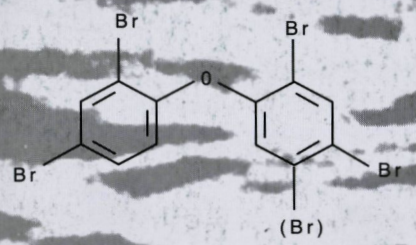
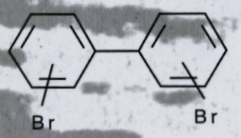


PBDEs



PBBs

In vitro biotransformatie van organohalogeenverbindingen in zeezoogdieren en vogels. Mogelijke gevolgen voor bioaccumulatie en genotoxiciteit

III. Gebromeerde vlamvertragers (polybroom difenylethers & polybroom bifenylen)

Beleidsgericht
ecologisch onderzoek
van de
Noordzee/Waddenzee

BEON

NIOZ
RIVO-DLO
RWS-RIKZ

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Uitgebreide Samenvatting:

Doel van het onderzoek:

Het onderzoek binnen dit BEON project beoogt een viertal doelen:

1. Ontwikkeling van een *in-vitro* bioassay voor de oxydatieve biotransformatie van verschillende groepen van lipofiele organische microverontreinigingen in mariene toppredatoren.
2. Validatie van de met deze assay behaalde resultaten door vergelijking met residupatronen in prooi en predator als gevolg van natuurlijke blootstelling in zee.
3. Bestuderen van de invloed van biotransformatie op de genotoxiciteit van de verbindingen in een bacteriële standaard bio-assay (mutatox®).
4. Beleidsadvisering naar aanleiding van de behaalde resultaten in combinatie met literatuurgegevens.

Probleemstelling

Van een aantal groepen van organische microverontreinigingen wordt de kans op het optreden van biomagnificatie (bioaccumulatie via de voedselketen) van kieuwademende prooidieren naar longademende predatoren zoals vogels en zoogdieren, bijzonder groot wordt geacht op grond van hun hoge vetoplosbaarheid. Hierbij wordt er impliciet vanuit gegaan, dat een stof in het lichaam niet reaktief is en dus niet van structuur verandert. De bioaccumulatie van stoffen die wèl enzymatisch worden omgezet, laat zich dus niet volgens deze regels

beschrijven. Dit aspect speelt een belangrijke rol bij de doorvergiftigingsproblematiek in de normstelling (1). Het is echter nog niet mogelijk om vanuit de theorie te voorspellen of bepaalde klassen van verbindingen door verschillende diergroepen al of niet kunnen worden omgezet. Ook het verkrijgen van informatie door middel van experimenten met levende dieren (*in-vivo* experimenten) is vanwege het grote benodigde aantal dieren om ethische en logistieke redenen slechts in hoge uitzonderingsgevallen mogelijk en bovendien erg duur. Doel van het onderzoek in het kader van dit project is de ontwikkeling van een alternatief voor zulke *in-vivo* experimenten in de vorm van een *in-vitro* bioassay om de oxidatieve biotransformatiecapaciteit van verschillende soorten mariene toppredatoren voor verschillende klassen van organische microverontreinigingen te kunnen meten. Deze assay maakt gebruik van speciaal geprepareerd levermateriaal (microsomen) van één enkel dier of slechts enkele individuen per soort. Voordat er met 'nieuwe' stoffen werd begonnen, is de gebruikte *in vitro* assay eerst gevalideerd voor de polychloorbiphenylen (PCBs), omdat het bioaccumulatie- en biotransformatiegedrag hiervan in zeezoogdieren en zeevogels al goed bekend is (resultaten beschreven in BEON rapport 95-4 (2)).

In dit meerjarige BEON project worden een aantal stofgroepen onderzocht, waarvan op grond van hun molecuulstructuur ophoping in de voedselketen kan worden verwacht, maar waarover nog weinig gegevens bekend zijn omtrent hun bioaccumulatie in top-predatoren uit de Noordzee en Waddenzee. Het betreft hier de insecticiden toxafeen (resultaten reeds verschenen in de BEON rapporten 95-4 (2)en 96-1 (3)) en chloordaan, de polychloor terfenylen (PCTs; een stofgroep met in essentie dezelfde toepassingen als PCBs in wassen, drukinkt, verf, lak, electrische apparatuur, hydraulische oliën, smeermiddelen, als weekmakers in plastics en als vlamvertragers), alsmede de als vlamvertragers gebruikte polybroom biphenylen (PBBs) en polybroom difenylethers (PBDEs). In dit rapport wordt het onderzoek naar de biotransformatie van deze beide laatstgenoemde groepen van gebromeerde vlamvertragers beschreven. De resultaten van chloordaan en de PCTs zullen hierna nog als BEON rapporten verschijnen.

Resultaten en conclusies van het uitgevoerde onderzoek met betrekking tot het te voeren beleid.

- Doorvergiftigings problematiek: In de *in vitro* assays bleken alle onderzochte PBDEs en PBBs op één enkele uitzondering niet te worden omgezet. Tevens werden in alle onderzochte monsters residuen van beide klassen van gebromeerde vlamvertragers gevonden in weefsels van de potvis, witsnuitdolfijn, zeehond, eidereend en kanoetstrandloper. Op grond van de geconstateerde hoge resistentie tegen enzymatische afbraak en de hoge lipofiliteit van deze stoffen is dit niet onverwacht. De vondst van deze groepen stoffen in alle drie de bij Kijkduin aangespoelde potvissen heeft hierbij een bijzondere betekenis. Gezonde potvissen bevinden zich namelijk in principe uitsluitend in de diepzee; op onze breedte zijn dit de Noord Atlantische Oceaan en de Middellandse Zee. Zij leven daar voornamelijk van inktvis, maar ook diep levende vissoorten worden gegeten. Zowel de prooidieren als de predator komen dus slechts bij hoge uitzondering in kustgebieden voor. Uit het feit, dat beide groepen gebromeerde vlamvertragers ook in deze potvissen werden gevonden, kan dus worden geconcludeerd dat we hier te maken hebben met wereldwijd verspreide groepen van stoffen, net zoals dit voor de PCBs en DDT-achtige verbindingen het geval is. Wel waren hierbij de concentraties van PBDEs duidelijk hoger dan die van de PBBs, dus de PBDEs vormen het grootste probleem.

- Normering: Beide groepen gebromeerde vlamvertragers zijn thans nog onbeperkt toegelaten en worden ook algemeen worden gebruikt in (huishoudelijke) electronische apparatuur. In een steekproef van de consumentengids bleek, dat 4% van alle onderzochte apparaten PBDE's bevatte, PBBs waren zeldzamer (4). Aangezien deze stoffen in hoge mate persistent waren in de bio-assay en tevens een PCB-achtige structuur bezitten, dient hiervoor vanuit milieu oogpunt bezien zo snel mogelijk een **meer restrictief beleid** te worden ingezet, ondanks dat er met betrekking tot de giftigheid van met name de polybroom diphenylether nog vele vragen liggen (zie ook onder *"aanbevelingen voor toekomstig onderzoek"*).

- Genotoxiciteit: In de mutatox assay werd voor zowel de PBDEs als de PBBs geen genotoxische werking vastgesteld. Dit is in overeenstemming met de meeste literatuur gegevens. Wel kunnen PBBs en PBDEs de mutagene werking van andere stoffen potentiëren (promotorwerking; zie ook hieronder bij punt 3 'sublethale effecten').

Aanbevelingen voor toekomstig onderzoek

1. Modellering Milieugedrag van Toxische Stoffen: Voor de modellering van het gedrag van contaminanten in voedselketens is het van groot belang dat het vermogen tot biotransformatie van stoffen binnen verschillende diergroepen beter kan worden voorspeld. Indien de biotransformatie van bepaalde groepen stoffen goed gerelateerd kan worden aan de aanwezigheid en de induceerbaarheid van bepaalde biotransformatie enzymen in alle belangrijke taxonomische groepen organismen, dan is daarmee een genetische basis aanwezig voor een systematische classificatie van soorten ten aanzien van hun biotransformatiecapaciteit en gevoeligheid voor bepaalde klassen van lichaamsvreemde stoffen. Het recent beschikbaar komen van specifieke anti-lichamen en specifieke remmers voor verschillende iso-enzymen van het cytochroom P450 systeem kan hierbij van groot nut zijn (5, 6). Met name de iso-enzymen behorende tot de subfamilies cytochroom P450 1A (CYP1A), CYP2B, en CYP3A lijken intensief te zijn betrokken bij de omzetting van lichaamsvreemde organische stoffen.

   Met behulp van *in vitro* assays zou in de toekomst een systematisch overzicht kunnen worden verkregen tussen de moglijkheden van verschilllene diersoorten om bepaalde groepen stoffen te kunnen metaboliseren en de concentraties van bepaalde iso-enzymen van cytochroom P450. (Als vervolg op het huidige BEON project zijn met dit type werk voor de PCBs en gechloreerde bornanen (toxafeen componenten) al een aantal interessante resultaten geboekt bij zowel het NIOZ als het RIVO-DLO).

2. Congeneer-specifiek onderzoek naar het voorkomen en de toxiciteit van PBBs en PBDEs. De resultaten van deze studie geven aan dat zowel PBDEs als PBBs wijdverspreid in het milieu lijken voor te komen. Gegeven hun op PCB gelijkende eigenschappen is dat een reden om zowel het voorkomen van deze stoffen als hun toxiciteit beter in kaart te brengen. De huidige gegevens van slechts een beperkt aantal veldmonsters geven aanleiding om de verspreiding en de toxiciteit van deze nog dagelijks in Nederland toegepaste stoffen veel uitgebreider te bestuderen. Naar analogie met het PCB onderzoek ligt het voor de hand om te kiezen voor een congeneer-specifieke benadering. Hierbij is het allereerst nodig om het voorkomen van de verschillende congeneren, inclusief andere dan de reeds bestudeerde, in kaart te brengen. Het gaat daarbij met name om het voorkomen van de verschillende congeneren te inventariseren en de eventuele degradatie van hoger

gebromeerde tot lager gebromeerde verbindingen in kaart te brengen.

3. Sublethale effecten: Gezien het algemene voorkomen van gebromeerde vlamvertragers is het noodzakelijk om meer kennis te verzamelen omtrent de toxiciteit van de individuele congeneren en hun eventuele omzettingsproducten. In het kader van haar studie biologie aan de Rijksuniversiteit Groningen is door mw. M.K. de Boer onder begeleiding van J.P. Boon en J. de Boer een literatuurscriptie gemaakt getiteld "Brominated Biphenyl and Diphenylether Flame Retardants". Hierin is een samenvatting opgenomen van de recent verschenen literatuur. Uit het overzicht blijkt, dat de in deze studie gevonden afwezigheid van genotoxische eigenschappen wordt ondersteund door veel overige literatuur. Wel kunnen PBBs en PBDEs de mutagene werking van andere stoffen potentiëren (promotorwerking). Omdat in het zeemilieu de gebromeerde vlamvertragers vaak voorkomen met bijvoorbeeld polyaromatische koolwaterstoffen (PAKs) waarvan er een aantal zeer carcinogeen zijn, lijkt dit een zeer relevant toxisch effect. Tevens zijn er indicaties voor effecten van gebromeerde vlamvertragers op de reproductie van laboratorium organismen en de regulatie van geslachts- en schildklierhormonen. Het belang van beide werkingsmechanismen zou voor verschillende congeneren in detail onderzocht kunnen worden door middel van toepassing van effectgerichte bioassays speciaal ontworpen voor bestudering van deze hormonale effecten.

***In vitro* Biotransformation of Cyclic Organohalogen Compounds in Marine Mammals and Birds. Possible Consequences for Bioaccumulation and Genotoxicity.**
III: Brominated Flame Retardants (Polybromodiphenylethers & Polybromobiphenyls).

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Running-title: Consequences of PBB and PBDE Biotransformation.

Key words: PBBs, PBDEs, *in vitro* Biotransformation, Residue Patterns, Genotoxicity, Wildlife.

Abstract

Since little is known about the persistence to biotransformation by the cytochrome P-450 system of the brominated flame retardants polybrominated diphenyl ethers (PBDEs) and polybrominated biphenyls (PBBs), an existing *in vitro* bioassay was adapted in order to investigate these characteristics in samples of marine mammals and sea birds. Hepatic microsomes of harbour seal (*Phoca vitulina*), whitebeaked dolphin (*Lagenorhynchus albirostris*), sperm whale (*Physeter macrocephalus*), and eider duck (*Somateria mollissima*) were incubated with standard PBDE and PBB mixtures.

The microsomal preparations were incubated with a standard PBDE mixture, containing 2,4,2',4'-tetra-, 2,4,5,2',4'-penta- and 2,3,4,5,6,2',3',4',5',6'-decabromodiphenyl ether. Assays to which NADPH was added as an electron source allowing for the occurrence of biotransformation were compared to reference samples without NADPH. Three metabolisable chlorobiphenyl congeners (CB-26, CB-28 and CB-101) were added as positive controls, whereas CB-153 was added as an internal standard. The positive controls and EROD activity measurements confirmed the ability of the microsomes to perform cytochrome P-450 mediated biotransformation reactions. All reactions did not show significant biotransformation of the BDE congeners. Although PBDEs seem to have affinity to the same biological receptors as the PCBs, they did not inhibit metabolism of the CB congeners used as positive control.

In case of the PBBs, the microsomes of all three marine mammals were able to biotransform the BB-15, in contrast to the microsomes of an eider duck. BB-15 (4,4'-dibromobiphenyl) contains vicinal hydrogen atoms exclusively at the *ortho-meta* positions. All four test animals in this study were incapable of metabolizing the remaining BB congeners with vicinal H atoms only at *meta-para* positions (BB-52, BB-49 and BB-101) or those without any vicinal H atoms (BB-155, BB-153 and BB-169).

