Detection of polybrominated diphenyl ethers and polychlorinated biphenyls in fatty tissues by gas chromatography combined with tandem mass spectrometry

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Thesis submitted in fulfilment of the requirements for the degree of Doctor in Pharmaceutical Sciences

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LIST OF ABBREVIATIONS

2,3,7,8-TCDD  2,3,7,8-tetrachlorodibenzo-p-dioxin
ABS           acrylonitrile-butadiene-styrene
AhR           aryl hydrocarbon-receptor
ASE           accelerated solvent extraction
atm           standard atmosphere
BDE 28        2,2,4′-tribromodiphenyl ether
BDE 47        2,2′,4,4′-tetrabromodiphenyl ether
BDE 51        2,2′,4,6′-tetrabromodiphenyl ether
BDE 75        2,4,4′,6-tetrabromodiphenyl ether
BDE 77        3,3′,4,4′-tetrabromodiphenyl ether
BDE 99        2,2′,4,4′,5-pentabromodiphenyl ether
BDE 100       2,2′,4,4′,6-pentabromodiphenyl ether
BDE 101       2,2′,4,5,5′-pentabromodiphenyl ether
BDE 118       2,3′,4,4′,5-pentabromodiphenyl ether
BDE 119       2,3′,4,4′,6-pentabromodiphenyl ether
BDE 126       3,3′,4,4′,5-pentabromodiphenyl ether
BDE 153       2,2′,4,4′,5,5′-hexabromodiphenyl ether
BDE 154       2,2′,4,4′,5,6′-hexabromodiphenyl ether
BDE 183       2,2′,3,4,4′,5′,6-heptabromodiphenyl ether
BDE 209       decabromodiphenyl ether
BFRs          brominated flame retardants
CALUX         chemically activated luciferase expression
CCα           decision limit
CCβ           detection capability
CI             chemical ionization
CID           collision induced dissociation
CITAC         Co-Operation on International Traceability in Analytical Chemistry
CRM           certified reference material
CYP450        cytochrome P450
ECD           electron capture detection
EI            electron ionization
EST           estrogen sulfotransferase
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC</td>
<td>gas chromatography</td>
</tr>
<tr>
<td>GPC</td>
<td>gel permeation chromatography</td>
</tr>
<tr>
<td>HETP</td>
<td>height equivalent to one theoretical plate</td>
</tr>
<tr>
<td>HBCD</td>
<td>hexabromocyclododecane</td>
</tr>
<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
</tr>
<tr>
<td>HRGC</td>
<td>high resolution gas chromatography</td>
</tr>
<tr>
<td>HRMS</td>
<td>high resolution mass spectrometry</td>
</tr>
<tr>
<td>IARC</td>
<td>International Agency for Research on Cancer</td>
</tr>
<tr>
<td>ICES</td>
<td>International Council for the Exploration of the Sea</td>
</tr>
<tr>
<td>id</td>
<td>internal diameter</td>
</tr>
<tr>
<td>IUPAC</td>
<td>International Union of Pure and Applied Chemistry</td>
</tr>
<tr>
<td>$K_{ow}$</td>
<td>octanol-water partitioning coefficient</td>
</tr>
<tr>
<td>LD$_{50}$</td>
<td>lethal dose for 50 % of subjects</td>
</tr>
<tr>
<td>LRMS</td>
<td>low resolution mass spectrometry</td>
</tr>
<tr>
<td>lw</td>
<td>lipid weight</td>
</tr>
<tr>
<td>m/z</td>
<td>mass-to-charge ratio</td>
</tr>
<tr>
<td>MAE</td>
<td>microwave assisted extraction</td>
</tr>
<tr>
<td>MC</td>
<td>3-methylcholantrene</td>
</tr>
<tr>
<td>MeO-PBDE</td>
<td>methoxylated polybrominated diphenyl ether</td>
</tr>
<tr>
<td>MeSO$_2$-PCB</td>
<td>methylsulfonylated polychlorinated biphenyl</td>
</tr>
<tr>
<td>MS</td>
<td>mass spectrometry</td>
</tr>
<tr>
<td>MS/MS</td>
<td>tandem mass spectrometry</td>
</tr>
<tr>
<td>MSPD</td>
<td>matrix solid-phase dispersion</td>
</tr>
<tr>
<td>N</td>
<td>number of theoretical plates</td>
</tr>
<tr>
<td>NCI</td>
<td>negative chemical ionization</td>
</tr>
<tr>
<td>OH-PCB</td>
<td>hydroxylated polychlorinated biphenyl</td>
</tr>
<tr>
<td>PB</td>
<td>phenobarbital</td>
</tr>
<tr>
<td>PBB</td>
<td>polybrominated biphenyl</td>
</tr>
<tr>
<td>PBB 103</td>
<td>2,2',4,5',6-pentabromobiphenyl</td>
</tr>
<tr>
<td>PBB 155</td>
<td>2,2',4,4',6,6'-hexabromobiphenyl</td>
</tr>
<tr>
<td>PBDD</td>
<td>polybrominated dibenzodioxin</td>
</tr>
<tr>
<td>PBDE</td>
<td>polybrominated diphenyl ether</td>
</tr>
<tr>
<td>PBDF</td>
<td>polybrominated dibenzofuran</td>
</tr>
<tr>
<td>PCB</td>
<td>polychlorinated biphenyl</td>
</tr>
<tr>
<td>PCB 25</td>
<td>2,3',4-trichlorobiphenyl</td>
</tr>
</tbody>
</table>
PCB 28 2,4,4’-trichlorobiphenyl
PCB 31 2,4’,5-trichlorobiphenyl
PCB 52 2,2’,5,5’-tetrachlorobiphenyl
PCB 101 2,2’,4,5,5’-pentachlorobiphenyl
PCB 118 2,3’,4,4’,5-pentachlorobiphenyl
PCB 138 2,2’,3,4,4’,5’-hexachlorobiphenyl
PCB 143 2,2’,3,4,5,6’-hexachlorobiphenyl
PCB 153 2,2’,4,4’,5,5’-hexachlorobiphenyl
PCB 180 2,2’,3,4,4’,5,5’-pentachlorobiphenyl
PCB 209 decachlorobiphenyl
PCDD polychlorinated dibenzodioxin
PCDF polychlorinated dibenzofuran
PCI positive chemical ionization
POP persistent organic pollutant
PTV programmable temperature vaporization
QISTMS quadrupole ion storage tandem mass spectrometry
R resolution
RF radio frequency
RoHS Restriction of certain Hazardous Substances in electrical and electronic equipment
RRT relative retention time
SEC size exclusion chromatography
SFE supercritical fluid extraction
SIM selected ion monitoring
SPE solid phase extraction
T3 triiodothyronine; 3,3’,5-triiodothyronine
T4 thyroxine; 3,3’,5,5’-tetraiodothyronine
TBBPA tetrabromobisphenol A
TEF toxic equivalency factor
TEQ total toxic equivalent
THR thyroid hormone receptor
TSH thyroid stimulating hormone
TTR transthyretin
U_{Dc} direct potential
UDPGT uridine diphosphate glucuronyl transferase
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>USE</td>
<td>ultrasonic extraction</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
<tr>
<td>V&lt;sub&gt;RF&lt;/sub&gt;</td>
<td>amplitude of the oscillating potential</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>ww</td>
<td>wet weight</td>
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</table>
CHAPTER 1. POLYBROMINATED DIPHENYL ETHERS
1.1. **INTRODUCTION TO BROMINATED FLAME RETARDANTS**

Fires are worldwide significant causes of death, injury and property damage. They cost society directly 0.07 % (Singapore) - 0.28 % (Norway) of the gross domestic product (2001-2003) (The Geneva Association, 2006). New technologies, new processes and new applications introduce new fire hazards. During the past several decades, modern technology has responded to this challenge by introducing flame retardant chemicals which reduce the chances of ignition and burning of a wide range of textiles, plastics, building materials and electronic equipment.

Flame retardants have already been in use since Egyptian times where alum was used to reduce the flammability of wood (Hindersinn, 1990). Nowadays, 175 different flame retardants are on the market and they can be divided into four major groups: inorganic, halogenated organic, organophosphorous and to lesser extent nitrogen based compounds (Environmental Health Criteria 192, 1997).

Halogenated organic flame retardants represent approximately 25 % of the worldwide flame retardant production and are generally classified as either chlorinated or brominated compounds. Fluorinated compounds are ineffective because of their strong C-F bond, and iodinated compounds because of their instability. The brominated flame retardants (BFRs) (Figure I.1) are often the more cost-effective flame retardants and can be divided again into reactive and additive BFRs. The reactive BFRs, e.g. tetrabromobisphenol A (TBBPA), are chemically bound to the polymer during synthesis. Additive BFRs, which include polybrominated diphenyl ethers (PBDEs), polybrominated biphenyls (PBBs) and hexabromocyclododecane (HBCD), are dissolved into the polymer and are believed to be more easily released in the environment than the reactive BFRs (Alaee and Wenning, 2002).
Figure I.1 Chemical structures of brominated flame retardants

Hexabromocyclododecane (HBCD)  Polybrominated diphenyl ether (PBDE)

Polybrominated biphenyl (PBB)  Tetrabromobisphenol A (TBBPA)

The total global market demand in 2003 of BFRs was estimated at 223,482 metric tons of which 56,418 metric tons constituted of Deca-BDE and 145,113 metric tons constituted of TBBPA. Major producers of BFRs are Albemarle Corporation, ICL industrial products, Chemtura and Tosoh Corporation (Bromine Science and Environmental Forum, 2006).
1.2. **MECHANISM OF ACTION OF HALOGENATED FLAME RETARDANTS**

Solid materials do not burn immediately, but they first have to be decomposed by heat (pyrolysis) to release flammable gases. After ignition, visible flames appear when these flammable gases react with oxygen to form CO\textsubscript{2} and generate more heat (burning phase). If sufficient heat is generated and radiated back to the material to continue the decomposition process, combustion becomes self-propagating. Halogenated flame retardants act by removing the H and OH radicals in the gas phase. This slows down or prevents the burning phase, thus reducing heat generation and so the production of further flammable gases (Environmental Health Criteria 192, 1997).

When exposed to high temperatures, the flame retardant molecules release Br or Cl radicals which react with hydrocarbon molecules of the flammable gases to form HBr or HCl. These compounds then react with OH and H radicals to form H\textsubscript{2}O, H\textsubscript{2} and again Br or Cl radicals:

\[
\begin{align*}
RX & \rightarrow R' + X' \\
X + RH & \rightarrow R' + HX \\
HX + OH & \rightarrow H_2O + X' \\
HX + H & \rightarrow H_2 + X'
\end{align*}
\]

The effectiveness of the flame retardant depends on the quantity of halogen atoms it contains and on its control of halogen release. Bromine is released in a narrow temperature range, thus resulting in optimal concentrations in the flame zone. In contrast, chlorine is released over a wider temperature range, making it less effective (Environmental Health Criteria 192, 1997).
CHAPTER 1. POLYBROMINATED DIPHENYL ETHERS

1.3. POLYBROMINATED DIPHENYL ETHERS

1.3.1. Identity, use and production

Polybrominated diphenyl ethers (PBDEs) represent a large group of the BFRs. A major incident with polybrominated biphenyls (PBBs) in Michigan (USA) in 1973 resulted in a substantial decrease of PBB production and the widespread use of PBDEs.

PBDEs have a structure similar to the PCBs, except for the oxygen atom between the aromatic rings. Theoretically there are 209 PBDE congeners which are divided into 10 congener groups (mono- to decabromodiphenyl ethers). The numbering of the congeners is analogous to the IUPAC nomenclature and the systematic numbering system for PCBs (Ballschmiter and Zell, 1980; Ballschmiter et al., 1992). The IUPAC nomenclature specifies the total number of bromine substituents and the position of each bromine. The systematic numbering system for PCBs, first proposed by Ballschmiter and Zell in 1980 and almost universally adopted, applies a number (from 1 to 209) to each congener after the congeners have been sorted on the basis of their structural names.

Commercial PBDE mixtures are produced by brominating diphenyl oxide in the presence of a Friedel-Craft catalyst. Each of the three existing commercial products contains a mixture of various congeners. The general composition of these products is given in Table I.1. The Penta-product is mainly used in polyurethane foams for upholstery and furnishings and that in concentrations up to 30%. The Octa-product is used in acrylonitrile-butadiene-styrene (ABS)-plastics of hard casings for televisions, computers and home appliances. The Deca-product is primarily used in textiles and in denser plastics (high impact polystyrene) of televisions and circuit boards (Wenning, 2002).

Table I.1 General composition of three commercially produced PBDE mixtures given in percent of BDE congeners present (Environmental Health Criteria 192, 1997)

<table>
<thead>
<tr>
<th>Product</th>
<th>Composition</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>TrBDEs</td>
</tr>
<tr>
<td>Penta-BDE</td>
<td>0-1%</td>
</tr>
<tr>
<td>Octa-BDE</td>
<td>10-12%</td>
</tr>
<tr>
<td>Deca-BDE</td>
<td>0.3-3%</td>
</tr>
</tbody>
</table>
PBDEs are very lipophilic, with log $K_{\text{ow}}$s (octanol-water partitioning coefficients) ranging from 3.7 (tetra-BDEs) to 11 (BDE 209) (Palm et al., 2002). The log $K_{\text{ow}}$ may give an indication of the possible degree of accumulation. The higher this value, the more likely it is that the compound tends to accumulate in fatty tissues and oily components of biota. PBDEs have low vapour pressures. The larger the number of bromine atoms, the heavier the molecule and consequently the less volatile the molecule is.

Although PBDEs are similar to PCBs, they are believed to enter the environment in different ways. PCBs, in general, enter the environment directly from point sources, e.g. from broken capacitors or by accidental discharge. PBDEs, which are not bound to the polymers that are used in plastics and foams, may not only leach in the environment during natural operating life of television sets, computers and upholstery, but also during processing, recycling or combustion (D'Silva et al., 2004). Detection of PBDEs in home and in work environments indicates that we all may be chronically exposed to PBDEs (Gevao et al., 2006; Jones-Otazo et al., 2005; Wilford et al., 2005).

Highly toxic polybrominated dibenzodioxins (PBDDs) and polybrominated dibenzofurans (PBDFs) may be formed during the combustion of PBDEs and of other BFRs. During this process the released bromine is combined with organic matter and forms brominated dioxins and furans. Formed levels depend on the type of flame retardant, type of polymer matrix, processing conditions (temperature and presence of oxygen) and presence of $\text{Sb}_2\text{O}_3$. Maximum concentrations of PBDFs and/or PBDDs were observed in a temperature range of 400-800 °C but 2,3,7,8-substituted compounds were only formed in very low concentrations (Environmental Health Criteria 162, 1994).

### 1.3.2. Breakdown in the environment

Because of the increasing use of Deca-BDE, it is important to understand its degradation and its relation to the occurrence of the more persistent lower brominated BDE congeners in the environment. PBDEs are resistant towards acids and bases and also towards reducing or oxidizing compounds (Pijnenburg, 1995). Due to the C-Br bond being weaker than the C-Cl bond, they are likely to be more susceptible to environmental degradation than PCBs. Eriksson et al. (2001; 2004) showed that the higher brominated congeners decomposed more rapidly in methanol/water (8:2) under influence of UV light than the less brominated ones. The photochemical degradation half-life of BDE 209 ($t_{1/2} = 0.5$ h) was more than 500
times shorter than that of the environmentally abundant BDE 47 ($t_{1/2} = 12$ d). In soil and sediment BDE 209 had a half-life in sunlight of 200 and 100 h respectively. Photolytic stability of BDE 209 has also been investigated by Soderstrom et al. (2004). Results showed that BDE 209 was photolytically unstable and forms nona- to tetra-BDEs in all investigated matrices (toluene, silica gel, sand, sediment and soil). No differences in debromination patterns were seen between different light conditions and different matrices. Rayne et al. (2006) reported also short photochemical half-life for BDE 153 with BDE 99, BDE 101 and BDE 118 as primary photodebromination products. If these degradation processes are significant in the environment, the degradation of higher brominated BDE congeners may contribute to the levels of the lower brominated BDE congeners, which are known to accumulate.

Research by Gerecke et al. (2005) showed that BDE 209 can also undergo microbially mediated reductive debromination under anaerobic conditions to nona- and octa-BDEs. He et al. (2006) also reported degradation of Deca-BDE and of an Octa-BDE mixture by anaerobic bacteria to lower brominated BDEs.

### 1.3.3. Bioaccumulation and biomagnification

Since Andersson and Blomkvist first reported PBDE contamination in the environment in 1981, there have been many indications of increased environmental and human PBDE accumulation over the last 10-15 years (Koizumi et al., 2005; Rayne et al., 2003). Long range transport of PBDEs is even suggested because these substances have been found at very remote areas (de Wit et al., 2006; Hale et al., 2003; Wania and Dugani, 2003).

PBDEs are very hydrophobic and therefore they are strongly adsorbed to soil, sediment and sludge. In these matrices, the higher brominated compounds are prevalent and the congener patterns reflect those of the utilized commercial mixtures. In biota however, these higher brominated congeners are normally present at lower levels and congener patterns differ considerably from those found in commercial products. This might be due to differences in bioaccumulation or biomagnification potential and differences in biotransformation between congeners. Biomagnification may be defined as the bioaccumulation of a substance up the food chain.

In biota, there is a clear tendency of increasing PBDE concentrations towards animals higher in the food chain (Law et al., 2006). Concentrations in invertebrates are lower than
those in fish, which themselves are much lower than those in marine mammals (Johnson-Restrepo et al., 2005). As stated before, the log $K_{ow}$ can give a prediction of biomagnification potential of a compound. However, it has been shown by Burreau et al. (2004; 2006) that tri- to hepta-BDEs do biomagnify but that a maximum for biomagnification was shown for penta- BDEs. The biomagnification of hexa- to hepta-BDEs was negatively correlated with the degree of bromination, likely due to their larger molecular size. Octa-BDEs, nona-BDEs and BDE 209 were detected, but did not biomagnify. On the other hand, debromination of higher brominated congeners due to environmental degradation or biotransformation can also highly influence the determination of bioaccumulation and biomagnification potential (Isosaari et al., 2005; Sormo et al., 2006; Tomy et al., 2004). High concentrations of BDE 209 in liver of the red fox (Vulpes vulpes) (up to 760 ng g$^{-1}$ lipid weight in liver) found by Voorspoels et al. (2006) have demonstrated that also BDE 209 might biomagnify in terrestrial top predators. Recently, Law et al. (2006) also found a strong positive linear relationship between BDE 209 concentrations and trophic level in the Lake Winnipeg food web.

1.3.4. Toxicokinetics and metabolism

Absorption, distribution, metabolism and excretion of PBDEs are highly congener and species dependent. The absorption after oral administration of BDE 209 (dissolved in dimethylamide/polyethylene glycol 400/water: 4/4/1) in rats was at least 26 % (Sandholm et al., 2003) and that of BDE 47 (dissolved in corn oil) in mice was at least 80 % (Staskal et al., 2005). So it seems that the bioavailability of BDE 209 is rather low.

Also in rats, at least 70 % and at least 50 % of the radio-labelled oral dose of BDE 100 and BDE 99 respectively, was retained after 72 h (Hakk et al., 2002; Hakk et al., 2006). Several studies indicate that lipophilic tissues are preferred sites for PBDE disposition (Chen et al., 2006; Darnerud and Risberg, 2006; Hakk et al., 2006; Sanders et al., 2006; Staskal et al., 2006).

Staskal et al. (2006) investigated the toxicokinetics of BDE 47, BDE 99, BDE 100 and BDE 153 in mice after intravenous injection. BDE 47 was excreted as the parent compound in both urine and faeces and that to a much greater extent than the other congeners in this study. This suggests that BDE 47 is less metabolized than BDE 99, BDE 100 and BDE 153. In contrast metabolites of BDE 99, BDE 100 and BDE 153 dominated over parent compounds in urine and faeces. These metabolites were congener specific and included mainly hydroxylated
and debrominated derivatives. Hydroxylated metabolites have also been detected in rat faeces after oral administration of BDE 47 (Marsh et al., 2006), BDE 100 (Hakk et al., 2006) and BDE 209 (Sandholm et al., 2003). The presence of these hydroxylated metabolites suggests metabolism via the cytochrome P450 (CYP) enzyme system. In vitro studies with DE-71 (58.1 % penta-BDE and 24.6 % tetra-BDE) and DE-79 (30.7 % octa-BDE and 45.1 % hepta-BDE) revealed increased activities of the hepatic detoxification isoenzymes CYP 1A1 (10 - 20 fold) and CYP 2B (30 - 40 fold) (Zhou et al., 2001). This means that PBDEs may induce their own metabolism. Greater rate of metabolism for BDE 99 than for BDE 100 was seen by Staskal et al. (2006). These differences in metabolism may be explained by differences in enzyme induction between congeners.

Half-lives in human serum of occupationally exposed workers seemed to decrease with increasing number of bromine substituents. Half-lives ranged from 15 days for BDE 209, 18 - 39 days for nona-BDEs, to 37 - 91 days for octa-BDEs (Thuresson et al., 2006b). Human whole-body half-lives of BDE 47, BDE 99 and BDE 153 are estimated between 1.8 - 3 years, 2.9 - 5.4 years and 6.5 - 11.7 years respectively (Geyer et al., 2004).

Recently, methoxylated diphenyl ethers (MeO-PBDEs) have been identified in salmon, in guillemot and Arctic cod liver (Sinkkonen et al., 2004), in liver from dolphins (Pettersson et al., 2004), in blubber from California sea lions (Stapleton et al., 2006), in dolphin and whale species from Japan (Marsh et al., 2005) and in plasma samples from glaucous gulls and polar bears (Verreault et al., 2005). There is evidence that MeO-PBDEs and also PBDEs and OH-PBDEs may also be naturally occurring molecules that originate from sponges (e.g. Dysidea sp.) (Bowden et al., 2000; Carte and Faulkner, 1981; Fu and Schmitz, 1996; Vetter et al., 2002) and red algae (Malmvar et al., 2005). In how far these compounds could be a contributory factor to the environmental pollution is not known.

In rats, 14.5 % of orally administered $^{14}$C-labelled BDE 47 was excreted (14 % in faeces and 0.5 % in urine), 79 % of which was the parent compound and 21 % corresponded to metabolites (Örn and Klasson-Wehler, 1998). In mice however, 53 % of orally administered $^{14}$C-labelled BDE 47 was excreted (20 % in faeces and 33 % in urine), 15 % of which was the parent compound and 85 % corresponded to metabolites (Örn and Klasson-Wehler, 1998). Similar differences have been seen for BDE 99 by Hakk et al. (2002) and Staskal et al. (2006). Rats are thus considerably less efficient in metabolizing BDE 47 and BDE 99 than mice.
1.3.5. Human exposure

The high degree of inter-individual variability in the concentration of PBDEs observed in human adipose tissue samples may be due to different factors related to exposure including diet, occupation, age and use of consumer products (e.g. computers, television sets, hair dryers) (Branchi et al., 2003).

1.3.5.1. Intake through food

The diet is seen as the main contributing factor, when it comes to total PBDE body burden. An overview of the dietary intake of PBDEs in different countries is listed in Table I.2. These data should be interpreted with caution because not all studies included the same number of PBDE congeners. Food of animal origin, e.g. fatty fish, dairy products and meat, with high fat content are major contributors to our dietary PBDE intake (Darnerud et al., 2001). BDE 47 and BDE 99 are the predominant congeners in food. A market basket study in the US has also reported that BDE 209 can be a major contributor in several food samples (Schecter et al., 2004).

Table I.2 Dietary intake of PBDEs in different countries

<table>
<thead>
<tr>
<th>Country</th>
<th>Intake (ng day⁻¹)ᵃ</th>
<th>Main congeners</th>
<th>Main contributing food groups</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Belgium</td>
<td>23</td>
<td>not indicated</td>
<td>Fish and seafood (39%), meat products (30%), cheese-butter-eggs (27%) and fast food (5%)</td>
<td>Voorspoels et al. (2007)</td>
</tr>
<tr>
<td>Finland</td>
<td>40</td>
<td>BDE 47 and BDE 99</td>
<td>Fish (53%), fats (17%) and beverages, sweets and spices (9%)</td>
<td>Kiviranta et al. (2004)</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>72</td>
<td>BDE 47 and BDE 183</td>
<td>Oils and fats (25%), milk (19%) and fish (13%)</td>
<td>de Mul et al. (2005)</td>
</tr>
<tr>
<td>Spain</td>
<td>82</td>
<td>Tetra-BDEs and penta-BDEs</td>
<td>Fish and shellfish (31%), oils and fats (24%) and meat (21%)</td>
<td>Bocio et al. (2003)</td>
</tr>
<tr>
<td>Sweden</td>
<td>35</td>
<td>BDE 47</td>
<td>Fish (47%), dairy products (17%) and meat (14%)</td>
<td>Darnerud et al. (2006)</td>
</tr>
<tr>
<td>United States</td>
<td>63</td>
<td>BDE 47 and BDE 99</td>
<td>Fish, meat, dairy</td>
<td>Schecter et al. (2006)</td>
</tr>
</tbody>
</table>

ᵃ Daily intake was estimated for an adult of 70 kg body weight using lower-bound intake levels.
Mainly workers in the manufacturing business of BFRs and workers in electronic dismantling plants are occupationally exposed. A survey by Sjödin et al. (1999) reported significantly higher PBDE concentrations (BDE 47, BDE 153, BDE 154, BDE 183, BDE 209) in the serum of personnel working at an electronic dismantling plant as compared to the levels found in two control groups (hospital cleaners and clerks). Moreover, electronic dismantling workers had 70 times higher blood levels of BDE 183 and only 1.8 times higher blood levels of BDE 47 compared to cleaners. This difference suggests that the exposure route for BDE 47 is mainly through food whereas airborne uptake is more pronounced for BDE 183. This theory is reinforced by the presence of higher brominated congeners in the ambient air of the plant (Sjödin et al., 2001). Similar observations have been made by Thomsen et al. (2001) and Thuresson et al. (2005). In response to these findings, industrial hygiene measures to reduce PBDE exposure were taken at the plant. As a result BDE 183 and BDE 209 blood levels of workers decreased significantly (Thuresson et al., 2006a).

PBDEs are also released in the home and work environment and they originate from commercial products such as furniture, electronic equipment (e.g. computers and televisions) and small motor appliances (e.g. hair dryers) during their use. Computer work is associated with elevated serum levels of BDE 153, BDE 183 and BDE 209 (Jakobsson et al., 2002). Inhalation and dust ingestion may be the second most important contributing factor, after dietary exposure, to the total human exposure of PBDEs. Some studies suggest that this might even be the most important exposure route, except for infants who are breast-fed (Gevao et al., 2006; Jones-Otazo et al., 2005). Wu et al. (2007) found a significant positive association between PBDE concentrations in breast milk of first-time mothers living in the Boston area and PBDE levels in dust from these women’s homes. Estimated daily human exposure to PBDEs through dust ingestion in Singapore was estimated between 4.8 and 116 ng day\(^{-1}\) for adults and between 64 and 232 ng day\(^{-1}\) for children (6 months – 2 years). The most abundant PBDEs in the dust samples were BDE 209, BDE 99 and BDE 47 (Tan et al., 2007). Wilford et al. (2005) calculated that ingestion of dust in the home environment (Ottawa, Canada) represents 14 % of the total daily exposure to PBDEs for adults. For toddlers however, this value increases to 80 % of the daily intake. Maximum daily human exposure via the inhalation pathway is estimated at 4 % (Wilford et al., 2004).
1.3.5.3. **Human milk**

Research performed by Noren and Meironyté (2000) and Meironyté et al. (1999) showed that PBDE levels in breast milk increased exponentially from 1972 to 1997 (0.07 - 4.02 ng g\(^{-1}\) lipid). Another time-trend study also showed an increase in PBDE levels from 1973 to 1988 (<0.01 - 1.6 ng g\(^{-1}\) lipid). After a decrease at the beginning of the 1990s, the concentration increased again and started levelling off (Akutsu et al., 2003). Jones- Otazo et al. (2005) calculated that human milk contributes the most to the breast-fed infant’s exposure. This importance of human milk is not surprising given the relatively high PBDE concentrations reported in literature (Table I.3).

Table I.3 PBDE concentrations in human milk from different countries

<table>
<thead>
<tr>
<th>Country</th>
<th>Year</th>
<th>n</th>
<th>Sum PBDEs (ng g(^{-1}) lipid weight)(^a)</th>
<th>Main congeners</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central Taiwan</td>
<td>2000 - 2001</td>
<td>20</td>
<td>3.9</td>
<td>BDE 47 and BDE 153</td>
<td>Chao et al. (2007)</td>
</tr>
<tr>
<td>Faroe Islands</td>
<td>1999</td>
<td>9</td>
<td>7.2</td>
<td>BDE 47 and BDE 153</td>
<td>Fängström et al. (2005)</td>
</tr>
<tr>
<td>Italy</td>
<td>1998 - 2001</td>
<td>39</td>
<td>2.8</td>
<td>BDE 47, BDE 99 and BDE 153</td>
<td>Ingelido et al. (2007)</td>
</tr>
<tr>
<td>Japan</td>
<td>2004</td>
<td>105</td>
<td>1.3</td>
<td>BDE 47</td>
<td>Eslami et al. (2006)</td>
</tr>
<tr>
<td>Japan</td>
<td>2005</td>
<td>89</td>
<td>1.6</td>
<td>BDE 47 and BDE 153</td>
<td>Inoue et al. (2006)</td>
</tr>
<tr>
<td>Poland</td>
<td>2004</td>
<td>22</td>
<td>2.0</td>
<td>BDE 47 and BDE 153</td>
<td>Jaraczewska et al. (2006)</td>
</tr>
<tr>
<td>Russia</td>
<td>2003 - 2004</td>
<td>10</td>
<td>1.0</td>
<td>BDE 153, BDE 197</td>
<td>Tsydenova et al. (2006)</td>
</tr>
<tr>
<td>(Buryatia)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>South China</td>
<td>not indicated</td>
<td>27</td>
<td>3.5</td>
<td>BDE 47 and BDE 153</td>
<td>Bi et al. (2006)</td>
</tr>
<tr>
<td>United States</td>
<td>2002</td>
<td>47</td>
<td>74</td>
<td>BDE 47</td>
<td>Schecter et al. (2006)</td>
</tr>
<tr>
<td>United States</td>
<td>2003</td>
<td>40</td>
<td>96</td>
<td>BDE 47 and BDE 153</td>
<td>She et al. (2007)</td>
</tr>
</tbody>
</table>

\(^a\)The number of congeners taken into account for calculation of the sum is different for each study.
1.3.6. Toxicology

Limited data is available about the toxicity of PBDEs. Most of the studies have been carried out using commercial PBDE mixtures. In some cases, little is known about the congener composition or possible presence of dioxin-like impurities.

1.3.6.1. Acute toxicity and subacute/subchronic toxicity

The acute toxicity of commercial PBDEs (administered orally, dermally or by inhalation) for laboratory animals is low (LD$_{50}$ > 0.5 – 28 g kg$^{-1}$ body weight). Penta-BDE appeared to be more toxic after oral administration than Octa- and Deca-BDE. Subacute/subchronic oral toxicity studies in rats revealed that effects were less pronounced with Deca-BDE than with the other two congener groups. Liver, kidney and thyroid gland were generally enlarged with or without some degenerative changes (Darnerud et al., 2001; Environmental Health Criteria 192, 1997).

