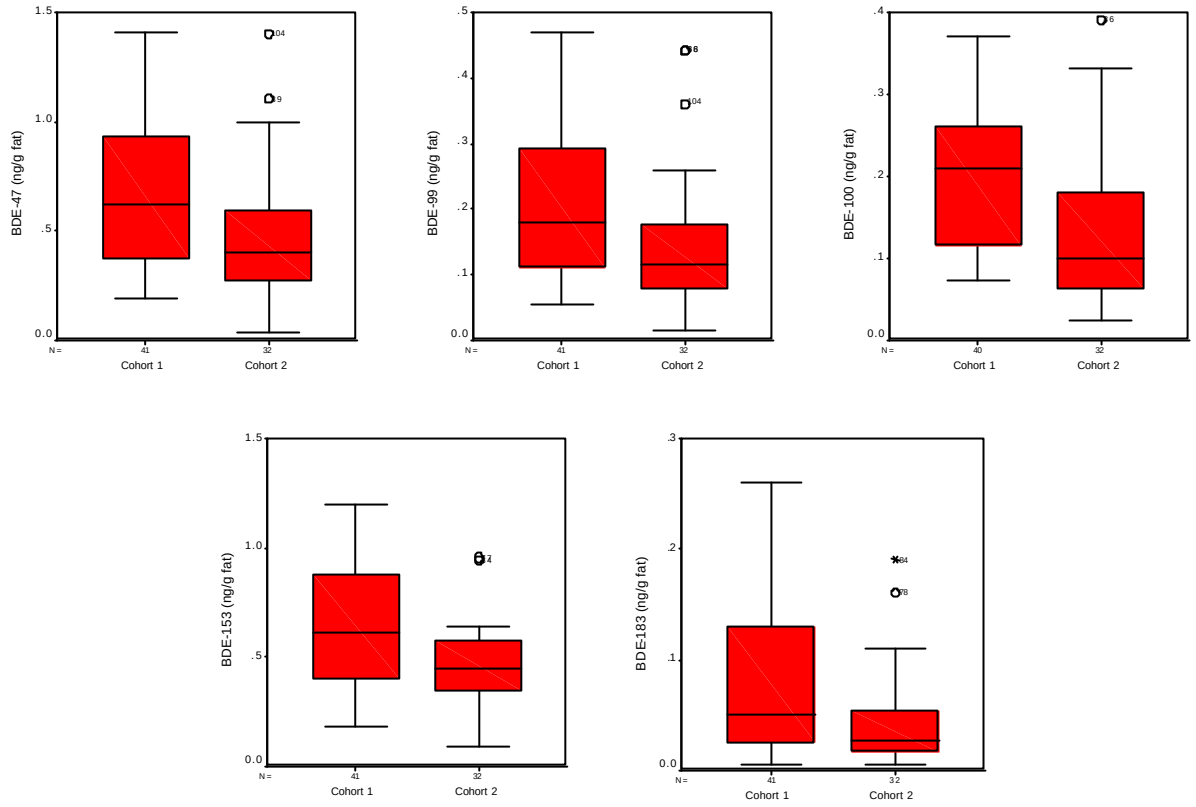

Fig. 5: Comparison of levels of BDE-47, BDE-99, BDE-100, BDE-153 and BDE-183 between Cohorts 1 and 2 by means of box-whisker plots

The study hypothesis I, stating that vegetarians exhibit statistically significantly lower PBDE levels in their breast milk than omnivores, has been confirmed by means of the t test. Therefore, a relationship between the consumption of foods of animal origin and the PBDE body burden is probable for the majority of congeners.

As explained in Chapter 5.1, however, the group of vegetarians includes a higher share of mothers breastfeeding their second or third child. This issue is discussed in more detail with regard to the influence on the PBDE levels under 5.5.2 and in Chapter 5.6.

5.5 Influence of breastfeeding on PBDE levels

5.5.1 Testing of study hypothesis II: Changes in PBDE levels during the lactation period

The levels of organochlorine compounds in breast milk are known to decrease during the lactation period. Reductions of ca. 30 % within the first 3 months were reported (Beck, 1992). It was unknown at the beginning of this study whether this phenomenon also applies to PBDEs in breast milk. Therefore, the study hypothesis II was generated stating

that PBDE levels are significantly lower after a 3-month lactation period than in the 2nd week p.p. The number of samples included had to be sufficient to verify significant differences of ca. 30 %. The testing of this hypothesis was based on the samples from those women who had exclusively breastfed their child for 12 weeks and who had collected a sample both at the first and at the second time of sampling. Due to non-compliance with the protocol (e.g. first samples were delayed in four cases), only 35 of the total of 39 paired samples could be included in this evaluation. The group consisted of 19 omnivores and 16 vegetarians.

The mean values of the PBDE levels of the 1st and 2nd sample were compared on the basis of the data in their logarithmic form using the one-tailed t test for paired samples. Table 11 summarizes the mean values of the congeners for both times of sampling and also the result of the t test.

Table 11: Mean values of PBDE levels in breast milk sampled at the 1st and 2nd time of sampling, and results of the paired t test.

	1st sample N=35 (ng/g fat)	2nd sample N=35 (ng/g fat)	t test p value
BDE-28	0.04	0.04	0.24
BDE-47	0.81	0.74	0.47
BDE-66	0.012	0.009	0.03*
BDE-99	0.24	0.24	0.18
BDE-100	0.18	0.18	0.22
BDE-153	0.51	0.48	0.06
BDE-154	0.02	0.02	0.21
BDE-183	0.06	0.07	0.06
BDE-209	0.14	0.12	0.43
Total PBDE	2.03	1.89	0.42

* Difference is significant

A statistically significant difference could only be demonstrated for the minor component BDE-66, which is of no relevance due to its minimal share in the total PBDE level. Slightly lower mean levels were detected of BDE-47, BDE-153 and BDE-209, and total PBDE after 12-weeks of breastfeeding. However, the differences are too little and statistically irrelevant with regard to the variability of data and the number of samples tested.

The scatter diagrams illustrate the results of the t test for total PBDE levels.

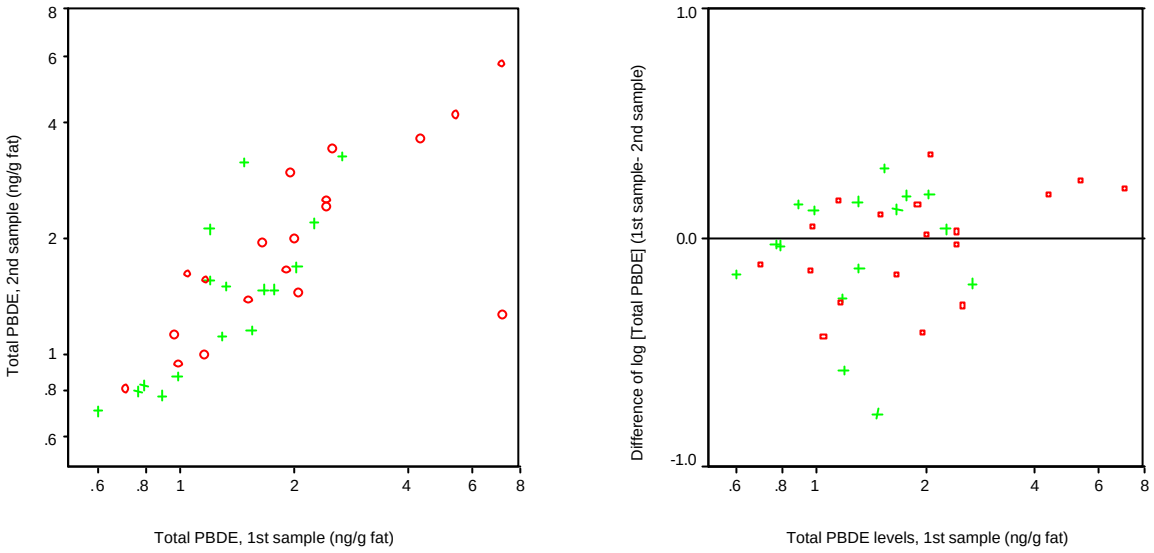

Fig. 6: Comparison of the individual total PBDE levels of the 1st and 2nd sample; left: representation of the levels; right: representation of the differences (circles = Cohort 1, crosses = Cohort 2)

The correlation between the total PBDE levels of the 1st and 2nd times of sampling is depicted in Fig. 6, left. Also the differences observed between the individual paired samples do not show a uniform trend, as illustrated in the scatter diagram right.

The study hypothesis II stating that after a 12-week lactation period, the PBDE levels are significantly lower than at the beginning (2nd week post partum), cannot be confirmed.

5.5.2 Influence of the number of breastfed children on PBDE levels in breast milk

Extended periods of breastfeeding and breastfeeding of more than one child resulted in a marked reduction of the body burden of dioxins in the mother (4th Report by the Bund/Länder AG Dioxine, 2002). Thus, the postnatal exposure of the second or third child is lower than that of a first-born child. This could not be confirmed for PBDEs in previous studies.

The extension of the test criteria and inclusion of mothers breastfeeding their second or third child provided the chance to examine the influence of breastfeeding with regard to its relevance for PBDE contamination of breast milk in another way and to possibly verify the minimal and insignificant differences observed after a 12-week lactation period (study hypothesis II).

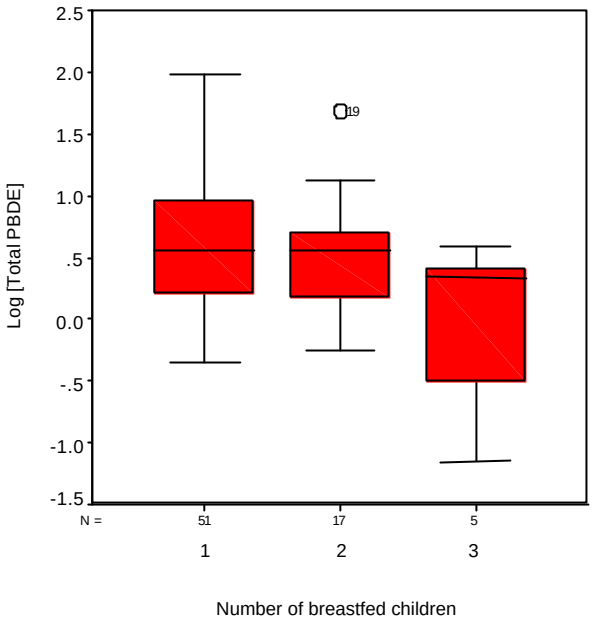

Fig. 7 Box-whisker plot of total PBDE levels (in logarithmic form) depending on the number of breastfed children (1st time of sampling)

The box-whisker plot illustrates that the total PBDE contamination of breast milk shows a decreasing tendency with increasing number of breastfed children.

Testing of the statistical significance of this difference was performed by means of the one-tailed t test and using the logarithmic PBDE levels detected at the first time of sampling. Samples available for evaluation included those from 51 mothers breastfeeding their first child, from 17 mothers breastfeeding their second child and from 5 mothers breastfeeding their third child. For the statstical test, the samples from mothers breastfeeding their second and third child were assigned to one evaluation group because otherwise, the number of samples would have been insufficient. Also a separate evaluation for the samples from omnivores and vegetarians was impossible due to the low numbers of samples. The results are summarized in Table 12.

The PBDE levels in the breast milk samples from multiparae are lower than in those from primiparae by up to 42 %. Significant differences could be shown for the predominant congener BDE-47, and the congeners BDE-99, BDE-100 and BDE-154 and the total PBDE levels. The congeners BDE-47, BDE-99 and BDE-100 altogether account for 57 % of the total PBDE level, on average, while BDE-154 is a minor component only. The decrease found for the second predominant congener BDE-153, is strikingly low: No significant difference can be established. Therefore, the order of the predominant congeners in the samples from multiparae shifts to BDE-153 > BDE-47 > BDE-99.

Table 12: Mean values of PBDE levels in breast milk from mothers breastfeeding their first child vs. those from mothers breastfeeding their second or third child, and results of comparison of mean values by the t test

	Primiparae N = 51 (ng/g fat)	Multiparae N = 22 (ng/g fat)	Difference of MV	t test p value
BDE-28	0.04	0.04	-6%	0.100
BDE-47	0.87	0.51	-41%	0.005*
BDE-66	0.012	0.008	-30%	0.066
BDE-99	0.26	0.15	-42%	0.007*
BDE-100	0.22	0.14	-36%	0.005*
BDE-153	0.61	0.58	-5%	0.180
BDE-154	0.023	0.018	-24%	0.025*
BDE-183	0.09	0.07	-22%	0.160
BDE-209	0.16	0.17	6%	0.250
Total PBDE	2.29	1.69	-26%	0.03*

* Significant difference found

A higher total number of children breastfed by the mother results in significantly lower total PBDE levels, with differences existing between the single congeners tested. In addition to the testing of study hypothesis II it can be stated that a significant influence of breastfeeding on the PBDE body burden is found if the number of breastfed children is evaluated as an influencing factor.

5.5.3 Influence of the duration of the lactation period on PBDE levels in extended breastfeeders

Among the subjects of the total cohort were 4 extended breastfeeders. These were not included in the study cohort, but are discussed below as individual cases. Description of the mothers:

1.) 36-year-old vegetarian breastfeeding her third child for 20 months.
2.) 26-year-old vegetarian breastfeeding her second child for almost 8 months, i.e. a lactation period to be referred to as extended breastfeeding only through the time window of this study.
3.) 26-year-old vegetarian breastfeeding her second child for 19 months.
4.) 38-year-old vegetarian breastfeeding her third child for 23 months.

The PBDE levels in the milk samples from these four women have been summarized in Table 13.

