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Identification and trophic transfer of contaminants in estuarine food webs

State of the art report 2008-2010

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Report

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Client	
Title	Identification and trophic transfer of contaminants in estuarine food webs

Abstract

This report describes the state-of-the-art of knowledge on food webs in estuarine environments and the trophic transfer of contaminants in these webs. These processes have been illustrated with two case studies in the Westerschelde estuary: a food web with a fish-eating bird, the common tern, as top predator, and a food web with a marine mammal, the harbour seal, as top predator. The results of these case studies have been used to identify possible implications for achievements of national goals as set for European directives.

References	(2010). Identification and trophic transfer of contaminants in estuarine food webs; state of the art report 2008-2010. Deltares report Z4635/1200235.002.

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Samenvatting (Nederlands)

In 2005 heeft in opdracht van RWS Dienst Zeeland een verkennende studie plaatsgevonden naar de aanwezigheid van dioxineachtige stoffen en andere mogelijke probleemstoffen in sediment, visserijproducten en voedselwebs van het Westerschelde estuarium. Resultaten van deze studie lieten zien dat een aantal vervuilende stoffen, zoals polychloorbifenylen (PCB's), gebromeerde vlamvertragers (BFR's), geperfluoreerde verbindingen (PFC's) en organotinverbindingen (OTC's) werden aangetroffen in deze monsters, soms in hoge gehalten.

Top predatoren van aquatische systemen, zoals visetende vogels en zeehonden, zijn belangrijke doelsoorten voor diverse internationale richtlijnen. Deze soorten zijn mogelijk belast met hoge gehalten aan vervuilende stoffen. Populaties van deze soorten zijn mogelijk nog niet stabiel in de Westerschelde, terwijl dit voor internationale richtlijnen als Natura2000 wel gewenst is.

Het doel van dit rapport is om:
1. De huidige kennis over de samenstelling van voedselwebs en doorgifte van vervuilende stoffen in estuariene voedselwebs te presenteren;
2. Deze processen aan de hand van twee case studies uit de Westerschelde te illustreren (voedselweb van de visdief en voedselweb van de gewone zeehond);
3. Eventuele gevolgen van doorgifte en accumulatie van vervuilende stoffen te benoemen voor doelen, zoals gesteld voor internationale richtlijnen (Kaderrichtlijn Water, Natura2000, Kaderrichtlijn Mariene Strategie, OSPAR).

Processen die doorgifte en ophoping (bioaccumulatie) van stoffen in voedselwebs sturen zijn:
1. De biobeschikbaarheid van stoffen, d.w.z. de aanwezigheid van vervuilende stoffen in het abiotische milieu in een dusdanige vorm dat ze kunnen worden opgenomen door organismen;
2. De opname van vervuilende stoffen door organismen via verschillende routes, zoals lucht, water, sediment en voedsel;
3. De uitscheiding van vervuilende stoffen door organismen via verschillende routes, zoals ademhaling, diffusie via de huid, urine/feces, groeiverdunning en afbraak van vervuilende stoffen door o.a. de lever.

De potentie van stoffen om op te hopen in het milieu en in voedselwebs kan worden uitgedrukt aan de hand van zogenaamde bioaccumulatiefactoren.

Ten behoeve van de twee casestudies in de Westerschelde zijn diersoorten verzameld in mei 2007 (voedselweb visdief) en september 2008 (voedselweb zeehond). Om een goed beeld te krijgen van de voedselwebs zijn voor elk voedselweb twee tot drie keer (binnen een maand) op twee locaties in de Westerschelde monsters verzameld. Hierdoor is spreiding in ruimte en tijd meegenomen. In deze monsters zijn stabiele isotopen en vervuilende stoffen gemeten om informatie te krijgen over de structuur van de voedselwebs en belasting van deze voedselwebs met vervuilende stoffen.

Resultaten uit de case studie rondom het voedselweb van de visdief laten zien dat dit voedselweb slechts een beperkte link naar de waterbodem heeft en het merendeel van de prooisoorten hun energie uit mariene koolstofbronnen haalt. Visdieven migreren jaarlijks wat invloed heeft op hun positie in het voedselweb van de Westerschelde. Ze kunnen ook vervuilende stoffen opnemen tijdens hun jaarlijks migratie naar Afrika en terug. Stoffen die ophopen in dit voedselweb zijn PCBs, PBDE's, HBCD, PFC's (m.n. PFOS) en TPT. PCB's en PBDE's worden goed doorgegeven van moedervogel naar eieren en zijn in een zelfde gehalte meetbaar. Hierdoor kunnen metingen in eieren ook gebruikt worden voor het bepalen van gehalten in oudervogels. PFC's, HBCD en TBT worden ofwel in hoge mate ofwel (vrijwel) niet doorgegeven, waardoor metingen in eieren een onjuist beeld geven van de belasting van ouderdieren. Huidige PFC-gehalten in visdiefeieren kunnen mogelijk reproductie-effecten veroorzaken.

De case studie van het voedselweb van gewone zeehonden in de Westerschelde laat zien dat platvissen waarschijnlijk de belangrijkste prooisoorten van de gewone zeehond in dit estuarium vormen. Stoffen die ophopen in dit voedselweb zijn PCBs, PBDE's, HBCD, PFC's (m.n. PFOS) en mogelijk TPT. Zowel platvissen als zeehonden bevatten hoge gehalten aan PFC's, vergeleken met zeehonden uit de Baltische zee. Beperkte resultaten laten zien dat PCB-gehalten mogelijk hoger zijn in Westerschelde zeehonden dan die uit de Oosterschelde en Waddenzee. Uit literatuur blijkt dat stoffen als PCB's en PFC's mogelijk effect kunnen hebben op de reproductie en het immuunsysteem van zeezoogdieren. Platvissen uit de Westerschelde bevatten gehalten aan PCB's die hoger zijn dan een norm voor toxiciteit die is afgeleid voor PCB's in voedsel van zeezoogdieren. Deze norm kan niet worden gebruikt als consumptienorm voor de mens. Voor andere stoffen zijn normen voor voedselkwaliteit t.b.v. zeezoogdieren niet beschikbaar.

Eventuele gevolgen van doorgifte en accumulatie van vervuilende stoffen voor doelen, zoals gesteld voor internationale richtlijnen zijn:

1. KRW:

- PBDE's en TBT zijn beiden opgenomen op de lijst van prioritaire stoffen onder de KRW. PCB's zijn opgenomen als stroomgebied relevante stof voor het Schelde stroomgebied. Een directe vergelijking tussen stofgehalten uit de casestudies en KRW-normen is echter niet mogelijk, aangezien KRW-normen voor water zijn opgesteld en in de case studies in sediment en biota is gemeten.
- Bioaccumulerende stoffen kunnen indirect een effect hebben op het behalen van een Goede Ecologische Toestand of een Goed Ecologisch Potentieel van een watersysteem. Dit komt omdat deze stoffen effecten kunnen veroorzaken op individueel niveau (een organisme), wat vervolgens weer effect kan hebben op soortenrijkdom, biodiversiteit en mogelijk de draagkracht van systemen.
- Geadviseerd wordt om PFC's (m.n. PFOS) toe te voegen aan de prioritaire stoffenlijst dan wel deze als stroomgebiedrelevante stof voor het Scheldestroomgebied aan te merken. Dit advies is gebaseerd op het feit dat a) de stof sterk accumuleert in voedselwebs van de Westerschelde en hierdoor in hoge gehalten in top predatoren van de Westerschelde wordt aangetroffen, b) eerste onderzoeken laten zien dat de stof mogelijk al bij lage concentraties effecten op o.a. reproductie en het immuunsysteem kan geven, c) er maatregelen mogelijk zijn om de emissie van PFC's terug te dringen.
- Geadviseerd wordt om PBDE's, OTC's en PCB's te blijven monitoren en PFC toe te voegen aan het monitoringsprogramma. Weliswaar zijn voor PBDE's, OTC's en PCB's al maatregelen getroffen, maar door o.a. nalevering en ophoping blijven deze stoffen in hoge gehalten aanwezig in voedselwebs van de Westerschelde. Door monitoring kunnen trends van deze stoffen worden gevolgd, kan worden bepaald of maatregelen effect hebben (via afnemende trends) en kan worden ingeschat of er risico's zijn voor milieu en mens.
- Geadviseerd wordt om voor KRW-monitoring PBDE's, OTC's en PCB's in sediment, biota of SPMD's te meten in plaats van in water en hiervoor een milieukwaliteitsnorm af te leiden. Dit advies is gebaseerd op het feit dat a) deze

stoffen slecht meetbaar zijn in water, b) afgeleide waternormen zo laag zijn dat toetsing aan deze normen vaak niet mogelijk is, c) deze stoffen wel in hoge gehalten in biota worden aangetroffen. Monitoring in biota heeft de voorkeur, aangezien zowel de biobeschikbaarheid als bioaccumulatie in de meting is meegenomen. Monitoring in SPMD geeft wel weer wat er beschikbaar is, maar niet wat er ophoopt in biota. Dit is middels modellen eventueel te voorspellen. Monitoring in sediment meet alleen wat er aan totaal gehalte in het sediment aanwezig is, maar niet wat daadwerkelijk beschikbaar is voor opname en hoeveel er ophoopt in biota.

Momenteel vindt binnen het MWTL-programma reeds monitoring plaats in zout water biota, te weten in bot (een platvis) en in mosselen o.a. uit de Westerschelde. In mosselen worden jaarlijks PCB’s, TBT en TPT gemeten, terwijl in bot PCB’s worden gemeten en eenmalig PBDE’s zijn geanalyseerd. Geadviseerd wordt om deze monitoring in biota te handhaven en voor wat betreft prioritaire en gebiedsrelevante KRW stoffen uit te breiden met PBDE’s in mosselen en OTC’s en PBDE’s in bot. Gezien bovenstaand advies omtrent PFC’s wordt geadviseerd om deze stofgroep ook op te nemen in de jaarlijkse monitoring in mosselen en bot. Daarnaast wordt geadviseerd monitoring in vogeleieren in de Westerschelde te hervatten, aangezien deze voor m.n. PCB’s, PBDE’s en mogelijk PFC’s een goed beeld geven van de belasting in top predatoren.

2. Natura2000:

- In N2000 soorten (m.n. top predatoren) van de Westerschelde kunnen bioaccumulerende stoffen in hoge gehalten aanwezig zijn (m.n. PCB’s en PFC’s). Van deze stoffen is uit de literatuur bekend dat ze effecten op de reproductie of het immuunsysteem kunnen uitoefenen. Zo kunnen ze N2000 doelen zoals een instandhoudingdoel of groei voor doelsoorten direct beïnvloeden.
- Geadviseerd wordt om verder te onderzoeken of vervuilende stoffen (m.n. PFC’s en PCB’s) invloed hebben op het behalen van de N2000 doel voor de gewone zeehond in de Westerschelde, gezien de hoge gehalten aan deze stoffen in zeehonden uit de Westerschelde en mogelijke effecten van deze stoffen.
- Geadviseerd wordt om verder te onderzoeken waarom platvissen en de gewone zeehond hoge gehalten aan PFC’s in zich opnemen in vergelijking tot andere biota uit de Westerschelde.

3. KRMS:

- De KRMS wordt momenteel geïmplementeerd in nationaal beleid. In 2012 dient elke lidstaat een Initiële Beoordeling, een beschrijving voor een Goede Milieu Toestand en de bijbehorende Doelen & Indicatoren opgeleverd te hebben. Hier wordt in 2010 een begin mee gemaakt. Lidstaten moeten voorkomen dat gehalten aan vervuilende stoffen in het mariene milieu dusdanig hoog zijn dat ze effecten uitoefenen op het ecosysteem (soorten, voedselweb en diversiteit) en mensen (door consumptie van visserijproducten). Monitoring van vervuilende stoffen en effecten in biota zijn van groot belang om de status van een (sub)regio vast te stellen, een effectief maatregelenpakket op te stellen en te bepalen of maatregelen effect hebben en doelen worden behaald.
- Geadviseerd wordt om voor PFC’s (in vissen), OTC’s (in schelpdieren) en PBDE’s (in vissen) normen op te stellen voor consumptie van visserijproducten. Deze zijn nodig omdat a) gehalten aan deze stoffen in de desbetreffende visserijproducten hoog zijn, b) zonder norm geen risico-inschatting gemaakt kan worden voor de mens.

1 Introduction

In 2005 RWS Dienst Zeeland commissioned a pilot study to assess the presence of dioxin like compounds and other contaminants in sediment, fishery products and food webs of the Westerschelde estuary (Van den Heuvel-Greve e.a., 2006). Results showed that a variety of contaminants, such as PolyChlorinated Biphenyls (PCBs), Brominated Flame Retardants (BFRs), PerFluorinated Compounds (PFCs) and OrganoTin Compounds (OTCs), could be found in samples of the Westerschelde estuary. At the same time, only limited information was available on the distribution and accumulation of most of these 'old' and 'new' contaminants, whereas from scientific literature it is known that these contaminants can locally pose hazards to environmental and human health.

Contaminants are problematic once introduced into aquatic food webs, particularly if they do not break down either chemically or biologically, and may persist for many years. Examples of bioaccumulating substances causing ecological damage include chlorinated hydrocarbons, especially certain groups of PCBs. Biomagnification of these compounds was noted in double-breasted cormorants (*Phalacrocorax auritus*) in the Great Lakes in the 1970s, with reproductive impairment and embryonic deformities (Ludwig *et al.*, 1984). Also, the use of TriButylTin (TBT) and related compounds as biocides in anti-fouling coatings with bioaccumulation and subsequent impact on mollusks (*i.e*., growth abnormalities to imposex) provide an exemplar of such behaviour in marine systems (Bryan et al, 1986; Gibbs and Bryan, 1996). In the Netherlands, related effects have been found on fish eating birds (common terns and eider ducks)(Koeman et al, 1968, Koeman & van Genderen, 1972), harbour seals (Reijnders, 1986) and marine snails (Mensink, 1999), which could be related to pollution by a.o. DichloroDiphenylTrichloroethane (DDT), PCBs and TBT.

Top predators of aquatic systems, such as fish-eating birds, seals and porpoises form an important part of policy measures in Dutch coastal areas. After being diminished in the twentieth century, populations of these top predators seem to be on the increase in the Dutch coastal areas nowadays. However, top predators of estuarine systems are particular at risk from contamination and questions can be posed on the stability of the current populations.

This project on the 'Identification and trophic transfer of contaminants in estuarine food webs' got started to answer two questions from RWS Dienst Zeeland:
1. What are the causes of a decreased reproductive success in the common tern, *Sterna hirundo*, of Terneuzen?
2. What are the causes of a decreased reproductive success in harbour seals, *Phoca vitulina*, in the Delta region of the Westerschelde estuary as compared to reproductive succes of these animals in the Wadden sea?
The information is analysed in a way to make it also applicable to other similar related environments within the Netherlands.

The objectives of this report are:
- To present the state-of-the-art of knowledge on food web structures and trophic transfer of contaminants in estuarine food webs;

- To illustrate the processes of trophic transfer and bioaccumulation using two case studies: the food webs of the common tern and the harbour seal in the Westerschelde estuary;
- To analyse the implications of trophic transfer and bioaccumulation of contaminants for national goals as set for European guidelines (Water Framework Directive, Natura2000, Marine Strategy Framework Directive, OSPAR), based on these case studies.

1.1 Reading guide

The focus lays on three major organic contaminant groups (PCBs, BFRs and PFCs) and one organo-metallic group (OTCs). Characteristics of these contaminants are presented in chapter 2. The presence of contaminants in sediment or water is the first step for these contaminants to become available to food webs. A description of what makes them available to biota is given in chapter 3. In chapter 4 the biological processes through which contaminants can be taken up, metabolised, accumulated or eliminated by organisms are described. The current knowledge on the ecology of food webs and on trophic transfer of contaminants is reviewed in chapter 5. The results of a case study on food webs of the common tern (pelagic food web) and of the harbour seal (bentho-pelagic food web) of the Westerschelde estuary, collected in 2007-2008, is described and discussed in chapter 6. This is important since field data can be used to update current accumulation and food web models and translate these results to other areas. Insight into the most relevant EU guidelines affecting food web health is presented in chapter 7. Lastly, conclusions and recommendations for policy and research are given in chapter 8.

2 Target contaminants

Pollution is the introduction of contaminants into an environment that causes instability, disorder, harm or discomfort to the ecosystem i.e. physical systems or living organisms. Persistent organic pollutants (POPs) are substances with low water solubility, high preference to bind to lipids and are resistant to biodegradation, which have toxic effects on biota (Green et al. 2003). Their complex structure makes long-time presence and wide distribution in the environment possible. Therefore many contaminants, which are not in use anymore, can still be found in high concentrations in the environment.

This study focuses on three groups of organic contaminants: PolyChlorinated Biphenyls (PCBs), Brominated Flame Retardants (BFRs) and PerFluorinated Compounds (PFCs) and a group of organo-metallic compounds: OrganoTin Compounds (OTCs).

2.1 General properties of target contaminants

2.1.1 PolyChlorinated Biphenyls (PCBs)

PCBs are a group of synthetic compounds and are part of the PolyHalogenated Aromatic Hydrocarbons (PHAHs). A total of 209 individual chemical forms (congeners) exist, with different degrees of chlorination (more or less chlorine atoms attached to the main molecule). 150 to 160 congeners are found in the environment (OSPAR 2000). The PCB congeners differ in physico-chemical properties and biological activities, caused by their distinctive structures. However a common distinction of PCBs is made by the dioxin and non-dioxin like PCBs, depending on the form of the congeners structure. In the literature it is common to use the $\sum$7PCBs, which adds up the seven main PCB congeners (including the dioxin-like congener 118).

2.1.2 Brominated Flame Retardants (BFRs)

Polybrominated Biphenyl Ethers (PBDEs) are BFRs with up to 209 different congeners, varying in their degree of halogenation (more or less bromine atoms attached to the main molecule) (Pijnenburg et al, 1995). The major commercial products contain mixtures of (mainly) penta-, octa-, and decaBDEs which give them their name, although this varies among authors in the literature (Table 2.1).

HexaBromoCycloDodecane (HBCD) is another BFR with environmental relevance. It can have up to 12 different stereoisomers (same molecular formula and atoms attached to the main molecule, but atoms can have a different orientation) and therefore separated in three different groups the α-, β- and γ-HBCD. In commercial mixtures the γ-HBCD seems to be predominant (Alaee et al, 2003).

Table 2.1 Overview of commercial PBDEs names (most commonly used in literature) and constitution

Commercial Names	PBDE congeners present in commercial mixture
Penta-mix/PeBDE/penta-BDE	TetraBDEs; PentaBDEs and HexaBDEs
Octa-mix/OcBDE/octa-BDE	HexaBDEs; HeptaBDEs; OctaBDEs; NonaBDEs and DecaBDEs
Deca-mix/DeBDE/deca-BDE	NonaBDEs and DecaBDEs

2.1.3 PerFluorinated Compounds (PFCs)

PFCs are compounds with relatively high molecular weights, constituted by carbon and fluor atoms which generate strong bonds between each other forming the surface-active properties of PFCs. In virtue of their structure, they are extremely resistant to biodegradation (Lewandowski et al. 2006). Their main particularity is to repel both oil and water. PerFluoroOctane Sulphonate (PFOS) is given particular attention since it is the most widely distributed PFC in the environment.

2.1.4 OrganoTin Compounds (OTCs)

OTCs are a group of organo-metalic compounds. Since they are constituted by a group of organic and metallic (tin) chemicals, these compounds present unique properties. The most well known OTC is TriButylTin (TBT), that has been widely used as biocide in marine antifouling paints. It can degrade in DiButylTin (DBT) and MonoButylTin (MBT) in the environment.

Table 2.2 Main use, actual sources and distribution of PCBs, BFRs, PFCs and OTCs in the environment

	PCBs	BFRs	PFCs	OTCs
Use	Additive in chemical industry since 1929 (e.g. fluid in electrical systems, hydraulic lubricants, sealants) [1]	Additive in fire retardants: since 1960s in polymers and textiles and 1970s in electronic equipment [2]	Additive used since 1950s. Wide range of applications (e.g. surfactant, refrigerant, fire retardant, pharmaceuticals) [3]	Additive since 1960s as biocide, fungicide and stabilizer (e.g. anti-fouling paints, wood preservation, polymers) [4]
Sources: EU	Diffuse; Leaks from closed systems	Direct; through leaching from plastics, electronic equipment and textiles.	Direct; municipal and industrial wastewater discharges and waste disposal	Paint removal, industrial effluents; leaching and runoff from agricultural fields
Sources: Non-EU	Same as in EU; direct input from non-industrialized countries (Aguilar et al. 2002)	Same as in EU	Same as in EU	Same as in EU; coatings on shipping vessels in some developing countries
Main storage in estuarine environment	Coastal sediments, especially in the carbonaceous matter (McLeod et al. 2004)	Sediment and suspended matter	Sediment and water	Sediment, suspended matter and water
Distribution	Global and present in every compartment (van Scheppingen et al. 1996; Echarri et al. 1998)	Global with increasing concentrations since extensively in use, especially the deca-mix	Global and present in every compartment	Global, affecting mainly harbor areas and main shipping routes
Relevant compounds in the environment	Dioxine-like: PCB 77, 105, 118, 126, 156, 157, and 169. Non-dioxine-like: PCB-153	HBCD, BDE 28, BDE 47, BDE 99, BDE 100, BDE 153, BDE 154 and BDE 209	PFOS and PFOA (PerFluoroOctanoic Acid)	TBT and breakdown products (DBT and MBT) and TPT

1. More information on use of PCBs see: (Green et al. 2003;Füll 2001;OSPAR 2000)
2. More information on use of BFRs see:(de Wit 2002;Costa & Giordano 2007)
3. More information on use of PFCs see:(Lewandowski et al. 2006).
4. More information on use of OTCs see: (Green et al. 2003;Fent 2004;OSPAR 2000)

Another important OTC is TriPhenylTin (TPT), which has gained popularity in various applications, with increasing environmental concern.

2.2 Sources and distribution of contaminants

An overview of the use, sources and distribution of the studied contaminants is presented in Table 2.2. All these substances have been used in industry due to their unique properties. PCBs became popular due to their chemical stability and high thermal and electrical resistance. BFRs increased commercial and domestic safety and PFCs have the property to repel both oil and water. OTCs were used as biocides on coatings of shipping vessels, reducing greatly the undesired growth of biota (algae, barnacles, shellfish, etc.).

Nevertheless the use of these chemicals has shown negative effects in the marine and estuarine environment. Some bans exist in the EU for PCBs, TBT, PFOS (voluntarily) and certain PBDEs, whereas others are still in use in the EU, without regulations to their production, use, emission or release to the environment.

2.3 Effects on estuarine biota

Table 2.3 gives an overview of the main effects of the considered contaminants on estuarine biota. Some contaminants have effects in very low concentrations. TBT is already toxic to aquatic invertebrates at concentrations as low as 1-10 ng/L (Fent 2004). Scarce amount of information is available on the effects of PFCs on estuarine biota and the available information derived mainly from laboratory tests. A summarizing report on aquatic ecotoxicology of PFOS has been recently published (Beach et al. 2006). Most toxic effects of PFOS are related to morphological alterations.

Marine mammals and fish eating birds of marine and estuarine systems are the groups of animals which are most affected by organic contaminants such as PCBs and BFRs. Kannan et al. (2004) summarized the main features which make marine mammals particularly vulnerable to contamination. The most important feature is that organic chemicals have a high potential to bind to lipids and marine mammals have large amount of fatty tissue that efficiently retains and accumulates lipophilic contaminants. It is in light of their warm-blooded nature, high trophic level and high feeding rate that they accumulate such high concentrations of contaminants. These contaminants generally pass onto the offspring (Debier et al. 2006;Beckmen et al. 2003). Concentrations of contaminants are measured in blubber, liver, tissues and in blood. Effects of contaminants can also be identified by ‘biomarkers’. A biomarker, or biological marker, is in general a substance used as an indicator of a biological state. It is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or responses to contaminant exposure. Biomarkers for contaminant exposure can be e.g. thyroid hormones, vitamin A, cytochrome P450 enzymes. Top predators from fresh water systems (such as otters and cormorants) may also be at risk from exposure to these contaminants, but these are not addressed here.

OTCs form an exception. OTCs are organo-metallic compounds that are not lipophilic and will accumulate in other parts within an organism. These contaminants are more toxic to organisms lower in a food web, such as invertebrates and fish. Recent studies indicate uptake and transfer of OTCs in estuarine food webs and accumulation to higher trophic levels (Strand & Jacobsen 2005; Hu et al. 2006).

Table 2.3 Main observed effects of contaminants in estuarine biota: (↓) indicates decrease, (↑) increase and (↕) indicates alterations. Effects in non-estuarine biota are reflected as grey coloring of text. n.i. – no information.