1.3.6.2. Disruption of thyroid hormone homeostasis

Rats, which were orally exposed to three commercial PBDE mixtures, DE-71 (58.1% penta-BDE and 24.6 % tetra-BDE), DE-79 (30.7 % octa-BDE and 45.1 % hepta-BDE) and DE-83R (98 % BDE 209) for 4 days, were examined for alterations in thyroid hormone homeostasis. Serum concentrations of 3,3′,5,5′-tetraiodothyronine (thyroxine, T4) were depressed in a dose-response related trend with a maximum of 80 % for DE-71 and 70 % for DE-79 in the highest dose of 300 mg kg$^{-1}$ day$^{-1}$ and 100 mg kg$^{-1}$ day$^{-1}$ respectively. Dose related effects in serum 3,3′,5-triiodothyronine (T3) levels were less apparent and DE-83R had no effect on any of the measured parameters (Zhou et al., 2001). The same was seen by Fernie et al. (2005) in American kestrels exposed to BDE 47, BDE 99 and BDE 100. BDE 47, BDE 99 and BDE 100 were negatively associated with plasma T4 levels but they did not alter T3 levels.

The mechanism of thyroid hormone disruption by PBDEs has not been fully characterized, but different mechanisms have been suggested. Firstly, it has been reported that PBDEs accelerate hepatic clearance of T4 because they induce the activity of uridine diphosphate glucuronyl transferase (UDPGT) in the liver (Hallgren and Darnerud, 2002; Zhou et al., 2001). Secondly, the chemical structure of PBDEs closely resembles that of the thyroid
hormones T3 and T4. Recent studies have shown that hydroxylated metabolites of PBDEs bind with high affinity to the thyroid hormone transport protein, transthyretin (Meerts et al., 2000). Transthyretin carries T4 in the plasma to the target tissues, where T4 is deiodinated to 3,3',5–triiodothyronine (T3). T3 then interacts with two types of thyroid hormone receptors (THRs), α and β. The T3-THR complex binds to response elements on the DNA that regulate the transcription of thyroid activated genes. Transthyretin is not the major transport protein in humans but it may play a critical role in transporting T4 from the mother to the foetus as well as delivering T4 across the blood-brain barrier (reviewed in Brouwer et al., 1998). This might lead to decreased levels of T4 in the foetus with consequences for foetal brain development (reviewed in Legler and Brouwer, 2003).

Evidence of direct effects on the thyroid gland function is limited. Increased incidences of thyroid follicular cell hyperplasia as well as slightly increased incidences of follicular cell adenomas and carcinomas were observed in mice that were chronically exposed to Deca-BDE (ATSDR, 2004).

1.3.6.3. Neurobehavioural effects

Studies (Eriksson et al., 2001; Eriksson et al., 2002; Eriksson et al., 2006; Eriksson and Fredriksson, 1998; Viberg et al., 2003; Viberg et al., 2006) in mice which were exposed as newborns to PBDEs (1.4 µmol – 21.1 µmol kg\(^{-1}\) body weight) indicate that PBDEs cause changes in spontaneous behaviour together with learning and motor deficits, and that these effects worsen as the animals grow older.

There are at least three possible mechanisms by which PBDEs can adversely affect the brain function. Thyroid hormones regulate brain development in both the foetal and neonatal periods (de Escobar et al., 2000). Recent studies have shown that relatively small thyroid abnormalities can have a negative impact on the intelligence and psychomotor skills of children (de Escobar et al., 2000; Haddow et al., 1999; Pop et al., 1999). Thus, pregnant women, developing foetuses and infants should be viewed as sensitive populations for exposure to PBDEs. Secondly, the observed neurotoxicity of PBDEs in rodent studies could also be a result of alterations in neurotransmitter systems, e.g. the cholinergic system. It has been shown in mice that neonatal exposure to PBDEs reduces the density of nicotinic and muscarinic receptors in the hippocampus at adult age (Viberg et al., 2003; Viberg et al., 2004; Viberg et al., 2005). Thirdly, PBDEs may function by disturbing intracellular second messenger systems, e.g. Ca\(^{2+}\) homeostasis in neuronal cells, inositol phosphates and protein...
kinase C, which play a vital role in neuronal growth and normal cellular physiology (Kodavanti and Ward, 2005). PBDEs were also shown to release arachidonic acid in cerebellar granule cells via activation of the phospholipase A2 pathway which has been associated with learning and memory processes (Kodavanti and Derr-Yellin, 2002).

1.3.6.4. Receptor mediated effects

Polyhalogenated aromatic hydrocarbons exert many different mechanisms of toxicity. One mechanism they share in common may be the arylhydrocarbon-receptor (AhR) binding effects. The AhR can be found in the cytoplasm of almost all vertebrate cells and its activation can lead to a variety of biological and/or toxic effects, e.g. induction of cytochrome P450 enzymes (CYPs). Chen and Bunce (2003) investigated whether PBDEs can act as AhR agonists or antagonists at sequential stages of the AhR signal transduction pathway leading to CYP 1A1. BDE 77, BDE 119 and BDE 126, and to a lesser extent, BDE 100, BDE 153 and BDE 183, showed ability to activate the AhR. The environmentally important congeners, BDE 47 and BDE 99, on the other hand showed very low activity. It was concluded that the relative induction potencies of the most active PBDEs towards CYP 1A1 were approximately 0.0001 compared to 2,3,7,8-tetrachlorodibenzodioxin (TCDD).

Some studies have indicated that PBDEs also interfere with estrogen pathways. A recently developed in vitro estrogen-receptor mediated reporter gene assay (ER-CALUX) has been used to determine estrogenic potency of a number of PBDEs. Eleven of 17 PBDEs demonstrated estrogenic activity with lowest observed effect concentrations from 0.05 to 5 µM and the most potent ones being BDE 100, BDE 75 and BDE 51 (Meerts et al., 2001). However, these congeners were still 250000 to 390000 times less potent than 17β-estradiol. In contrast, some hydroxylated BDEs had estrogenic potencies that were almost similar to that of 17β-estradiol (lowest observed effect concentrations beginning from 2.0 x 10^-5 µM). The ranking order for estrogenicity of the hydroxylated PBDEs was the reverse order found for binding to the human thyroid receptor and human transthyretin.

1.3.6.5. Carcinogenicity

In chronic toxicity studies (103 weeks) with rats and mice, tumour incidence was observed after Deca-BDE exposure via the diet. Hepatic and pancreatic adenomas and carcinomas were observed, but only at very high doses (1200 mg kg\(^{-1}\) body weight day\(^{-1}\) and
above) (reviewed by Hooper and McDonald, 2000). One study implied an association between adipose tissue levels of BDE 47 and the risk of non-Hodgkin lymphoma among Swedish hospital cancer patients (Hardell et al., 1998).

1.3.7. **Legislation**

In 1976, the Directive 76/769/EEC was issued to establish limitations on the marketing and use of dangerous substances and preparations in the European Union (EU). This directive has been amended several times in order to update its scope of application to other dangerous substances. The 24th amendment of 6 February 2003 restricts the use of Penta-BDE and Octa-BDE in all applications for the EU market from 15 August 2004 on (Directive 2003/11/EC). Directive 2002/95/EC or also called “the Restriction of certain Hazardous Substances in electrical and electronic equipment (RoHS)” desires to phase out the use of some substances, e.g. Penta-BDE and Octa-BDE, which are deemed to be hazardous in electrical and electronic equipment from 1 July 2006 on. Deca-BDE was excluded from the RoHS-Directive on the basis of a 10-year environmental and human health risk assessment (Commission Decision 2005/717/EC). Although Penta-BDE and Octa-BDE are being phased out, a large number of products containing these flame retardants are still in use, resulting in a continuous release of these types of flame retardants into the environment.

In 2001, the EU identified a priority list of 33 substances in the field of water policy (Decision 2455/2001 of the European Parliament and of the Council). Out of the 33 substances some will be monitored or reviewed for identification as potentially hazardous substances, while others were identified as hazardous substances to be phased out within 20 years. PBDEs are listed among the substances to be monitored, while only Penta-BDE is listed as a hazardous substance (Bromine Science and Environmental Forum, 2006).

Currently there are no federal bans on PBDEs in the United States. However, a number of states have or are proposing restrictions on the use of Penta-BDE and Octa-BDE. States with existing bans and effective dates are: California, Hawaii, Illinois, Maine, Maryland, Michigan, New York, Oregon and Rhode Island (Bromine Science and Environmental Forum, 2006). Washington is the first state to ban the use of Deca-BDE in mattresses by 1 January 2008 and in upholstered furniture, televisions or computers by 1 January 2011 (Washington State Legislature, 2007). In China, the so called China RoHS legislation also limits the use of Penta-BDE and Octa-BDE in electric and electronic equipment (Kirschner, 2007). In July
2006, PBDEs were added to the List of Toxic Substances in Schedule 1 of the Canadian Environmental Protection Act of 1999. As a consequence the manufacture of PBDEs and the use of tetra-BDE, penta-BDE and hexa-BDE is prohibited in Canada (Environment Canada, 2007).
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CHAPTER 2. POLYCHLORINATED BIPHENYLS
2.1. INTRODUCTION TO POLYCHLORINATED BIPHENYLS

Polychlorinated biphenyls (PCBs) are a class of man-made compounds, first commercially manufactured as mixtures in the early 1930s by the US manufacturer Monsanto (Environmental Health Criteria 140, 1992) under the name Aroclor (e.g. Aroclor 1260). The last two digits in the numerical designation of the name indicate the percentage of chlorine content. Similar commercial PCB mixtures have been produced by other manufacturers and these include Clophens (Bayer, Germany), Phenoclors and Pyralenes (Prodelec, France), Fenclors (Caffaro, Italy) and Kanechlors (Kanegafuchi, Japan). In the mid 1960s, Soren Jensen (1966) first detected PCBs in Swedish environmental samples and since then they have been identified in almost every compartment of the global ecosystem. In 1968, an accidental human poisoning episode in Japan (Yusho) occurred, which was initially attributed to the consumption of rice bran oil contaminated with PCBs. This incident and a similar one in Taiwan (Yucheng) in 1979 increased concern over the safety of PCBs (Ikeda, 1996).

2.2. IDENTITY, USE AND PRODUCTION

PCBs are made up of a biphenyl nucleus with 1-10 chlorine atoms (Figure II.1). Theoretically, 209 congeners are possible, but only 130 congeners are likely to occur in commercial PCB mixtures. PCBs are numbered according to the IUPAC numbering which specifies the sites where chlorines are attached to the two benzene rings, e.g. 2,2’,4,4’,5,5’-hexachlorobiphenyl. Another numbering system developed by Ballschmitter and Zell (1980) assigns separate numbers, from 1 to 209, to each of the specific PCB congeners, e.g. PCB 153.

The benzene rings of PCBs can rotate freely around the bond that is connecting them. The degree of planarity is largely determined by the number of substitutions in ortho position. When hydrogen atoms in ortho position are substituted by chlorine atoms, which are larger, the benzene rings are forced out of their planar conformation. The benzene rings of non-ortho substituted PCBs, as well as of mono-ortho substituted PCBs, may assume a planar conformation and are referred to as planar or coplanar congeners. The benzene rings of other
congeners can not assume a planar or coplanar conformation and are referred to as non-planar congeners.

The physicochemical properties of PCBs include chemical inertness, low electrical conductivity, heat-resistance and low vapour pressure. They are very lipophilic, with log $K_{ow}$ values ranging from 4.3 to 8.3 (Environmental Health Criteria 140, 1992). Because of their broad range of physicochemical properties, PCBs have been used in a variety of applications, e.g. as organic diluents, plasticizers, adhesives, heat transfer and dielectric fluids in transformers, dielectric fluids in capacitors, hydraulic lubricants and in carbonless copy paper (Safe, 1994).

The manufacturing process for Aroclors involved the chlorination of biphenyl with anhydrous chlorine in the presence of a catalyst, such as ferric chloride. The degree of chlorination, which determines the nature of the Aroclor, was controlled by the chlorine-contact time (range 12-36 hours) in the reactor. During production, Aroclor mixtures were contaminated by small amounts of polychlorinated dibenzofurans (PCDFs) (ATSDR, 2000). PCDFs and polychlorinated dibenzodioxins (PCDDs) may also be formed during combustion of PCBs (Hutzinger et al., 1985).

In contrast to PBDEs, there are no natural sources of PCBs. Applications in open systems, e.g. in inks, inherently resulted in widespread low-level release of PCBs in the environment. Use in closed systems could have contributed to the environmental burden by spills, improper handling or disposal (Erickson, 1997). Once in the environment, PCBs can travel long distances in the air and can be deposited in areas far away from where they were released (Montone et al., 2003).
2.3. **BREAKDOWN IN THE ENVIRONMENT**

Wong and Wong (2006) investigated the degradation of five PCBs (PCB 25, PCB 52, PCB 101, PCB 153 and PCB 180) by UV in organic solvents. The main degradation products were lesser chlorinated congeners and the removal efficiency of chlorine increased when UV intensity increased. As UV is the energy source that drives dechlorination, increased UV intensity provides more photons for the reaction and as a result increases removal efficiency. PCB 52 was the most resistant to photochemical degradation due to the stability of its symmetrical configuration. It was shown that symmetrical, coplanar and lower chlorinated PCB congeners are more stable than others. Miao et al. (1999) and Yao et al. (1997) reported that reactivity of the chlorine atoms of the PCB rings is generally in the order: ortho>meta>para.

Anaerobic and aerobic micro-organisms can also participate in the degradation of polychlorinated biphenyls. The biodegradation process consists of the removal of chlorine from the phenyl ring by anaerobic bacteria and the subsequent cleavage and oxidation of the resulting compound by aerobic bacteria. Effectiveness of biodegradation depends on various conditions, e.g. pollutant concentration, structure, solubility, temperature and pH (Borja et al., 2005).

2.4. **BIOACCUMULATION AND BIOMAGNIFICATION**

Biomagnification potential of PCBs was studied in food webs from the Baltic Sea and the Northern Atlantic Ocean (Burreau et al., 2006). A trophic level correlated increase for all studied PCBs (except for PCB 209 in salmon) was seen. Biomagnification in fish at the highest trophic levels was influenced by body size rather than by position in the food chain (trophic position). In contrast to PBDEs, a positive correlation was found between biomagnification power of PCBs and their hydrophobicities. Similar results were obtained by Borga et al. (2001), Kwon et al. (2006) and Metcalfe et al. (1997). According to Stapleton and Baker (2003), BDE 47 and PCB 153 have a comparable potential for bioaccumulation.
Biomagnification of some PCB congeners appears to be lower than would be predicted from their $K_{ow}$s. It seems that the potential for biomagnification of a PCB congener is also highly dependent on its substitution pattern. PCBs with low biomagnification potential are all non-ortho or mono-ortho substituted and have all meta-para and to a lesser extent ortho-meta vicinal hydrogens. This indicates that these congeners are more easily metabolized. PCB 153 and PCB 180, which have no vicinal hydrogens, are considered to be highly persistent congeners (Andersson et al., 2001; Buckman et al., 2006; Leonards et al., 1998; Looser and Ballschmiter, 1998; McFarland and Clarke, 1989; Metcalfe and Metcalfe, 1997).

2.5. TOXICOKINETICS AND METABOLISM

Toxicokinetics of PCBs in higher animals is influenced by numerous factors including type of congener and type of species. Due to their high lipophilicity, it may be assumed that PCB congeners are easily absorbed in the intestine through diffusion. Gastrointestinal absorption of individual congeners in rats has been reported to vary between 66 % and 96 % and increases with chlorination degree (Tanabe et al., 1981). Subsequently, they are rapidly distributed to all tissues (Matthews and Dedrick, 1984). PCBs initially accumulate in the more highly perfused tissues, e.g. liver and muscle. With time, the more persistent congeners will accumulate in lipid-rich tissues (Maervoet et al., 2004).

The general pathway of PCB metabolization consists of an introduction of oxygen via the cytochrome P450 (CYP) enzyme system which results in the formation of hydroxylated metabolites or epoxides (James, 2001). The hydroxylated metabolites can be further hydroxylated or conjugated with glucuronide or sulphate. The epoxide may be metabolized to dihydrodiol or be conjugated with glutathione. Further metabolization leads ultimately to the formation of a large number of metabolites, e.g. OH-PCBs, MeSO$_2$-PCBs and glucuronide or sulphate conjugates. Some OH-PCBs and MeSO$_2$-PCBs are persistent and toxic (Letcher et al., 2000). They have been identified in blood and tissues of laboratory and wild animals (Buckman et al., 2006; Houde et al., 2006; Sandala et al., 2004), as well as in humans (Fangstrom et al., 2002; Guvenius et al., 2002; Hovander et al., 2006; Soechitram et al., 2004; Weiss et al., 2006).
The formation of these metabolites depends on the degree of chlorination (Haraguchi et al., 1997) on the substitution pattern (Looser and Ballschmiter, 1998) and on the species-specific metabolic capacity (Haraguchi et al., 2005; Safe, 1994). It has been suggested that non-ortho or mono-ortho PCBs are the preferred substrates for CYP 1A enzymes and that the para position is the preferred site of hydroxylation. In addition, these coplanar PCBs have also been shown to interact with the arylhydrocarbon-receptor (AhR) which results in induction of CYP 1A enzymes. This means that some PCB congeners contribute to their own biotransformation. Other non-coplanar PCBs are more readily metabolized by CYP 2B and 3A enzymes with hydroxylation at the meta position (Bandiera, 2001). This is also the reason why greater biotransformation of PCBs with vicinal hydrogen atoms in meta-para or meta-ortho position has been seen and why highly chlorinated non-coplanar PCBs (e.g. PCB 153) are metabolized more slowly (Looser and Ballschmiter, 1998; Metcalfe and Metcalfe, 1997).

2.6. **HUMAN EXPOSURE**

Intake of PCBs through food is estimated to account for more than 90% of the human total exposure (Liem et al., 2000). Dietary exposure and main contributing food groups are highly dependent on the local eating habits. Food groups that contribute the most to the human exposure of PCBs are fish, meat and dairy products. In Table II.1 the dietary intake of PCBs in different countries is compared. Studies which exclusively measured dioxin-like PCB congeners and studies which expressed intake results in total toxic equivalents (TEQs) have not been included in this comparison.
Table II.1 Dietary intake of PCBs in different countries

<table>
<thead>
<tr>
<th>Country</th>
<th>Intake (ng day(^{-1})) (^a)</th>
<th>Number of congeners</th>
<th>Main congeners</th>
<th>Main contributing food groups</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Finland</td>
<td>930</td>
<td>34</td>
<td>PCB 110, PCB 138, PCB 153, PCB 118</td>
<td>Fish (68%)</td>
<td>Kiviranta et al. (2004)</td>
</tr>
<tr>
<td>Italy</td>
<td>260</td>
<td>60</td>
<td>PCB 101, PCB 138, PCB 153</td>
<td>Bread, cereals and potatoes (45%), protein composite (33%), fruits and vegetables (19%)</td>
<td>Turci et al. (2006)</td>
</tr>
<tr>
<td>Sweden</td>
<td>482</td>
<td>23</td>
<td>PCB 153</td>
<td>Fish (57%), dairy products (14%) and meat (12%)</td>
<td>Darnerud et al. (2006)</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>392</td>
<td>7</td>
<td>not indicated</td>
<td>Meat products (27%), fish (26%), industrial oils and fats (18%) and dairy products (17%)</td>
<td>Bakker et al. (2003)</td>
</tr>
</tbody>
</table>

\(^a\) Daily intake was estimated for an adult of 70 kg body weight.

PCBs are found in human breast milk in comparatively high concentrations (Table II.2). Infants with human milk as the dominant food source will consequently have higher PCB intake than adults. The levels found depend on dietary habits and also on the place of residence. PCB levels in breast milk of women living in industrialized countries are higher than of those living in developing countries (Minh et al., 2004).

Due to the stop in PCB use and authority measures taken, PCB levels in breast milk have decreased significantly in the past decades. Shade and Heinzow (1998) found that PCB levels in breast milk from Germany showed a 60% decrease in the time frame 1986 – 1997. Noren and Meironyté (2000) also noticed that PCB levels in 1997 were reduced to 30% of those found in 1972. Similar results were also seen in breast milk samples from Japan (Konishi et al., 2001).
Table II.2 PCB concentrations in human milk from different countries

<table>
<thead>
<tr>
<th>Country</th>
<th>Year</th>
<th>n</th>
<th>Sum PCBs (ng g⁻¹ lipid weight)</th>
<th>Main congeners</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia</td>
<td>1995</td>
<td>60</td>
<td>500</td>
<td>not indicated</td>
<td>Quinsey et al. (1995)</td>
</tr>
<tr>
<td>Czech Republic</td>
<td>1996</td>
<td>17</td>
<td>937</td>
<td>PCB 153</td>
<td>Schoula et al. (1996)</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>and PCB 180</td>
<td></td>
</tr>
<tr>
<td>United States</td>
<td>2003</td>
<td>40</td>
<td>147</td>
<td>PCB 138, PCB 153 and PCB 180</td>
<td>She et al. (2007)</td>
</tr>
</tbody>
</table>

*The number of congeners taken into account for calculation of the sum is different for each study.

2.7. **TOXICOLOGY**

2.7.1. **Arylhydrocarbon receptor mediated toxic effects: The toxic equivalent approach**

One of the more sensitive indicators of PCB exposure is the induction of CYP450 enzymes. Inducers of these types of enzymes can be divided into two main classes typified by phenobarbital (PB) and 3-methylcholanthrene (MC). Coplanar PCBs which are substituted in both para positions, in at least two meta positions and unsubstituted in ortho position (Figure II.2) are structurally related to 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) and are therefore able to bind with high affinity to the cytosolic arylhydrocarbon-receptor (AhR). This results in the induction of MC type inducible enzymes (e.g. CYP 1A) and in many other toxic (e.g. immunotoxicity, endocrine effects, reproductive and developmental toxicity) or carcinogenic responses (McFarland and Clarke, 1989; Safe, 1993).
Figure II.2 Chemical structures of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) and of the coplanar PCBs with the highest AhR binding affinity

Eight analogues of these four non-ortho coplanar PCBs with a single chlorine atom in ortho position can also bind to the AhR. Their binding affinities are however much lower. At relatively high concentrations, many di-ortho substituted PCBs have also appeared to be weak AhR agonists (Mcfarland and Clarke, 1989; Safe, 1993).

This structure-activity relationship demonstrates the complex nature of PCB mixtures and the difficulties that arise for human risk evaluation. For this purpose, the concept of toxic equivalency factors (TEFs) has been developed which is based on the ability of any dioxin-like compound to elicit AhR mediated responses, and that in relation to the most potent dioxin-like compound, 2,3,7,8-TCDD. TEF values for individual congeners in combination
with their chemical concentration can be used to calculate the total 2,3,7,8-TCDD toxic equivalent (TEQ):

\[
\text{TEQ}_{\text{PCB}} = \sum_{i=1}^{n} [\text{PCB}_i \times \text{TEF}_i]
\]

Table II.3 WHO TEFs for human risk assessment based on the conclusions of the 2005 World Health Organization meeting in Geneva (van den Berg et al., 2006)

<table>
<thead>
<tr>
<th>Congener</th>
<th>TEF Value</th>
<th>Congener</th>
<th>TEF Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCB 77</td>
<td>0.0001</td>
<td>PCB 105</td>
<td>0.00003</td>
</tr>
<tr>
<td>PCB 81</td>
<td>0.0003</td>
<td>PCB 114</td>
<td>0.00003</td>
</tr>
<tr>
<td>PCB 126</td>
<td>0.1</td>
<td>PCB 118</td>
<td>0.00003</td>
</tr>
<tr>
<td>PCB 169</td>
<td>0.03</td>
<td>PCB 123</td>
<td>0.00003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PCB 156</td>
<td>0.00003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PCB 157</td>
<td>0.00003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PCB 167</td>
<td>0.00003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PCB 189</td>
<td>0.00003</td>
</tr>
</tbody>
</table>

Twelve PCB congeners (4 non-ortho PCBs and 8 mono-ortho PCBs) have been assigned with a TEF value (Table II.3). While the use of the TEF concept for human risk assessment is the most feasible approach, other non-additive AhR interactions or non-AhR mediated mechanisms of action should not be ignored (Bonefeld-Jorgensen, 2004; Guvenius et al., 2002; Safe, 1994).

2.7.2. Non-arylhydrocarbon mediated toxic effects

Many PCBs elicit PB-like induction activity which also results in induction of PB type inducible enzymes (e.g. CYP 2B) and in a number of adverse health effects (e.g. liver and prostate cancer). The most active PB-like inducers contain at least two ortho and two para chlorine atoms (e.g. PCB 153 – Figure II.3).
PCBs interfere with the thyroid signalling pathway by reducing circulating levels of thyroid hormones. Correlation studies in blood from wildlife and humans reported that PCBs decrease levels of thyroid hormones 3,3',5-triiodothyronine (T3) and 3,3',5,5'-tetraiodothyronine (T4) and increase thyroid stimulating hormone (TSH) levels (Chiba et al., 2001; Debier et al., 2005; Hagmar et al., 2001; Osius et al., 1999; Sala et al., 2001; Skaare et al., 2001; Sormo et al., 2005). Some research suggests that the reduced T4 levels might be due to MeSO₂-PCBs which induce uridine diphosphate glucuronyl transferase (UDPGT) (Hood et al., 2003; Kato et al., 1998; Kato et al., 2000a; Kato et al., 2000b; Kato et al., 2005) and as a consequence increase T4 glucuronidation and T4 clearance. PCBs have been reported to alter the structure of the thyroid gland and by that maybe also its ability to respond to TSH (Kilic et al., 2005).

As already described for PBDEs, OH-PCB metabolites are also known to be T4 competitors for transthyretin (TTR). OH-PCB metabolites with a para or a meta substituted hydroxyl group and with adjacent chlorine atoms in the ortho and meta positions have the highest affinity for TTR (Lans et al., 1993; Lans et al., 1994). Due to the ability of TTR to pass the placental and blood–brain barriers, OH-PCBs can be preferentially transferred to the fetal compartment and cause hypothyroidism in the brain tissue. It has recently been shown that despite the reduced levels of T4 in the fetal brain, T3 levels are maintained by increased deiodination of T4 (Meerts et al., 2002; Morse et al., 1996).

PCB 138, PCB 153 and PCB 180 have been shown to have low affinities for the estrogen and androgen receptor (Bonefeld-Jorgensen et al., 2001). In contrast to PCBs however, OH-PCBs are extremely potent inhibitors of the human estrogen sulfotransferase (EST). This might result in an increased bioavailability of estrogens in tissues that express EST (Kester et al., 2000).
PCBs have been classified by the IARC as probably carcinogenic to humans (group 2A) (IARC, 1987). They have been suspected as possible contributors to the incidence of several types of cancer, e.g. pancreatic cancer, prostate cancer, testicular cancer and non-Hodgkin lymphoma (De Roos et al., 2005; Hardell et al., 2004; Hardell et al., 2006; Pavuk et al., 2004; Prince et al., 2006; Ritchie et al., 2003). In a study by Moysich et al. (1999), a relationship has been found between elevated PCB levels combined with CYP 1A1 polymorphism and breast cancer. However, no significant positive association between exposure to PCBs and risk of breast cancer has been found (Negri et al., 2003). Higher PCB body burdens have also been associated with increased incidence of diabetes (Fierens et al., 2003; Longnecker et al., 2001; Louis et al., 2005; Porpora et al., 2006; Vasiliu et al., 2006) and increased risk of endometriosis (Porpora et al., 2006; Reddy et al., 2006). Several reports have also shown that PCBs exhibit neurotoxic effects, e.g. alteration of neurotransmitter (dopaminergic and cholinergic) processes, Ca\(^{2+}\) homeostasis, signal transduction and cell death of neuronal cells (Mariussen and Fonnum, 2006).

2.8. LEGISLATION

Sweden (1970) and Japan (1972) were the first to ban the manufacturing, use and import of PCBs (Borja et al., 2005). In the US, the manufacturing, processing and distribution of PCBs were banned under the 1976 Toxic Substances Control Act.

The use of PCBs in open applications, e.g. inks, was banned in the European Community in 1976 by Council Directive 76/403/EEC, which stipulated that measures should be taken to prohibit the uncontrolled discharge and dumping of PCBs. The use of PCBs as a raw material or chemical intermediate was prohibited in 1985 by Council Directive 85/467/EEC (6th amendment of Directive 76/769/EEC). PCBs are also one of the 12 persistent organic pollutants that have been listed by the Stockholm Convention. Countries that have ratified this convention must make determined efforts to identify, label and remove PCB containing equipment from use by 2025.

Commission Regulation (EC) N° 1881/2006 (amendment of Commission Regulation (EC) N° 466/2001) has set maximum levels for the sum of dioxins and dioxin-like PCBs in a variety of foodstuffs. According to Belgian legislation (KB 19th of May, 2000), it is prohibited
to commercialize milk or derived products, with a fat percentage of over 2%, which contain PCB levels (sum of PCB 28, PCB 52, PCB 101, PCB 118, PCB 138, PCB 153, PCB 180) of over 100 ng g\(^{-1}\) lipid weight. There is also a prohibition to commercialize meats (beef, pork or poultry), eggs, animal fat or oils, with a fat percentage of over 2%, which contain PCB levels (sum of PCB 28, PCB 52, PCB 101, PCB 118, PCB 138, PCB 153, PCB 180) of over 200 ng g\(^{-1}\) lipid weight. For fish (including crustaceans and shellfish) PCB levels should not exceed 75 µg kg\(^{-1}\) product (KB 6\(^{th}\) of March, 2002).
2.9. REFERENCES


CHAPTER 2. POLYCHLORINATED BIPHENYLS


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CHAPTER 3. CURRENT ANALYTICAL METHODS
In this chapter, a general outline of the analytical methods and techniques which are commonly utilized for the determination of PCBs and PBDEs in fatty tissues is given. A detailed review of the recent developments regarding PBDE analysis can be found in Covaci et al. \(\textit{in press}\).

### 3.1. Extraction

During the extraction step, the contaminants are isolated from the matrix and transferred to an organic solvent. The yield of the extraction is determined by several factors such as the solubility of the analytes in the extraction mixture, the accessibility of the matrix to the extraction solvent and the extraction time and temperature. The absence of water in the samples makes the sample matrix more accessible to organic solvents. That is why the sample is often dried before extraction by means of evaporation, grinding with anhydrous sodium sulphate or freeze-drying.

#### 3.1.1. Soxhlet extraction

Liquid-solid extraction, using a Soxhlet apparatus, is widely used as a standard extraction technique because of its robustness and low cost. With this technique, the dried sample is continuously extracted by a non-polar solvent or a mixture of non-polar and polar solvents. Extraction times with a regular Soxhlet apparatus may vary between 6 h and 24 h. With hot Soxhlet extraction, the solvent is distilled into an extraction chamber which is heated below the boiling point of the solvent. In this way, the sample is continuously in contact with hot, but not boiling solvent, resulting in a reduction of the extraction time. General disadvantages of this method are the requirement of large volumes of solvent, the long extraction times and the laborious nature of the method (Covaci et al., 2003; Erickson, 1997).
3.1.2. Supercritical fluid extraction (SFE)

Carbon dioxide (CO\textsubscript{2}) that is brought above its critical pressure (73 atm) and critical temperature (31.1 °C) results in supercritical CO\textsubscript{2}, which diffuses through solids like a gas and dissolves matrix components like a liquid. PCBs and PBDEs dissolve easily in supercritical CO\textsubscript{2} and are extracted together with lipids (Hartonen et al., 1997). After extraction the analytes need to be collected or trapped into a collection device, e.g. a vessel containing a small volume of organic solvent or a column filled with adsorbing material (Focant et al., 2004a; Turner et al., 2002). Sometimes fat retainers (e.g. florisil, silica or basic alumina) are added directly into the extraction cell to achieve a fat-free extract (Jaremo et al., 2000).

3.1.3. Accelerated solvent extraction (ASE)

In contrast to SFE, ASE is more extensively used and differs in a way that an organic solvent or a combination of solvents has replaced CO\textsubscript{2} and that increased pressures and temperatures are used to speed up the extraction time. By pressuring the extraction cell, it is possible to keep the organic solvent as a liquid at relatively high extraction temperatures. With this technique simultaneous extraction and clean-up is also possible (Abrha and Raghavan, 2000; Covaci et al., 2003; Focant et al., 2004a).