Table 13: PBDE levels in milk samples from extended breastfeeders

	1st test person		2nd test person		3rd test person		4th test person	
	ng/g fat	%	ng/g fat	%	ng/g fat	%	ng/g fat	%
BDE-28	0.25	1.4 %	0.0079	0.9 %	0.0069	0.7 %	n.d.(0.01)[1]	0.4 %
BDE-47	6.8	38.3 %	0.26	30.2 %	0.19	20.4 %	0.24	21.2 %
BDE-66	0.2	1.1 %	0.012	1.4 %	n.d.(0.01)[1]	0.5 %	n.d.(0.01)[1]	0.4 %
BDE 99	6.4	36.0 %	0.11	12.8 %	0.077	8.3 %	0.17	15.0 %
BDE-100	2.2	12.4 %	0.069	8.0 %	0.039	4.2 %	0.100	8.8 %
BDE-153	1.3	7.3 %	0.30	34.8 %	0.52	55.9 %	0.46	40.6 %
BDE-154	0.35	2.0 %	n.d.(0.01)	0.6 %	n.d.(0.01)	0.5 %	0.019	1.7 %
BDE-183	0.068	0.4 %	0.052	6.0 %	0.012	1.3 %	0.13	11.5 %
BDE-209	0.2	1.1 %	0.046	5.3 %	0.075	8.1 %	n.d.(0.01)	0.4 %
Total PBDE	17.8	100 %	0.85	100 %	0.92	100 %	1.1	100 %

[1] n.d. = not detectable, data in brackets are the experimental limits of detection

The first test person shows an extraordinary total PBDE level of 18 ng/g fat. This is the highest level detected in this study. Another striking fact is the different pattern of congeners. While the common order in the samples of the multiparae is BDE-153 (34 %) > BDE-47 (30 %) >> BDE-99 (9 %), the share of BDE-99 (36 %) is markedly higher in the milk sample from this test person. Additionally, BDE-153 (7 %) accounts for an even lower percentage in the total PBDE level than BDE-100. The order observed in the case of the first test person, i.e. BDE 47 ˜ BDE 99 >> BDE 100 > BDE 153, is found to differ markedly from the characteristic patterns of congeners of the samples from the study cohort and from the multiparae, but also from the other three extended breastfeeders. This could be an indication of other routes of exposure relevant in this special case. However, the case history of the woman concerned does not give any indications of a plausible explanation for the very high levels and the different pattern of congeners: Being a housewife, she stated that she had not been aware of any contacts with the substances in question (plastic materials, plastic foam etc.), and had used neither computer nor TV. There were no industrial areas in her housing environment within a radius of ca. 5 km.

In contrast, the PBDE levels both of the congeners and of total PBDE levels detected in the samples from the remaining three extended breastfeeders are clearly below the mean values detected in the samples from multiparae. This applies likewise to almost all

congeners, but is less pronounced for BDE-153. The order of congeners observed in the test persons 2 – 4, namely BDE-153 > BDE-47 > BDE-99, complies with that observed in the multiparae.

Although the PBDE levels detected in the samples from the test persons 2 – 4 suggest markedly lower body burdens in extended breastfeeders, no substanciated evaluation can be made due to the very low number of samples and also due to the extremely high levels in test person 1, which could not be plausibly explained so far. However, it has to be pointed out that these extended breastfeeders have already breastfed more than one child (i.e. they are also repeat breastfeeders) and therefore, the changes in the pattern of congeners are similar to those of mothers having nursed more than one child (repeat breastfeeders).

5.6 Joint evaluation of dietary habits and number of breastfed children

At first, the dietary habits, i.e. the consumption of foods of animal origin, and the total number of breastfed children were independently tested and identified as significant influencing factors. Both a lower share of foods of animal origin in the diet and a higher number of breastfed children result in lower body burdens.

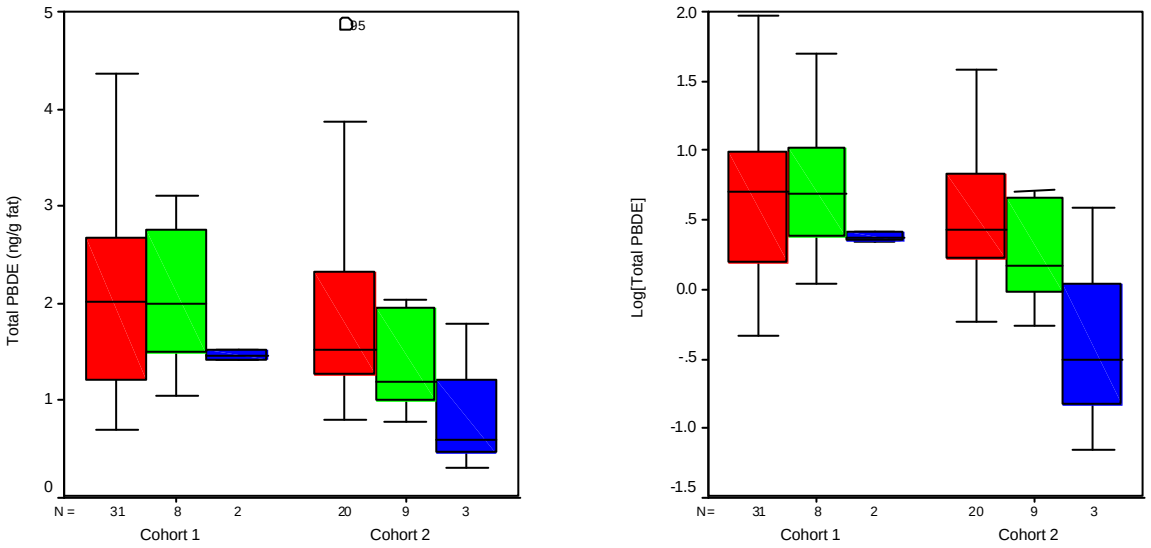

Fig. 8 Comparison of total PBDE levels depending on the dietary habits and the number of breastfed children (box-whisker plots; 1st time of sampling); left: normal values, right: logarithmic values, red: 1 breastfed child, green: 2 breastfed children, blue: 3 breastfed children

The box-whisker plot (Fig. 8) illustrates the influences of the two factors on the total PBDE level, by assigning test persons to groups by the number of breastfed children separately

for the two cohorts. Thus, it becomes clear that obviously, the influence of the number of breastfed children on the PBDE body burden is more pronounced in Cohort 2 (vegetarians) than in Cohort 1 (omnivores).

Since on average, the vegetarians had breastfed more children than the omnivores (see Table 7), the question was raised whether the difference found between the onmivores and the vegetarians could be explained predominantly by the dietary habits and to what extent a contribution was made by the number of breastfed children.

For an assessment of the influences of both factors compared with each other, a multiple linear regression was performed on the basis of the levels in their logarithmic form.

$$\text{Log [BDE level]} = A + c \times C + d \times D$$

A = constant for the background exposure
c = Number of breastfed children (1, 2 or 3)
d = Indicator for the type of diet (0 = omnivore, 1 = vegetarian / vegan)
C and D = Factors for the power of each influencing parameter

In this equation, c and d are characteristics of the indicidual women, i.e. known factors; A, C and D are model parameters to be estimated.

For the non-logarithmic PBDE levels, the following adequate multiplicative regression equation is obtained:

$$\text{BDE level} = A' \times C'^{\,c} \times D'^{\,d}$$

The coefficients, C' and D' were obtained by regression for the single congeners and for the total level. The suitability of the model is supported in each case by its significance. The coefficient of determination R^2 is a measure for the model's quality of the fit. The value shows how much of the variance of data can be explained by this model, i.e. by the factors considered.

The model calculations were performed for those congeners that exhibited significant differences between the groups during the separate testing of the two study hypotheses and that are found in breast milk in relevant quantities; minor components have not been taken into account.

Table 14 below shows the result for the total PBDE level and for the predominant congeners, BDE-47, BDE-99, BDE-100, BDE-153 and BDE-183; the 95 % confidence intervals of the calculated model parameters, C' and D' have been given in brackets.

Table 14: Estimated model parameters for the model, diet and number of breastfed children including the 95 % confidence intervals for C' and D'

	Significance of the model	R^2	Parameter C' Number of breastfed children	Parameter D' Diet
BDE-47	0.000	22.7 %	0.60 (0.45 – 0.80)	0.66 (0.46 – 0.95)
BDE-99	0.000	23.5 %	0.63 (0.47 – 0.84)	0.61 (0.43 – 0.86)
BDE-100	0.000	25.7 %	0.65 (0.50 – 0.84)	0.62 (0.45 – 0.86)
BDE-153	0.081	7.3 %	0.94 (0.77 – 1.14)	0.78 (0.61 – 0.99)
BDE-183	0.110	6.3 %	0.79 (0.52 – 1.21)	0.65 (0.38 – 1.09)
Total PBDE	0.003	15.7 %	0.79 (0.64 – 0.97)	0.73 (0.56 – 0.94)

The model is highly significant for the congeners, BDE-47, BDE--99 and BDE-100 and for total PBDE levels. According to the calculated coefficient of determination, R^2 , ca. 25 % of the variance of BDE-47, BDE-99 and BDE-100 levels is explained by means of this model, i.e. by the influencing factors of the number of breastfed children and of the dietary habits. The calculated values of the parameters, C' and D' are comparable for each of the 3 congeners, i.e. the influences of the factor of the number of breastfed children and of the factor of the dietary habits on the levels of these congeners are in a comparable order of magnitude.

In contrast, the significances of the model calculated for BDE-153 and BDE-183 are 0.08 and 0.11, respectively, thus exceeding the threshold value of 0.05. Also the quality of fit of the model (R^2) of ca. 6 and 7 %, respectively, is unsatisfactory. For these two congeners, this multiplicative model is unsuitable. Significances for these two congeners could only be observed if the dietary habits were tested separately (Table 10).

5.7 Testing of other potential confounders

Other possible counfounders tested included: the mother's age, body mass index, hours of screen exposure per week (calculated as sum of hours spent watching TV and working on the computer), and smoking habits. The testing whether these factors indeed have an influence on PBDE levels was performed by means of scatter diagrams and box-whisker plot (smoking habits) and by calculating the correlation coefficient and two-tailed test of significance. For the metric parameters, age, BMI and hours of screen exposure, the correlation coefficient after Pearson and for the categorial parameter of smoking, the Spearman correlation coefficient were calculated.

A visual impression is given by the scatter diagrams below. For a discrimination between the cohorts, the circles represent Cohort 1, and the crosses, Cohort 2.

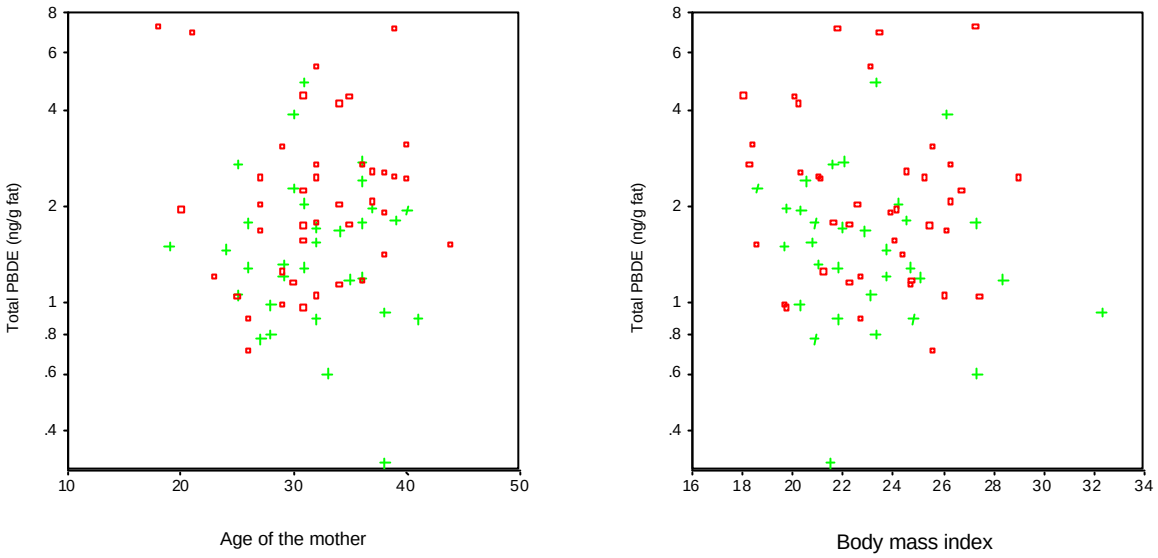

Fig. 9: Scatter diagrams of total PBDE levels with age and body mass index of the mother

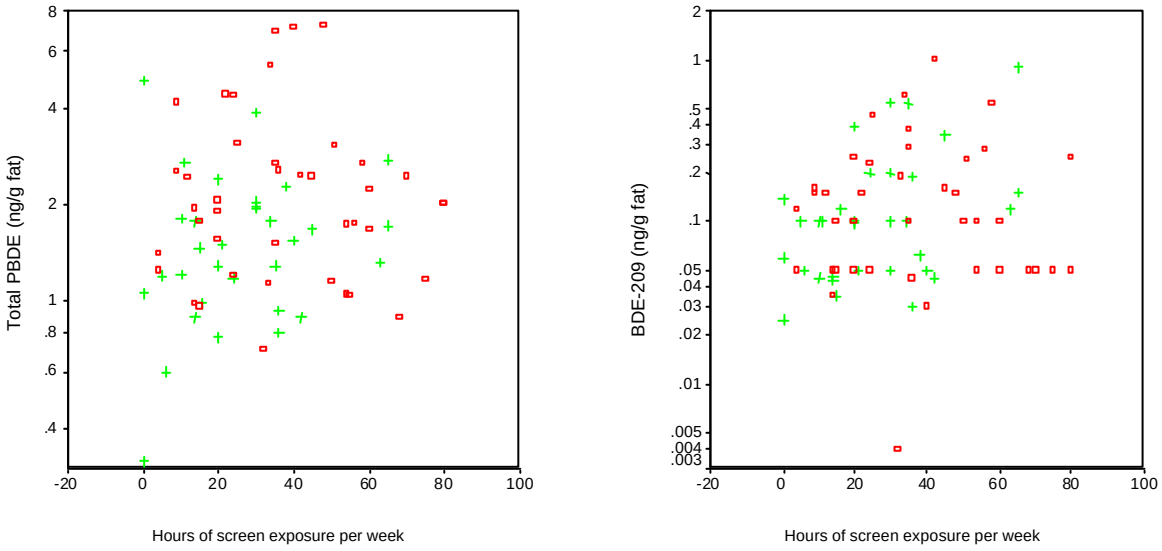

Fig. 10: Scatter diagrams of total PBDE and BDE-209 with hours of screen exposure per week

Due to the specific use of the technical DecaBDE in computers, the test result for the congener, BDE-209 has been depicted in addition to the total PBDE level for the potential confounder of hours of screen exposure per week.