The apparent persistence of both classes of brominated flame retardants was clearly reflected in fact that environmental residues of compounds belonging to both classes of flame retardants were found in all samples analyzed. The PBDE levels were generally more than 50 times higher than those of the PBBs. Since residues were also found in blubber and liver of all three sperm whales investigated, it was concluded that both classes of compounds can considered to be global pollutants, since healthy sperm whales generally stay in deep water and their occurrence in shelf seas is considered to be accidental.

The concentrations of the fully brominated congeners decabromodiphenylether or

decabromobiphenyl were below the limits of detection. Since these compounds are unlikely to be metabolised by the cytochrome P450 system, this most likely results from the fact that these molecules are too large to be taken up, or their debromination by other processes in the environment.

Both classes of compounds did not show a genotoxic response in the mutatox® assay. The absence of a direct mutagenicity of these compounds is supported by the existing literature, which also includes experiments with laboratory rodents. However, PBBs and PBDEs can potentiate the carcinogenic properties of other compounds. Since they often occur together with polyaromatic hydrocarbons (PAHs), of which a number of compounds is highly carcinogenic, this may be a highly relevant mechanism in the field situation. In other studies, PBBs were shown to affect reproductive processes and the regulation of steroid and thyroid hormones in laboratory rodents, birds and monkeys. PBDEs affected reproductive processes in fish.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) and polybromobiphenyls (PBBs) are used as additive flame retardants, which are incorporated into the matrix of various plastic materials like polystyrenes, polyesters and copolymerisates of acrylonitrile, butadienes and styrenes (ABS) to prevent them from catching fire (7). Additive flame retardants are much more prone to leaching or escape from the finished polymer product than reactive flame retardants, because they are only mixed with, or dissolved in, the material and can therefore migrate out of the product (8, 9, 10). Since their emission is diffuse and difficult to control, a substantial part of these compounds will eventually reach the marine environment, where they are likely to accumulate due to their high lipophilicity ($\log K_{ow}$ values generally >6) and resistance to degradation (11). The main environmental properties and mechanisms of toxicity of PBDEs and PBBs are similar to those of the polychlorobiphenyls (PCBs) and chlorinated dibenzodioxins (PCDDs) (11, 12). These classes of compounds are considered to be major causes for the reproductive failure and immunological impairment that has been observed in marine mammals (13).

In view of these properties, the policy measurements taken to prevent environmental exposure to brominated flame retardants have been quite weak when compared to those taken against the important classes of chlorinated compounds mentioned above. In the Netherlands, 4% of all electronic equipment randomly tested was shown to contain PBDEs, but PBBs were not found (4). The environmental occurrence of PBBs is very seldom reported. In 1973 large amounts of PBBs were accidentally mixed in dairy food in Michigan (USA), which resulted in the destruction of nearly 30,000 cattle plus thousands of other farm animals (14, 15). As a result PBB concentrations were detected in adipose tissue, blood, and breast milk of exposed humans. This high exposure resulted in impairment of the immune system, diverse neurological effects such as headaches, numbness and fatigue, hepatic damage including liver enlargement and porhyria, and reproductive damage like low birth weights (16). After this, the production of PBBs slightly decreased (17). Still decabromobiphenyl (BB-209) and possible other PBBs are produced commercially but alternative chemicals have been introduced to replace them as flame retardants, in particular the PBDEs.

As a result of this, the production and use of brominated flame retardants has even increased in the past decades (12, 18, 19, 20). Also in the Netherlands, brominated flame retardants are still impregnated in all kinds of materials, e.g. electronical equipment and clothing. The

presence of PBDEs has already been reported in different environmental compartments of the marine environment: in sediments (21, 22), fish (18, 22, 23, 24, 25), marine mammals (18, 26), and birds (24, 26, 27, 28). The presence of PBDEs in samples from remote areas indicates a global distribution pattern similar to the related chlorinated chemicals (26).

However, like PCB congeners, some PBDE and PBB congeners may be enzymatically biotransformed. The oxidative biotransformation pathway of these polyaromatic halogenated hydrocarbons (PHAHs) is mediated by the cytochrome P-450 dependent monooxygenase (HMO) system. This complex metabolises endogenous and exogenous compounds in order to facilitate the excretion of these compounds (29). From a toxicological point of view, formation of water soluble metabolites, that are excreted, is advantageous, but occasionally biotransformation generates metabolites that are more toxic than the parent compounds (30, 31).

The present study on brominated fire retardants is part of a larger BEON project which aims to:

1. Investigate the ability of hepatic microsomal preparations of different species of marine mammals and birds to metabolise halogenated organohalogen compounds in an *in vitro* bioassay.
2. Obtain information on residue patterns of the same classes of compounds in environmentally exposed marine mammals and birds, in relation to the results of the *in vitro* assays.
3. Obtain information on the genotoxicity of the compounds in a standard microbial assay (mutatox®), both of the parent compounds and after allowing for biotransformation to occur.
4. To advise with respect to the development of policy measurements for these compounds on the basis of the results obtained and literature data.

2. Materials and methods

2.1 In vitro assays

2.1.1 Glassware: All glassware was thoroughly washed with soap, rinsed with demineralised water, heated overnight at 350°C and rinsed with hexane prior to use.

2.1.2. Organic Solvents: Hexane, pentane and 2,2,4-trimethylpentane (TMP) (Chemproha) were purified as described previously (32). All other solvents used were nanograde quality (Mallinckrodt), except methanol (BAR-grade, Baker).

2.1.3. Organohalogens:

Throughout this report, the systematic numbering system developed by Ballschmiter and Zell (33) for the 209 theoretically possible polychlorinated biphenyl (PCB) congeners has also been adopted for the corresponding PBDE and PBB congeners. The general structures of the compounds investigated are given in **fig. 1**.

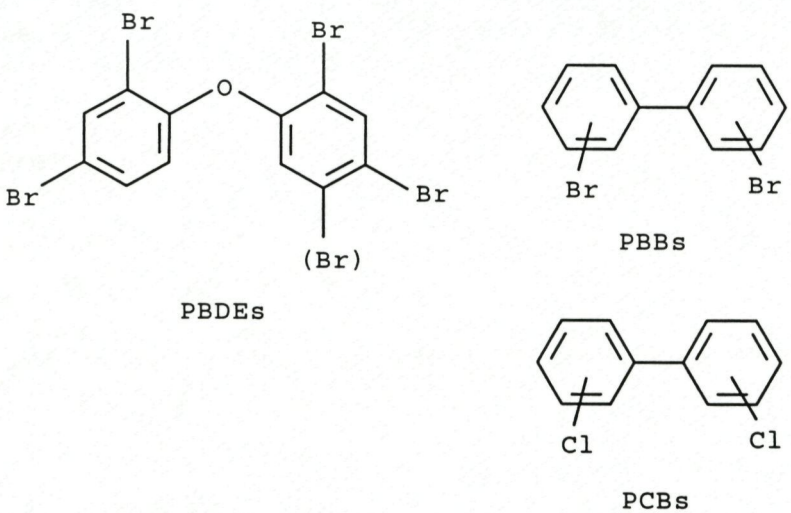


Fig. 1: General structures of the brominated diphenylethers (PBDEs), and the polybrominated (PBBs), and polychlorinated (PCBs) biphenyls.

2.1.3.1. PBDEs

A standard incubation mixture was made, consisting of the technical mixture Bromkal® 70-5DE and deca-BDE. Four CB-congeners were added to this standard mixture: CB-26, CB-28, CB-101 were added as positive controls for the occurrence of biotransformation during

incubation, whereas CB-153 was added as an internal standard. CB-153 is known to be highly resistant to enzymatic attack by the cytochrome P-450 system. The solvent was acetone, because the mixture has to be soluble in aqueous solutions. Calculated concentrations in this incubation mixture are given in table 1:

Table 1: Composition of incubation mixture to test for the biotransformation of brominated diphenylethers (BDEs). CBs -26, -28, and -101 were added as positive controls. CB-153 was added as internal reference compound.

Systematic no.	Halogenation pattern	Concentration(ug/ml)
Bromkal® 70-5DE	[1)]	1.73
BDE-209 [2)]	2,2',3,3',4,4',5,5',6,6'-decaBDE	30.8
CB-26	2,3',5-triCB	1.43
CB-28	2,4,4'-triCB	1.35
CB-101	2,2',4,5,5'-pentaCB	0.66
CB-153	2,2',4,4',5,5'-hexaCB	0.60

[1)]: Bromkal® 70-5DE contains 41.7% BDE-47 (2,2',4,4'-tetraBDE), 44.4% BDE-99 (2,2',4,4',5-pentaBDE) and 13.9% of BDE-*xy* (a penta BDE congener with an unknown substitution pattern.

[2)]: the solution of this compound contained also minor amounts of 3 nonabromo diphenyl ethers, of which the small peaks were neglected

2.1.3.2 PBBs

The incubation mixture used in the *in-vitro* assay consisted of a combination of seven different BB congeners and three different CB congeners that were dissolved in acetone. The concentrations of the congeners employed in the standard mixture are listed in Table 2. The concentration of BB-15 is so much higher than the other compounds because of its low response factor on an electron capture detector (see also paragraph *2.1.10 'analysis of organobromines'*).

Table2: Composition of the incubation mixture to test for the biotransformation of brominated biphenyls.

Systematic no.	Halogenation pattern	Concentration (µg/ml)
BB15	4,4'-dibromobiphenyl	25.44
BB49	2,2',4,5'-tetrabromobiphenyl	1.42
BB52	2,2',5,5'-tertabromobiphenyl	1.93
BB101	2,2',4,5,5'-pentabromobiphenyl	1.24
BB153	2,2',4,4',5,5'-hexabromobiphenyl	1.06
BB155	2,2',4,4',6,6'-hexabromobiphenyl	1.41
BB169	3,3',4,4',5,5'-hexabromobiphenyl	3.75
CB26	2,3',5-trichlorobiphenyl	1.77
CB101	2,2',4,5,5'-pentachlorobiphenyl	7.08
CB153	2,2',4,4',5,5'-hexachlorobiphenyl	0.33

2.1.4. Origin of samples

The type of research described in this report in it's entity, not just this project in particular, depends greatly upon the availability of dead animals. To isolate useful enzymes, the liver of the dead animals should be in a good condition. The liver samples used for the *in vitro* assay should therefore be taken as soon after death as possible. Since the availability of the studied species almost always is coincidental, it has been attempted to study the most important animal groups. When the possibility arises to analyze the biotransformation ability of unique species, it will most certainly be done. For the study of the brominated flame retardants, samples were taken from the following four species:

(i) Harbour seal (*Phoca vitulina*)

PBDE assays: Microsomes originated from a female juvenile animal found dead in the TESO ferry harbour of Texel at 5th January 1994. Animal was tagged with number N3213 of the seal rehabilitation centre at Pieterburen. Length: 1.10 m; weight: 31 kg; thickness of blubber layer: 4.0 cm, measured ventrally directly below sternum. NIOZ sample code: 94PVa. The cause of death was an torn body cavity, probably by the propeller of the ferry. No indications of scavenging were present. Blood still poured out of the heart when this was punctured. Peculiarities: Apparently good condition, time of death unknown.

PBB-assays: A female seal found dead in Camperduin was sampled was on the 10th of October 1996. The age was estimated to be 4 years. Weight 49,5 kg. The animal was just moulting and had a lot of inflammations on her skin. Considering her age, she was late with her moulting, which was probably due to a bad condition. The blubber layer was smaller than 0.5 cm. The NIOZ code was: $96PVd_1$

(ii) Whitebeaked dolphin (*Lagenorhynchus albirostris*)

Pregnant female specimen. Stranded alive at 24th January 1995, 13:45 at Texel. Animal died at 14:15. Autopsy at 17:00. Samples were frozen in liquid nitrogen by 18:00. Thickness of blubber layer: 2.1 cm, measured ventrally at the incision from the sternum downward to obtain liver samples. Length of animal: 2.5m. Animal lost milk and was missing some teeth. The NIOZ identification code for this sample is $95LAa_3$. (the microsomes were used for the PBDE and PBB assays).

(iii) Sperm whale (*Physeter macrocephalus*).

Male specimen stranded alive at Kijkduin (near the Hague) at 12th of January 1995. First sighting at 9:00 am. Upon arrival at the beach at 19:00 the body cavity had already been opened by Ir. R.A. Kastelein from the Harderwijk Marine Mammal Park. The liver samples for biochemical purposes were frozen in liquid nitrogen at 20:15,. Length of animal: 14.40 m; estimated age: 15-25 years; estimated weight: 30 tonnes. The NIOZ identification code: $95PMb_3$.

iv. Minke whale (*Balaenoptera acutorostrata*).

Samples of this baleen whale were only used for residue analyses. It concerns a juvenile male animal that stranded alive around 10:00 on 25th November 1994 at Zoutelande in the province of Zeeland in the southwest of the Netherlands. The animal died at 12:30; sampling occurred within three hours after the moment of death. Length of animal 4.53 m; width of the tail: 1.18 m. Estimated weight: 1100 kg. Animal was lean (thickness of blubber layer at the neck *dorso-laterally*: 3 cm; halfway between the dorsal fin and the tail: 2 cm) and dried-out; most probably it was an infant that had lost its mother. Sampling was carried out by Ir. R.A. Kastelein of the Harderwijk Marine Mammal Park.

(v) Common eider duck (*Somateria mollissima*).

