
Organotin and seals in the Dutch Wadden Sea

juni 2004

Werkdocument RIKZ/AB/2004.611W

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Preface

For the study environmental science I was obliged to fulfill a final project. With the help of contacts at the Van Hall Institute in Leeuwarden, the Netherlands, I managed to get a subject. Since I had spent my time in Spain to do an internship until several days before the period started the contact restricted to email. In the weeks before the 15th of March, which was the official start, I was able to gather information. After an introduction conversation at the office of RIKZ in Haren I was able to put all my enthusiasm in the work. The project had started officially and I could begin immediately. Although the enormous amount of information I was able to concentrate on the part that I needed. The weeks went by and many articles and publications are read. The project started to get more and more interesting and when the results of the laboratory became available the real interesting part started. Now, 4 months later a report is presented. During the period of work it became obvious that a total literature study was unable to fulfill and therefore there is concentrated on the most recent information. Although it was hard work I have enjoyed my work at the RIKZ in Haren. I would like to thank all my collegians for a good atmosphere and the offers to help. A special thanks is for my supervisor from as well RIKZ, Kees van de Ven and Joop Bakker, and for the supervisors of the Van Hall Institute, Martin Jansen and Peter Hofman. These people made it possible for me to work at this project. I hope we will be able to mean something for each other in the future.

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English summary

Already for decades the quality of the water in the Dutch Wadden Sea is an important issue for research, discussions and legislation. Although the amount of well-known pollutants has been decreasing an optimum has not been reached. The question if the present pollutions can cause immunosuppression for animals is still unknown. The concentrations of heavy metal and other pollutants like PCB's have been decreasing. Never the less some substances are present higher than the maxima acceptable risk.

One of these substances is organotin. Organotin represents a group of organic compounds that contain tin. Butyl and phenyl groups are the most well known. Examples of widely produced organotins are TBT (tributyltin) and TPT (triphenyltin). TBT is mainly used as paint additive in the shipping industry to prevent the fouling of ship hulls. TPT is also used in this matter but only in small amount. The major use was as fungicide in agriculture, especially potato culture. Although the use of TBT and TPT are prohibited for several years still quantities can be found in the environment. TBT and TPT seem to be comparable in toxicity and bioaccumulative behaviour but can be distinguished in degradability. TPT appeared to be more persistent than TBT for as well as in sediment as in organisms. Because their hydrophobic quality it is hard to solve in water and within a short period after release they are fixated to sediment and suspended matter.

Earlier research has shown that imposex can occur already at concentrations as low as a billionth of gram per liter. Because of the large differences in doses effect relations between different trophic levels no assumption can be made that the concentration in which imposex occurs also establishes negative consequences for the harbour seal. In this report analyses of organotin in suspended matter and in harbour seal livers reported. Further more a research check is performed to find results of negative effects of organotin for sea mammals and other organisms.

After comparing organotin concentrations in several organisms it became obvious that an animals that stand high in a food chain perform better in the biotransformation process to degrade organotins to less toxic substances. In order to look at the organotin pollution of the Dutch Wadden Sea a monitoring program of the RIKZ was used to see the development in pollution for several years (1998-2003). Suspended matter samples are chosen to look at the most recent pollution. A decreasing amount of the most toxic organotins (TBT and TPT) was the conclusion. Never the less still extending the maximal acceptable risk.

Harbour seal liver analyses show large differences between the amount of TBT and TPT. TBT is only present for a few percentages of butyltins in the liver of the Harbour seal. This suggested the good ability to biotransform TBT to DBT and MBT. For TPT the results show the opposite. Because TPT is present in the highest concentration compared to its metabolites DPT and MPT it indicates the difficulties in the biotransformation process. This confirms the higher persistency.

Because the concentrations of organotin don't say anything on itself a focus is put on the food chain. No uptake of organotins by gills or skin is possible for sea mammals the food is the main source of intake. This daily intake was calculated with the concentrations of fish from the North Sea that are reported in a report from RIVO. The estimation is 8 ug/kg per kg bodyweight per day. Compared with a No Observed Adverse Effect Level (NOAEL) in weanling rats the intake seemed to be 3 times lower. This suggests no harmful effects of organotin for the Harbour seal in the Dutch Wadden Sea.

Due to the biotransformation by seals and the lowering concentrations of organotin in the Dutch Wadden Sea no effects on seals are expected in the future. The legislation, which minimises the use of organotins, will lower the possibilities for negative effects. Never the less very low concentrations can have negative effects on other organisms.