3.1.4. Other extraction techniques

Other extraction techniques include matrix solid-phase dispersion (MSPD), ultrasonic extraction (USE) and microwave-assisted extraction (MAE). MSDP allows for extraction and clean-up to be carried out in one step. The sample is dispersed with a fat retaining solid support, e.g. florisil. This mixture is then placed into a column and PCBs and PBDEs are eluted with organic solvents, e.g. dichlormethane:hexane (1:1) (Covaci et al., 2002; Valsamaki et al., 2006). With USE, the sample is immersed in a vessel with an appropriate organic solvent and placed into an ultrasonic bath. (Bayen et al., 2004; Li et al., 2006). USE has the advantage that several extractions can be done simultaneously and no sophisticated laboratory material is required. With MAE, extraction solvent and sample are subjected to
radiation from a magnetron in either a sealed or an open vessel. A disadvantage of USE and of MAE is that an additional off-line clean-up procedure must be executed.

3.2. **CLEAN-UP**

3.2.1. **Non-destructive lipid removal**

3.2.1.1. *Gel permeation chromatography (GPC) or size exclusion chromatography (SEC)*

Gel permeation chromatography (GPC) is used to separate the analytes of interest from other components, based on their difference in molecular size. A column is filled with a polymeric gel, e.g. Bio-Beads S-X3, which will only allow the admittance of small molecules. The larger molecules in a mixture will move rapidly through the column, the smaller molecules permeate the pores of the polymer and take longer to pass the column. As a consequence the mixture is fractioned into its components by size. Gel permeation chromatography has been successfully applied as a clean-up method but additional clean-up by means of solid phase extraction with silica gel or florisil was still needed to eliminate small amounts of impurities (Alaee et al., 2001b; Saito et al., 2004; Vetter et al., 1998).

3.2.1.2. **Adsorption chromatography**

In adsorption chromatography, a solvent elutes a sample extract from a column which contains at least one adsorbent. Separation is based on the difference between adsorption affinities of the sample components for the adsorbent. Silica gel, alumina and florisil exhibit similar abilities for fat adsorption and may also be used for fractionation purposes. Silica gel was used by Martinez et al. (2005) to separate PCBs and PBDEs into two separate fractions based on the difference in polarities. Both activated alumina and florisil may be used to separate PCBs from polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzodioxines (PCDDs) and to separate non-ortho substituted PCBs from the other PCBs (Liu et al., 2006; Loos et al., 1997). Carbon columns exhibit high selectivity for those PCBs which can assume a planar conformation. They can be used as a second clean-up after major interferences have been removed by another technique (Erickson, 1997; Pirard et al., 2002).
High performance liquid chromatography (HPLC) has a number of advantages over open-column techniques, e.g. resolution, speed, reproducibility and the use of less solvent. However, HPLC is more expensive and has a low capacity for dirty samples (e.g. fat extracts) (Covaci et al., 2002; Erickson, 1997).

3.2.2. Destructive lipid removal

Chemical degradation techniques should be used with caution to ensure that target analytes are not destroyed along with the interferences. Sulphuric acid treatment can be used to remove lipids and other interferences. The most efficient and easiest way is to elute the sample extract through a column filled with sulphuric acid-treated silica. PCBs and PBDEs were found to be stable under these conditions (Covaci and Voorspoels, 2005; de Boer et al., 2001; Ramos et al., 2004; Valsamaki et al., 2006). The treatment of the sample extract with a strong base can also degrade interferences. However, degradation of both PCBs and PBDEs has been reported with this technique (Covaci et al., 2003; Covaci et al., in press; Kannan et al., 1993).

3.3. GAS CHROMATOGRAPHY

3.3.1. Introduction

In 1941, Martin and Synge were the first to suggest the possibility of gas as a mobile phase to separate volatile substances. In 1952, James and Martin reported the separation of volatile fatty acids by partition chromatography with nitrogen gas as the mobile phase. Since then, gas chromatography (GC) has developed rapidly. Nowadays helium or hydrogen are used as carrier gas and instead of packed columns, which were packed with a solid support that was coated with the stationary phase, capillary columns have been introduced. In a capillary column, the stationary phase is coated on the inner wall, either as a thin film or impregnated into a porous layer on the inner wall (Bartle and Myers, 2002).
The lay-out of a modern instrument is shown schematically in Figure III.1. A gas chromatograph generally consists of a system to control the flow of the carrier gas, an inlet system and a column oven with capillary column.

Gas chromatographic separations are achieved by a series of partitions between the moving gas phase and the stationary liquid phase. The more affinity a component has for the stationary phase, the more it will be retained and the longer its retention time will be. How well a chromatographic separation has succeeded is measured by the resolution (R) or the ratio of peak separation to the average base width of the peaks. Peak width is dependent upon the number of partitions of a component between the gas phase and the liquid phase in the column, also called the number of theoretical plates (N). Since N depends on the column length, the height equivalent to one theoretical plate (HETP) was introduced. It has been shown that HETP depends on the average gas velocity ($\bar{u}$), the Eddy diffusion (A) or with other words the effect of multiple gas pathways through a packed column bed, the longitudinal diffusion in the gas phase (B) and the resistance to mass transfer in the gas and liquid phases (C) (Bartle, 1993). This is summarized in the Van Deemter equation:

$$\text{HETP} = A + \frac{B}{\bar{u}} + C \cdot \bar{u}$$

Capillary columns are more efficient than packed columns because they are much more permeable to gas flow ($A = 0$) and their film thickness is less ($C$ is lower). This also means that analysis speed is greatly increased for a given resolution (Bartle, 1993).
3.3.2. Gas chromatography columns for PCB and PBDE analysis

Due to the large number of different PCB and PBDE congeners and their similar physical and chemical characteristics, the complete separation of all congeners on a single capillary column is impossible. Generally, separations of PCBs and PBDEs are achieved on non-polar columns such as 100 % dimethyl polysiloxane (type DB-1ms) and 5 % diphenyl-95% dimethyl polysiloxane (type DB-5ms) with lengths of 25 to 60 m (Covaci et al., 2003). The separation of PCBs on twenty capillary columns (Frame, 1997) and the separation of PBDEs on seven capillary columns (Korytar et al., 2005) has been previously investigated. Choice of column-type largely depends on the possible co-elutions of target compounds that might occur. Pirard et al. (2006) compared the separation of 12 PBDE congeners on an Rtx®-5Sil-MS and on an Stx®-500 capillary column. Notwithstanding that a better separation is achieved between BDE 100 and BDE 119 with an Rtx®-5Sil-MS column, a greater isolation of BDE 47 is observed with the Stx®-500.

In this work, the separation efficiency of the commonly used DB-5ms column was investigated, but PCB 28 and PCB 31 could not be sufficiently separated. In addition, co-elution of BDE 154 and PBB 153; BDE 153 and TBBPA (Korytar et al., 2005); PCB 180 and BDE 47 (Alaee et al., 2001a) has been reported on this type of column in literature. Since a HT8 (8% diphenyl-polycarborane siloxane) column, already in use in our laboratory for the routine analysis of PCBs, gave good resolution results in above mentioned studies and separation of PCB 28 and PCB 31 was achieved, this type of column was chosen for the present study. A carborane group is incorporated into the polysiloxane backbone of the stationary phase of the HT8 column (Figure III.2).

![Chemical structure of siloxane-carborane](image-url)
This carborane group has a high affinity for chlorinated biphenyls with a low degree of ortho substitution. This is a result of the high degree of rotational freedom of non-ortho and mono-ortho congeners that allows for a coplanar conformation and for a closer $\pi-\pi$ interaction with the carborane group (Larsen et al., 1995). As a consequence retention time order of PCBs is more disordered in regard to the degree of chlorination than is the case for PBDEs.

The retention time of PBDEs generally increases with bromine substitution. Little co-elutions of BDE congeners with different bromine numbers occur, but co-elution within homologue groups is frequent (Korytar et al., 2005). This is a result of the ether linkage in the diphenyl oxide molecule which introduces a high barrier to rotation and prevents the two aromatic rings from assuming a planar conformation (Hardy, 2002). As a consequence, the number of bromine substituents outweigh the effect of planarity on GC retention (Wang et al., 2006).

Recently, heart-cut and comprehensive multidimensional gas chromatography may resolve the problem of co-eluting components in complex environmental mixtures (Bucheli and Brandli, 2006; Focant et al., 2004b; Focant et al., 2004c; Haglund et al., 2001; Harju et al., 2003; Horii et al., 2005; Korytar et al., 2005). In heart-cut multidimensional GC, co-eluting congeners are transferred to a second column of different selectivity. In comprehensive multidimensional GC, the effluent from a first column is introduced continuously into a secondary column, in the form of narrow pulses, providing an orthogonal system. The secondary column should be a short, high speed column. The new generation of fast scanning time of flight-mass spectrometry instruments are capable of working at high scan rates and offer the ideal detection technique to couple to GC x GC.

Because of its high sensitivity for higher temperatures, BDE 209 should receive special attention to avoid degradation in the GC system. The GC column should be relatively short, preferably 10 - 15 m, to reduce as much as possible its residence time in the column (de Boer et al., 2001). This means that the analysis of BDE 209 should occur separately from the analysis of the other PBDEs and a maximum oven temperature of 300°C should only be applied for a short time at the end of the temperature programme. High speed gas chromatography like narrow-bore chromatography could be a solution for this kind of problem. Reducing the internal diameter of the capillary column improves separation by affecting the mass transfer term $C$ in the van Deemter equation, resulting in an improvement in column efficiency due to the lowering of the height of the theoretical plates ($H$). This means that this technique allows for fast analysis, high separation efficiency and low detection limits. Covaci et al. (2002; 2001) used narrow-bore (0.10 mm id) capillary
chromatography on a 10 m AT-5ms column to separate PCBs and PBDEs. Sufficient attention should be paid to the injection technique, which could attribute considerably to band broadening and outweigh the benefits of high speed GC.

3.3.3. Injection techniques

The three most frequently used injection systems in PCBs and PBDE analysis are splitless injection, programmable temperature vaporization (PTV) and on-column injection. With splitless injection, small sample volumes (1 - 2 µL) are injected in an injector at a high temperature (250 – 300 °C). The split flow is stopped during injection and is continued after the sample is transferred to the column, this to purge residual traces of solvent from the liner (Tipler, 1993). To avoid band broadening, secondary focusing mechanisms, e.g. solvent effect, must be employed (Figure III.3).

Figure III.3 Refocusing by means of solvent effect in splitless injection (Hübschmann, 2001)

The solvent effect uses an initial oven temperature below the boiling point of the solvent. As the sample is heated within the injector, the solvent is the first to vaporize and enter the column. Some of the solvent will condense and collect on the column walls. As the components of interest pass into the column, they will dissolve into the condensed solvent, thus slowing down and giving a focusing effect (Figure III.3-A). The column oven is then
temperature programmed and the solvent vaporizes, leaving the components deposited in a narrow band (Figure III.3-B and C) (Hübschmann, 2001). Highly brominated diphenyl ethers which are high boiling compounds can easily be discriminated in the splitless injector. Furthermore, the high injection temperatures used with splitless injection may also increase thermal degradation of PBDEs and especially of the thermally labile BDE 209.

With temperature programmed volatilization (PTV – split injection), the sample (< 5 µl – 125 µl) is injected at a temperature that corresponds to the boiling point of the solvent. During injection, the split valve is open and the oven temperature is kept below the PTV temperature. The maximum injection rate depends on how much solvent per unit time can be evaporated in the insert and carried out through the split. After the split valve is closed, the temperature of the liner is rapidly increased. As each compound is vaporized, it is swept from the liner onto the column and thus will not be subjected to a temperature higher than necessary. With this technique large volume multiple injections can also be made (Bailey, 2005; Hübschmann, 2001; Stapleton, 2006). Tollbäck et al. (2003) developed and optimized a large volume PTV injection-fast GC-MS method for the determination of PBDEs, including BDE 209. The high boiling points of PBDEs decrease the chance of losses through the solvent vent and the lower injection temperatures limit the probability of degradation.

In cool on-column injection, the extract is injected directly onto the column. This technique causes less degradation and discrimination. The extracts should be as clean as possible, as otherwise the GC column may quickly deteriorate due to disposition of involatile material. The use of a retention gap is particularly recommended when larger volumes (10 – 250 µl) need to be injected. It also acts as a guard column which protects the analytical column from involatile residues (Bailey, 2005; Hübschmann, 2001; Stapleton, 2006).

3.4. DETECTION BY MEANS OF MASS SPECTROMETRY

3.4.1. Introduction

Electron capture detection (ECD) is a common detection method in PCB analysis because it is sensitive to molecules which contain highly electronegative atoms. When an electrophilic molecule enters the ECD detector, it will react with free thermal electrons which are formed by ionization of a carrier gas. As a consequence, the standing current, resulting
from the collection of these electrons, is reduced. This reduction in standing current is proportional to the analyte concentration. Although electron capture detection has been proved useful several drawbacks have reduced its application. Sensitivity is not only influenced by the number of halogens but also by the halogen substitution pattern (Oliver et al., 1989; Sellström, 1999). Additionally, any halogen containing molecule will produce a signal. This means that no distinction can be made between co-eluting halogen containing compounds. Therefore results of PBDE analysis can be greatly influenced when PCB congeners are present at high concentrations (Covaci et al., 2003).

In order to evaluate the global distribution of PCBs and PBDEs in the environment, a sensitive and interference-free analytical method is required. Combination of the separation efficiency of gas chromatography (GC) and the qualitative information and high sensitivity of mass spectrometry (MS) has resulted in a very high number of applications. High resolution MS has the advantage of increased sensitivity and selectivity but is also more costly, labour intensive and requires experienced users. In this work, an ion trap mass spectrometer has been used for detection purposes. As a result, only low resolution mass spectrometry (LRMS) will be further discussed in this chapter.

3.4.2. Ionization techniques

3.4.2.1. Electron ionization

Several ionization techniques are used in GC-MS. Among them, electron ionization (EI) is generally the most frequently used because it often produces both molecular and fragment ions. In EI, gas analyte molecules are bombarded by energetic electrons at 70 electron volts. Energy is transferred to the neutral molecule and is thereby able to eject one of its own electrons. This process leads to the generation of a molecular radical ion (M⁺):

\[
M + e^- \rightarrow M^+ + 2e^-
\]

The molecular ion still has a considerable excess of energy and much of this energy can be dissipated by fragmentation of its chemical bonds which results in the generation of ionized fragments. This type of fragmentation can be useful because it provides structural information for structure elucidation of an unknown analyte. (Covaci et al., 2003; de Hoffman and Stroobant, 2001; Erickson, 1997; Watson, 1985).
3.4.2.2. Chemical ionization

In some cases, EI does not provide the sensitivity required for the analysis of very small amounts of compounds in environmental samples. This is mainly due to the extensive fragmentation. Chemical ionization (CI) produces ions with little excess energy and yields spectra in which the molecular ion is easily recognized due to reduced fragmentation.

In contrast to EI, a reagent gas is used in CI. As a result, the amount of sample molecules in the ion source is very small compared to the amount of reagent gas molecules. The reagent gas molecules will be preferentially ionized through electron ionization and the resulting ions collide with other reagent gas molecules, creating ionization plasma which contains product ions and low energy electrons. The type of ions formed depends on the type of reagent gas. The major reaction in positive chemical ionization (PCI) is proton transfer and it is typical for sample molecules which have a higher proton affinity than the reagent gas ions. When analyte molecules M are introduced in the ionization plasma, the protonated reagent gas ions GH\(^+\) can transfer a proton to the molecules M and produce protonated molecular ions MH\(^+\) (quasimolecular ion). Dependent on the type of analyte and reagent gas, also other types of reactions (e.g. hydride abstraction and adduct formation) may occur. The degree of further fragmentation is much less than in EI and may be controlled by varying the nature of the reagent gas. CI reagent gases vary from application to application, but the most common are methane, isobutane and ammonia. In CI, also negative ions can be produced (negative chemical ionization or NCI). Low-energy electrons, present in the plasma, react with electrophilic molecules (e.g. PCBs and PBDEs) to form negative molecular ions.

3.4.2.3. Electron ionization and chemical ionization in PBDE and PCB analysis

Both EI and NCI have been used for the detection of PCBs and PBDEs (Dirtu et al., 2006; Donato et al., 2006; Meneses et al., 1999; Voorspoels et al., 2006). In this work, EI-MS full scan mass spectra of PBDEs were dominated by the product ions due to the loss of Br\(_2\) and to a lesser degree by the ions of the molecular ion cluster \([M]^+\) (Figure III.4). These results are in accordance with results obtained by Pirard et al. (2003) and Alaee et al. (2001b) who reported that bromine in ortho position favoured the formation of the \([M-Br_2]^+\) species over \([M]^+\). The prominent \([M-Br_2]^+\) ion cluster can be explained by the formation of a relatively stable oxonium ion that is generated by the loss of two bromine atoms (Cooper et
Ikonomou (2002) and Riu et al. (2006) reported that the stability of the [M]$^+$ is decreased with increasing number of ortho bromine atoms.

Figure III.4 EI-MS full scan mass spectrum of BDE 99 obtained with a GCQ Plus ion trap mass spectrometer (ThermoFinnigan)

The mass spectra of PCBs, which we have obtained in EI-MS full scan mode, were dominated by the molecular [M]$^+$ and to a lesser degree by [M-Cl$_2$]$^+$ or [M-Cl]$^+$ (Figure III.5). The loss of a single chlorine atom is specific for the chlorinated biphenyls with two or more ortho substituents on different rings (Lausevic et al., 1995). This may be explained by the fact that the removal of one ortho chlorine atom can reduce the steric hindrance that prevents the biphenyl rings from becoming coplanar (Erickson, 1997; Levy and Oswald, 1976).

Figure III.5 EI-MS full scan mass spectra of PCB 118 (left) and PCB 153 (right) obtained with a GCQ Plus ion trap mass spectrometer (ThermoFinnigan)
Eljarrat et al. (2002) investigated the NCI-MS spectra of 40 PBDEs and concluded that NCI (SIM mode) gives 15 times higher responses for PBDEs than EI (SIM mode). The NCI-MS spectra were dominated by the mass fragment \([\text{Br}]^-\) (m/z 79, 81) and the molecular cluster was not observed or constituted only a minor peak. This is in contrast with the NCI spectra for PCBs, in which the molecular cluster is predominant. So a general drawback of the NCI technique is that for PBDEs, only the ions due to bromine can be monitored, which results in lower selectivity. A solution to this problem is the combination of the sensitivity of large volume injection with the higher selectivity of EI-LRMS (Covaci et al., 2002). Several studies have also compared sensitivities of EI-LRMS and NCI-LRMS for PCB analysis (Raverdino et al., 1996; Rothweiler and Berset, 1999; Turci et al., 2003). They concluded that NCI offers higher sensitivity. Lower chlorinated PCBs however show lower S/N ratios and should be determined with EI-LRMS.

3.4.3. Mass analysers

Once the ions have been produced, they need to be separated according to their masses. Since a ThermoFinnigan GCQ ion trap instrument was used for this work, the following discussion of mass analysers will be limited to the linear quadrupole and the quadrupole ion trap.

3.4.3.1. Linear quadrupole

The principle of the quadrupole was first described by Paul and Steinwedel in 1953 at the Bonn University. The linear quadrupole mass analyser can be considered as a mass filter and it consists of four hyperbolic rods placed parallel in a radial array (Figure III.6). These rods are applied with a direct potential \(U_{DC}\) at which an oscillating (radiofrequency or RF) potential, equal to \(V_{RF} \cos \omega t\), is superposed. Two opposed rods are applied with a potential \(\Phi_0 = + (U_{DC} - V_{RF} \cos \omega t)\) and the other two with a potential \(\Phi_0 = - (U_{DC} - V_{RF} \cos \omega t)\). In this equation, \(\omega\) is the angular frequency of the RF field \((\omega = 2\pi \nu\) and constant for a given quadrupole\) and \(V_{RF}\) is the amplitude of the RF voltage.
A positive ion, that enters the space between the rods, will be drawn to the negative rod. If the rod’s potential changes sign before the ion discharges itself on this rod, the ion will change direction. This means that an appropriate combination of $U_{DC}$ and $V_{RF}$, applied to the four rods, induces an oscillatory motion of the ions that enter the space between the rods. For well-defined values of $U_{DC}$ and $V_{RF}$, regions of stable oscillation can be identified for ions of different $m/z$ values. These regions can be schematically represented by a stability diagram. The area under the curve corresponds to the region of stable oscillation (Figure III.7).

As can be seen from the diagram, changing $U_{DC}$ linearly, while keeping the $U_{DC}/V_{RF}$ ratio constant, allows for the successive detection of ions of different $m/z$ values. The resolution of the quadrupole increases with higher $U_{DC}/V_{RF}$ ratios. Usually, quadrupole mass
spectrometers are operated at a resolution that is sufficient to separate two peaks with one mass unit apart.

3.4.3.2. Quadrupole ion trap

The ion trap mass spectrometer is a member of the quadrupole instrument family, first described by Paul and Steinwedel in 1960. They developed a method for mass analysis by trapping a range of ion masses in the ion trap and detecting the ion masses while stored. The ion trap is in fact the three dimensional analogue of the linear quadrupole and consists of two ‘end cap’ electrodes at either side of a central ring electrode (Figure III.8).

![Figure III.8 Quadrupole ion trap mass spectrometer (ThermoFinnigan GCQ Plus operating manual)](image)

The superimposition of a direct potential ($U_{DC}$) with an oscillating potential (RF) on the ring electrode creates a ‘three-dimensional quadrupole’ in which the ions of all masses are trapped in a three-dimensional trajectory. Because ions in the trap repel each other, their trajectories expand as a function of time. To avoid losses of ions by this expansion, a continuous pressure of helium gas is maintained inside the trap. Collision between the ions and the helium gas removes excess energy from the ions and as a result stabilizes their trajectories (de Hoffman and Stroobant, 2001).

As for quadrupole mass spectrometers, stability diagrams can also be made for ion trap mass spectrometers. An ion will be stably trapped depending upon its m/z value, the direct potential ($U_{DC}$), the oscillating potential (RF) and the dimensions of the trap (Figure III.9).
CHAPTER 3. CURRENT ANALYTICAL METHODS

Commercial ion traps work along the line $U_{\text{DC}} = 0$. As $V_{\text{RF}}$ is increased, the motion of the ions becomes progressively more energetic and develops unstable trajectories. Using this technique, trapped ions are sequentially ejected from the trapping cavity towards an external detector in increasing m/z order, thus generating a mass spectrum (March, 1997). In normal mode of use, an auxiliary oscillating potential with high amplitude is applied across the end cap electrodes. If its frequency is equal to the secular frequency at which certain ions oscillate in the trap, the ion will come in resonance, will be destabilized and ejected from the trap (resonance ejection). This means that ions with higher m/z values can be ejected from the trap or that ions can be ejected at lower $V_{\text{RF}}$ values. This also means that the mass range of the ion trap is extended (de Hoffman and Stroobant, 2001).

Most of today’s ion trap instruments have an external ion source which inhibits ion-molecule interactions in the trap. Electron ionization (EI) in combination with full scan acquisition mode is preferentially used because ion trap analysers are more sensitive in full scan mode than linear quadrupoles. As a result, qualitative full scan information is combined with sensitivity. Selected ion monitoring (SIM) is not frequently used in ion traps because no improvement in sensitivity is obtained with respect to full scan mode (Santos and Galceran, 2003).

Enhanced selectivity and sensitivity can be obtained in MS/MS mode with the additional advantage that the full scan spectra of product ions are also collected. First, ions of
a single m/z ratio are selected by expelling all the others from the trap. This is done by ramping the $V_{RF}$ on the ring electrode in normal scan manner such that all ions with lower m/z values than the m/z value of the target ion are removed. Consecutively a broadband RF field, that includes all secular frequencies of the ions with an m/z value which is higher than the m/z value of the target ion, is imposed on the end caps (resonance ejection). As a consequence all ions will be eliminated from the trap except for the target ion. After isolation of the target ion, the kinetic energy of target ions that are left in the trap is increased by imposing their resonant frequency on the end cap electrodes. The amplitude of this resonant frequency voltage should be weak enough not to expel the ions from the trap (resonant excitation). As a result collisions with the helium buffer gas atoms in the trap will result into fragmentation (collision induced dissociation or CID).

Recently several methods, using quadrupole ion storage tandem mass spectrometry (QISTMS), for the determination of PCBs and PBDEs have been developed (de Hoffman and Stroobant, 2001; Gomara et al., 2006; Malavia et al., 2004; Mandalakis et al., 2001; Pirard et al., 2003; Pirard et al., 2006; Yusa et al., 2006). Malavia et al. (2004) even stated that QISTMS analysis of PCBs could be an interesting low-cost alternative for GC-HRMS. In this study, the optimization of the MS/MS method for the detection of PCBs has previously been executed by de Saeger et al. (2005). For both PBDEs and PCBs, the most abundant ion of the molecular cluster was chosen as the precursor ion for subsequent application of MS/MS. In order to achieve as much sensitivity as possible, the mass isolation window was set at 3 m/z with an isolation time of 16 ms. Precursor ions were fragmented using collision-induced dissociation and the two most abundant product ions were chosen for identification and quantification purposes. Excitation time was set at its maximum value of 15 ms. MS/MS parameters, e.g. $q$-value and excitation voltage are further optimized and discussed in following chapters. The MS/MS mass spectra of PBDEs were dominated by product ions due to the loss of two bromine atoms. Product ions due to the loss of two chlorine atoms were predominant in the MS/MS spectra of PCBs. Product ions corresponding to the loss of a single chlorine atom were practically non existent in the MS/MS spectra of the mono-ortho substituted PCBs 28 and 118.
3.4.3.3. Detector

The ions that pass the mass analyser are detected and transformed into a usable signal by the detector. Different types of detectors exist, but only the electron multiplier (Figure III.11) was used in this work. When a positive or negative ion leaves the mass analyser, it strikes the conversion dynode and secondary particles (positive ions, negative ions, electrons and neutrals) are formed. Thus, when negative ions need to be detected, a positive potential is applied to a conversion dynode. These negative ions strike the positive high voltage conversion dynode and positive ions are emitted. These secondary particles strike the surface of the electron multiplier and electrons are emitted. As these secondary electrons pass further into the electron multiplier and strike the surface, more and more electrons are emitted. This finally results in a measurable current at the end of the multiplier (de Hoffman and Stroobant, 2001).

Figure III.11 Schematic representation of an electron multiplier (de Hoffman and Stroobant, 2001)
3.5. REFERENCES


chromatography analytical method for measuring persistent organohalogen compounds in adipose and organ tissue analysis. *Chemosphere* (57) 373-381.


CHAPTER 4. DEVELOPMENT OF A GAS CHROMATOGRAPHY/ION TRAP MASS SPECTROMETRY BASED METHOD FOR THE QUANTIFICATION OF POLYBROMINATED DIPHENYL ETHERS AND POLYCHLORINATED BIPHENYLS IN ADIPOSE TISSUE
4.1. BACKGROUND AND OBJECTIVES

Since PBDEs accumulate in fatty tissues, the first objective of this work was to develop a clean-up method in combination with gas chromatography-tandem mass spectrometry (GC-MS/MS) for the detection and quantification of seven PBDE congeners (BDE 28, BDE 47, BDE 99, BDE 100, BDE 153, BDE 154 and BDE 183) in adipose tissue. We have selected GC-MS/MS over GC-MS because of the improved selectivity and sensitivity inherent to tandem mass spectrometry. In order to compare detected PBDE levels with levels of other comparable persistent organic pollutants, we have also included the seven marker PCB congeners which are mentioned in the Belgian legislation in our method development. It was our intention to determine PBDEs and PCBs in one single GC-MS/MS run, but due to software related limitations it was not possible to identify and quantify more than two closely eluting peaks in MS/MS mode. As a result we chose to perform two separate injections per sample. Afterwards, the developed clean-up and detection method was subjected to an extensive validation procedure. In order to assess the applicability of the developed method to real samples, five human adipose tissue samples were analyzed.

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4.2. DEVELOPMENT OF A GAS CHROMATOGRAPHY/ION TRAP MASS SPECTROMETRY BASED METHOD FOR THE QUANTIFICATION OF POLYBROMINATED DIPHENYL ETHERS AND POLYCHLORINATED BIPHENYLS IN ADIPOSE TISSUE

4.2.1. Abstract

A combined gas chromatographic mass spectrometric (GC-MS/MS) method for the determination of 7 polybrominated diphenyl ethers (PBDEs) and 7 marker polychlorinated biphenyls (PCBs) in adipose tissue has been developed. Adipose tissue was melted and filtrated through anhydrous sodium sulphate to obtain pure fat. Clean-up was performed using a glass column containing acidified silica, deactivated alumina and anhydrous sodium sulphate. Polybrominated biphenyl (PBB) 155 and Mirex were added as internal standards for PBDEs and PCBs respectively. Injection standards, PBB 103 and PCB 143, for PBDEs and PCBs respectively, were added before analysis with GC-MS/MS. The developed GC-MS/MS method has the advantage of being more selective than single MS methods because matrix effects are largely eliminated. Validation of this method was done according to Commission Decision 2002/657/EC. Decision limits for PBDEs and PCBs ranged from 0.06 ng g\(^{-1}\) - 0.15 ng g\(^{-1}\) and from 0.35 ng g\(^{-1}\) - 1.22 ng g\(^{-1}\) respectively. Detection capabilities were all between 0.23 ng g\(^{-1}\) - 0.55 ng g\(^{-1}\) for PBDEs and between 0.98 ng g\(^{-1}\) - 2.29 ng g\(^{-1}\) for PCBs. Precision, recovery, bias and selectivity were tested with satisfactory results.

4.2.2. Introduction

The annual production of brominated flame retardants is estimated at 200 000 metric tons of which 70 000 are polybrominated diphenyl ethers (PBDEs)\(^1\). The reason for using brominated compounds as flame retardants is based on the ability of the organobromine compound to capture free radicals in the gas flame phase and retard both ignition and rate of combustion\(^2\). PBDEs are a group of additive flame retardants used in plastics, electronic equipment (TV sets, computers and household appliances), textiles and printed circuit boards. Additive flame retardants are mixed with or dissolved in polymers and are therefore able to leach from the products and into the environment.
Polychlorinated biphenyls (PCBs) have been widely used as complex mixtures in heat transfer fluids, dielectric fluids in capacitors and transformers and as additives in paints, pesticides, copy paper, adhesives and plastics. PCBs are introduced into the environment by escaping from ‘closed’ systems (e.g. capacitors or transformers) or by accidental loss from ‘open’ systems during incineration of industrial or municipal waste.

PBDEs and PCBs are commonly very similar, and their basic structures differ only by the presence of an ether linkage in PBDEs. Both classes consist of 209 congeners with different numbers and positions of the bromine atoms on the diphenyl ether part and chlorine atoms on the biphenyl part, respectively.

Three technical PBDE mixtures are produced, known commercially as the Penta-mix, Octa-mix and Deca-mix. These three mixtures contain fewer congeners than commercial PCB mixtures. As a consequence the number of PBDE congeners found in environmental samples is much lower than the number of PCB congeners. PBDEs and PCBs, which are persistent and lipophilic, have high binding affinity to particles and a tendency to bioaccumulate through the food chain.

They have been detected in sediments, air, biota and human blood, adipose tissue and breast milk. The main human exposure route is through the diet, via the aquatic food chain and food products rich in lipids. Highest concentrations have been found in oils, fats, fish and shellfish, meat and eggs. After exposure, the PBDEs and PCBs accumulate in the adipose tissue and are transported via the blood throughout the body compartments and different organs.

Several animal studies indicate that PBDEs are potentially toxic substances. Certain congeners have been shown to cause neurotoxic effects, to interfere with the brain development, to bind to the thyroid and aryl hydrocarbon (Ah) receptor and to have endocrine disrupting properties. However, overall toxicity is lower than for PCBs.