In contrast to the previously mentioned animals, the liver samples of the Eider duck were taken from animals which had been euthanised. Eider ducklings were captured in the 'Kroonpolders' on the island of Vlieland (in the north of the Netherlands). The ducklings were very young, approximately 1 day of age. They were brought to large outdoor birdhouses at the NIOZ where they were marked individually and kept in groups. This was done to imitate a natural raising situation. The young birds were fed dried food pellets (Poultry pellets, Koopmans BV, Leeuwarden, Netherlands) *ad libitum*. An acclimatisation period of 27 days was allowed before treatment was started. In order to induce the total cytochrome P-450 system of these animals, the ducklings were treated with CB-77 at 50 mg/kg body weight. CB-77 was used because it is one of the most potent PCB congeners to induce cytochrome P-450 1A (a subfamily of the cytochrome P-450 system). This incubation period lasted for 10 days. The birds were then tranquillised with diethyl ether, and then euthanised by exsanguination via cardiac puncture. Subsequently the liver was removed and the necessary samples were taken.
NIOZ sample code of the individual used: 94SM51D for PBDE assays and 94SM51A for the PBB assays.

vi. Knot (*Calidris canutus*).

Samples of this species were only used for residue analyses. Since the individual birds contained very little adipose depot tissue, the material of 6 animals was pooled. These birds had been caught in the western Wadden Sea and had been held in captivity at NIOZ for a period of XX weeks. Date of sacrifice: 17th April 1996. Tissue was frozen and stored at -20°C for further analysis.
NIOZ lab. code:96CCa1-6.

Preparation of liver homogenates:

All procedures with liver tissue were conducted at 0-5°C. Liver lobes were cut to small pieces on site and immediately homogenised with an Ultra Turrax TP 18/10 after the addition of half a volume of ice-cold glycerol. During homogenisation the beaker containing the liver material was cooled by placing it into a larger beaker filled with crushed ice. The homogenate was divided over a number of 10 ml nalgene tubes, directly frozen on site in liquid nitrogen,

and subsequently transported to the laboratory were it was stored in -80°C freezer for the preparation of the microsomal fraction at a later date.

2.1.5. Preparation of microsomes:

The thawed liver homogenate was mixed with three times its volume of a 0.1 M phosphate buffer (pH 7.6) with 10% glycerol, 0.15M KCl, 1mM EDTA, and 1mM dithiothreitol (DTT) and homogenized with Ultra-turrax and Potter-Elvehjem tube. The homogenate was then centrifuged at 12,500 g for 15 min. Subsequently, the supernatant was centrifuged at 100,000 g for 1 h. The pellets were redissolved in 0.1 M phosphate resuspension buffer (pH 7.6) containing 1mM EDTA, 1mM DTT and 20% glycerol and homogenized in a Potter-Elvejhem tube. The homogenate was centrifuged at 100,000 g for 1 h to wash out excess blood and the pellet was resuspended in the resuspension buffer in a Potter-Elvejhem tube. It was then aliquoted in 0.5ml portions in cryovials and frozen in liquid nitrogen. Storage at -80°C.

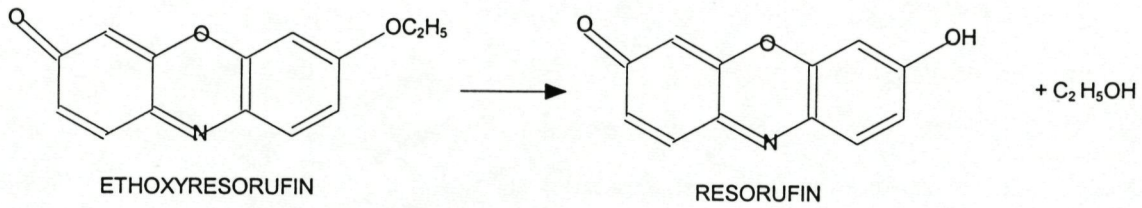
2.1.6. Quality criteria for the microsomal preparations: Liver samples were taken from the bodies of dead animals, but only when samples could be taken within a short time span (2-6 hours) after the moment of death. This precaution was taken to reduce the danger that *post-mortem* decay processes would have adversely affected the condition of the tissues (34). As a further precaution it was established whether the microsomal preparations still contained a viable cytochrome P450 system by measurement of the ethoxyresorufin-O-deethylase (EROD) activity. Although this is strictly speaking a measurement for the activity of the iso-enzyme cytochrome P450 1A1 only, it was used as a general quality index for the activity of the microsomes. In view of the range of EROD activities published in the literature for marine mammals (35), it was decided that the EROD activity of the microsomal preparations should be at least 10 pmol • mg^{-1} protein • min^{-1} for use in the *in-vitro* assays.

2.1.7. Protein Analysis: Total protein concentrations were determined with the Biorad assay based on the method developed by Bradford (36). The analysis was conducted on a Biorad model 3550 microplate reader at a wavelength of 595 nm. Biorad protein assay dye reagent was used to stain the proteins. Bovine serum albumin was used as an external standard.

2.1.8. EROD activity measurements:

Ethoxyresorufin-*O*-deethylase (EROD) is one of the model reactions to establish the activity of the cytochrome P-4501A mediated MO-system (37, 38). This reaction is shown in **fig. 2**. In the context of this paper, EROD activity was used as a general index of the viability of the microsomes used next to the behaviour of the chlorobiphenyl congeners added as positive controls in the assays.

Fig.2. The *O*-deethylation of ethoxyresorufin to the fluorescent product resorufin



The EROD activities were determined fluorimetrically according to the method of Eggens and Galgani (39) using a Flow Laboratories Titertek fluoroskan II plate reader. The temperature was 37°C. The pH was 7.6 for marine mammals and 7.4 for eiderduck microsomes. Assays were performed in a 0.1 M phosphate buffer containing 0.9 µM ethoxyresorufin and 150 µM NADPH. The resorufin concentration in the external standard was calculated with the extinction coefficient 73 $mM^{-1} \cdot cm^{-1}$ (40).

2.1.9. In vitro bioassay

With the bioassay, the transformation of the BDE and BB congeners by the microsomal fractions of the liver is monitored. The basic methodology has been described previously (41): 0.1 ml of a microsomal suspension containing 10 mg protein/ml was added to 1 ml of a 0.08 M $NaH_2PO_4*H_2O$ buffer with 1 mM EDTA in a 25 ml Erlenmeyer flask (pH 7.6 for marine mammals, pH7.4 for sea birds). 3 µl solutions of mixtures of the brominated flame retardants and a number of chlorinated biphenyls dissolved in acetone were added with a 10 µl Hamilton syringe to the erlenmeyer flasks for incubation. Substrate concentrations are mentioned above (refer to "organohalogens"). Assays were carried out in a water bath at 37°C. After 3 min pre-incubation, 0.1 ml of a 11 mM NADPH solution was added every 10 min to four assay samples; four other samples that did not get NADPH served as reference samples where no reaction could occur. The reaction was terminated after 90 min. or less by the addition of 1 ml ice-cold methanol and 1 ml of milli-Q water to the samples. In total, each

assay consisted of 9 samples: 4 reference samples without NADPH, and 4 assay samples with NADPH, and one blank without either incubation mixture or NADPH, which was used to check for interfering or co-eluting peaks due to the analytical procedure. All samples were run simultaneously.

2.1.10: Analysis of organobromines:

Extraction and clean-up: the samples were extracted after the incubation with hexane/methanol by the addition of 2 ml hexane, followed by 30 seconds of intensive shaking by a Vortex mechanical shaker and 5 minutes of centrifugation at 2500 rpm. The upper hexane fraction was transported to a test tube. This procedure was repeated three times. Subsequently, the 8 ml of hexane was treated with 0.5 ml portions of concentrated sulphuric acid to remove lipids (23). The PBDE, PBB and PCB congeners were found to be resistant to this acid treatment. The test tube was shaken twenty times. The lower sulphuric acid fraction was removed and the hexane was washed six times with approximately 8 ml water each time. After each washing step, the increase in the pH was checked. When the pH had risen above a value of 6, approximately 0.4 ml of 2,2,4-trimethylpentane (TMP) was added and the hexane was evaporated, using a micro-Snyder column. The remaining TMP was transferred to a 1ml GC vial for injection (final sample volume 0.5 ml).

GC analysis: separation and identification of the CB and BB congeners was performed on a Carlo-Erba 5160 gas chromatograph with an electron capture detector (GC-ECD). A 25m*0.32 mm * 0.12 μm CP-Sil-8CB semi wide bore column was used (Chrompak No. 907243). The initial chromatograms of the PBDE mixture as obtained with a narrow bore column, showed strongly tailing peaks of especially BDE-209, whereas the high separation power offered by this column was unnecessary for the relatively simple mixtures used in this study. The carrier gas was hydrogen; pressure 100 kPa, linear gas velocity 107 cm/sec. The injection port and detector temperatures were kept at 250°C and 340°C respectively. The oven temperature programme used for the PBDEs was: initial temperature 90°C for 2 minutes, followed by a temperature increase with 20°C min^{-1} to a final temperature of 300°C, which was kept for 15.5 min. For the PBB mixture a slightly different temperature programme was used: initial temperature 90°C for 2 minutes, followed by a temperature increase of 10°C min^{-1} to 215°C, which was kept for 5 minutes, followed by another temperature increase of 10°C min^{-1} to a final temperature of 250°C, which was kept for 16 minutes. 1 μl injections were made with an autosampler.

2.1.11. Statistical treatment

With the analytical results, calculations were made to determine a difference in peak heights between the reference samples and the assay samples. The following calculations were made: first the ratio of each compound with the internal standard (CB-153) was calculated (Ratio-153) according to the following formula.

$$\text{Ratio-153} = \frac{H_{\text{Compound X}}}{H_{\text{CB-153}}},$$

where H is peak height. The ratio-153 values of the reference samples were then divided by the ratio-153 values of the standard mixture to check their recovery. It was stated that there should be a minimal recovery of 75 % of each individual congener in the reference sample. The significance of the difference in Ratios-153 of PCBs, PBDEs and PBBs between assay and reference samples was tested with analysis of variance (ANOVA) or student's t-tests on log-transformed data (to obtain homogeneity of variance), using the SYSTAT 5.0.4 programme.

For a graphical display in bar diagrams, the remaining fraction of each compound in the assay was determined by dividing its Ratio-153 value in a randomly chosen assay sample by the value in a randomly chosen reference sample according to the equation:

$$Rf = \frac{Ratio-153^{assay}_{compoundX}}{Ratio-153^{reference}_{compoundX}}$$

R*f* is the remaining fraction of compound X after biotransformation. Means were calculated for the four combinations of assay and reference samples.

2.2: Wildlife residues

2.2.1: Analysis of organobromines: PBBs and PBDEs were determined in 16 wildlife samples of lung-breathing animals feeding on invertebrates and/or fish: whitebeaked dolphin (*Lagenorhynchus albirostris*; main food source fish), sperm whale (*Physeter macrocephalus*; main food source squid and fish from deep water), minke whale (*Balaenoptera acutorostrata*), harbour seal (*Phoca vitulina*; main food source fish), eider duck (*Somateria*

mollissima; main food source invertebrates) and knot (*Calidris canutus*; main food source bivalves). A mackerel sample from the southern North Sea was also analysed as a proxy for PBDE and PBB concentrations and patterns in the food of seals and dolphins.

2.2.2. Extraction and clean-up:

The initial extraction procedure with dichloromethane/pentane resulted in low recoveries, particularly of the decabrominated congeners BB 209 and BDE 209, which were not found at all after extraction. Soxhlet extraction with hexane/acetone (3:1 v/v) at 70° C on a water bath resulted in much better recoveries. After silica gel fractionation the major part of the PBBs was found in the first fraction, whereas the PBDEs eluted in the second fraction. However, BDE-209 eluted completely in the first silica fraction. With this procedure, all PBB recoveries were > 85%, whereas all PBDE recoveries were > 70% in comparison to untreated standard solutions. All results reported in this paper have been corrected for recovery.

Following extraction, the samples were treated with concentrated sulphuric acid to remove the lipids. The silica gel fractionation was carried out in the following way. Columns (6 mm i.d.), 1.6 g SiO_2. 1.5%H_2O (Merck no. 7754, 63-200 µm, elution: 11 ml TMP (fraction 1) and 10 ml di-ethylether/TMP (1:5, v/v).

2.2.3. GC/MS analysis:

The samples were analysed on a HP-5988A GC/MS in the negative chemical ionization (NCI) mode. Bromine containing compounds were selectively detected by selective ion monitoring (SIM) at the masses of both existing isotopes of bromine with masses of 79 and 81 occurring at frequencies of 50.69% and 49.31 % respectively.