Dutch summary

Sinds decennia is de waterkwaliteit in de Nederlandse Waddenzee een belangrijk punt voor onderzoek, discussie en regelgeving. De waterkwaliteit is de laatste jaren sterk verbeterd met betrekking tot de traditionele bekende verontreinigingen, maar optimale omstandigheden zijn nog niet bereikt. De concentraties zware metalen en andere vervuilende stoffen als PCB's zijn verminderd. Onzekerheid is er of de vervuilende stoffen een weerstandsvermindering (immunosuppressie) teweegbrengen bij organismen zoals de zeehond. Desalniettemin zijn er nog steeds stoffen aanwezig in concentraties die hoger zijn dan de MTR.

Een van die stoffen is organotin. Organotin is de verzamelnaam voor organische tinverbindingen, waarvan die met butyl of fenyl-groep(en) het meest worden toegepast. De meest giftige organotinverbindingen zijn TBT(TriButylTin) en TFT (TriFenylTin) (In het engels TPT). TBT is een stof die voornamelijk is verwerkt in verf voor schepen. Deze stof in de verf zorgt ervoor dat algen en schelpdieren niet aan de romp groeien (anti-fouling) zodat er minder wrijving optreedt tijdens het varen. TPT wordt ook in kleine hoeveelheden in verf gebruikt maar is voornamelijk toegepast als bestrijdingsmiddelen tegen schimmels in de aardappelteelt. Ondanks dat TBT en TFT al enige tijd niet meer zijn toegestaan, worden er nog steeds aanzienlijke concentraties in het milieu teruggevonden. De stoffen TBT en TFT hebben een vergelijkbaar bioaccumulerend gedrag en giftigheid maar onderscheiden zich in de afbreeksnelheid. TFT blijkt persistenter dan TBT in zowel sediment als organisme. Organotins zijn slecht oplosbaar in water en hechten zich aan sediment en zwevend stof.

Uit eerder uitgevoerd onderzoek blijkt dat concentraties van 1 ng/l TBT in een marien milieu al imposex bij Wulken tot gevolg kan hebben. Doordat de stof grote verschillen in dosis effect-relaties tussen organismen van andere trofische niveaus heeft kan er niet zonder meer gezegd worden dat de aanwezige concentraties in de Nederlandse Waddenzee effect hebben op alle organismen.

Door de resultaten van organotinconcentraties in verschillende organismen samen te voegen wordt duidelijk dat organismen hoger in trofische niveaus over het algemeen beter in staat zijn organotin af te breken in minder giftige stoffen. Om vervolgens naar de concentraties in de Waddenzee te kijken zijn resultaten van een monitoringsprogramma, uitgevoerd door het RIKZ van meerdere jaren (1998-2003), verwerkt tot trendlijnen. Er is gekozen voor zwevend stof, omdat dit een goed beeld geeft van de huidige concentraties in de Waddenzee. Hieruit blijkt dat de concentraties van de giftigste componenten van organotin (TBT en TFT) de laatste jaren langzaam afnemen, alhoewel ze nog ver boven de MTR liggen.

Uit de resultaten van de analyses op organotin in de zeehondenlevers blijkt dat er grote verschillen zijn tussen TBT en TFT concentraties. TBT is slechts in enkele procenten aanwezig in vergelijking met de afbraakproducten DBT en MBT. Dit duidt op een goed vermogen tot afbreken van TBT. Voor TFT zijn de resultaten omgekeerd. Hier zijn de concentraties van TFT hoog in tegenstelling tot de afbraakproducten DFT en MFT. Dit bevestigt de in literatuur aangetoonde hogere persistentie.

Omdat de concentratie organotin in de lever op zichzelf niks zegt over de mate van effecten die kunnen optreden bij zeehonden is er naar de voedselketen van de zeehond gekeken om een dagelijkse inname te kunnen berekenen. Omdat zeehonden de stof niet via de huid opnemen is aangenomen dat organotin met name via vis, de voedselbron, wordt

ingenomen. Met bekende concentraties van organotin in vis, is een dagelijkse opname berekend. De dagelijkse opname van organotin is geschat op 8 µg/kg lichaamsgewicht. Doordat in het verleden, voor zover bekend, geen onderzoeken op de zeehond zijn uitgevoerd is deze dagelijkse opname vergeleken met een effectconcentratie bij ratten en muizen. Omdat de stoffen voornamelijk effect hebben op jonge organismen door een minder ontwikkeld afweersysteem zijn resultaten bij jonge ratten gebruikt. Een concentratieniveau van 25 µg/kg lichaamsgewicht geeft geen merkbare negatieve effecten. Dit niveau is meer dan 3 maal hoger dan de geschatte concentratie die per dag door de zeehond wordt opgenomen en op basis van deze verhouding worden geen directe immunosuppressieve effecten verwacht.