	Immune function	Reproductive success	Neurology	Morphology	DNA	Feeding behaviour
PCBs	↓ in harbour seal (deSwart et al. 1995;Levin et al. 2005;Mos et al. 2006) ↓ in fur seal (Beckmen et al. 2003) ↓ in Southern sea otter (Kannan et al. 2007)	↓ in harbour seal (Reijnders 1980;Reijnders 1986) ↓ in American mink (Kihlstrom et al. 1992)	↕ in rat and human, in particular ortho-substituted PCBs summarized by (Hale & La Guardia 2002)	↕ of skull of grey seal (Zakharov & Yablokov 1990)	↕ in harbour seal summarized by: (Thompson et al. 2007)	↕ in harbour seal (Tabuchi et al. 2006)
BFRs	↕ in harbour seal, by PBDEs (Neale et al. 2005)	↓ in common tern, by PBDEs (Sulu-Gambari 2007) ↓ in grey seal, by PBDEs (Hall et al. 2003)	↕ in rats and mice, by PBDEs (Darnerud 2003) ↕ in mice, by PBDE 209 (Johansson et al. 2008)	↕ of liver and kidney of rats and rabbits, by PBDEs (Darnerud 2003)	↕ in rats and mice, by PBDEs (McDonald 2002) ↕ in *in vitro* mammalian cells, by HBCD (Helleday et al. 1999)	↕ in harbour porpoise, by PBDEs summarized by (Thron et al. 2004)
PFCs	↓ in mice, by Sulfluramid (Peden-Adams et al. 2007)	↓ in bottlenose dolphin (Houde et al. 2006a)	*n.i.*	↑ in liver weight of ♀ bobwhite & ↓ in body weight of ♂ mallard, by PFOS (Newsted et al. 2005)	↕ in rats (Hu et al. 2002)	*n.i.*
OTCs	↓ in sea otter (Kannan et al. 1998) ↓ murray cod (Harford et al. 2007) ↓ in oyster (Alzieu 1998;Alzieu 1991)	↓ in gastropods, by TBT (Oehlmann et al. 1991)	↕ in rat (Boyer 1989)	↕ of oyster shell, by TBT (Alzieu 1998;Alzieu 1991)	*n.i.*	↕ in shell fish and tunicates (Cima & Ballarin 1999; Cima et al. 1998)

2.4 Legislation on contaminants

The international and EU regulations concerning contaminants in European water are presented in this section (Table 2.4).

Table 2.4 EU legislation and International conventions affecting EU countries. The status of each group (or part of the group) of contaminants, is defined. Namely if there is any regulation (+), if regulation is in proceedings or consideration (±) or if there isn't any regulation (-).

	PCBs	PBDEs	PFCs	OTCs
WFD or Directive 2000/60/EC	+	+	-	+
Directive 2006/11/EC	+	-	-	+
Directive 76/769/EEC	±	+	+	+
OSPAR	+	+	+	+
Stockholm convention	+	±	±	-
Aarhus protocol	+	-	-	-
Rotterdam convention	+	-	-	±
AFS convention	-	-	-	+
MSFD or Directive 2008/56/EC	-	-	-	-

Water Framework Directive

The EU Water Framework Directive (WFD) or Directive 2000/60/EC (22 December 2000), established a framework for community action in the water policy field, namely to protect inland surface waters, transitional waters, coastal waters and groundwater and improve water quality in European waters.

A priority list of hazardous substances was established, that includes TBT(organotins) and PentaBDE (PBDEs) compounds.

Directive 2006/11/EC

The Directive 2006/11/EC (15 February 2006), on pollution caused by certain dangerous substances discharged into the aquatic environment of the community, is a codified version of the Council Directive 76/464/EEC (4 May 1976). It takes into account the changes brought by the WFD.

OTCs are part of the priority list of hazardous substances and PCBs are regulated by terms on List II of the directive.

Directive 76/769/EEC

The Directive 76/769/EEC (27 July 1976) focuses on the approximation of the laws, regulations, and administrative provisions of the member states relating to restrictions on the marketing and use of certain dangerous substances and preparations.

Directive 2003/11/EC (6 February 2003) added Penta/octabromodiphenyl ether to the list of priority substances (Annex I). Directive 2006/122/EC (12 December 2006) added PFOS to Annex I of the Directive 76/769/EEC, limiting it's release and use, due to its persistence, bioaccumulation potential and toxicity to mammalian species. PFOA is kept under review. OTCs have also been mentioned in this regulation (Gipperth in press).

By 2009, this legislation has been substituted by the regulation EC 1907/2006 or the REACH (Registration, Evaluation, Authorization and Restriction of Chemicals) regulation.

OSPAR

The Convention for the Protection of the marine Environment of the North-East Atlantic or simply the OSPAR Convention entered into force on 25 March 1998. It replaced all decisions, recommendations and all other agreements from the 1972 Oslo and 1974 Paris Conventions. It was signed by all EU countries of the North-eastern Atlantic and also by Iceland, Norway and Switzerland (for further information see http://www.ospar.org/). The OSPAR Commission prepared a list of chemicals for priority action, under which all contaminant groups of this study are listed. Some OTCs and a few congeners of PCBs were considered less important and have therefore been excluded from the list.

Stockholm convention on Persistent Organic Pollutants (UNEP)

The UNEP Stockholm convention on persistent organic pollutants was adopted on the 22 May 2001 and it entered in force on 17 May 2004. The aim of this convention is to reduce or eliminate the production and use of several POPs (see http://chm.pops.int/). The Stockholm convention prohibits the production and use of PCBs. At present new POPs are considered to be added by January 2009 (Fiedler 2008), these are pentaBDE (PBDEs), octaBDE (PBDEs) and PFOS (PFCs).

Aarhus protocol on persistent organic pollutants (UNECE)

The United Nations Economic Commission for Europe (UNECE) '1998 Aarhus protocol on persistent organic pollutants' entered into force on 23 October 2003. This protocol was established due to the growing concern of environmental effects of POPs and banned the production and use of some contaminants and the elimination of others in a later stage (see http://www.unece.org/env/lrtap/pops_h1.htm). The protocol prohibits the production and use of PCBs. Lohmann et al. (2007) claims that PentaBDEs (PBDEs), OctaBDEs (PBDEs), PFOS (PFCs) and related chemicals were considered for future inclusion in the UNECE protocol in 2007.

Rotterdam convention (UNEP/FAO)

The United Nations Environment Programme (UNEP) and the Food and Agriculture Organization of the United Nations (FAO) jointly implemented the ‘Rotterdam convention on the prior informed consent procedure for certain hazardous chemicals and pesticide in international trade’. The convention was adopted on 10 September 1998 and entered into force on 24 February 2004. For further information see: http://www.pic.int.

Under this convention PCBs are classified as ‘severely restricted in use’ and TBT compounds are notified as potential chemicals to be severely restricted in use.

AFS convention (IMO)

The international convention on the control of harmful anti-fouling systems on ships or ‘AFS convention’ proposed by the International Maritime Organization (IMO) was adopted on 5 October 2001. It prohibits the application and forces the elimination of harmful anti-fouling systems (such as OTCs) on ships since 1 January 2008. In the EU the convention is additionally regulated by Regulation (EC) No 782/2003 and Commission Regulation (EC) No 536/2008.

Marine Strategy Framework Directive (MSFD)

A further EU legislation is the ‘Marine Strategy Framework Directive’ or Directive 2008/56/EC (17 June 2008). It’s goal is to improve the quality of marine waters in the EU by 2021 and to protect economic and social activities of marine areas. However, at present, the legislation doesn’t have any regulation on contaminants. Although it defines in Annex I that concentrations of contaminants should not be at levels to cause to pollution effects (referring to descriptor 8 of the ‘Qualitative descriptors for determining good environmental status’).

3 Bioavailability

Contaminants enter estuarine environments via several pathways (Figure 3.1). From there on they can take different routes, until they become bioavailable for uptake and enter food webs. Bioavailability generally refers to a contaminant or a group of contaminants, present in the environment (biotic or abiotic), in such state that it is accessible for uptake by biota. The following chapter will elucidate the major processes affecting bioavailability, since recent studies show that bioavailability of organic and organo-metallic compounds is still not well understood (Eggleton & Thomas 2004).

3.1 Processes controlling bioavailability

In the estuarine environment, contaminants can be directly bioavailable to biota through water and sediment. If this is not the case, they can be transformed or transported from one to another compartment until they eventually become available (Table 3.1). Bioavailability is an interaction of physical and biogeochemical processes (Table 3.2), environmental factors (Table 3.3) and contaminant properties (Table 3.4).

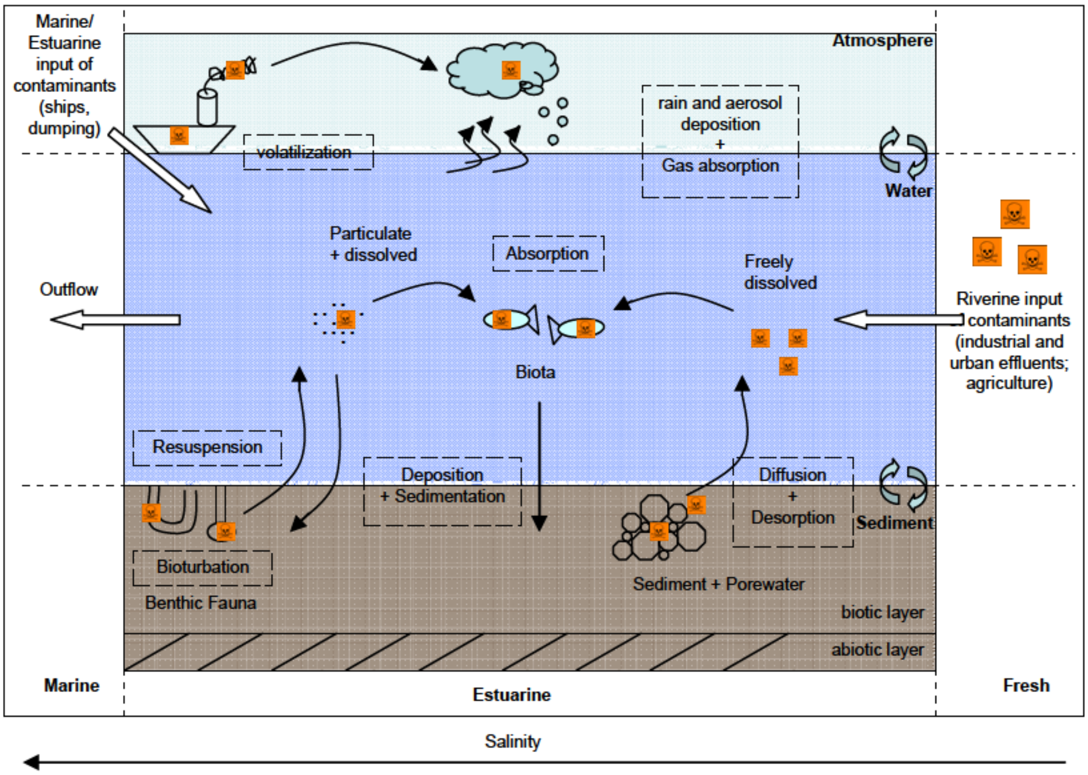

Figure 3.1 Major contaminant fluxes in the estuarine environment, concerning bioavailability. Horizontal separation indicates variations in salinity (from fresh water up to marine). Vertical separation indicates compartment types: sediment (brown), water (dark blue) and atmosphere (light blue). Sediment is divided in a biotic (biologically active) and abiotic (generally biological inactive) layer. Plain text indicates storage possibilities of contaminants and text with frame indicates involving processes.

Table 3.1 Description of how contaminants are stored in water and sediment

Contaminant storage in water	
Dissolved in water	Represents the freely dissolved contaminants in the water column and dissolved particles to which contaminants are adsorbed (<0,45µm).
Suspended matter	Composed of particulate organic and inorganic material (>0,45µm), to which contaminants can absorb. Generally in high concentration in estuarine waters (Lee 2002) and these are often retained in the upper water layers.
Pelagic biota	Organisms that live in the water column and can incorporate contaminants through feeding processes.
Contaminant storage in sediment	
Sediment	Constituted of materials with different sizes and compositions. Contaminants preferentially sorb to the fine fraction, particle with size <63µm (Villars et al. 2001) with high organic carbon content (Lee 2002), especially to carbonaceous matter (Cornelissen et al. 2005).
Pore water	Refers to the interstitial water in sediment. The properties of contaminants in pore water are similar to that dissolved in water.
Benthic fauna	Fauna that inhabits the seabed (such as worms, shellfish). They have the ability to redistribute contaminants in the sediment layer and to the overlying water layer so as to adsorb contaminated particles to their body or incorporate them by ingestion of particles.

Table 3.2 Processes of contaminant exchange in water (w) and sediment (s). Arrows (→) indicate fluxes between compartments.

Physical processes	
Deposition (w) → (s)	Particles that gain a certain size or weight precipitate into greater depths reaching, after some time, the sediment layer.
Sedimentation (w) → (s)	Refers to the deposition of (only) sediments.
Resuspension (s) → (w)	Variations in the water flow (during tidal movement, storms, dredging events, etc.) lead to resuspension of sediments.
Chemical processes	
Absorption (s),(w) → (s), biota	Process in which a contaminant is incorporated into the interior of a particle or organism.
Adsorption (w) → (s)	Process in which chemicals bind to the outer surface of inorganic and organic particles.
Desorption (s) → (w)	Occurs when molecules detach from the interior or surface of inorganic and organic particles. Generally divided into fast and slow desorption. Rate of desorption decreases with time.
Diffusion (s) ↔ (w)	Defined by the transport of a compound between two compartments, in order to equal compound concentrations on the basis of equilibrium partitioning.
Biological processes	
Bioturbation (s) → (w)	Mixing process of the upper sediment layer, on account of biological activity (e.g. burrowing, feeding). Through the mixing process contaminants can be redistributed in the sediment layer and to the overlying water and an input of oxygen reaches the sediment.
Spawning biota → (w) → (s)	Release of eggs into the environment, which have incorporated contaminants (Le Gall et al. 2003).

Table 3.3 Environmental factors: effect on estuarine bioavailability process

Environmental factor	Description	General effect
Salinity	In estuaries salinity ranges from 0 – >30‰	Increasing salinity, higher solubility; controls mixing of water, flocculation and sedimentation
Temperature	Variation of temperature are mainly spatial (from upper to lower water layers and from rivers to the sea) and temporal (changes with seasons)	Higher temperatures generally accelerate chemical reactions
Contact time	Duration of a contaminant being present in a compartment	Longer presence, higher entrapment, lower bioavailability
Concentration effect	Quantity of contaminant in environment	In general: higher concentration, higher bioavailability
Dissolved oxygen	Freely dissolved oxygen in a compartment	High oxygen content, high reactivity and bioavailability
Redox potential	Oxydo-reduction reactions	breakdown of highly reactive molecules, enhancing bioavailability
pH	The range of pH in estuaries, 6-7 in fresh water to slightly basic ~8 in seawater	Lower pH, higher bioavailability

Table 3.4 Contaminant properties: effect on bioavailability process. Consider that the intrinsic compound properties can vary from one specific compound to another

Contaminant property	Description	General effect
Molecular size	Size of the compound	Increase in molecular size decreases bioavailability.
Molecular weight	Sum of all atomic weights that constitute a compound	Low molecular weight favours bioavailability.
Structure	Molecular geometry and ligands of a compound	Less complex structure, more reactivity.
Substitution groups	Part of the molecular structure can be substituted by another compound	Absence of substitution groups facilitates chemical break down;
Octanol-water partioning coefficient (Kow)	Describes solubility of a compound: the affinity of a chemical to bind to lipids (lipophilic or hydrophobic) or distribute in water (hydrophilic or lipophobic)	Bioavailability depends on preferred uptake route by biota; high Kow → lipophilic → bioavailable in sediment or particles (ingestion) Low Kow → hydrophilic → bioavailable in water (diffusion).

Estuarine sediments serve as a filter for many organic contaminants between land and sea. Once organic contaminants enter estuaries they will preferentially adhere to suspended matter in water and eventually deposit into the sediment. There they become trapped and stay immobilized in the abiotic layer, except if the upper layer is removed (e.g. dredging). This 'sink' mechanism renders estuaries to be more susceptive to contamination (Chapman & Wang 2001).

Since estuaries are transition areas between fresh water and marine environments, certain environmental properties have strong spatial variations. The increase of salinity is identified as the main feature of interaction in estuaries (Villars et al. 2001). This interaction is far from simple. Water with different physical and chemical properties can form layers and reduce mixing processes. Salinity can also increase deposition rate of suspended particles. This generally happens in the transition part of rivers to estuaries, where salinity starts to increase and compounds tend to aggregate. Temperature also varies along estuaries with higher warm water influx from rivers in summer and influx of

Table 3.5 Relevant biogeochemical processes affecting PCBs

Relevant processes affecting PCBs	
Aging effect	Higher contact time, increases sorptivity to organic matter (Bucheli & Gustafsson 2001)
Sorption/desorption	Desorption is slower in sediment than in suspended matter, due to particle sizes (Bucheli & Gustafsson 2001)
Biodegradation	Slow process, even for congeners with low substitution (Villars et al. 2001)

colder water in winter. Variations of pH along estuaries, controlling the acid-base reactions, additionally influence chemical availability of many compounds (Eggleton & Thomas 2004). Estuary therefore may on one side bind contaminants more effectively to silt particles under the influence of an increase of salinity, but on the other hand, because of higher concentrations and turbid environmental conditions, enhance bioavailability of contaminants.

3.2 Bioavailability of contaminants

PCBs

The main features affecting bioavailability of PCBs is presented in Table 3.5.

BFRs

BFRs, especially highly brominated BDEs have a high log Kow and are therefore very hydrophobic and lipophilic (Hale & La Guardia 2002;de Wit 2002). PBDEs seem resistant to environmental degradation processes (Zegers et al. 2003). Similar to PCBs, individual PBDE congeners can vary in their physical and chemical properties.
HBCD have low water solubility and relatively large molecules (solubility: α-HBCD < β-HBCD < γ-HBCD) and therefore will tend to bind to lipids and accumulate in sediment (Covaci et al. 2006). Particularly the α-HBCD isomer is most widely distributed in the environment and tends to accumulate in biota (Janak et al. 2005).

PFCs

Studies on properties, distribution and effects of PFCs are still rare, which is in part due to lacking reliable analytical methods (Giesy & Kannan 2001). It was believed for many years that these compounds were too stable to react or have a toxic effect in the environment.

The main group of PFOS is hydrophilic and only a minor part is both hydrophobic and lipophobic (de Vos et al. 2008). The octanol water partitioning coefficient cannot be determined since PFOS aggregate at surface interfaces (Beach et al. 2006). Yet, it is known that the size of PFCs (length of perfluorinated tail) influences sorptivity to sediment (de Vos et al. 2008).

OTCs

The lipohilic nature of organotins leads to rapid adsorption by sorptive matter, but can desorb rapidly from sediments during storms and dredging events (Fent 2004), since this allows to remobilize sediments from anoxic sediment where organotins tend to accumulate (Eggleton & Thomas 2004). It is known that bioavailability of organotins is mainly controlled by pH and salinity of water (Veltman et al. 2006) affecting sorption and partitioning processes. The octanol-water distribution coefficients of TBT and TPT are higher, with increasing pH (Fent 2004) which is related to increasing salinity. Therefore at higher pH and salinity, these compounds will have a hydrophobic behaviour.

4 Uptake and elimination of contaminants

4.1 General uptake and elimination mechanisms

The bioaccumulation of contaminants depends, besides on bioavailability of contaminants as described in the previous chapter, on how they are taken up and elimated by organisms. The most important mechanisms are described in Mackay & Fraser (2000). In the general case of an organism, such as a fish, mammal or bird, there are three possible uptake and six possible elimination, clearance or loss mechanisms (see Figure 4.1). These mechanisms also apply to invertebrates and vertebrate animals.

Organisms obtain oxygen by respiration, either from air or water. This respiration can result both in uptake and clearance of contaminants, usually by a passive diffusion process. Besides respiration, there may also be dermal or surface exchange. For small organisms, this can be a large uptake or clearance route, whereas for larger animals this route is less important. Both routes via respiration and dermal uptake, are reversible transfer processes, because they are mainly based on diffusion. The other important uptake route is food ingestion as a source of energy, and inadvertently, contaminants. This route is most important for larger organisms and contaminants that are highly lipophilic. Besides respiration and dermal loss, there may be loss by egestion of fecal matter and urine. Within the animal, metabolic conversion of contaminants can take place, changing the contaminant in other chemicals. There may also be clearance or reduction in concentration by growth dilution. Female animals can lose contaminants by reproductive processes, such as birth of offspring, egg laying, or lactation.

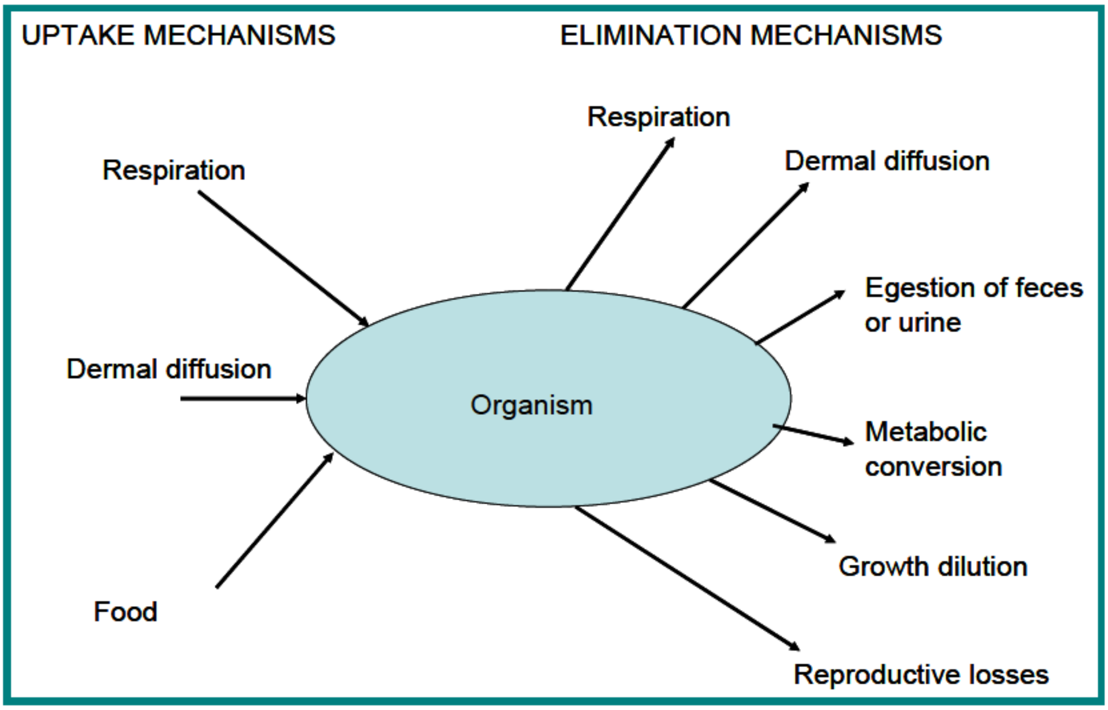

Figure 4.1 Uptake and elimination processes applicable to a general organism (adopted from Mackay & Fraser, 2000).

4.2 Uptake routes

4.2.1 Water

Water is one of the main routes for water respiring animals to take up water-soluble contaminants. The contaminants enter the animal through the gills or skin. Even less water-soluble contaminants can enter an organism through this route, although it depends on the actual concentration in water and within the organism. This however will not be the main route of entrance for more lipophilic and less hydrophilic contaminants.

4.2.2 Sediment

For sediment dwelling organisms, pore water is a major exposure medium. This means that concentrations in pore water strongly influences the uptake of contaminants in benthic species. As described in chapter 5, organic lipophilic contaminants tend to sorb to particulate (organic) matter in water and sediment. Particulate organic matter is ingested by organisms, such as filter feeders (e.g. shell fish) and worms. The binding strength of contaminants to particulate organic matter mainly determines the bioavailability of these contaminants when ingested by these organisms. However, bioavailability is also influenced by gut digestion processes within an organism.

4.2.3 Food

Food is ingested by animals to obtain energy and mass. However, food can also be loaded with contaminants through uptake from the surrounding environment (water and sediment). For instance, flat fish feed e.g. on shell fish that have collected contaminants through their filter feeding behaviour. After ingestion, food is digested and contaminants are taken up in the gastrointestinal tract. Food as uptake route becomes more and more important when contaminants are more lipophilic and an organism is placed higher in the trophic system.

4.3 Elimination routes

4.3.1 Respiration and dermal diffusion

Both these routes are based on diffusion processes and important for water-soluble contaminants. Contaminants will diffuse out of an organism when the concentration in the surrounding environment (water) is lower than the internal concentration of the organism.

4.3.2 Egestion

Organisms dispose of nondigested food items and waste products through feces and urine. This route is also used for egestion of contaminants, either when not taken up from undigested matter, or as waste product.

4.3.3 Metabolic conversion

Within an organism, contaminants can be metabolised (broken down) by the organism itself. The breakdown products are usually more oxygenated and water-soluble than the parent compound. These breakdown product can generally be egested more readily. Metabolism of contaminants takes place in the gut or internal organs (notably the liver) of an animal. Enzymes play an important role in this process. Whether contaminants can be metabolised depends on the chemical structure of the contaminant and the species involved, because the capacity to metabolise contaminants is highly species specific. In general, animals higher in the food web can metabolise more contaminants than organisms at the base of a food web. PAHs can only be marginally metabolised by invertebrates, whereas they are rather easily broken down in fish and higher trophic animals. This largely explains why PAHs do not bioaccumulate in food web above the level of fish. However, even between animals at a similar trophic levels, such as fish-eating birds and harbour porpoises, a large variety in metabolic capacity exist.