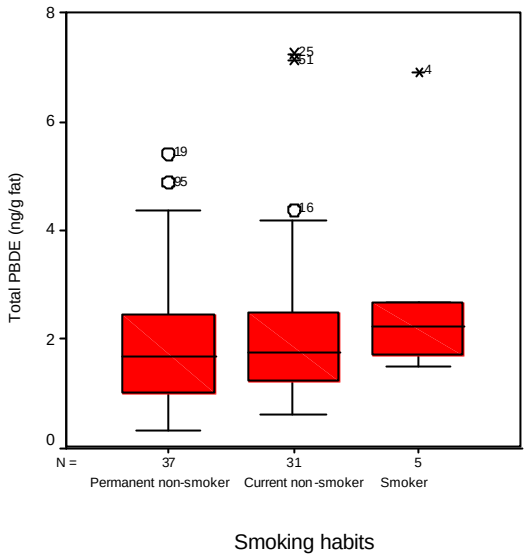

Fig. 11: Box-whisker plot for total PBDE levels depending on the smoking habits

For the potential confounders discussed, the correlation coefficients and the corresponding significances were calculated for all congeners and total PBDE on the basis of the data of the first time of sampling in their logarithmic form. Selected data have been condensed into Table 15.

Table 15: Parameters of the correlation calculation for the potential confounders

Confounder	Study cohort		Cohort 1		Cohort 2	
	Correlation coefficient	p value	Correlation coefficient	p value	Correlation coefficient	p value
Age						
Log [Total PBDE]	0.002 [1]	0.990	0.045 [1]	0.779	0.039 [1]	0.833
BMI						
Log [Total PBDE]	-0.115 [1]	0.337	-0.109 [1]	0.503	-0.236 [1]	0.202
Hours of screen exposure / week						
Log [Total PBDE]	0.128 [1]	0.280	-0.052 [1]	0.746	0.094 [1]	0.617
Log [BDE-209]	0.163 [1]	0.171	0.007 [1]	0.964	0.373 [1]	0.039
Smoking						
Log [Total PBDE]	0.164 [2]	0.165	0.199 [2]	0.213	0.065 [2]	0.728

[1] Pearson correlation coefficient
[2] Spearman rank correlation coefficient

The scatter diagrams and the box-whisker plot as well as the parameters of correlation demonstrate no correlations to exist between the mother's age and body mass index, her smoking habits, hours of screen exposure per week and the PBDE levels . No influence of

these parameters could be shown neither on the single congeners nor on the total PBDE level.

5.8 Estimate of PBDE intake levels of the breastfed infant

The postnatal PBDE exposure was estimated for a 4-month-old and exclusively breastfed infant on the basis of the PBDE levels detected in this study. A probabilistic estimate of the quantities of PBDE ingested by the infant during breastfeeding would very well reflect their variability, however, it could not be performed due to a lacking basis of data. Therefore, the PBDE intake levels were performed as point estimates. For calculation, the same model was used as in the risk assessment by the European Union (EU Risk Assessment Report, 2000). The equation used for calculation ot the intake levels of contaminants through breast milk is:

$$ADU = \frac{Cfat \times F1 \times F2 \times MI}{b.w.}$$

Where:

ADU	Amount of the contaminant ingested by the infant in ng/kg b.w. /day
C_{fat}	Concentration of the contaminant in breast milk in ng/g fat
F1	Fat content in the breast milk
F2	Share of the absorbed quantity of the contaminant ingested
MI	Milk intake of the infant (mL/day)
b.w.	Body weight of the infant (kg)

The calculation of the mean intake level was based on the mean PBDE levels and the mean fat content of the breast milk samples from the first time of sampling for the study cohort, for Cohort 1 and Cohort 2. In order to include worst-case considerations, the 95th percentiles of the PBDE levels and the 95th percentiles of the fat content in the breast milk samples were used in addition. The latter scenario is expected to rather result in an overestimation of exposure. Since it is completely unknown how much of the PBDE ingested during breastfeeding is indeed absorbed, an absorption rate of 100 % was assumed in the sense of a worst-case assumption.

Intake calculations were performed for the predominant congener, BDE-47 and for total PBDE, and for the sum of the congeners, BDE-47, BDE-99 and BDE-153. These 3 congeners are the predominant congeners in breast milk and they are also contained in relevant amounts in the technical product, pentabromodiphenyl ether.

The values used for the calulation included:

C_{fat} Mean concentration and 95th percentile (in ng/g fat)

F1 Fat content in the breast milk samples:

Study cohort:	MV = 3.9 %;	95th percentile = 6.6 %
Cohort 1:	MV = 3.4 %;	95th percentile = 5.6 %
Cohort 2:	MV = 4.35 %;	95th percentile = 7.9 %

F2 1 (100 % absorption assumed)

MI Mean daily milk intake of a 4-month-old, exclusively breastfed infant = 821 mL/day (Wallgren, 1945)

b.w. Mean body weight of a 4-month-old infant = 6.5 kg (Brand, 1979)

The intake levels calculated by this way have been condensed into Table 16.

Table 16: Daily PBDE intake levels of a 4-month-old, exclusively breastfed infant based on the mean value and the 95th percentile of the levels detected in breast milk at the first time of sampling and the mean values and the 95th percentile of the fat content of the samples of each cohort.

	Intake levels (ng/kg b.w./d)					
	Study cohort		Cohort 1		Cohort 2	
	MV[1]	95th perc.[2]	MV[1]	95th perc.[2]	MV[1]	95th perc.[2]
BDE-47	3.7	23.9	4.1	26.9	3.0	17.2
Sum (47+99+153)[3]	9.5	37.5	8.1	41.0	6.8	33.0
Total PBDE	10.3	49.1	10.6	50.4	9.3	42.5

[1] Mean value of PBDE levels and mean fat content of breast milk samples

[2] 95th percentile of PBDE levels and 95th percentile of fat contents of breast milk samples

[3] Sum of BDE-47, BDE-99, BDE-153

For the study cohort, the mean intake calculated for BDE-47 is 3.7 ng/kg b.w. per day, and the worst-case estimate made, based on the corresponding 95th percentiles, is 23.9 ng/kg b.w. per day. For the sum of the 3 predominant congeners, this range was found to be between 9.5 and 37.5 ng/kg b.w. and day. The levels calculated for total PBDEs are 10.3 and 49.1 ng/kg b.w. and day. The worst-case intake levels are higher than the mean daily intake levels by a factor of approximately 4 – 6.5. As expected, the infants breastfed by omnivores have slightly higher PBDE intake levels than the children of vegetarians.

5.9 Results of PBDE detection in human blood and comparison with the breast milk data

The primary plan intended to compare the patterns and levels of PBDEs in human milk with those in human blood. However, no more than 7 blood samples could be collected. The final report of analysis of the human blood samples is enclosed as Annex 7.

Due to the low volumes of the blood sampled and the considerably lower fat content of 0.5 %, the limits of analytical feasibility were in part exhausted. Thus, levels of the low-brominated congeners, BDE-28, BDE-47, BDE-99 and BDE-100 and also of the hepta- and decabrominated congeners were too low to be quantified in the majority of samples. Therefore, evaluation could only be performed for the congeners, BDE-100, BDE-153 and BDE-154.

The quotients from the levels in milk and those in blood show a great variability, with values of 0.3 - 2.2 for BDE-100, of 0.6 – 1.8 for BDE-153 and of 0.2 – 0.8 for BDE-154. There is no uniform pattern to be seen for the distribution between milk and blood. A cause to be mentioned is seen in the analytical problems concerning the blood samples.

Table 17: Comparison of PBDE levels in blood and breast milk (in ng/g fat)

	Test person 1		Test person 2		Test person 3	
	Blood	Breast milk	Blood	Breast milk	Blood	Breast milk
BDE-28	n.d.(<0.09)	0.059	n.d.(<0.07)	0.046	n.d.(<0.09)	0.036
BDE-47	n.d.(<0.7)	0.79	n.d.(<0.5)	0.53	n.d.(<0.7)	0.45
BDE-66	n.d.(<0.03)	0.017	n.d.(<0.02)	0.0074	n.d.(<0.03)	0.0092
BDE-99	n.d.(<0.3)	0.27	n.d.(<0.3)	0.12	n.d.(<0.3)	0.1
BDE-100	0.11	0.24	0.14	0.21	0.060	0.14
BDE-153	0.44	0.71	0.52	0.92	0.57	0.35
BDE-154	0.035	0.029	0.063	0.029	0.052	0.020
BDE-183	n.d.(<0.08)	0.17	n.d.(<0.07)	0.26	n.d.(<0.08)	0.026
BDE-209	n.d.(<1)	0.16	n.d.(<1)	n.d.(<0.2)	n.d.(<1)	0.12

	Test person 4		Test person 5		Test person 6		Test person 7	
	Blood	Breast milk	Blood	Breast milk	Blood	Breast milk	Blood	Breast milk
BDE 28	n.n. (<0,3)	0,021	n.n. (<0,07)	0,035	n.n. (<0,07)	0,083	n.n. (<0,09)	
BDE 47	n.n. (<2)	0,27	n.n. (<0,5)	0,34	2,7	0,75	n.n. (<0,7)	
BDE 66	n.n. (<0,1)	n.n. (<0,007)	n.n. (<0,02)	0,031	n.n. (<0,02)	0,041	0,036	n.n.
BDE 99	n.n. (<1)	0,12	n.n. (<0,3)	0,13	0,56	0,19	n.n. (<0,3)	
BDE 100	0,30	0,096	0,18	0,13	0,55	0,25	0,13	0,036
BDE 153	0,71	0,87	0,54	0,34	1,0	1,2	0,42	0,36
BDE 154	0,10	0,022	0,023	0,015	0,091	0,053	0,025	0,0039
BDE 183	n.n. (<0,3)	0,068	n.n. (<0,07)	n.n. (<0,035)	0,066	0,10	n.n. (<0,08)	
BDE 209	n.n. (<5)	0,28	n.n. (<1)		n.n. (<1)	0,45	n.n. (<1)	

[1] n.d. = not detectable, data in brackets are the limits of detection

6 Discussion and assessment

6.1 PBDE levels in breast milk from Germany, and international comparison

In the context of this study, a total of 128 breast milk samples from 89 women were collected at 2 defined times of sampling. Comparable numbers of breast milk samples were collected from omnivorous women and from vegetarians. Thus, this study is one of the most comprehensive studies ever performed worldwide on PBDE in breast milk (see Table 1). Owing to the target-oriented structure of the study design, an assessment can be made of selected influencing factors.

A questionnaire was filled in by each woman providing both personal details and information on dietary habits, possible occupational and other potential exposure and influencing factors. The breast milk samples were analyzed for 9 congeners.

The questionnaires did not present any indications of occupational exposure. Therefore, the PBDE levels detected in these samples were to reflect the background exposure.

The mean total PBDE level for the first samples from all 89 women is 2.49 ng/g fat, while the median, which is less influenced by extreme values, is 1.72 ng/g fat. Since, however, the share of the vegetarians included in this group (32 out of 89) is above their average share in the general population, a characterization of the background exposure in Germany should rather be performed based on the PBDE levels detected in the samples from omnivores. These presented a mean value of 2.47 ng/g fat, a median of 2.01 ng/g fat and a 95^{th} percentile of 7.11 ng/g fat. Thus, the PBDE levels detected in breast milk are lower by a factor of 20 – 100 than the mean levels of DDT, HCB, ß-HCH and PCB, and higher by a factor of 500 than the mean levels of dioxin in breast milk from Germany.

The mean PBDE contamination of the breast milk samples of this comprehensive study is comparable to the mean level for 7 breast milk samples stated by Fürst (2001). In contrast, the mean total PBDE level of 7.2 ng/g fat (sum of 7 congeners) in 8 breast milk samples reported by Weber and Heseker (2004) is higher by a factor of 3. It has remained unclear whether a causal relationship might exist between regional factors and the differences observed, the more so since some of the samples analyzed by Weber and Heseker originated from mothers from North Rhine-Westphalia, i.e. the same federal Land as those examined by Fürst.

Schröter-Kermani et al. (2000) reported on an increasing trend in blood specimens from Germany between 1985 and 1999. In 1999, the mean total PBDE level was 5.6 ng/g fat and thus more than twice as high than the mean total PBDE levels in breast milk samples

detected in the present study, which had been collected between 2001 and 2004. It has to be doubted, however, whether this can be interpreted as a reversion of trends in the background exposure similar to that observed in breast milk from Sweden owing to the abandonment of the use of technical pentabromodiphenyl ether in that country (Meironyte Guvenius, 2001). No such abandonment of use has been practised in Germany. Instead, it has to be assumed that the difference between the PBDE levels detected by Schröter-Kermany in the blood and those detected in this study in breast milk are rather to be attributed to the different analytical detection methods used than to the different matrices.