Door de biotransformatie door de zeehond zullen de concentraties van TBT en TFT ook niet dusdanig hoog worden dat effect kan worden verwacht. Mede door de dalende concentraties organotins in de Waddenzee wordt niet verwacht dat deze effecten nog zullen optreden. Wel kunnen de aanwezige concentraties nadelige gevolgen hebben voor andere organismen in de Nederlandse Waddenzee.

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1. Introduction

In the year 2002 the population of seals in the Wadden Sea has managed to sustain a significant number of seals despite a virus attack. The population has been increasing during the last decades, that started discussion if there still is a need for seal day care centres. A strict policy, using guidelines for rehabilitation should solve this problem. Governmental bodies such as the ministry of 'Agriculture, Nature conservation & Food Safety' and 'Transport, Public and Water management', seal rehabilitation centres, recreation and fishery have a different opinions on what the best solution is.

To monitor the condition of the seal population in the Netherlands, a platform was established in the autumn of 2002. This platform has the opinion that the population of seals is strong enough because of their health and size of the population. Their main motivation is that nature should develop the population by natural selection. It is thought that negative effects will arise when the rehabilitation of weak and sick animals proceeds. Weakened animals that return to the wild after their rehabilitation can possess risks. Exotic pathogens, a weakening of the population strength and the decrease of domesticated animals in the wild population can threaten the population. Opponents, like rehabilitation centre 'Pieterburen', note that day care activities facilitate a good health monitoring. In addition it is seen as a moral duty to help animals in need. Rehabilitation centre 'Pieterburen' suggests that seals are less resistant against disease more quickly (immunosuppression) due to anthropogenic pollution (www.zeehondencreche.nl). Substances considered as potential danger are the polychlorobiphenyls (PCB's), organotins and brominated flame-retardants. The missing knowledge of potential effects of these compounds inhibits proper political decision making. A good example is the substance PCB. It took 20 years before this substance was qualified as a cause of harmful effects.

After publications in the media of possible relations between water quality and health conditions of the seals research was funded. Organisations as RIKZ (National Institute for Coastal and Marine Management), NIOZ (National Institute for Sea Research), Alterra and Ecomare agreed to analyse seal material. The outbreak of the virus in 2002 had provided a lot of seal material. Organotin (e.g. tributyltin and triphenyltin) was chosen as substance for analyses and research because it is considered a problematic substance in the Wadden Sea (Hofstede & van de Ven, 2001).

This report will start with chapters about the Wadden Sea, Seals, organotin and effects of tin in biota, providing a better background for results that will be presented later in this report. In chapter 6 a sketch of organotin pollution in the Dutch Wadden Sea is described followed by the analyses of organotin in seal livers in chapter 7. Organotin in the food chain of the seal will be discussed in chapter 8. Based on the information that is gathered, a daily intake of organotin for the harbour seal is calculated in chapter 9. The most important relevant facts found in literature are summarised in chapter 10. Uncertainties within this research are described in the discussion and finally the conclusions are presented. A short advisory will follow before the abbreviation and technical terms in this report are explained.

In this report an attempt will be made to answer the following questions. They are divided into a main question and sub-questions. The sub-questions are worked out in the report and they lead to the answer of the main question.

Main question

Is a sufficient amount of information present to establish a relationship between organotins and immunosuppression in seals in the Dutch Wadden Sea?