Belgian legislation has set maximum limits for the total sum of seven marker PCB congeners (PCBs 28, 52, 101, 118, 138, 153, 180) in different food matrices. For beef, pork, chicken, eggs, animal fat or oil and products thereof, a limit of 200 ng g\(^{-1}\) fat has been set for products with a fat content above 2 %. According to Council Directive 2003/11/EC, the use of Penta- and Octa-BDE and the placing on the market of articles containing one or both of these substances was banned within the European Union by the 15\(^{th}\) of August 2004. A risk reduction strategy will be established for Deca-BDE. Although Deca-BDE has been found in a large number of environmental samples, very low levels were detected in biological
samples. This is probably due to the very poor bioavailability and fast metabolism of this highly brominated compound\textsuperscript{31}.

The aim of this study was to develop a gas chromatography/ion trap mass spectrometry method for the determination of 7 PBDE congeners and 7 marker PCB congeners by means of a single clean-up step.

4.2.3. Experimental

4.2.3.1. Reagents and solvents

All reagents and solvents were of analytical reagent grade. Isooctane, n-hexane Suprasolv\textsuperscript{®} and anhydrous sodium sulphate were obtained from Merck (Darmstadt, Germany). Nonane was purchased from Sigma Aldrich nv/sa (Bornem, Belgium).

Acidified silica was prepared by adding 35.5 mL concentrated sulphuric acid p.a. (Merck) to 100 g silica gel (0.063 - 0.200 mm, Merck) and mixing thoroughly. Water was obtained by a Milli Q gradient system (Millipore, Brussels, Belgium). Preparation of deactivated alumina was performed by adding 5 mL water to 45 g alumina B activity I (ICN Biomedicals, Eschwege, Germany). Silane-treated glass wool was obtained from Alltech Associates (Deerfield, IL, USA).

4.2.3.2. Standard solutions

Individual PBDE standards, IUPAC Nos. 28, 47, 99, 100, 153, 154 and 183 (see Table IV.1), were purchased from Wellington Laboratories (Ontario, Canada). Individual PCB standards, IUPAC Nos. 28, 52, 101, 118, 138, 153 and 180 (see Table IV.1), together with PCB-Mix 3 (10 ng µL\textsuperscript{-1} of each congener), internal standards (Mirex and polybrominated biphenyl (PBB) 155) and injection standards (PCB 143 and PBB 103) were purchased from Dr. Ehrenstorfer (Augsburg, Germany).

A standard stock solution containing all seven PBDE congeners was prepared at a concentration of 1 ng µL\textsuperscript{-1}. Standard stock solutions of PBB 155, PBB 103, PCB 143 and Mirex were prepared at 10 ng µL\textsuperscript{-1}. All stock solutions were stored between 2 °C and 8 °C, protected from light. Suitable working PBDE and PCB solutions were prepared daily by dilution in nonane and isooctane respectively.
Table IV.1 IUPAC numbering of investigated PBDE and PCB congeners

<table>
<thead>
<tr>
<th>IUPAC No.</th>
<th>Structure</th>
<th>IUPAC No.</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCB 28</td>
<td>2,4,4’</td>
<td>BDE 28</td>
<td>2,4,4’</td>
</tr>
<tr>
<td>PCB 52</td>
<td>2,2’,5,5’</td>
<td>BDE 47</td>
<td>2,2’,4,4’</td>
</tr>
<tr>
<td>PCB 101</td>
<td>2,2’,4,5,5’</td>
<td>BDE 99</td>
<td>2,2’,4,4’,5</td>
</tr>
<tr>
<td>PCB 118</td>
<td>2,3’,4,4’,5</td>
<td>BDE 100</td>
<td>2,2’,4,4’,6</td>
</tr>
<tr>
<td>PCB 138</td>
<td>2,2’,3,4,4’,5’</td>
<td>BDE 153</td>
<td>2,2’,4,4’,5,5’</td>
</tr>
<tr>
<td>PCB 153</td>
<td>2,2’,4,4’,5,5’</td>
<td>BDE 154</td>
<td>2,2’,4,4’,5,6’</td>
</tr>
<tr>
<td>PCB 180</td>
<td>2,2’,3,4,4’,5,5’</td>
<td>BDE 183</td>
<td>2,2’,3,4,4’,5,6’</td>
</tr>
</tbody>
</table>

4.2.3.3. Apparatus

Analysis of the adipose tissue samples was performed using a Finnigan GCQ gas chromatograph coupled to a Finnigan GCQ mass spectrometer (Austin, Texas, USA). The autosampler was a CTC 200 series injector (Zwingen, Switzerland). Separation of the PBDE and PCB congeners was performed using a HT8 capillary column (25 m x 0.22 mm x 0.25 µm, SGE, Achrom, Zulte, Belgium). Carrier and collision gas was Alphagaz 2 helium (Air Liquide, Liege, Belgium). Instrument set points and data acquisition were under control of GCQ software.

4.2.3.4. Sample collection and storage

Fat tissue samples taken within the frame of the Belgian national monitoring programme for PCBs were collected and stored at –20°C. Only fat samples that contained PCB levels lower than the decision limit (CCα) were used to prepare a pool of blank extracted fat for the validation procedure. Out of this pool a sample was analyzed and a new pool was prepared if PBDE levels were above the decision limit (CCα).

Five human adipose tissue samples from the abdominal fat region were obtained from autopsy and stored at -20°C.
4.2.3.5. Extraction and clean-up

In order to prevent contamination, glassware was washed extensively and rinsed twice with hexane (Acros, Geel, Belgium). Plastic materials were not used in order to avoid contamination. Approximately 10 g of adipose tissue sample was brought onto a folded Ederol filter paper (VWR, Leuven, Belgium) in a glass funnel together with anhydrous sodium sulphate (10 g). The fat was melted in a microwave oven (600 W – 2 min) and received in a glass vessel. Internal standards PBB 155 (40 µL, 0.1 ng µL⁻¹), Mirex (20 µL, 1 ng µL⁻¹) and n-hexane (5 mL) were added to the melted fat (2 g). A glass column containing a wad of silane-treated glass wool at the bottom was used for clean-up. This column was subsequently filled with n-hexane (25 mL), acidified silica (12 g), deactivated alumina (3 g) and anhydrous sodium sulphate (3 g). After removal of any excess n-hexane from the column, the fat solution was brought onto the column and elution was performed with n-hexane (40 mL). The eluate was evaporated in a rotary evaporator at 40 °C to ca. 4 mL. This solution was transferred to a graduated glass vial (Egilabo, Kontich, Belgium). Injection standards, PBB 103 (40 µL, 0.1 ng µL⁻¹) and PCB 143 (20 µL, 1 ng µL⁻¹) and keeper solvent (isooctane) were added. This mixture was concentrated under nitrogen at 40 °C to 100 µL. This condensed isooctane solution was split over 2 GC-MS vials.

4.2.3.6. Gas chromatography-mass spectrometry

A 2 µL aliquot of the final sample extract was injected in the splitless mode; the splitless period following injection was 1 min. Injection temperature was set at 300 °C and transferline temperature was 275 °C. The oven was programmed from 70 °C for 1 min to 170 °C at a rate of 30 °C min⁻¹, then to 300 °C (15 min) at a rate of 8 °C min⁻¹.

Optimization of GC-MS/MS parameters for PCB analysis was previously done by De Saeger et al. In this study optimization of GC-MS/MS parameters was limited to PBDEs. In order to achieve optimal mass spectrometric conditions ionisation energy was varied between 35 eV and 70 eV. The mass spectrometer was operated in MS/MS mode. Ideal fragmentation conditions were also determined by varying q-value and excitation voltage.
Validation of the method was conducted according to Commission Decision 2002/657/EC. This document gives a description of the performance characteristics that have to be verified for a specific method. Specificity, decision limit (CC$_\alpha$), detection capability (CC$_\beta$), recovery and precision need to be determined for a quantitative confirmatory method. All validation parameters, except for specificity were determined on the most abundant ion. This ion was also used for quantification purposes. CC$_\alpha$ or decision limit was established by the calibration curve procedure and was repeated 5 times. Blank fat samples were fortified with a mixture of seven PBDE congeners at a level of 0, 0.5, 1, 2, 5 ng g\(^{-1}\) per congener; and seven PCB congeners at 0, 1, 5, 10, 20 ng g\(^{-1}\) per congener. After identification, the signal was plotted against the added concentration. The corresponding concentration at the y-intercept plus 2.33 times the standard deviation of the within-laboratory reproducibility of the intercept is defined as the decision limit (\(\alpha = 1\ %\)). CC$_\beta$ or detection capability was determined by using the same calibration curves. CC$_\beta$ equals the corresponding concentration at the decision limit plus 1.64 times the standard deviation of the within-laboratory reproducibility of the mean measured at the decision limit (\(\beta = 5\ %\)). Specificity was checked by analyzing 20 different blank fat samples for interferences. Recovery is defined as that fraction of mass of the analyte added to the sample which is present in the final extract. For the determination of the recovery 18 blank fat samples were fortified before clean-up at 1, 2 and 4 ng g\(^{-1}\) per congener for PBDEs and 5, 10 and 20 ng g\(^{-1}\) per congener for PCBs. The responses of these fortified samples were compared with samples fortified after clean-up. Within day and between day precision were calculated as a coefficient of variation (CV %) by fortifying blank fat samples at 3 nominal levels (1, 2 and 4 ng g\(^{-1}\) per congener for PBDEs and 5, 10 and 20 ng g\(^{-1}\) per congener for PCBs). Bias was determined as an approximation for trueness and was assessed by fortifying 18 blank fat samples at 1, 2 and 4 ng g\(^{-1}\) per congener for PBDEs and 5, 10 and 20 ng g\(^{-1}\) per congener for PCBs.

Measurement uncertainty was determined according to the EURACHEM/CITAC guide (2000). The global uncertainty was calculated as a combination of the uncertainty associated with precision and the uncertainty associated with accuracy. Although this is the combined uncertainty, the results are expressed as an expanded uncertainty, which corresponds to twice this value.
4.2.4. Results and discussion

4.2.4.1. Extraction and clean-up

Because this method only applies to adipose tissue samples, no particular fat isolation step was necessary except melting. Sodium sulphate extracted any excess water during the melting process. To obtain enough sensitivity it was necessary to extract a minimum of 2 g of fat. Clean-up by sulphuric acid treatment was used to remove potential chromatographic interferences, e.g. fatty acids and organic macromolecules. Chromatographic columns were filled with various quantities of acidified silica, alumina and sodium sulphate. Best results were obtained with 12 g acidified silica, 3 g alumina and 3 g anhydrous sodium sulphate. The columns were eluted with various volumes of n-hexane. To obtain sufficient recoveries 40 mL of n-hexane was necessary. After addition of the injection standard, the eluate was evaporated to 100 µL. A procedure was tested whereby all of the eluate was evaporated and redissolved in isoctane; this approach was discarded because of volatilization of BDE 28 and irreversible adsorption of PCBs to the glassware.

4.2.4.2. Optimization of GC-MS/MS parameters

The HT8 column, characterised by a carborane phase, was used to separate the seven marker PCB congeners and seven PBDE congeners. This type of column was chosen because fewer co-elutions of target analytes occur. The seven PBDEs were selected because they are the main reported congeners in the literature. It was decided to not include BDE 209, which is commercially the most important congener, in this study due to significant difficulties in its analysis. BDE 209 is light and heat sensitive and requires a specific GC column owing to its very long retention time.

PBDEs and PCBs had to be determined in two consecutive runs because PCB 180, Mirex and BDE 47 could not be separated in a single run. The chromatograms in Figs. IV.1 and IV.2 show that PBDEs, PCBs, internal standards and injection standards are well separated.
The use of isotopically labelled internal standards was considered but due to software related problems this approach was discarded and Mirex and PBB 155 were chosen as alternatives.
CHAPTER 4. DEVELOPMENT OF A GAS CHROMATOGRAPHY/ION TRAP MASS SPECTROMETRY BASED METHOD FOR THE QUANTIFICATION OF POLYBROMINATED DIPHENYL ETHERS AND POLYCHLORINATED BIPHENYLS IN ADIPOSE TISSUE

Detection of all analytes used electron impact (EI) MS/MS. This combination was favoured because less interferences occur and it achieves a higher degree of selectivity. In most studies using EI-MS the electron energy was either 70 eV or between 30 - 40 eV\textsuperscript{31}. In this work the electron energy was varied between 30 eV and 70 eV and no difference was noticed, and therefore the default value of 70 eV was used. As previously reported by Pirard \textit{et al.}\textsuperscript{31}, the EI spectra were dominated by M\textsuperscript{+} and [M-Br\textsubscript{2}]\textsuperscript{+} for the lower brominated and higher brominated PBDEs respectively. The molecular ion was chosen as the MS/MS precursor ion because it offers more structural information. Precursor ions were then isolated in the ion trap and fragmented by collision-induced dissociation (CID) resulting into fragment ions. Excitation voltage, excitation time and $q$-value were optimised and fragment ions were detected in full scan mode.

Table IV.2 Ion trap mass spectrometric conditions and ions monitored (more intense ions in bold font)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Excitation voltage (V)</th>
<th>$q$-value</th>
<th>Precursor Ion (m/z)</th>
<th>Product Ions (m/z)</th>
<th>Retention time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCB 28</td>
<td>1.80</td>
<td>0.450</td>
<td>256</td>
<td>186 151</td>
<td>11.50</td>
</tr>
<tr>
<td>PCB 52</td>
<td>1.80</td>
<td>0.450</td>
<td>292</td>
<td>257 222</td>
<td>12.29</td>
</tr>
<tr>
<td>PCB 101</td>
<td>2.00</td>
<td>0.450</td>
<td>326</td>
<td>256 291</td>
<td>14.45</td>
</tr>
<tr>
<td>PCB 118</td>
<td>2.00</td>
<td>0.450</td>
<td>326</td>
<td>256 254</td>
<td>16.35</td>
</tr>
<tr>
<td>PCB 138</td>
<td>1.80</td>
<td>0.450</td>
<td>360</td>
<td>290 325</td>
<td>17.41</td>
</tr>
<tr>
<td>PCB 153</td>
<td>1.80</td>
<td>0.450</td>
<td>360</td>
<td>290 325</td>
<td>16.57</td>
</tr>
<tr>
<td>PCB 180</td>
<td>1.80</td>
<td>0.450</td>
<td>394</td>
<td>324 359</td>
<td>19.21</td>
</tr>
<tr>
<td>Mirex</td>
<td>1.50</td>
<td>0.450</td>
<td>272</td>
<td>237 235</td>
<td>19.32</td>
</tr>
<tr>
<td>PCB 143</td>
<td>2.00</td>
<td>0.450</td>
<td>360</td>
<td>325 290</td>
<td>16.23</td>
</tr>
<tr>
<td>BDE 28</td>
<td>1.50</td>
<td>0.300</td>
<td>406</td>
<td>246 248</td>
<td>16.33</td>
</tr>
<tr>
<td>BDE 47</td>
<td>1.50</td>
<td>0.300</td>
<td>486</td>
<td>326 328</td>
<td>19.34</td>
</tr>
<tr>
<td>BDE 99</td>
<td>1.50</td>
<td>0.300</td>
<td>566</td>
<td>406 408</td>
<td>22.23</td>
</tr>
<tr>
<td>BDE 100</td>
<td>1.50</td>
<td>0.300</td>
<td>566</td>
<td>406 408</td>
<td>21.44</td>
</tr>
<tr>
<td>BDE 153</td>
<td>1.50</td>
<td>0.300</td>
<td>644</td>
<td>484 482</td>
<td>26.19</td>
</tr>
<tr>
<td>BDE 154</td>
<td>1.50</td>
<td>0.300</td>
<td>644</td>
<td>484 482</td>
<td>24.29</td>
</tr>
<tr>
<td>BDE 183</td>
<td>2.00</td>
<td>0.450</td>
<td>722</td>
<td>564 562</td>
<td>32.38</td>
</tr>
<tr>
<td>PBB 155</td>
<td>1.50</td>
<td>0.300</td>
<td>628</td>
<td>470 549</td>
<td>21.13</td>
</tr>
<tr>
<td>PBB 103</td>
<td>1.50</td>
<td>0.300</td>
<td>548</td>
<td>469 390</td>
<td>19.17</td>
</tr>
</tbody>
</table>
Fragmentation of the molecular ion only generated a loss of two bromine atoms for all PBDE congeners. As stipulated by Commission Decision 2002/657/EC the two most abundant fragment ions were chosen as diagnostic ions. An overview of the ions monitored and the spectrometric conditions are given in Table IV.2. Quantification was done with the most abundant fragment ion except for BDE 183 where the sum of m/z 561 to 566 was used. This was necessary because quantification of BDE 183 with a single fragment ion did not generate reproducible results.

### 4.2.4.3. Validation of the method

Because no interfering peaks could be detected when analyzing 20 blank fat samples, it could be decided that the method was specific. Results of the different validation parameters obtained at 2 ng g\(^{-1}\) for PBDEs and 10 ng g\(^{-1}\) for PCBs are presented in Table IV.3.

<table>
<thead>
<tr>
<th>Compound</th>
<th>CC(\alpha) (ng g(^{-1}))</th>
<th>CC(\beta) (ng g(^{-1}))</th>
<th>Repeatability (CV %)</th>
<th>Bias(^a)% ((n = 6))</th>
<th>Recovery(^a)% ((n = 6))</th>
<th>Expanded measurement uncertainty(^a)%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Within day(^a) ((n = 6))</td>
<td>Between day(^a) ((n = 6))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCB 28</td>
<td>0.56</td>
<td>1.75</td>
<td>5.07</td>
<td>2.69</td>
<td>+ 3.2</td>
<td>98.1</td>
</tr>
<tr>
<td>PCB 52</td>
<td>0.55</td>
<td>1.70</td>
<td>5.12</td>
<td>3.98</td>
<td>+ 2.9</td>
<td>91.7</td>
</tr>
<tr>
<td>PCB 101</td>
<td>0.38</td>
<td>1.25</td>
<td>2.22</td>
<td>2.43</td>
<td>- 2.4</td>
<td>91.7</td>
</tr>
<tr>
<td>PCB 118</td>
<td>0.35</td>
<td>0.98</td>
<td>3.33</td>
<td>4.30</td>
<td>- 0.9</td>
<td>90.5</td>
</tr>
<tr>
<td>PCB 138</td>
<td>0.35</td>
<td>1.28</td>
<td>5.45</td>
<td>0.68</td>
<td>- 3.0</td>
<td>89.4</td>
</tr>
<tr>
<td>PCB 153</td>
<td>0.95</td>
<td>2.21</td>
<td>2.70</td>
<td>4.79</td>
<td>- 7.6</td>
<td>93.2</td>
</tr>
<tr>
<td>PCB 180</td>
<td>1.22</td>
<td>2.29</td>
<td>4.34</td>
<td>2.52</td>
<td>- 3.0</td>
<td>88.9</td>
</tr>
<tr>
<td>BDE 28</td>
<td>0.06</td>
<td>0.23</td>
<td>2.53</td>
<td>3.60</td>
<td>+ 0.50</td>
<td>86.9</td>
</tr>
<tr>
<td>BDE 47</td>
<td>0.06</td>
<td>0.31</td>
<td>3.75</td>
<td>4.98</td>
<td>-1.50</td>
<td>88.4</td>
</tr>
<tr>
<td>BDE 99</td>
<td>0.09</td>
<td>0.30</td>
<td>2.10</td>
<td>5.03</td>
<td>0</td>
<td>95.3</td>
</tr>
<tr>
<td>BDE 100</td>
<td>0.09</td>
<td>0.40</td>
<td>2.39</td>
<td>4.37</td>
<td>+ 1.0</td>
<td>86.5</td>
</tr>
<tr>
<td>BDE 153</td>
<td>0.10</td>
<td>0.55</td>
<td>5.20</td>
<td>3.88</td>
<td>- 3.0</td>
<td>83.0</td>
</tr>
<tr>
<td>BDE 154</td>
<td>0.08</td>
<td>0.46</td>
<td>3.13</td>
<td>4.14</td>
<td>- 1.0</td>
<td>81.5</td>
</tr>
<tr>
<td>BDE 183</td>
<td>0.15</td>
<td>0.53</td>
<td>5.70</td>
<td>7.89</td>
<td>- 6.0</td>
<td>85.4</td>
</tr>
</tbody>
</table>

\(^a\) Data were obtained at 2 ng g\(^{-1}\) level for PBDEs and 10 ng g\(^{-1}\) level for PCBs
Since PBDEs occur in lower concentrations in biological samples compared to PCBs, calibration curves were determined in a lower linear range resulting in lower decision limits (CCα) and detection capabilities (CCβ). Decision limits for PBDEs and PCBs ranged from 0.06 to 0.15 ng g⁻¹ and from 0.35 ng g⁻¹ to 1.22 ng g⁻¹ respectively. Detection capabilities of PBDEs and PCBs varied between 0.23 - 0.55 ng g⁻¹ and 0.98 - 2.29 ng g⁻¹ respectively. Precision, expressed as the coefficients of variation, did not exceed 7.89 % accounting for the good repeatability of the developed method. In the concentration range 0.5 to 5 ng g⁻¹ and 1 to 20 ng g⁻¹ for PBDEs and PCBs respectively, the correlation coefficients (R²) were all higher than 0.995. Recoveries ranged between 88.9 and 98.1 % for PCBs and between 81.5 and 95.3 % for PBDEs. As shown in Table IV.3 the percent bias was within the -30 % till + 10 % range and -20 % till +10 % range, as prescribed by Commission Decision 2002/657/EC, for concentrations ranging from 1-10 ng g⁻¹ and >1-10 ng g⁻¹ respectively. Expanded measurement uncertainty varied between 23.5 and 42.8 % for PCBs and 16.5 and 28.2 % for PBDEs. All identification criteria mentioned by Commission Decision 2002/657/EC were fulfilled. These criteria include the specification that the signal-to-noise ratio for each diagnostic ion is larger than or equal to three. Relative retention times (RRTs) of the analyte did not differ by more than +/- 0.5 % of the RRT of the calibration standard. The relative intensities of the detected ions, expressed as a percentage of the intensity of the most abundant ion, corresponded to those of the samples fortified at comparable concentrations within the tolerances mentioned by Commission Decision 2002/657/EC.

4.2.4.4. Analysis of human adipose tissue samples

In order to evaluate the applicability of the developed method, five human adipose tissue samples from the abdominal fat region were analyzed. Concentrations of the seven PBDE and seven marker PCB congeners are presented in Table IV.4.
### Table IV.4 Concentrations of the different PCB and PBDE congeners in human adipose tissue samples

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>PCB (ng g(^{-1}))</th>
<th>PBDE (ng g(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>28</td>
<td>52</td>
</tr>
<tr>
<td>2000/219</td>
<td>&lt;CCβ</td>
<td>&lt;CCβ</td>
</tr>
<tr>
<td>2001/158</td>
<td>3.1</td>
<td>2.4</td>
</tr>
<tr>
<td>2002/129</td>
<td>5.8</td>
<td>&lt;CCβ</td>
</tr>
<tr>
<td>2002/130</td>
<td>3.1</td>
<td>2.0</td>
</tr>
<tr>
<td>2002/3</td>
<td>&lt;CCβ</td>
<td>&lt;CCβ</td>
</tr>
</tbody>
</table>
4.2.5. Conclusions

The GC-MS/MS method described in this paper provides a reliable procedure for the determination of above mentioned PCBs and PBDEs. All validation criteria mentioned by Commission Decision 2002/657/EC\textsuperscript{33} were fulfilled. The analysis of five human adipose tissue samples demonstrates the applicability of the described method to real samples.
4.2.6. References


CHAPTER 5. OCCURRENCE OF POLYCHLORINATED BIPHENYLS AND POLYBROMINATED DIPHENYL ETHERS IN BELGIAN HUMAN ADIPOSE TISSUE SAMPLES
5.1. **BACKGROUND AND OBJECTIVES**

Because of the fact that PBDEs accumulate in fatty tissues and because scientific research has reported that PBDE levels in humans have risen significantly over the past decades, it was our objective to determine the levels and congener pattern of PBDEs in a large number of Belgian human adipose samples. In this chapter, fifty three human adipose tissue samples were analyzed with the newly developed GC-MS/MS method which is described in Chapter 4. In order to examine if PBDE levels correlated with levels of other similar persistent pollutants, samples were simultaneously analyzed for PCBs. In addition, determined levels were compared to PCB and PBDE levels in adipose tissue of humans living in other countries. Following table gives an overview of the PBDE and PCB concentrations in human adipose tissue:

<table>
<thead>
<tr>
<th>Country</th>
<th><strong>Sum (ng g(^{-1}) lipid weight)</strong>(^{a})</th>
<th><strong>Main congeners</strong></th>
<th><strong>Reference</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PBDE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Belgium</td>
<td>4.8 (mean)</td>
<td>BDE 153 and BDE 47</td>
<td>Covaci et al. (2002)</td>
</tr>
<tr>
<td>Japan</td>
<td>1.3 (median)</td>
<td>BDE 47 and BDE 153</td>
<td>Choi et al. (2003)</td>
</tr>
<tr>
<td>Singapore</td>
<td>5.2 (mean)</td>
<td>BDE 47, BDE 154 and BDE 99</td>
<td>Li et al. (2006)</td>
</tr>
<tr>
<td>Spain</td>
<td>3.8 (mean)</td>
<td>BDE 153, BDE 47 and BDE 183</td>
<td>Fernandez et al. (2007)</td>
</tr>
<tr>
<td>Sweden</td>
<td>5.4 (mean)</td>
<td>BDE 47, BDE 99 and BDE 153</td>
<td>Guvenius et al. (2001)</td>
</tr>
<tr>
<td>United States (CA)</td>
<td>17 (median)</td>
<td>BDE 47</td>
<td>Petreas et al. (2003)</td>
</tr>
<tr>
<td>United States (CA)</td>
<td>86 (mean)</td>
<td>BDE 47, BDE 153 and BDE 154</td>
<td>She et al. (2002)</td>
</tr>
<tr>
<td>United States (NY)</td>
<td>399 (mean), 77 (median)</td>
<td>BDE 47, BDE 99 and BDE 100</td>
<td>Johnson-Restrepo et al. (2005)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>PCB</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Belgium</td>
<td>841 (median)</td>
<td>PCB 153, PCB 180 and PCB 138</td>
<td>Covaci et al. (2002)</td>
</tr>
<tr>
<td>Canada</td>
<td>890 (mean)</td>
<td>PCB 138, PCB 153 and PCB 156</td>
<td>Paris-Pombo et al. (2003)</td>
</tr>
<tr>
<td>Hong Kong</td>
<td>190 (mean)</td>
<td>Hexa- and heptachlorinated PCBs</td>
<td>Poon et al. (2005)</td>
</tr>
<tr>
<td>Spain</td>
<td>560 (mean)</td>
<td>PCB 180, PCB 153 and PCB 138</td>
<td>Costabeber and Emmanuelli (2003)</td>
</tr>
<tr>
<td>Sweden</td>
<td>1227 (mean)</td>
<td>PCB 153</td>
<td>Guvenius et al. (2001)</td>
</tr>
<tr>
<td>Turkey</td>
<td>383 (mean)</td>
<td>PCB 153, PCB 180 and PCB 138</td>
<td>Çok and Satiróğlu (2004)</td>
</tr>
<tr>
<td>United States (NY)</td>
<td>295 (median)</td>
<td>PCB 153, PCB 138 and PCB 118</td>
<td>Stellman et al. (2000)</td>
</tr>
</tbody>
</table>

\(^{a}\)The number of congeners taken into account for calculation of the sum is different for each study.
CHAPTER 5. OCCURRENCE OF POLYCHLORINATED BIPHENYLS AND POLYBROMINATED DIPHENYL ETHERS IN BELGIAN HUMAN ADIPOSE TISSUE SAMPLES

Taking into account that PCBs and PBDEs accumulate in the adipose tissue throughout an entire human lifetime, we also wanted to investigate if there was an age-dependency in PCB and PBDE levels. To conclude, we examined if there was a significant difference in levels between males and females.

References


The content of this chapter is reproduced from the paper published in *Archives of Environmental Contamination and Toxicology* 50 (2006) 290-296, written by C. Naert, M. Piette, N. Bruneel and C. Van Peteghem.
5.2. OCCURRENCE OF POLYCHLORINATED BIPHENYLS AND POLYBROMINATED DIPHENYL ETHERS IN BELGIAN HUMAN ADIPOSE TISSUE SAMPLES

5.2.1. Abstract

Levels of polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) were measured in 53 human adipose tissue samples. The samples consisted of adipose tissue from 31 men and 22 women having a mean age of 53 years. No information about diet or occupational exposure was provided.

Clean-up was performed using a glass column containing acidified silica, deactivated alumina and anhydrous sodium sulphate. Subsequently, samples were analyzed by high resolution gas chromatography/tandem mass spectrometry (HRGC-MS/MS).

PBDE concentrations (sum of BDE 28, BDE 47, BDE 99, BDE 100, BDE 154, BDE 153 and BDE 183) ranged between 1.23 ng g\(^{-1}\) and 57.2 ng g\(^{-1}\) lipid weight and were comparable with levels in other European countries. The sum of seven International Council for the Exploration of the Sea (ICES) indicator PCB congeners (PCB 28, PCB 52, PCB 101, PCB 118, PCB 138, PCB 153 and PCB 180) ranged from 126 ng g\(^{-1}\) to 2090 ng g\(^{-1}\) lipid weight. No age dependency was found for PBDEs (Pearson correlation = -0.023, Sig. = 0.873) while PCBs showed higher correlation coefficients with age (Pearson correlation = 0.613, Sig. < 0.0005). There was no relationship between PBDE and PCB levels (Pearson correlation = -0.010, Sig. = 0.943).

5.2.2. Introduction

Polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) are pollutants that have been identified worldwide in environmental and biological matrices i.e., air (Sjödin et al. 2001a, Montone et al. 2003), water (Binelli et al. 2004, Law et al. 2003), sediment (Allchin et al. 1999, de Boer et al. 2003, Sapozhnikova et al. 2004), fish (Focardi et al. 1996, Hites 2004, Johnson and Olson 2001, Akutsu et al. 2001), wildlife (Law et al. 2003) and human adipose tissue (Guvenius et al. 2001, Meneses et al. 1999, She et al. 2002, Pauwels et al. 2000, Costabeber and Emmanuelli 2003), blood (Sjödin et al. 1999, Sjödin et
PCBs have been widely used as electric insulators in transformers and capacitors, as heat transfer fluids and as additives in pesticides, adhesives, plastics and paints (Safe, 1994). A substantial part of the environmental burden of these compounds has resulted from disposal practices or leakage from industrial facilities and chemical waste disposal sites.

PBDEs are a group of additive flame retardants that are mixed with or dissolved into potentially flammable materials. The quantity and type of flame retardants employed depends on the application and fire protection requirements (Sjödin et al. 2001a). PBDEs are commercially produced as three mixtures, i.e. Penta-BDE, Octa-BDE and Deca-BDE, indicating their degree of bromination (Sjödin et al. 2003). These three mixtures each contain fewer congeners than the former commercial PCB mixtures and their general compositions are given in Table V.1. As a consequence the number of PBDE congeners found in environmental samples is much lower than the number of PCB congeners.

Table V.1 General composition of three commercially produced PBDE mixtures given in percent of BDE congeners present (WHO/IPCS, 1994).

<table>
<thead>
<tr>
<th>Product</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TrBDEs</td>
</tr>
<tr>
<td>Penta-BDE</td>
<td>0-1%</td>
</tr>
<tr>
<td>Octa-BDE</td>
<td>10-12%</td>
</tr>
<tr>
<td>Deca-BDE</td>
<td>0.3-3%</td>
</tr>
</tbody>
</table>

PCBs and PBDEs are chemically stable, lipophilic and have been shown to be ubiquitous environmental pollutants. They have a tendency to accumulate in lipid-rich tissues and magnify up the food chain, increasing in concentration at each successively higher trophic level. Octa-, nona- and deca-BDEs are detected in biota and humans but show no biomagnification potential (Burreau et al. 2004). The major human exposure route appears to be through intake of various kinds of fish, meat and dairy products (Sjödin et al. 2003), although consensus on this point has not yet been reached. Occupational exposure may on certain occasions contribute significantly to the body burden of PBDEs (Sjödin et al. 1999, Thomsen et al. 2001b).