When compared with current data on breast milk reported from other European countries, the levels detected in the present study are rated among the lower ranges of PBDE contamination, being similar to current data from Sweden or Finland, while the levels reported from Italy, Belgium, Norway or the Netherlands are somewhat higher (Guvenius, 2003; Strandman, 2000; Ingelio, 2004; Pirard, 2003; Polder, 2004; Baumann, 2003). Altogether, however, it has to be assessed that the PBDE levels detected in breast milk in these European countries are in a comparable order of magnitude, which is an indication of comparable exposure situations. In contrast, the levels detected in samples from the United Kingdom or the Faeroe Islands, which are higher by a factor of 2-3, could suggest a higher relevance of other routes of exposure or elevated PBDE levels in the staple foods relevant in these countries, such as seal meat (Kalantzki, 2003; Fängström, 2004).

PBDE levels in breast milk samples from North America are higher by a factor of 10 – 30 than those detected in Germany as a background exposure level (Schecter, 2003; Ryan, 2004a). So far, no causes have been identified yet for this difference. Another route of exposure discussed is inhalative or ingestive PBDE uptake through dust, which has been reported to be contaminated to a markedly higher degree in North America than in Europe, according to Sjödin, 2004b. In general, 95 % of the world production of the technical pentabromodiphenyl ether is used in North America, which could result in a markedly higher background exposure of the population in the USA.

The congener pattern of the dioxins accumulated in human fat and analyzed in breast milk allows to draw conclusions as to possible sources of exposure. Regarding PBDE, no results have been available so far in this respect. Nevertheless, similarities in the pattern of congeners should suggest comparable sources of exposure. As far as the order of predominant congeners is concerned, the pattern of PBDE congeners in the breast milk samples analyzed in the present study, i.e. BDE-47 > BDE-153 > BDE-99, complies with that identified by Fürst (2001) and in most European countries (Guvenius, 2003; Strandman, 2000; Pirard, 2003; Polder, 2004; Baumann, 2003; Kalantzki, 2003). This fact

could be an indication of similar sources of exposure. In contrast, although the tetrabrominated congener, BDE-47 is the main component also in breast milk samples from North America, the share of the hexabrominated congener, BDE-153 is essentially lower than the shares of the pentabrominated congeners, BDE-99 and BDE-100, being another indication of a clearly different type of exposure prevailing in Europe. In the breast milk samples from the Faeroe Islands, BDE-153 has even been the main component.

6.2 The decabrominated congener (BDE-209) in breast milk

Particular importance has been attributed to the decabrominated congener, BDE-209, detected for the first time in this study in breast milk samples, which reflect the essentially lower European background exposure as compared to North America. Despite its lower absorption rate after oral ingestion compared with the lower brominated compounds and in contrast to other breast milk studies from Europe published so far, BDE-209 was quantified in 44 out of 89 samples of the total cohort, i.e. in ca. 50 % of all samples. Thus, BDE-209 accounts for ca. 8 % of the total BDE levels, which is a higher share than that of BDE-28, BDE-66 or BDE-154. Its bioavailability in humans had been disputed for a long time both due to the low oral absorption rate in rats and to the difficult analytical method (de Boer, 2002; EU Risk Assessment Report, 2002). Up to the present, BDE-209 has been detected in human samples with clearly higher total PBDE burdens, thus e.g. in the blood of workers (Table 2, Sjödin 1999, 2001; Thuresson, 2002; Jakobsson, 2002) and in 7 out of 23 breast milk samples from the USA (Table 1, Schecter, 2003). Simultaneously with the results of the present study, comparable levels of BDE-209 in Norwegian breast milk were reported on the 24th International Symposium on Halogenated Environmental Organic Pollutants and POPs held in September 2004 in Berlin (Vieth, 2004; Polder, 2004a). Such findings have also been supported by current data from Mexico on BDE-209 detected in 7 breast milk samples. Table 18 shows a compilation of the levels of BDE-209 detected in breast milk.

The data of the present study have demonstrated that in spite of the low bioavailability, the decabrominated congener can be quantified in breast milk samples, which reflect the low levels of background exposure in Europe, and that an exposure takes place of the breastfed infant. This is relevant also against the background of the fact that the technical product, decabromodiphenyl ether accounts for ca. 81 % of the PBDE world market. In 1999 a quantity produced of 55,000 t per year DBDE was produced. After the bans on penta- and octabromodiphenyl ethers were set into force in the EU in 2004, the quantities

used of the decabrominated product and thus, exposure of humans will possibly continue to increase.

Table 18: Levels of BDE-209 in breast milk – Current data

		BDE 209			Total PBDE (ng/g fat)	Reference
		No. of samples	Mean (ng/g fat)	Range (ng/g fat)		
Germany, Total cohort [5]	2001-2004	44[1] / 89[2]	0.21	n.d.[3] – 4.5	2.49	Present study
Germany, Cohort 1 [5]	2001-2004	20[1] / 41[2]	0.17	n.d. [3] – 1.0	2.47	Present study
Norway, 2003		22[1] / 38[2]	0.3	0.08 – 1.91	2.96	Polder, 2004a
USA, 2002 [5]		7[1] / 23[2]	0.92	n.d. [3]– 8.24	73.5	Schecter, 2003
Mexico, 2004		n.s.[4] / 7[2]	0.3	0.1 – 0.6	4.4	Lopez, 2004

[1] Number of positive values measured, [2] Total number of samples analyzed, [3] n.d. = not detectable, [4] n.s. = not stated, [5] Levels not detectable were included using half the value of the detection limit.

6.3 The influence of dietary habits on PBDE levels

It has been assumed in scientific literature that, similar to dioxins or PCBs, food is probably a main route of exposure to PBDE for the general population (Domingo, 2004; Darnerud, 2001; Bocio, 2003). This assumption has been supported by the persistence of this class of compounds, their lipophilic character and accumulation in the food chain, which has been demonstrated through the several trophic levels of the aquatic chain (Darnerud, 2001; deWit, 2002). Market basket studies including the calculation of PBDE intake levels based on the PBDE levels in relevant food groups were meanwhile carried out in Sweden, Canada, Spain, the United Kingdom and USA (Darnerud, 2000; Lind, 2001, 2002; Ryan, 2001; Bocio, 2003; Wijesekera, 2002; Schecter, 2004a, 2004b).

Nevertheless, direct evidence had been missing so far for the relationship between the internal exposure, i.e. the body burden, and the external, i.e. oral exposure to PBDE. Vegetarians had never been included in the studies with regard to their PBDE body burden.

The present estimates of the daily dietary PBDE intake show the comsumption of meat and fish to be the main factor of PBDE intake. In their sum, they account for 50 – 80 % of oral PBDE intake (Table 3). It had been unknown before to what extent refraining from meat/meat products and fish/fish products as typical of the vegetarian diet would indeed result in lower PBDE body burdens and thus, lower levels in breast milk. This relationship

was to be tested in the context of hypothesis I of the present study. To this aim, samples from vegetarian women were collected and the corresponding data on consumption frequencies for the different foods of animal origin recorded in the questionnaire to be filled in by all participants.

Owing to the target-oriented study design, i.e. inclusion of vegetarians and vegans and the statistically derived numbers of samples, it was possible for the first to demonstrate a direct relationship between dietary habits and PBDE budy burden. The predominant congeners, BDE-47, BDE-99 and BDE-153, but also BDE-100 and BDE-183, as well as the minor components, BDE-66, BDE-154 and the sum of all congeners analyzed were found to be present at significantly lower levels in the samples from the vegetarians/vegan than in the samples from onmivores. With all restrictions applying to the evaluation of one single sample, this result is also supported by the PBDE levels detected in the sample from a woman having been on a vegan diet for more than 10 years. The total PBDE level of 0.31 ng/g fat detected was by far the lowest level observed in this study. Thus, it is shown that partial or complete refraining from the consumption of foods of animal origin over a period of at least 5 years has indeed resulted in significantly lower PBDE body burdens, i.e. significantly lower PBDE levels in breast milk samples from Cohort 2.

As expected, the frequencies of consumption of the food groups in question differed between the two cohorts regarding meat and fish consumption, while the frequencies of consumption of milk/milk products and of eggs were comparable. Therefore, the clear mean level differences between the two dietary groups observed of 20 – 50 % depending on the congener can be associated with the different frequencies of consumption of these two food groups. This is in compliance with the calculations of the daily PBDE intakes in other countries and the high contribution made by meat and fish consumption concluded from these. Obviously, this is also true of the dietary habits in Germany.

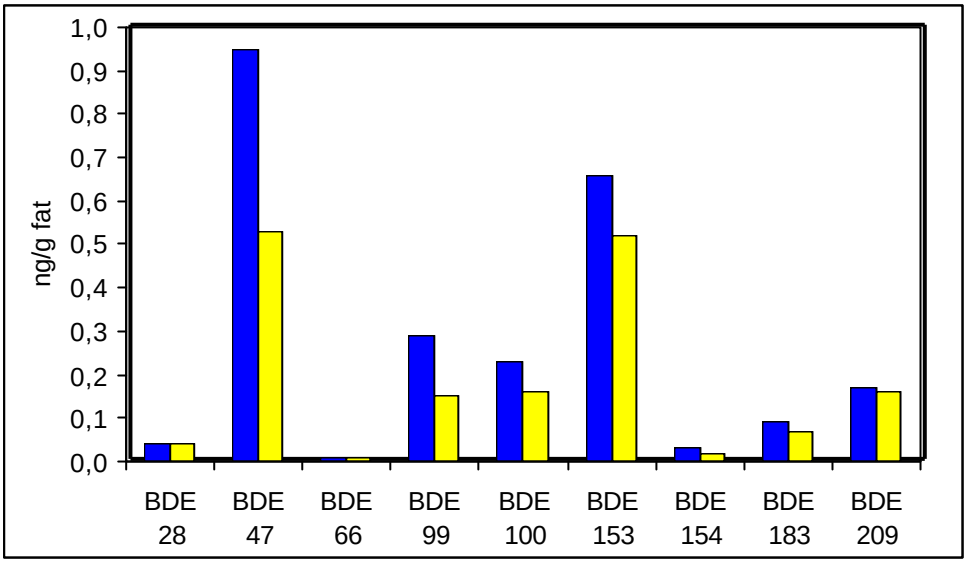

Fig. 12: Comparison of PBDE levels in breast milk samples from omnivores (Cohort 1, blue) and vegetarians (Cohort 2, yellow)

However, it has to be taken into account in addition that also the number of breastfed children proved to have an influence on the current PBDE levels in breast milk (Table 12). Since in this respect, there were differences in the composition of the cohorts (the share of mothers breastfeeding their 2nd or 3rd child was higher in Cohort 2 than in Cohort 1), the differences observed between the cohorts regarding PBDE levels could not be attributed to the different dietary habits alone but only to the combined effect of both influencing factors. This is discussed in detail in Chapter 6.5.

No influences of other possible confounders, such as age, BMI, hours of screen exposure per week and smoking habits could be demonstrated. There was no statistically significant difference between the two cohorts regarding these parameter values. Furthermore, it was demonstrated that no correlation exists between these factors and the PBDE levels in breast milk. Also other studies reported age, BMI and working hours on the PC to have no significant influence on PBDE levels (Schecter, 2003; Darnerud, 1998; Petreas, 2003; Thomsen, 2002; Lind 2001; Sjödin, 2001). In contrast to Lind et al. (2001), who found a weak association between PBDE levels and smoking habits, the data of the present study do not show any relationship between the smoking habits and PBDE levels detected in breast milk. This means that no bias or confounder is given in this respect.

Owing to the study design chosen, it was possible for the first time to reliably demonstrate that the diet, i.e. the consumption of foods of animal origin, is a relevant factor of exposure with regard to the PBDE body burden. At least, this applies to the background exposure, i.e. the total exposure and the dietary habits characteristic for Germany. However, it remains unclarified to what extent this conclusion also applies to countries with a

markedly higher background exposure, such as the USA and Canada. The different pattern of congeners in breast milk observed in these countries has suggested a different exposure situation. The latter may result from a different distribution of congeners in the relevant food groups. However, also other routes of exposure such as inhalation or ingestion of PBDEs via dust have been discussed in addition to the diet. Comparative studies by Sjödin et al. (2004b) on PBDE levels in dust from German and American households suggested a clearly higher PBDE concentrations in American samples. However, such data were not confirmed by Knoth (2002, 2003). The actual contribution of inhalative or ingestive PBDE intake to the background exposure has remained unclarified so far. With regard to persistent organochlorine compounds, which have been subject to comprehensive studies, such contribution has not been confirmed up to the present.

For a further and more detailed assessment of the quantities of PBDE taken up through the different food groups, based on the dietary habits characteristic of Germany, representative data on PBDE levels in foods are required. From Germany, however, no such data on food that would be suitable for evaluation are available so far. For this reason, market basket studies as carried out in Sweden or Spain are indispensible also for Germany.

6.4 The influence of breastfeeding on PBDE levels

Due to their highly lipophilic character, PBDEs are accumulated in human fatty tissue. These fat stores are mobilized during breastfeeding. A flush-out effect will occur. As a result, the levels of contaminants stored in the fatty tissue and detectable in breast milk will decrease in the course of the lactation period. This has been confirmed by monitoring of persistent organochlorine compounds: After a 12-weeks lactation period, their levels are on average by ca. 30 % lower than at the beginning of lactation. Also the number of lactation periods has an influence on the reduction of the body burden. Thus, dioxin levels detected in mothers breastfeeding their 2nd child were found to be lower by 20 %, and those detected in mothers breastfeeding their 3rd or 4th child, even by 30 – 40 % compared with breast milk samples from primiparae. (4th Report by the Bund/Länder AG Dioxine, 2002).

In contrast to findings made for dioxins, studies performed so far on PBDEs in breast milk could neither find any correlation between PBDE levels and the duration of the lactation period nor between PBDE levels and the number of lactation periods (Schecter, 2003). In the past, correlations between PBDE levels and the duration of lactation periods were tested by means of single samples from different mothers with different durations of

lactation periods. However, in these studies using data from the USA, this evaluation was considerably interfered by the great individual variability of PBDE levels (Schecter, 2003). The approach chosen in the present study was fundamentally different because the testing of study hypothesis II, stating that PBDE levels would be significantly lower after a 3-month lactation period, was based on the intraindividual decrease between the two defined times of sampling.