Sub-questions

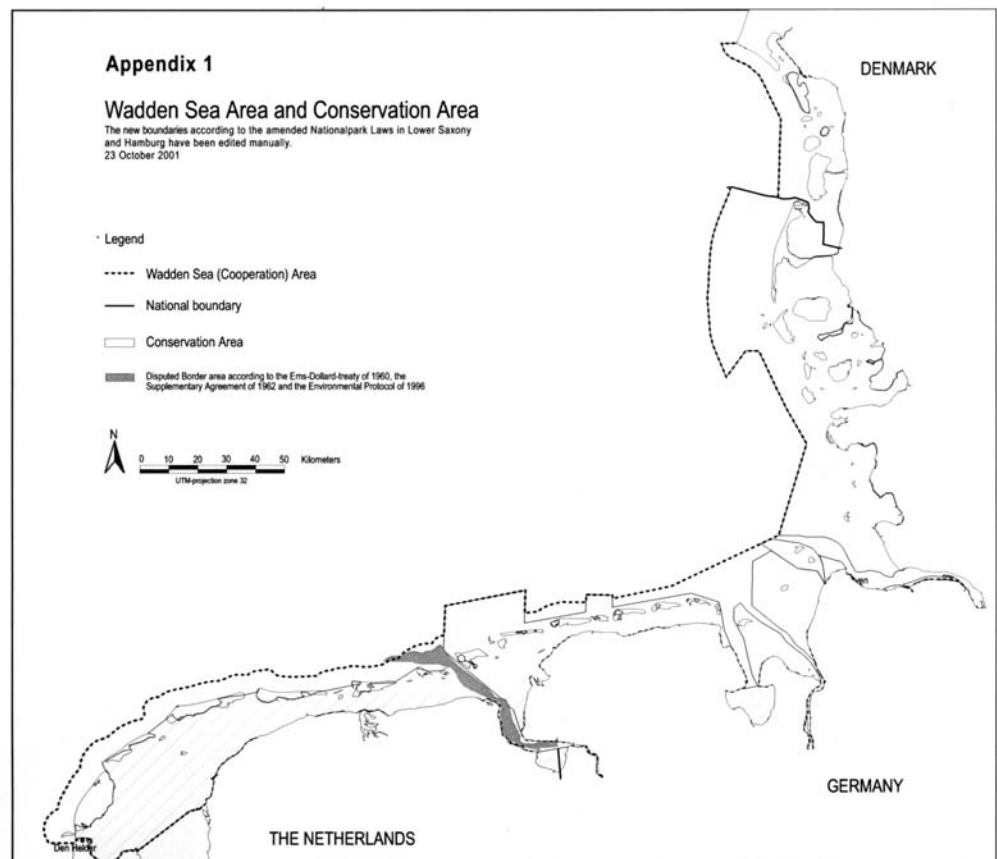
- a. What kind of relevant research in relation to organisms, with special attention to sea mammals, and organotins is performed and what are the results?
- b. What can be concluded on the results of the seal liver analyses for organotins?
- c. How does organotin behave in different species of organisms, sediment and suspended matter?
- d. To what extent is pollution of organotins present in the Dutch Wadden Sea?
- e. What are organotins and what are their effects on different trophic levels?
- f. How can the differences in organotin concentrations in different species be explained?
- g. To what levels and in which way does organotin accumulate in the food chain of the seal?

2. Wadden Sea

The possible relation between organotins and effects on seals that is discussed in this report is confined to the Wadden Sea. To better understand this complex ecosystem, background information, regarding both nature and anthropogenic activities, is given in this chapter. The Wadden Sea is a unique piece of art produced by mother nature that is used in many different ways. The policy must be strict to preserve this complex ecosystem from influences that can endanger the preservation of this unique system. To get a short impression of what is considered as Wadden Sea and what elements are important in relation to nature and policy, relevant information is summarized in this chapter.

2.1 Nature and scenery

The Wadden Sea is a shallow sea extending along the North Sea coasts of the Netherlands, Germany and Denmark (see map 1). It is a highly dynamic ecosystem with tidal channels, sands, mud flats, salt marshes, beaches, dunes, river mouths and a transition zone to the North Sea, the offshore zone. The area of the trilateral cooperation of The Netherlands, Germany and Denmark, the so-called Cooperation Area is 13,500 km² large.



Map 1: Wadden Sea area

Source: Quality Status Report 1999 (de Jong *et al.*, 1999).

The transition zone to the North Sea covers about 4000 km², the islands about 1000 km², the tidal area some 7,500 km², the salt marshes and summer polders some 350 km². The four estuaries, the Varde Å, the Elbe, the Weser and the Ems have a total surface area of 260 km². Also some areas on the mainland, which are important for birds, are part of the cooperation area and cover about 250 km².