4.3.4 Growth dilution

Reduction in concentration of a contaminant in an organism can happen by growth dilution. This means that the concentration falls as a result of the same quantity of a contaminant being distributed in a larger volume of tissue (when the organism has grown).

4.3.5 Reproductive losses

Female animals can loose a substantial portion of their contaminant loading through reproductive processes, such as production of offspring and lactation. Especially lactation is a very important process for marine mammals such as seals and dolphins, because the milk of these animals is rich in fat and may contain high concentrations of lipophilic contaminants. These maternal losses however represent uptake by the offspring.

4.4 Bioaccumulation Factors

In Table 4.1 a variety of factors is described that can be used to assess bioaccumulation potential of contaminants. These factors are estimated from both field and laboratory experiments. Especially BCFs are used for regulatory purposes, such as the approval of the production of new substances.

Table 4.1 Parameters that are relevant in expressing processes involved in trophic transfer of contaminants.

Parameter	Explanation	Definition
BCF	BioConcentration Factor: the uptake from a contaminant by absorption from the water (via respiratory and dermal uptake mechanisms)	The ratio of the contaminant concentration in organism tissue to that in water to which the organism has been exposed.
BSAF	Biota Sediment Accumulation Factor: the bioaccumulation potential of sediment-bound contaminants to benthic species	The ratio of the contaminant concentration in organism tissue to that in sediment to which the organism has been exposed. For neutral organic chemicals, BSAFs are typically expressed by normalising tissue concentrations to a lipid basis and sediment concentrations to an organic carbon basis.
BMF	BioMagnification Factor: the increase in contaminant concentration from predator to prey	The ratio of the concentration in a predator organism to the concentration in its prey or contaminated laboratory diet.
BAF	BioAccumulation Factor: the uptake potential of a contaminant taking into account both the respiratory, dermal an dietary absorption routes	The ratio of a contaminant in organism tissue to that in water taking into account both uptake routes (BCF + BMF).
TMF	Trophic Magnification Factor: the mean concentration change per trophic level in the food web	The TMF is based on the slope from the regression of chemical concentration in an organism onto its trophic position, where the chemical concentration is normalized depending on its sorption properties. TMFs for pelagic and benthic food webs at a particular site should be calculated separately as their carbon sources differ.

5 Food web composition and trophic transfer

To understand trophic transfer it is necessary to understand food web composition and how contaminants act inside this structure.

5.1 Composition of food webs

Food webs describe the predator-prey relationships between species within an ecosystem. Organisms are connected to the organisms they consume by lines representing the direction of organism or energy transfer. It also shows how the energy from the producer is given to the consumer. Organism will possess a certain trophic level within a food web. Shell fish for instance will have a relatively low trophic level, whereas mammals will generally possess a high trophic level.

5.1.1 Measuring food web relations

Composition of food webs can be determined by analysing the carbon and nitrogen stable isotope signature of the various components. Isotopes are atoms having the same number of protons, but different number of neutrons, thus only differing in mass and not in chemical properties (Hansson et al. 1997). For example, carbon normally occurs with a nuclear mass of 12 (^{12}C), but a small fraction in the environment will exist of its stable isotope ^{13}C. Similarly, nitrogen normally occurs as ^{14}N, but a small fraction will be ^{15}N. The ratio of the stable isotope to the standard isotope in the environment may differ per site. Primary producers taking up nitrogen in the form of nutrients and carbon in the form of CO_2 will reflect the ratio in the environment to a certain extent, but depending on their photosynthetic pathways may take up one or the other isotope with a certain bias. When consumers in the food web subsequently assimilate the primary producers their carbon isotope ratio will strongly reflect that of their food. The nitrogen isotopes are taken up with a certain preference for the heavier isotope, resulting in an enrichment of the $^{15}N/^{14}N$ ratio (or $\delta^{15}N$) in higher trophic levels.

Numerous studies have used the difference in nitrogen isotope ratios ($\delta^{15}N$) between two or more systems (i.e. lakes) to quantify the trophic position of individual species and food web length and complexity.

5.1.2 Calculation of trophic level

For δ15N values one expects an enrichment of 3-4‰ per trophic level; a widely accepted average in estuarine systems is 3.4‰ (Peterson and Fry, 1987). Trophic level is a relative scale and to get to a specific value generally a specific species is chosen as a standard. Commonly the standard is a general, non discriminatory filter feeder, such as the cockle (*Cerastoderma edule*). Trophic levels (TL) of all other species are subsequently calculated relative to this benchmark, according to the following equation:

$$TL = 2 + (\delta^{15}N_{species} - \delta^{15}N_{cockle}) / 3.4 \qquad (1.1)$$

5.2 Trophic transfer

Trophic transfer (or dietary accumulation) refers to the passage of chemicals from lower to higher trophic levels. During trophic transfer chemicals can biomagnify along the food web. Biomagnification occurs when the concentration of a chemical in an organism is higher than the chemicals concentration in the organism's dietary uptake (Gobas & Morrison 2000). Trophic magnification takes into account bioaccumulation and trophic transfer, and quantifies the magnification of contaminants through the food web. How some of these processes work is illustrated in Figure 5.1 and Figure 5.2.

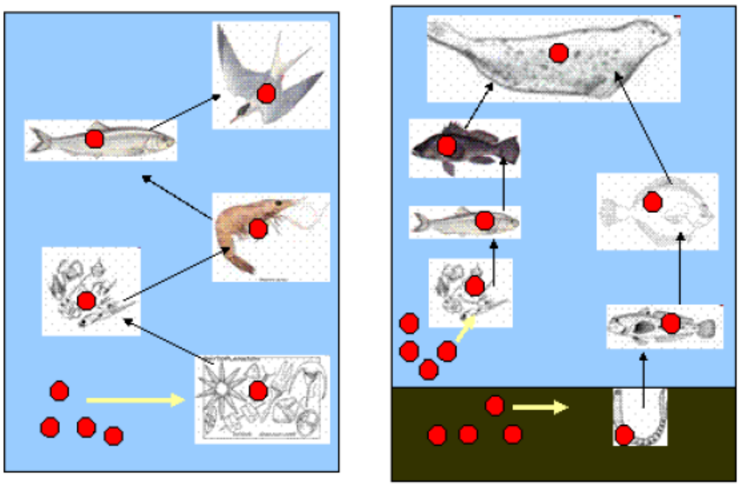

Figure 5.1 *Example of trophic transfer in a pelagic (left image) and benthopelagic (right image) food web. In the pelagic food web the contaminant (red dots) is bioavailable in water and the route of transfer (water → phytoplankton → zooplankton → shrimp → herring → common tern). In the bentho-pelagic food web there can be two different sources of contaminants: water(zooplankton → hering → seabass → harbour seal) and sediment (sediment → goby → flatfish → harbour seal).*

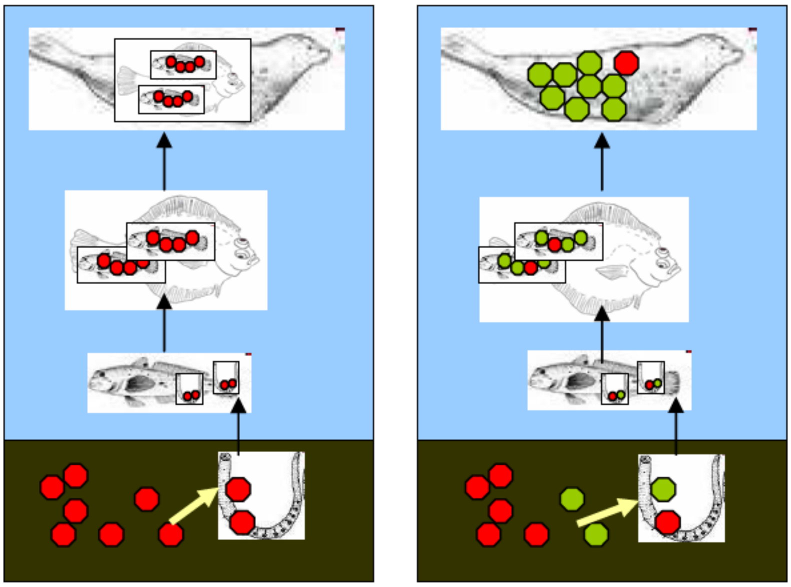

Figure 5.2 *Example of trophic magnification (left) and selective trophic magnification (right) in a benthopelagic food web. The red and green dots are an example of an hydrophobic contaminants. In the left picture the contaminant (type red) is accumulated and multiplied along the trophic web. In (c), the contaminants (type red and green) are passed on from on trophic level to another and two processes occur: 1) the contaminants biomagnify along the food web 2) there is a selective uptake of one contaminant (type green) and elimination of another contaminant (type red).*

Top level predators, such as marine mammals and fish-eating birds, feed at high trophic level. Since food intake is the main source of gaining energy, contaminants that have been accumulated in their food web will be taken up. In a very simple approach it could be assumed that the contaminants available in high concentrations in sediment or water would end up in the top predators of the food web, depending on their feeding habit. This is not necessarily the case. Some contaminants accumulate, while others are easily eliminated. The same applies to each group of contaminants, where congeners can have different physical and chemical properties changing their environmental fate. Nonetheless, contaminant concentrations in water and sediment can give an indication of what is available for uptake in the environment. To understand what happens in the whole environment a top-down perspective, such as the food basket approach, gives a measure of how contaminants pass along the food web.

Factors controlling trophic transfer in food webs

The following factors controlling trophic transfer are based on a review of Borga et al. (2004). The chemical factor with most influence seems to be the lipophilicity of contaminants, since it influences the storage of organic chemicals inside the body. The most relevant biological factors for estuarine areas are:

- lipid content: the storage of organic contaminants is possible through the presence of lipid content in biota. Variety in lipid content therefore influences concentrations of lipophilic contaminants in biota;
- seasonality: in estuaries seasonality affects directly environmental parameters such as temperature and salinity (see description in chapter 3). These do not only influence bioavailability, but also organism properties, and provoke shifts in food availability;
- body state: contaminant concentrations are influences by size, age, sex and life cycle of biota (see Corsolini et al. 2007);
- habitat use: benthic species tend to accumulate higher contaminant concentrations than pelagic species (Van den Heuvel-Greve et al. 2006);
- migration: sessile organisms will better reflect represent a contaminant picture of a local site, whereas migrating animals will collect contaminants from all places they visit and show an environmental picture of a region. Estuaries are important areas for migrating species (from coast to open sea, from river to estuary/sea, and vice versa);
- feeding ecology: the choice of prey items determines contaminant exposure. Depending on the contaminant accumulation may be higher in top predators of either benthic or pelagic food webs (Van den Heuvel-Greve et al. 2006; Kidd et al. 2001);
- trophic level. Cold-blooded species low in the food web usually show contaminant concentrations and pattern that are very different from that found in warm-blooded species at higher trophic levels (Borgå et al. 2004).

Those contaminants which have the ability to be transferred from one trophic level to another and eventually biomagnify in food webs are of particular concern (Mackay & Fraser 2000). A short evaluation on the contaminants focused in this study, concerning food web processes, is presented in Table 5.1. Scarce information was available on trophic transfer of organotins (Veltman et al. 2006) and no information on trophic magnification.

Table 5.1 Properties of contaminants in food web processes. Results of studies on trophic transfer and trophic magnification.

	PCBs	BFRs	PFCs
Trophic transfer	Harbour seal (Ruus et al. 2002a;Ruus et al. 2002b;Cullon et al. 2005) Polar bear (Chiu et al. 2000)	Westernschelde food chain: PBDEs (Voorspoels et al. 2003) Harbour seal and harbour porpoise: PBDEs (Boon et al. 2002) various aquatic organisms: HBCD (Covaci et al. 2006)	Marine mammals: PFOS (Van de Vijver et al. 2003) Westernschelde food chain: PFOS(de Vos et al. 2008)
Trophic transfer and trophic magnification	Bluefin tuna: most PCB congener (Corsolini et al. 2007) Spider crab: especially 2,4,5-substitution congener (Bodin et al. 2008)	Beluga whale: (OH-) and (MeO-) PBDEs (Kelly et al. 2008) Polar bear and beluga whale: α-HBCD (Tomy et al. 2008)	Bottlenose dolphin: PFOS and C_8-C_{11} perfluorinated carboxylates (Houde et al. 2006b) Arctic marine food web: PFOS (Tomy et al. 2008)

An example of selective trophic transfer, was found in chemical profiles of PFCs of the common tern food web in 2007 in the Westerschelde estuary (more information on the sampling and results is presented in the next chapter). In Figure 5.3 the results illustrate differences in water and sediment ratios, concerning PFCs. This shows well how some PFCs partition preferentially to water or to sediment. PFOS and PFOA are the main PFC compound in sediment. In water the main PFCs are perfluorobutane sulfonate (PFBS) and perfluorobutyric acid (PFBA). However these ratios in water and sediment do not represent the concentrations of PFCs which are accumulated to higher trophic levels (see Figure 5.4). In the chemical profile of the herring sample, perfluorooctane sulfonamide (PFOSA) is the dominant compound and in the common tern this position is taken by PFOS. This shows that not all contaminants which seem to be bioavailable and taken up by biota are necessarily bioaccumulated or transferred to higher trophic levels.

5.3 Trophic Magnification Factor (TMF)

The Trophic Magnification Factor (TMF) was introduced in by Fisk et al (2001) and may eventually be considered the gold standard metric for understanding the biomagnification potential of a chemical. The TMF describes the average increase (biomagnification) or decrease (biodilution) of a contaminant per trophic level in a food web. Basically, a TMF is derived by regressing the pollutant’s lipid-adjusted chemical concentration in log scale onto the trophic level of three or more sampled organisms (Borgå et al. 2004). The slope of the resulting regression line is called “bioaccumulation rate” and its antilog is called “trophic magnification factor (TMF)” (Fisk et al. 2001). A positive slope of the lipid-normalized concentration to trophic level regression line indicates that the substance biomagnifies in the food web, while a negative slope is an indication of trophic dilution, which is likely due to metabolism, food web dynamics, or limited uptake potential (Mackintosh et al. 2004). The TMF-regression assumes that diet is the main pollutant exposure route, although direct partitioning is important in the uptake of more water-soluble pollutants in water-respiring animals. TMF calculation for pelagic and benthic food webs at a particular site should be calculated separately as their carbon sources differ.

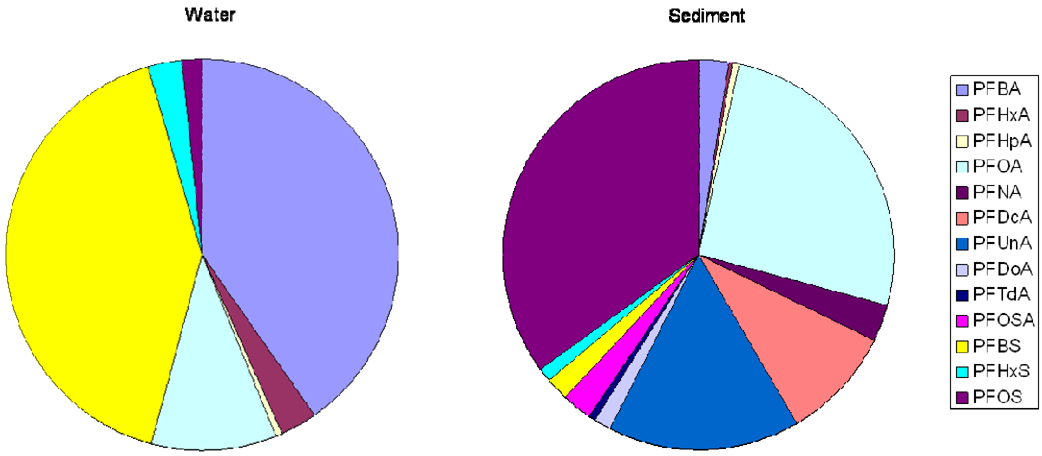

Figure 5.3 *Average chemical profiles of PFCs in water and sediment samples from the Middelplaat of the Westerschelde estuary in 2007.*

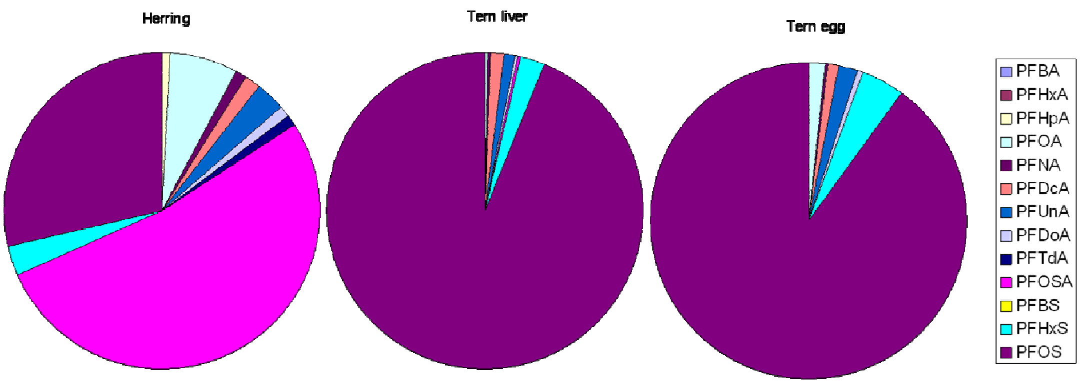

Figure 5.4 *Average chemical profiles of PFCs in samples of herring, common tern liver and common tern eggs of the Westerschelde estuaryin 2007.*

6 Study case: Westerschelde estuary

6.1 Case study area: the Westerschelde estuary

The Westerschelde estuary is a nursery for fish and shrimp stocks, an important area for birds, and a typical habitat for harbour seals. The estuary is strongly influenced by human activities, both physically and chemically. The system contains several contaminants with potential hazards for the populations of birds and marine mammals.

The aim of this case study is to characterise the food webs of a number of important predator species in this ecosystem and the contaminant transfer within these food webs. Two toppredators were selected: the common tern (*Sterna hirundo*) and the harbour seal (*Phoca vitulina*). The common tern is forming a part of a mainly pelagic food web, whereas harbour seals belongs to a benthopelagic food web, which means that it feeds on both pelagic and benthic species.

A number of contaminants that are found of importance for the Westerschelde estuary in earlier studies (Van den Heuvel-Greve et al. 2006; 2007), were studied. These are PCBs, BFRs (PBDEs and HBCD), PFCs (such as PFOS) and OTCs (TBT and TPT).

6.2 Common tern food web

6.2.1 Research questions

The Westerschelde estuary is a nesting area for common terns. There are several breeding colonies in this area, one located on a small pier in the harbour of Terneuzen. This colony at the sluice complex near the town of Terneuzen in the southwest Netherlands was established in 1979. Until 1994, the population increase in this colony followed the same pattern as that in adjacent colonies. However, between 1994 and 1999 a sharp decline in number of breeding pairs and reproduction success was observed (see Figure 6.1), with eggs not hatching and chicks having deformities or diseases resulting in death. None of these effects has been observed in any other common tern colony in the region. After 1999, the number of breeding pairs at Terneuzen steadily increased again without any sign of disease. At this moment, the colony is again one of the main breeding sites of the common tern in the Delta area. More than a decade of research showed that contaminants could have contributed to the reproductive problems of this colony (Van den Heuvel-Greve et al. 2003).

As described above, it is well known that the Westerschelde system contains relatively large amounts of contaminants that are not natural to the system. It is also documented that some of these contaminants can accumulate in relatively high concentrations in top predators, such as the Common Tern. Before contaminants can be identified that need further policy measures more information is needed on how these contaminants are taken up from the environment, transferred through different trophic levels and accumulate in these species.

As described above, it is well known that the Westerschelde system contains relatively large amounts of contaminants that are not natural to the system. It is also documented that some of these contaminants can accumulate in relatively high concentrations in top predators, such as the common tern. Before contaminants can be identified that need further policy measures more information is needed on how these contaminants

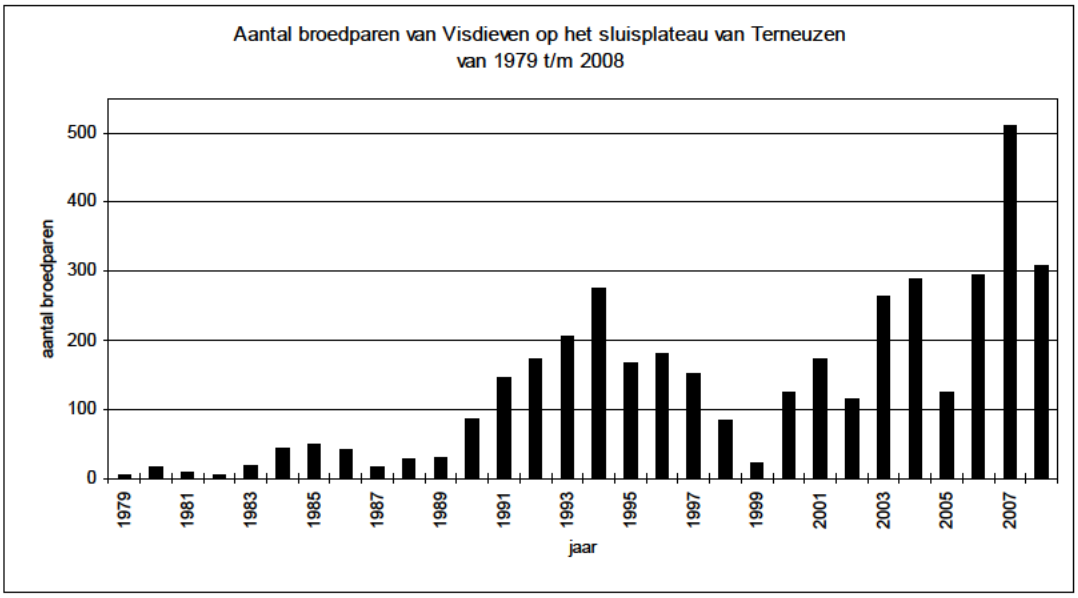

Figure 6.1 Number of breeding pairs at the Common Tern colony in Terneuzen. Reproductive effects were observed in the period 1994-1997.

are taken up from the environment, transferred through different trophic levels and accumulate in these species.

Specific questions of the current case study were:
1) What is the structure of the food web of the common tern near Terneuzen?
2) Which of the selected contaminants are being transferred in the common tern food web near Terneuzen?
3) Can contaminants be transferred from mother bird to egg and how suitable are eggs as indicators for concentrations of the selected contaminants in adult birds?

6.2.2 Sampling

In 2005 a pilot study was carried out at the Middelplaat near a colony of common terns in Terneuzen. The results showed that a selection of contaminants showed bioaccumulation patterns in food items of the common tern (Van den Heuvel-Greve et al. 2003). In May 2007 a full sampling campaign was conducted on two locations in the Westerschelde (Middelplaat and Terneuzen Harbour) to sample the food web of common terns (Figure 6.2). In the same period egg samples were also collected from common terns in the colony of Terneuzen Harbour and from a colony further upstream at Saeftinge. Sampling was conducted by RWS Meet- en Informatiedienst (suspended particulate matter (SPM)), Grontmij|AquaSense and the fishing Vessel “Harlingen 10” (Cor Fondse). This vessel is equipped with several types of fishing gear, including a pelagic seine net, a beam trawling net and a set bag net (“raamkuil”), a type of fishing gear that is positioned stationary in the tidal flow. This type of net is particularly suitable to catch small fry, such as juvenile herring and sprat. Details can be found in Burger (2007).

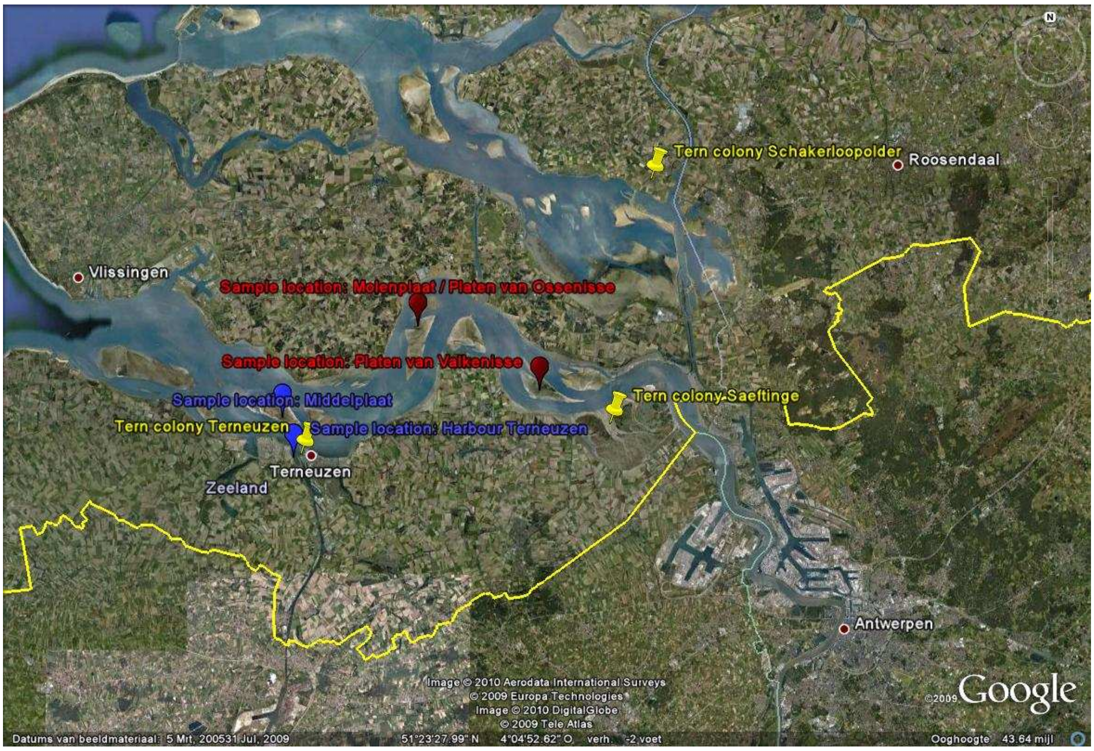

Figure 6.2 Overview of the sampling locations of the common tern (blue and yellow) and harbour seal (red) food webs in the Westerschelde estuary (2007 and 2008).