For total PBDE and some congeners, a tendency towards lower PBDE levels after a 12-week lactation period could be observed. Significance could only be demonstrated for BDE-66, which, however, is a minor component accounting for less than 1 % of the total PBDE level and therefore being irrelevant. Possible changes in weight during the lactation period, i.e. changes in the BMI, can be excluded as influencing factors since the BMI was demonstrated not to be a confounder. However, a 3-month lactation period may possibly be too short to demonstrate a significant decrease in PBDE levels. This assumption has been supported by the relatively low PBDE levels detected in 3 out of 4 extended breastfeeders (> 8 months). However, due to lacking significance, study hypothesis II had to be rejected.

Nevertheless, the present study could demonstrate for the first time that breastfeeding causes a significantly lower body burden also of PBDEs in the mother and thus in breast milk because PBDE levels detected were lower in the samples from multiparae than in those from primiparae. A higher number of breastfeeding periods resulted in significantly lower levels of the congeners, BDE-47, BDE-99, BDE-100 and BDE–154 as well as of total PBDE levels.

However, such evidence could only be provided owing to the specific design of this study, i.e. by including vegetarians. Fig. 8 shows that this effect was essentially more pronounced in the group of vegetarians than in that of omnivores. This also explaines why the studies performed before, that used to include mothers without any differentiation with regard to their dietary habits (i.e. mothers eating a mixed diet as typical of their country of residence), could not establish any relationship between the number of breastfed children and the PBDE levels in breast milk.

There are two reasons why the influence of preceding lactation periods is more pronounced in vegetarians: Firstly, the preceding lactation periods of vegetarians were longer than those of omnivores. In addition, in omnivorous women, the partial flush-out of PBDEs from the body fat as a result of breastfeeding is compensated during non-lactation periods due to their dietary PBDE intake, which is significantly higher than that of vegetarians. Since in contrast, vegetarians take in essentially lower quantities of PBDEs

through their food, the stores are only partly refilled in this cohort resulting in the significant differences regarding PBDE levels. Obviously, this is an effect accumulating with an incrasing number of breastfed children. Thus, the difference between the PBDE levels detected in the two cohorts is essentially larger for mothers breastfeeding their 3rd child than for mothers breastfeeding their first child.

In addition, however, this observation confirms the diet to be a dominating source of PBDE background exposure in Germany. The influence of the number of breastfed children on the PBDE levels in breast milk from vegetarians could only be demonstrated provided that other routes of exposure were less relevant.

Longer durations of lactation periods as well as repeated periods of breastfeeding are found to result in a shift of the pattern of congeners. While the levels of the lower brominated congeners, BDE-47, BDE-99 and BDE-100 show a clear decrease, those of the higher-brominated congeners are influenced less markedly. Particularly the levels of the hexabrominated congener, BDE-153, being a predominant congener, remain relatively unchanged. Accordingly, a change in the order of predominant congeners can be observed in the samples both from multiparae and from 3 out of 4 of extended breastfeeders: BDE-153 dominates over BDE-47, followed by BDE-99. This trend is illustrated in Fig. 13.

The above phenomenon can be explained taking into account the short elimination half-lives of the heptabrominated congener, BDE-183 (110 days) and the decabrominated congener, BDE-209 (7-14 days). These cause the steady state to be reached again within the limited period between the lactation periods (Sjödin, 1999, 2000b). Markedly longer half-life times required for elimination from human fat were reported for the lower brominated congeners, BDE-47 (3 years), BDE-99 (5.4 years) and BDE-100 (2.9 years) (Geyer, 2004). Due to these very long half-lives, a return to the steady state between the lactation periods cannot be expected, and hence, lower levels of these congeners are detected in samples from women brestfeeding their 2nd or 3rd child.

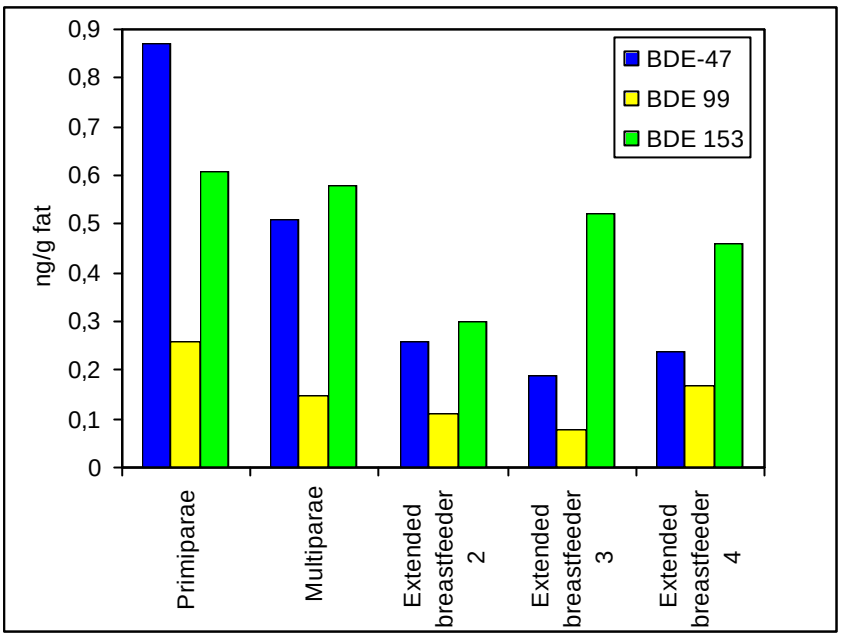

Fig. 13: Changes in the pattern of congeners due to extended breastfeeding or repeated lactation periods

The situation is found to be somewhat different for BDE-153. Its half-life time for elimination from human fat amounting to 11.7 years is the longest compared with all other congeners. The more surprising it is found that the levels of this hexabrominated congener are hardly influenced in samples from multiparae and extended breastfeeders. Which specific properties of BDE-153 are the cause of this phenomenon has remained unclear and can only be speculated. Thus, for example, a metabolic debromination of the decabrominated congener, BDE-209 to BDE-153, as was found in rainbow trout, might possibly result in an internal refilling of the stores in human body fat partially emptied by breastfeeding (Kierkegaard, 1995).

6.5 Common model for the influencing factors of dietary habits and number of breastfed children

The simultaneous effect of the factors influencing the PBDE levels that proved to be statistically significant, i.e. the dietary habits and the number of breastfed children, was comparatively estimated by means of multiple linear regression in order to characterize the real situation. While obviously, the levels of BDE-153 and BDE-183 were significantly influenced only by the dietary habits, the model is significant for BDE-47, BDE-99 and BDE-100, as well as total PBDE. The influence of the two factors on the concentrations of congeners is approximately similar. By means of this model it can be estimated that the concentrations of the corresponding congeners, BDE-47, BDE-99 and BDE-100 should be lower by ca. 40 % in breast milk from mothers breastfeeding their 2nd child and by ca.

65 % in breast milk from mothers breastfeeding their 3rd child compared with those in breast milk from primiparae. Differences of 20 and 38 %, respectively are calculated by the corresponding comparison for total PBDE levels.

If on the other hand, a hypothetical comparison is made between the samples from two women differing only with regard to their dietary habits, the result obtained by means of the model will be that the concentrations of BDE-47, BDE-99, BDE-100 and of total PBDE detected in the sample from the vegetarian are lower by ca. 35 – 40 % and 25 %, respectively.

Although the model is highly significant for the congeners, BDE-47, BDE-99 and BDE-100 as well as for total PBDE, the calculated coefficients of determination R^2 suggest that the two influencing variables included in the model account for no more than 23 – 26 % of the variability of the levels of congeners and 16 % of the variability of the total PBDE level. From this result it can be concluded that factors not taken into account and possibly unknown so far make an essential contribution to the variability of the levels. The question whether such factors may also include inhalative or ingestive intake via dust cannot be answered.

6.6 Assessment of PBDE intake of the breastfed infant

Particular attention is required with regard to the PBDE intake of the exclusively breastfed infant: Against the background of the toxic potential of PBDEs and bearing in mind the still developing, vulnerable newborn, an unfavourable effect of breastfeeding has to be excluded with a sufficient degree of certainty.

The PBDE intake levels were calculated by means of a equation used in the EU Risk Assessment Report for pentabromodiphenyl ethers. Estimates included both the mean intake levels based on mean PBDE levels and mean fat contents in breast milk, and worst-case estimates based on the respective 95th percentile of PBDE and fat content. The values calculated by this method are 10 and 50 ng/kg b.w./d, respectively, for the mean and the worst-case intake of total PBDEs. Thus, they are lower by one order of magnitude than the intake levels calculated for the USA amounting to 355 ng/kg b.w./d (Schecter, 2003).

For an assessment, the intake levels calculated by this way for an exclusively breastfed infant aged 4 months have to be compared with the sensitive toxicological parameters. Pentabromodiphenyl ether is the most potent of the three technical products with regard to potential toxic effects. Particularly sensitive endpoints are effects on the liver due to

chronic exposure, for which a NOAEL of 0.45 mg/kg b.w./d was derived from animal experiments (EU Risk Assessment Report, 2000). For the total PBDE level, Darnerud (2001) estimated an ADI of 1mg/kg b.w./d, which has also been used for the present assessment.

The margin of safety (MOS) calculated from the comparison of the intake levels of the breastfed infant with the NOAEL and ADI was taken as a basis for the assessment whether these intake levels are safe in terms of health, based on the current state of knowledge.

For the sum of BDE-47, BDE-99 and BDE-153, which are the predominant congeners of the technical PeBDE, the MOS calculated on the basis of the mean intake levels and the NOAEL was 5×10^{4}, and the MOS for the corresponding worst-case intake levels was 1×10^{4}. Even when comparing the somewhat higher intake levels for total PBDE with the corresponding ADI, the MOS calculated by this way, being 8×10^{4} for the mean intake level and 2×10^{4} for the worst case, are within a range providing a very safe margin. The quantities of PBDEs absorbed by a 4-month-old infant through breastfeeding are lower by a factor of ca. 10,000 than the lowest levels obtained in animal experiments at which no adverse effects have been observed.

However, it has to be pointed out that the composition of congeners of the technical products used for the toxicological studies does not exactly correspond to those in breast milk to which the infant is exposed. Therefore, the comparison with the NOAEL or ADI should only be taken as an orientating basis for the assessment. Based on the very large margins of safety that have been estimated it can be concluded, according to the current state of knowledge, that for the infant, no health risks are associated with the quantities of PBDE absorbed through breastfeeding.

Also taking into account the PBDE intake of the breastfed infant, the breastfeeding recommendation by the German National Breastfeeding Committee can be maintained without reservation. It recommends to exclusively breastfeed the infant for at least 4 – 6 months (Breastfeeding Recommendation, 1995).

7 Summary

It had been the aim of the present study to characterize, on the one hand, the PBDE background exposure in Germany and to assess whether any health risks could be involved in PBDE intake by the infant through breastfeeding. On the other hand, it had also been intended to test the influence of the mother's diet and of breastfeeding in the context of 2 study hypotheses since no unambiguous evidenve had been available so far in literature. For this purpose, a special study design was developed including 2 groups of participants, i.e. omnivores and vegetarians, and 2 defined sampling times. The numbers of samples required for testing of the study hypotheses had been derived by statistical methods in advance. In addition, each participating woman filled in a questionnaire stating personal data, lifestyle factors, frequencies of consumption of foods and other possible influencing factors. Inclusion and exclusion criteria for test persons were defined in advance.

In the period from November 2001 to March 2004, 128 breast milk samples were collected from 89 mothers on a German national level. The samples were examined for 9 congeners, which were evaluated together with the sum of congeners referred to as the total PBDE level.

An average background burden of total PBDE of 2.47 ng/g fat was calculated from the breast milk samples tested. The median is 2.11 ng/g fat, and the 95th percentile, 7.11 ng/g fat. An extreme value of 17.8 ng/g fat was quantified, but no indications of any specific exposure could be identified. Compared with data from other European countries (mean PBDE levels between 2.1 and 7.2 ng/g fat), the background exposure in Germany has been assigned to the lower range. Also the order of predominant congeners, i.e. BDE-47 > BDE-153 > BDE-99, was found to be identical in most European countries, which is a fact suggesting similar sources of exposure. In contrast, PBDE concentrations detected in breast milk samples from North America amounting to levels between 22 and 73 ng/g fat are higher by a factor of 10 – 30 than those found in the present study.

Particular importance has been attributed to the detection of the decabrominated compound, BDE-209 in breast milk samples with a low background exposure. BDE-209 was quantified in 50 % of all samples of this study. So far, this congener could be detected only in human specimens with clearly higher total PBDE levels, e.g. in the blood of exposed workers and in the highly contaminated breast milk samples from the USA. The data have shown that BDE-209 is absorbed and can be detected also in human specimens with lower PBDE background burdens despite its low bioavailability compared

with the lower brominated congeners. BDE 209 is the predominant congener of the technical decabromodiphenyl ether, which has been used in large quantities and has been the only commercial PBDE product approved within the EU since the EU ban was set into force.

The PBDE levels detected in breast milk are by 1 to 2 orders of magnitude below the levels detected of the organochlorine pesticides, DDT, HCB or ß-HCH and PCB. However, they exceed those of dioxins and dioxin-like PCBs in breast milk from Germany by ca. 2 to 3 orders of magnitude.

For the background exposure observed in Germany, foods are a relevant route of exposure. For the first time, the influence of the diet on the PBDE body burden could be demonstrated by including vegetarians in the study. Partial or complete refraining from the consumption of foods of animal origin results in statistically significantly lower levels of the congeners, BDE-47, BDE-66, BDE-99, BDE-100, BDE-153, BDE-154 and BDE-183 and also of total PBDE levels (study hypothesis I).