Most parts of the Wadden Sea, in particular in the Netherlands and Lower Saxony, are sheltered by barrier islands and contain smaller or wider areas of intertidal flats. Between the Weser estuary and the island Amrum, the area is relatively broad and open to the North Sea. Because of embankments only four large sheltered bays have remained in the total area; the Ho Bugt in Denmark, the Jadebusen and the Leybucht in Lower Saxony and the Dollard in the Dutch-German border area.

Twenty-three islands with sand dunes, as well as fourteen high sands without dunes form a barrier to the North Sea. The present form of the Wadden Sea is the result of both natural forces and action by man.

Of greatest influence on the shape and functioning are the daily tides. Twice a day, on average, 15 km³ of seawater enter the Wadden Sea. This doubles the volume to some 30 km³. With the water from the North Sea, large amounts of sand and silt are imported which settle in places with little water movement. During low tides large parts of the Wadden Sea emerge. These so-called tidal flats cover about two-thirds of the tidal area and are one of its most characteristic features. Nowhere in the world can such a large unbroken stretch of tidal flats be found. They account for 60 percent of all tidal areas in Europe and North Africa.

Since the middle ages Man has changed the Wadden Sea landscape: dikes were built and land reclaimed. The natural 'wandering' of the islands as a result of accretion and erosion has been considerably reduced during the last century through the construction of dikes and groynes and through beach nourishment (www.waddensea.org).

2.2 Biological importance

The importance of the Wadden Sea as habitat for birds, seals, shellfish and fish species stems from the high growth rate of algae, the so-called primary production. Two factors are essential for the high primary production. Because the water is shallow, there is sufficient light for algae to grow. Secondly the water of the Wadden Sea contains many nutrients which are also essential for algal growth. In addition algae are imported from the North Sea. These account for almost half of the food resources in the Wadden Sea.

The Wadden Sea ecosystem is very dynamic with regular and unexpected changes from one extreme situation to another. Factors such as temperature with the possibility of ice, salinity, storms, waves and currents vary greatly. Only species, which have adapted to these extreme conditions, can exist here. That is why the Wadden Sea species, and consequently the ecosystem itself, have a large potential for survival.

The Wadden Sea provides a multitude of transitional zones to the land, the sea and freshwater environment, which is the basis for exceptional species richness. This includes 2,000 species of spiders, insects and other invertebrates in the salt marshes and 1,800 in the marine and brackish areas. Among these organisms, there is a high degree of ecological specialisation.

On the tidal flats, to the contrary, only a few species of flora and fauna have adapted to the extreme environment. Of these however exceptionally high numbers can be found. The high biological productivity of the tidal flats is comparable with that of tropical rain forests (www.waddensea.org).

2.2.1 Birds

The Wadden Sea is vital for about 50 bird species originating from a large part of the northern hemisphere. Among these are many rare and threatened species. The area is of international importance for at least 52 geographically distinct populations of 41 species. In about 20 populations more than half of the individuals utilize the Wadden Sea at some stage of their annual life cycle. For about 10 species almost the entire populations occurs in the Wadden Sea. Every year an average of 10 to 12 million birds pass through this area on their migration route from the breeding grounds in Siberia, Iceland Greenland and Northeast Canada to their wintering grounds in Europe and Africa. They feed on the tidal flats, which are the most nutritious areas of the Wadden Sea.

For more than 30 species of birds, the Wadden Sea is an indispensable reproduction area. For some endangered species, like little tern (*Sterna albifrons*) and kentish plover (*Charadrius alexandrinus*), the Wadden Sea has special significance. The most important breeding areas are the salt marshes, and, to a lesser extent, the dunes and beach plains of the islands. Typical Wadden Sea birds are redshank (*Tringa totanus*), black-tailed godwit (*Limosa limosa*), oystercatcher (*Haematopus ostralegus*), ringed plover (*Charadrius hiaticula*), avocet (*Recurvirostra avosetta*) and a number of species of ducks, geese, gulls and terns (www.waddensea.org).

2.3 Anthropogenic activities

The Wadden Sea is not only used by nature. People use the Wadden Sea already for many ages in many different ways. This use is expanding more and more. The community of interest must be controlled properly to minimize negative effects. In table 1 is showed what communities of interest are 'using' the Wadden Sea and for what purpose (www.waddensea.org).