In spite of the fact that PBDE concentrations in humans are significantly lower than those of PCBs, they are a growing reason for concern because PBDE levels have increased
exponentially since the late 1970s (Alaee and Wenning 2002). A study by Meironyté (2002) showed decreasing levels of BDE 47 in breast milk from 1998 to 2000 but no large differences were noticed in levels of higher brominated PBDEs. In contrast PCB levels have declined in the past decades (Schecter et al. 2004).

Toxic effects of PBDEs and PCBs include immunotoxicity, reproductive effects, teratogenicity, endocrine disruption and carcinogenicity (Meerts et al. 2000, Legler and Brouwer 2003, Gill et al. 2004). However, because of the wide variation in physicochemical properties of individual congeners, generalized statements about their toxicology are difficult to make. Generally, the technical Penta-BDE products seem to cause effects at comparably the lowest dose, whereas much higher doses are needed for effects of the Deca-BDE (Darnerud, 2003).

The European Union has recently closed a 10-year scientific risk assessment of Deca-BDE and concluded that it poses an acceptably low risk to human health and no further risk reduction measures beyond those which are being applied are necessary. According to Council Directive 2003/11/EC (2003), the use of Penta- and Octa-BDE and the placing on the market of articles containing one or both of these substances is banned within the European Union from the 15th of August 2004 on. The Belgian legislation has set maximum limits for the total sum of seven International Council for the Exploration of the Sea (ICES) indicator PCB congeners (PCBs 28, 52, 101, 118, 138, 153, 180) in different food matrices.

The main objective of this work was to determine the levels and profiles of PCBs and PBDEs in adipose tissue of people living in Belgium.

5.2.3. Materials and methods

5.2.3.1. Samples

Fifty three human adipose tissue samples from the abdominal fat region were obtained by autopsy between 2001 and 2003 in two different Belgian laboratories from deceased individuals who died from natural or accidental causes. The mean age of the deceased subjects was 53 years, ranging from 19 to 84 years (Table V.2). There were 31 men and 22 women. For each subject, the province of residence in Belgium was known, but no information was available about the type of residence area (rural or industrial). No information about diet or occupational exposure was provided. Animal fat tissue samples,
taken within the frame of the Belgian national monitoring programme for PCBs and containing PCB levels lower than the decision limit \((C_C^\alpha)\), were used to prepare a pool of blank extracted animal fat for quality control purposes. Out of this pool a sample was analyzed and a new pool was prepared if PBDE levels were above the decision limit \((C_C^\alpha)\). Samples were stored at -20°C till analysis.

5.2.3.2. Materials and reagents

Individual PBDE standards, IUPAC Nos. 28 (2,4,4'-tribromodiphenyl ether), 47 (2,2',4,4'-tetrabromodiphenyl ether), 99 (2,2',4,4',5-pentabromodiphenyl ether), 100 (2,2',4,4',6-pentabromodiphenyl ether), 153 (2,2',4,4',5,5'-hexabromodiphenyl ether), 154 (2,2',4,4',5,6'-hexabromodiphenyl ether) and 183 (2,2',3,4,4',5,6-heptabromodiphenyl ether), were purchased from Wellington Laboratories (Ontario, Canada). Individual PCB standards, IUPAC Nos. 28 (2,4,4'-trichlorobiphenyl), 52 (2,2',5,5'-tetrachlorobiphenyl), 101 (2,2',4,5,5'-pentachlorobiphenyl), 118 (2,3',4,4', 5-pentachlorobiphenyl), 138 (2,2',3,4,4',5'-hexachlorobiphenyl) and 180 (2,2',3,4,4',5,5'-heptachlorobiphenyl) together with PCB-Mix 3 (10 ng µL\(^{-1}\) of each congener) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Internal standards, Mirex, PCB 143 (2,2',3,4,5,6'-hexachlorobiphenyl) polybrominated biphenyl (PBB) 155 (2,2',4,4',6,6'-hexabromobiphenyl) and injection standard PBB 103 (2,2',4,5',6-pentabromobiphenyl) were also purchased from Dr. Ehrenstorfer (Augsburg, Germany).

A standard stock solution containing all 7 PBDE congeners was prepared at a concentration of 1 ng µL\(^{-1}\) in nonane. Individual standard stock solutions of PBB 155 and PBB 103 in nonane and PCB 143 and Mirex in isooctane were prepared at 10 ng µL\(^{-1}\).

All reagents and solvents were of analytical reagent grade. Isooctane, n-hexane Suprasolv\(^\circledR\) and anhydrous sodium sulphate were obtained from Merck (Darmstadt, Germany). Nonane was purchased from Sigma Aldrich nv/sa (Bornem, Belgium). Acidified silica was prepared by adding 35.5 mL concentrated sulphuric acid p.a. (Merck) to 100 g silica gel (0.063 – 0.200 mm, Merck) and mixing thoroughly. Water was obtained by a Milli Q gradient system (Millipore, Brussels, Belgium). Preparation of deactivated alumina was done by adding 5 mL water to 45 g alumina B activity I (ICN Biomedicals, Eschwege, Germany). Silane treated glass wool was obtained from Alltech Associates (Deerfield, IL, USA).
5.2.3.3. Clean-up of adipose tissue

The analysis method was evaluated and fully validated and is described in detail elsewhere (Naert et al. 2004). Ten grams of adipose tissue was brought onto a folded Ederol filter paper (VWR, Leuven, Belgium) in a glass funnel together with anhydrous sodium sulphate (10 g). The fat was melted in a microwave oven (600 W for 2 minutes) and received in a glass recipient. Internal standards PBB 155 (40 µL 0.1 ng µL⁻¹), Mirex (20 µL 1 ng µL⁻¹) and PCB 143 (20 µL 10 ng µL⁻¹) were added to the melted fat (2 g). A glass column filled with acidified silica, deactivated alumina and anhydrous sodium sulphate was used for clean-up. PBDEs and PCBs were eluted with 40 mL n-hexane. The eluate was evaporated in a rotary evaporator (Büchi, Switzerland) at 40 °C to ca. 4 mL. This solution was transferred to a graduated glass vial (Egilabo, Kontich, Belgium). Injection standard PBB 103 (40 µL 0.1 ng µL⁻¹) and keeper solvent isooctane were added. This mixture was concentrated under nitrogen at 40 °C to 100 µL and divided over 2 GC-MS vials.

5.2.3.4. Gas-chromatography-mass spectrometry

Analysis of the adipose tissue samples was performed on a Finnigan GCQ gas chromatograph coupled to a Finnigan GCQ mass spectrometer (Austin, Texas, USA) operating in electron impact – tandem mass spectrometry (MS/MS) mode. A 25 m x 0.22 mm x 0.25 µm HT8 capillary column (SGE, Achrom, Zulte, Belgium) was used for the separation of the PCB and PBDE congeners.

A 2 µL aliquot of the final sample extract was injected in the splitless mode (CTC 200 series injector, Zwingen, Switzerland). The oven was programmed from 70°C for 1 min to 170 °C at a rate of 30 °C min⁻¹, then to 300 °C (15 min) at a rate of 8 °C min⁻¹.

5.2.3.5. Quality control

A six-point calibration curve in matrix was made with every sample sequence, by fortifying blank animal fat samples with a mixture of 7 PBDE congeners at a level of 0.2, 0.5, 1, 2, 5, 10 ng g⁻¹ per congener and 7 PCB congeners at 5, 10, 20, 100, 300, 500 ng g⁻¹ per congener. Since PBDEs were expected to occur in a lower concentration range than PCBs, PBDE calibration curves were determined in a lower linear range.
CHAPTER 5. OCCURRENCE OF POLYCHLORINATED BIPHENYLS AND POLYBROMINATED DIPHENYL ETHERS IN BELGIAN HUMAN ADIPOSE TISSUE SAMPLES

Given that levels of PCB 138, PCB 153 and PCB 180 were significantly higher than levels of PCB 28, PCB 52, PCB 101 and PCB 118, concentrations of PCB 138, PCB 153 and PCB 180 were calculated with internal standard PCB 143. Concentrations of PCB 28, PCB 52, PCB 101 and PCB 118 were calculated with internal standard Mirex.

In order to be acceptable (Beltest I014-Rev4-17/7/2000-17 2000), the correlation coefficient ($R^2$) of the calibration curves had to be at least 0.995 for each congener. In every sequence a blank animal fat sample was subjected to the entire analytical procedure to determine possible contamination.

Retention times, ion chromatograms and intensity ratios were used as identification criteria. According to Commission Decision 2002/657/EC (2002) relative retention times (RRTs) of the analyte may not differ more than $+/- 0.5\%$ of the RRT of the calibration standard. The relative intensities of the detected ions, expressed as a percentage of the intensity of the most abundant ion, have to correspond to those of the samples fortified at comparable concentrations within the tolerances mentioned by Commission Decision 2002/657/EC (2002). The signal-to-noise ratio for each diagnostic ion has to be more than 3:1.

5.2.4. Results

PCB and PBDE chromatograms of a human adipose tissue sample extract are shown in Figures V.1-V.2. A summary of the PCB and PBDE levels found in Belgian human adipose tissue is listed in Tables V.2 and V.3.

Figure V.1 PCB chromatogram of a human adipose tissue sample extract (sample 20)
The total PBDE concentration (sum of BDE 28, BDE 47, BDE 99, BDE 100, BDE 153, BDE 154 and BDE 183) in Belgian human adipose tissue ranged between 1.23 ng g\textsuperscript{-1} and 57.2 ng g\textsuperscript{-1} lipid weight with a median value of 5.32 ng g\textsuperscript{-1}. BDE 47, BDE 153 and BDE 183 were the predominant PBDE congeners accounting for 83 % (ranging from 63 % to 100 %) of the total PBDE content. BDE 153 was present in all of the adipose tissue samples. Levels of BDE 47 and BDE 183 were higher than BDE 153 in nine and five samples respectively.

Table V.2 Concentrations (ng g\textsuperscript{-1} lipid weight) of PBDEs and PCBs in Belgian adipose tissue samples (n = 53)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>PCB Sum</th>
<th>PBDE Sum</th>
<th>Sample</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>PCB Sum</th>
<th>PBDE Sum</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>19</td>
<td>M</td>
<td>171</td>
<td>7.70</td>
<td>28</td>
<td>50</td>
<td>M</td>
<td>1118</td>
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<tr>
<td>2</td>
<td>21</td>
<td>M</td>
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<td>2.83</td>
<td>29</td>
<td>55</td>
<td>F</td>
<td>1049</td>
<td>3.52</td>
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<tr>
<td>3</td>
<td>22</td>
<td>F</td>
<td>144</td>
<td>5.93</td>
<td>30</td>
<td>60</td>
<td>M</td>
<td>151</td>
<td>5.30</td>
</tr>
<tr>
<td>4</td>
<td>23</td>
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<td>31</td>
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<td>F</td>
<td>1063</td>
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<td>3.74</td>
<td>32</td>
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<td>1077</td>
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<td>6</td>
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<td>M</td>
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<td>6.07</td>
<td>33</td>
<td>65</td>
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</tr>
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<td>M</td>
<td>1570</td>
<td>6.50</td>
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</table>
Table V.2 Concentrations (ng g\(^{-1}\) lipid weight) of PBDEs and PCBs in Belgian adipose tissue samples  
(n = 53) (Continued)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>PCB Sum</th>
<th>PBDE Sum</th>
<th>Sample</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>PCB Sum</th>
<th>PBDE Sum</th>
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<tbody>
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<td>M</td>
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</tr>
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<td>M</td>
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<td>3.59</td>
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<td>F</td>
<td>1100</td>
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<td>F</td>
<td>251</td>
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<td>49</td>
<td>77</td>
<td>M</td>
<td>542</td>
<td>23.3</td>
</tr>
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<td>57.2</td>
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<td>M</td>
<td>868</td>
<td>3.26</td>
</tr>
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<td>49</td>
<td>M</td>
<td>1092</td>
<td>29.0</td>
<td>53</td>
<td>84</td>
<td>F</td>
<td>869</td>
<td>6.05</td>
</tr>
</tbody>
</table>

Table V.3 Distribution of the different PBDE and PCB congeners in Belgian adipose tissue samples  
(n = 53)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mean (ng g^{-1} lipid weight)</th>
<th>Median</th>
<th>Range</th>
<th>Mean (ng g^{-1} lipid weight)</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCB 28</td>
<td>7.2</td>
<td>4.0</td>
<td>&lt; CC(\alpha) - 52</td>
<td>BDE 28</td>
<td>0.40</td>
<td>0.20 &lt; CC(\alpha) - 2.03</td>
</tr>
<tr>
<td>PCB 52</td>
<td>2.4</td>
<td>2.3</td>
<td>&lt; CC(\alpha) - 3.6</td>
<td>BDE 47</td>
<td>2.12</td>
<td>0.88 &lt; CC(\alpha) - 14.3</td>
</tr>
<tr>
<td>PCB 101</td>
<td>4.1</td>
<td>2.6</td>
<td>&lt; CC(\alpha) - 11</td>
<td>BDE 99</td>
<td>1.56</td>
<td>0.47 &lt; CC(\alpha) - 7.98</td>
</tr>
<tr>
<td>PCB 118</td>
<td>27</td>
<td>23</td>
<td>1.9 - 84</td>
<td>BDE 100</td>
<td>0.80</td>
<td>0.72 &lt; CC(\alpha) - 1.91</td>
</tr>
<tr>
<td>PCB 138</td>
<td>181</td>
<td>149</td>
<td>19 - 543</td>
<td>BDE 153</td>
<td>3.68</td>
<td>2.40 0.70 - 25.1</td>
</tr>
<tr>
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<td>310</td>
<td>274</td>
<td>55 - 848</td>
<td>BDE 154</td>
<td>0.93</td>
<td>0.93 &lt; CC(\alpha) - 1.28</td>
</tr>
<tr>
<td>PCB 180</td>
<td>232</td>
<td>205</td>
<td>11 - 931</td>
<td>BDE 183</td>
<td>1.62</td>
<td>0.78 &lt; CC(\alpha) - 15.4</td>
</tr>
</tbody>
</table>
The sum of seven ICES PCB congeners (PCB 28, PCB 52, PCB 101, PCB 118, PCB 138, PCB 153 and PCB 180) ranged from 126 ng g\(^{-1}\) to 2090 ng g\(^{-1}\) lipid weight with a median of 605 ng g\(^{-1}\). The sum of PCB 138, PCB 153 and PCB 180 constituted 76 % to 100 % of the total sum of PCBs. PCB 153 was the dominant PCB congener in 43 samples while PCB 180 was the most abundant in the remaining 10 samples.

No age-dependency was found for PBDEs (Pearson correlation = -0.023, Sig. = 0.873), while PCBs showed higher correlation coefficients with age (Pearson correlation = 0.613, Sig. < 0.0005) (Figure V.3). PBDEs showed no correlation with the sum of PCBs (Pearson correlation = -0.010, Sig. = 0.943). High Pearson correlation coefficients were found between PCB 138 (Pearson correlation = 0.909, Sig. < 0.0005), PCB 153 (Pearson correlation = 0.960, Sig. < 0.0005), PCB 180 (Pearson correlation = 0.913, Sig. < 0.0005) and the sum of PCBs, while correlations between BDE 47 (Pearson correlation = 0.685, Sig. < 0.0005), BDE 153 (Pearson correlation = 0.815, Sig. < 0.0005), BDE 183 (Pearson correlation = 0.809; Sig. < 0.0005) and the sum of PBDEs were lower.

There was no sex related difference for PBDEs (t = -0.526, Sig. = 0.601) and PCBs (t = 1.297, Sig. = 0.201). Analysis of variance showed no significant province related difference in total PBDE (F = 2.937, Sig. = 0.102) and PCB (F = 2.427, Sig. = 0.144) concentration.
5.2.5. Discussion

5.2.5.1. Method

The extraction, clean-up and GC-MS/MS procedures were fully evaluated and validated elsewhere (Naert et al. 2004). Validation of the method was done according to Commission Decision 2002/657/EC (2002). Specificity, decision limit (CCα), detection capability (CCβ), recovery and precision were determined. Decision limit (CCα) is defined as the limit at and above which it can be concluded with an error probability of α that a sample is non-compliant (α = 1%). Detection capability (CCβ) means the smallest content of the substance that may be detected, identified and/or quantified in a sample with an error probability of β (β = 5 %). Decision limits for PBDEs and PCBs ranged from 0.06 ng g⁻¹ to 0.15 ng g⁻¹ lipid weight and from 0.35 ng g⁻¹ to 1.22 ng g⁻¹ lipid weight respectively. Detection capabilities were all between 0.23 ng g⁻¹ and 0.55 ng g⁻¹ lipid weight for PBDEs and between 0.98 ng g⁻¹ and 2.29 ng g⁻¹ lipid weight for PCBs (see Table V.4).

Measurement uncertainty was determined according to the EURACHEM/CITAC guide (Ellison et al. 2000). Expanded measurement uncertainty varied between 23.5 and 42.8 % for PCBs and between 16.5 and 28.2 % for PBDEs (see Table V.4).

<table>
<thead>
<tr>
<th>Compound</th>
<th>CCα</th>
<th>CCβ</th>
<th>Expanded measurement uncertaintya (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCB 28</td>
<td>0.56</td>
<td>1.75</td>
<td>32.4</td>
</tr>
<tr>
<td>PCB 52</td>
<td>0.55</td>
<td>1.70</td>
<td>42.8</td>
</tr>
<tr>
<td>PCB 101</td>
<td>0.38</td>
<td>1.25</td>
<td>23.5</td>
</tr>
<tr>
<td>PCB 118</td>
<td>0.35</td>
<td>0.98</td>
<td>27.4</td>
</tr>
<tr>
<td>PCB 138</td>
<td>0.35</td>
<td>1.28</td>
<td>27.7</td>
</tr>
<tr>
<td>PCB 153</td>
<td>0.95</td>
<td>2.21</td>
<td>32.9</td>
</tr>
<tr>
<td>PCB 180</td>
<td>1.22</td>
<td>2.29</td>
<td>28.4</td>
</tr>
<tr>
<td>BDE 28</td>
<td>0.06</td>
<td>0.23</td>
<td>23.1</td>
</tr>
<tr>
<td>BDE 47</td>
<td>0.06</td>
<td>0.31</td>
<td>28.2</td>
</tr>
<tr>
<td>BDE 99</td>
<td>0.09</td>
<td>0.30</td>
<td>17.6</td>
</tr>
<tr>
<td>BDE 100</td>
<td>0.09</td>
<td>0.40</td>
<td>16.5</td>
</tr>
<tr>
<td>BDE 153</td>
<td>0.10</td>
<td>0.55</td>
<td>19.8</td>
</tr>
<tr>
<td>BDE 154</td>
<td>0.08</td>
<td>0.46</td>
<td>18.8</td>
</tr>
<tr>
<td>BDE 183</td>
<td>0.15</td>
<td>0.53</td>
<td>24.7</td>
</tr>
</tbody>
</table>

a Data were obtained at 2 ng g⁻¹ level for PBDEs and 10 ng g⁻¹ level for PCBs.
5.2.5.2. Levels of PBDEs in human adipose tissue samples

Concentrations of PBDEs in 53 adipose tissue samples from the Belgian population were comparable to previously reported data in Europe (Guvenius et al. 2001, Covaci et al. 2002, Meneses et al. 1999) but were considerably lower than PBDE concentrations in human adipose tissue samples from California, US (She et al. 2002, Petreas et al. 2003). According to Petreas et al. (2003) this might be due to California regulations mandating that all polyurethane foam and textiles used in furnishings pass a flammability test. Another explanation might be that the relative use of the commercial Penta-BDE compared to Deca-BDE is the highest within the American continent (Bromine Science and Environmental Forum 2003).

BDE 47 was the predominant congener in human adipose tissue samples from Sweden (Guvenius et al. 2001) and the USA (She et al. 2002, Petreas et al. 2003). In contrast BDE 153 was the major PBDE congener in the examined Belgian adipose tissue samples. High BDE 153 levels were also present in adipose samples from Spain (Meneses et al. 1999), Belgium (Covaci et al. 2002), Japan (Choi et al. 2003) and human milk samples from the Faroe Islands (Fängström et al. 2004).

According to Covaci et al. (2002) this might be due to the difference in diets containing food items in which the higher brominated (hexa- to deca-BDE) congeners are preferentially bioaccumulated. According to Bocio et al. (2003) tetra-BDEs and penta-BDEs constitute 69 % of the total dietary intake of PBDEs. In contrast hexa-BDEs represent only 16 % of the total dietary intake. As a consequence other exposure routes e.g. dermal uptake and inhalation could also be at the origin of the more elevated BDE 153 levels. The occurrence of these higher BDE 153 levels compared to BDE 47 may also reflect elevated exposure to the octa-BDE, which contains more of the higher brominated PBDE congeners (Gill et al. 2004).

In accordance with other previously reported data (Sjödin et al. 1999, Sjödin et al. 2003, Covaci et al. 2002, Kazda et al. 2004, Schecter et al. 2003) no significant relationship with age could be observed. This lack of correlation might indicate that PBDE levels are increasing in the West-European environment.
5.2.5.3. Levels of PCBs in human adipose tissue samples

PCB profiles and levels were in harmony with profiles found in Sweden (Guvenius et al. 2001), Italy (Mariottini et al. 2000), Wales (Duarte-Davidson et al. 1994), Spain (Costabeber and Emmanuelli 2003) and Belgium (Covaci et al. 2002). On the other hand, concentrations (mean = 756 ng g\(^{-1}\) lipid weight) were higher than levels found by Pauwels et al. (2000) (mean = 334 ng g\(^{-1}\) lipid weight) in Belgian female adipose tissue samples. In this study, the mean age of the subjects (32 years) was considerably lower than in our study (53 years). This supports findings in studies by Costabeber and Emmanuelli (2003), Covaci et al. (2002), Duarte-Davidson et al. (1994) and in our present study that the PCB body burden increases with age.

It is known that the PCB pattern shifts from lower to higher chlorinated congeners when the organisms move to higher trophic levels. The higher concentrations of PCB 153 and PCB 180 (persistent congeners) and the absence of lower chlorinated congeners indicate that the principal source of contamination with PCBs was from the diet and not from direct exposure (Chu et al. 2003).

However, the PCB profile found in the Belgian human adipose tissue samples was different from that found in Turkey (Çok and Satiroğlu 2004). The concentrations of PCB 28, 52, 101 in the Turkish study were found to be higher than those found in studies from industrialized countries. According to Çok and Satiroğlu (2004) this could be attributed to the fact that exposure to PCBs still continues in Turkey.

Although the province of residence was known, it was not known for how long and in what kind of area (rural or industrialized) the subjects have resided. Therefore no assumption could be made in relation to the correlation between place of residence and PCB or PBDE levels. The lack of correlation between PCBs and PBDEs indicate other exposure pathways of PBDEs compared to PCBs and suggests that there could be other important sources of PBDE exposure than food.

5.2.6. Acknowledgements

The authors wish to thank E. Van Royen for her technical support and enthusiasm.
5.2.7. References


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Burreau S, Zebühr Y, Broman D, Ishaq R (2004) Biomagnification of polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) studied in pike (Esox lucius), perch (Perca fluviatilis) and roach (Rutilus rutilus) from the Baltic Sea. Chemosphere 55: 1043-1052


CHAPTER 6. DISTRIBUTION OF POLYCHLORINATED BIPHENYLS AND POLYBROMINATED DIPHENYL ETHERS IN BIRDS OF PREY FROM SWITZERLAND
6.1. **BACKGROUND AND OBJECTIVES**

As already described in Chapters 1 and 2, one of the most important toxicological endpoints of PBDEs and PCBs is their toxicity towards the central nervous system. In this chapter, it was our objective to study the levels of PBDEs and PCBs in the brain tissue of birds of prey (buzzard and sparrow hawk) from Switzerland. This to establish how well the central nervous system is protected from these type of persistent organic pollutants. An additional terrestrial (blackbird) and aquatic (cormorant) bird species was also included in this study. Subsequently, PBDE and PCB levels in brain tissue were compared to levels found in the corresponding adipose tissue. In conclusion, we investigated the influence of nutrition status and bird species on the levels of PBDEs and PCBs in the brain and the adipose tissue.

In order to extract the PBDEs and PCBs from the brain tissue, an additional extraction step with n-hexane was added to the procedure described in Chapter 4. Subsequently this procedure was fully validated. Because the supplied sample amounts of adipose tissue were small, PCBs and PBDEs were also extracted with n-hexane instead of melting the fat in a microwave oven. Concentrations of both matrices were expressed on a wet weight basis. For this reason, decision limit ($CC_\alpha$) and detection capability ($CC_\beta$) for adipose tissue needed to be verified once again. A further optimization of MS/MS parameters was carried out by means of experimental design.

6.2. DISTRIBUTION OF POLYCHLORINATED BIPHENYLS AND POLYBROMINATED DIPHENYL ETHERS IN BIRDS OF PREY FROM SWITZERLAND

6.2.1. Abstract

Polychlorinated biphenyls (PCBs) and the structurally related polybrominated diphenyl ethers (PBDEs) have been associated with chronic neurotoxicity involving reduced motor activity and impaired attentiveness. Such neurobehavioural effects indicate that the central nervous system may represent an important target organ for the action of these persistent contaminants in wildlife. As a consequence, the brain of different terrestrial and aquatic birds collected in Switzerland was analyzed for PCBs and PBDEs. In parallel, the same contaminants were examined in the accompanying adipose tissue. After clean-up by means of glass columns containing acidified silica, deactivated alumina and anhydrous sodium sulphate, the samples were analyzed by high resolution gas chromatography/tandem mass spectrometry (HRGC-MS/MS).

Median PCB concentrations in the brain (sum of PCB 28, PCB 52, PCB 101, PCB 118, PCB 138, PCB 153 and PCB 180) ranged between 13 ng g\(^{-1}\) wet weight (ww) in blackbirds (\textit{Turdus merula}) and 428 ng g\(^{-1}\) ww in sparrow hawks (\textit{Accipiter nisus}). Median PBDE concentrations in the brain (sum of BDE 28, BDE 47, BDE 99, BDE 100, BDE 153, BDE 154 and BDE 183) ranged from below the decision limit in buzzards (\textit{Buteo buteo}) and blackbirds, to 14 ng g\(^{-1}\) ww in sparrow hawks.

After correction for the respective lipid content, higher PCB or PBDE concentrations in brain compared to adipose tissue, were found in three sparrow hawks, four buzzards and in all investigated blackbirds. These results suggest that a deficit in the neuroprotective function of the blood-brain barrier may cause unexpected levels of PCBs and PBDEs in the central nervous system.

6.2.2. Introduction

PCBs (polychlorinated biphenyls) and PBDEs (polybrominated diphenyl ethers) are distinct members of the group of persistent organic pollutants (POPs). In the past, PCBs have
been used as electric insulators in transformers and capacitors, as heat transfer fluids as well as additives in pesticides, adhesives, plastics and paints (Safe, 1994). PCBs are likely to enter the environment directly from point sources due to inappropriate disposal practices, leakage from industrial facilities or chemical waste disposal sites and, to a lesser extent, from global recycling (Meijer et al., 2003). PBDEs are widely used as flame retardants in textiles, plastics, cars and building materials. The commercial products consist of three distinct technical mixtures, i.e. Penta-BDE, Octa-BDE and Deca-BDE, reflecting the varying degree of bromination of the diphenyl ethers. As these flame retardants are not permanently bound to the polymer matrix, they leach out during natural operational life as well as during processing, recycling or combustion of the polymeric material (D’Silva et al., 2004). Due to their lipophilicity and resistance to chemical and biological degradation, PCBs and PBDEs are nearly ubiquitously distributed in the environment, food, biota and humans. In addition, PCBs and also some PBDEs are characterized by their tendency to accumulate in lipid-rich tissues and magnify along the food chain, increasing in concentration at each successively higher trophic level (Muir et al., 1992).

Predatory birds respond to relatively low levels of POPs, but their sensitivity varies considerably with respect to compound and species (Herzke et al., 2005). Organochlorine contaminants may impair reproduction, affect the ability to compete for food and habitat, and contribute to reduced parental attentiveness (Ulfstrand et al., 1971; Fry et al., 1995; Savinova et al., 1995; Vorkamp et al., 2004). For PBDEs, several studies indicate that the most critical effects result from their disturbance of the thyroid hormone homeostasis and from their chronic neurotoxicity, generating behavioural alterations such as deficits in spontaneous motor activity (Porterfield, 2000; Branchi et al., 2002; Mc Donald, 2002; Darnerud, 2003; Gill et al., 2004; Kodovanti and Ward, 2005).

Since birds of prey are highly positioned on the food chain, they are very suitable to study the bioaccumulation in specific target organs (Fox and Lock, 1978). PCB levels in unhatched eggs of Goshawks from Northern Germany have decreased since the 1970s and are relatively constant during the last 8-10 years (Scharenberg and Looft, 2004). This trend is also visible in gull eggs from the Great Lakes (Stow, 1995). Braune et al. (2001) observed a significant decline of PCB levels in seabird eggs from the Canadian Arctic from 1975 until 1998. In contrast, PBDE levels (sum of BDE 47, BDE 99 and BDE 100) in herring gull eggs from the Great Lakes region have risen exponentially (Norstrom et al., 2002). Similarly, PBDE levels in the eggs of several marine and freshwater birds from British Columbia are increasing with doubling times of 5-6 years (Elliott et al. 2005). This general increase of
PBDE levels is also detectable in fish, marine mammals and humans (Hites, 2004). However, few data exist on the level of PCBs and PBDEs in the central nervous system, which constitutes a sensitive target for the toxicological action of both types of contaminants. As a consequence, we determined the PCB and PBDE concentrations in the brain of birds of prey (*Accipiter nisus* and *Buteo buteo*) that were found dead or dying from accidental causes, and compared the values with those from adipose tissue of the same animals. Also the PCB and PBDE levels in these predatory birds were compared with those from an additional terrestrial and an aquatic species. It was our objective to investigate if there is a relationship between brain and adipose tissue concentrations and how efficiently the central nervous system is protected from the deposition of persistent organic pollutants.

### 6.2.3. Experimental

#### 6.2.3.1. Sample collection

Sixty eight brain and fifty adipose tissue samples of buzzards (*Buteo buteo*), sparrow hawks (*Accipiter nisus*), cormorants (*Phalacrocorax carbo sinensis*) and blackbirds (*Turdus merula*) from different places in Switzerland were obtained from 2003 until 2005 by the Swiss Ornithological Institute. Sample details are given in Table VI.1. All birds included in this study were found dead or dying from accidental causes. No bird was killed for the purpose of this study. Due to food deprivation, adipose tissue could not be collected from every specimen. Samples were stored at -20°C until analysis.