During this sampling trip there was a very strong bloom of jelly fish, both the moon jelly (*Aurelia aurita*) and the comb jelly (*Pleurobrachia pluteus*) were found in extremely large numbers (Burger, 2007). This hampered the collection of zooplankton, such as mysids, which are known to be a common prey item for many juvenile fish species. As a result an important link in the food web is missing. Of the fish species, we only collected individuals that were smaller than about 12 cm. This is more or less the maximum size that common terns can physically handle. Larger fish can therefore not be part of the common tern food web.

Five female common terns were captured for further laboratory analysis to determine tissue and organ concentration of contaminants. The capture of terns was only possible under a special permission by the Animal Experiment Commission (DEC), which was granted guided by the knowledge that a.o. only a few individuals are collected, their collection is conducted by licensed personnel, approved protocols are applied and that the conservation status of these birds is of ‘least concern’. In order to be able to determine transfer of contaminants from parent to egg, the corresponding three eggs of each female were collected as well. As reference, 10 eggs were collected from random nests at the Saeftinge and Schakerloo colonies.

Permission for the entire sampling campaign was received under the ‘Flora and Fauna’ legislation and the Province of Zeeland.

Analyses of contaminants was conducted by IVM, Amsterdam and IMARES IJmuiden using standard protocols.

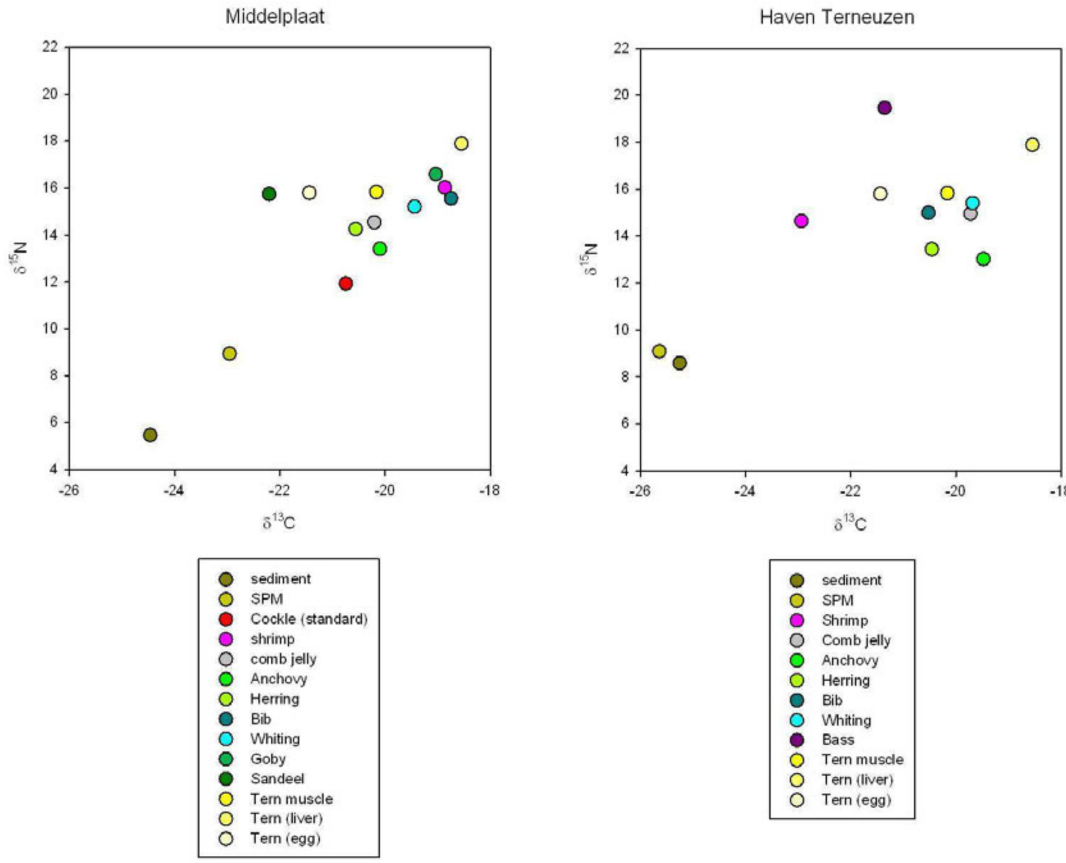

Figure 6.3 *Average δ13C and δ15N ratios of samples taken at both sampling sites. The data for the common tern samples from the Terneuzen harbour colony are included in both figures. SPM=Suspended Particulate Matter.*

6.2.3 Food web composition

Cockles were collected on the Middelplaat to use as a standard for the calculation of trophic level. It was not possible to collect a similar filter feeder at the Terneuzen harbour site. When algae (primary producers) are considered to be trophic level 1, the cockle is by definition trophic level 2.

Figure 6.3 shows the δ13C and δ15N ratios of all samples taken at both sampling sites. Both show a clear trend of fractionation between trophic levels in the δ15N signature. In all samples Seabass (caught only at one of the sampling days in the harbour) has the highest value of δ15N. Higher trophic levels also tend to have a slightly heavier δ13C signature than the sediment and suspended matter, which should both be strongly influenced by algae.

There are some differences between the two sites. In the harbour of Terneuzen the sediment and SPM values for both isotopes are quite similar. At the Middelplaat site there is a clear separation between the samples, with the SPM showing a distinctly higher δ15N level and a marginally higher δ13C. The fish samples (particularly herring, whiting, anchovy and bib) are very similar at both sites for both isotopes. The same is true for the comb jellies. The suspended matter and the shrimp samples in the harbour have a lower δ13C ratio.

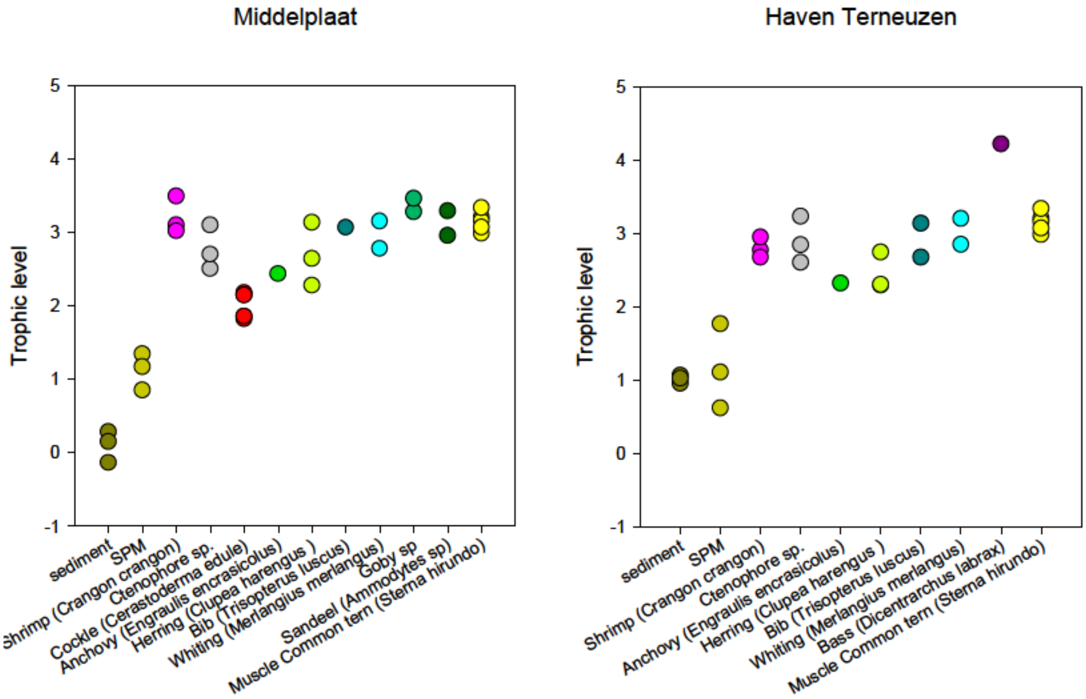

Figure 6.4 *Trophic levels of the samples taken at both sampling sites of the common tern food web. The data for the common tern samples from the Terneuzen harbour colony are included in both figures. SPM=Suspended Particulate Matter.*

When the staple isotope information is presented as trophic level, the figure (Figure 6.4) gives similar results as in Figure 6.3. However in terms of trophic level SPM is similar at both sites and it looks like there is a difference of a full trophic level in the sediment signature between the two sites. At the Middelplaat the sediment has a trophic level rank of 0 (so below what one would expect for the primary producer source for the standard (here the cockles), while in the harbour both sediment and SPM rank at level 1. Here it is very important to remember that we did not have a “non-discriminatory filter feeder” suitable to use as a standard available in the harbour. For both sites the cockles collected at the Middelplaat were used as the benchmark.

It is noteworthy that the signature of the Common terns, expressed as trophic level, was lower than that of the bass (*Dicentrarchus labrax*). Another significant result is that shrimps (*Crangon crangon*), which are known to be prey items for many fish, are ranked at a similar trophic level as most of the fish species in our samples.

The data set contains an indication of two possible food sources: sedimentary organic material and suspended organic material. Both sources are characterised by a δ15N of ~ 7.5 ‰, a typical value for detritus in the Dutch coastal zone (Middelburg and Herman 2007). The sediment δ13C is about -25 ‰. The suspended particular organic matter of the Terneuzen Harbour location is comparable, however the material on the Middelplaat is heavier (~ -23 ‰). These values are consistent with previous studies (Middelburg and Nieuwenhuize 1998; Middelburg and Herman 2007). The Terneuzen Harbour site is strongly influenced by a fresh water influx from the Gent-Terneuzen Canal. Freshwater algal species, and therefore also consumers living of these algae tend to have a lower δ 13C level (Middelburg and Herman 2007).

According to the principle “you are what you eat” one would expect that consumers have similar δ 13C values to their food sources. One would therefore expect δ 13C values for animals to range between -25 and -23 ‰ and δ 15N values to exceed 11 ‰. In this study the animal consumers appear to be enriched in δ 13C. This can indicate the presence and consumption of an additional local food source rich in 13C, such as microphytobenthos (Herman et al. 2000; Middelburg et al. 2000). Another possibility lies in temporal or spatial variability in δ13C values in the estuary (Middelburg and Herman 2007) and / or migration of animals from downstream areas to upstream areas. The ctenophores (comb jellies) as well as most fish species (herring, whiting etc) are typical marine species and it is very likely that they do originate from further downstream. Most of these are also known to forage predominantly pelagically, and a strong link with an additional benthic source is less likely for these species. The latter explanation appears therefore the most likely. The location of Terneuzen and the Middelplaat are relatively close to the mouth of the system. The Westerschelde has a significant tidal amplitude and tidal exchange amounts to about 45,000 m3 s^{-1} is much more important than freshwater discharge about 100 m3 s^{-1} (Middelburg and Nieuwenhuize 1998). It is therefore not surprising that at this location the species in the food web that draw on pelagic carbon production are characterised by a marine signature.

From the fact that the samples of pelagic species at both sites had very similar isotope signatures, despite the fact that the Terneuzen Harbour has such a relatively large influx of fresh water, we can determine that these species tend to move in and out of the harbour freely. The signature of all of these species was typical for the Westerschelde estuary around the Middelplaat. Local food production inside the harbour appears therefore not important for these pelagic species, such as juvenile herring. This would also imply that any relatively high local contamination levels in the harbour, due to the heavy shipping traffic, are of relatively little importance for the Common terns. This is different for the shrimps. The δ13C values in the harbour were clearly lower than outside. This implies that the shrimp population utilises local, probably benthic primary production. From the fact that the shrimps in both locations ranked at a similar trophic level as the fish species which are known to be preferred prey items for common terns we have to conclude that shrimps do not constitute a major link in the tern food web.

At first glance it appears strange that common tern samples appear to have a similar signature and therefore a similar trophic level as most fish species. Seabass, a known predator that even at juvenile sizes is capable of handling very large prey, ranks a full trophic level higher than the terns. The lower trophic level as measured in muscles of common terns is a consequence of the fact that these are migrating species. Muscle tissue has been built up not only during the nesting period in the Westerschelde, but also in (sub) tropical areas where this species spends the winter. Material produced in (sub) tropical areas is less enriched with the heavier isotopes, resulting in a lowering of the trophic level. When comparing the liver isotope with the muscle isotope of common terns, indeed the former shows a higher trophic level. Liver has a faster turn-over rate and therefore represents better the local food web situation of the Westerschelde estuary. Interestingly, eggs of common terns have N signatures that resemble their muscle signature. This shows that for the production of eggs also stored energy is used as opposed to only energy directly ingested from food. Isotope analysis of feather material in common terns also showed lower δ15N values in feathers collected just after the birds returned from their southern winter areas than feathers that were known to be grown in the nesting area (Nisbet et al., 2002). The muscle signature is probably the result of a mix of tropical and Westerschelde material.

6.2.4 Contaminant transfer

To be able to assess bioaccumulation potential of contaminants in the Westerschelde food web, the trophic level (expressed as δ15N value) was used to plot contaminant levels against. Of the selected contaminants PCBs were found in the highest concentrations in the Westerschelde food web (up to 1000 ng/g PCB-153 in lipid of biota, except for common tern) as compared to other lipophilic contaminants (up to 75 ng/g PBDE-47 in lipid of biota, and up to 100 ng/g HBCD in lipid of biota).

PCBs

Contaminant transfer of PCBs can be seen in Figure 6.5. The relation between the line and dots is weak (R^2=0.1). The R^2 value represents the strength of the correlation between the drawn line and the dots. This can be explained by the outlier with concentrations around 2000-4000 ng/g PCB-153 lipid of common terns (subcutaneous fat). This means that concentrations in common terns are much higher than what could be expected from the local environment near Terneuzen. As discussed in paragraph 6.2.3., common terns only spend between April and August in the Westerschelde to breed. The remaining time common terns are migrating to and from their wintering area and can collect contaminants in these areas.

If the data of common terns are omitted from the graph, a strong relation (R^2=0.7) can be found between trophic level and contaminant concentration (see Figure 6.6). PCB-153 is bioaccumulating in the food web of the common tern in the Westerschelde.

BFRs

Also PBDEs accumulate in the Westerschelde food web, as can be seen from the BDE-47 figure (Figure 6.7). Bioaccumulation potential of alpha-HBCD is shown in Figure 6.8.

PFCs

Of the non-lipophilic contaminants, PFCs shows similar concentrations in the common tern food web (on average up to 25 ng/g PFOS wet weight, except for one value at 100 ng/g PFOS wet weight) as compared to organotin compounds (up to 25 ng Sn/g TBT wet weight).

Bioaccumulation of PFCs does not show a strong correlation (R^2=0.2) at first hand (see Figure 6.9). However, also here common terns have much higher concentrations (as measured in liver and eggs) than species lower in the food web. When removing the common tern data, a stronger relation (R^2=0.5) can be found and bioaccumulation potential is shown (see Figure 6.10).

OTCs

Even though TBT can be found in several types of biota, there appears no correlation (R^2=0.1) between trophic level and TBT concentration (see Figure 6.11). Uptake and accumulation of TBT therefore seems to be species specific. TPT however does show a rather weak relation (R^2=0.4) between trophic level and TPT concentration (see Figure 6.12).

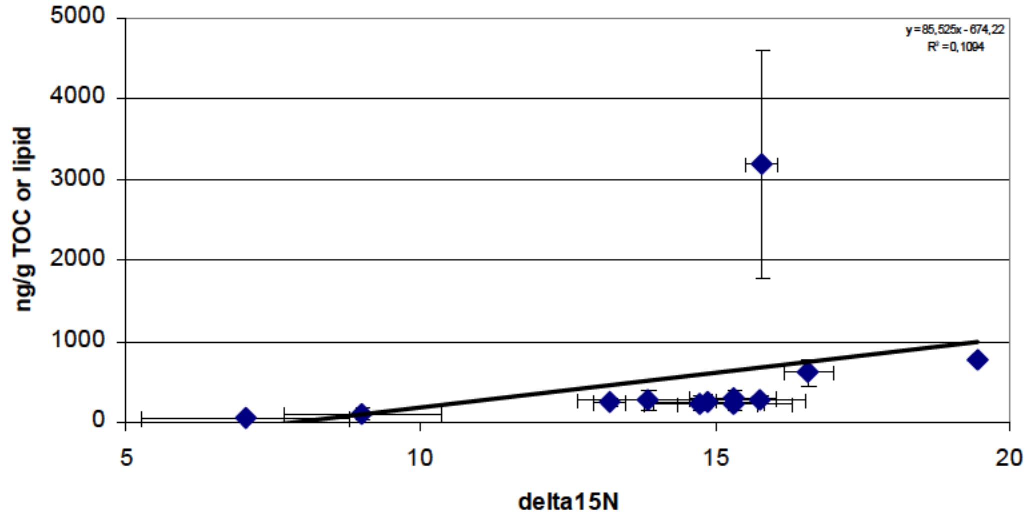

Figure 6.5 *Transfer of PCBs (expressed as PCB-153 ng/g TOC (for sediment and suspended matter samples) or lipid (biota samples)) in the common tern food web of the Westerschelde estuary.*

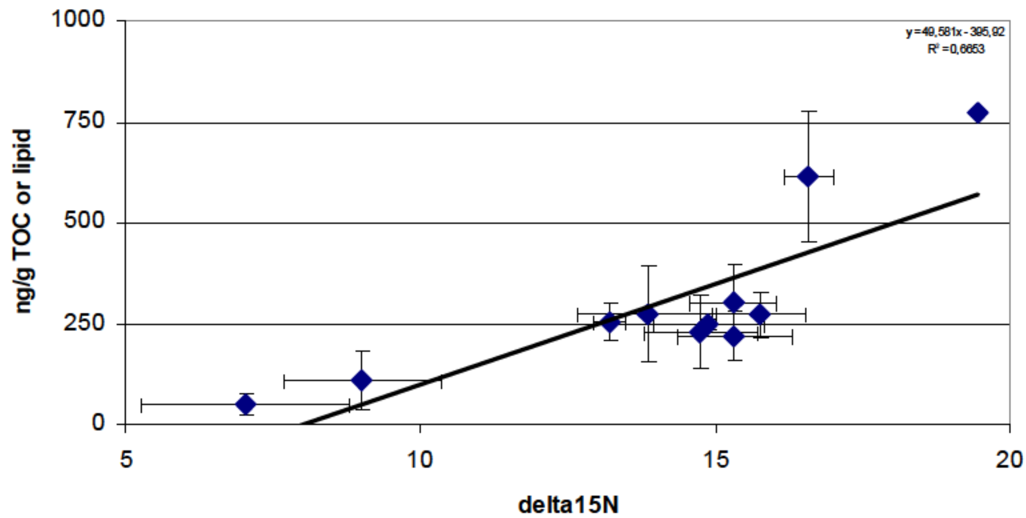

Figure 6.6 *Transfer of PCBs (expressed as PCB-153 ng/g TOC (for sediment and suspended matter samples) or lipid (biota samples)) in the common tern food web of the Westerschelde estuary. The PCB-153 results of the common tern (subcutaneous fat) are left out in the graph.*

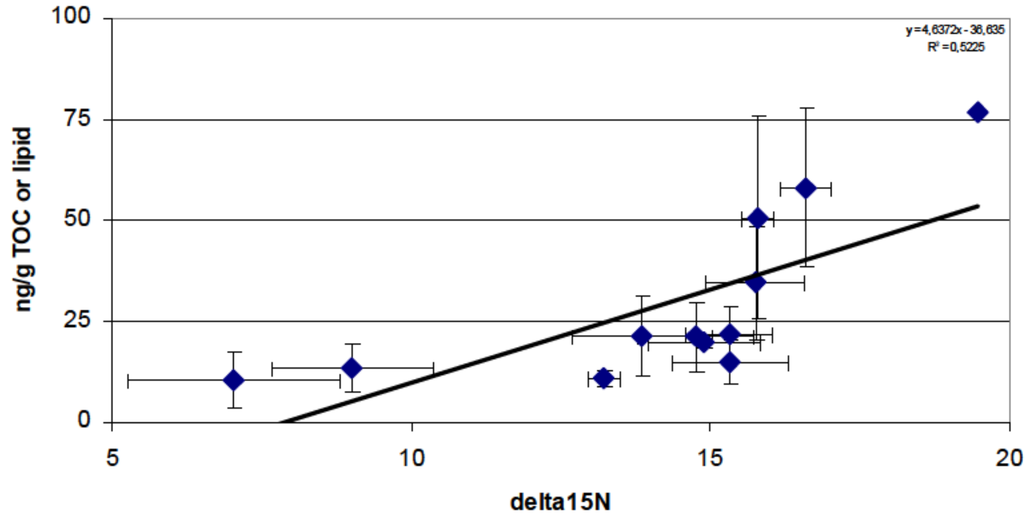

Figure 6.7 *Transfer of PBDEs (expressed as PBDE-47 ng/g TOC (for sediment and suspended matter samples) or lipid (biota samples)) in the common tern food web of the Westerschelde estuary.*

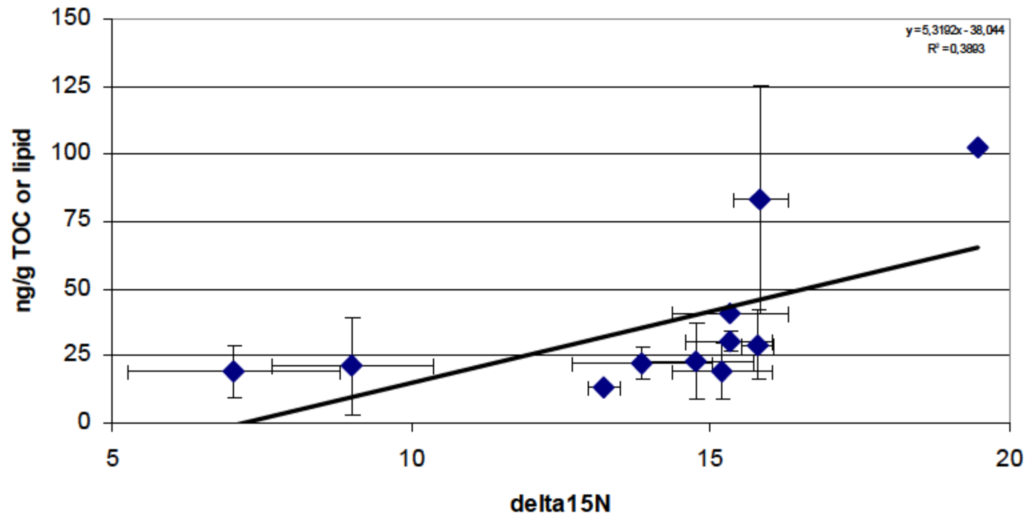

Figure 6.8 *Transfer of HBCD (expressed as alpha-HBCD ng/g TOC (for sediment and suspended matter samples) or lipid (biota samples)) in the common tern food web of the Westerschelde estuary.*

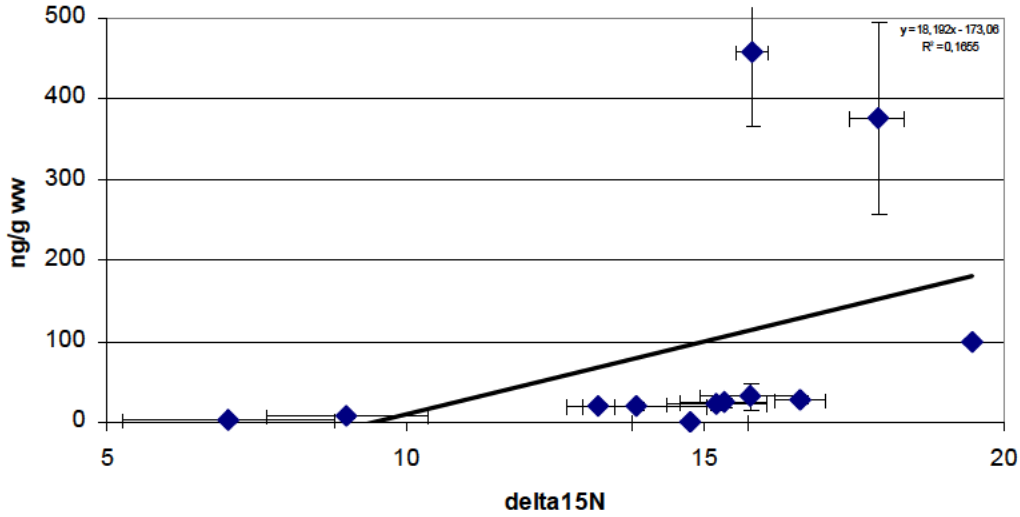

Figure 6.9 *Transfer of PFCs (expressed as PFOS ng/g wet weight) in the common tern food web of the Westerschelde estuary.*