GC-conditions:

Method of injection: splittles, splitter open after 3.0 minutes

Column: DB5; length* internal diameter * film thickness = 20 m * 0.25 mm * 0.25 µm

Temperature program: 90 °C (3'), 30 °C/min. -> 180 °C, 5 °C/min. -> 285 °C (50'); post run at 290 °C (5').

carrier gas : helium

linear gas velocity : ~41 cm/sec t°= 48 sec

MS-conditions:

Method of ionisation : negative chemical ionisation

Reagent gas : methane

T source : 100 °C

T injector : 285 °C

T analysator : 120 °C front and 75 °C back

T transferline : 280 °C

P column : 160 kPa

P analysator : ~5 • 10^{-6} torr (- reagent gas)

P source : ~4 • 10^{-6} torr (- reagent gas)

: ~3 • 10^{-4} torr (+ reagent gas)

P methane plasma : 1 torr

m/z ratios: 79 and 81 (Br^-)

2.3: Genotoxicity

A positive genotoxic response in the Mutatox® assay can be caused by five different modes of action (42): base substitution, frame-shift agents, DNA-damaging agents, DNA synthesis inhibitors, and DNA-intercalating agents. Only the first two processes result in genetically stable revertants. This bio-assay uses a dim variant of the normally photoluminescent bacterium *Vibrio fisheri*. The presence of genotoxic compounds may result in mutations and consequently in a restoration of photoluminescence (*direct genotoxicity*). A rat liver S9 homogenate is available from the supplier to test for the presence of compounds that need biotransformation to express genotoxicity (*indirect or induced genotoxicity).*

Procedure: In the present study, the genotoxicity of PBDEs and PBBs was evaluated. According to the protocol of the Mutatox® test (43), all compounds or mixtures should be tested over a range of concentrations. Stock solutions of contaminants or mixtures thereof were made in acetone. The highest concentration tested (initial concentration) was made by adding 10 µl of a stock solution to 500 µl of growth medium. Of a given initial concentration, a series of nine serial 1:1 dilutions with growth medium was made. Thus, the minimum concentration tested was always $1/2^9$=1/512 of the initial concentration. Of the PBDEs, the technical mixture Bromkal® 70-5DE was tested in two series with initial concentrations of 0.035 and 0.89 µg.ml^{-1}, whereas BDE-209 was tested in two series with initial concentrations of 0.35 and 9.86 µg.ml^{-1}. Of the PBBs, a mixture containing only the brominated congeners listed in table 2 was tested in two series with initial concentrations of 0.863 and 8.63 µg.ml^{-1}. Cuvettes with S9 were pre-incubated for 45 minutes at 35.0 ± 0.1 ° C to activate the

biotransformation enzymes. All cuvettes, both with and without S9 were incubated at 27.0 ± 0.1 ° C for 22 hours, recording the light levels at intervals of one hour. Blank controls (acetone + media) were run to determine spontaneous mutations (i.e. not induced by the test compound). Positive controls with phenol and benzo[a]pyrene dissolved in acetone were run according to the protocol. A positive genotoxic test result in the assay was scored when the test response was higher than 4 times the response of the solvent + media blank (44).

Quantification: the original test protocol allows for qualitative results only ("+" or "-", i.e. yes or no genotoxicity). Measuring light levels from 10 to 24 hours after incubation at one hour intervals allows for a more quantitative interpretation. The test compound concentration that gives the maximum response (CMR) is calculated by quadratic regression through the three data points that construct the top of the dose-response curve. The lowest concentration that still gives a positive test result (LOEC) is determined by linear interpolation between the two data points that gave responses just below and above the 4 times the blank value.

3. Results

3.1. In vitro *Assays*

3.1.1. PBDEs:

3.1.1.1. EROD activity of the microsomal preparations.

Table 3 shows the microsomal protein content and the EROD activities. The harbour seal microsomes were the most active. The value of 537pmol/min/mg protein is an average one for harbour seal when compared to previous measurements (35). The whitebeaked dolphin, common eider duck and sperm whale displayed EROD activities of more than an order of magnitude lower than the harbour seal. The sperm whale was just above the minimum of 10 $pmol \bullet mg^{-1}$ $protein \bullet min^{-1}$.

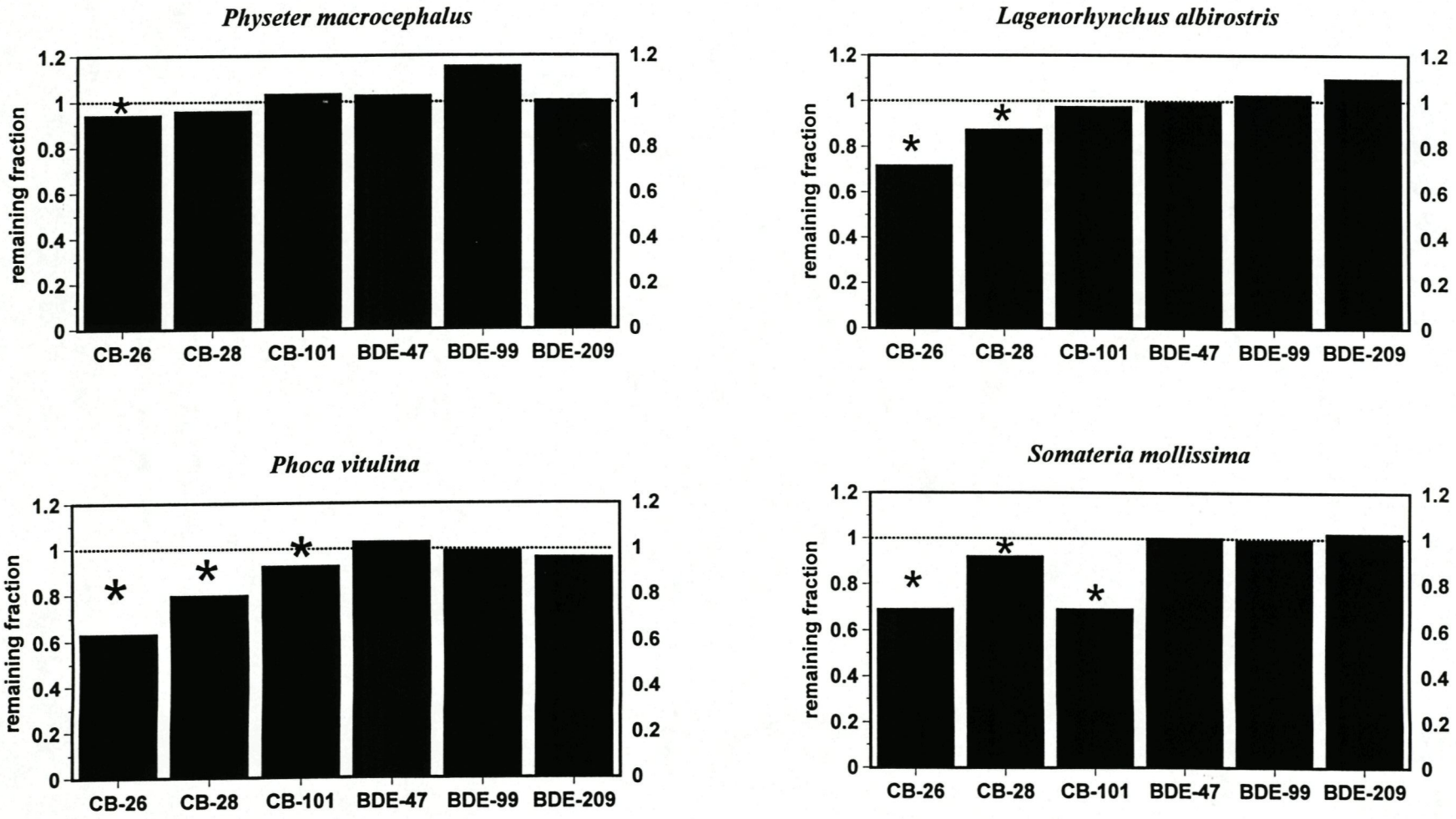
Table 3. Protein content and EROD activity in liver microsomes of marine mammals and birds used for measurements of PBDE metabolism.

Species	Protein content ($mg \bullet ml^{-1}$)	EROD activity ($pmol \bullet mg^{-1}$ $protein \bullet min^{-1}$)
Harbour seal	11.9	537
Whitebeaked dolphin	41.8	25.6
Sperm whale	8.2	10.6
Eider duck	14.3	33.6

3.1.1.2. In vitro bioassays

The results of the biotransformation assays are presented in **fig. 3**. The harbour seal, whitebeaked dolphin and common eider duck showed a significant decrease of CB-26 and CB-28. The common eider duck was also able to reduce CB-101 significantly. In contrast to this behaviour of the positive controls, no significant decrease of the BDE congeners in any of the investigated marine mammals and birds was visible. The sperm whale only showed a small but significant (P=0.012), decrease of CB-26.

Fig. 3: Biotransformation of BDE & CB congeners



3.1.1.3. Competitive substrate inhibition

Although the PBDE congeners investigated were apparently not metabolised by the cytochrome P-450 system, their presence may still interact with the metabolism of other compounds. To investigate whether the PBDEs competitively inhibited the metabolism of the chlorobiphenyl congeners used as positive controls, the decrease of CBs was compared between assays incubated with CBs only and assays incubated with a mixture of CBs and BDEs. **Fig. 4** shows that there was no difference in the biotransformation rates of the CBs of harbour seal microsomes.

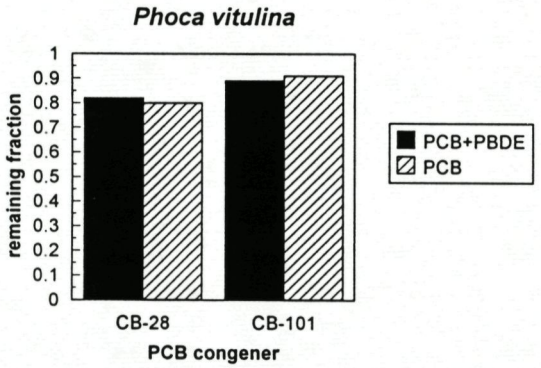


Fig. 4: Inhibition of the in vitro biotransformation of two CB congeners in the presence of PBDEs.

3.1.2: PBBs

3.1.2.1 EROD Activity of microsomal preparations

The results of the EROD activity determination and the protein content of the microsomes are shown in Table 4. As can be seen in the table, the seal had the highest activity whereas the eider duck showed the lowest enzyme activity.

Although the enzyme activity of the seal was the highest, it is still quite low compared with an earlier measured value for the seal samples used for studies on PBDE metabolism.

In contrast, the enzyme activity of the sperm whale sample is rather high in comparison to previously measured values; 10.6 pmol• mg^{-1} protein•min^{-1} for the PBDE assays and 17 pmol• mg^{-1} protein•min^{-1} for previous assays on the biotransformation of chlorobornanes

(toxaphene; (3).

The activity value of the whitebeaked dolphin is extremely close to the value for the samples used for the PBDE studies. (49.3 pmol•mg^{-1} protein•min^{-1}) but low when compared to the range reported for the striped-dolphin (35).

Table 4. Values of the EROD activity and the protein content of the microsomal preparations used for the *in vitro* assays of PBB metabolism.

Animal	Protein content (mg•ml^{-1})	EROD activity (pmol•mg^{-1} protein•min^{-1})
Harbour seal	39.0	149.3
Whitebeaked dolphin	57.2	48.9
Sperm whale	11.3	41.9
Eider duck	14.3	33.6

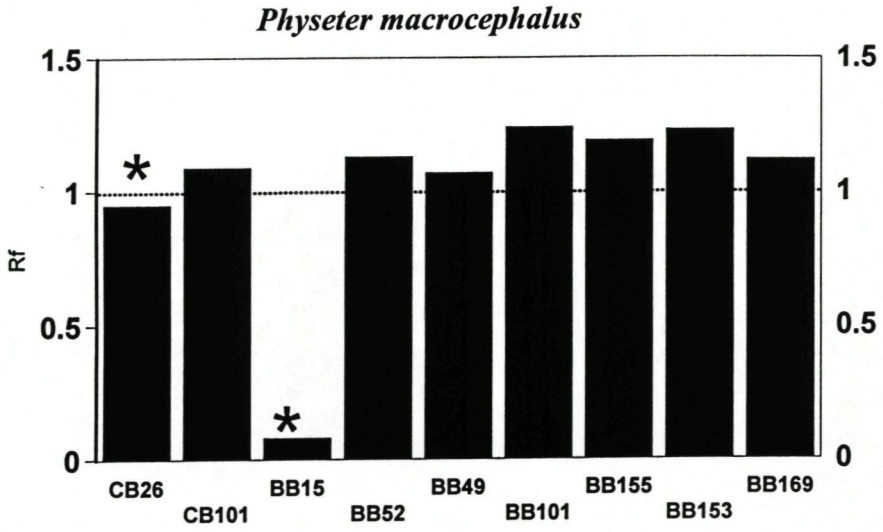
3.1.2.2. In-vitro Bioassay

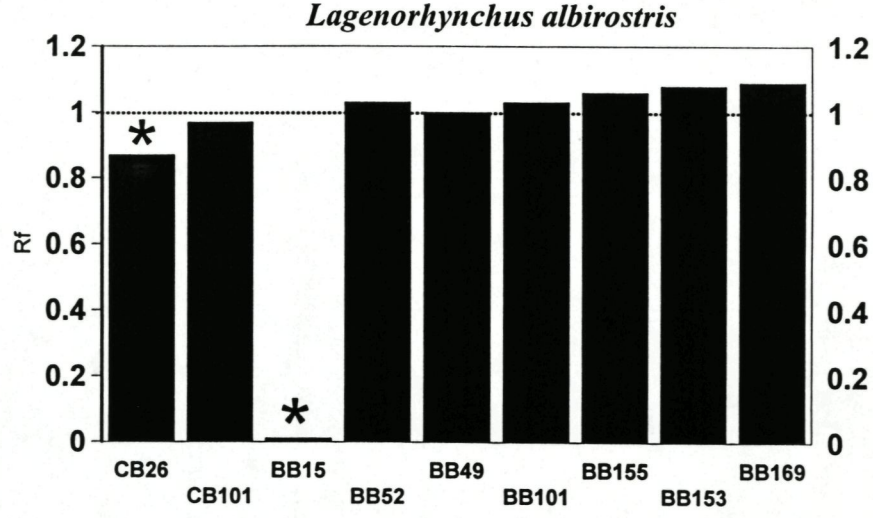
The results of the *in-vitro* assays are shown in **fig. 5**. As can be seen, the marine mammals were able to metabolize the BB-15 congener whereas the eider duck could not. Furthermore, all the animals showed a significant decrease of CB-26 except for the sperm whale. This is in contrast to the PBDE assays, where the decrease of CB-26 was significant. Only the eider duck was capable of reducing CB-101 significantly. All four animals were unable to metabolize the other BB congeners. Thus, in the eider duck CB-101 seems to be more easily metabolisable than BB-101.