Table 1: Human activities in the Wadden Sea

Human activity	Way of use
Fishery of fish and shellfish	Fishing is an important economic activity in the Wadden Sea. The main catches are cockles, mussels, seed mussels and shrimps. Eel, grey mullet, roundfish and flatfish are economically less important.
Tourism	The diversity of nature and animal attracts many people to the area in boats and by foot.
Shipping	Transportation by boat for industries.
Research	Governmental institutions and universities are carrying out reasearch to monitor pollution and give prospectives for nature.
Oil & Gas	Drilling Oil & Gas for energy consumption.
Salt mining	In some parts rock salt is produced.
Military	Military uses the area for practicing bombing and shooting.

2.4 Pollution

The relatively high level of contamination of the Wadden Sea is caused by three main factors:

A number of rivers, the catchment areas of which are highly industrialized and agronomised, flow into the Wadden Sea. The catchment area adds up to some 231,000 km². It extends to the southeast as far as the Chechian-Austrian border. Among the largest rivers are the Elbe and the IJssel, an outflow of the Rhine. In addition a substantial part of the Rhine water enters the Wadden Sea via the North Sea through a coastal flow along the Dutch coast.

The Wadden Sea imports more sediment than it exports. The sediments originate almost completely from the North Sea and are carriers of heavy metals and other contaminants. Due to the net North Sea current, a substantial part of North Sea sediments -and consequently polluting substances- is deposited into the Wadden Sea.

The Wadden Sea lies at the rim of northwestern Europe. An important part of its contamination is caused by rain and dust that originate from the highly industrialized northwest and central European countries.

Rivers are by far the largest carrier of polluting substances from the land to the Wadden Sea. The German rivers Elbe, Weser and Ems, together with the Dutch IJsselmeer, discharge each year at average 60 km³ of polluted water into the Wadden Sea. The rivers transport heavy metals, PCBs and pesticides like lindane and large amounts of nutrients. The amount of polluting substances is to an important degree determined by the amount of water that is discharged by the rivers. This discharge shows large yearly fluctuations as a result of differences in rain and snowfall in the catchment areas. That is why it is so difficult to determine whether or not the pollutant loads have decreased over the past years.

In the Wadden Sea itself a general reduction in the concentration of pollutants can be observed. Since 1983 almost everywhere in the sediments of the Wadden Sea concentrations of heavy metals have decreased.

Besides the 'large' amounts of contaminations that are gathered in the Wadden Sea there are also some pollutants that have another source. Organotin is one of those contaminants. In chapter 4 a special part will attentively be discussed because the source and knowledge of a pollutant is from high importance to discuss and predict the consequences (www.waddensea.org).

2.5 Policies

Already at the beginning of the last century smaller uninhabited islands were protected as nature reserves for birds. Later, this was followed by protection of salt marshes and, to a limited extent, also tidal areas. But in the 70s it became evident that the whole ecosystem would have to be protected including the tidal flats and sub tidal areas. This resulted in the establishment of protected areas, national parks and nature and wildlife reserves. The major part of the area between Den Helder in the Netherlands and Blåvandshuk in Denmark is now under legal protection. The areas under conservation are indicated on the [map 1](#) as Trilateral Conservation Area. Regulations in different countries are given below (www.waddensea.org).

2.5.1 The Dutch Wadden Sea

Since 1980 the Netherlands Wadden Sea is protected according to the key planning decision Wadden Sea (PKB), also called the Wadden Sea Memorandum, which is a national physical planning document defining the overall objectives of conservation, management and use of the Wadden Sea (amended 1993). The objectives and conditions of the Wadden Sea

Memorandum are binding upon all state, regional and local authorities. The area, for which the Wadden Sea Memorandum is valid, is, with the exception of the major shipping lanes and areas directly south of the islands, also a nature protection area.

According to the Dutch nature protection law it is prohibited without permission to undertake activities, which destroy and damage the protected area including its flora and fauna or its scenic importance. Within the protected area some areas have been closed for the whole or part of the year. This concerns areas are mainly important for seals and breeding birds. About a quarter of the tidal flats has been closed for cockle and mussel fishery (www.waddensea.org).

2.5.2 The German Wadden Sea

In Germany the coastal federal states are responsible for the implementation of the Federal Nature Conservation act. Schleswig-Holstein, Lower Saxony and Hamburg have established national parks for the major parts of the Wadden Sea in 1985, 1986 and 1990 respectively. Within the federal state Bremen a small part of the Wadden Sea is situated which has been partly designated as a nature reserve.