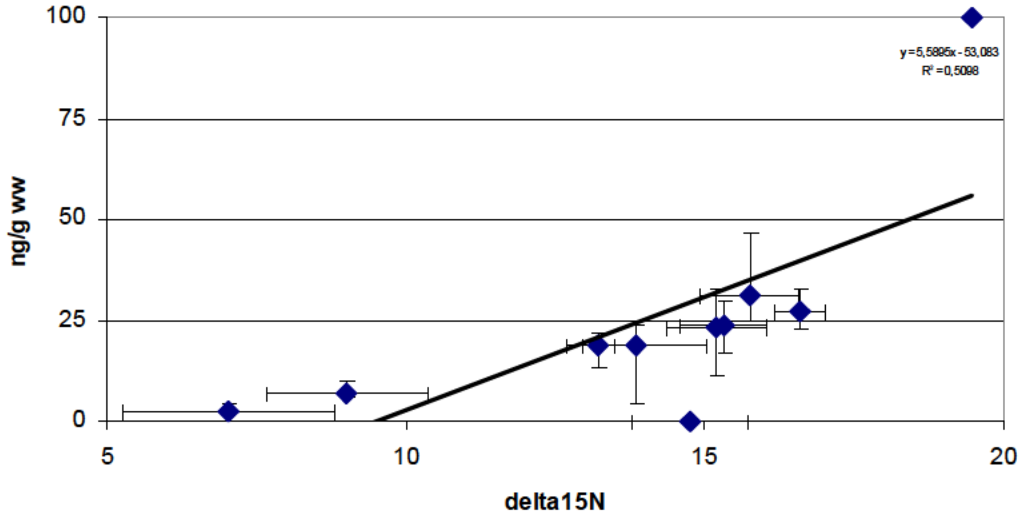

Figure 6.10 *Transfer of PFCs (expressed as PFOS ng/g wet weight) in the common tern food web of the Westerschelde estuary. The PFOS results of the common tern (liver and egg) are left out in the graph.*

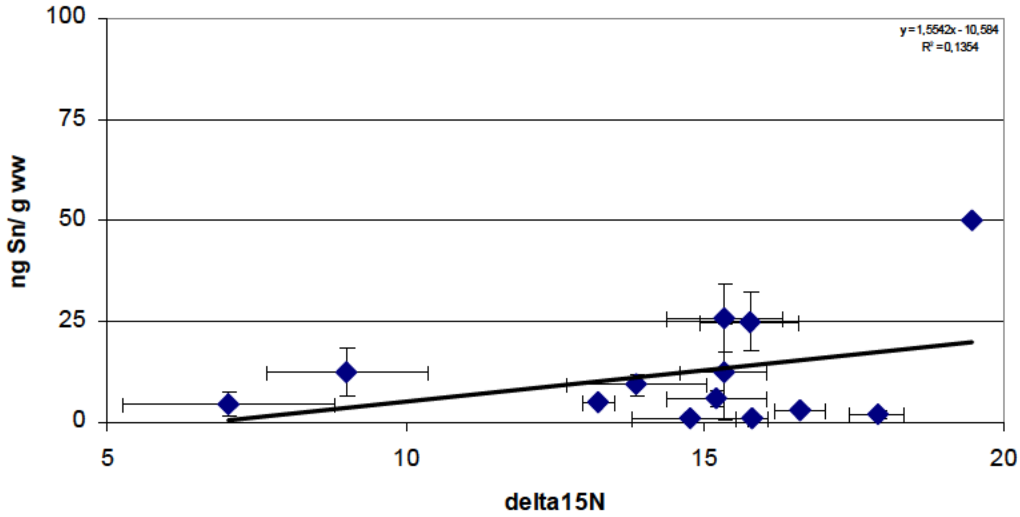

Figure 6.11 *Transfer of TBT (expressed as TBT ng Sn/g wet weight) in the common tern food web of the Westerschelde estuary.*

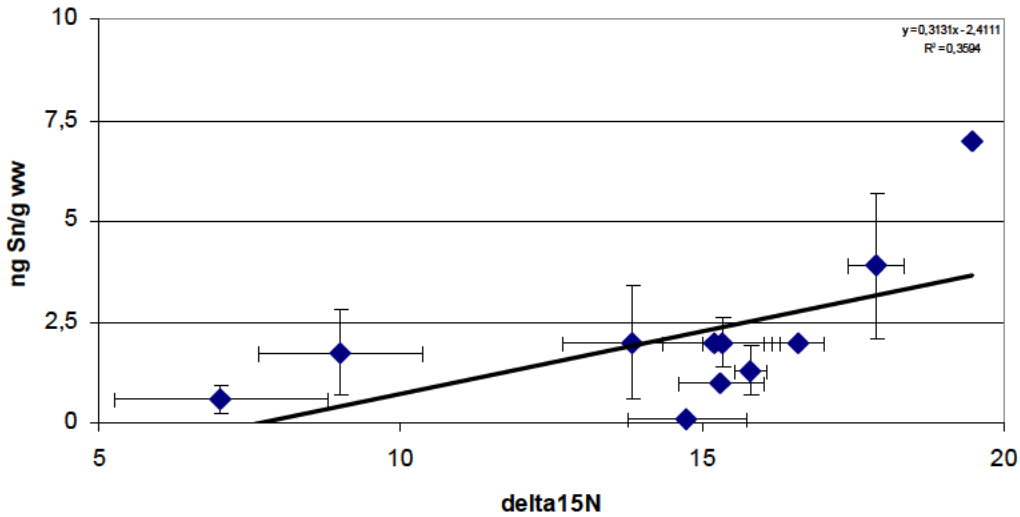

Figure 6.12 *Transfer of TPT (expressed as TPT ng Sn/g wet weight) in the common tern food web of the Westerschelde estuary.*

Table 6.1 Average concentrations of contaminants in adult female common terns in comparison to their eggs. PCB-, PBDE- and HBCD-concentrations are measured in subcutaneous fat of adult female common terns. PFOS- and TBT-concentrations are measured in livers of adult female common terns.

Compound	Concentration in adults	Concentration in eggs
PCBs	9.61 μg/g lipid (+/- 0.74)	9.43 μg/g lipid (+/- 4.45)
PBDEs	146 ng/g lipid (+/- 117)	125 ng/g lipid (+/- 45)
HBCD	83.0 ng/g lipid (+/- 42.3)	29.2 ng/g lipid (+/- 13.0)
PFOS	376 ng/g wet (+/- 119)	457 ng/g wet (+/- 90)
TBT	4 ng TBT/g wet (+/- 3.7)	1.6 ng TBT/g wet (+/- 1.3)

6.2.5 Maternal transfer of contaminants in common terns

Results are presented in Table 6.1. PCB and PBDE concentrations appear to be almost similar in female subcutaneous fat and corresponding eggs. Concentrations of HBCD and TBT are lower in eggs as compared to respectively female subcutaneous fat and liver. PFOS concentrations however are slightly elevated in eggs as compared to female livers but the difference is not statistically significant.

PCBs

In general, the concentrations of PCBs measured in adult terns in 2007 are comparably lower than those measured in studies carried out 6 years ago (Van den Brink & Bosveld 2001). There is no significant difference in the amount between parent birds and eggs ($p>0.05$).

Oviparous transfer of PCBs has been observed in glaucous gulls, opportunistic feeders that incorporate fish in its diet (Verreault et al. 2006). Incorporation is suggested to occur during egg yolk formation and the degree of incorporation varies with the amount of lipid that is sequestered by the parent to the egg in the process. The mechanism of the transfer is dependent on biological factors such as clutch size, energy demand and yolk content, and physicochemical factors such as the properties of the contaminant, its activity, lipid solubility induction of xenobiotic metabolising enzymes such as cytochrome P450 (CYP) monooxygenases. It has been observed that metabolically resistant, higher-chlorinated congeners were readily retained in parent birds and less readily transferred to eggs (Verreault et al. 2006, Bargar et al. 2001).

Survivability is not equivalent to hatchability though the latter does influence the chance of survival. Survivability here means the ability of young terns to remain alive following hatching in spite of toxicological effects. Whether or not the survival of tern offspring is prevented cannot be fully established without the LD50 terns for each contaminant. The LD50 would be compared to the levels measured in tern embryos from the various collated studies. However, this was not found available in a literature search.

PBDEs

PBDE concentrations in 2007 show the same trend as is seen with PCBs. There is no significant difference between parent terns and eggs, although notable differences have been reported in literature (Verreault et al. 2006, Van den Brink et al. 2007). Again, this reflects a balance in concentrations between parent and offspring. A trend cannot easily be established by comparison of the concentrations measured between years as differing congeners were measured in each annual study, with the exception of PBDE 47. A decrease in the measured concentration of this congener is seen between 2005–2007. PBDE analyses have not been part of a monitoring programme in mussels in the Westerschelde, so these trends cannot be confirmed in mussels.

Oviparous transport of PBDEs from avian parents to their offspring is also reported in field studies by Verreault et al. (2006) on glaucous gulls.

Concentrations of α-HBCD did show a difference in concentrations between parent birds and eggs. There were considerably lower concentrations of the contaminant in eggs than in adults (29.2 ng/g in egg as compared to 83.0 ng/g in adult, on average). This shows that there is an imbalance in the concentration of HBCDs towards adult birds and that these compounds are not readily transferred from adult to egg based on this study in 2007. The measured concentrations in eggs were also found to be lower than those measured in 2005 and 2006 (Van den Brink et al. 2007). HBCD analyses have not been part of a monitoring programme in mussels in the Westerschelde, so these trends cannot be confirmed in mussels.

PFOS

The PFOS concentrations in eggs in 2007 exceeded those measured in parent birds, though it is concluded that this difference is not statistically significant (Van den brink et al. 2007). What is significant though, is the difference in concentrations as compared to those measured in 2006, which are lower in current results by about three-fold. Yet, a trend is not easily established as in there was an observed ten-fold increase in concentration between 2005 and 2006 (Van den Brink et al. 2007). The lowest concentration found in this study in eggs is 4.09 µg/g wet weight, which already exceeds concentrations seen to cause developmental effects in chicken embryos (Molina et al. 2006) and is also comparable to the LD50 for chickens.

It has been observed that PFOS preferentially binds to egg albumin, thereby denoting the oviparous transfer of the compound from female parents to their eggs. Concentrations in eggs tend were also higher than concentrations in adult birds in another study (Kannan et al. 2005). The oviparous transfer of PFOS from parent birds to offspring has been monitored in chicken, northern bobwhites and mallards, all of which are not fish-eating birds. The results have shown that there chicken embryos were more sensitive to PFOS than either bobwhites or mallards (Molina et al. 2006). This was additionally observed in the fish-eating common merganser, where there was a gender-specific difference in the level of PFOS measured in liver with lower levels in females as compared to males (Sinclair et al. 2006).

A comparative sensitivity to PFOS of common terns with chickens (the typical model) is not available in literature.

OTCs

These compounds were generally lower in eggs as compared to adults in 2007. As such it can be suggested that there is an imbalance in the concentrations of organotin compounds towards adult terns. Specifically, the concentrations of TBT and TPT in adults are on average about 3- and 20-fold greater as compared to offspring. Low concentrations in eggs can be explained by the behaviour of OTCs. OTCs accumulate in organs rather than fatty tissue and are therefore not well represented in lipid stores that are used in egg formation (Veltman et al. 2006).

6.3 Harbour seal food web

6.3.1 Research questions
The current policy question consists of:
1) What is the cause of a lower reproductive success in harbour seals of the Delta region (<1 %) as compared to that in the Wadden Sea (~15%)?

Specific questions of the current case study were:
1) What do harbour seals in the Westerschelde estuary eat and what is the structure of the harbour seal food web in the Westerschelde estuary?
2) Which of the selected contaminants are being transferred in the harbour seal food web near Terneuzen?
3) What are the current contaminant concentrations and toxicological profiles in harbour seals of the Westerschelde?

6.3.2 Sampling
Feces samples of harbour seals in the Westerschelde estuary were collected monthly by IMARES for a period of 6 months in 2007. Sand banks, where harbour seals were known to rest, were frequented after low tide to collect samples. The samples were analysed at IMARES.

In September 2008 an extensive sampling campaign was conducted at two locations in the Westerschelde for the study on the food web of harbour seals in this estuary: Molenplaat, as part of the Platen van Ossenisse, and Platen van Valkenisse (Figure 6.2). Sampling was conducted by RWS Meet- en Informatiedienst (suspended particulate matter (SPM) and silicon sheets), Grontmij|AquaSense and the fishing vessel “BRU45” (Iman and Jaap Deurloo). This vessel was equipped with several types of fishing gear, including a pelagic seine net, a beam trawling net and a set bag net (“raamkuil”). Details can be found in Burger & Vanagt (2008). Samples were analysed by IVM (PFCs) and Wageningen IMARES (PCBs, PBDEs, Organotin compounds).

Sampling took place under the following permissions: NBW-permission, Flora & Fauna exemption, approval of Animal Experiment Committee (DEC), and an exemption of the Fishery Directive (ministry of LNV).

6.3.3 Food web composition
As can be seen in Figure 6.13 the main diet of harbour seals in the Westerschelde estuary (>80%) is composed of flat fish species, such as plaice, sole and flounder.

To determine the trophic positions of of species, the peppery furrow shell (*Scrobicularia plana*) was collected at the Molenplaat and Platen van Valkenisse. Originally, cockles were chosen as a standard for the calculation of trophic level, but no cockles could be collected at either sand flats. When algae (primary producers) are considered to be trophic level 1, the peppery furrow shell is by definition at level 2.

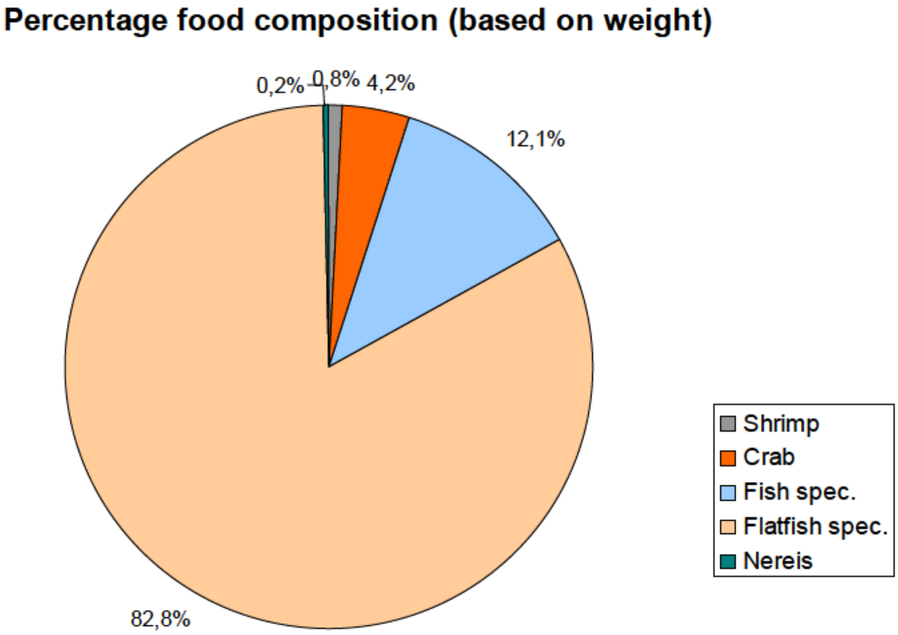

Figure 6.13 *Percentage food composition of Harbour Seals in the Westerschelde estuary (based on weight).*

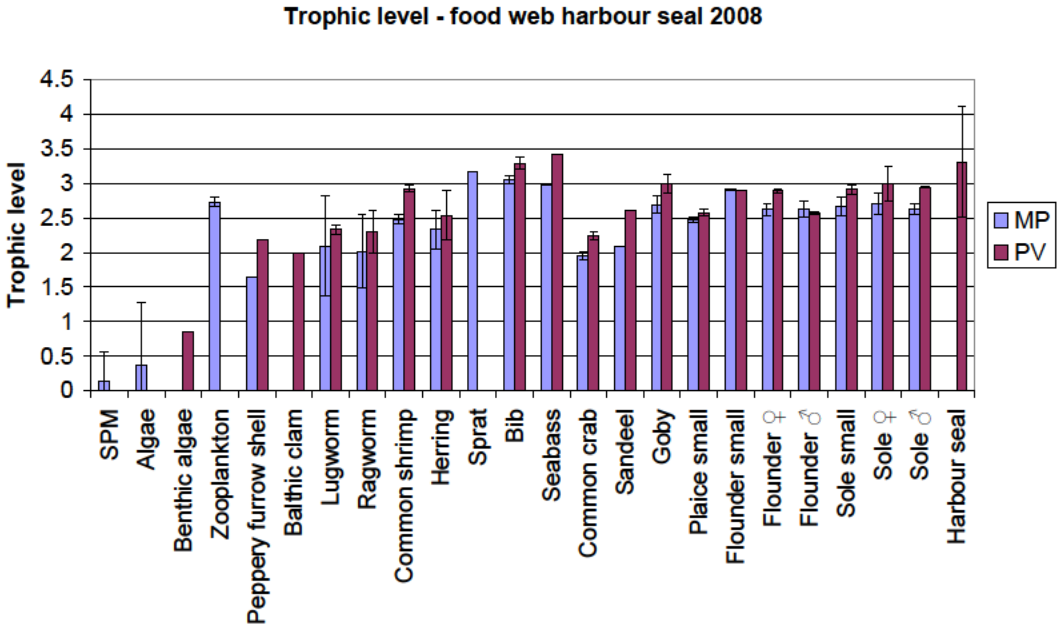

Figure 6.14 *Trophic levels of the samples taken at Molenplaat (MP) and Platen van Valkenisse (PV) of the harbour seal food web. SPM=Suspended Particulate Matter.*

The composition of the food web can be seen in Figure 6.13. SPM and benthic algae are around a trophic level of 1. Shell fish are found around trophic level 2, whereas worms and crabs can be found around level 2-2,5. Shrimps are up to a trophic level of 3. All fish species are between trophic level 2 and 3, with bib and seabass occupying the trophic levels up to 3.5. Highest trophic levels are found in harbour seals, although variation is relatively high compared to the other species (3.3 ± 0.8). Trophic levels are in general slightly higher for each species at the Platen van Valkenisse (more upstream). When compared to the trophic levels of the common tern food web, the levels are quite comparable. Because larger prey items were selected and caught, higher trophic levels were expected in the harbour seal food web. Direct comparison however is not possible since samples were taken at a different time during the year (common tern food web in May, harbour seal food web in September) and at different locations (harbour seal samples were collected further upstream).

6.3.4 Contaminant transfer

To be able to assess bioaccumulation potential of contaminants in the Westerschelde food web, the trophic level was used to plot contaminant levels against. PCB concentrations were the contaminants that were found in the highest concentrations in the Westerschelde food web (up to 114267 ng/g in lipid of harbour seal blood and up to 9843 ng/g PCB-153 in lipid of other biota (common crab)) as compared to BFRs. Concentrations were up to 41 ng/g in lipid of harbour seal blood and up to 188 ng/g PBDE-47 in lipid of biota (flounder). Concentrations were up to 91 ng/g HBCD in lipid of biota (flounder), whereas no data were derived for harbour seal. Of the non-lipophilic compounds, PFOS concentrations were highest (up to 11000 ng/g in harbour seal blood and up to 500 ng/g ww in other biota (flounder)), followed by TBT (up to 100 ng Sn/g ww (common shrimp)) and TPT (up to 25 ng Sn/g ww (seabass)). OTC concentrations in harbour seal blood were below detection limits. Harbour seal data will be further discussed in chapter (6.3.5).

When compared to fish samples of the common tern food web, the samples of the harbour seal food web contain contaminant concentrations that are roughly three times higher than concentrations found in the common tern food web, except for HBCD which has comparable levels in both food webs. Higher concentrations in the harbour seal food web can be explained by a variety of factors such as: 1) samples were collected further upstream, whereas contaminant levels tend to be higher upstream (Van den Heuvel-Greve et al. 2006), 2) body sizes of species collected were larger than samples from the common tern food web, which may point at older individuals with a longer exposure time to contaminants, 3) samples of the harbour seal food web contained many benthic species, and benthic species may collect higher contaminant levels than pelagic species (of the common tern food web), 4) samples were taken in a different season of the year (September for the harbour seal food web versus May for the common tern food web). Interestingly however, is that stable isotope data do not differ greatly between these two food webs (see chapter 6.3.3), which may point at the fact that both location (further upstream) and composition of the (benthic) food web may have influenced differences in contaminant levels.

PCBs

Contaminant transfer of PCBs can be seen in Figure 6.15. Even though an increase in Sum-7PCB concentrations can be seen from left to right, the most striking is the large variety between samples. PCB-153 surprisingly does not show a strong bioaccumulation potential in these samples.

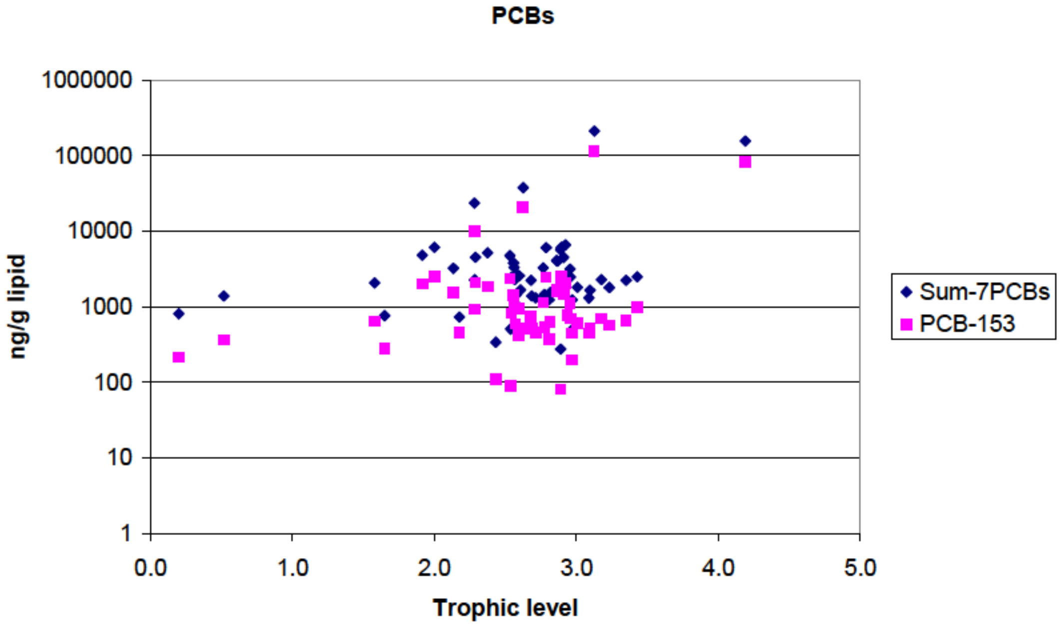

Figure 6.15 *Transfer of PCBs (expressed as ng Sum-7PCB or PCB-153 /g TOC or lipid weight) in the harbour seal food web of the Westerschelde estuary. Note: the Y-axis is plotted on a logarithmic scale.*

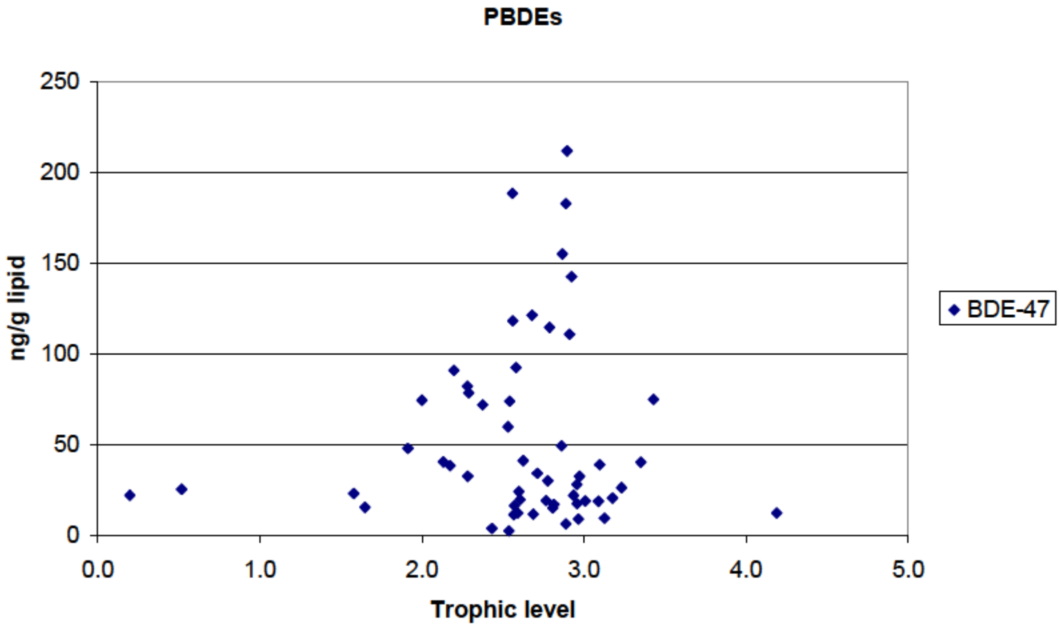

Figure 6.16 *Transfer of PBDEs (expressed as ng PBDE-47 /g TOC or lipid weight) in the harbour seal food web of the Westerschelde estuary.*