The slight decrease of PBDE levels during a 3-month lactation period was statistically tested in the context of study hypothesis II. Obviously, the 3-month period of observation was too short, because no statistically significant difference could be shown. However, it could be demonstrated for the first time that breastfeeding during repeated lactation periods results in significantly lower levels of BDE-47, BDE-99, BDE-100 and BDE-154 and also of total PBDE levels. This effect is particularly pronounced in the group of vegetarians. In contrast, there was hardly any influence seen on the levels of BDE-153. Shifts of the pattern of congeners towards the higher brominated congeners are observed.

The simultaneous influence of the two factors identified as statistically significant, i.e. the dietary habits and the number of breastfed children, was modelled and estimated by means of multiple linear regression. For the predominant congeners, BDE-47 and BDE-99 as well as for BDE-100 and total PBDE levels, the model is highly significant. In these cases, the influence of the two factors is estimated to be comparable. No significance of the model was found for the predominant congener, BDE-153, and for BDE-183. Obviously, only the influence of the diet is relevant for these congeners. However, the dietary habits and the number of breastfed children can explain no more than ca. 25 % of the variability of the congener levels and 16 %, respectively, of the total PBDE level. It remains open which other factors contribute to the variability of the PBDE levels.

No statistically significant influence on PBDE levels in breast milk could be demonstrated for the potential confounders, age, BMI, smoking habits and hours of screen exposure (TV and PC) per week. This complies with the results of other studies.

Based on the current state of knowledge, the intake of PBDEs by breastfeeding do not carry any health risks for the infant. Estimates of the PBDE intakes by the breastfed infants arrive at a mean value of 10 ng/kg b.w./d and a worst-case value of 50 ng/kg b.w./d. Hence, the margin of safety (MOS) is generally $>10^4$ in comparison to the NOAEL for the most sensitive toxicological endpoint or to the ADI, respectively.

The study presented here is one of the most comprehensive studies on PBDE levels in breast milk performed worldwide up to now. A great power of this study is seen in its structured approach, i.e. the target-oriented study design forming the basis for carrying out the study. This approach differs clearly from that of all studies performed before on PBDE in breast milk and formed a prerequisite for the successful testing of influencing factors. For the first time, evidence could be provided for the statistically significant influence of the diet, i.e. the consumption of foods of animal origin, and the statistically significant influence of the number of lactation periods on the PBDE body burden and the PBDE levels, respectively, in breast milk For a long time, such influence had been discussed and assumed in scientific literature, however, no clear verification had been provided so far. Significant test results with regard to these influencing factors could only be obtained by including a required number of milk samples of vegetarians, as stipulated in the study design.

8 Conclusions and outlook

Due to the EU directives to ban the technical pentabromodiphenyl ether and the technical octabromodiphenyl ether that have meanwhile set into force, changes in the exposure scenario have to be expected which should also be reflected in PBDE levels detected in breast milk. In order to check the effectiveness of such regulatory measures, analyses of breast milk for PBDEs should be performed at regular intervals, e.g. in the context of targeted monitoring programmes. It has remained subject to speculation so far whether the EU bans will result in a use of higher quantities of the still approved decabrominated product. In order to demonstrate a possibly increasing exposure of humans to BDE-209, this congener should at any rate be included in the spectrum of congeners to be analyzed in breast milk. This, however, will also require an appropriate analytical quality.

By demonstrating the influence of the diet, i.e. the consumption of foods of animal origin, on PBDE body burdens, the present study has identified a relevant route of exposure in Germany. This should give rise to launching analyses of PBDE levels in different groups of foods in the sense of market basket studies. In this respect, data from Germany suitable for evaluation have been lacking completely so far. Such data would, however, be required to evaluate the daily dietary PBDE intake and to identify the groups of foods accounting for the largest share in the PBDE body burden.

It has to be examined to what extent in addition to the diet, also other routes of exposure, such as the discussed inhalation or ingestion of dust, are relevant for the background exposure in Germany. Starting points for such examinations should include strikingly high single levels in human specimens that cannot be attributed to a recognizable special exposure so far. For this purpose, analyses of human specimens for PBDEs should be accompanied by inquiries by means of questionnaires.

In addition, studies are required with regard to other brominated flame retardants, like tetrabromobisphenol A (TBBPA) and hexabromocyclododecane (HBCD), which meanwhile have also been detected in human specimens and are both lipophilic and persistent. On these substances, no data at all are available from Germany.

In a summarizing view, it can be assessed that at present, scientific attention is focussed on the range of brominated flame retardants in order to be able to comprehensively evaluate human exposure and, if required, to initiate measures reducing exposure for precautionary reasons.

9 Acknowledgement

In particular, we wish to thank all participating mothers who made this study possible by contributing their breast milk samples.

The indispensible support in the technical performance of the study provided by Ms. Kramer as an assistant is gratefully acknowledged.

We also wish to thank Mr. Herrmann, head of the analytical service of the ERGO Forschungsgesellschaft m.b.H., for the high-quality analysis.

Thanks are due to Mr. Lindtner for the important calculations of PBDE intake levels in infants.

10 References

4. Bericht der Bund/Länder Arbeitsgruppe Dioxine, Dioxine - Daten aus Deutschland, Dioxin-Referenzmeßprogramm, Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit, 2002, ISBN 3-00-009326-5

Akutsu, K., Kitagawa, M., Nakazawa, H., Makino, T., Iwazaki, K., Oda, H., Hori, S.: Time trend (1973-2000) of polybrominatd diphenyl ethers in japanese mother milk. Chemosphere, 53, 2003, 645-654

Alexy, U., Kersting, M.: Was Kinder essen und was sie essen sollten. Die DONALD-Studie und die Ernährungskonzepte des FKE. Hans Marseille Verlag, München 1999, ISBN 3 - 886 16 - 095 – 5.

Ausschuss für Umwelthygiene (AUH) Arbeitsgemeinschaft der leitenden Medizinalbeamtinnen und -beamten der Länder, Behörde für Arbeit, Gesundheit und Soziales, Eigenverlag Hamburg, 2000

Bahn, A.K., Mills, J.L., Snyder, P.J., Gann, P.H., Houten, L., Bialik, O., Hollmann, L., Utiger, R.D.: Hypothyroidism in workers exposed to polybrominated biphenyls. N Engl J Med. 302, 1980, 31-3.

Ballschmiter, K., Zell, M.: Analysis of polychlorinated biphenyls (PCB) by glass-capillary gas-chromatography. Composition of technical Aroclor-PCB and Colphen-PCB mixtures. Fresenius Z. Anal. Chem. 302, 1980, 20

Baumann, B., Hijman, W., van Beuzekom, S., Hoogerbrugge, R., Houwelling D., Zeilmaker, M.: PBDEs in human milk from the Dutch 1998 monitoring programme. Organohalogen Compd 61, 2003 (CD-ROM)

Beck, H., Droß, A., Mathar, W.: Dependence of PCDD ad PCDF levels in human milk of various parameters in Germany II. Chemosphere 25, 1992, 1015-1020

Bocio, A., Llobet, J.M., Domingo, J.L., Corbella, J., Teixido, A., Casas, C.: Polybrominated diphenyl ethers (PBDEs) in foodstuffs: human exposure through the diet. J. Agric. Food Chem. 51, 2003, 3191-3195

Brandt, I.: Perzentilkurven für die Gewichtsentwicklung bei Früh- und Reifgeborenen in den ersten fünf Lebensjahren. Der Kinderarzt 10, 1979, 713-718

Bundesratsdrucksache 97/01: URL: http://www.parlamentsspiegel.de/ dokumentenarchiv/ Bundesrat / 97/01

Bundesgesetzblatt: Siebte Verordnung zur Änderung chemikalienrechtlicher Verordnungen vom 15. August 2003, 2003, Teil I Nr. 44, 1697

Chen, Y.C., Yu, M.L., Rogan W.J., Gladen, B.C., Hsu, C.C.: A 6-year follow-up of behavior and activity disorders in the Taiwan Yu-cheng children. Am J Public Health. 84, 1994, 415-21.

Darnerud, P., Atuma, S., Aune, M., Cnattingus, S., Wernroth, M., Wicklund-Glyn, A.: Polybrominated diphenyl ethers (PBDEs) in breast milk of primiparous woman in Uppsala county Sweden. Organohalogen Compd. 35, 1998, 411-414

Darnerud, P.O., Atuma, S., Aune, M., Becker, W., Wicklund-Glynn, A., Petersson - Grawe, K.: New Swedish estimate of the dietary intake of PBDE, Dioxins, PCB and DDT, derived from market basket data. Toxicol. Lett. 116 (Suppl.) 2000, 28

Darnerud, P.O., Eriksen, G.S., Johannesson, T., Larsen, P.B., Viluksela, M.: Polybrominated diphenyl ethers: occurence, dietary exposure and toxicology. Environmental Health Perspect. 109, 2001, 49-68

De Boer, J., Wells, D.E., Noren, K.: BSEF/Quasimeme interlaboratory study on brominated flame retardants. Organohalogen Compd. 58, 2002, 197-204

De Winter-Sorkina, R., Bakker, M.I., Baumann, R.A., Hoogerbrugge, R., Zeilmaker, M.J.: Exposure assessment of Dutch nursing infants to brominated flame retardants via breast milk. Organohalogen Compd. 61, 2003, 187 -190

De Wit, C.A.: An overview of brominated flame retardants in the environment. Chemosphere 46, 2002, 583-624

Diphenyl Ether, Pentabromo Derivative (Pentabromodiphenyl Ether) Summary Risk Assessment Report Joint Research Centre, Special Publication I. 00.130, 2001

Domingo, J.: Human exposure to polybrominated diphenyl ethers through the diet. J. Chromatogr. A 1054, 2004, 321-326

Eriksson, P.: Developmental neurotoxicity of environmental agents in the neonate. Neurotoxicology, 18, 1997, 719-26.

Eriksson, P., Jakobsson, E., Fredriksson, A.: Developmental neurotoxicity of brominated flame retardants, polybrominated diphenyl ethers and tetrabromo-bis-phenol A. Organohalogen Compd. 35, 1998, 375-377.

Eriksson, P., H. Viberg, H.J., Jakobsson, E., Örn, U., Fredriksson, A.: PBDE, 2,2',4,4',5-pentabromodiphenyl ether, causes permanent neurotoxic effects during a defined period of neonatal brain development. Organohalogen Compd. 40, 1999, 333-336.

Europäische Union: Richtlinie 2003/108/EC des europäischen Parlaments und des Rates vom 08.12. 2003 zur Änderung der Richtlinie 2002/96/EC über Elektro- und Elektronik-Altgeräte, Amtsblatt der Europäischen Union, 31.12.2003, L 345/106

Europäische Union: Richtlinie 2003/11/EC des europäischen Parlaments und des Rates vom 06. Februar 2003 zur 24. Änderung der Richtlinie 76/769/EWG des Rates über Beschränkungen des Inverkehrbringens und der Verwendung gewisser gefährlicher Stoffe und Zubereitungen (Pentabromdiphenylether, Octabromdiphenylether), Amtsblatt der Europäischen Union. 42. 2003, 45.

European Union: Risk Assessment Report Bis(pentabromophenyl) ether CAS No.: 1163-19-5, European Chemicals Bureau. 17. 2002, URL: http://ecb.jrc.it/

European Union: Risk Assessment Report Diphenyl ether, octabromo deriv. CAS.: 3253-52-0, European Chemicals Bureau. 16. 2003, URL: http://ecb.jrc.it/

European Union: Risk Assessment Report Diphenyl ether, pentabromo deriv. CAS No.:32534-81-9, European Chemicals Bureau. 5. 2000, URL: http://ecb.jrc.it/

Fängström, B., Strid, A., Athanssiadis, I., Greandjeann, Ph., Weihe, P., Bergman, A.: A retrospective time trend study of PBDEs and PCBs in human milk from the faroe islands. Organohalogen Compd. 66, 2004, 2795-2799

Fürst, P.: Organochlorine pesticides, dioxins, PCB and polybrominated diphenylethers in human milk from Germany in the course of time. Organohalogen Compd. 52, 2001, 185-188

Geyer, H., Schramm, K.W., Darnerud, P., Aune, M., Feicht, E.A., Fried, K., Henkelmann, B., Lenoir, D., Schmidt, P., McDonald, P.: Terminal elimination half-lives of brominated flame retardants TBBPA, HBCD and lower brominated PBDEs in humans. Organohalogen Compd. 66, 2004, 3820-3825.

Guvenius, D.M., Aronsson, A., Ekman-Ordeberg, G., Bergman, A., Noren, K.: Human prenatal and postnatal exposure to polybrominated diphenyl ethers, polychlorinated biphenyls, polychlorobiphenylols and pentachlorophenol Environmental Health Perspect. 111, 2003, 1235-1241

Haddow, J.E., Palomaki, G.E., Allan, W.C., Williams, J.R., Knight, G.J., Gagnon, J., O`Heir, C.E., Mitchell, M.L., Hermos, R.J., Waisbren, S.E., Feix, J.D., Klein, R.Z.: Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. N Engl J Med. 341, 1999, 549-555

Hagmar, L., Bergmann, A.: Human exposure to BFRs in Europe. The second international workshop on brominated flame retardants, BFR 2001, Stockholm, 14-16 may 2001, conference proceedings 107-111

Hagmar, L., Sjödin, A., Höglund, P., Thuresson, K., Rylander, L., Bergmann, A.: Biological half-lives of polybrominated diphenyl ethers and tetrabromobisphenol A in exposed workers. Organohalogen Compd. 47, 2000, 198-201

Hallgren, S., Darnerud, P.: Effects of polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs) and chlorinated paraffins (CPs) on thyroid hormone levels and enzyme activities in rats. Organohalogen Compd. 1998, 391-394.