The objectives of the national parks are to protect the Wadden Sea and to allow natural process to take place with a minimum degree of disturbance and other detrimental effects of human activities. The national parks have been divided into two or three zones of which the zone I embraces ecological valuable areas. Therefore, strict regulations apply to the zone I including prohibition of public admittance. In zone II utilization and activities are allowed under such conditions that the overall protection objectives are not impaired. The national parks are managed by an administrative unity, the national park administrations, which are responsible for the implementation of the provisions of the national park instruments (www.waddensea.org).

2.5.3 The Danish Wadden Sea

In Denmark the Wadden Sea was declared a nature and wildlife reserve by Statutory Order in 1982. The order has been amended on two occasions; the last one was issued in 1999. The objective is to conserve the Wadden Sea as a nature area of national and international importance. It is, in general, prohibited to undertake activities which destroy or permanently change the natural environment of the Wadden Sea. Strict regulations apply to areas of special importance for seals and birds in which public admittance is prohibited. In other areas recreational boating and other recreational activities have been strictly regulated. Mussel and cockle fishery is prohibited in the major part of the tidal area. In the remaining areas, particularly the main shipping routes and the area offshore of the islands, no general restrictions apply (www.waddensea.org).

2.6 Ramsar Convention, EC Bird Directive, EC Habitat Directive

The Ramsar Convention 1971 is a worldwide treaty for the conservation of wetlands: shallow open waters and any land regularly or intermittently covered or saturated by water. In the framework of the Convention wetlands of international importance are designated by the contracting parties. Major parts of the Wadden Sea have been designated as Ramsar sites:

The Dutch Wadden Sea Memorandum Area is a Ramsar site. In Germany the Wadden Sea Ramsar sites are basically the national parks and a number of areas on the islands and the adjacent mainland. In Denmark the Wadden Sea Ramsar site is the Nature and Wildlife Reserve, the uninhabited parts of islands and the adjacent marsh areas on the mainland.

A more detailed description of the protection scheme and recent developments can be found in Chapter 1 of the Quality Status Report 1999 (de Jong *et al.*, 1999) and at the Esbjerg 2001 Conference.

The EC Bird Directive 1979 aims at the protection of all species of naturally occurring birds in the territory of the member states. According to the Bird Directive member states shall classify the most suitable territories for the conservation of these species including migratory species as special protection areas (SPAs). The Dutch and the Danish Wadden Sea Ramsar sites have also been designated as SPAs. In Germany major part of the Wadden Sea and a number of adjacent areas (offshore and inland areas) have been designated as SPA.

The EC Habitat Directive 1992 aims at the conservation of habitats of wild flora and fauna in the member states. In the framework of the Habitat Directive a coherent ecological network, called NATURA 2000, shall be established. NATURA 2000 will consist of Special Areas of Conservation (SACs) designated according to the Flora, Fauna and Habitat Directive, and the SPAs of the Bird Directive. Major parts of the Wadden Sea have been designated under the Habitats Directive and are included in NATURA 2000 (www.waddensea.org).

2.7 The Trilateral Wadden Sea cooperation

Already in the beginning of the 70's environmental scientists stated that the ecosystem of the Wadden Sea cannot be divided according to national borders. The Wadden Sea is, from an ecological point of view, one system. The politicians from the three Wadden Sea countries were called upon to work together in the protection and conservation of the area.

The first trilateral governmental conference on the protection of the Wadden Sea was held in 1978 in The Hague, The Netherlands. The second Wadden Sea Conference took place two years later in Bonn, Germany.

In 1982, at the third Conference in Copenhagen, Denmark a Joint Declaration was agreed upon by the three countries. According to the Joint Declaration the Wadden Sea countries declare their intention to coordinate their activities and measures to implement a number of international legal instruments in the field of natural environmental protection, amongst others the Ramsar Convention and the EC Bird Directive, for a comprehensive protection of the Wadden Sea region as a whole, including its flora and fauna.

Since 1982 four more Governmental Wadden Sea Conferences were held and the trilateral cooperation strengthened and intensified.

Other important trilateral events are the International Scientific Wadden Sea Symposia, which are held every three years. At the Symposia, scientists from the three Wadden Sea countries exchange relevant research findings and formulate recommendations to the politicians. The Symposia also deal with management issues. The findings of the scientific symposia have been and are important for the development of trilateral policies.