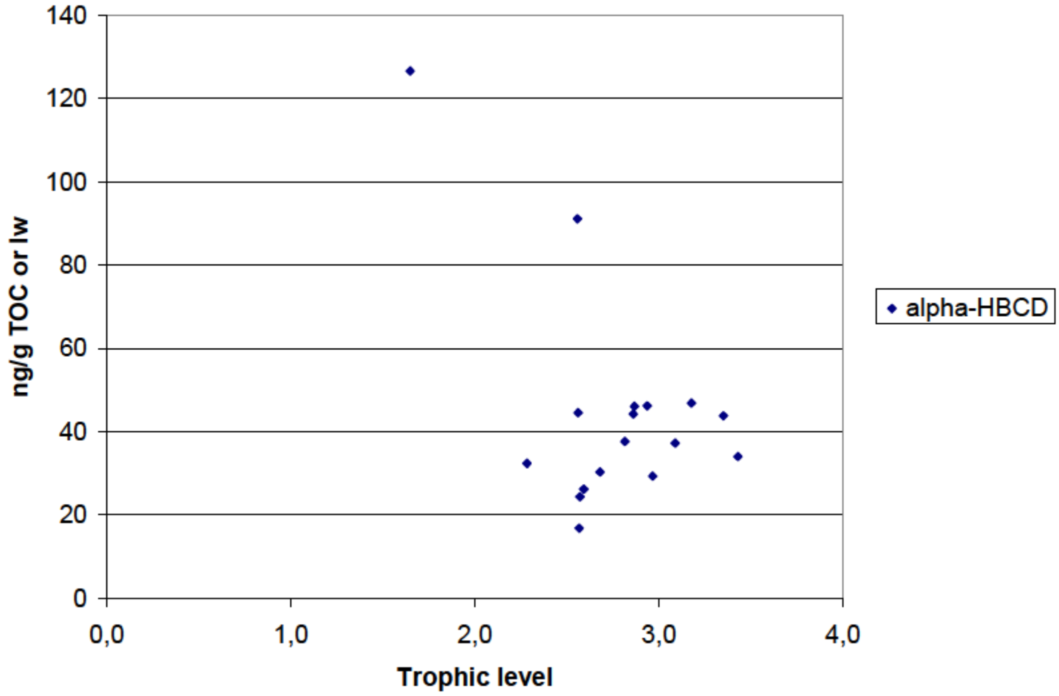

Figure 6.17 *Transfer of HBCD (expressed as ng/g TOC or lipid weight) in the harbour seal food web of the Westerschelde estuary.*

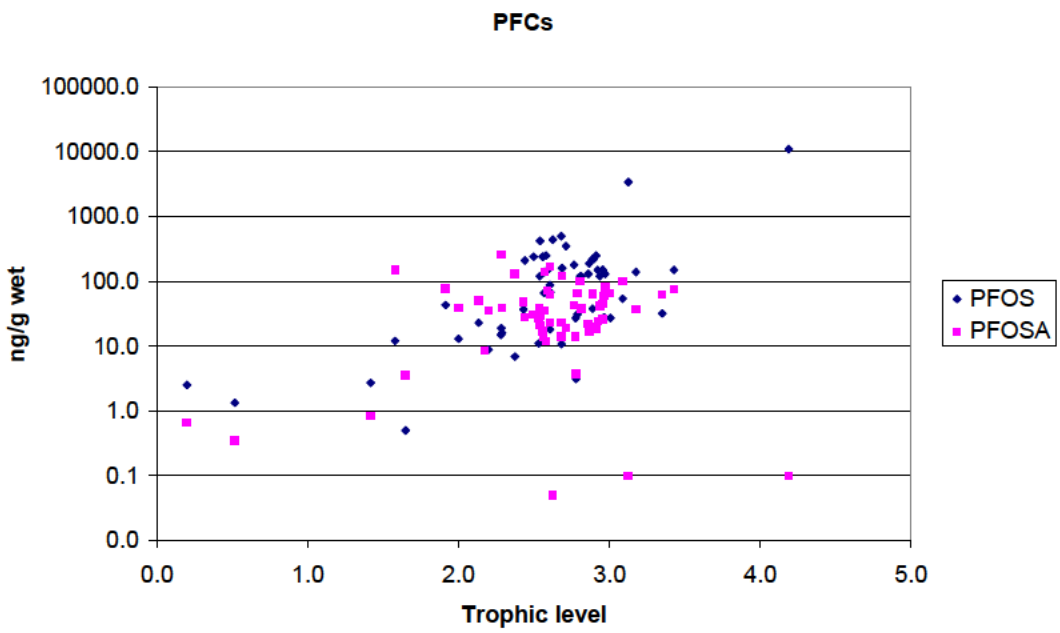

Figure 6.18 *Transfer of PFCs (expressed as PFOS and PFOSA ng/g wet weight) in the harbour seal food web of the Westerschelde estuary. Note: the Y-axis is plotted on a logarithmic scale.*

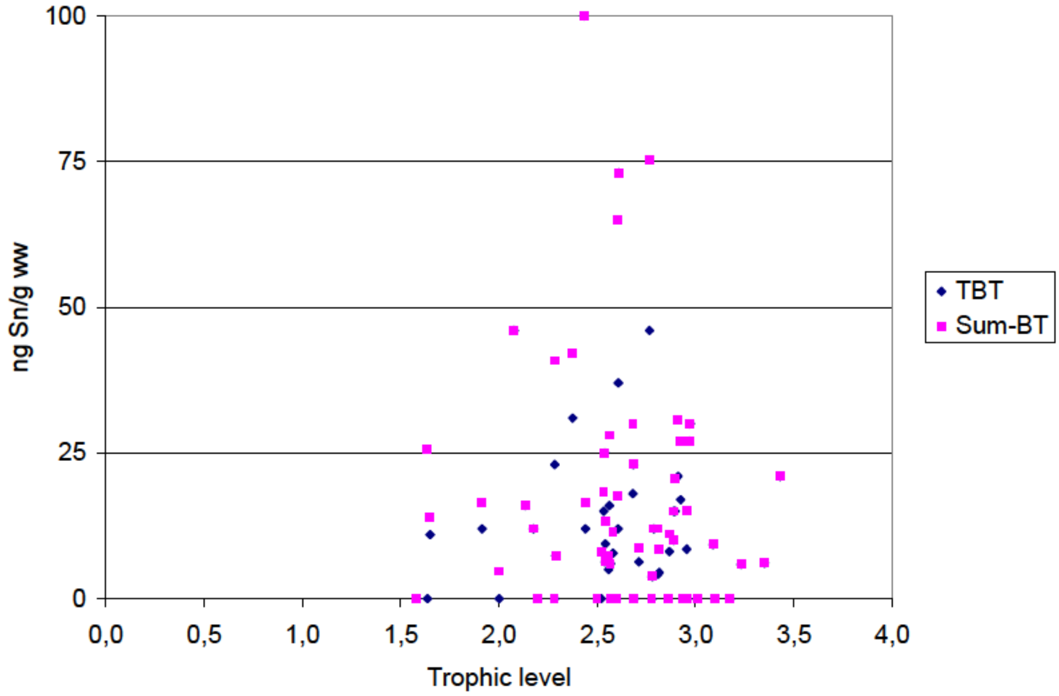

Figure 6.19 Transfer of butyltin compounds (expressed as TBT and sum-BT ng Sn/g wet weight) in the harbour seal food web of the Westerschelde estuary.

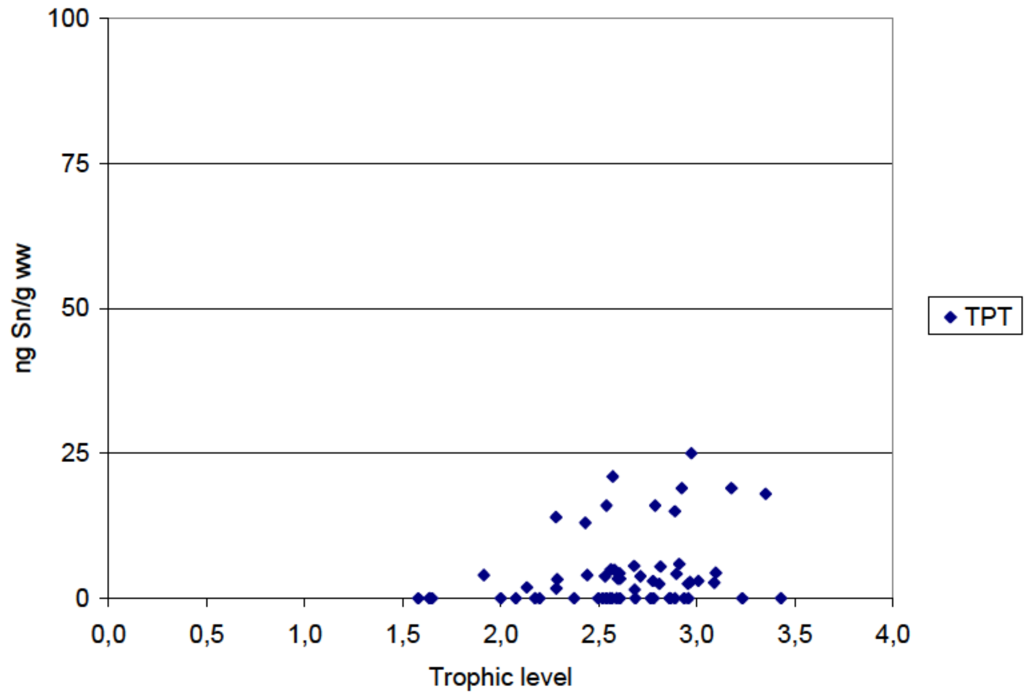

Figure 6.20 Transfer of phenyltin compounds (expressed as TPT ng Sn/g wet weight) in the harbour seal food web of the Westerschelde estuary.

BFRs

Also PBDEs accumulate in the Westerschelde harbour seal food web, as can be clearly seen from the BDE-47 data (Figure 6.16).

Bioaccumulation potential of HBCD in the sampled harbour seal food web is not strong according to Figure 6.17. Most samples were below detection limit, only samples above the limit are shown. Two outliers containing 127 and 91 ng alpha-HBCD /g lipid weight are for a peppery furrow shell and a plaice sample. HBCD was not analysed in harbour seal samples.

PFCs

In Figure 6.18 the bioaccumulation of PFOS and PFOSA in the harbour seal food web is shown. PFOS clearly show bioaccumulation, whereas PFOSA shows bioaccumulation up to a trophic level of around 2.5 after which dilution can be seen (mainly driven by the low PFOSA concentrations in harbour seal blood). Flat fish contain higher PFOS concentrations than expected from the position in the food web. In the common tern food web PFOS concentrations were on average up to 25 ng/g PFOS wet weight, except for one value at 100 ng/g PFOS wet weight. The samples harbour seal food web contain higher PFOS values. This can be explained by the larger size of biota sampled (food of harbour seals can be up to 30 cm in size as compared to a maximum of 12 cm for common terns) and the fact that the harbour seal food web is bentho-pelagic as compared to the pelagic common tern food web. It should be noted however that the food web of the harbour seals was sampled in September, whereas the food web of the common tern was sampled in May. Differences in concentration between seasons (spring versus autumn) cannot be excluded.

OTCs

Butyltin concentrations in the Harbour Seal food web are given in Figure 6.19. Butyltin compounds consist of TBT, DBT and MBT. In most samples TBT was the main compound, whereas in half of the samples also DBT was observed. MBT was only found in some samples, lower in de food web (e.g. worms and crab). Butyltin concentrations in harbour seal blood were below detection limits.

Transfer of Phenyltin compounds can be seen in Figure 6.20. More than half of the samples contained TPT concentrations above detection limit. No DPT concentrations were observed in any of the samples, whereas MPT was only found once in a seabass sample. Phenyltin concentrations in harbour seal blood were mainly below detection limits, except for one sample containing low levels of TPT.

6.3.5 Contaminant concentrations and toxicological profiles in harbour seals

A preliminary set of samples was analysed to assess the level of a selection of contaminants in harbour seals of the Westerschelde estuary. PCBs, PBDEs, chlorinated phenolics, chlorinated pesticides and metabolites of PCBs and PBDEs were analysed in nine blood clot samples of individual harbour seals sampled by Wageningen IMARES. Those samples were taken in the Westerschelde (three males, two females), Oosterschelde (two males) and Waddensea (two males). PFCs and OTCs were analysed in three blood plasma samples derived from Wageningen IMARES. These samples were taken from male individuals that were sampled in the Westerschelde estuary in September 2007.

Variation of contaminant levels in blood samples is high between individual harbour seals (see standard deviation in both Table 6.2 and Table 6.3). This is caused by the individual behaviour of harbour seals. Each harbour seal has it’s own preferred feeding strategy with regards to for example location and prey. Both these parameter influence the exposure to and uptake of contaminants.

Of the lipophilic compounds (PCBs, PBDEs and chlorinated phenols) PCBs were found in highest concentrations (see Table 6.2). When compared to all contaminants, PFOS concentrations were highest (see Table 6.3).

Sum-PCB concentrations of were in line with PCB levels in ringed seal blood plasma reported by Routti et al. (2008) from the Svalbard and Baltic Sea region, although one sample of a male seal of the Westerschelde is a factor three higher than others. Even though the number of samples is low, data show that PCB concentrations are highest in male and female harbour seals of the Westerschelde when compared to those of the Oosterschelde and Waddensea. Especially concentrations in male harbour seals of the Westerschelde are elevated. Elevated levels can also be seen in the first data on chlorinated phenolics (PentaChloroPhenol and related compounds). PCP-production and use is prohibited in Europe as of 1992, but can still be found in water due to it's persistency. Of the 43 PBDE congeners monitored, only a small number were detected sporadically and at low levels (i.e. 1.0 ng/g ww). Concentrations were in line with blood levels in ringed seals from the Balthic Sea (see Table 6.4). Various levels of a few OC pesticides were detected. Chlorinated pesticides were mainly dominated by the presence of p,p'-DDD followed by β-HCH.

Metabolites of PCB and PBDEs (OH-PCB, OH-PBDE and MeSO2-PCB) are in line with previous studies of seals in the Baltic Sea and Svalbard (Routti et al., 2008; 2009). MeSO2-PCB levels in blood were virtually non-detectable. As for OH-PBDEs, in the blood of recently collected ringed seal from the Baltic Sea and Svalbard, OH-PBDEs are essentially non-detectable, but with low frequency and sub-ppb detection of a few OH-PBDEs (e.g., 6-OH-BDE47) and only in Baltic seals (Routti et al., 2009).

PFC-results showed a high variety between the three samples (see standard deviations in Table 6.3). The highest concentrations were probably from a harbour seal that spend a long time near and in Antwerp harbour. When compared to concentrations in seals from other areas, PFOS concentrations in harbour seal samples of the Westerschelde are 0.5-12 times higher than the highest concentration in seals of the German Bight, 2-48 times higher than the highest concentration in seals of the Balthic Sea, and 9-220 times higher in the highest concentration in seals of the Arctic (see Table 6.4).

Organotin results showed TBT- and TPT-concentrations around background level (see Table 6.3), except for TPT that was above detection level in one animal. Concentrations are in range with those from seals from Canada (see Table 6.4).

All samples showed some dioxin like activity (see Table 6.3).

Table 6.2 Average concentrations (wet weight) in blood samples (clot) of harbour seals of several locations within the Netherlands (only these groups contaminants have been analysed at several locations within the Netherlands).

Compound	Westerschelde Female (n=2) Mean (±stdev)	Westerschelde Male (n=3) Mean (±stdev)	Oosterschelde Male (n=2) Mean (±stdev)	Waddensea Male (n=2) Mean (±stdev)
Sum-PCB (ng/g ww)	392 (417)	1514 (1087)	221 (48)	225 (218)
Sum-PBDE (ng/g ww)	0.23 (0.14)	0.12 (0.03)	0.34 (0.22)	0.03 (0.04)
Sum chlorinated phenolics (ng/g ww)	13.6 (0.3)	33.2 (16.3)	9.0 (2.0)	4.0 (3.0)

Table 6.3 *Average concentrations (wet weight) in blood samples (plasma) of three male harbour seals of the Westerschelde estuary.*

Compound	Mean (±stdev)
PFOS (ng/ml)	4947 (5447)
PFOA (ng/ml)	20 (16)
PFBS (ng/ml)	233 (297)
TPT (ng Sn/ml)	0.6 (0.3)
TBT (ng Sn/ml)	<3
DR-CALUX (ng TEQ/ml)	0.44 (0.37)

Table 6.4 *Average concentrations of PFOS in blood samples (plasma and whole blood) of seals of the Balthic Sea. Plasma is the liquid phase after centrifuging non-clotted blood. Whole blood is the entire blood sample. Serum is the liquid phase after centrifuging clotted blood.*

Species	Region	Sample type	Compound	Concentration	Reference
Ringed seal	Svalbard	Plasma	Sum-PCB (ng/g ww)	2.6-260	Routti et al. 2008
Ringed seal	Balthic Sea	Plasma	Sum-PCB (ng/g ww)	4.6-185	Routti et al. 2008
Baikal seal	Lake Baikal	(Whole) blood	Sum-PCB (ng/g ww)	6.4-130	Imaeda et al. 2009
Ringed seal	Svalbard	Plasma	Sum-PBDE (ng/g ww)	0.067-0.80	Routti et al. 2009
Ringed seal	Balthic Sea	Plasma	Sum-PBDE (ng/g ww)	0.24-1.0	Routti et al. 2009
Baikal seal	Lake Baikal	Serum	PFOS (ng/g ww)	1.3-17	Ishibashi et al. 2008
Harbour seal	German Bight	(Whole) blood	PFOS (ng/g ww)	48-887	Ahrens et al. 2009
Ringed and grey seal	Canadian & Norwegian Arctic	Blood	PFOS (ng/ml)	3-50	Kannan & Giesy 2002
Ringed and grey seal	Balthic Sea	Blood	PFOS (ng/ml)	14-230	Kannan & Giesy 2002
Harbour seal (pup)	St Lawrence estuary, Canada	Whole blood	Sum-BTs (ng Sn/g ww)	<detection-0.4	Frouin et al. 2008

Effects of contaminants on marine mammals

Results of a feeding experiment reported in literature showed significant effect of PCBs on reproduction in seals (see Table 6.5). The food during these experiments contained a mixture of contaminants, although PCB concentrations changed significantly between ‘clean’ and ‘contaminated’ food. Effects on reproduction were observed; the number of pregnant females was significantly lower in the group that was fed ‘contaminated fish’ as compared to ‘clean’ food. Further research showed that exposure to contaminants during implantation (the period straight after ovulation) was the cause of reduced fertility (Reijnders 1986).

In another feeding study experiment results also showed a significant effect of PCBs on immune functioning in seals (see Table 6.5). Threshold for immunotoxic effects were < 11-22 mg/kg PCBs in lipids, and <210 ng/kg TEQ/PCBs (non- and mono-ortho PCBs) in lipids. Effects on the immune system were also related to PCBs concentrations in field animals (Levin et al. 2005; Beineke et al. 2005).

Du et al (2008) summarised some of the chronic effects of PFOS. In mammals, PFOS has been shown in rats and mice throughout pregnancy, to severely affect postnatal survivor of neonatal rats and mice (Thibodeaux et al., 2003; Luebker et al., 2005a, b; Fuentes et al., 2006, 2007). Recently, Chang et al. (2008) studied PFOS exposure to rats and hypothesized that exposure to PFOS may increase free thyroxine (FT4) in the rat serum due to the ability of PFOS to compete with thyroxine for binding proteins and increase in FT4 would increase the availability of the thyroid hormone to peripheral tissues for utilization, metabolic conversation, and therefore increase T3. Since thyroid hormones play important roles in development, further investigations are necessary to elucidate the changes on thyroid hormone due to PFOS exposure.

Toxic thresholds of contaminants in seals

Pijnenburg and Van den Heuvel-Greve (2008) conducted a literature review on the effects of contaminants on marine mammal health. It was concluded that even though extensive research is conducted, toxic thresholds for marine mammals are still hard to establish. Only for PCBs toxic thresholds were derived (see Table 6.6). As contaminants were only measured in harbour seal blood, no direct comparison can be made to the threshold in marina mammal blubber. Flat fish of the Westerschelde contain sum-7PCB concentrations of 41-191 ng/g wet weight in this study, which is similar to the toxic threshold of 10-150 ng/g wet weight as derived for marine mammal diet. It should however be noted that effect concentrations can not directly be taken from one organism to another. Hammond (2005) observed for example toxicity of PCBs for the harbour seal, but at the same concentration no immunotoxicity was observed in grey seals.

There are not enough clear data available for other contaminants to base toxic thresholds on.

Table 6.5 An overview of effects of contaminants in seals. [1] PCB-99, -149, -153, -138, -180, -187.

Type of research	Contaminant	Mixture of contaminants?	Effect concentration	Effect	Reference
Feeding experiment	PCBs, DDT	Yes	Intake of 1,5 mg PCB and 0,4 mg DDT /day during 2 years t.o.v 0,22 mg PCB en 0,13 mg DDT	Significant decrease in reproduction	Reijnders 1986
Feeding experiment	PCBs, PCDD/F	Yes	< 11-22 mg/kg PCBs in lipid 16 µg/kg PCB wet weight in food	Effects on immune system LOAEL immune system	Ross, 1995/1996 Kannan 2000
Effects correlated with field concentrations	PCBs	Yes	2,5 mg/kg lipid weight in blubber	Increase immune functions	Levin, 2005
Effects correlated with field concentrations	PCBs	Yes, DDT, toxaphene, PBDEs	Average of 10,12 mg/kg PCB lipid weight in blubber Average of 9,48 mg/kg PCBs lipid weight in blubber	Effect on immune system: thymus atrophy Degradation of spleen	Beineke, 2005

Table 6.6 Toxic thresholds for PCBs in marine mammal tissue and diet of marine mammals (Kannan, 2000).

Sample type	Toxic threshold value for PCBs
Marine mammal (blubber)	17 mg/kg lipid weight
Marine mammal (food)	10-150 µg/kg wet weight (on average 89 µg/kg wet weight)

7 Importance of trophic transfer to EU guidelines

The process of bioaccumulation of contaminants in aquatic organisms is clearly vital to the proper understanding whether a substance could accumulate sufficiently *in vivo* to cause significant adverse effects in individual organisms and populations, and ultimately to ecosystem structure and function. Information on a chemical's capacity for accumulation is commonly used for the assessment of wildlife and human food chain exposure, *i.e*., evaluation of food web transfer and identification of potential POP compounds, chemical risk characterization, and development of environmental quality standards. However, it is not yet fully incorporated in environmental European directives and guidelines.

In chapter 2.4 several international directives and other guidelines legislation were briefly described. In this chapter we will further focus on a few directives that focus on the environmental quality of aquatic systems: the WFD, Natura2000, OSPAR and MSFD.

7.1 Water Framework Directive (WFD)

On 23 October 2000, the "Directive 2000/60/EC of the European Parliament and of the Council establishing a framework for the Community action in the field of water policy" or, in short, the EU Water Framework Directive (WFD) was finally adopted. The Directive was published in the Official Journal (OJ L 327) on 22 December 2000 and entered into force the same day. The purpose and contents of the WFD are (http://ec.europa.eu/environment/water/water-framework/info/intro_en.htm):

For each river basin district - some of which will traverse national frontiers - a "river basin management plan" will need to be established and updated every six years. There are a number of objectives in respect of which the quality of water is protected. The key ones at European level are general protection of the aquatic ecology, specific protection of unique and valuable habitats, protection of drinking water resources, and protection of bathing water. All these objectives must be, if applicable, integrated for each river basin. Ecological protection should apply to all waters: the central requirement of the Treaty is that the environment be protected to a high level in its entirety. For this reason, a general requirement for ecological protection, and a general minimum chemical standard, was introduced to cover all surface waters. These are the two elements "good ecological status" and "good chemical status".

Chemical protection
A good chemical status is defined in terms of compliance with all the quality standards established for chemical substances at European level. The Directive also provides a mechanism for renewing these standards and establishing new ones by means of a prioritisation mechanism for hazardous chemicals. This will ensure at least a minimum chemical quality, particularly in relation to very toxic substances, everywhere in the Community.