Hallgren, S., Sinjari, T., Hakansson, A., Darnerud, P.O.: Effects of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) on thyroid hormone and vitamin A levels in rats and mice. Arch Toxicol. 75, 2001, 200-8.

Harden, F., Toms, L.M., Ryan, J.J., Müller, J.F.: Determination of the levels of polybrominated diphenylethers (PBDEs) in pooled blood sera from Australians aged 31-45 years. The third international workshop on brominated flame retardants, June 6-9, 2004, Toronto, Canada

Hirai, T., Fujimine, Y., Watanabe, S., Nakamura, Y., Shimomura, H., Nagayama, J.: Maternal-infant transfer of polybrominated diphenyl ethers and polychlorinated biphenyl. Organohalogen Compd. 66, 2004, 2422-2425

Hooper, K., McDonald, T.A.: The PBDEs: an emerging environmental challenge and another reason for breast-milk monitoring programs. Environmental Health Perspect. 108, 2000, 387-92.

Huisman, M., Koopman-Esseboom, C., Lanting, C.I., van der Paauw, C.G., Tuinstra, L.G., Fidler, V., Weisglas-Kuperus, N., Sauer, P.J., Boersma, E.R., Touwen, B.C.: Neurological

condition in 18-month-old children perinatally exposed to polychlorinated biphenyls and dioxins. Early Hum Dev. 43, 1995, 165-76.

Huisman, M., Koopman-Esseboom, C., Fidler, V., Hadders-Algra, M., van der Paauw, C.G., Tuinstra, L.G., Weisglas-Kuperus, N., Sauer, P.J., Touwen, B.C., Boersma, E.R.: Perinatal exposure to polychlorinated biphenyls and dioxins and its effect on neonatal neurological development. Early Hum Dev. 41, 1995, 111-27.

Ingelido, A., DiDomenico, A., Ballard, T., De Felip, E., Dellatte, E., Ferri, F., Fulgenzi, A., Herrmann, T., Iacovella, N., Miniero, R., Päpke, O., Porpora, M.: Levels of polybrominated diphenyl ethers in milk from Italian woman living in Pome and Venice. Organohalogen Compd. 66, 2004, 2689-2694

Jakobsson, K., Thuresson, K., Rylander, L., Sjödin, A., Bergman, A.: Exposure to polybrominated diphenyl ethers and tetrabromobisphenol A among computer technicians. Chemosphere, 46, 2002, 709 – 716

Jakobsson, K., Thuresson, K., Höglund, P., Sjödin, A., Hagmar, L., Bergman, A.: A summary or exposure to polybrominated diphenyl ethers (PBDEs) in Swedish workers, and determination of half-lives of PBDEs. Organohalogen Compd. 2003, 61-65.

Kalantzki, O., Alcock, R.E., Martin, F., Thomas, G., Jones, K.: Polybrominated diphneyl ethers (PBDEs) and selected organochlorines in human breast milk samples from the United Kingdom. Organohalogen Compd. 61, 2003, 9-12

Kierkegaard, A., Balk, L., Sellström, U., Tjärnlund, U., Örn, U., de Wit, C., Jansson, B.: Uptake of decabromodiphenyl ether (DeBDE) in rainbow trout via administration in the diet. Poster presented at the 5th SETAC-Europe Congress, 25-28 June 1995, Copenhagen, Denmark.

Knoth, W., Mann, W., Meyer, R., Nebhuth, J.: Brominated diphenylether in indoor dust. Organohalogen Compd. 61, 2003, (CD-ROM)

Knoth, W., Mann, W., Meyer, R., Nebhuth, J.: Polybrominated diphenylether in house dust. Organohalogen Compd. 58, 2002, 213-216

Koopman-Esseboom, C., Weisglas-Kuperus, N., de Ridder, M.A., van der Paauw, C.G., Tuinstra, L.G., Sauer, P.J.: Effects of polychlorinated biphenyl/dioxin exposure and feeding type on infants' mental and psychomotor development. J. Pediatrics. 97, 1996, 700-6.

Krüger, C.: Polybromierte Biphenyle und polybromierte Biphenylether - Nachweis und Bestimmung in ausgewählten Lebensmitteln. Inaugural-Dissertation, Universität Münster, 1998

Lind, Y., Atuma, S., Aune, M., Bjerselius, R., Darnerud, P.O., Cnattingius, S., Glynn, A.: Polybrominated dipenyl ethers (PBDEs) in breast milk from Uppsala woman - extension and up-dating of data. The second international workshop on brominated flame retardants, 14.- 16.05.2001, Stockholm, Schweden, conference proceedings 117-120

Lind, Y., Aune, M., Atuma, S., Becker, W., Bjerselius, R., Glynn, A., Darnerud, P.O.: Food intake of the polybrominated flame retardants PBDE's and HBCD in Sweden. Organohalogen Compd. 58, 2002, 181-188

Lind, Y., Darnerud, P. O., Atuma, S., Aune, M., Becker, W., Bjerselius, R., Cnattingius, S., Glynn, A.: Polybrominated diphenyl ethers (PBDEs) in breast milk from Uppsala women extension and up-dating data. Environ. Res. 93, 2003, 186 - 194,

Lindström, G., Kärrman, A., van Bavel, B., Hardell, L., Hedlund, B.: Levels of persistent fluorinated, chlorinated and brominated compounds in human blood collected in Sweden in 1997-2000. Organohalogen Compd. 66, 2004, 2609-2612

Lopez, A., Athanasiadou, M., Athanassiades, I., Estrada, L.Y., Diaz-Barriga, F., Bergmann, A.: A preliminary study on PBDE and HBCDD in blood and milk from Mexican woman. The third international workshop on brominated flame retardants, June 6-9, 2004, Toronto, Canada

Marsh, G., Bergman, A., Bladh, L.G., Gillner, M., Jakobsson, E.: Synthesis of p-hydroxybromodiphenyl ethers and binding to the thyroid hormone receptor. Organohalogen Compd. 37, 1998, 305-150.

Mazdai, A., Dodder, N.G., Abernathy, M.P., Hites, R.A., Bigsby, R.M.: Polybrominated diphenyl ethers in maternal and fetal blood samples. Environmental Health Perspect. 111, 2003, 1249-1252

Meerts, I. A., van Zanden, J.J., Luijks, L.A., van Leeuwen-Bol, I., Marsh, G., Jakobsson, E., Bergman, A., Brouwer, A.: Potent competitive interactions of some brominated flame retardants and related compounds with human transthyretin in vitro. Toxicol Sci. 56, 2000, 95-104.

Meerts, I., Marsh, G., van Leeuwen-Bol, I., Luijks, L.A., Jakobsson, E., Bergman, A., Brouwer, A.: Interaction of polybrominated diphenylether metabolites (PBDE-OH) with human transthyrethrin in vitro. Organohalogen Compd. 37, 1998, 309-312.

Meironyte-Guvenius, D., Aronsson, A., Ekman-Ordeberg, G., Bergmann, A., Noren, K.: Human prenatal and postnatal exposure to polybrominated diphenyl ethers, polychlorinated biphenyls, polychlorobiphenylols and pentachlorophenol. Environmental Health Perspect. 111, 2003, 1235 – 1241.

Meironyte-Guvenius, D., Noren, K.: Polybrominated diphenyl ethers in Swedish human milk. The follow-up study. The second international workshop on brominated flame retardants, BFR 2001, Stockholm, 14-16 may 2001, conference proceedings 303-305.

Meironyte, D., Bergman, A., Noren, K.: Analysis of polybrominated diphenyl ethers in human milk - proceedings from polymer addditives and monomers. Organohalogen Compd. 36, 1998, 387-390

Meironyte, D., Noren, K., Bergmann, A.: Analysis of polybrominated diphenylethers in Swedish human milk - a time-related study 1972-1997. J. Toxicol. Environm. Health, Part A, 58, 1999, 329-341

Meironyte-Guvenius, D., Herrmann, T., Noren, K.: Determination of PBDEs in human milk from the United States. Organohalogen Compd. 51, 2001, 197 - 200

Morreale de Escobar, G.: Maternal hypothyroxinemia versus hypothyroidism and potential neurodevelopmental. Alterations of her offspring. Ann Endocrinol 64, 2003, 51-2

Morreale de Escobar, G., Obregon, M.J., Escobar del Ray, F.: Is neuropsychological development related to maternal hypothyroidism or to maternal hypothyroxinemia? J Clin Endocrinol Metab. 85, 2000, 3975-87.

Noren, K., Meironyte, D.: Certain organochlorine and organobromine contaminants in swedish human milk in perspektive of past 20 - 30 years. Chemosphere 40, 2000, 1111-1123.

NTP National Toxicology Program Toxicology and Carcinogenesis Studies of Decabromodiphenyl Oxide (CAS No. 1163-19-5) In F344/N Rats and B6C3F1 Mice (Feed Studies). Natl Toxicol Program Tech Rep Ser. 309, 1986, 1-242.

NTP Technical Report n° 309. US Department of Health and Human Services. Toxicology and carcinogenesis studies of decabromodiphenyl oxide in F344N rats and B6C3F1 mice (feed studies), 1986.

Ohta, S., Ishizuka, D., Nishimura, H., Nakao, T., Aozasa, O., Shimidzu, Y., Ochiai, F., Kida, T., Nishi, M., Miyata, H.: Comparision of polybrominated diphenyl ethers in fish, vegetables and meat and levels in human milk of nursing woman in Japan. Chemosphere 46, 2002, 689-696

Päpke, O., Bathe, L., Bergmann, A., Fürst, P., Guvenius, D.M., Herrmann, T., Noren, K.: Determination of PBDE's in human milk from the United States - comparison of results from three laboratories. Organohalogen Compd. 52, 2001, 197 – 200.

Päpke, O., Bathke, L., Bergman, A., Fürst, P.: Polybrominated diphenyl ethers (PBDEs) in U.S. mother's milk. Environmental Health Persp. 111, 2003, 1723 - 1729, USA, 2001

Päpke, O., Vieth, B., Ostermann, B., Herrmann, T.: Determination of PBDEs in human milk - analysis and quality control. Organohalogen Compd. 66, 2004, 552 - 558

Petreas, M., She, J., Brown, F., Winkler, J., Windham, G., Rogers, E.: High body burden of 2,2'4,4'-tetrabromdiphenylether (BDE-47) in Californien women. Environmental Health Perspect. 111, 2003, 1175-1179

Pirard, C., De Pauw, E., Focant, J-F.: Levels of selected PBDEs and PCBs in belgian human milk. Organohalogen Compd. 61, 2003, 263 – 266.

Polder, A., Thomson, C., Becher, G., Skaare, U.J., Loken, K., Eggesbo, M.: The Norwegian human milk study - HUMIS - variations in the levels of chlorinated pesticides, PCBs and PBDEs in Norwegian breast milk. Organohalogen Compd. 66, 2004, 2476-2481

Polder, A., Thomson, C., Becher, G., Skaare, U.J., Loken, K., Eggesbo, M.: (2004a) The Norwegian human milk study - HUMIS - variations in the levels of chlorinated pesticides, PCBs and PBDEs in Norwegian breast milk. Presentation at the 24th International Symposium on Halogenated Environmental Organic Pollutants and POPs, 6.-10.09.2004, Berlin, Deutschland

Porterfield, S.P.: Thyroidal dysfunction and environmental chemicals--potential impact on brain development. Environmental Health Perspect. 108 Suppl 3., 2000, 433-8.

Porterfield, S.P.: Vulnerability of the developing brain to thyroid abnormalities: environmental insults to the thyroid system. Environmental Health Perspect. 102 Suppl 2. 1994, 125-30.

Richtlinie 2002/95/ EG des Europäischen Parlaments und des Rates vom 27. Januar 2003 zur Beschränkung der Verwendung bestimmter gefährlicher Stoffe in Elektronikgeräten, verfügbar über URL: http://europa.eu.int

Ryan, J.J., Patry, B.: Body burden and food exposure in Canada for polybrominated diphenyl ethers (PBDEs). Organohalogen Compd. 51, 2001, 226-230

Ryan, J.J., van Oostdam, J.: Polybrominated diphenyl ethers(PBDEs) in maternal and cord blood plasma of several northern Canadian populations. Organohalogen Compd. 66, 2004, 2549-2555

Ryan, J.J., Patry, B.: Body burdens and exposure from food for polybrominated diphenyl ethers (PBDEs) in Canada. The second international workshop on brominated flame retardants, BFR 2001, Stockholm, 14-16 may 2001, conference proceedings 103-106

Ryan, J.J., Schecter, A., Pavuk, M., Päpke, O., Ryan, J., Birnbaum, L., Rosen, R.: Polybrominated diphenyl ethers (PBDEs) in human milk; occurence worldwide. The third international workshop on brominated flame retardants, June 6 - 9, 2004 Toronto, Canada conference proceedings, 17 – 21

Sarver, J.G., White, D., Erhardt, P., Bachmann, K.: Estimating xenobiotic half-lives in humans from rat data: influence of log P. Environmental Health Perspect. 105, 1997,1204-9.

Schecter, A., Vuk, M.P., Päpke, O., Ryan, J.J., Birnbaum, L., Rosen, R.: Polybrominated diphenyl ethers (PBDEs) in US mothers' milk. Environmental Health Perspect 111, 2003, 1723-1729.