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Species</th>
<th>Year of birth</th>
<th>Sex</th>
<th>Found in</th>
<th>Date</th>
<th>Cause of death</th>
<th>Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>buzzard</td>
<td>-</td>
<td>-</td>
<td>Sursee</td>
<td>23.05.2004</td>
<td>thudded</td>
<td>725</td>
</tr>
<tr>
<td>B3</td>
<td>buzzard</td>
<td>-</td>
<td>-</td>
<td>Ennetbürgen</td>
<td>24.01.2004</td>
<td>starvation</td>
<td>500</td>
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<td>B4</td>
<td>buzzard</td>
<td>-</td>
<td>-</td>
<td>Ebikon</td>
<td>16.01.2004</td>
<td>starvation</td>
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</tr>
<tr>
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<td>buzzard</td>
<td>-</td>
<td>-</td>
<td>Zell</td>
<td>11.05.2004</td>
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<td>860</td>
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<td>B6</td>
<td>buzzard</td>
<td>-</td>
<td>-</td>
<td>Schötz</td>
<td>26.04.2004</td>
<td>nerval wing trauma, infection</td>
<td>855</td>
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<td>B7</td>
<td>buzzard</td>
<td>-</td>
<td>-</td>
<td>Zürich</td>
<td>07.07.2004</td>
<td>nerval wing trauma</td>
<td>620</td>
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### Table VI.1 Details of different birds of prey samples investigated in this study (Continued)

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<th>Year of birth</th>
<th>Sex</th>
<th>Found in</th>
<th>Date</th>
<th>Cause of death</th>
<th>Weight (g)</th>
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<td>buzzard</td>
<td>2004</td>
<td>-</td>
<td>Rain</td>
<td>18.06.2004</td>
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<td>845</td>
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<tr>
<td>B10</td>
<td>buzzard</td>
<td>2004</td>
<td>-</td>
<td>Willisau</td>
<td>17.08.2004</td>
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<tr>
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<td>-</td>
<td>Emmen</td>
<td>15.07.2004</td>
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<td>-</td>
<td>Wallisellen</td>
<td>02.03.2004</td>
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<td>790</td>
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<tr>
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<td>buzzard</td>
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<td>-</td>
<td>Zug</td>
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<td>wing fracture</td>
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<td>-</td>
<td>Menziken</td>
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</tr>
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<td>-</td>
<td>Zug</td>
<td>04.03.2004</td>
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<td>-</td>
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<td>Luzern</td>
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<td>-</td>
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<td>buzzard</td>
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<td>-</td>
<td>Schüpfheim</td>
<td>03.02.2004</td>
<td>hit by car, poisoning</td>
<td>-</td>
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<td>buzzard</td>
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<td>-</td>
<td>Hildisrieden</td>
<td>05.01.2004</td>
<td>hit by car</td>
<td>-</td>
</tr>
<tr>
<td>B19</td>
<td>buzzard</td>
<td>2004</td>
<td>-</td>
<td>Luzern</td>
<td>06.01.2004</td>
<td>fracture</td>
<td>-</td>
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<td>Wauwilermoos</td>
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<td>Grosswangen</td>
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<td>-</td>
<td>Hellbrühl</td>
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<td>Neuenkirch</td>
<td>09.03.2005</td>
<td>head tilt (hit by car)</td>
<td>730</td>
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<td>-</td>
<td>Dietwil</td>
<td>07.05.2005</td>
<td>starvation</td>
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<td>buzzard</td>
<td>2004</td>
<td>-</td>
<td>Neuenkirch</td>
<td>01.03.2005</td>
<td>leg trauma</td>
<td>675</td>
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<td>buzzard</td>
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<td>-</td>
<td>Abtwil</td>
<td>04.03.2005</td>
<td>ratite (trauma)</td>
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<td>buzzard</td>
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<td>Littau</td>
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<td>-</td>
<td>Sarnen</td>
<td>24.05.2005</td>
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<td>B34</td>
<td>buzzard</td>
<td>2005</td>
<td>-</td>
<td>Kaltbach</td>
<td>26.03.2005</td>
<td>back and wing trauma</td>
<td>460</td>
</tr>
<tr>
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<td>buzzard</td>
<td>2005</td>
<td>-</td>
<td>Luzern</td>
<td>30.05.2005</td>
<td>open humerus fracture</td>
<td>550</td>
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<tr>
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<td>buzzard</td>
<td>2005</td>
<td>-</td>
<td>Altwis</td>
<td>18.03.2005</td>
<td>paralysis</td>
<td>685</td>
</tr>
<tr>
<td>B37</td>
<td>buzzard</td>
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<td>-</td>
<td>Wald ZH</td>
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<td>unknown</td>
<td>650</td>
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<tr>
<td>S1</td>
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<td>female</td>
<td>Neuenkirch</td>
<td>18.12.2003</td>
<td>wing fracture</td>
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<td>S2</td>
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<td>Neuenkirch</td>
<td>25.03.2004</td>
<td>starvation</td>
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<td>S3</td>
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<td>female</td>
<td>Weggis</td>
<td>29.10.2003</td>
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Table VI.1 Details of different birds of prey samples investigated in this study (Continued)

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Species</th>
<th>Year of birth</th>
<th>Sex</th>
<th>Found in</th>
<th>Date</th>
<th>Cause of death</th>
<th>Weight (g)</th>
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<tr>
<td>S4</td>
<td>sparrow hawk</td>
<td>2004</td>
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<td>Büron</td>
<td>07.01.2004</td>
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<td>S5</td>
<td>sparrow hawk</td>
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<td>-</td>
<td>Sacheln</td>
<td>19.01.2005</td>
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<tr>
<td>S6</td>
<td>sparrow hawk</td>
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<td>sparrow hawk</td>
<td>-</td>
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<td>Kleinwangen</td>
<td>22.04.2004</td>
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<td>S8</td>
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<td>Basel</td>
<td>21.11.2004</td>
<td>thudded</td>
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<td>male</td>
<td>Oberkirch</td>
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<td>125</td>
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<tr>
<td>S10</td>
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<td>male</td>
<td>Eschenbach</td>
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<td>S11</td>
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<td>-</td>
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<td>drowned</td>
<td>2000</td>
</tr>
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<td>Lake of Sempach</td>
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<td>-</td>
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<td>-</td>
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<td>3400</td>
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<td>C5</td>
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<tr>
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<td>cormorant</td>
<td>-</td>
<td>-</td>
<td>Lake of Sempach</td>
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<td>drowned</td>
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<td>cormorant</td>
<td>-</td>
<td>-</td>
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<td>08.02.2005</td>
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<td>2595</td>
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<td>Schlossried</td>
<td>10.11.2003</td>
<td>thudded</td>
<td>-</td>
</tr>
<tr>
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<td>-</td>
<td>-</td>
<td>Luzern</td>
<td>10.01.2004</td>
<td>victim of cat</td>
<td>-</td>
</tr>
<tr>
<td>BB3</td>
<td>blackbird</td>
<td>-</td>
<td>-</td>
<td>Horw</td>
<td>15.12.2003</td>
<td>hit by car</td>
<td>-</td>
</tr>
<tr>
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<td>blackbird</td>
<td>-</td>
<td>-</td>
<td>Sursee</td>
<td>30.01.2004</td>
<td>victim of cat</td>
<td>-</td>
</tr>
<tr>
<td>BB5</td>
<td>blackbird</td>
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<td>-</td>
<td>Luzern</td>
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<td>Zürich</td>
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<tr>
<td>BB8</td>
<td>blackbird</td>
<td>jung</td>
<td>-</td>
<td>Luzern</td>
<td>-</td>
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<td>60</td>
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<tr>
<td>BB9</td>
<td>blackbird</td>
<td>jung</td>
<td>-</td>
<td>Hochdorf</td>
<td>13.08.2005</td>
<td>victim of cat</td>
<td>30</td>
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<tr>
<td>BB10</td>
<td>blackbird</td>
<td>jung</td>
<td>-</td>
<td>Meggen</td>
<td>13.08.2005</td>
<td>euthanasia</td>
<td>22.5</td>
</tr>
<tr>
<td>BB11</td>
<td>blackbird</td>
<td>jung</td>
<td>-</td>
<td>Luzern</td>
<td>-</td>
<td>unknown</td>
<td>45</td>
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<td>BB12</td>
<td>blackbird</td>
<td>jung</td>
<td>-</td>
<td>Luzern</td>
<td>-</td>
<td>unknown</td>
<td>45</td>
</tr>
</tbody>
</table>

Age, sex and weight are not provided for all animals.

A pool of extracted fat, from blank samples taken within the framework of the Belgian monitoring programme for PCBs, was used as blank for validation and quality control purposes. A sample of this pool was analyzed for PBDEs and a new pool was prepared if PBDE levels were above the decision limit (CCα: the limit at and above which can be concluded with an error of probability of α that a sample is non-compliant). Similarly, a pool
of porcine brain tissue that contained PCB and PBDE levels lower than the respective decision limits (CCα) was prepared and used as blank. Decision limits (CCα) and detection capabilities (CCβ: the smallest content of the substance that may be detected, identified and/or quantified in a sample with an error probability of β) for PCBs and PBDEs are presented in Table VI.2.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Brain tissue</th>
<th>Adipose tissue</th>
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<tr>
<td></td>
<td>CCα</td>
<td>CCβ</td>
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<tr>
<td>PCB 28</td>
<td>0.81</td>
<td>1.10</td>
</tr>
<tr>
<td>PCB 52</td>
<td>0.84</td>
<td>1.01</td>
</tr>
<tr>
<td>PCB 101</td>
<td>0.46</td>
<td>0.54</td>
</tr>
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<td>PCB 118</td>
<td>0.43</td>
<td>1.62</td>
</tr>
<tr>
<td>PCB 138</td>
<td>0.47</td>
<td>0.84</td>
</tr>
<tr>
<td>PCB 153</td>
<td>0.46</td>
<td>0.57</td>
</tr>
<tr>
<td>PCB 180</td>
<td>0.93</td>
<td>1.24</td>
</tr>
<tr>
<td>BDE 28</td>
<td>0.33</td>
<td>0.50</td>
</tr>
<tr>
<td>BDE 47</td>
<td>0.33</td>
<td>0.49</td>
</tr>
<tr>
<td>BDE 99</td>
<td>0.47</td>
<td>0.66</td>
</tr>
<tr>
<td>BDE 100</td>
<td>0.42</td>
<td>0.76</td>
</tr>
<tr>
<td>BDE 153</td>
<td>0.67</td>
<td>1.14</td>
</tr>
<tr>
<td>BDE 154</td>
<td>0.65</td>
<td>0.92</td>
</tr>
<tr>
<td>BDE 183</td>
<td>0.76</td>
<td>0.93</td>
</tr>
</tbody>
</table>

6.2.3.2. Standard solutions

PBDE standards: IUPAC numbers 28 (2,4,4'-tribromodiphenyl ether), 47 (2,2',4,4'-tetrabromodiphenyl ether), 99 (2,2',4,4',5-pentabromodiphenyl ether), 100 (2,2',4,4',6-pentabromodiphenyl ether), 153 (2,2',4,4',5,5'-hexabromodiphenyl ether), 154 (2,2',4,4',5,6'-hexabromodiphenyl ether) and 183 (2,2',3,4,4',5,6-heptabromodiphenyl ether) were from Wellington Laboratories (Ontario, Canada). PCB standards: IUPAC numbers 28 (2,4,4'-trichlorobiphenyl), 52 (2,2',5,5'-tetrachlorobiphenyl), 101 (2,2',4,5,5'-pentachlorobiphenyl),
118 (2,3',4,4',5-pentachlorobiphenyl), 138 (2,2',3,4,4',5'-hexachlorobiphenyl), 153 (2,2',4,4',5,5'-hexachlorobiphenyl) and 180 (2,2',3,4,4',5,5'-heptachlorobiphenyl) together with PCB-Mix 3 (10 ng µL\(^{-1}\) of PCB 28, PCB 52, PCB 101, PCB 118, PCB 138, PCB 153 and PCB 180) were from Dr. Ehrenstorfer (Augsburg, Germany). Internal standards, Mirex, PCB 143 (2,2',3,4,5,6'-hexachlorobiphenyl) polybrominated biphenyl (PBB) 155 (2,2',4,4',6,6'-hexabromobiphenyl) and injection standard PBB 103 (2,2',4,5',6-pentabromobiphenyl) were also purchased from Dr. Ehrenstorfer (Augsburg, Germany). A stock solution containing all 7 PBDE congeners was prepared at a concentration of 1 ng µL\(^{-1}\) in nonane. Individual stock solutions of PBB 155 and PBB 103 in nonane as well as PCB 143 and Mirex in iso-octane were prepared at 10 ng µL\(^{-1}\).

6.2.3.3. Materials and reagents

All reagents and solvents were of analytical grade. Iso-octane, n-hexane Suprasolv\(^{\circledR}\) and anhydrous sodium sulphate were obtained from Merck (Darmstadt, Germany). Nonane was purchased from Sigma Aldrich (Bornem, Belgium). Acidified silica was prepared by adding 35.5 mL concentrated sulphuric acid p.a. (Merck) to 100 g silica gel (0.063 – 0.200 mm, Merck) and mixing thoroughly. Deactivated alumina was prepared by adding 5 mL water to 45 g alumina B activity I (ICN Biomedicals, Eschwege, Germany). Silane-treated glass wool was obtained from Alltech Associates (Deerfield, IL, USA).

6.2.3.4. Extraction and clean-up

To prevent contamination, we used only glassware that was washed extensively and rinsed twice with hexane (Acros, Geel, Belgium). Plastic materials were not used in order to avoid contamination. Four grams of homogenised brain tissue or two grams of homogenised adipose tissue were dried with anhydrous sodium sulphate (20 g). This mixture was transferred into a hexane-rinsed centrifugation tube. Internal standards PBB 155 (80 µL 0.1 ng µL\(^{-1}\)) and PCB 143 (40 µL 1 ng µL\(^{-1}\)) were added in the case of brain tissue and the internal standards PBB 155 (40 µL 0.1 ng µL\(^{-1}\)), PCB 143 (40 µL 5 ng µL\(^{-1}\)) and Mirex (40 µL 0.5 ng µL\(^{-1}\)) were added in the case of adipose tissue. PBDEs and PCBs were extracted in two steps, by thoroughly shaking the mixture in the centrifugation tube with 25 mL n-hexane. After centrifugation for 10 min at 3000 rpm, the hexane extract was evaporated in a rotary evaporator at 40 °C to about 5 mL and cleaned-up as described by Naert et al. (2004); using a
glass column that was subsequently filled with n-hexane (25 mL), acidified silica (12 g), deactivated alumina (3 g) and anhydrous sodium sulphate (3 g). PBDEs and PCBs were eluted from the column with 40 mL n-hexane. The eluate was evaporated in a rotary evaporator at 40 °C to ca. 4 mL. This solution was transferred to a graduated glass vial (Egilabo, Kontich, Belgium). Keeper solvent isoctane (100 µL), the injection standard PBB 103 (brain: 80 µL 0.1 ng µL⁻¹; adipose: 40 µL 0.1 ng µL⁻¹) and, in the case of brain tissue samples, also Mirex (40 µL 1 ng µL⁻¹) were added. This mixture was concentrated under nitrogen at 40 °C to 100 µL and divided over 2 GC-MS vials.

6.2.3.5. Gas chromatography-mass spectrometry

Analysis of the samples was done using a GCQ gas chromatograph coupled to a GCQ mass spectrometer (Finnigan, Austin, Texas, USA). The autosampler was a CTC 200 series injector (Zwingen, Switzerland). Separation of the PBDE and PCB congeners was performed using a HT8 capillary column (25 m x 0.22 mm x 0.25 µm, SGE, Achrom, Zulte, Belgium). Carrier and collision gas was Alphagaz 2 helium (Air Liquide, Liege, Belgium). Instrument set points and data acquisition were under control of the GCQ software.

A 2 µL aliquot of the final sample extract was injected with a splitless period after injection of 1 min. The injection temperature was set to 300 °C and transferline temperature was 275 °C. The oven was programmed from 70 °C for 1 min. to 170 °C at a rate of 30 °C min⁻¹, then to 300 °C (15 min.) at a rate of 8°C min⁻¹. The method for optimization of the excitation voltage and of the amount of energy needed to hold the precursor ion in the trap during excitation (q-value) has been described by De Saeger et al. (2005). Additional optimization of these parameters for PBDE analysis was carried out by a 3-level factorial design (Statgraphics Plus for Windows 3.0, Statistical Graphics Corporation, Englewood Cliffs, NY, USA). Peak area ratios of the most abundant ion in relation to PBB 103 were measured to generate response surface graphs for each component and to locate the optimal excitation voltage and q-value.

6.2.3.6. Validation

Validation of the method was carried out according to Commission Decision 2002/657/EC. A quantitative confirmatory method implies that specificity, decision limit (CCα), detection capability (CCβ), recovery and precision need to be determined. Since
validation of the method regarding adipose tissue has already been described by Naert et al. (2004) and only a different extraction step was applied, a limited validation procedure to determine decision limit (CC\(\alpha\)) and detection capability (CC\(\beta\)) for adipose tissue was carried out.

With exception of assay specificity, all other validation parameters regarding the analysis of brain tissue were determined on the most abundant ion. To determine decision limits and detection capabilities, 5 calibration curves with 6 data points ranging from 0 to 10 ng g\(^{-1}\) per PBDE congener and 0 to 40 ng g\(^{-1}\) per PCB congener were obtained. After identification, peak ratios (peak area/internal standard) were plotted against the added concentration. The concentration at the y-intercept plus 2.33 times the standard deviation of the within-laboratory reproducibility of the intercept is defined as the decision limit or CC\(\alpha\) (\(\alpha = 1\%\)). CC\(\beta\) or detection capability was determined by using the same calibration curves. CC\(\beta\) equals the concentration at the decision limit plus 1.64 times the standard deviation of the within-laboratory reproducibility of the mean measured at the decision limit (\(\beta = 5\%\)). To challenge the specificity of the method, at least 10 different blank samples were analyzed and checked for interfering compounds in the region of interest where the target analyte is expected to elute. Recovery was determined by fortifying blank samples before extraction and clean-up at 3 different levels (1, 2 and 4 ng g\(^{-1}\) per congener for PBDEs and 5, 20 and 40 ng g\(^{-1}\) per congener for PCBs). The results obtained with these samples were compared with those from blanks fortified after clean-up. Within-laboratory coefficients of variation (CV %) were calculated with blank samples fortified as outlined before. Measurement uncertainty was determined according to the EURACHEM/CITAC guide (Ellison et al., 2000). The global uncertainty was calculated as a combination of the uncertainty associated with precision and the uncertainty associated with bias. Although this is the combined uncertainty, the results are expressed as an expanded uncertainty, which corresponds to twice this value.

### 6.2.3.7. Quality Control

A six point calibration curve in matrix was made by fortifying blank animal fat and brain samples with a mixture of 7 PBDE congeners at a level of 0.5, 1, 2, 5, 10, 20 ng g\(^{-1}\) per congener. Brain was fortified with 7 PCB congeners at 1, 5, 10, 20, 40, 80 ng g\(^{-1}\) per congener, whereas adipose tissue was fortified with PCBs at a concentration of 1, 5, 20, 50, 100, 300 ng g\(^{-1}\) per congener. Since PBDEs occur in a lower concentration range than PCBs, PBDE calibration curves were determined in a lower linear range. Given that levels of PCB
138, PCB 153 and PCB 180 in adipose tissue were significantly higher than levels of PCB 28, PCB 52, PCB 101 and PCB 118, concentrations of PCB 138, PCB 153 and PCB 180 were calculated using, as internal standard, PCB 143. Concentrations of PCB 28, PCB 52, PCB 101 and PCB 118 were calculated using Mirex as the internal standard. The correlation coefficient for each congener was $> 0.995$ (Beltest I014-Rev4-17/7/2000-17, 2000). A blank adipose or brain tissue sample was subjected to full analysis to rule out possible contaminations. Retention times, ion chromatograms and intensity ratios were used as the identification criteria (Commission Decision 2002/657/EC).

### 6.2.4. Results and discussion

#### 6.2.4.1. Methodology and validation

Different extraction solvents, e.g. hexane, hexane/acetone (1/1), hexane/acetone/dichloromethane (1/1/1), were tested but only the use of hexane yielded satisfactory recoveries of $> 80\%$. The following clean-up of brain and adipose tissue extracts as well as the optimization of GC-MS/MS parameters has been described elsewhere (Naert et al., 2004). An additional optimization, using experimental design, was performed to select the most efficient GC-MS/MS settings for PBDE analysis. A response surface plot obtained for BDE 47 is presented in Figure VI.1. An overview of optimal excitation voltage and $q$-value for each PBDE congener is presented in Table VI.3.

![Figure VI.1 Response surface plot of BDE 47](image-url)
Table VI.3 Optimal excitation voltage and $q$-value for each PBDE congener

<table>
<thead>
<tr>
<th>PBDE congener</th>
<th>$q$-value</th>
<th>Excitation voltage (V)</th>
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</thead>
<tbody>
<tr>
<td>BDE 28</td>
<td>0.300</td>
<td>1.5</td>
</tr>
<tr>
<td>BDE 47</td>
<td>0.300</td>
<td>2</td>
</tr>
<tr>
<td>BDE 99</td>
<td>0.300</td>
<td>2</td>
</tr>
<tr>
<td>BDE 100</td>
<td>0.300</td>
<td>2</td>
</tr>
<tr>
<td>BDE 153</td>
<td>0.300</td>
<td>2.5</td>
</tr>
<tr>
<td>BDE 154</td>
<td>0.300</td>
<td>2.5</td>
</tr>
<tr>
<td>BDE 183</td>
<td>0.300</td>
<td>3</td>
</tr>
</tbody>
</table>

A different extraction step had been added to the previously published clean-up procedure for the analysis of adipose tissue (Naert et al., 2004). Thus, decision limits (CCα) and detection capabilities (CCβ) for PCBs and PBDEs in adipose tissue were reassessed. In parallel, a full validation procedure according to Commission Decision 2002/657/EC was carried out for the analysis of brain tissue. Decision limits and detection capabilities for PCBs and PBDEs in adipose and brain tissue are presented in Table VI.2. Specificity of the method was inferred from the fact that no interfering peaks were detected when analyzing 10 blank brain samples. Under repeatability conditions, within-laboratory coefficients of variation (CV %) were between 2.1 % and 14 % for PCB congeners and between 3.2 % and 12 % for PBDE congeners. Under within-laboratory reproducibility conditions, these coefficients ranged between 3.6 % and 15 % for PCBs and between 4.1 % and 16 % for PBDEs. Such low CV % values are in accordance with Commission Decision 2002/657/EC stating that for mass fractions $< 100 \mu g \text{kg}^{-1}$ simple application of the Horwitz equation would not be acceptable. Recovery of individual PCB congeners varied from 92 % to 107 % and recovery of individual PBDE congeners ranged from 85 % to 109 %. The expanded measurement uncertainty varied from 36 % to 65 % for PCBs and from 9.1 % to 16 % for PBDEs.

6.2.4.2. Influence of the nutrition status on the PCB and PBDE levels in brain and adipose tissue

Because in some cases the amount of sample was insufficient to determine the lipid content, a wet weight basis was chosen for calculating PCB and PBDE concentrations. Whenever possible, the percentages of the hexane extractable lipids in the tissue probes were determined. These measurements yielded a lipid content in the brain of 7.6 % $\pm$ 2.3 %.
proportion of lipid in adipose tissue was 78 % +/− 5 %. In starved animals, these lipid contents in brain and adipose tissue remain unchanged, but the amount of adipose tissue diminishes such that the lipophilic pollutants that were stored in the adipose compartment of the body are remobilised to other sites including the brain (Voorspoels et al., 2004; Wienburg and Shore, 2004). Indeed, many buzzards displayed only a limited amount of adipose tissue and the brain of these buzzards contained higher PCB and PBDE levels than the brain of well-fed counterparts (PCB: $t = 3.559$, Sig. = 0.003; PBDE: $t = 2.162$, Sig. = 0.037). In addition, partial correlation coefficients were calculated to explore a possible relationship between body weight, corrected for each species, and contaminant levels. In three bird species, e.g. buzzards, sparrow hawks and cormorants, negative correlations could be established between the overall body weight and the concentration of PCB and PBDE in both brain and adipose tissue.

6.2.4.3. PCB and PBDE levels in brain and adipose tissue

Because the PCB and PBDE concentrations did not follow normal distributions, median values were used for further comparisons (Table VI.4). The median PCB concentrations (sum of PCB 28, PCB 52, PCB 101, PCB 118, PCB 138, PCB 153 and PCB 180) in the brain ranged from 13 ng g$^{-1}$ wet weight (ww) in blackbirds to 428 ng g$^{-1}$ ww in sparrow hawks. In adipose tissue, the median PCB concentrations ranged from below the decision limit in blackbirds to 25951 ng g$^{-1}$ ww in sparrow hawks. The median PBDE concentrations (sum of BDE 28, BDE 47, BDE 99, BDE 100, BDE 153, BDE 154 and BDE 183) in brain ranged from below the decision limit in blackbirds to 14 ng g$^{-1}$ ww in sparrow hawks. Similarly, the median PBDE concentrations in adipose tissue ranged from below the decision limit in blackbirds to 709 ng g$^{-1}$ ww in sparrow hawks. A summary of the PCB and PBDE levels found in brain and adipose tissue of all animals from the 4 investigated species is presented in Table VI.5. Overall, significant correlations could be established between PCB and PBDE levels in brain tissue (Spearman’s rho = 0.753, Sig. < 0.0005) and between PCB and PBDE levels in adipose tissue (Spearman’s rho = 0.944, Sig. < 0.0005).
Table VI.4 Median PCB and PBDE levels (ng g\(^{-1}\) wet weight) in brain and adipose tissue samples from 3 different kinds of birds of prey species and blackbirds

<table>
<thead>
<tr>
<th>Compound</th>
<th>Buzzard</th>
<th>Sparrow hawk</th>
<th>Cormorant</th>
<th>Blackbird</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCB 28</td>
<td>&lt;CCa (&lt;CCa - 6.9)</td>
<td>2.8 (&lt;CCa - 22)</td>
<td>3.3 (&lt;CCa - 14)</td>
<td>2.9 (&lt;CCa - 36)</td>
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<tr>
<td>PCB 52</td>
<td>&lt;CCa (&lt;CCa - 41)</td>
<td>1.9 (&lt;CCa - 7.7)</td>
<td>7.4 (&lt;CCa - 56)</td>
<td>&lt;CCa</td>
</tr>
<tr>
<td>PCB 101</td>
<td>&lt;CCa (&lt;CCa - 11)</td>
<td>9.0 (&lt;CCa - 61)</td>
<td>6.2 (&lt;CCa - 85)</td>
<td>280 (11 - 1315)</td>
</tr>
<tr>
<td>PCB 118</td>
<td>2.0 (&lt;CCa - 56)</td>
<td>36 (7.1 - 285)</td>
<td>27 (61 - 7417)</td>
<td>1289 (&lt;CCa - 4.4 - 23)</td>
</tr>
<tr>
<td>PCB 138</td>
<td>9.1 (PCB - 246)</td>
<td>293 (50 - 1266)</td>
<td>142 (255 - 24019)</td>
<td>6700 (11 - 83)</td>
</tr>
<tr>
<td>BDE 28</td>
<td>&lt;CCa (&lt;CCa - 0.42)</td>
<td>6.2 (&lt;CCa - 1.3)</td>
<td>3.9 (&lt;CCa - 2.0)</td>
<td>199 (&lt;CCa - 1.3)</td>
</tr>
<tr>
<td>BDE 47</td>
<td>(BDE -6.9)</td>
<td>4.3 (&lt;CCa - 24)</td>
<td>4.4 (&lt;CCa - 1.3)</td>
<td>2 60 (15 - 553)</td>
</tr>
<tr>
<td>BDE 99</td>
<td>&lt;CCa (&lt;CCa - 7.8)</td>
<td>1.9 (&lt;CCa - 1.3)</td>
<td>2.0 (&lt;CCa - 2.0)</td>
<td>114 (&lt;CCa - 1.3)</td>
</tr>
<tr>
<td>BDE 100</td>
<td>&lt;CCa (&lt;CCa - 3.0)</td>
<td>5.0 (&lt;CCa - 8.2)</td>
<td>0.80 (&lt;CCa - 2.0)</td>
<td>78 (&lt;CCa - 1.3)</td>
</tr>
<tr>
<td>BDE 153</td>
<td>&lt;CCa (&lt;CCa - 10)</td>
<td>1.2 (&lt;CCa - 6.2)</td>
<td>1.3 (&lt;CCa - 6.2)</td>
<td>27 (&lt;CCa - 29)</td>
</tr>
<tr>
<td>BDE 154</td>
<td>1.0 (&lt;CCa - 0.4)</td>
<td>0.50 (&lt;CCa - 6.2)</td>
<td>1.0 (&lt;CCa - 2.0)</td>
<td>30 (&lt;CCa - 1.7 - 26)</td>
</tr>
<tr>
<td>BDE 183</td>
<td>&lt;CCa (&lt;CCa - 6.1)</td>
<td>1.0 (&lt;CCa - 2.0)</td>
<td>(4.1 - 90)</td>
<td>&lt;CCa</td>
</tr>
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</table>
Table VI.5 Concentrations (ng g\(^{-1}\) wet weight) of PCBs and PBDEs in birds of prey and blackbird from Switzerland

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Species</th>
<th>Brain tissue</th>
<th>Adipose tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sum PCB</td>
<td>Sum PBDE</td>
</tr>
<tr>
<td>B1</td>
<td>buzzard</td>
<td>17</td>
<td>&lt;CC(\alpha)</td>
</tr>
<tr>
<td>B3</td>
<td>buzzard</td>
<td>171</td>
<td>1.8</td>
</tr>
<tr>
<td>B4</td>
<td>buzzard</td>
<td>622</td>
<td>13</td>
</tr>
<tr>
<td>B5</td>
<td>buzzard</td>
<td>5.7</td>
<td>&lt;CC(\alpha)</td>
</tr>
<tr>
<td>B6</td>
<td>buzzard</td>
<td>9.5</td>
<td>&lt;CC(\alpha)</td>
</tr>
<tr>
<td>B7</td>
<td>buzzard</td>
<td>6.6</td>
<td>0.68</td>
</tr>
<tr>
<td>B8</td>
<td>buzzard</td>
<td>72</td>
<td>0.84</td>
</tr>
<tr>
<td>B9</td>
<td>buzzard</td>
<td>7.6</td>
<td>0.68</td>
</tr>
<tr>
<td>B10</td>
<td>buzzard</td>
<td>35</td>
<td>17</td>
</tr>
<tr>
<td>B11</td>
<td>buzzard</td>
<td>950</td>
<td>13</td>
</tr>
<tr>
<td>B12</td>
<td>buzzard</td>
<td>8.7</td>
<td>0.70</td>
</tr>
<tr>
<td>B13</td>
<td>buzzard</td>
<td>14</td>
<td>&lt;CC(\alpha)</td>
</tr>
<tr>
<td>B14</td>
<td>buzzard</td>
<td>11</td>
<td>&lt;CC(\alpha)</td>
</tr>
<tr>
<td>B15</td>
<td>buzzard</td>
<td>12</td>
<td>&lt;CC(\alpha)</td>
</tr>
<tr>
<td>B16</td>
<td>buzzard</td>
<td>6.9</td>
<td>11</td>
</tr>
<tr>
<td>B17</td>
<td>buzzard</td>
<td>12</td>
<td>&lt;CC(\alpha)</td>
</tr>
<tr>
<td>B18</td>
<td>buzzard</td>
<td>2.3</td>
<td>&lt;CC(\alpha)</td>
</tr>
<tr>
<td>B19</td>
<td>buzzard</td>
<td>261</td>
<td>5.6</td>
</tr>
<tr>
<td>B20</td>
<td>buzzard</td>
<td>12</td>
<td>&lt;CC(\alpha)</td>
</tr>
<tr>
<td>B21</td>
<td>buzzard</td>
<td>57</td>
<td>&lt;CC(\alpha)</td>
</tr>
<tr>
<td>B22</td>
<td>buzzard</td>
<td>181</td>
<td>3.9</td>
</tr>
<tr>
<td>B23</td>
<td>buzzard</td>
<td>127</td>
<td>0.74</td>
</tr>
<tr>
<td>B24</td>
<td>buzzard</td>
<td>121</td>
<td>0.87</td>
</tr>
<tr>
<td>B25</td>
<td>buzzard</td>
<td>52</td>
<td>0.96</td>
</tr>
<tr>
<td>B26</td>
<td>buzzard</td>
<td>15</td>
<td>&lt;CC(\alpha)</td>
</tr>
<tr>
<td>B27</td>
<td>buzzard</td>
<td>289</td>
<td>6.0</td>
</tr>
<tr>
<td>B28</td>
<td>buzzard</td>
<td>384</td>
<td>6.8</td>
</tr>
<tr>
<td>B29</td>
<td>buzzard</td>
<td>249</td>
<td>5.6</td>
</tr>
<tr>
<td>B30</td>
<td>buzzard</td>
<td>27</td>
<td>&lt;CC(\alpha)</td>
</tr>
<tr>
<td>B31</td>
<td>buzzard</td>
<td>5.6</td>
<td>&lt;CC(\alpha)</td>
</tr>
<tr>
<td>B32</td>
<td>buzzard</td>
<td>222</td>
<td>5.6</td>
</tr>
<tr>
<td>B33</td>
<td>buzzard</td>
<td>761</td>
<td>31</td>
</tr>
<tr>
<td>B34</td>
<td>buzzard</td>
<td>22</td>
<td>2.1</td>
</tr>
<tr>
<td>B35</td>
<td>buzzard</td>
<td>188</td>
<td>1.7</td>
</tr>
<tr>
<td>B36</td>
<td>buzzard</td>
<td>192</td>
<td>8.1</td>
</tr>
<tr>
<td>B37</td>
<td>buzzard</td>
<td>17</td>
<td>&lt;CC(\alpha)</td>
</tr>
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</table>
Table VI.5 Concentrations (ng g\(^{-1}\) wet weight) of PCBs and PBDEs in birds of prey and blackbird from Switzerland *(Continued)*