Of the compounds described in this report the following are listed as priority substance under the WFD: PBDEs and TBT. WFD monitoring takes place in either total water or suspended matter and are compared to environmental standards in total water. These standards are developed based on both toxicological effects and bioaccumulation. However, analyses of these compounds in water is difficult, which is especially the case for TBT but also for PBDEs on a routinely basis (Roex & Van den Heuvel-Greve, 2010). Roex & Van den Heuvel-Greve (2010) advise to monitor TBT in either sediment or biota in future monitoring until chemical analyses of these substances in water have been improved. In the Westerschelde case studies PBDEs and TBT have been analysed in biota or sediment. Therefore direct comparison of the levels of these contaminants in the case studies to WFD standards (in water) can not be made.

Addition of PCBs and PFCs to the WFD priority substances list has been proposed. When a decision will be reached on a possible incorporation of these substances is not known at this stage.

Besides environmental standards in water, the WFD has also set standards in biota for the following priority substances: hexachlorobenzene, hexachlorobutadiene and methylmercury. The EC set these standards in biota because high uncertainties in the application of bioaccumulation factors (in particular BCFs) while developing environmental standards in water. As mentioned above, environmental standards in biota may also be needed for TBT and possibly PBDEs due to difficulties in analysis of these compounds in water (Roex & Van den Heuvel-Greve 2010).

Ecological protection
A Good Ecological Status is defined in Annex V of the Water Framework Proposal, in terms of the quality of the biological community, the hydrological characteristics and the chemical characteristics. As no absolute standards for biological quality can be set which apply across the Community, the controls are specified as allowing only a slight departure from the biological community which would be expected in conditions of minimal anthropogenic impact.

Besides the selection of priority substances under a ‘Good Chemical Status’, the WFD also supports the selection of a set of additional ‘relevant substances’ under a Good Ecological Status specific for a river basin. For the Schelde river basin, these ‘other relevant substances’ are copper, zinc and PCBs. Monitoring of these substances is preferably done in total water (or suspended matter).

Bioaccumulative contaminants may affect the achievement of a Good Ecological Status as set for coastal & transitional waters by influencing the species abundance, biodiversity and carrying capacity of an ecosystem. Analysis of concentrations in either total water or suspended matter does not provide information on the bioavailability, trophic transfer and accumulation processes of a particular system. To be able to better assess possible impacts on ecology, either chemical analyses of these compounds in biota or effect assessments in biota are preferred.

7.2 Natura2000

The legal basis for the Natura 2000 network comes from the Birds Directive and the Habitats Directive. Council Directive 79/409/EEC on the conservation of wild birds, commonly referred to as the Birds Directive, is the EU’s oldest piece of nature legislation and one of the most important, creating a comprehensive scheme of protection for all wild bird species naturally occurring in the Union. Its was adopted unanimously by the Members States in 1979 as a response to increasing concern about the declines in Europe's wild bird populations resulting from pollution, loss of habitats as well as unsustainable use. It was also in recognition that wild birds, many of which are migratory, are a shared heritage of the Member States and that their effective conservation required international co-operation. The Habitats Directive (together with the Birds Directive) forms the cornerstone of Europe's nature conservation policy. It is built around two pillars: the Natura 2000 network of protected sites and the strict system of species protection. All in all the directive protects over 1.000 animals and plant

Table 7.1 Goals for a selection of Natura2000 species in Natura 2000 area 122 – Westerschelde & Saeftinghe.

Species number	Species name	Goal area	Goal population	Explanation
A193	Common tern	Maintain	Maintain	Originally around 1000 breeding pairs were found in the Westerschelde. This number decreased in the '60s down to a few hundred. After that, the number increased again. The goal for the entire Delta area is 6500 pairs. Growth in the Delta area is not needed, since the national population seems to increase slightly. Most important breeding areas in the Westerschelde: Hooge Platen and Saeftinghe.
H1365	Harbour seal	Increase	Growth	The Westerschelde estuary can contribute to the regional goal of 200 individual harbour seals in the Delta areas. Improvement of environmental quality is also dependent on measures taken in Belgium

species and over 200 so called "habitat types" (e.g. special types of forests, meadows, wetlands, etc.), which are of European importance. Natura 2000 and the WFD are closely connected, since all N2000 are located in a WFD river basin. All N2000 measures have to correspond with WFD measures and vice versa.

The Westerschelde and Saeftinghe (NL9803061) both fall under the Bird and Habitat Directives. Target species in this area are amongst others common terns and harbour seals (see Table 7.1). The conservation goal for common terns in this area is to maintain the current population size, whereas the goal for harbour seals is to grow up to 200 individuals in the entire Delta region. Contaminants can reach high levels in these top predators via trophic transfer and biomagnifications processes (see chapter 6). This may cause effects on reproduction as has been shown in Table 2.3 and hereby directly affect N2000 goals as set for these species.

7.3 Marine Strategy Framework Directive (MSFD)

The aim of the Marine Strategy Framework Directive (2008/56/EC) is to protect more effectively the marine environment across Europe, including all waters in the Mediterranean Sea, the Baltic Sea, the Black Sea and the North-east Atlantic Ocean, and waters surrounding the Azores, Madeira and the Canary Islands. Coastal waters, including their seabed and subsoil, are an integral part of the marine environment, and as such are covered by the Directive, in so far as particular aspects of the environmental status of the marine environment are not already addressed through the WFD, so as to ensure complementarity while avoiding unnecessary overlaps. The Directive establishes a framework within which Member States shall take the necessary measures to achieve or maintain good environmental status in the marine environment by the year 2020 at the latest. For that purpose, marine strategies shall be developed and implemented in order to:
(a) protect and preserve the marine environment, prevent its deterioration or, where practicable, restore marine ecosystems in areas where they have been adversely affected;

Table 7.2 MSFD incorporation of the subjects of trophic transfer and bioaccumulation of contaminants in food webs.

Annex	Relevant part
I - Qualitative descriptors for determining good environmental status (referred to in Articles 3(5), 9(1), 9(3) and 24)	(4) All elements of the marine food webs, to the extent that they are known, occur at normal abundance and diversity and levels capable of ensuring the long-term abundance of the species and the retention of their full reproductive capacity. (8) Concentrations of contaminants are at levels not giving rise to pollution effects. (9) Contaminants in fish and other seafood for human consumption do not exceed levels established by Community legislation or other relevant standards.
III - Indicative list of pressures and impacts	Contamination by hazardous substances | Introduction of synthetic compounds (e.g. priority substances under Directive 2000/60/EC which are relevant for the marine environment such as pesticides, antifoulants, pharmaceuticals, resulting, for example, from losses from diffuse sources, pollution by ships, atmospheric deposition and biologically active substances),introduction of non-synthetic substances and compounds (e.g. heavy metals, hydrocarbons, resulting, for example, from pollution by ships and oil, gas and mineral exploration and exploitation, atmospheric deposition, riverine inputs), introduction of radio-nuclides.

(b) prevent and reduce inputs in the marine environment, with a view to phasing out pollution, so as to ensure that there are no significant impacts on or risks to marine biodiversity, marine ecosystems, human health or legitimate uses of the sea.
The marine strategies to be developed by each Member State must contain a detailed assessment of the state of the environment, a definition of "good environmental status" at regional level and the establishment of clear environmental targets and monitoring programmes.

The goal of the MSFD is in line with the objectives of the WFD (http://ec.europa.eu/environment/water/water-framework/pdf/water_note11_marine_strategy.pdf). Member States will also need to coordinate the implementation of the MSFD with their actions on the WFD since the two pieces of legislation are closely linked. The WFD will reduce pollution from land-based sources from reaching Europe's seas to improve marine conditions. The directive also protects coastal waters as well as transitional waters such as estuaries and coastal lagoons. These provide spawning grounds for many marine fish species and are a crucial link between freshwater and marine ecosystems. Together the two directives provide a complete structure for the protection and management of Europe's freshwater and marine waters.

Even though the implementation of the MSFD in member states has just started and details are not known yet, the Directive does focus on goals for both food web quality, trophic transfer and bioaccumulation of contaminants (see Table 7.2). This implies that member states have to prevent contaminant levels in the marine environment that can cause either effects in the environment (species, food web abundance and diversity) and/or in humans (after consumption of fishery products). Both chemical and effect analysis in biota are of high importance to assess the status of a (sub)region. Monitoring (both chemical and effect analysis in biota) is necessary to determine whether measurements succeed and goals are reached.

7.4 OSPAR

OSPAR is the mechanism by which fifteen Governments of the western coasts and catchments of Europe, together with the European Community, cooperate to protect the marine environment of the North-East Atlantic (www.ospar.org). It started in 1972 with the Oslo Convention against dumping. It was broadened to cover land-based sources and the offshore industry by the Paris Convention of 1974. These two conventions were unified, up-dated and extended by the 1992 OSPAR Convention. The new annex on biodiversity and ecosystems was adopted in 1998 to cover non-polluting human activities that can adversely affect the sea.

The fifteen Governments are Belgium, Denmark, Finland, France, Germany, Iceland, Ireland, Luxembourg, The Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and United Kingdom. Finland is not on the western coasts of Europe, but some of its rivers flow to the Barents Sea, and historically it was involved in the efforts to control the dumping of hazardous waste in the Atlantic and the North Sea. Luxembourg and Switzerland are Contracting Parties due to their location within Rhine catchments.

OSPAR's work is organised under six strategies, applying the ecosystem approach to deliver on the Ministerial Declarations and Statements made at the adoption of the Convention and at subsequent Ministerial meetings of the OSPAR Commission. For each strategy a programme of work is designed and implemented annually. Five thematic strategies address the main threats that it has identified within its competence:
-the Biodiversity and Ecosystem Strategy
-the Eutrophication Strategy
-the Hazardous Substances Strategy
-the Offshore Industry Strategy
-the Radioactive Substances Strategy
The last strategy consists of Joint Assessment and Monitoring Programme, which assesses the status of the marine environment and follows up implementation of the strategies and the resulting benefits to the marine environment. These six strategies fit together to underpin the ecosystem approach.

The strategy on hazardous substances is focused on contaminants. For OSPAR purposes, hazardous substances are defined as substances which are persistent, liable to bioaccumulate and toxic (PBT substances), or which give rise to an equivalent level of concern as the PBT substances. This might for example be concern that they can interfere with the hormone system of organisms. OSPAR established in 2002 a List of Substances of Possible Concern and revised the List of Chemicals for Priority Action. The substances on this list are those which require priority action, based primarily which substances represent the highest concern due to the amount produced, the degree of hazardous properties and/or the actual occurrence in the marine environment. All contaminant groups described in this report are mentioned on the List of Chemicals for Priority Action.

In the light of developments in the chemicals sector in the European Community (WFD and REACH), OSPAR’s work on the selection and prioritisation of substances has been put on hold. Instead, OSPAR collaborates with the EC on these issues.

With regards to the Biodiversity and Ecosystem Strategy, OSPAR has accumulated 15 years of experience in developing a conceptual framework for ecological indicators and objectives, and in applying the framework to the North Sea as a test case. These Ecological Quality Objectives (EcoQOs) have become a model for the implementation of the MSFD.

8 Conclusions and Recommendations

8.1 Conclusions

This report describes the state-of-the-art of knowledge on food webs in estuarine environments and trophic transfer of contaminants in these webs. These processes have been illustrated with two case studies in the Westerschelde estuary: a food web with a fish-eating bird, the common tern, as top predator, and a food web with a marine mammal, the harbour seal, as top predator. Each of these food webs have their own specific research questions. Conclusions on these research questions are given below. The results of these case studies have been used to identify possible implications for achievements of national goals as set for European directives. Conclusions on implications on these directives are presented below.

8.1.1 Case study - common tern food web

Conclusions with regards to the research questions of the case study ‘common terns’ are:

1) What is the structure of the food web of the common tern near Terneuzen?

- The food web of the terns seems to have limited links to benthic components.
- Most of the pelagic prey appear to draw their carbon from marine resources.
- Terns are migratory, which is also reflected in their isotope signatures. Liver δ15N isotope are highest and fit best in the food web picture of the Westerschelde estuary.

2) Which of the selected contaminants are being transferred in the common tern food web near Terneuzen?

- PCBs, PBDEs, HBCD, PFOS, TPT.

3) Can contaminants be transferred from mother bird to egg and how suitable are eggs as indicators for concentrations of the selected contaminants in adult birds?

- Contaminants can be transferred from mother bird to egg, however not all compounds are transferred in a similar way.
- Good indicators for contaminants in mother birds: PCBs, PBDEs
- Higher contaminant concentration in eggs as compared to mother birds: PFOS
- Lower contaminant concentration in eggs as compared to mother birds: HBCD, TBT
- PFOS can reach contamination levels in eggs, that has caused reproductive effects in several bird species under laboratory conditions.

8.1.2 Case study - harbour seal food web

Conclusions with regards to the research questions of the case study ‘harbour seals’ are:

1) What do harbour seals in the Westerschelde estuary eat and what is the structure of the harbour seal food web in the Westerschelde estuary?

- Based on this preliminary study on harbour seal feces, harbour seals in the Westerschelde estuary mainly feed on flat fish, such as plaice, flounder and sole.
- Based on this preliminary study on contaminants in prey items of harbour seals, harbour seals in the Westerschelde estuary mainly feed on flat fish (especially when looking at PFC data).

2) Which contaminants are being transferred in the harbour seal food web near Terneuzen?

- PCBs, PBDEs, PFCs (particularly PFOS), and possibly TPT.
- High PFOS concentrations were found in flat fish (whole body) and harbour seal.

3) What are the current contaminant concentrations and toxicological profiles in harbour seals of the Westerschelde?

- Based on this preliminary study, PFOS concentrations in harbour seals of the Westerschelde estuary were high, up to 50 times higher than concentrations in seals of the polluted Baltic Sea and up to 220 times higher than seals from the Arctic. High concentrations of PFOS were also found in flat fish (whole body) as important food source of harbour seals of the Westerschelde estuary.
- Based on this preliminary study, Sum-PCB concentrations in harbour seals of the Westerschelde are in line with levels in ringed seals from the Svalbard and Baltic Sea region.
- Based on this preliminary study, Sum-PCB and Sum-chlorinated phenolics may be elevated in harbour seals feeding in the Westerschelde as compared to the Oosterschelde and Waddensea.
- Based on literature, observed concentrations of both PCBs and PFOS in this preliminary study can potentially exert effects on reproduction and the immune system.
- Based on this preliminary study, flat fish (whole body) of the Westerschelde contain PCB concentrations in the range of the toxic threshold as derived for marine mammal diet. These toxic thresholds are set up for marine mammals and do not correspond with human consumption thresholds.

8.1.3 Implications for national goals as set for European directives

Conclusions with regards to possible implications for European directives are:

WFD:

- PBDEs and TPT are both listed as priority substance under the WFD, whereas PCBs are listed as ‘other relevant substance’ for the Schelde river basin. Direct comparison to WFD standards is not possible because environmental standards for these substances have been set for water, whereas these substances in the case studies have been analysed in either sediment or biota.
- Bioaccumulative contaminants may hamper reaching a Good Ecological Status as set for coastal & transitional waters. This is based on the fact that contaminants may cause effects on an organism level, which in turn may ultimately affect species richness, biodiversity and possible carrying capacity of a system.

Natura2000:

- Bioaccumulative contaminants can reach high levels in N2000 species of the Westerschelde, as has been shown for common tern and harbour seals. Results from literature show that these contaminants may affect reproduction and functioning of the immune system. Therefore, contaminants may affect N2000 goals such as maintenance or growth of a population size of these species.

MSFD:

- This Directive is currently in the implementation phase. In 2012 each Member State will present their Initial Assessment, description of a Good Ecological Status and description of Goals & Indicators. In 2010 work on these reports has been initiated.
- Member states have to prevent contaminant levels in the marine environment that can cause either effects in the environment (species, food web abundance and diversity) and/or in humans (after consumption of fishery products). Both chemical and effect analysis in biota are of high importance to assess the status of a

(sub)region. Monitoring (both chemical and effect analysis in biota) is necessary to determine whether measurements succeed and goals are reached.

8.2 Recommendations

Based on this preliminary study, the following recommendations are given:

Monitoring:

- To continue WFD monitoring of PBDEs, OTCs and PBDEs, and add PFCs to the programme. Even though measures have been take to reduce the emission of PBDEs, OTCs and PCBs, high levels of these contaminants are still present in Westerschelde food webs. Monitoring gives the possibility to follow trends of these contaminants, determines whether measures have effects (by decreasing time trends) and helps determining risks for both the environment and humans.
- To analyse PBDEs, OTCs and PCBs in sediment, SPMDs or biota instead of in water for WFD monitoring purposes. This is based on the facts that a) these substances are difficult to analyse in water, b) environmental standards for the WFD in water are low making it hard to compare levels in waters to these standards, c) these substances can reach high levels in biota. Monitoring in biota is preferred as it covers both bioavailability and bioaccumulation processes. Monitoring in SPMD does largely cover bioavailability, but not bioaccumulation, although this may be roughly predicted using models. Monitoring in sediment only shows total concentrations in sediments, but not which portion is available for uptake and if bioaccumulation in biota occurs.
- To monitor PBDEs, OTCs, PCBs and PFCs annually in mussel and flounder of the Westerschelde. Currently annual monitoring takes places along the Dutch coast (MWTL programme) using two estuarine/marine species (mussels and flounder). PCBs, TBT and TPT are analysed in mussels of a.o. the Westerschelde estuary, whereas PCBs are monitored in flounder. PBDEs were analysed in flounder once.
- To restart monitoring of contaminants (PBDEs, OTCs, PCBs and PFCs) in common tern eggs of the Westerschelde to get a better understanding of the exposure of these top predators to contaminants.

Policy:

- To consider PFCs (and especially PFOS) as potential additional ‘priority substance’ or ‘river basin substance’ for the Westerschelde river basin under the WFD. This is based on the fact that: a) PFCs strongly accumulate in food webs, b) effects may arise at low concentrations and c) measurements against further emissions of PFCs can be taken.
- To study whether contaminants such as PCBs and PFCs influence the N2000 goals for harbour seals in the Westerschelde estuary. This is based on the fact that a) harbour seals contain high levels of these contaminants and, b) these contaminants may affect reproduction (and perhaps pup survival) and functioning of the immune system.
- To study why flat fish (such as flounder, plaice and sole) and harbour seals contain particular high levels of PFCs compared to the other species of the Westerschelde estuary.

Consumption:

- To set up consumption standards for PFCs (in fish), OTCs (in shell fish and shrimp) and PBDEs (in fish). This is based on the fact that a) levels of these contaminants are high in these fishery products of the Westerschelde, b) consumption standards are needed to be able to make an risk assessment for humans who consume fishery products of the Westerschelde estuary.

9 References

Aguilar,A., Borrell,A. & Reijnders,P.J.H. (2002) Geographical and temporal variation in levels of organochlorine contaminants in marine mammals. *Marine Environmental Research*, **53**, 425-452.

Ahrens, L., U. Siebert, R. Ebinghaus (2009). Total body burden and tissue distribution of polyfluorinated compounds in harbor seals (*Phoca vitulina*) from the German Bight. *Marine Pollution Bulletin* **58**: 520-525.

Alzieu,C. (1991) Environmental-Problems Caused by Tbt in France - Assessment, Regulations, Prospects. *Marine Environmental Research*, **32**, 7-17.

Alzieu,C. (1998) Tributyltin: case study of a chronic contaminant in the coastal environment. *Ocean & Coastal Management*, **40**, 23-36.

Bargar, T.A., G.I. Scott, G.P. Cobb (2001). Maternal Transfer of Contaminants: Case Study of the Excretion of Three Polychlorinated Biphenyl Congeners and Technical-Grade Endosulfan Into eggs by White Leghorn Chickens (*Gallus domesticus*). *Environmental Toxicology and Chemistry* **20**(1): 61-67.

Beach,S.A., Newsted,J.L., Coady,K. & Giesy,J.P. (2006) Ecotoxicological evaluation of perfluorooctanesulfonate (PFOS). *Reviews of Environmental Contamination and Toxicology, Vol 186*, **186**, 133-174.

Beckmen,K.B., Blake,J.E., Ylitalo,G.M., Stott,J.L. & O'Hara,T.M. (2003) Organochlorine contaminant exposure and associations with hematological and humoral immune functional assays with dam age as a factor in free-ranging northern fur seal pups (*Callorhinus ursinus*). *Marine Pollution Bulletin*, **46**, 594-606.

Beineke, A., U. Siebert, M. McLachlan, R.Bruhn, K.Thron, en K. Failing (2005). Investigations of the potential influence of environmental contaminants on the Thymus and spleen of Harbour porpoises (*Phocoena phocoena*). *Environmental Science and Technology* **39**: 3933 -3938.

Bodin,N., Le Loc'h,F., Caisey,X., Le Guellec,A.M., Abarnou,A., Loizeau,V. & Latrouite,D. (2008) Congener-specific accumulation and trophic transfer of polychlorinated biphenyls in spider crab food webs revealed by stable isotope analysis. *Environmental Pollution*, **151**, 252-261.

Boon,J.P., Lewis,W.E., Tjoen,A.C., Allchin,C.R., Law,R.J., De Boer,J., ten Hallers-Tjabbes,C.C. & Zegers,B.N. (2002) Levels of polybrominated diphenyl ether (PBDE) flame retardants in animals representing different trophic levels of the North Sea food web. *Environmental Science & Technology*, **36**, 4025-4032.

Borga,K., Fisk,A.T., Hoekstra,P.F. & Muir,D.C.G. (2004) Biological and chemical factors of importance in the bioaccumulation and trophic transfer of persistent organochlorine contaminants in arctic marine food webs. *Environmental Toxicology and Chemistry*, **23**, 2367-2385.

Boyer,I.J. (1989) Toxicity of Dibutyltin, Tributyltin and Other Organotin Compounds to Humans and to Experimental-Animals. *Toxicology*, **55**, 253-298.

Bucheli,T.D. & Gustafsson,O. (2001) Ubiquitous observations of enhanced solid affinities for aromatic organochlorines in field situations: Are in situ dissolved exposures overestimated by existing partitioning models? *Environmental Toxicology and Chemistry*, **20**, 1450-1456.

Burger, E.M. (2007). Sampling of fish, shrimp, sediment, suspended matter and water from the Westerschelde near Terneuzen. I&M-99387821-EB. Colijnsplaat, Grontmij | Aquasense (*in Dutch*).

Burger, E.M., T.J. Vanagt (2008). Sampling of biota, sediment, suspended particulate matter and water from the Westerschelde estuary. 249510-EBU. Colijnsplaat, Grontmij | Aquasense.

Chang, S. C., Thibodeaux, J. R., Eastvold, M. L., Bjork, D. J., Froehlich, J. W., Lau, C., Singh, R. J., Wallace, K.B., Butenhoff, J. L. (2008). Thyroid hormone status and pituitary function in adult rats given oral doses of perfluorooctanesulfonate (PFOS). *Toxicology*, **243**, 330–339.

Chapman,P.M. & Wang,F.Y. (2001) Assessing sediment contamination in estuaries. *Environmental Toxicology and Chemistry*, **20**, 3-22.

Chiu,A., Chiu,N., Beaubier,N.T., Beaubier,J., Nalesnik,R., Singh,D., Hill,W.R., Lau,C. & Riebow,J. (2000) Effects and mechanisms of PCB ecotoxicity in food chains: algae double right arrow fish double right arrow seal double right arrow polar bear. *Journal of Environmental Science and Health Part C-Environmental Carcinogenesis & Ecotoxicology Reviews*, **18**, 127-152.

Cima,F. & Ballarin,L. (1999) TBT-induced apoptosis in tunicate haemocytes. *Applied Organometallic Chemistry*, **13**, 697-703.

Cima,F., Marin,M.G., Matozzo,V., Da Ros,L. & Ballarin,L. (1998) Immunotoxic effects of organotin compounds in Tapes philippinarum. *Chemosphere*, **37**, 3035-3045.

Cornelissen,G., Gustafsson,O., Bucheli,T.D., Jonker,M.T.O., Koelmans,A.A. & van Noort,P.C.M. (2005) Extensive sorption of organic compounds to black carbon, coal, and kerogen in sediments and soils: Mechanisms and consequences for distribution, bioaccumulation, and biodegradation. *Environmental Science & Technology*, **39**, 6881-6895.

Corsolini,S., Sara,G., Borghesi,N. & Focardi,S. (2007) HCB, p,p '-DDE and PCB ontogenetic transfer and magnification in bluefin tuna (*Thunnus thynnus*) from the Mediterranean Sea. *Environmental Science & Technology*, **41**, 4227-4233.

Costa,L.G. & Giordano,G. (2007) Developmental neurotoxicity of polybrominated diphenyl ether (PBDE) flame retardants. *Neurotoxicology*, **28**, 1047-1067.

Covaci,A., Gerecke,A.C., Law,R.J., Voorspoels,S., Kohler,M., Heeb,N.V., Leslie,H., Allchin,C.R. & De Boer,J. (2006) Hexabromocyclododecanes (HBCDs) in the environment and humans: A review. *Environmental Science & Technology*, **40**, 3679-3688.

Cullon,D.L., Jeffries,S.J. & Ross,P.S. (2005) Persistent organic pollutants in the diet of harbor seals (Phoca vitulina) inhabiting Puget Sound, Washington (USA), and the Strait of Georgia, British Columbia (Canada): A food basket approach. *Environmental Toxicology and Chemistry*, **24**, 2562-2572.