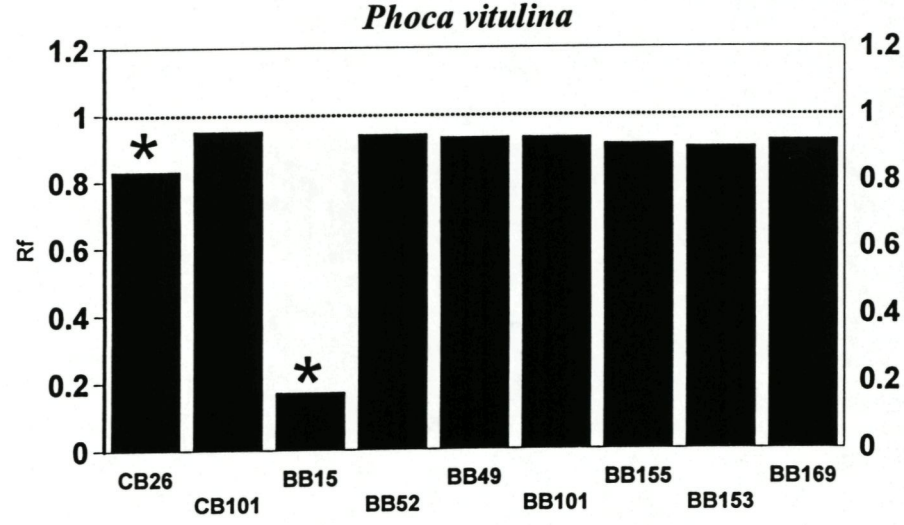
3.2. Wildlife residues

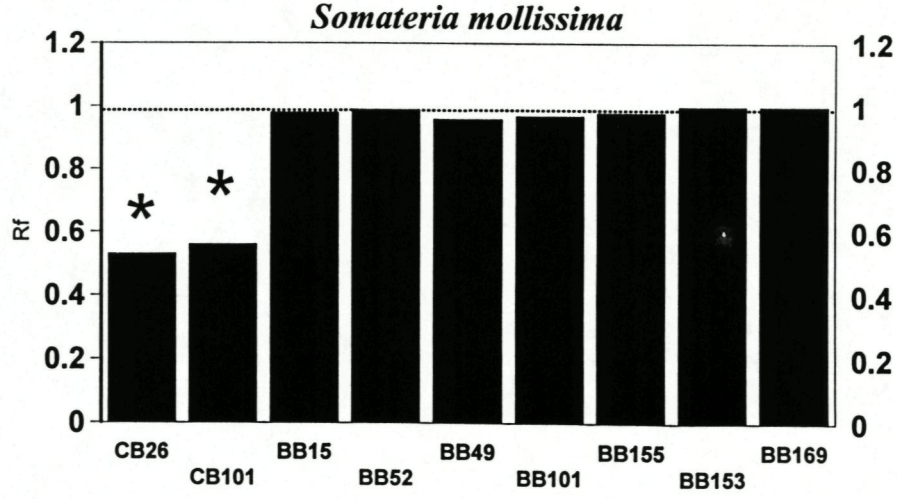
Representatives of both groups of brominated flame retardants were found in all samples investigated, showing that these compounds are widely distributed in the marine environment (**Table 5; figures 6 and 7**). Samples of blubber of a minke whale (*Balaenoptera acutorostrata*) and adipose tissue of knot (*Calidris canutus*) have been analysed additionally to the species used for *in vitro* biotransformation studies. Minke whale is a representative of the baleen whales (*mysticeti*). The knot is a wading bird species that migrates over enormous distances between tropical areas in West Africa in winter to breeding areas in the Arctic in

Fig. 5: In vitro Biotransformation of BB & CB congeners









■ = Average remaining fraction;

* = Significant decrease in assay ($P<0.05$)

Table 5: Concentrations of polybrominated biphenyls (BBs) and bromodiphenyl ethers (BDEs) in marine wildlife. All samples were taken on the Dutch coast of the North Sea or the Wadden Sea. Concentrations below the detection limit are printed in italics. (B&D): Extraction according to Bligh and Dyer. BDE-xy is a penta-BDE with an unknown bromine substitution pattern. *: Graphical representation of this sample in figure 6 (PBDEs) and 7 (PBBs).

Species	Tissue	NIOZ Lab-code	RIVO LIMS-no.	% free Fat	% Fat (B&D)	BB-15	BB-52	BB-49	BB-101	BB-153	BB-169	BB-209	BDE-47	BDE-xy	PDE-99	BDE-209
Odontocetes (Cetaceans)																
Physeter macrocephalus	blubber	95PM1	15920	56.40	72.2	0.06	0.24	0.40	0.91	1.90	*<0.08*	*<0.5*	95.0	14.8	26.0	*<6*
Physeter macrocephalus *	blubber	95PM2	15871	25.40	23.4	0.04	0.13	0.21	0.40	0.73	0.052	*<0.3*	58.0	8.1	15.0	*<3*
Physeter macrocephalus	liver	95PM2	15872	4.01	2.33	*<0.01*	*<0.01*	*<0.01*	0.63	17.50	*<0.04*	*<0.3*	2.70	0.54	0.91	*<3*
Physeter macrocephalus	blubber	95PM3	15870	35.80	31.7	0.07	0.20	0.36	0.70	1.10	*<0.07*	*<0.4*	61.0	7.50	10.20	*<5*
Lagenorhynchus albirostris *	blubber	95 LA1	15926	96.10	99	0.2	7.50	4.10	8.30	13.10	*<0.15*	*<0.9*	5500	1200	1000	*<10*
Lagenorhynchus albirostris	liver	95 LA1	15927	2.80	2.7	*<0.005*	0.06	0.03	0.74	19.20	*<0.02*	*<0.1*	21.80	5.80	3.00	*<1*
Mysticetes																
Balaenoptera acutorostrata *	blubber	94BA1	15921	12.90	14	0.11	0.27	0.24	0.54	0.82	*<0.02*	*<0.1*	88.00	11.20	23.00	*<1*
Pinnipeds																
Phoca vitulina	blubber	92PVa	15922	24.40	n.b.	<0.05	34	5.70	9.30	61.1	12.0	*<1.3*	1200	110	160	*<15*
Phoca vitulina	liver	94PVa	15923	2.70	3	<0.005	0.90	0.14	0.44	13.0	*<0.02*	*<0.1*	19.71	0.070	0.53	*<2*
Phoca vitulina *	blubber	96PVa	15928	82.60	96.3	*<0.04*	3.10	2.30	1.40	1.50	*<0.15*	*<0.9*	1200	100	40	*<10*
Phoca vitulina	liver	96PVa	15929	5.20	3.5	*<0.005*	0.10	0.05	0.62	17.50	*<0.02*	*<0.1*	20.70	0.93	0.85	*<2*
Phoca vitulina	blubber	96PVc	19488	72.20	n.b.	*<0.03*	3.00	0.52	1.10	13.1	*<0.13*	*<0.8*	280	18.40	140	*<10*
Phoca vitulina	liver	96PVc	19489	5.10	n.b.	*<0.004*	0.10	0.03	0.04	0.82	*<0.014*	*<0.09*	12.30	0.33	5.10	*<1*
Pisces																
Scomber scombrus *	muscle		19490-1	15.20	15.2	0.02	0.01	0.01	*<0.007*	0.040	*<0.03*	*<0.2*	5.60	0.59	1.60	*<2*
Scomber scombrus	muscle		19490-2	14.00	15.2	0.007	0.01	0.01	*<0.007*	0.030	*<0.025*	*<0.2*	5.20	1.00	2.10	*<2*

table 5 (continued)

Species	Tissue	NIOZ Lab-code	RIVO LIMS number	% free Fat	% Fat (B&D)	BB-15	BB-52	BB-49	BB-101	BB-153	BB-169	BB-209	BDE-47	BDE-xy	PDE-99	BDE-209
Aves																
Somateria mollissima	liver	97SMa	20331	2.10	2.6	*<0.003*	0.10	0.02	*<0.006*	0.61	*<0.01*	*<0.06*	0.78	3.20	0.72	*<0.7*
Somateria mollissima	liver	96SMa	20332	4.00	4.4	*<0.003*	0.07	0.04	*<0.003*	1.88	*<0.011*	*<0.07*	0.46	21.50	2.60	*<0.8*
Somateria mollissima	liver	97SMb	20333	3.10	2.7	*<0.002*	0.23	0.03	0.06	1.90	*<0.01*	0.20	2.30	13.70	8.80	*<0.7*
Somateria mollissima	liver	97SMc	20334	2.10	1.9	*<0.002*	0.05	0.020	0.02	1.60	*<0.01*	0.20	0.62	6.80	2.10	*<0.7*
Somateria mollissima *	liver	97SMd	20335	3.10	2.5	*<0.003*	0.15	0.040	0.07	3.20	*<0.011*	*<0.07*	1.50	9.20	4.80	*<0.8*
Calidris canutus *	adipose depots	96CCal-6	15512	49.50	38.4	*<0.02*	0.03	*<0.02*	0.05	1.80	*<0.09*	*<0.6*	49.00	4.40	5.60	*<6*