Schecter, A., Päpke, O., Ryan, J.J., Rosen, R., Tung, K.C., Pavuk, M., Staskal, D., Binrbaum, L., Quynh, H.T., Constable, J.D. (2004a): PBDEs in U.S. milk, blood and food and temporal trends for PBDEs, PCDDs and PCBs in US blood. Organohalogen Compd. 66, 2004a, 2834-2840

Schecter, A., Päpke, O., Ryan, J.J., Rosen, R., Tung, K.C., Pavuk, M., Staskal, D., Binrbaum, L., Quynh, H.T., Constable, J.D. (2004b): PBDEs in U.S. milk, blood and food and temporal trends for PBDEs, PCDDs and PCBs in US blood. Presentation at the 24th International Symposium on Halogenated Environmental Organic Pollutants and POPs, 6.-10.09.2004, Berlin, Deutschland

Schecter, A., Quynh, H.T., Päpke, O., Malisch, R., Constable, J.D., Tung, K.C. (2004c): Halogenated organics in Vietnamese and in Vietnam food: Dioxins, dibenzofurans, PCBs, polybrominated diphenyl ethers and selected pesticides. Organohalogen Compd. 66, 2004, 3634-3639

Schröter-Kermani, C., Helm, D., Herrmann, T., Päpke, O.: The German Environmental Specimen Bank - application in trend monitoring of polybrominated diphenylethers in human blood. Organohalogen Comp. 47, 2000, 49-52.

Senatskommission zur Prüfung von Rückständen in Lebensmitteln der Deutschen Forschungsgemeinschaft Mitteilung XII 1984, URL: http//www.dfg.de

She, J., Petreas, M., Winkler, J., Visita, P., McKinney, M., Kopec, D.: PBDEs in the San Francisco Bay area: measurements in harbour seal blubber and human breast adipose tissue. Chemosphere 46, 2002, 697-707.

Siebte Verordnung zur Änderung Chemikalienrechtlicher Verordnungen vom 29. August 2003 BGBL. I Nr.44 vom 04.09.2003, S. 1697

Sjödin, A., Hagmar, L., Klasson-Wehler, E., Kronholm-Diab, K., Jakobsson, E., Bergman, A.: Flame retardant exposure: polybrominated diphenyl ethers in blood from Swedish workers. . Environmental Health Perspect. 107, 1999, 643- 648.

Sjödin, A. Hagmar, L., Klasson-Wehler, E., Björk, J., Bergman, A.: Influence of the consumption of fatty baltic sea fish on plasma levels of halogenated environmental contaminats in Latvian and Swedish men. Environmental Health Perspect. 108, 2000a, 1035-1041.

Sjödin, A.: Occupational and dietary exposure to organohalogen substances with special emphasis on polybrominated diphenyl ethers [PhD Thesis]. Department of Environmental Chemistry, Universität Stockholm, 2000b, (ISBN 91-7265-052-4) Stockholm.

Sjödin, A., Carlsson, H., Thuresson, K., Sjödin, S., Bergman, A., Östman, C.: Flame retardants in indoor air at an electronics recycling plant and at other work environments. Environ. Sci. Technol. 35, 2001, 448 – 454.

Sjödin, A., Jones, R.S., Lapeza, Ch., Focant, J.-F., Wang, R., Turner, W.E., Needham, L.L., Patterson, D.G.: Retrospective time trend of brominated flame retardants and polychlorinated biphenlys in human serum from various regions of the United States 1985-2002. Organohalogen Compd., Vol. 60-65, 2003.

Sjödin, A., Jones, R.S., Focant, J., Lapeza, Ch., Wang, R.Y., McGahee. E., Zhang, Y., Turner, W., Slazyk, B., Needham, L., Patterson, D.: Retrospektive time-trend study of polybrominated diphenyl ether and polybrominated and polychlorinated biphenyl levels in human serum from the United States. Environmental Health Perspect. 112, 2004a, 654-658.

Sjödin, A., Päpke, O., McGahee, E., Jones, R., Foant, J-F., Pless-Mulloli, T., Toms, L.-M., Wang, R., Zhang, Y., Needham, L., Herrmann, T., Patterson, D.: Concentration of polybrominated diphenyl ethers (PBDEs) in house hold dust from various countries - Inhalation a potential route of human exposure. Organohalogen Compd. 66, 2004b, 3770-3775.

Stanley, J.S., Cramer, P.H., Thornburg, K.R., Remmers, C.J., Breen, J.J., Schwemberger, J.: Mass spectral confirmation of chlorinated and brominated diphenylethers in human adipose tissues. Chemosphere 23, 1991, 1185-1195.

Stillempfehlung: Beschluß der Nationalen Stillkommission vom 20.11.1995, Rückstände in Frauenmilch. Bundesgesundheitsblatt 39, 1995, 87

Strandman, T., Koistinen, J., Vartiainen, T.: Polybrominated diphenyl ethers (PBDEs) in placenta and human milk. Organohalogen Compd. 47, 2000, 61-65.

Strandmann, T., Kiviranta, H., Kumpulainen, J., Koistinen, J., Vartiainen, T.: Polybrominated diphenyl ethers (PBDEs) in Finnish food items. The second international

workshop on brominated flame retardants, BFR 2001, Stockholm, 14-16 may 2001, conference proceedings 303-305

Thomsen, C., Lundanes, E., Becher, G.: Brominated flame retardants in archived serum samples from Norway: a study on temporal trends and the role of age, Environ Sci Technol., 36, 2002, 1414-1418

Thomson, C., Frohaug, M., Leknes, H., Becher, G.: Brominated flame retardants in breast milk from Norway. Organohalogen Compd. 2003, 61, 33 – 36.

Thuresson, K., Jakosson, K., Hagmar, L., Englyst, V., Bergman, A.: Work related exposure to brominated flame retardants when recycling metals from printed circuit boards, Organohalogen Compd. 58, 2002, 249.

Bromine Science and Environmental Forum 2001 und 2003: URL:http://www.bsef.com

Viberg, H., Fredriksson, A., Jakobsson, E., Örn, U., Eriksson, P.: Brominated flame retardant uptake, retention and developmental neurotoxic effects of decabromodiphenyl ether (PBDE 209) in the neonatal mouse. Poster presentation, Second International Workshop on Brominated Flame Retardants, May 14-16, Stockholm University. (2001).

Viberg, H., Fredriksson, A., Jakobsson, E., Örn, U., Eriksson, P.: Developmental neurotoxic effects of 2,2',4,4',5,5'-pentabromodiphenyl ether (PBDE 99) in the neonatal mouse. Toxicologist, 54, 2000, 290.

Vieth, B. (2001) in "4. Bericht der Bund/Länder-Arbeitsgruppe DIOXINE, Dioxin-Referenzmeßprogramm", Kapitel Humandaten

Vieth, B., Stillen und unerwünschte Fremdstoffe in Frauenmilch, Teil 1: Datenlage und Trends in Deutschland. Umweltmedizinischer Informationsdienst (UMID) 2, 2002, 20-23.

Vieth, B., Heinrich-Hirsch, B., Mathar, W.: Trends in dioxin intake and human milk levels in Germany. 20th International symposium on Halogenated Environmental Pollutants and POPs, Organohalogen Compd. 47, 2000, 300.

Vieth, B., Herrmann, T., Mielke, H., Ostermann, B., Päpke, O., Rüdiger, T.: PBDE levels in human milk: The situation in Germany and potential influencing factors - a controlled study. Organohalogen Compd. 66, 2004, 22613-2618

von Meyerinck, L., Hufnagel, B., Schmoldt. A., Benthe, H.F.: Induction of rat liver microsomal cytochrome P-450 by the pentabromo diphenyl ether Bromkal 70 and half-lives of its components in the adipose tissue. Toxicology. 61,1990, 259-74.

Wallgren, A.: Breast milk consumption of healthy full term infants, Acta Pediatr. 32, 1945, 778 - 790.

Weber, H., Heseker, H.: Bestimmung von polybromierten Flammschutzmitteln in Frauenmilch deutscher Frauen. Ernährungsumschau 51, 2004, 4-9.

Weiss. J., Meijer, L., Sauer, P., Linderholm, L., Athanassiadis, I., Bergman, A.: PBDE and HBCDD levels in blood from Dutch mothers and infants - analysis of a Dutch Groningen infant cohort. Organohalogen Compd. 66, 2004, 2647-2652.

Wijesekera, R., Halliwelli, C., Hunter, S., Harrad, S.: A preliminary assessment of UK human exposure to polybrominated diphenyl ethers (PBDEs). Organohalogen Compd. 55, 2002, 239-241

11 Lists

11.1 Abbreviations used

ADI	Acceptable daily intake
BDE	Brominated diphenyl ethers
BDE-28	2,4,4´-tribromodiphenyl ether
BDE-47	2,2´,4,4´-tetrabromodiphenyl ether
BDE-66	2,3´,4,4´-tetrabromodiphenyl ether
BDE-85	2,2´3,4,4´-pentabromodiphenyl ether
BDE-99	2,2´4,4´,5-pentabromodiphenyl ether
BDE-100	2,2´4,4´,6-pentabromodiphenyl ether
BDE-153	2,2´,4,4´,5,5´-hexabromodiphenyl ether
BDE-154	2,2´,4,4´,5,6´-hexabromdiphenyl ether
BDE-183	2,2´,3,4,4´,5,6-heptabromodiphenyl ether
BDE-209	2,2´, 3,3´, 4,4´, 5,5´, 6,6´-decabromodiphenyl ether
LOD	Limit of determination
DBDE	Decabromodiphenyl ether, technical product
EI	Electron impact ionization
EU	European Union
GC	Gas chromatography
HRMS	High resolution mass spectrometry
Max	Maximum
Med	Median
Min	Minimum
MOS	Margin of Safety
MV	Mean value
N	Number of samples

NOAEL	No observed adverse effect level
OBDE	Octabromodiphenyl ether, technical product
p.p.	post partum (after delivery)
PBDE	Polybrominated diphenyl ether
PBBs	Polybrominated biphenyls
PCBs	Polychlorinated biphenyls
PCDD/PCDF	Polychlorinated dibenzodioxins and dibenzofurans („dioxins“)
PeBDE	Pentabromodiphenyl ether, technical product
RAR	Risk Assessment Report
RSD	Relative standard deviation
SD	Standard deviation
SOP	Standard Operation Procedure
Total PBDE	Sum of all PBDE congeners
T3	Triiodthyronine
T4	Tetraiodthyronine (thyroxine)

11.2 List of Tables

Page

Table 1: Mean PBDE levels (congeners analyzed and total PBDE levels) in human specimens with background exposure – Current international data (in ng/g fat)…. 18

Table 2: PBDE levels in the blood of exposed workers (medians and ranges; Sjödin 1999, 2001, Thuresson, 2002, Jakobsson, 2002) 20

Table 3: Dietary intake levels of PBDE and percentage share of the different food groups – International data 24

Table 4: Schedule of the sampling plan 28

Table 5: ^{13}C-labelled internal standards used, mass traces (m/z) for detection and quantification and mean limits of determination of the breast milk method 33

Table 7: Characterization of the evaluation groups, total cohort, study cohort, omnivores, and vegetarians with regard to age, height, weight, body mass index (BMI), hours of screen exposure, smoking habits, number of breastfed children, country of birth 38

Table 8: Comparison of frequencies of consumption of foods of animal origin between omnivores (Cohort 1) and vegetarians (Cohort 2) 39

Table 9: PBDE concentrations in breast milk samples from the total cohort and the study cohort (in ng/g fat; levels < LOD included as 50 % of LOD level; 1st time of sampling) 40

Table 10: Comparison between the mean values of PBDE levels in breast milk from omnivores (Cohort 1) and those from vegetarians/vegan (Cohort 2), and results of the one-tailed t test (1st time of sampling) 44

Table 11: Mean values of PBDE levels in breast milk sampled at the 1st and 2nd time of sampling, and results of the paired t test 46

Table 12: Mean values of PBDE levels in breast milk from mothers breastfeeding their first child vs. those from mothers breastfeeding their second or third child, and results of comparison of mean values by the t test 49

Table 13: PBDE levels in milk samples from extended breastfeeders 50

Table 14: Estimated model parameters for the model, diet and number of breastfed children including the 95 % confidence intervals for C' and D' 53

Table 15: Parameters of the correlation calculation for the potential confounders 55

Table 17: Comparison of PBDE levels in blood and breast milk (in ng/g fat) 58

Table 18: Levels of BDE-209 in breast milk – Current data 63

11.3 List of Figures

Page

Fig. 1 Shares of single PBDE congeners in the total PBDE level in breast milk samples from German 41

Fig. 2: Histograms for total PBDE levels and log [total PBDE] in breast milk from the first time of sampling including the curve of the normal distribution (study cohort; N = 73)…… 42

Fig. 3 Histograms for total PBDE levels and log [total PBDE] in breast milk from the second time of sampling including the curve of the normal distribution (study cohort; N = 35)…… 42

Fig. 4: Box-whisker plot for total PBDE levels in breast milk samples from omnivores, vegetarians and one vegan (1st time of sampling) 43

Fig. 5: Comparison of levels of BDE-47, BDE-99, BDE-100, BDE-153 and BDE-183 between Cohorts 1 and 2 by means of box-whisker plots 45

Fig. 6: Comparison of the individual total PBDE levels of the 1st and 2nd sample; left: representation of the levels; right: representation of the differences (circles = Cohort 1, crosses = Cohort 2) 47

Fig. 7 Box-whisker plot of total PBDE levels (in logarithmic form) depending on the number of breastfed children (1st time of sampling) 48

Fig. 8 Comparison of total PBDE levels depending on the dietary habits and the number of breastfed children (box-whisker plots; 1st time of sampling); left: normal values, right: logarithmic values, red: 1 breastfed child, green: 2 breastfed children, blue: 3 breastfed children.. 51

Fig. 9: Scatter diagrams of total PBDE levels with age and body mass index of the mother…….. 54

Fig. 10: Scatter diagrams of total PBDE and BDE-209 with hours of screen exposure per week 54

Fig. 11: Box-whisker plot for total PBDE levels depending on the smoking habits 55

Fig. 12: Comparison of PBDE levels in breast milk samples from omnivores (Cohort 1, blue) and vegetarians (Cohort 2, yellow).....65

Fig. 13: Changes in the pattern of congeners due to extended breastfeeding or repeated lactation periods 69