Darnerud,P.O. (2003) Toxic effects of brominated flame retardants in man and in wildlife. *Environment International*, **29**, 841-853.

de Vos,M.G., Huijbregts,M.A.J., Van Den Heuvel-Greve,M., Vethaak,A.D., de Vijver,K.I.V., Leonards,P.E.G., Van Leeuwen,S.P.J., de Voogt,P. & Hendriks,A.J. (2008) Accumulation of perfluorooctane sulfonate (PFOS) in the food chain of the Western Scheldt estuary: Comparing field measurements with kinetic modeling. *Chemosphere*, **70**, 1766-1773.

de Wit,C.A. (2002) An overview of brominated flame retardants in the environment. *Chemosphere*, **46**, 583-624.

Debier,C., Chalon,C., Le Boeuf,B.J., de Tillesse,T., Larondelle,Y. & Thome,J.P. (2006) Mobilization of PCBs from blubber to blood in northern elephant seals (Mirounga angustirostris) during the post-weaning fast. *Aquatic Toxicology*, **80**, 149-157.

deSwart,R.L., Ross,P.S., Timmerman,H.H., Vos,H.W., Reijnders,P.J.H., Vos,J.G. & Osterhaus,A.D.M.E. (1995) Impaired Cellular Immune-Response in Harbor Seals (*Phoca vitulina*) Feeding on Environmentally Contaminated Herring. *Clinical and Experimental Immunology*, **101**, 480-486.

Du, Y., Shi, X., Yu, K., Liu, C., Zhou, B. (2008). Chronic Effects of Waterborne PFOS Exposure on Growth, Development, Reproduction and Hepatotoxicity in Zebrafish. In: Interdisciplinary Studies on Environmental Chemistry - Biological Responses to Chemical Pollutants. Eds., Y. Murakami, K. Nakayama, S.-I. Kitamura, H. Iwata, S. Tanabe, pp. 37-54.

Echarri,I., Nerin,C., Wells,D.E., Domeno,C. & Cacho,J. (1998). Multivariate analysis applied to the study of the distribution of chlorobiphenyls (CBs), including the non-ortho-CBs, in fish and sea mammals. *Analyst*, **123**, 421-427.

Eggleton,J. & Thomas,K.V. (2004). A review of factors affecting the release and bioavailability of contaminants during sediment disturbance events. *Environment International*, **30**, 973-980.

Fent,K. (2004). Ecotoxicological effects at contaminated sites. *Toxicology*, **205**, 223-240.

Fiedler,H. (2008). Stockholm convention on POPs: Obligations and implementation. *Fate of Persistent Organic Pollutants in the Environment*, 3-12.

Fisk, A.T., K.A. Hobson, R.J. Norstrom (2001). Influence of Chemical and Biological Factors on Trophic Transfer of Persistent Organic Pollutants in the Northwater Polynya Marine Food Web. *Environmental Science Technology* **35** (4): 732–738.

Frouin, H., M. Lebeuf, R. Saint-Louis, M. Hammill, E. Pelletier, M. Fournier (2008). Toxic effects of tributyltin and its metabolites on harbour seal (*Phoca vitulina*) immune cells *in vitro*. *Aquatic Toxicology* **90**: 243-251.

Fuentes, S., Colomina, M. T., Rodriguez, J., Vicens, P., Domingo, J. L. (2006). Interactions in developmental toxicology: Concurrent exposure to perfluorooctane sulfonate (PFOS) and stress in pregnant mice. *Toxicol. Lett.* **164**, 81–89.

Fuentes, S., Colomina, M. T., Vicens, P., Franco-Pons, N.,Domingo, J. L. (2007). Concurrent exposure to perfluorooctane sulfonate and restraint stress during pregnancy in mice: effects on postnatal development and behavior of the offspring. *Toxicol. Sci.* **98**, 589–598.

Giesy,J.P. & Kannan,K. (2001). Global distribution of perfluorooctane sulfonate in wildlife. *Environmental Science & Technology*, **35**, 1339-1342.

Gipperth,L. (*in press*). The legal design of the international and European Union ban on tributyltin antifouling paint: Direct and indirect effects. *Journal of Environmental Management*, In Press, Corrected Proof.

Gobas,F.A.P.C. & Morrison,H.A. (2000). Bioconcentration and Biomagnification in the Aquatic Environment. *Handbook of Property Estimation Methods for Chemicals: Environmental and Health Sciences* (eds R. S. Boethling & D. Mackay), CRC Press LLC, Boca Raton.

Green, N., Bjerkeng, B., Hylland, K., Ruus, A., Ryyg, B., and Kuenitzer, A. (2003). Hazardous substances in the European marine environment: Trends in metals and persistent organic pollutants. EEA Reports 2/2003.

Hale,R.C. & La Guardia,M.J. (2002). Emerging contaminants of concern in Coastal and Estuarine Environments. *Coastal and Estuarine Risk Assessment* (eds M. C. Newman, M. H. Roberts Jr. & R. C. Hale), CLC Press LLC, Boca Raton.

Hall,A.J., Kalantzi,O.I. & Thomas,G.O. (2003). Polybrominated diphenyl ethers (PBDEs) in grey seals during their first year of life - are they thyroid hormone endocrine disrupters? *Environmental Pollution*, **126**, 29-37.

Hansson, S., J.E. Hobbie, R. Elmgren, U. Larsson, B. Fry, S. Johansson (1997). The stable nitrogen isotope ratio as a marker of food-web interactions and fish migration. *Ecology* Vol. **78**, No. 7: 2249-2257.

Harford,A.J., O'Halloran,K. & Wrightt,P.F.A. (2007). Effect of in vitro and in vivo organotin exposures on the immune functions of murray cod (*Maccullochella peelii peelii*). *Environmental Toxicology and Chemistry*, **26**, 1649-1656.

Helleday,T., Tuominen,K.L., Bergman,A. & Jenssen,D. (1999). Brominated flame retardants induce intragenic recombination in mammalian cells. *Mutation Research-Genetic Toxicology and Environmental Mutagenesis*, **439**, 137-147.

Herman, P.M.J., J.J. Middelburg, J. Widdows, C.H. Lucas, C.H.R. Heip (2000). Stable isotopes as trophic tracers: combining field sampling and manipulative labelling of food resources for macrobenthos. Mar Ecol Prog Ser **204**: 79-92.

Houde,M., Balmer,B.C., Brandsma,S., Wells,R.S., Rowles,T.K., Solomon,K.R. & Muir,D.C.G. (2006a). Perfluoroalkyl compounds in relation to life-history and reproductive parameters in bottlenose dolphins (*Tursiops truncatus*) from Sarasota Bay, Florida, USA. *Environmental Toxicology and Chemistry*, **25**, 2405-2412.

Houde,M., Bujas,T.A.D., Small,J., Wells,R.S., Fair,P.A., Bossart,G.D., Solomon,K.R. & Muir,D.C.G. (2006b). Biomagnification of perfluoroalkyl compounds in the bottlenose dolphin (*Tursiops truncatus*) food web. *Environmental Science & Technology*, **40**, 4138-4144.

Hu,J.Y., Zhen,H.J., Wan,Y., Gao,J.M., An,W., An,L.H., Jin,F. & Jin,X.H. (2006). Trophic magnification of triphenyltin in a marine food web of Bohai Bay, north China: Comparison to tributyltin. *Environmental Science & Technology*, **40**, 3142-3147.

Hu,W.Y., Jones,P.D., Upham,B.L., Trosko,J.E., Lau,C. & Giesy,J.P. (2002). Inhibition of gap junctional intercellular communication by perfluorinated compounds in rat liver and dolphin kidney epithelial cell lines in vitro and sprague-dawley rats in vivo. *Toxicological Sciences*, **68**, 429-436.

Imaeda, D., K. Nomiyama, T. Kunisue, H. Iwata, O. Tsydenova, S. Takahashi, M. Amano, E.A. Petrov, V.B. Batoe, S. Tanabe (2009). Residue levels of OH-PCBs and PCBs in the blood of Baikal Seals (*Pusa sibirica*). Interdisciplinary Studies on Environmental Chemistry - Environmental Research in Asia. Eds. Y. Obayashi, T. Isobe, A. Subramanian, S. Suzuki, S. Tanabe, pp. 111-117.

Ishibashi, H., H. Iwata, E-Y Kim, L. Tao, K. Kannan, M. Amano, N. Miyazaki, S. Tanabe, V.B. Batoev, E.A. Petrov (2008). Contamination and effects of perfluorochemicals in Baikal seal (*Pusa sibirica*). 1. Residue level, tissude distribution, and temporal trend. *Environ.Sci.Technol*. **42**: 2295-2301.

Janak,K., Covaci,A., Voorspoels,S. & Becher,G. (2005). Hexabromocyclododecane in marine species from the Western Scheldt Estuary: Diastereoisomer- and enantiomer-specific accumulation. *Environmental Science & Technology*, **39**, 1987-1994.

Johansson,N., Fredriksson,A. & Eriksson,P. (2008). Neonatal exposure to perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) causes neurobehavioural defects in adult mice. *Neurotoxicology*, **29**, 160-169.

Kannan,K., Guruge,K.S., Thomas,N.J., Tanabe,S. & Giesy,J.P. (1998). Butyltin residues in southern sea otters (*Enhydra lutris nereis*) found dead along California coastal waters. *Environmental Science & Technology*, **32**, 1169-1175.

Kannan, K., J.P. Giesy (2002). Global distribution and bioaccumulation of perfluorinated hydrocarbons. *Organohalogen Compounds* **59**: 267170.

Kannan, K., et al. (2005). Perfluorinated Compounds in Aquatic Organisms at Various Trophic Levels in a Great Lakes Food Chain. *Archives of Environmental Contamination and Toxicology* **48**: 559-566.

Kannan,K., Perrotta,E., Thomas,N.J. & Aldous,K.M. (2007). A comparative analysis of polybrominated diphenyl ethers and polychlorinated biphenyls in southern sea otters that died of infectious diseases and noninfectious causes. *Archives of Environmental Contamination and Toxicology*, **53**, 293-302.

Kelly,B.C., Ikonomou,M.G., Blair,J.D. & Gobas,F.A.P.C. (2008). Bioaccumulation behaviour of polybrominated diphenyl ethers (PBDEs) in a Canadian Arctic marine food web. *Science of the Total Environment*, **401**, 60-72.

Kidd, K.A., H.A. Bootsma, R.H. Hesslein, D.C.G. Muir, R.E. Hecky (2001). Biomagnification of DDT through the Benthic and Pelagic Food Webs of Lake Malawi, East Africa: Importance of Trophic Level and Carbon Source. *Environ. Sci. Technol.* **35** (1): 14–20.

Kihlstrom,J.E., Olsson,M., Jensen,S., Johansson,A., Ahlbom,J. & Bergman,A. (1992). Effects of Pcb and Different Fractions of PCB on the Reproduction of the Mink (*Mustela vison*). *Ambio*, **21**, 563-569.

Koeman, J.H., J. Veen, E. Brouwer, L. Huisman-de Brouwer, J.L. Koolen (1968). Residues of chlorinated hydrocarbon insecticides in the North Sea environment. *Helgoländer Wiss. Meersunters.* **17**: 375-380.

Koeman, J. H., van Genderen, H. (1972). Tissue levels in animals and effects caused by chlorinated hydrocarbon insecticides, chlorinated biphenyls and mercury in the marine environment along the Netherlands coast. Marine pollution and sea life. Fishing News, Surrey, pp. 428-35.

Le Gall, A. C., Loizeau, V., Abarnou, A., van Hattum, B., and Romaña, L.-A. (2003). Generic Estuary Modelling system to evaluate transport, fate and impact of contaminants – the food web model. DEL/EC/03.01, pp. 1-159. Brest, France, IFREMER.

Lee,R.F. (2002). Bioavailability, Biotransformation and Fate of Organic Contaminants in Estuarine Animals. *Coastal and Estuarine Risk Assessment* (eds M. C. Newman, M. H. Roberts Jr. & R. C. Hale), CLC Press LLC, Boca Raton.

Levin,M., De Guise,S. & Ross,P.S. (2005). Association between lymphocyte proliferation and polychlorinated biphenyls in free-ranging harbor seal (*Phoca vitulina*) pups from British Columbia, Canada. *Environmental Toxicology and Chemistry*, **24**, 1247-1252.

Lewandowski,G., Meissner,E. & Milchert,E. (2006). Special applications of fluorinated organic compounds. *Journal of Hazardous Materials*, **136**, 385-391.

Lohmann,R., Breivik,K., Dachs,J. & Muir,D. (2007). Global fate of POPs: Current and future research directions. *Environmental Pollution*, **150**, 150-165.

Luebker, D.J., Case, M. T., York, R. G., Moore, J. A., Hansen, K. J., Butenhoff, J. L. (2005a). Two generation reproduction and cross-foster studies of perfluorooctanesulfonate (PFOS) in rats. *Toxicology*, **215**, 126–148.

Luebker, D. J., York, R. G., Hansen, K. J., Moore, J. A., Butenhoff, J. L. (2005b). Neonatal mortality from in utero exposure to perfluorooctanesulfonate (PFOS) in Sprague-Dawley rats: Doseresponse, and biochemical and pharamacokinetic parameters. *Toxicology*, **215**, 149–169.

Mackay,D. & Fraser,A. (2000). Bioaccumulation of persistent organic chemicals: mechanisms and models. *Environmental Pollution*, **110**, 375-391.

McDonald,T.A. (2002). A perspective on the potential health risks of PBDEs. *Chemosphere*, **46**, 745-755.

Mackintosh, C.E., J. Maldonado, J. Hongwu, N. Hoover, A. Chong, M.G. Ikonomou, F.A.P.C. Gobas (2004). Distribution of Phthalate Esters in a Marine Aquatic Food Web: Comparison to Polychlorinated Biphenyls. *Environ. Sci. Technol.* **38** (7): 2011–2020.

McLeod, P.B., M.van Den Heuvel-Greve, R.M. Allen-King, S.N. Luoma, R.G. Luthy (2004). Effects of particulate carbonaceous matter on the bioavailability of benzo[a]pyrene and 2,2 ',5,5 '-tetrachlorobiphenyl to the clam, *Macoma balthica*. *Environmental Science & Technology*, **38**, 4549-4556.

Mensink, B. (1999). Imposex in the common whelk, *Buccinum undatum*. Proefschrift Universiteit van Wageningen, 125 pgs.

Middelburg, J.J., J. Nieuwenhuize, (1998). Carbon and nitrogen stable isotopes in suspended matter and sediments from the Schelde Estuary. *Marine Chemistry* **60**: 217-225.

Middelburg, J.J., C. Barranguet, H.T.S. Boschker, P.M.J. Herman, T. Moens, C.H.R. Heip (2000). The fate of intertidal microphytobenthos carbon: An in situ C-13-labeling study. *Limnol Oceanogr* **45**: 1224-1234.

Middelburg, J.J., P.M.J. Herman (2007). Organic matter processing in tidal estuaries. *Marine Chemistry* **106**: 127-147.

Molina, E.D., et al. (2006). Effects of air cell injection of Perfluorooctane Sulfonate before incubation on development of the white leghorn chicken (*Gallus domesticus*) embryo. *Environmental Toxicology and Chemistry* **25**(1): 227-232.

Mos,L., Morsey,B., Jeffries,S.J., Yunker,M.B., Raverty,S., De Guise,S. & Ross,P.S. (2006). Chemical and biological pollution contribute to the immunological profiles of free-ranging harbor seals. *Environmental Toxicology and Chemistry*, **25**, 3110-3117.

Neale,J.C.C., Gulland,F.M.D., Schmelzer,K.R., Harvey,J.T., Berg,E.A., Allen,S.G., Greig,D.J., Grigg,E.K. & Tjeerdema,R.S. (2005). Contaminant loads and hematological correlates in the harbor seal (*Phoca vitulina*) of San Francisco Bay, California. *Journal of Toxicology and Environmental Health-Part A-Current Issues*, **68**, 617-633.

Newsted,J.L., Jones,P.D., Coady,K. & Giesy,J.P. (2005). Avian toxicity reference values for perfluorooctane sulfonate. *Environmental Science & Technology*, **39**, 9357-9362.

Nisbet, I.C.T., J.P. Montoya, J. Burger, J.J. Hatch (2002). Use of stable isotopes to investigate individual differences in diets and mercury exposures among common terns *Sterna hirundo* in breeding and wintering grounds. *Marine Ecology Progress Series* **242**: 267-274.

Oehlmann,J., Stroben,E. & Fioroni,P. (1991) The Morphological Expression of Imposex in Nucella-Lapillus (Linnaeus) (Gastropoda, Muricidae). *Journal of Molluscan Studies*, **57**, 375-390.

OSPAR (2000). Convention for the protection of the marine environment of the North-East Atlantic. Chapter 4 - chemistry. OSPAR Commission.

Peden-Adams,M.M., Eudaly,J.G., Dabra,S., EuDaly,A., Heesemann,L., Smythe,J. & Keil,D.E. (2007). Suppression of humoral immunity following exposure to the perfluorinated insecticide sulfluramid. *Journal of Toxicology and Environmental Health-Part A-Current Issues*, **70**, 1130-1141.

Peterson, B.J., B. Fry (1987). Stable Isotopes in Ecosystem Studies. *Annual Review of Ecology and Systematics* Vol. **18**: 293-320.

Pijnenburg A.M., Everts J.W., de Boer J., Boon J.P. (1995). Polybrominated biphenyl and diphenylether flame retardants: analysis, toxicity, and environmental occurrence. *Rev Environ Contam Toxicol.* **141**:1-26.

Pijnenburg, J., M. Van den Heuvel-Greve (2009). Effects of contaminants on marine mammals. Working report RWS Waterdienst and Deltares (*in Dutch*).

Reijnders,P.J.H. (1980). Organochlorine and Heavy-Metal Residues in Harbor Seals from the Wadden Sea and Their Possible Effects on Reproduction. *Netherlands Journal of Sea Research*, **14**, 30-65.

Reijnders,P.J.H. (1986). Reproductive Failure in Common Seals Feeding on Fish from Polluted Coastal Waters. *Nature*, **324**, 456-457.

Roex, E., M. van den Heuvel-Greve (2010). Monitoring of bioaccumulative WFD priority substances; in water or in biota? Deltares report 1001-0154 (*in Dutch*).

Routti, H., Letcher, R.J., Arukwe, A., van Bavel, B., Yoccoz, N.G., Chu, S.G., Gabrielsen, G.W. (2008). Biotransformation and metabolites of PCBs in relation to Phase I and II xenobiotic-metabolizing enzyme activities in ringed seals (*Phoca hispida*) from Svalbard and the Baltic Sea. *Environmental Science & Technology* **42**:8952–8958.

Routti, H., Letcher, R.J., van Bavel, B., Chu, S.G., Gabrielsen, G.W. (2009). Polybrominated diphenyl ethers and their hydroxylated analogues in ringed seals (*Phoca hispida*) from Svalbard and the Baltic Sea. *Environmental Science & Technology* **43**: 3494-3499.

Ruus,A., Sandvik,M., Ugland,K.I. & Skaare,J.U. (2002a). Factors influencing activities of biotransformation enzymes, concentrations and compositional patterns of organochlorine contaminants in members of a. marine food web. *Aquatic Toxicology*, **61**, 73-87.

Ruus,A., Ugland,K.I. & Skaare,J.U. (2002b). Influence of trophic position on organochlorine concentrations and compositional patterns in a marine food web. *Environmental Toxicology and Chemistry*, **21**, 2356-2364.

Sinclair, E., et al. (2006). Occurrence of Perfluoroalkyl Surfactants in Water, Fish, and Birds from New York State. *Archives of Environmental Contamination and Toxicology* **50**: 398-410.

Strand,J. & Jacobsen,J.A. (2005) Accumulation and trophic transfer of organotins in a marine food web from the Danish coastal waters. *Science of the Total Environment*, **350**, 72-85.

Sulu-Gambari, F. (2007). Characterisation of the food chain of the common tern (*Sterna hirundo*) in the Western Scheldt estuary; accumulation and risk analysis of toxic substances in fish-eating birds. BSc thesis Roosevelt Academy, Middelburg, the Netherlands.

Tabuchi,M., Veldhoen,A., Dangerfield,N., Jeffries,S., Helbing,C.C. & Ross,P.S. (2006). PCB-related alteration of thyroid hormones and thyroid hormone receptor gene expression in free-ranging Harbor seals (*Phoca vitulina*). *Environmental Health Perspectives*, **114**, 1024-1031.

Thibodeaux, J. R., Hanson, R. G., Rogers, J. M., Grey, B. E., Barbee, B. D., Richards, J. H., Butenhoff, J. L., Stevenson, L. A., Lan, C. (2003). Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. I: Maternal and prenatal evaluations. *Toxicol. Sci.*, **74**, 369–381.

Thompson,B., Adelsbach,T., Brown,C., Hunt,J., Kuwabara,J., Neale,J., Ohlendorf,H., Schwarzbach,S., Spies,R. & Taberski,K. (2007). Biological effects of anthropogenic contaminants in the San Francisco Estuary. *Environmental Research*, **105**, 156-174.

Thron,K.U., Bruhn,R. & McLachlan,M.S. (2004). The influence of age, sex, body-condition, and region on the levels of PBDEs and toxaphene in harbour porpoises from European waters. *Fresenius Environmental Bulletin*, **13**, 146-155.

Tomy,G.T., Pleskach,K., Oswald,T., Halldorson,T., Helm,P.A., Macinnis,G. & Marvin,C.H. (2008). Enantioselective bioaccumulation of hexabromocyclododecane and congener-specific accumulation of brominated diphenyl ethers in an eastern Canadian Arctic marine food web. *Environmental Science & Technology*, **42**, 3634-3639.

Van den Brink, N.W., A.T.C. Bosveld (2001). PCB concentrations and metabolism patterns in common terns (*Sterna hirundo*) from different breeding colonies in the Netherlands. *Marine Pollution Bulletin* **42** (4): 280-285.

Van den Brink, N.W., et al. (2007). Relations between contaminant concentrations in eggs and mother birds of common terns. Alterra, WUR: Wageningen. p. 1-18 (*in Dutch*).

Van den Heuvel-Greve, M.J., M.S.J. Hoekstein, F.O.B. Lefèvre, P.J. Meininger, A.D. Vethaak (2003). Possible causes of breeding failure in the common tern colony near Terneuzen; current status and recommendations. Report RIKZ/2003.037. RWS National Institute for Coastal and Marine Management: Middelburg, the Netherlands (*in Dutch*).

Van den Heuvel-Greve, M.J., P.E.G. Leonards, A.D. Vethaak (2006). Dioxin survey Westerschelde; analyses of dioxins, dioxin like substances and other contaminants in fishery products, sediment and food chains of the Westerschelde. Report RIKZ/2006.011. RWS National Institute for Coastal and Marine Management: Middelburg, the Netherlands (*in Dutch*).

Van den Heuvel-Greve, M., L. Withagen, P.E.G.Leonards (2007). Follow up survey contaminants in fishery products of the Westerschelde; dioxines (including furans), PCBs, brominated flame retardants and perfluorinated compounds, 2006. Report RIKZ/2007.007. RWS National Institute for Coastal and Marine Management: Middelburg, the Netherlands (*in Dutch*).

Van de Vijver,K.I., Hoff,P.T., Das,K., Van Dongen,W., Esmans,E.L., Jauniaux,T., Bouquegneau,J.M., Blust,R. & De Coen,W. (2003). Perfluorinated chemicals infiltrate ocean waters: Link between exposure levels and stable isotope ratios in marine mammals. *Environmental Science & Technology*, **37**, 5545-5550.

Van Scheppingen,W.B., Verhoeven,A.J.I.M., Mulder,P., Addink,M.J. & Smeenk,C. (1996). Polychlorinated biphenyls, dibenzo-p-dioxins, and dibenzofurans in harbor porpoises (*Phocoena phocoena*) stranded on the Dutch coast between 1990 and 1993. *Archives of Environmental Contamination and Toxicology*, **30**, 492-502.

Veltman,K., Huijbregts,M.A.J., Van Den Heuvel-Greve,M., Vethaak,A.D., Hendriks,A.J. (2006). Organotin accumulation in an estuarine food chain: Comparing field measurements with model estimations. *Marine Environmental Research*, **61**, 511-530.

Verreault, J., et al. (2006). Maternal transfer of organohalogen contaminants and metabolites to eggs of Arctic-breeding glaucous gulls. *Environmental Pollution* **114**: 1053-1060.

Villars, M.T., Devigne, G.A.L. (2001). Estuarine processes: A literature review. WL|Delft Hydraulics. Z2725. Delft, The Netherlands.

Voorspoels,S., Covaci,A. & Schepens,P. (2003). Polybrominated diphenyl ethers in marine species from the Belgian North Sea and the Western Scheidt Estuary: Levels, profiles, and distribution. *Environmental Science & Technology*, **37**, 4348-4357.

Zakharov,V.M. & Yablokov,A.V. (1990). Skull Asymmetry in the Baltic Gray Seal - Effects of Environmental-Pollution. *Ambio*, **19**, 266-269.

Zegers,B.N., Lewis,W.E., Booij,K., Smittenberg,R.H., Boer,W., De Boer,J. & Boon,J.P. (2003). Levels of polybrominated diphenyl ether flame retardants in sediment cores from Western Europe. *Environmental Science & Technology*, **37**, 3803-3807.