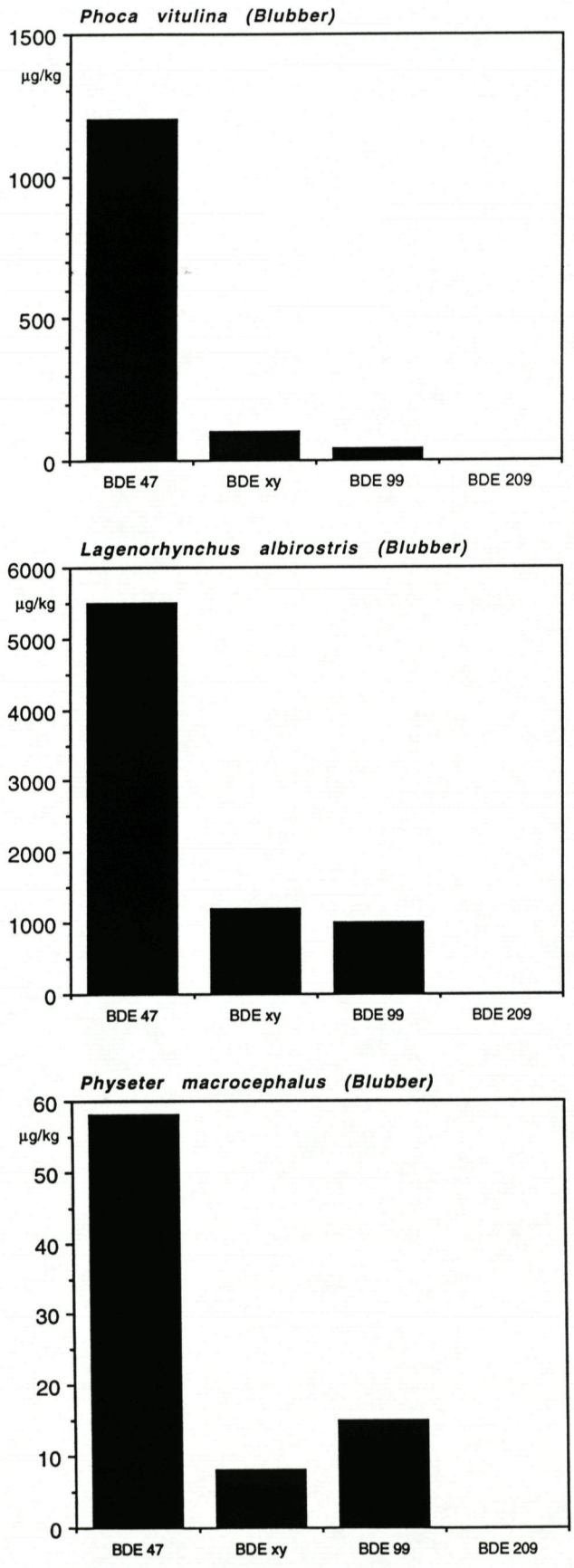


Fig. 6: PBDEs in marine wildlife tissues

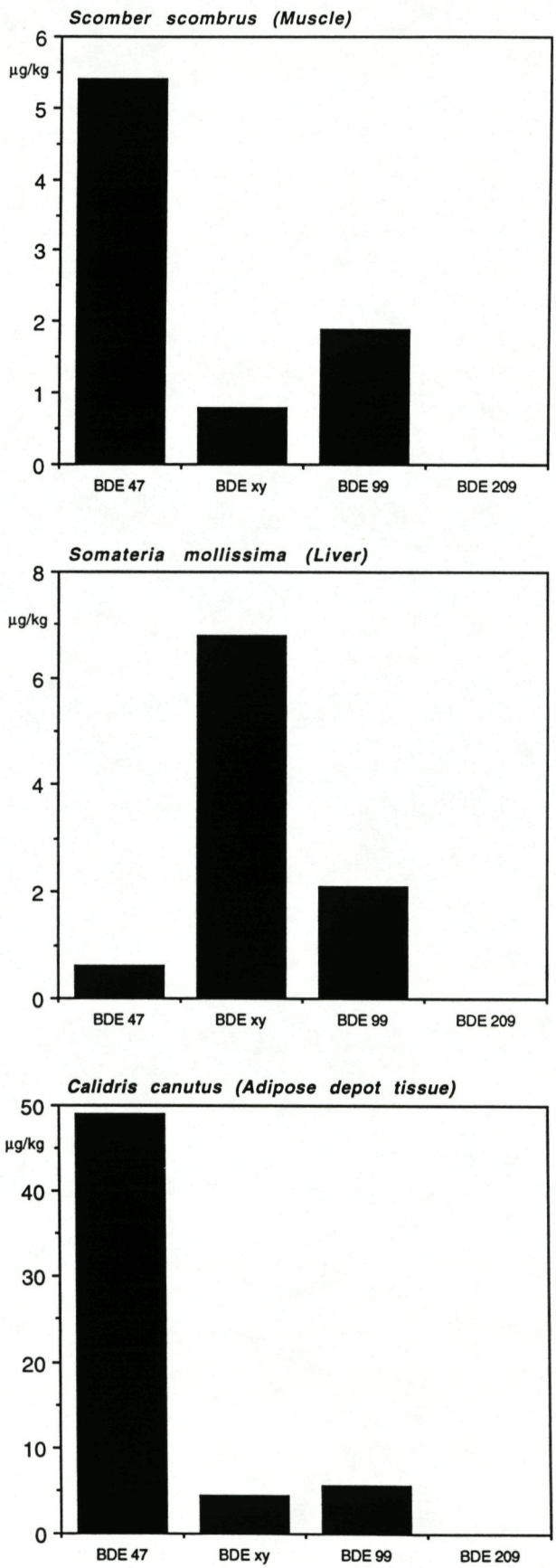


Fig. 6 (continued): PBDEs in marine wildlife tissues

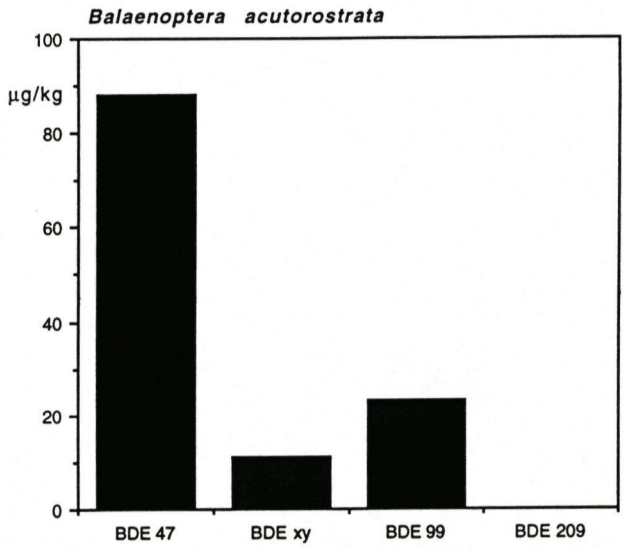


Fig. 6 (continued): PBDEs in marine wildlife tissues.

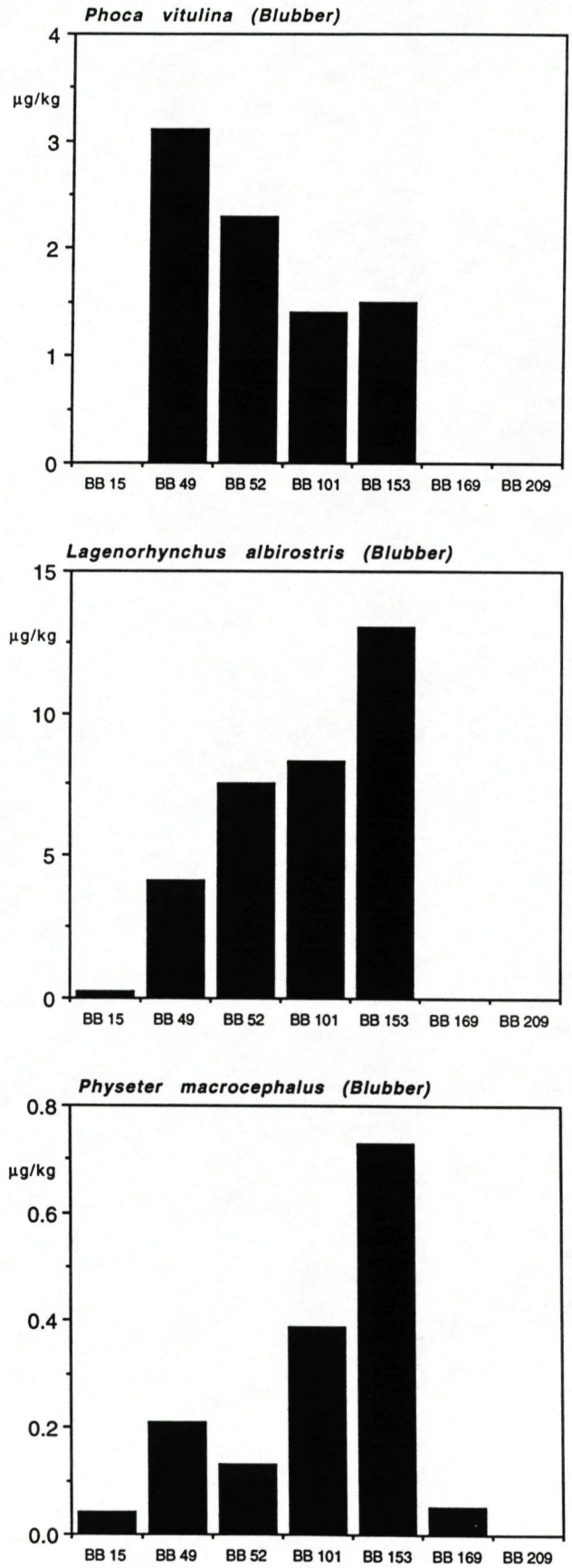


Fig. 7: PBBs in marine wildlife tissues.

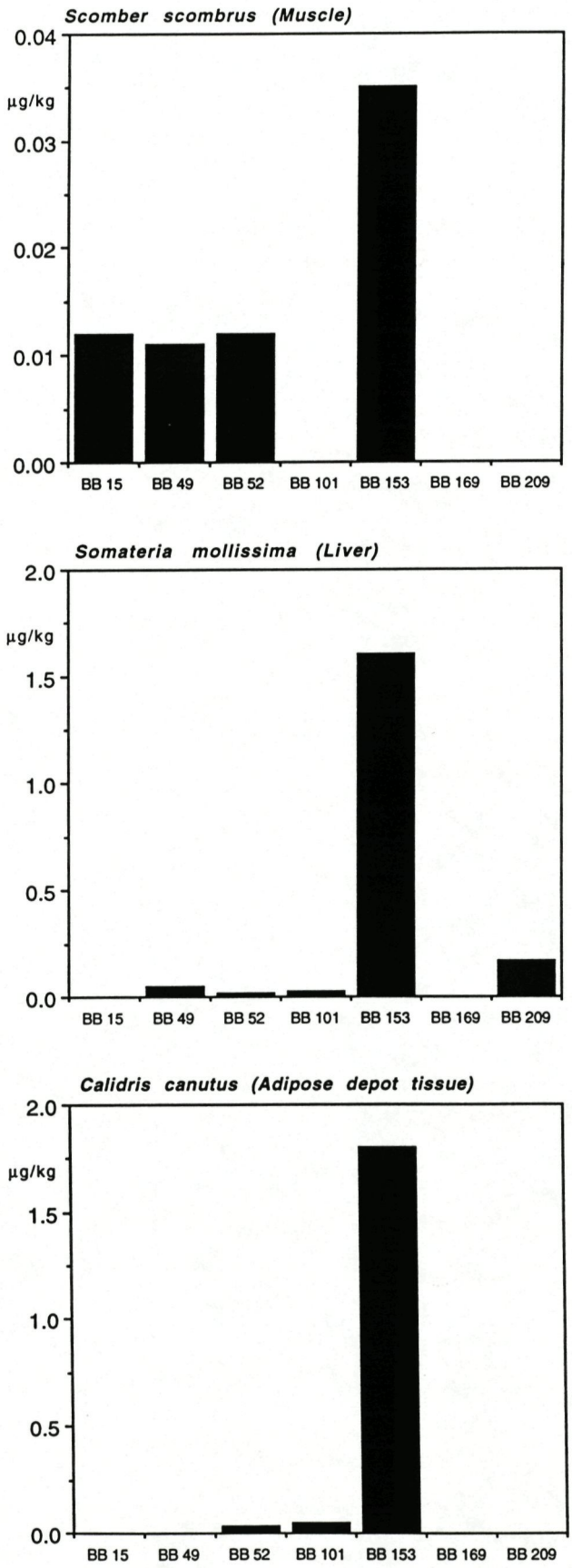


Fig. 7 (continued): PBBs in marine wildlife tissues.

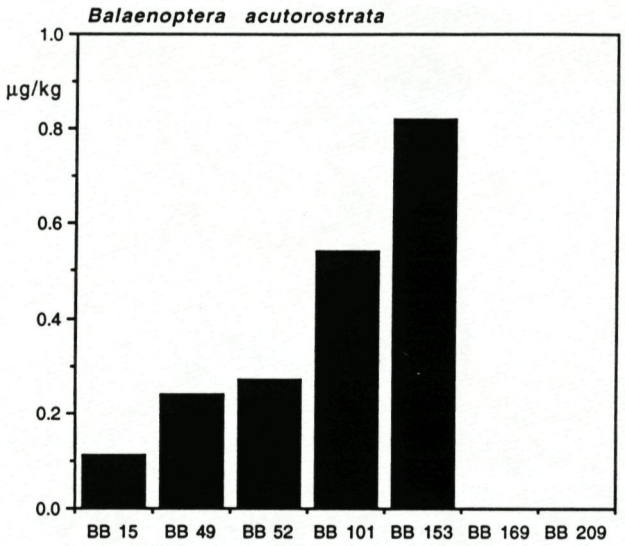


Fig. 7 (continued): PBBs in marine wildlife tissues.

summer, for which the availability of the Wadden Sea as a halfway refuelling station is essential to obtain enough energy for the whole journey (45). Samples of mackerel (*Scomber scombrus*) from the North Sea were added to get a first impression of the level and pattern in fish that may serve as food for marine mammals.

The PBDE concentrations in mackerel varied from <1 to 5.4 µg/kg wet weight per congener (**fig. 6**), which is considerably higher than the PBB concentrations (fig. 7). BDE 47 (2,4,2',4') shows the highest concentration, followed by BDE 99 (2,4,5,2',4') and BDE-*xy*, a penta-BDE with an unknown substitution pattern. The concentration of BDE 209 is below the detection limit. The PBDE concentrations are highest in the whitebeaked dolphin blubber sample, where BDE-47 reaches a level of 5500 µg/kg wet weight. BDE-47 is the dominating congener in all marine mammal samples. The BDE concentrations in the livers are clearly lower than in the blubber samples. BDE 209 is not found above its detection limits. The PBDE concentrations in seal and dolphin are at least 50-fold higher than the PBB concentrations. The PBDE pattern in birds differed quite considerably between eider duck liver and knot adipose tissue. In knot the pattern was similar to that of the marine mammals, but in eider duck BDE-*xy* dominated over BDE-99 and -47. BDE 209 was not present above detection limits.

The BB concentrations in mackerel are below 0.04 µg/kg and relatively low compared to the marine mammals and birds (**fig. 7**). The PBB concentrations in seal and dolphin blubber and liver vary between <0.01 and 13 µg/kg wet weight per congener. The PBB concentrations in the livers are generally lower than in the blubber samples. BB-153 shows the highest concentrations in all samples except seal blubber. The high BB 153 concentration is comparable to the dominating chlorobiphenyl (CB) 153 concentrations in PCB patterns. BB-209 was not found above detection limit; whereas BB 169 was only found once. Compared to mackerel and the marine mammals, the dominance of BB-153 in the chromatograms of eider duck liver and knots adipose tissue is much stronger. In contrast with all other samples, BB 209 could be quantified in two eider duck livers. BB-15 shows higher relative concentrations in mackerel than in the marine mammals. This is in accordance with the observed metabolism in the *in vitro* assays. However, BB-15 was also relatively low in the bird samples; but it was not metabolisable by the eider duck microsomes.

Besides the compounds reported here, another hexabromobiphenyl was often detected. This congener showed an almost equal retention as BB-155 on a CP Sil8 column, but small differences suggest that there is some doubt on its identity. In addition, the substitution