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Species</th>
<th>Brain tissue</th>
<th>Adipose tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sum PCB</td>
<td>Sum PBDE</td>
</tr>
<tr>
<td>B38</td>
<td>buzzard</td>
<td>192</td>
<td>2.0</td>
</tr>
<tr>
<td>B39</td>
<td>buzzard</td>
<td>31</td>
<td>&lt;CC(\alpha)</td>
</tr>
<tr>
<td>S1</td>
<td>sparrow hawk</td>
<td>207</td>
<td>4.6</td>
</tr>
<tr>
<td>S2</td>
<td>sparrow hawk</td>
<td>7439</td>
<td>168</td>
</tr>
<tr>
<td>S3</td>
<td>sparrow hawk</td>
<td>344</td>
<td>4.6</td>
</tr>
<tr>
<td>S4</td>
<td>sparrow hawk</td>
<td>776</td>
<td>12</td>
</tr>
<tr>
<td>S5</td>
<td>sparrow hawk</td>
<td>231</td>
<td>13</td>
</tr>
<tr>
<td>S6</td>
<td>sparrow hawk</td>
<td>4452</td>
<td>154</td>
</tr>
<tr>
<td>S7</td>
<td>sparrow hawk</td>
<td>428</td>
<td>8.9</td>
</tr>
<tr>
<td>S8</td>
<td>sparrow hawk</td>
<td>2327</td>
<td>62</td>
</tr>
<tr>
<td>S9</td>
<td>sparrow hawk</td>
<td>2105</td>
<td>57</td>
</tr>
<tr>
<td>S10</td>
<td>sparrow hawk</td>
<td>333</td>
<td>14</td>
</tr>
<tr>
<td>S11</td>
<td>sparrow hawk</td>
<td>390</td>
<td>15</td>
</tr>
<tr>
<td>C1</td>
<td>cormorant</td>
<td>71</td>
<td>1.3</td>
</tr>
<tr>
<td>C2</td>
<td>cormorant</td>
<td>91</td>
<td>0.63</td>
</tr>
<tr>
<td>C3</td>
<td>cormorant</td>
<td>145</td>
<td>0.60</td>
</tr>
<tr>
<td>C4</td>
<td>cormorant</td>
<td>32</td>
<td>&lt;CC(\alpha)</td>
</tr>
<tr>
<td>C5</td>
<td>cormorant</td>
<td>38</td>
<td>0.54</td>
</tr>
<tr>
<td>C6</td>
<td>cormorant</td>
<td>119</td>
<td>&lt;CC(\alpha)</td>
</tr>
<tr>
<td>C7</td>
<td>cormorant</td>
<td>210</td>
<td>&lt;CC(\alpha)</td>
</tr>
<tr>
<td>BB1</td>
<td>blackbird</td>
<td>41</td>
<td>27</td>
</tr>
<tr>
<td>BB2</td>
<td>blackbird</td>
<td>198</td>
<td>15</td>
</tr>
<tr>
<td>BB3</td>
<td>blackbird</td>
<td>8.8</td>
<td>1.0</td>
</tr>
<tr>
<td>BB4</td>
<td>blackbird</td>
<td>62</td>
<td>1.4</td>
</tr>
<tr>
<td>BB5</td>
<td>blackbird</td>
<td>34</td>
<td>&lt;CC(\alpha)</td>
</tr>
<tr>
<td>BB6</td>
<td>blackbird</td>
<td>60</td>
<td>&lt;CC(\alpha)</td>
</tr>
<tr>
<td>BB7</td>
<td>blackbird</td>
<td>9.2</td>
<td>&lt;CC(\alpha)</td>
</tr>
<tr>
<td>BB8</td>
<td>blackbird</td>
<td>11</td>
<td>&lt;CC(\alpha)</td>
</tr>
<tr>
<td>BB9</td>
<td>blackbird</td>
<td>4.5</td>
<td>&lt;CC(\alpha)</td>
</tr>
<tr>
<td>BB10</td>
<td>blackbird</td>
<td>13</td>
<td>&lt;CC(\alpha)</td>
</tr>
<tr>
<td>BB11</td>
<td>blackbird</td>
<td>2.7</td>
<td>&lt;CC(\alpha)</td>
</tr>
<tr>
<td>BB12</td>
<td>blackbird</td>
<td>13.3</td>
<td>&lt;CC(\alpha)</td>
</tr>
</tbody>
</table>
Among the four bird species tested, sparrow hawks showed the highest PCB and PBDE levels. The lowest PCB and PBDE contamination was found in blackbirds. In general, PCB and PBDE concentrations in the brain were lower than in the adipose tissue. This overall trend was retained when lipid-normalized concentrations between brain and adipose tissue were compared. The heterogeneous partitioning between brain and adipose tissue could be ascribed to the blood-brain barrier, which is thought to protect against the accumulation of persistent organic pollutants. In fact, a key role of the blood-brain barrier in preventing the deposition of contaminants in the brain is indicated by the relative uniform concentration of PCBs throughout the various organs of fish, which are known to display a much more permeable blood-brain barrier (Bachour et al., 1998). Alternatively, the lower PCB or PBDE levels in the brain may be related to the different proportion of neutral lipids such as triglycerides. While in fat triglycerides constitute > 90% of the total lipid content, in the brain the main lipid constituents are cholesterol and phospholipids (Covaci et al., 2004). Intriguingly, however, at least one sparrow hawk (S6) and three blackbirds (BB1, BB2 and BB4) displayed higher absolute PCB and PBDE concentrations in the brain tissue than in adipose tissue. One buzzard (B27) and an additional blackbird (BB3) displayed higher PBDE levels in the brain than in the adipose tissue. Another four blackbirds (BB7, BB8, BB10 and BB11) contained higher absolute concentrations of PCB in the brain than in adipose tissue. Moreover, after correction for the varying total lipid content, higher PCB levels in brain fat compared to adipose fat were detected in three buzzards (B1, B27 and B35), three sparrow hawks (S2, S3 and S6) and in all investigated black birds. In some cases, the same animals also contained higher PBDE concentrations in brain fat compared to adipose fat. These observations indicate that there are important interspecies and individual differences in the susceptibility for accumulation of persistent organic pollutants in the central nervous system.

**6.2.4.4. Differences in PCB and PBDE levels between species**

ANOVA and Bonferroni-adjusted comparisons were performed to determine differences in mean PCB and PBDE levels between species. Significant differences in PCB and PBDE levels could be established between sparrow hawks, on the one hand and buzzards, cormorants and blackbirds on the other hand. Significant interspecies disparities in tissue levels have also been reported by Hoshi et al. (1998), Senthilkumar et al. (2002), Tanabe et al. (1998), and Wienburg and Shore (2004). Despite the fact that both buzzards and sparrow hawks are feeding in a terrestrial environment, the differences in PCB and PBDE levels and
congener patterns between buzzard and sparrow hawk are much more pronounced than those between buzzard and fish eating cormorants.

PCB 138, PCB 153 and PCB 180 were the predominant PCB congeners in adipose and brain tissue of all species accounting for 91% (ranging from 56% to 100%) and 93% (ranging from 82% to 98%) of the total PCB content, respectively (Figure VI.2). This finding was comparable to congener patterns found in eggs from Norwegian predators (Herzke et al., 2005), in tissues from British birds (Boumphrey et al., 1993) and in fulmars from the Faroe Islands (Fängström et al., 2005). It is known that the PCB pattern shifts from lower to higher chlorinated congeners when these pollutants are transferred to organisms of higher trophic levels (Chu et al., 2003). BDE 47, BDE 99, BDE 100 and BDE 153 were the major congeners in adipose and brain tissue of all species. This observation is reminiscent of the PBDE profiles found in birds of prey from Australia (Symons et al., 2004), in eggs of little owls from Belgium (Jaspers et al., 2005), in eggs of Norwegian predatory birds (Herzke et al., 2005) and in birds of prey of Flanders (Voorspoels et al., 2004). Nevertheless, specific differences in the contribution of the different PBDE congeners between species could be observed in the present study (Figure VI.2). In fact, BDE 99 was the most abundant congener in samples from sparrow hawks, in contrast to cormorants where BDE 47 was the most abundant congener. It has been suggested by Law et al. (2003) that birds feeding in terrestrial environments may be more exposed to the higher brominated BDE congeners than aquatic species. The heptabrominated diphenyl ether congener 183 was detected only in terrestrial birds of prey (Figure VI.2).
Median PBDE - adipose tissue

Median PCB - adipose tissue

Median PBDE - brain tissue

Median PCB - brain tissue

Figure VI.2 Median PCB and PBDE patterns in adipose and brain tissue of birds of prey and blackbird from Switzerland.
6.2.5. Acknowledgements

The authors would like to thank Consella Ochieng for her technical support and enthusiasm.
6.2.6. References


Beltest I014-Rev4-17/7/2000-17 (2000) Determination of polychlorinated biphenyls in animal feed, animal fat, egg and egg products, milk products and other foodstuffs. Beltest – Belac (Belgian Organisation for Accreditation)


CHAPTER 6. DISTRIBUTION OF POLYCHLORINATED BIPHENYLS AND POLYBROMINATED DIPHENYL ETHERS IN BIRDS OF PREY FROM SWITZERLAND


CHAPTER 7. DEVELOPMENT AND APPLICATION OF A SIMPLIFIED CLEAN-UP PROCEDURE FOR THE DETERMINATION OF POLYCHLORINATED BIPHENYLS AND POLYBROMINATED DIPHENYL ETHERS IN HORSE FAT BY GAS CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY
7.1. BACKGROUND AND OBJECTIVES

The aim of the work in this chapter was to develop a new clean-up procedure which was faster, less laborious and consumed less organic solvent than the clean-up method described in Chapter 4. In order to do so we preferred to use disposable solid phase extraction columns (SPE). Different commercial and in-house made SPE columns were assessed in different combinations. The developed method was subsequently validated and CRM material (ERM-BB446) was analyzed. To examine the applicability of the newly developed clean-up method to real samples, we decided to analyse 44 horse fat samples. PCB and PBDE levels in horse fat were compared to levels in other meat products. We could conclude that the newly developed method allowed us to analyze much more samples in a much shorter space of time, consumed less organic solvent and gave acceptable validation results.
7.2. DEVELOPMENT AND APPLICATION OF A SIMPLIFIED CLEAN-UP PROCEDURE FOR THE DETERMINATION OF POLYCHLORINATED BIPHENYLS AND POLYBROMINATED DIPHENYL ETHERS IN HORSE FAT BY GAS CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

7.2.1. Abstract

For the determination of polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in adipose tissue, a simplified clean-up procedure was developed in combination with gas chromatography-tandem mass spectrometry (GC-MS/MS). Clean-up was performed by the successive application of a Mega Bond Elut® silica column and a Bond Elut® PCB column. Validation of the method was conducted according to EU Commission Decision 2002/657/EC. In order to evaluate the applicability of the developed method, 44 horse fat samples were analyzed. The total PCB concentration (sum of PCBs 28, 52, 101, 118, 138, 153, 180) ranged from 5.35 to 140 ng g\(^{-1}\) lipid weight. The total PBDE concentration (sum of BDEs 28, 47, 99, 100, 153, 154, 183) ranged from below the decision limit to 6.34 ng g\(^{-1}\) lipid weight.

7.2.2. Introduction

Polybrominated diphenyl ethers (PBDEs) have been used to protect the public from accidental fires by reducing the flammability of combustible materials, e.g. plastics and synthetic polymers (WHO – EHC 162, 1994). Nowadays, they are a matter of concern because they have been identified worldwide, in both aquatic and terrestrial compartments, as newly identified environmental contaminants (Gama et al., 2006; Law et al., 2006; Hites, 2004).

Polychlorinated biphenyls (PCBs) have been mainly produced from the 1930s to the 1970s as complex mixtures, containing many different congeners. Their good thermal and chemical stability and their electrical insulating properties made them used in a variety of industrial applications (WHO – EHC 140, 1992).
Both PCBs and PBDEs bioaccumulate through the food chain (Domingo, 2004; Sjödin et al., 2003) and as a consequence humans are significantly exposed to both types of contaminants through their diet. The toxicological profile of PBDEs and especially of PCBs is mainly dependent on the type of congener. Generally, PBDEs have been shown to act as thyroid hormone disruptors (Meerts et al., 2000) and neuro-developmental toxicants (Branchi et al., 2003). The IARC has classified PCBs as probably carcinogenic to humans (Group 2A) (IARC, 1987). Moreover, they have also been shown to cause a number of non-cancerous health effects, e.g. immunotoxicity (Levin et al. 2005), reproductive toxicity (Pocar et al., 2006), neurotoxicity (Pabello et al., 2006) and endocrine disruption (Tabuchi et al., 2006).

According to Directive 2002/95/EC or the RoHS Directive (OJ L 37, 2003), member states should ensure that from the 1st of July 2006 on, new electrical and electronic equipment put on the market, does not contain any PBDEs. Deca-BDE was exempted from the RoHS Directive on the 15th of October 2005, on the basis of the conclusions of a 10-year European Union environmental and human health risk assessment (OJ L 271, 2005). Prior to this, the use of Penta-BDE and Octa-BDE in all applications for the European market has been banned since the 15th of August 2004 (OJ L 42, 2003). PCBs are one of the 12 persistent organic pollutants (POPs) that have been listed by the Stockholm Convention. Belgian legislation has set maximum limits for the total sum of seven marker PCB congeners (PCBs 28, 52, 101, 118, 138, 153, 180) in different food matrices.

There is a need for simple and sensitive analytical methodology for these two groups of components. Most methods, developed for their determination in biological samples and more specific in adipose tissue, have the disadvantage of being very laborious. They usually consist of a lipid extraction step using a non-polar and/or polar solvent. Further clean-up and removal of co-extracted lipids is performed by gel permeation chromatography, adsorption chromatography and/or multilayer silica clean-up with sulphuric acidic treatment. The final determination is generally carried out by gas chromatography (GC) coupled with high or low resolution mass spectrometry (MS) or electron capture detection (ECD) (de Boer et al., 2001; de Boer et al., 2002; Pirard et al., 2002; Ramos et al., 2004; Saito et al., 2004).

It was the intention of the authors to develop a practical, non-laborious, yet sensitive analysis method for the determination of PCBs and PBDEs in adipose tissue. In order to evaluate the newly developed method, horse fat samples were analyzed. Within the European Union the Italians are consuming most horse meat per capita, followed by the Belgians (Hertrampf, 2003). In 2004, Belgium was the main importer of horse meat and the second largest exporter of horse meat, in terms of quantity (Food and Agricultural Organization of
the United Nations, 2004). Horses for human consumption have been mainly working animals or horses that were reared for recreational riding or racing (Martuzzi et al., 2001; Gill, 2005). Horses may be exposed to environmental contaminants through the ingestion of contaminated feed, contaminated soil or contaminated water (Rhind, 2002). Considering that horses for human consumption have had a long life-span and come from different backgrounds makes them interesting cases to study POPs.

7.2.3. Experimental

7.2.3.1. Chemicals

All reagents and solvents were of analytical grade. Isooctane, n-hexane Suprasolv® and anhydrous sodium sulphate were obtained from Merck (Darmstadt, Germany). Nonane was purchased from Sigma Aldrich nv/sa (Bornem, Belgium). Bond Elut® SPE cartridges were purchased from Varian Inc. (Sint-Katelijne-Waver, Belgium).

Individual PBDE standards (IUPAC Nos. 28, 47, 99, 100, 153, 154, and 183) were purchased from Wellington Laboratories (Guelph, Ontario, Canada). Individual PCB standards (IUPAC Nos. 28, 52, 101, 118, 138, 153 and 180) together with PCB-Mix 3 (10 ng µL⁻¹ of each congener), internal standards (polybrominated biphenyl (PBB) 103 and PCB 143) and injection standards (Mirex and PBB 155) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Certified reference material, ERM-BB446, pork fat (sum of 7 PCBs: 207 ± 11 ng g⁻¹) was obtained from the Institute for Reference Materials and Measurements (IRMM, Geel, Belgium). Stock solutions were stored between 2 and 8°C, protected from light. Suitable PBDE and PCB working solutions were prepared daily by dilution in nonane and isooctane, respectively.

7.2.3.2. Apparatus

Analysis of cleaned-up extracts was carried out using a Finnigan GCQ gas chromatograph coupled to a Finnigan GCQ mass spectrometer (Austin, Texas, USA). The autosampler was a CTC 200 series injector (Zwingen, Switzerland). PCB and PBDE congeners were separated on a HT8 capillary column (25 m x 0.22 mm x 0.25 µm, SGE, Achrom, Zulte, Belgium). Carrier and collision gas was Alphagaz 2 helium (Air Liquide,
7.2.3.3. Sample collection and storage

Horse fat samples were collected from March until June 2006 from a local slaughterhouse and stored at -20°C. Details of sample collection, e.g. age and slaughter dates are presented in Table VII.1. A pool of extracted chicken fat, from samples taken within the framework of the Belgian monitoring programme for PCBs, was used as blank for validation and quality control purposes. A sample of this pool was analyzed for PBDEs and a new pool was prepared if PBDE levels were above the decision limit (CCα). Because levels of PCB 118, PCB 138, PCB 153 and PCB 180 in the blank pool were occasionally higher than the decision limit, validation and sample results were corrected accordingly.

7.2.3.4. Extraction and clean-up

To prevent contamination, glassware was washed extensively and rinsed twice with hexane (Acros, Geel, Belgium). Approximately 10 g of adipose tissue was placed on a folded Ederol filter paper (VWR, Leuven, Belgium) in a glass funnel together with anhydrous sodium sulphate (10 g). The fat was melted in a microwave oven (600 W, 2 min) and collected in a glass vessel. Internal standards PCB 143 (50 µL 0.1 ng µL⁻¹) and PBB 103 (20 µL 0.1 ng µL⁻¹), together with 1 mL n-hexane were added to 0.5 g of melted fat.

A Mega Bond Elut® silica column (2 g) was conditioned with 12 mL of n-hexane. Subsequently, the sample was passed through the column without applying vacuum. Another 9 mL of n-hexane were used to elute the analytes from the column. The eluate was evaporated under a gentle nitrogen stream at 40°C to approximately 1 mL. A Bond Elut® PCB column was conditioned with 4 mL of n-hexane. Consequently the reduced eluate was applied to the column and eluted once more without applying vacuum. Two mL of n-hexane were used to elute the analytes under light vacuum. This step was repeated three additional times to an accumulated volume of 9 mL. After evaporation to approximately 4 mL and transfer to a graduated vial, injection standards PBB 155 (20 µL 0.1 ng µL⁻¹) and Mirex (50 µL 0.1 ng µL⁻¹) and keeper solvent isooctane were added. This mixture was concentrated under a gentle nitrogen stream at 40 °C to 100 µL and put into a GC-MS vial.
Table VII.1 Sample collection details and concentrations (ng g\(^{-1}\) lipid weight) of PCBs and PBDEs in horse fat samples (n = 44)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Furnisher</th>
<th>Slaughter date</th>
<th>Age</th>
<th>Sum PCB(^a)</th>
<th>Sum PBDE(^a)</th>
<th>Sample</th>
<th>Furnisher</th>
<th>Slaughter date</th>
<th>Age</th>
<th>Sum PCB(^a)</th>
<th>Sum PBDE(^a)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>27.03.06</td>
<td>-</td>
<td>26.6</td>
<td>0.98</td>
<td>23</td>
<td>4</td>
<td>02.05.06</td>
<td>foal</td>
<td>23.2</td>
<td>1.37</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>10.04.06</td>
<td>-</td>
<td>31.4</td>
<td>1.74</td>
<td>24</td>
<td>2</td>
<td>06.06.06</td>
<td>-</td>
<td>9.84</td>
<td>&lt;CC(\alpha)</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>04.04.06</td>
<td>-</td>
<td>27.7</td>
<td>1.09</td>
<td>25</td>
<td>5</td>
<td>08.05.06</td>
<td>adult</td>
<td>7.43</td>
<td>&lt;CC(\alpha)</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>14.04.06</td>
<td>-</td>
<td>20.2</td>
<td>1.02</td>
<td>26</td>
<td>6</td>
<td>02.05.06</td>
<td>foal</td>
<td>20.5</td>
<td>0.93</td>
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<td>1.32</td>
<td>28</td>
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<td>foal</td>
<td>8.98</td>
<td>&lt;CC(\alpha)</td>
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<td>29</td>
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<td>24.06.06</td>
<td>-</td>
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<td>30</td>
<td>2</td>
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<td>foal</td>
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<td>31</td>
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<td>adult</td>
<td>8.15</td>
<td>1.65</td>
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<tr>
<td>10</td>
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<td>27.03.06</td>
<td>-</td>
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<td>1.14</td>
<td>32</td>
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<td>06.06.06</td>
<td>-</td>
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<td>11</td>
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<td>3.45</td>
<td>33</td>
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<td>-</td>
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<td>1.87</td>
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<td>-</td>
<td>17.2</td>
<td>6.34</td>
<td>34</td>
<td>4</td>
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<td>-</td>
<td>32.6</td>
<td>1.23</td>
<td>35</td>
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<td>foal</td>
<td>5.35</td>
<td>&lt;CC(\alpha)</td>
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<td>-</td>
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<td>1.56</td>
<td>36</td>
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<td>1.03</td>
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<td>5</td>
<td>16.05.06</td>
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<td>73.8</td>
<td>2.98</td>
<td>37</td>
<td>2</td>
<td>29.06.06</td>
<td>foal</td>
<td>7.81</td>
<td>0.87</td>
</tr>
<tr>
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<td>foal</td>
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<td>1.92</td>
<td>38</td>
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<td>79.4</td>
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<td>foal</td>
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<td>3.63</td>
<td>39</td>
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<td>adult</td>
<td>30.2</td>
<td>0.68</td>
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<td>foal</td>
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<td>1.92</td>
<td>40</td>
<td>5</td>
<td>24.04.06</td>
<td>adult</td>
<td>49.3</td>
<td>1.23</td>
</tr>
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<td>19</td>
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<td>adult pony</td>
<td>19.6</td>
<td>2.11</td>
<td>41</td>
<td>2</td>
<td>13.06.06</td>
<td>-</td>
<td>36.2</td>
<td>1.93</td>
</tr>
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<td>15.05.06</td>
<td>foal</td>
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<td>foal</td>
<td>12.4</td>
<td>0.93</td>
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<td>foal</td>
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<td>07.06.06</td>
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<td>23.5</td>
<td>0.84</td>
</tr>
</tbody>
</table>

\(^a\) Concentrations below the decision limit (CC\(\alpha\)) were not taken into account for the calculations of the sum.
Two µL of the final extract was injected in the splitless mode with a splitless period of 1 min following injection. Injection temperature was set at 300°C and the transferline temperature was 275°C. The oven was programmed from 70 °C for 1 min to 170 °C at a rate of 30 °C min⁻¹, then to 300°C (15 min) at a rate of 8 °C min⁻¹. Optimization of GC-MS/MS parameters has been elaborately described elsewhere (Naert et al., 2004; Naert et al., 2006; Naert et al.; accepted for publication in Chemosphere). Detection of all analytes was done in electron ionization MS/MS mode. In order to achieve as much selectivity as possible, the most abundant ion of the molecular ion cluster was chosen as precursor ion for collision induced dissociation. Excitation voltage, excitation time and q-value are presented in Table VII.2.

<table>
<thead>
<tr>
<th>PCB congener</th>
<th>q-value</th>
<th>Excitation voltage (V)</th>
<th>Excitation time (ms)</th>
<th>PBDE congener</th>
<th>q-value</th>
<th>Excitation voltage (V)</th>
<th>Excitation time (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCB 28</td>
<td>0.450</td>
<td>1.8</td>
<td>15</td>
<td>BDE 28</td>
<td>0.300</td>
<td>1.5</td>
<td>15</td>
</tr>
<tr>
<td>PCB 52</td>
<td>0.450</td>
<td>1.8</td>
<td>15</td>
<td>BDE 47</td>
<td>0.300</td>
<td>2.0</td>
<td>15</td>
</tr>
<tr>
<td>PCB 101</td>
<td>0.450</td>
<td>2.0</td>
<td>15</td>
<td>BDE 99</td>
<td>0.300</td>
<td>2.0</td>
<td>15</td>
</tr>
<tr>
<td>PCB 118</td>
<td>0.450</td>
<td>2.0</td>
<td>15</td>
<td>BDE 100</td>
<td>0.300</td>
<td>2.0</td>
<td>15</td>
</tr>
<tr>
<td>PCB 138</td>
<td>0.450</td>
<td>1.8</td>
<td>15</td>
<td>BDE 153</td>
<td>0.300</td>
<td>2.5</td>
<td>15</td>
</tr>
<tr>
<td>PCB 153</td>
<td>0.450</td>
<td>1.8</td>
<td>15</td>
<td>BDE 154</td>
<td>0.300</td>
<td>2.5</td>
<td>15</td>
</tr>
<tr>
<td>PCB 180</td>
<td>0.450</td>
<td>1.8</td>
<td>15</td>
<td>BDE 183</td>
<td>0.300</td>
<td>3.0</td>
<td>15</td>
</tr>
</tbody>
</table>

7.2.3.6. Validation

Validation of the method was carried out according to Commission Decision 2002/657/EC (OJ L 221, 2002). Specificity, decision limit (CCα), detection capability (CCβ), recovery, precision, bias and trueness were determined as required for a quantitative confirmatory method. Because levels of PCB 118, PCB 138, PCB 153 and PCB 180 in the blank pool were occasionally higher than the decision limit, a blank subtraction was applied in order to correct validation and sample results accordingly.

CCα and CCβ were established by calibration curve procedure and this was repeated 5 times. Blank fat samples were fortified with a mixture of 7 PBDE congeners at a level of 1, 2, 4, 8, 15 ng g⁻¹ per congener and a mixture of 7 PCB congeners at 1, 5, 10, 20, 50 ng g⁻¹ per
congener. Subsequently, the signal was plotted against the added concentration. The decision limit or $CC_\alpha$ ($\alpha = 1\%$) corresponds to the concentration at the y-intercept plus 2.33 times the standard deviation of the within-laboratory reproducibility of the intercept. Detection capability or $CC_\beta$ ($\beta = 5\%$) equals the concentration at the decision limit plus 1.64 times the standard deviation of the within-laboratory reproducibility of the mean measured at the decision limit.

Specificity for all congeners, except for PCB 118, PCB 138, PCB 153 and PCB 180, was investigated by analyzing different blank samples for interferences. For the determination of recovery, bias, within day and between day precision, 18 blank fat samples were fortified at 3 levels (2, 4 and 8 ng g$^{-1}$ per congener for PBDEs and 5, 10 and 20 ng g$^{-1}$ per congener for PCBs). The recovery is defined as that fraction of mass of the analyte added to the sample which is present in the final extract. The responses of the fortified samples were compared with samples fortified after clean-up. Within day and between day precision were expressed as a coefficient of variation (CV %). Trueness was determined by means of analysis of purchased CRM material (ERM-BB446). Certified values for PCB 28, 52, 101, 118, PCB 138, PCB 153 and PCB 180 and an indicative value for BDE 47 were given. Measurement uncertainty was determined according to the EURACHEM/CITAC guide (Ellison et al., 2002). The global uncertainty was calculated as a combination of the uncertainty associated with precision and the uncertainty associated with accuracy. Although this is the combined uncertainty, the results are expressed as an expanded uncertainty, which corresponds to twice that value.

7.2.4. Results and discussion

7.2.4.1. Extraction and clean-up

An extensive clean-up procedure using glass columns filled with sodium sulphate, alumina and sulphuric acid was previously described elsewhere (Naert et al., 2004; Naert et al., 2006; Naert et al., accepted for publication in Chemosphere). Since this method was laborious and large volumes of solvent were consumed, it was the authors’ intention to simplify this clean-up procedure by using disposable solid phase extraction (SPE) columns. Fat removal properties of silica, florisil and alumina (activity III) were compared and the Mega Bond Elut® silica column (2 g) yielded best results. Still, further clean-up was required. A number of additional commercial SPE columns, e.g. Bond Elut® PCB (Varian, Inc.), Mega
Bond Elut® SCX (2 g) (Varian, Inc.), Sep-Pak® florisil cartridge (Waters, Zellik, Belgium), Mega Bond Elut® silica (2 g) (Varian, Inc.), PCB-A (Malinckrodt Baker, Boom, Diegem, Belgium), and an in-house made column (2 g) filled with silica, acidified silica or alumina were assessed. Best results and cleanest chromatograms were obtained with a Mega Bond Elut® silica column (2 g) in combination with the Bond Elut® PCB column. This column is a dual phase SPE column consisting of a strong cation exchange bed and a silica bed. It has been developed for the extraction of PCBs in transformer oil and organic matrices.

7.2.4.2. Validation

A summary of the validation results is presented in Table VII.3. Decision limits for PCBs and PBDEs ranged from 0.53 - 1.73 ng g⁻¹ lipid weight and from 0.30 - 1.07 ng g⁻¹ lipid weight, respectively. Detection capabilities for PCBs and PBDEs varied between 1.23 - 3.65 ng g⁻¹ lipid weight and between 0.78 - 1.90 ng g⁻¹ lipid weight, respectively.

<table>
<thead>
<tr>
<th>Compound</th>
<th>CCα (ng g⁻¹ lipid weight)</th>
<th>CCβ (ng g⁻¹ lipid weight)</th>
<th>Within day (n = 6)</th>
<th>Between day (n = 6)</th>
<th>Bias (%) (n = 6)</th>
<th>Recovery (%) (n = 6)</th>
<th>Expanded measurement uncertainty (%)</th>
<th>Trueness (%) (n = 4)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCB 28</td>
<td>0.53</td>
<td>1.25</td>
<td>4.77</td>
<td>11.4</td>
<td>-3.4</td>
<td>78.1</td>
<td>19.1</td>
<td>101</td>
<td>15.6</td>
</tr>
<tr>
<td>PCB 52</td>
<td>0.65</td>
<td>1.52</td>
<td>8.40</td>
<td>8.67</td>
<td>-2.3</td>
<td>82.5</td>
<td>27.4</td>
<td>98.7</td>
<td>15.1</td>
</tr>
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<td>0.59</td>
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<td>9.94</td>
<td>-1.6</td>
<td>81.6</td>
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<td>-0.1</td>
<td>83.2</td>
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<td>1.46</td>
<td>8.02</td>
<td>7.58</td>
<td>-1.9</td>
<td>86.6</td>
<td>30.9</td>
<td>102</td>
<td>10.1</td>
</tr>
<tr>
<td>PCB 153</td>
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<td>3.65</td>
<td>13.1</td>
<td>16.4</td>
<td>2.0</td>
<td>85.6</td>
<td>37.2</td>
<td>103</td>
<td>6.78</td>
</tr>
<tr>
<td>PCB 180</td>
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<td>1.29</td>
<td>9.46</td>
<td>7.44</td>
<td>-5.1</td>
<td>87.8</td>
<td>24.7</td>
<td>108</td>
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</tr>
<tr>
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<td>0.41</td>
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<td>5.74</td>
<td>7.02</td>
<td>0.5</td>
<td>94.3</td>
<td>18.1</td>
<td>-</td>
<td>-</td>
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<td>BDE 47</td>
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<td>98.8</td>
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<td>10.9</td>
<td>12.1</td>
<td>-1.7</td>
<td>99.3</td>
<td>29.4</td>
<td>-</td>
<td>-</td>
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<tr>
<td>BDE 183</td>
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<td>1.90</td>
<td>10.1</td>
<td>10.5</td>
<td>-2.4</td>
<td>84.4</td>
<td>34.9</td>
<td>-</td>
<td>-</td>
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</table>

*Data obtained at the 4 ng g⁻¹ level for PBDEs and the 10 ng g⁻¹ level for PCBs.