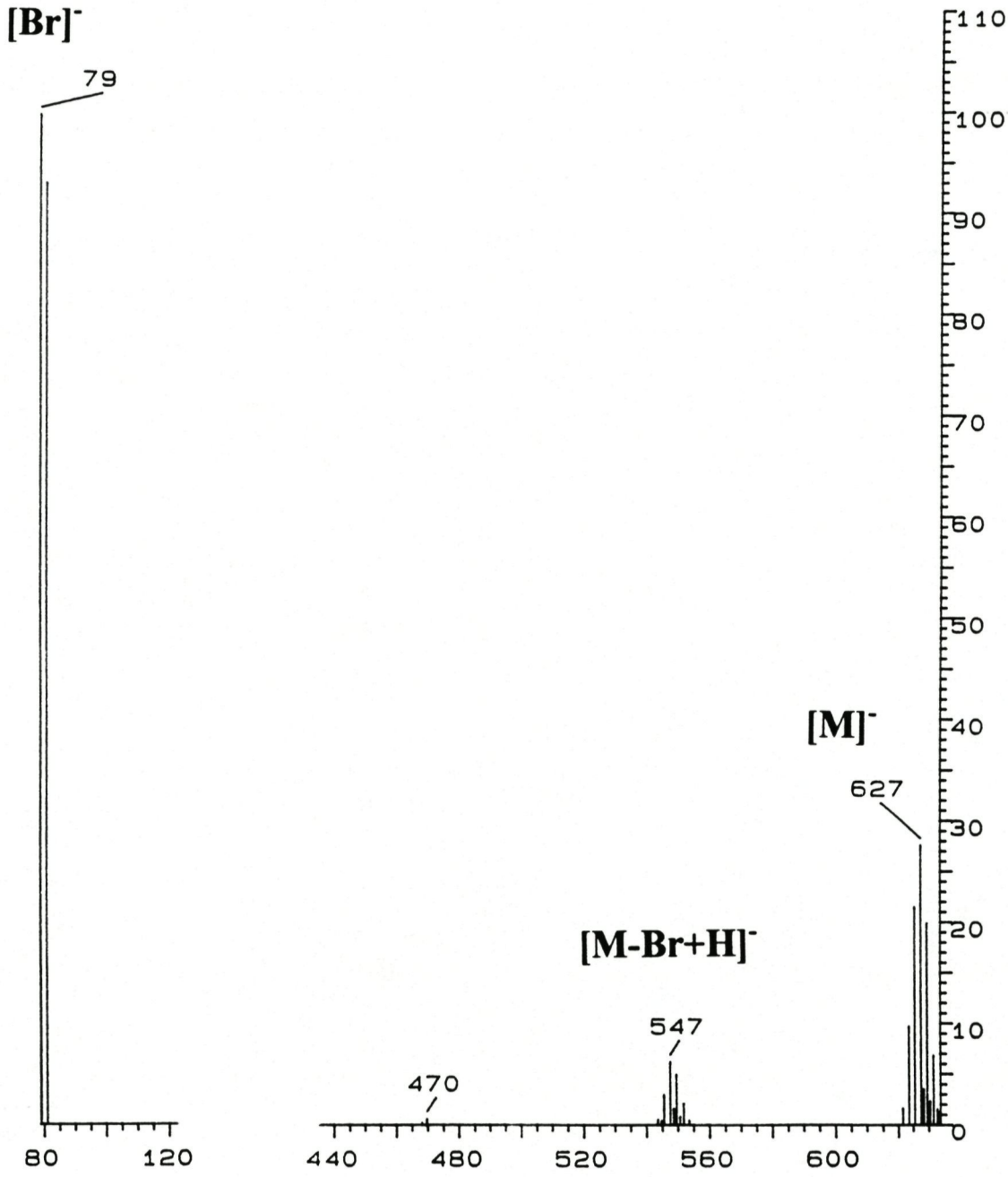
pattern of BB 155 (2, 2', 4, 4',6, 6' hexabromo) is an unfavourable one, and this congener is not found in the technical bromobiphenyl mixtures Firemaster BP-6 and FF-1 (46). However, a mass-spectrum of the compound in wildlife tissue confirmed that it is indeed a hexabromobiphenyl (**fig. 8**). Analysis on a CP Sil19 column with electron capture detection showed equal retention times of this peak and a BB 155 standard. However, a strong correlation exists between CP Sil8 and CP Sil19 for *ortho* substituted PCBs (47). Therefore, the co-elution of an unknown hexabromobiphenyl with BB-155 can not be excluded. A GC-column with a stationary phase that does not correlate so strong with CP Sil8 should be used in combination with mass spectrometric detection to obtain more information on possible differences in retention times between this compound and BB-155.

The PBDE concentrations in the knot sample were about 10-20-fold higher than the PBB concentrations, which is similar to the marine mammals. The PBDE concentrations in eider duck livers however, were comparable or only slightly higher than the PBB concentrations.

3.3. Genotoxicity

No genotoxic responses were observed for PBDEs and PBBs in either the direct Mutatox® assay or in the presence of the rat S9 fraction.

Fig. 8: Full-scan NCI mass spectrum of the unknown hexabromobiphenyl. (Extracted from eider duck liver).



4. Discussion

4.1. Biotransformation

According to the results of the three metabolisable CB-congeners and the EROD activity measurements, microsomes of all investigated marine mammals and birds, even the sperm whale, were able to perform at least one cytochrome P-450 mediated biotransformation reaction. When comparing the measured EROD activity values from the harbour seal, whitebeaked dolphin, and eider duck as found in this study with those of previously used samples from different animals, it becomes clear that the EROD values cover a wide range (2).

Still, the results support the pattern observed earlier for PCBs (48) and toxaphene (3), that harbour seal hepatic microsomes are more active in metabolising xenobiotics than hepatic microsomal preparations from cetaceans. Strikingly, only the avian hepatic microsomes were able to biotransform CB-101, but not BB-101, significantly. However, earlier results obtained with microsomes of another harbour seal and a harbour porpoise showed, that CB-101 is metabolisable (49). This is also supported by the occurrence of methylsulfone metabolites derived from this congener in grey seal (*Halichoerus grypus*) and beluga whale (*Delphinapterus leucas*) (50, 51), and by the results of pharmacokinetic modelling of wildlife residues in blubber of seals and cetaceans (48). Thus, a negative result in the assay does not guarantee that the compound is totally persistent in the environment, and a threshold in the sensitivity of the *in vitro* assay has to be taken into account. A proper way to avoid such 'false negative results' is to complement the results of the *in vitro* assays with the results of residue pattern comparisons of mammals, birds, and their food sources (48).

No evidence was obtained for any reduction of the three BDE congeners (BDE-47, -99, -209) investigated in the *in vitro* assay. Of the PBBs tested, only BB-15 was metabolized to a significant extent. When it is assumed that PBDEs and PBBs follow the same "rules" for biotransformation as the structurally related chlorinated biphenyls (table 6) (35), the result for the PBDEs could be expected, since BDE-47, BDE-99 and BDE-209 all possess a molecular configuration which is highly resistant to enzymatic attack in seals, whales and birds (32). For the PBBs tested, this is also true for the congeners BB-153, BB-155, and BB-169. In contrast, the apparent lack of metabolism was unexpected for BB-49, BB-52,

Table 6. Structural characteristics of all individual halogenated biphenyl congeners and diphenylethers determining their assignment to metabolic groups I-VI as defined by Boon *et al.* (32). This list can be used for PBBs, PCBs and PBDEs.

Syst. no.[1)]	Halogen substitution at	nr. pairs *o,m* vic. H-atoms	nr. pairs *m,p* vic. H-atoms	nr. *ortho*-Cl atoms	metabolic group [2)]	P450 induction[3)]
15	4,4'	4	-	0	III	2B
26	2,3',5	1	2	1	VI	
28	2,4,4'	3	-	1	III	
47	2,2',4,4'	2	-	2	II	
49	2,2',4,5'	1	2	2	IV	none
52	2,2',5,5'	-	2	2	IV	none
99	2,2',4,4',5	1	-	2	II	
101	2,2',4,5,5'	-	1	2	IV	2B
153	2,2',4,4',5,5'	-	-	2	I	2B
155	2,2',4,4',6,6'	-	-	4	I	
169	3,3',4,4',5,5'	-	-	0	I	1A
209	2,2',3,3',4,4',5,5',6,6'	-	-	4	I	

1): Systematic numbering of congeners according to Ballschmiter and Zell, 1980 (33).

2): Criteria for the assignment of chlorobiphenyl congeners to one of six structural groups with regards to biotransformation (32):

I: Congeners without any vicinal hydrogen (H) atoms

II: Congeners with vicinal H atoms **exclusively** in the *ortho*- and *meta*-positions in combination with ≥ 2 *ortho*-Cl substituents.

III: Congeners with vicinal H atoms in the *ortho*- and *meta*-positions in combination with ≤ 1 *ortho* Cl.

IV: Congeners with vicinal H atoms in the *meta*- and *para*-positions in combination with ≤ 2 *ortho*-Cl.

V: Congeners with vicinal H atoms in the *meta*- and *para*-positions in combination with ≥ 3 *ortho*-Cl.

VI: Congeners with vicinal H atoms **both** in the *ortho*- and *meta*-positions and *meta*- and *para*-positions in combination with ≤ 1 *ortho*-Cl.

3): Experimental evidence on induction of cytochrome P450 1A or 2B subfamilies in laboratory rodents. When left blank, no data are available (35).

and BB-101, which all possess *meta-, para* vicinal H atoms in combination with two *ortho*-bromine substituents. In rats, metabolism of BBs -49, -52 and -101 was observed especially in animals that were induced with phenobarbital (PB) a few days prior to dosing with PBBs. This treatment specifically induces the iso-enzyme cytochrome P450 2B (CYP2B). Metabolism of BB-15 was strongly enhanced in animals induced with 3-methylcholanthrene (3-MC) in a similar manner. 3-MC is a model inducer for the iso-enzyme cytochrome P450 1A (CYP1A). It was concluded that CYP2B was responsible for the attack on *meta, para* unsubstituted positions of PBBs whereas CYP1A catalysed the oxidation at *ortho, meta* unsubstituted positions (52). A decreased rate of hydroxylation of brominated compared to chlorinated biphenyls has also been reported (53). This may be related to the larger molecular mass of bromine (two isotopes with molecular masses of 79 and 81 respectively) compared to chlorine (two isotopes with molecular masses of 35 and 37 respectively), which would make brominated biphenyls less favourable substrates for P-450 enzymes than chlorinated biphenyls (54). ^{14}C-labelled BDE-47 was eliminated at a very low rate (86% of the dose remaining in the body 5 days after exposure) in orally dosed rats, but in mice the excretion was much more rapid with 53% of the dose being excreted after 5 days (55). Since this occurred mainly via the urine, it was concluded that water soluble metabolites were formed, but their structure was not further elucidated. These results imply, that some of the PBB and the PBDE congeners tested can sometimes be quite rapidly metabolised in laboratory rodents. However, in such studies, the animals were often pre-induced with model compounds before the animal was dosed with a brominated fire retardant.

4.2. Environmental residues.

The BDE 47 concentration in mackerel is ca. 3-fold lower than its concentration reported in herring from the southern North Sea (28). However, the BDE 47 concentration in seal blubber nr. 15928 is higher than tetra-BDE concentrations reported in seal from the Baltic Sea and Northern Ice Sea (24). Jansson and co-workers (18, 26) also reported higher PBDE concentrations than PBB concentrations in the same set of samples (herring, seal, and guillemot). Total PBB concentrations in seal from the Baltic Sea and from waters around Spitsbergen were 90 and 40 µg/kg lipid weight (18), which is somewhat higher than total PBB concentrations in the presently studied seals. PBB concentrations in herring from the Skagerrak, 0.27 µg/kg lipid weight (26) were comparable to PBB concentrations in the presently studied mackerel from the southern North Sea.

Bioconcentration factors (BCFs) for seal/mackerel for PBBs are in the range of 10-1,000. BCFs for seal/mackerel for PBDEs are in the range of 10-100. The differences in biomagnification between the different congeners are relatively small, with exception of the congeners BB 15, BB 169, BB 209 and BDE 209, which were only occasionally found above the detection limits in the biota samples.

In samples from the Baltic of herring and seal, the PBDE residues were enriched in BDE-47 compared to the technical mixture Bromkal 70-5DE and sewage sludge (Table 7) (9, 10). A similar observation was made by Watanabe and co-workers (22). In theory this can either be explained by higher rates of uptake of lower brominated congeners or lower rates of excretion. However, the latter is unlikely in view of the general increase in lipophilicity with increasing bromine content (11) and the observed persistence of both congeners in the present study. Thus, a higher uptake rate of BDE-47 is the most probable explanation. This may be explained by a decreasing membrane passage velocity of bromodiphenyl ether

Table 7. Percentages of PBDE congeners in Bromkal® 70-5 DE and some environmental samples) (9, 10).

	BDE-47	xy-pentaBDE (not defined)	BDE-99
Bromkal® 70-5 DE	44	8	48
Sewage sludge	40	9	51
Seal (Baltic Sea)	89-92	3-5	2-6
Herring (Baltic Sea)	62-80	6-11	9-21

molecules with increasing Br substitution (7). Bioaccumulation coefficients of polybrominated biphenyls have been reported to decrease with increasing bromine substitution (56). The compounds with the highest degree of bromination, such as BDE-209, could hardly pass the cell membrane and were therefore unable to bioaccumulate. On the other hand, Kierkegaard and co-workers (57) did find an increase of BDE-209 in rainbow trout after 120 days of experimental exposure to this congener. This indicates that BDE-209 can be accumulated, but not as fast as lower brominated congeners. After 49 days of depuration the concentration of BDE-209 was decreased in the rainbow trout, whereas the levels of BDE-153 increased, indicating debromination of BDE-209. This type of reductive

dehalogenation occurred probably by enzymes in the intestinal micro flora under anaerobic conditions (19). Another process that may decrease the degree of bromination is photochemical degradation (19). The importance of these processes in the marine environment could be derived from a comparison of BDE patterns in sediment with those of the technical mixture. (9, 10). The relative amounts of the three analysed BDE congeners in sewage sludge and surface sediments of the Bornholm deep in the Baltic Sea were quite similar to the pattern of the technical Bromkal mixture; sewage sludge and Bromkal were even almost identical. This indicates, that microbial degradation is apparently not an important process. Also, samples derived from herbivorous terrestrial mammals showed a pattern similar to Bromkal, but concentrations were generally lower than in aquatic organisms. In fish, the relative amounts of the three BDEs varied between species, but this was attributed to different technical products as sources or differences in uptake and excretion rates for the different congeners.

This means that, when the BDE congeners have entered an animal, the larger part will bioaccumulate because of their lipophilic characteristic, especially the lower brominated diphenyl ethers like BDE-47.

4.3 . A literature review of the toxic effects of brominated fire retardants

4.3.1. PBBs: Most available data about the effects of PBBs on organisms in the environment are data on farm animals from the Michigan disaster. The estimated average exposure of cows at a high contaminated farm was 250 $mg \cdot kg^{-1}$ body weight (58). A few weeks after ingestion of contaminated cattle food, clinical signs were a reduction of about 50% in food consumption (anorexia) and a decrease of around 40% in milk production. Some cows showed an increased frequency of urination and lacrimation, and developed haematomas, abscesses, abnormal hoof growth, lameness, alopecia, hyperkeratosis, and cachexia. Several cows died within 6 months of exposure (59). The death rate in 6- to 18- months old calves was much higher, 50% died within 6 weeks (59, 60).