Precision, expressed as the coefficient of variation (CV %), did not exceed 16.4 %, indicating the good repeatability of the developed method. Recoveries were between 78.1 - 87.8 % for PCBs and between 84.4 - 99.3 % for PBDEs. Ratios of the peak area of the analyte...
spiked to a blank extract after sample clean-up and of the peak area obtained for standards were calculated and ranged from 63 % to 105 % for PCBs and from 66 % to 97 % for PBDEs.

No interfering peaks for PBDEs and PCB 28, PCB 52 and PCB 101 could be detected when analyzing different blank fat samples. Specificity for PCB 118, PCB 138, PCB 153 and PCB 180 could not be established because of the difficulty to obtain blank animal fat for these congeners. To ensure that separation of PCBs and PBDEs was adequate, separation of PCB 28 and PCB 31 was verified with every sample sequence. In the concentration ranges 1 - 15 ng g$^{-1}$ and 1 - 50 ng g$^{-1}$ for PBDEs and PCBs, respectively, correlation coefficients ($R^2$) were all higher than 0.990. The percent bias was within the - 30 % to + 10 % range and - 20 % to + 10 % range, as prescribed by Commission Decision 2002/657/EC, for concentrations ranging from >1-10 ng g$^{-1}$ and $\geq$ 10 ng g$^{-1}$, respectively. Trueness, established by analysis of certified reference material (ERM-BB446) varied between 98.7 % and 108 % for PCBs. Trueness for BDE 47 was 109 %. Expanded measurement uncertainty ranged from 19.1 to 37.2 % for PCBs and from 18.1 to 34.9 % for PBDEs.

Identification criteria, as laid down by Commission Decision 2002/657/EC (OJ L 221, 2002), were fulfilled. Signal-to-noise ratios for each diagnostic ion were larger than or equal to three. Relative retention times (RRTs) of the analyte did not differ by more than $\pm$ 0.5 % of the RRT of the calibration standard. The relative intensities of the detected ions, expressed as a percentage of the intensity of the most abundant ion, corresponded within the tolerances specified by Commission Decision 2002/657/EC.

7.2.4.3. Analysis of horse fat samples

In order to evaluate the newly developed method, 44 horse fat samples from a local slaughterhouse were analyzed for 7 indicator PCBs (PCB 28, 52, 101, 118, 138, 153 and 180) and 7 PBDE congeners (BDE 28, 47, 99, 100, 153, 154 and 183). PCBs were identified in all samples and PBDEs were identified in all but 6 samples. A summary of the PBDE and PCB levels is listed in Tables VII.1 and VII.4. A PCB and a PBDE chromatogram of a horse adipose tissue extract (sample n° 38) are presented in Figures VII.1 and VII.2.
CHAPTER 7. DEVELOPMENT AND APPLICATION OF A SIMPLIFIED CLEAN-UP PROCEDURE FOR THE DETERMINATION OF POLYCHLORINATED BIPHENYLs AND POLYBROMINATED DIPHENYL ETHERs IN HORSE FAT BY GAS CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

Figure VII.1 PCB chromatogram of a horse fat sample extract (sample n° 38)

Figure VII.2 PBDE chromatogram of a horse fat sample extract (sample n° 38)
Table VII.4 PCB and PBDE concentrations in horse fat samples (n = 44)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mean</th>
<th>Median</th>
<th>Range</th>
<th>Compound</th>
<th>Mean</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(ng g(^{-1}) lipid weight)</td>
<td></td>
<td></td>
<td></td>
<td>(ng g(^{-1}) lipid weight)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCB 28</td>
<td>1.13</td>
<td>1.15</td>
<td>&lt;CC(\alpha) – 4.87</td>
<td>BDE 28</td>
<td>&lt;CC(\alpha)</td>
<td>&lt;CC(\alpha)</td>
<td>&lt;CC(\alpha)</td>
</tr>
<tr>
<td>PCB 52</td>
<td>&lt;CC(\alpha)</td>
<td>&lt;CC(\alpha)</td>
<td>&lt;CC(\alpha) – 2.78</td>
<td>BDE 47</td>
<td>1.56</td>
<td>1.23</td>
<td>&lt;CC(\alpha) – 4.37</td>
</tr>
<tr>
<td>PCB 101</td>
<td>&lt;CC(\alpha)</td>
<td>&lt;CC(\alpha)</td>
<td>&lt;CC(\alpha) – 4.62</td>
<td>BDE 99</td>
<td>&lt;CC(\alpha)</td>
<td>&lt;CC(\alpha)</td>
<td>&lt;CC(\alpha) – 1.97</td>
</tr>
<tr>
<td>PCB 118</td>
<td>5.96</td>
<td>4.98</td>
<td>1.11 – 18.3</td>
<td>BDE 100</td>
<td>&lt;CC(\alpha)</td>
<td>&lt;CC(\alpha)</td>
<td>&lt;CC(\alpha)</td>
</tr>
<tr>
<td>PCB 138</td>
<td>6.57</td>
<td>3.97</td>
<td>&lt;CC(\alpha) – 37.2</td>
<td>BDE 153</td>
<td>&lt;CC(\alpha)</td>
<td>&lt;CC(\alpha)</td>
<td>&lt;CC(\alpha) – 0.74</td>
</tr>
<tr>
<td>PCB 153</td>
<td>14.2</td>
<td>12.0</td>
<td>2.06 – 52.9</td>
<td>BDE 154</td>
<td>&lt;CC(\alpha)</td>
<td>&lt;CC(\alpha)</td>
<td>&lt;CC(\alpha)</td>
</tr>
<tr>
<td>PCB 180</td>
<td>4.95</td>
<td>3.35</td>
<td>&lt;CC(\alpha) – 30.7</td>
<td>BDE 183</td>
<td>&lt;CC(\alpha)</td>
<td>&lt;CC(\alpha)</td>
<td>&lt;CC(\alpha) – 1.18</td>
</tr>
<tr>
<td>SUM</td>
<td>33.1</td>
<td>28.9</td>
<td>5.35 – 140</td>
<td>SUM</td>
<td>1.84</td>
<td>1.35</td>
<td>&lt;CC(\alpha) – 6.34</td>
</tr>
</tbody>
</table>

The sum of the seven PCB congeners ranged between 5.35 and 140 ng g\(^{-1}\) lipid weight (median 28.9 ng g\(^{-1}\) lipid weight). No sample exceeded the maximum permitted level of 200 ng g\(^{-1}\) fat as laid down by the Belgian legislation. Levels were higher than levels found in meat and meat products from Brazil (median 10.30 ng g\(^{-1}\)) (Costabeber et al., 2006), in pork fat (range: 2.6 – 6.3 ng g\(^{-1}\)) and pork meat (range: 8.6 – 17.5 ng g\(^{-1}\)) from Romania (Covaci et al., 2004) and in beef (median 0.284 ng g\(^{-1}\)), pork (median 0.234 ng g\(^{-1}\)) and chicken (median 0.241 ng g\(^{-1}\)) from South Korea (Kim et al., 2004). Levels corresponded extremely well with a study by Pirard et al. (2002). In this study poultry, horse, pork and beef samples were analyzed and horse samples also showed higher background levels of marker PCBs (21.6 ng g\(^{-1}\) fat). It has been suggested that this might be due to the longer life-span of horses compared to pigs, cattle or chickens (Focant et al., 2002). In the present study, no significant difference in PCB concentration has been found between adult horses or ponies and foals (t-test = -1.070; Sig. = 0.315). Perhaps diet can also be a major contributing factor because the composition of the diet may vary according to age, breed and type of labour. This conclusion should however be interpreted with caution because only eight adult animals were included in this study. PCB 153 was the predominant congener in every sample accounting for 43 % of the total PCB concentration (range: 31 - 74 %). This is in accordance with the notion that the higher chlorinated PCB congeners are also the more persistent congeners.

The total PBDE concentration ranged from below the decision limit (CC\(\alpha\)) to 6.34 ng g\(^{-1}\) lipid weight (median 1.35 ng g\(^{-1}\) lipid weight). Comparisons with other studies are difficult because most studies are market basket based studies. PBDE levels found in this study are comparable to levels found by Huwe et al. (Huwe et al., 2002) in chicken and higher than levels found in meat products from Catalonia (Bocio et al., 2003). BDE 47 was the
predominant congener in all but one sample (range: 64 – 100 %). This is in contrast with a market basket based study from the US where BDE 99, followed by BDE 47, predominates in meat samples (Schecter et al., 2004). No significant difference in PBDE concentration was found between adult horses or ponies and foals (t-test = 0.735; Sig. = 0.849). Significant but low correlation could be established between the sum of PCBs and PBDEs (Spearman’s rho = 0.531, Sig. < 0.0005).

7.2.5. Acknowledgements

The authors wish to thank Dr. H. De Brabander for the supply of the horse fat samples.
7.2.6. References


CHAPTER 7. DEVELOPMENT AND APPLICATION OF A SIMPLIFIED CLEAN-UP PROCEDURE FOR THE DETERMINATION OF POLYCHLORINATED BIPHENYLS AND POLYBROMINATED DIPHENYL ETHERS IN HORSE FAT BY GAS CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY


SUMMARY AND CONCLUSIONS
The objective of this work was the development of a gas chromatography – ion trap/mass spectrometry (GC-ITMS) based method for the identification and quantification of 7 polybrominated diphenyl ethers (BDEs 28, 47, 99, 100, 153, 154 and 183) in fatty tissues. Polybrominated diphenyl ethers (PBDEs) are widely used as flame retardants in a variety of products, e.g. polyurethane foams, high-impact polystyrene and ABS, furniture textiles and printed circuit boards. Because of their high lipophilicity and their resistance to degradation, they accumulate in lipid-rich tissues and biomagnify in the food chain. As a consequence, humans are mainly exposed to PBDEs through their diet by the intake of foods of animal origin (e.g. fish, meat and dairy products). Other major exposure pathways are the ingestion of house dust (especially for toddlers) and the intake of mother’s milk for newborns. PBDEs are linked to adverse health effects, e.g. interference with the neurobehavioural development and the thyroid function. For this reason, the use of two commercial PBDE mixtures, Penta-BDE and Octa-BDE, has been banned in the European Union since 15 August 2004. General aspects regarding PBDEs are described in Chapter 1. Since it was our intention to compare PBDE levels with levels of other similar compounds, we have also included the determination of the 7 marker polychlorinated biphenyls (PCBs 28, 52, 101, 118, 138, 153 and 180) in our method development. A short description of PCBs is given in Chapter 2. Chapter 3 provides a summary of the analytical methods which are currently used for the determination of PBDEs and PCBs in fatty tissues.

Chapter 4 describes the method development of the clean-up and the GC-ITMS method for the determination of the above mentioned PBDE and PCB congeners in adipose tissue. Adipose tissue is melted and filtered over anhydrous sodium sulphate. Subsequent clean-up is achieved using a column filled with acidified silica, deactivated alumina and anhydrous sodium sulphate. PBDE and PCB congeners are separated on a HT8 column. Due to software related limitations, PBDE and PCB congeners could not be determined in the same GC-MS/MS run. For that reason, each sample was analyzed in two consecutive runs. For the detection of both groups of contaminants, the electron ionization-tandem mass spectrometry (EI-MS/MS) mode was preferred because of its enhanced selectivity and sensitivity over other detection methods. The most abundant ion of the molecular ion cluster was chosen as precursor ion. Collision induced dissociation of PBDEs only generated product ions due to the loss of two bromine atoms. MS/MS spectra of PCBs were dominated by product ions due to loss of a single and two chlorine atoms. Product ions corresponding to the loss of a single chlorine atom were practically nonexistent in the MS/MS spectra of the mono-ortho substituted PCBs 28 and 118. The developed method was subjected to an extensive...
validation procedure. In order to assess the applicability of the method, five human adipose tissue samples were analyzed. Because it was our intention to determine the PBDE and PCB levels in a large number of Belgian human adipose tissue samples, the number of samples was extended to a total of 53 samples. The results of this study are reported in Chapter 5. PBDE concentrations (sum of BDEs 28, 47, 99, 100, 153, 154 and 183) ranged between 1.23 ng g\(^{-1}\) and 57.2 ng g\(^{-1}\) lipid weight and were comparable with levels in other European countries but they were lower than levels of humans living in the United States. BDE 153 was the predominant congener in the majority of the analyzed samples, followed by BDE 47 and BDE 183. These results are consistent with findings of other recent studies which also reported a shift in the most predominant congener from BDE 47 to BDE 153. This recent shift may be the result of the longer half-life of BDE 153 compared to the half-lives of the other PBDEs. The sum of the seven indicator PCB congeners (PCBs 28, 52, 101, 118, 138, 153 and 180) ranged from 126 ng g\(^{-1}\) to 2090 ng g\(^{-1}\) lipid weight. The predominant congeners were PCB 153, PCB 138 and PCB 180 which are also the most persistent PCB congeners. These profiles and levels were comparable to levels found in other European countries. No age-dependency was found for PBDEs while PCBs showed higher correlation coefficients with age. In addition, no relationship could be established between PBDE and PCB levels. These findings could be explained by the fact that humans were or are still exposed to increasing levels of PBDEs or by the fact that humans are exposed to PBDEs and PCBs through different pathways. Lower half-lives of PBDEs compared to PCBs may also account for the lack of correlation between PBDEs on the one hand and PCBs and age on the other hand.

Because PBDEs and PCBs have been associated with neurotoxicity, we studied their levels in the brain tissue of birds of prey (buzzard and sparrow hawk) from Switzerland (Chapter 6). This to assess how well the central nervous system is protected from the disposition of these types of contaminants. An additional terrestrial (blackbird) and aquatic (cormorant) bird species was also included into this study. A negative correlation could be established between the overall body weight and the PCB and PBDE levels in both brain and adipose tissue. This means that in case of starvation, the amount of adipose tissue diminishes and the pollutants that were stored in this body compartment are distributed to other sites including the brain. The median PCB concentrations (sum of PCBs 28, 52, 101, 118, 138, 153 and 180) in the brain ranged from 13 ng g\(^{-1}\) wet weight (ww) in blackbirds to 428 ng g\(^{-1}\) ww in sparrow hawks. In adipose tissue, the median PCB concentrations ranged from below the decision limit in blackbirds to 25951 ng g\(^{-1}\) ww in sparrow hawks. The median PBDE
concentrations (sum of BDEs 28, 47, 99, 100, 153, 154 and 183) in brain ranged from below the decision limit in blackbirds to 14 ng g\(^{-1}\) ww in sparrow hawks. Similarly, the median PBDE concentrations in adipose tissue ranged from below the decision limit in blackbirds to 709 ng g\(^{-1}\) ww in sparrow hawks. Sparrow hawks showed the highest PCB and PBDE levels and significant differences in levels could be established between sparrow hawks on the one hand and buzzards, cormorants and blackbirds on the other hand. This means that the difference in PBDE and PCB levels between buzzard and sparrow hawks, which are both feeding in a terrestrial environment, is greater than the difference between buzzards and fish eating cormorants. PCB 138, PCB 153 and PCB 180 were the predominant PCB congeners and BDE 47, BDE 99, BDE 100 and BDE 153 were the major PBDE congeners in adipose and brain tissue of all species. Nevertheless, specific differences in the contribution of different PBDE congeners between species could be observed (e.g. BDE 99 was the most abundant in sparrow hawks and BDE 47 was the most abundant in cormorants). In general, PCB and PBDE concentrations in the brain were lower than those in the adipose tissue. This heterogeneous partitioning of PCB and PBDE levels between brain and adipose tissue can be ascribed to the blood-brain barrier which protects the brain from accumulation of persistent organic pollutants. Another explanation may be found in the different lipid composition of adipose and brain tissue. At least one sparrow hawk and three blackbirds displayed higher absolute PCB and PBDE concentrations in the brain tissue than in adipose tissue. After correction for the respective lipid content, higher PCB or PBDE concentrations in brain compared to adipose tissue, were found in three sparrow hawks, four buzzards and in all investigated blackbirds. These results suggest that a deficit in the neuroprotective function of the blood-brain barrier may cause unexpected levels of PCBs and PBDEs in the central nervous system. Further research would be needed to examine the influence of the blood-brain barrier on the contaminant levels in the brain tissue.

Finally, in Chapter 7, the development of a clean-up procedure was investigated which was faster, less laborious and consumed less organic solvent than the procedure described in Chapter 4. It was our intention to use disposable solid phase extraction (SPE) columns. Best results were obtained with a Mega Bond Elut\textsuperscript{®} silica column (2 g) in combination with a Bond Elut\textsuperscript{®} PCB column. The developed method was subsequently validated and CRM material was analyzed. In order to evaluate the newly developed clean-up method, 44 horse fat samples were analyzed for PCBs and PBDEs. The sum of the seven PCB congeners ranged between 5.35 and 140 ng g\(^{-1}\) lipid weight (median 28.9 ng g\(^{-1}\) lipid weight). This means that no sample exceeded the maximum permitted level of 200 ng g\(^{-1}\) fat as laid down by the
Belgian legislation. However, PCB and PBDE levels seem to be higher in horse samples than in samples from poultry, pork and beef. PCB 153 was the predominant congener in every sample. The total PBDE concentration ranged from below the decision limit to 6.39 ng g$^{-1}$ lipid weight (median 1.35 ng g$^{-1}$ lipid weight) and BDE 47 was the major congener. Low correlations could be established between PCB and PBDE levels, but no significant difference could be found in PCB and PBDE levels between adult horses or ponies and foals.

In conclusion, we can state that we have developed a clean-up procedure and an on gas chromatography - ion trap/mass spectrometry based detection method for the determination of polybrominated diphenyl ethers and polychlorinated biphenyls in fatty tissues. To accommodate for the laborious nature of the developed clean-up procedure, an alternative method using disposable solid phase extraction columns was also developed. Human adipose tissue, brain and adipose tissue from 4 different bird species, and equine adipose tissue were analyzed with these developed methods. PBDE concentrations should be continuously monitored in humans to identify future trends and more specifically to examine the influence of the recent legal bans of Penta-BDE and Octa-BDE in different countries. Additional studies are also necessary to determine whether different exposure routes are on the basis of the divergent PBDE concentrations which are found in humans living in different regions of the world. For this purpose correlation studies between PBDE concentrations in humans and in the house dust of the homes they are living in may be useful.
SAMENVATTING EN CONCLUSIES
De doelstelling van dit werk was de ontwikkeling van een op gaschromatografie – ion trap/massaspectrometrie (GC-ITMS) gebaseerde methode voor de identificatie en gehaltebepaling van 7 polygebroemde difenyl ethers (BDE’s 28, 47, 99, 100, 153, 154 en 183) in vetrijke weefsels. Polygebroemde difenyl ethers (PBDE’s) worden wijdverspreid gebruikt als brandvertragers en dit in een groot assortiment van producten (vb. polyurethaanschuim, hoog impact polystyreen en ABS, gedrukte circuits en textiel voor meubilair). Omwille van hun sterke lipofiliciteit en hun weerstand tegen afbraak, accumuleren zij in weefsels die rijk zijn aan vet en bioaccumuleren zij doorheen de voedselketen. De mens wordt hoofdzakelijk blootgesteld aan PBDE’s via de voeding en dit voornamelijk via de inname van voedingsmiddelen van dierlijke oorsprong (vb. vis, vlees en zuivelproducten). Andere belangrijke blootstellingsroutes zijn de inname van huisstof (voornamelijk bij peuters) en de inname van moedermelk door zuigelingen. PBDE’s worden in verband gebracht met een aantal ongunstige gezondheidseffecten zoals de verstoring van het gedrag, het geheugen en het leervermogen, en de ontregeling van de schildklierhormoonhuishouding. Als gevolg van deze toxische effecten is het gebruik van twee commerciële PBDE-mengsels, nl. de Penta- en Octa-mix, verboden in de Europese Unie sinds 15 augustus 2004. Deze algemene aspecten betreffende PBDE’s worden beschreven in Hoofdstuk 1.

Aangezien het onze intentie was om PBDE-gehalten te vergelijken met deze van andere gelijkaardige componenten, werd de bepaling van de 7 merker polychloorbifenylen (PCB’s 28, 52, 101, 118, 138, 153 en 180) geïntegreerd in onze methodeontwikkeling. Een korte beschrijving van polychloorbifenylen (PCB’s) is weergegeven in Hoofdstuk 2. Hoofdstuk 3 geeft een samenvatting van de analytische methoden die gebruikt worden voor de bepaling van PBDE’s en PCB’s in vetrijke weefsels.

Hoofdstuk 4 beschrijft de ontwikkeling van de methode voor staalopzuivering en de ontwikkeling van de GC-ITMS methode voor het bepalen van de bovenvermelde PBDE’s en PCB’s in vetrijke weefsels. Eerst werd het vetweefsel afgesmolten en gefiltreerd door anhydrisch natriumsulfaat. Vervolgens gebeurde de staalopzuivering aan de hand van een chromatografische kolom gevuld met aangezuurde silica, gedeactiveerd aluminiumoxide en anhydrisch natriumsulfaat. PBDE- en PCB-congeneren werden gescheiden op een HT8 capillaire kolom. Door beperkingen van de GCQ-software konden PBDE- en PCB-congeneren niet in één enkele GC-MS/MS run gescheiden worden. Daarom werd elk staal geanalyseerd in twee opeenvolgende runs. Omwille van de betere selectiviteit en gevoeligheid werd voor detectie van beide groepen contaminanten de voorkeur gegeven aan elektron ionisatie – tandem massaspectrometrie. Hierbij werd het meest intense ion van de moleculaire
cluster gekozen als precursor ion. “Collision induced dissociation” van PBDE’s genereerde enkel productionen ten gevolge van het verlies van 2 bromatomen, waar de MS/MS spectra van PCB’s werden gedomineerd door productionen ten gevolge van het verlies van 1 of 2 chlooratomen. Produktionen ten gevolge van het verlies van één enkel chlooratoom waren niet aanwezig in de MS/MS spectra van de mono-ortho gesubsstitueerde PCB’s 28 en 118. De ontwikkelde methode werd vervolgens onderworpen aan een uitgebreide validatieprocedure. Om de toepasbaarheid van de methode na te gaan werden vijf menselijke vetweefselstalen geanalyseerd.

Aangezien het onze bedoeling was om de PBDE- en PCB-gehalten te bepalen in een groot aantal menselijke vetweefselstalen werd de totale hoeveelheid monsters uitgebreid tot 53. De resultaten van deze studie zijn opgenomen in Hoofdstuk 5. De PBDE gehalten (som van BDE’s 28, 47, 99, 100, 153, 154 en 183) situeerden zich tussen 1.23 en 57.2 ng g⁻¹ vet en waren vergelijkbaar met de concentraties van personen uit andere Europese landen maar lager dan deze van personen uit de Verenigde Staten. BDE 153 was de belangrijkste congenere in de meerderheid van de geanalyseerde stalen, gevolgd door BDE 47 en BDE 183. Deze resultaten zijn consistent met de bevindingen van andere recente studies, die eveneens een verschuiving rapporteerden van de meest voorkomende congenere, namelijk van BDE 47 naar BDE 153. Deze verschuiving kan het gevolg zijn van de hogere halfwaardetijd van BDE 153 ten opzichte van de halfwaardetijd van de andere PBDE-congeneren. De som van de 7 merker PCB-congeneren (PCB’s 28, 52, 101, 118, 138, 153 en 180) was gelegen tussen 126 en 2090 ng g⁻¹ vet en de meest abundante congenere waren PCB 153, PCB 138 en PCB 180. Deze congeneerprofiliën en gehalten zijn vergelijkbaar met deze geregistreerd in andere Europese landen. De teruggevonden PBDE-concentraties waren niet leeftijdsgebonden, dit in tegenstelling tot de PCB-concentraties die wel een hogere leeftijdsafhankelijkheid vertoonden. Bovendien kon er geen correlatie worden vastgesteld tussen PCB- en PBDE-concentraties. Deze bevindingen kunnen worden verklaard doordat de mens wordt blootgesteld aan steeds stijgende hoeveelheden PBDE’s. Een andere verklaring kan liggen in het feit dat de blootstelling van de mens aan PBDE’s en PCB’s op verschillende manieren gebeurt. De lagere halfwaardetijden van PBDE’s, in vergelijking met deze van PCB’s, kunnen eveneens aan de basis liggen van het gebrek aan correlatie tussen PBDE-concentraties enerzijds en PCB-concentraties en leeftijd anderzijds.

Aangezien PBDE’s en PCB’s geassocieerd worden met neurotoxiciteit, werden hun concentraties bestudeerd in het hersenweefsel van roofvogels (buizerd en sperwer) uit Zwitserland (Hoofdstuk 6). Dit om te evalueren in welke mate het centrale zenuwstelsel
beschermd is tegen de afzetting van dit type componenten. Aanvullend werden een zich op het land voedende vogelsoort (merel) en een visetende vogelsoort (aalscholver) in deze studie opgenomen. Een negatieve correlatie kon worden vastgesteld tussen het lichaamsgewicht en de PBDE- en PCB-concentraties die in hersen- en vetweefsel werden teruggevonden. Dit kan betekenen dat in geval van verhongering, de hoeveelheid vetweefsel vermindert en de contaminanten die in dit lichaamscompartiment opgestapeld waren naar andere lichaamsdelen, zoals de hersenen, worden verspreid. De mediane PCB-concentraties (som van PCB’s 28, 52, 101, 118, 138, 153 en 180) in de hersenen lagen tussen 13 ng g⁻¹ weefsel in merels en 428 ng g⁻¹ weefsel in sperwers. In vetweefsel schommelden de mediane PCB-concentraties van beneden de beslissingsgrens in merels tot 25951 ng g⁻¹ weefsel in sperwers. De mediane PBDE-concentraties (som van BDE’s 28, 47, 99, 100, 153, 154 en 183) in het hersenweefsel varieerden van beneden de beslissingsgrens in merels tot 14 ng g⁻¹ weefsel in sperwers. Vergelijkbaar strekten de mediane PBDE-concentraties in vetweefsel zich uit van beneden de beslissingsgrens in merels tot 709 ng g⁻¹ weefsel in sperwers. Sperwers vertoonden de hoogste PCB- en PBDE-concentraties en een significant concentratieverschil kon vastgesteld worden tussen sperwers enerzijds en buizerds, aalscholvers en merels anderzijds. Dit betekent dat het verschil in PBDE- en PCB-concentraties tussen buizerds en sperwers, die zich beide voeden op het land, groter is dan het verschil tussen buizerds en visetende aalscholvers. PCB 138, PCB 153 en PCB 180 en BDE 47, BDE 99, BDE 100 en BDE 153 waren de meest voorkomende PCB- en PBDE-congeneren in hersenweefsel en vetweefsel van alle vogelsoorten. Toch konden specifieke verschillen vastgesteld worden in het aandeel van de congeneren tussen de species (vb. BDE 99 was meest voorkomend in sperwers en BDE 47 was meest voorkomend in aalscholvers). Algemeen kon gesteld worden dat de PCB- en PBDE-concentraties in het hersenweefsel lager waren dan deze in het vetweefsel. Dit verschil kan toegeschreven worden aan de bloed-hersenbarrière die de hersenen beschermt tegen de accumulatie van persistente organische polluanten. Een andere verklaring kan liggen in de verschillende vetsamenstelling van het hersen- en vetweefsel. Ten minste één sperwer en drie merels vertoonden hogere PCB én PBDE concentraties in hersenweefsel dan in het vetweefsel. Na correctie voor de respectievelijke vetconcentraties werden van drie sperwers, vier buizerds en alle onderzocht merels, hogere PBDE- én PCB-concentraties teruggevonden in hersenweefsel dan in vetweefsel. Deze resultaten suggereren dat de oorzaak van deze onverwachte PCB- en PBDE-concentraties kan liggen in een gebrek in de neuroprotectieve functie van de bloed-hersenbarrière. Verder onderzoek is nodig om de
invloed van de bloed-hersenbarrière na te gaan op de concentraties van deze contaminanten in de hersenen.

**Hoofdstuk 7** ten slotte beschrijft de ontwikkeling van een methode voor staalopzuivering die sneller en minder arbeidsintensief is en ook minder organisch solvent verbruikt dan de procedure beschreven in Hoofdstuk 4. Het was onze intentie om wegwerp vaste fase extractiekolommen te gebruiken. De beste resultaten werden verkregen met een Mega Bond Elut® silica kolom (2 g) in combinatie met een Bond Elut® PCB kolom. De ontwikkelde methode werd vervolgens gevalideerd en gecertificeerd referentiemateriaal werd geanalyseerd. Om de toepasbaarheid van de nieuw ontwikkelde methode na te gaan, werden 44 vetstalen afkomstig van paarden geanalyseerd op PBDE’s en PCB’s. De som van de 7 merker PCB-congeneren was gelegen tussen 5.35 en 140 ng g⁻¹ vet (mediaan 28.9 ng g⁻¹ vet). Dit betekent dat geen enkel staal het maximaal toelaatbare gehalte van 200 ng g⁻¹ vet, zoals beschreven in de Belgische wetgeving, overschreed. Desalniettemin blijkt dat de PCB- en PBDE-gehalten in vet van paarden hoger liggen dan de overeenkomstige gehalten in monsters van gevogelte, varkens of runderen. PCB 153 was de meest voorkomende congener in elk staal. De totale PBDE-concentratie varieerde van beneden de beslissingsgrens tot 6.39 ng g⁻¹ vet en dit met BDE 47 als meest voorkomende congener. Er werd slechts een lage correlatie vastgesteld tussen de PCB- en de PBDE-gehalten. Tussen de PCB- en PBDE-gehalten in volwassen paarden of pony’s en veulens kon geen significant verschil worden geconstateerd.

Tot slot kan gesteld worden dat een methode voor staalopzuivering en een op gaschromatografie – ion trap/massaspectrometrie gebaseerde methode voor de bepaling van PBDE’s en PCB’s in vetrijke weefsels werden ontwikkeld. Om tegemoet te komen aan de arbeidsintensieve aard van de ontwikkelde opzuiveringsmethode werd een alternatieve methode ontwikkeld die gebaseerd is op het gebruik van wegwerp vaste fase extractiekolommen. Humaan vetweefsel, hersenweefsel en vetweefsel afkomstig van 4 verschillende vogelsoorten en vetweefsel afkomstig van paarden werden onderzocht met de ontwikkelde analysemethoden. De PBDE-concentraties in de mens zouden continu opgevolgd moeten worden om toekomstige trends te identificeren en ook om de invloed van het recentelijk verbod van de Penta- en Octa-mix na te gaan. Meer studies zijn ook noodzakelijk om te bepalen of de verschillende blootstellingsroutes aan de basis liggen van de uiteenlopende PBDE-concentraties die in de mens worden teruggevonden. Correlatiestudies tussen de PBDE-concentraties in mensen enerzijds en in het huisstof van hun respectievelijke woningen anderzijds kunnen hierbij nuttig zijn.