In contrast of the observed toxicity in cattle in Michigan, in the Michigan population no definitive health effects that could be correlated with PBB exposure have been identified. However, the follow-up period has not been long enough for the development of cancer. In industry it appears that chloro-acne like lesions may develop in workers producing PBB, and hypothyroidism in workers exposed to BB-209 (17). Further *in vitro* studies are necessary to identify toxic effects of PBBs. Controlled long term feeding studies on cattle exposed to low

doses of Fire Master did not reveal any adverse effects as indicated by food intake, clinical signs, clinicopathological changes, or performance. Minks, guinea-pigs, and monkeys appeared to be more susceptible to PBB toxicity (17).

Fire Master BP-6 appears to have a similar acute toxicity to rats as the PCB mixtures Aroclor 1254 and Kanechlor 500 (11). The LD_{50} values of commercial mixtures show a relatively low order of acute toxicity ($LD_{50} > 1$ g•kg^{-1} body weight) in rats, rabbits and quails, following oral or dermal administration. The toxicity of PBBs was higher with multiple dose rather than single dose administration. The few studies performed with commercial Octabrominated bromobiphenyl mixtures and BB-209 did not result in mortality in rats and fish. On the basis of limited, available data, octabrominated biphenyls and BB-209 appear to be less toxic and less absorbed than other PBB mixtures (17). The toxicity of PBB congeners strongly depends on their molecular structure (11, 17). Induction of the P450 1A subfamily of cytochrome P450 is the precursor of a whole spectrum of possible effects at more integrated levels of biological structure: weight loss, thymus atrophy, and changes in the liver such as proliferation of the smooth endoplasmatic reticulum (location of the P450 system), increased RNA and protein content, decreased DNA content, cell necrosis, liver enlargement, and hepatic porphyria (61, 62, 63).

The more toxic congeners cause a decrease in thymus and/or body weight and produce pronounced histological changes in the liver and thymus. Categorisation of halogenated has been made on a structural basis. Category 1 comprises isomers and congeners lacking *ortho*-substituents (coplanar PBBs). Mono-*ortho*-substituted derivatives constitute the second category. Other PBBs (mainly those with two or more *ortho*-bromine substituents) have been organised into the third category. Congeners of Category 1 tend to elicit the most severe effects, while the congeners of the second and third categories show decreasing toxicological changes. Within these categories, the degree of bromination may also influence toxicity. In all combinations tested, 3,3',4,4',5,5'-hexaBB (BB-169) was found to be the most toxic PBB (17).

Several reports on the carcinogenicity of PBBs and PCBs have concluded that there are strong indications that these compounds are not mutagenic in itself, but do promote the carcinogenicity of mutagenic compounds, such as nitrosamine and certain polyaromatic hydrocarbons (PAHs) (11, 16, 46, 63, 64). This is highly relevant, since in the marine environment halogenated compounds often co-occur with PAHs.

The only lifetime study with a technical nonabromobiphenyl mixture was conducted on rats and mice in a recent bioassay. The lowest dose tested that still produced carcinogenic effects on

rodents was 0.5 mg/kg body weight per day (orally), and no observed effect level in a rat was 0.15 $mg \cdot kg^{-1}$ body weight per day (65). The carcinogenicity of technical octabrominated biphenyl mixture and BB-209 has not been studied, although a number of chronic effects have been observed in experimental animals at doses of around 1 $mg \cdot kg^{-1}$ body $weight \cdot day^{-1}$ during long term exposure (17).

After the ingestion of a Fire Master mixture, hyperkeratosis and hair loss were seen in cattle, and lesions resembling chloro-acne were seen in rhesus monkeys (50 $mg \cdot kg^{-1}$ in the diet) (16). After 20 wk, exposure at a dose of 2 $mg \cdot animal^{-1}$ twice a week, Fire Master FF-1 also caused skin papillomas in previously initiated mice (66).

Fire Master BP-6 caused chronic and subchronic neuronal symptoms, such as irritation, changed behaviour and decreased muscular control (16). Immuno-suppression by PBBs occurs at levels that also cause a number of the other toxic effects described (11).

Fire Master FF-1 caused a longer sexual cycle in monkeys (11), and PBBs PBB caused decreased egg production and nesting behaviour in Japanese quail (15). One recent study reported that in mice PBB (di-BBs and tetra-BBs) reduced the *in vitro* fertilisation rate at higher dosages. Furthermore, an increased incidence of abnormal two-cell embryos and degenerative oocytes was observed at the 1 and 10 $mg \cdot ml^{-1}$ concentration of PBB (67).

PBBs also affect the regulation of steroid hormones. The extent depends on the species as well as the dose and duration of exposure. Furthermore, PBBs interact with thyroid hormone production e.g. rats and pigs showed dose related decreases in serum thyroxine and triiodothyronine (17). PBBs produced porphyria in rats and male mice at doses as low as 0,3 $mg \cdot kg^{-1}$ body weight per day (no observed effect level was 0,1 $mg \cdot kg^{-1}$ body weight day^{-1}). There was a pronounced influence of PBBs on vitamin A storage as well as effects on intermediary metabolism (17).

PBDEs: **There is no information available on the toxicity of PBDEs in organisms in the environment.** Commercial DeBDE, OcBDE and PeBDE have been the subject of laboratory toxicological studies. Toxicological data on one of these compounds do not reflect the toxicity of all PBDE congeners. The acute toxicity of DeBDE and OcBDE for laboratory animals is low ($LD_{50} > 1$ $g \cdot kg^{-1}$ body weight). The acute oral toxicity of PeBDE is low in rats, and dermal toxicity in rabbits is also low. DeBDE and OcBDE are not irritant to the skin, and DeBDE is not irritant to the eyes of a rabbit, but OcBDE gives minor eye irritation (19).

BDE-209 has no effect on survival, body weight or food consumption, and no gross or

microscopic pathological effects in feeding studies on rats and mice have been found (19). However in short term toxicity studies with Octa-BDEs, rats administered dietary levels of 100 $mg \cdot kg^{-1}$ had increased liver weights and showed microscopal changes of liver tissue. These liver changes were more severe at higher dose levels, i.e. 1000 and 10,000 $mg \cdot kg^{-1}$ diet. In addition, hyperplasia of the thyroid was seen (Great Lakes Chemical Corporation, 1987). Similar observations have been reported for Penta-BDEs (19) and BDE-209 (68). Penta-BDEs increased liver/body weight ratio with 64%, Octa-BDEs with 45%, and BDE-209 with 25% in a study where a dose of 0.1 $mM \cdot kg^{-1} \cdot day^{-1}$ was administered to male rats during 14 days (69). Further more penta-BDEs increased cytochrome P450 to a higher extend than Octa-BDEs, while BDE-209 did not significantly increase cytochrome P450. As with BB congeners, the toxicity of BDE congeners strongly depends on their molecular structure, and BDEs induce the same isoenzymes of cytochrome P450 (11).

In a carcinogenicity study in rats and mice, DeBDE was administered at dietary levels of up to 50 $g \cdot kg^{-1}$. An increased incidence of adenomas (but no carcinomas) was found in the livers of male rats receiving 25 $g \cdot kg^{-1}$ and female rats receiving 50 $g \cdot kg^{-1}$. In male mice, increased incidences of hepatocellular adenomas and/or carcinomas (combined) were found at 25 $g \cdot kg^{-1}$ and an increasing thyroid follicular cell adenomas/carcinomas (combined) at both dose levels. Female mice did not show any increase in tumour incidence. There was equivocal evidence for carcinogenicity in male and female rats and male mice only at dose levels of 25-50 g BDE-209 $\cdot kg^{-1}$ diet (70, 71). Since the results of all mutagenicity tests have been negative, it was concluded that BDE-209 is not a genotoxic carcinogen (19). In 1990 the International Agency for Research on Cancer (IARC) concluded that there was limited evidence for carcinogenicity, indicating that BDE-209, at present exposure levels, does not present a carcinogenic risk for humans (19).

The results for mutagenicity of penta-BDEs and BDE-209 were negative. Results of the mutagenicity tests of octa-BDEs including an unscheduled DNA assay, *in vitro* microbial assays, and an assay for sister chromatid exchange with Chinese hamster ovary cells were also negative (Pers. Comm. Great Lakes Chemical Corporation published in (19).

DeBDE caused no teratogenic response in fetuses of rats intubated with 10-1000 $mg \cdot kg^{-1}$ day^{-1} on gestation days 6-15. Fetal toxicity only occurred at 1000 $mg \cdot kg^{-1}$ as subcutaneous edema and a delayed ossification of normally developed bones of the fetal skull (68). At high dose levels of octa-BDEs (25 and 50 $mg \cdot kg^{-1}$ body weight) in rats, resorptions or delayed ossification of different bones and fetal malformations were observed (Pers. Comm. Great Lakes Chemical

Corporation published in (19). In rabbits there was no evidence for teratogenic activity, but fetotoxicity was seen at a maternally toxic dose level of 15 mg octa-BDE•kg^{-1} body weight (no observed effect level 2.5 mg•kg^{-1} body weight) (72). Test results for teratogenicity of PeBDE were negative (19). Oral administration of a dietary dose of approximately 0.5 mg Bromkal 70-5 DE for 3.5 months to female sticklebacks, *Gasterosteus aculeatus*, resulted in a decreased spawning success (73).

5. Conclusions:

- No biotransformation of the three BDE congeners investigated was observed in the *in vitro* bioassays. Thus, the observed enrichment of lower brominated congeners in residues of marine biota is most likely due to higher uptake rates instead of a more rapid elimination.
- The microsomes of harbour seal, whitebeaked dolphin, and sperm whale were able to biotransform 4,4'-bromobipheynyl (BB-15) to a considerable extent. BB-15 possesses a bromine substitution pattern with four pairs of vicinal H atoms at *ortho-meta* positions. In contrast, eider duck microsomes did not show this ability. All four test species in this study were incapable of metabolising the remaining BB congeners with vicinal H atoms at *meta-para* positions (BB-52, BB-49 and BB-101) or no vicinal H atoms at all (BB-155, BB-153 and BB-169). Only the latter can be anticipated on the basis of the structural rules developed for PCBs.
- The presence of PBDEs did not inhibit the rates of metabolism of the PCBs.
- The analytical method developed is suitable for the identification and quantification of individual PBB and PBDE congeners at low concentrations (0.01 $\mu g \cdot kg^{-1}$ wet weight).
- Relatively high PBB and PBDE concentrations were found in marine mammals and birds. Bioconcentration factors (BCFs) of about 100 for PBDEs and 1,000 for PBBs were calculated for transfer from mackerel to seal. It may therefore be concluded that most PBDE and PBB congeners investigated, bioaccumulate very easily. Despite the lower estimated BCFs for the PBDEs compared to the PBBs, PBDE concentrations were about 50-fold higher than the PBB concentrations in most samples analysed, indicating that the PBDE contamination of the aquatic environment is of considerable concern. Given the PBB and PBDE concentrations found in the sperm whales, it may be concluded that PBBs and PBDEs have reached the the deep waters of the Atlantic Ocean.
- The patterns of PBBs and PBDEs in marine mammals and birds have many similarities. An interesting difference is the relatively low concentration of BDE-47 in eider duck livers.
- No genotoxicity was observed for both classes of brominated fire retardants in the Mutatox® assay. This is in accordance with literature data on experiments with higher organisms.
- In view of the results obtained and the available literature data , a more restrictive policy is advised with respect to the legislation of both classes of brominated fire retardants. For

PBBs this advice is based on the environmental persistence of the compounds and information on their toxic properties. For the PBDEs this advise is mainly based on the observed persistence of the compounds in this study an d their levels in marine wildlife, because much is still unknown about their toxicity.

6. Recommendations for further Research

1. Congener-specific investigations on the occurrence and the toxicity of PBBs and PBDEs.
   The still fragmentary results of this study indicate that both PBDEs and PBBs are widely distributed in the marine environment. Besides their presence in a number of marine mammals and birds from the North Sea and Wadden Sea, they were also encountered in three sperm whale specimens, indicating that they have also reached the open ocean environment. In view of their structural resemblance to PCBs this is a reason to put more effort into the investigations of the occurrence and toxicity of these compounds, which are still impregnated in many materials as diverse as electrical equipment or clothing. Analogous to PCBs it would be preferable choose a congener-specific method since only this allows for the analyses of compounds not present in the known technical formulations, formed e.g. by degradation of higher brominated to lower brominated congeners.
2. Not much is known yet about the toxicity of PBDEs. The absence of a genotoxic response as found in this study is supported by other reports.. On the other hand PBDEs and PBBs may interfere with the mutagenicity of other compounds. The scarce information shows that these compounds interfere with the regulation of cytochrome P450 1A enzyme in fish which is responsible for the formation of carcinogenic metabolites of polyaromatic hydrocarbons (PAHs). Since brominated flame retardants occur together with PAHs in coastal environments like the North Sea and Wadden Sea, they are likely to interfere with these compounds. There are also indications that brominated flame retardants interfere with reproduction and the regulation of steroid and thyroid hormones. The importance of both mechanisms of action could be investigated in detail by the use of bio-assays which are at momentarily being developed to study these hormonal effects.
3. In order to find out which BB congeners can be metabolised by which iso-enzyme of the cytochrome P450 system, it is suggested that activity assays for other iso-enzymes than CYP1A should also be executed in order to establish whether or not iso-enzymes of other families of cytochrome P450 are also active. Especially the iso-enzymes belonging to the subfamilies CYP2B or CYP3A also seem to be involved in the biotransformation of xenobiotics. Selective inhibition of the different iso-enzymes can be a useful tool as competitive substrate inhibitors, specific antibodies or specific inhibitors can inhibit single iso-enzymes at a time. In the end such investigations can lead to the establishment of 'cytochrome P450 maps' of single species from which it can be deduced which groups of

compounds can be metabolised. In this way, differences in sensitivity between species can be explained on a biochemical basis.

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