1988 - 5th Wadden Sea Conference, Bonn, D Adoption of the Agreement on the Protection of Seals

The Trilateral Working Group (TWG) is a permanent working group installed by the collaboration, which meets, on average, four times a year. The TWG is composed of civil servants of the responsible ministries and other relevant ministries as well as regional authorities. The TWG is commissioned with the overall implementation of the decisions of the Governmental Conferences, the overall coordination of the work of the cooperation and the preparation of the Governmental Conferences. The TWG can establish ad hoc working groups to execute special tasks. Also operating under the responsibility of the TWG, is

the permanent Trilateral Monitoring and Assessment Group (TMAG). This group is responsible for the trilateral monitoring and assessment program (TMAP).

The Common Wadden Sea Secretariat (CWSS) was established in 1987 in Wilhelmshaven, Germany, as the secretariat for the trilateral cooperation. Its primary task is to support, initiate, facilitate and coordinate the activities of the collaboration.

The CWSS is responsible for the preparation of the meetings held in the framework of the cooperation. It is also responsible for the collection and assessment of information with regard to Wadden Sea protection, management and monitoring including progress in the implementation of the decisions of the ministerial conferences. Furthermore the CWSS collects information on activities that have, or may have, significant effects on the natural environment of the Wadden Sea and give suggestions for appropriate actions. Finally, the secretariat coordinates trilateral initiatives in relevant international organizations.

Reports and documents produced by the trilateral cooperation are published by the CWSS.

The work of the secretariat is supervised by a board of representatives composed of one representative of each of the responsible national ministries (www.waddensea.org) and (www.waddenseasecretariat.org).

3. Seals

3.1 Morphology

The harbour seal (*Phoca vitulina*) can grow to a length of approximately 1.60 m and a weight of 120 kg. It can grow as old as 40 years. Their daily life cycle is determined by the tides. During high tide, they hunt for fish; during low tide, they like to bask in the sun upon the sandbanks. During the summer, it is here upon the tidal banks where the young are born (www.waddensea.org).



Fig 1: Harbour and grey seal

(source: <http://www.vlieberg.nl/dieren/zeehonden/foto.htm>)

3.2 Distribution of harbour seals

One rarely spots a seal in the open sea. They are typical coastal residents. The harbour seal is found along almost all of the shorelines along the northern Atlantic Ocean, and therefore also in the North Sea. Tidal areas and river basins, where isolated sandbanks lie above the water surface during low tide, is their preference. Thirty percent of the 'British' seals are found by the Orkney Islands. British nature organizations are examining the possibility of placing the island Sanday under the European Habitat Guidelines, in the interest of the seals (www.waddensea.org).

3.3 Food choice

Harbour seals feed in general on benthic fish, however most of the animals are fairly opportunists where eating is concerned. Some harbour seals eat in areas lying more than 20 km from their resting area; other animals eat fish found in their direct vicinity. Adult seals in captivity eat an average of 3 to 4 kg of mackerel or herring per day. Animals that eat lean flatfish need almost two times as much weight in food per day than animals that eat fatty herring or mackerel.

The harbour seals in the Wadden Sea usually migrate through the sea passages during the winter to hunt for fish. The flatfish flee the cold wintry water of the tidal flats and find refuge in the deeper North Sea. Observations from ships have shown that most of the seals are found at around a depth of 10 metres. Occasionally, seals will hunt as deep as 20 metres (www.waddensea.org).

3.4 Population developments

Many young seals were born in the summer of 1989. The first step to recovery after the epidemic was off to a quick start: 535 seals were counted in the autumn of 1989. This rapid recovery did not continue in 1990: in that year, 562 harbour seals were counted in the whole Dutch Wadden Sea.

In 1991, there was talk of a 'pup' boom: 150 pups were born. There was probably also some immigration of seals from Germany and Denmark, because a total of 750 seals were counted. In 1992, the researchers counted approximately 965 seals in the Wadden Sea. In July 1992 there were more than 50 dead seal pups found in the eastern tidal area - they were only a few days old. Normally, only a few seal pups are found dead. Up to this day, there is still no good explanation to this phenomenon.

In 1994 1230 harbour seals were counted on the Dutch tidal flats, 1410 in 1995 and 2400 in 1999. In that way, the seal population has been recovering more quickly than one ever dared to dream of in 1988. It is supposed that a strict, natural selection took place during the virus epidemic; only the most exceptionally vital animals possessing the greatest resistance against the seal sickness could survive.

Remarkable is that the amount of dead harbour seals in 2002 is far higher compared with the amount of died grey seal (*Halichoerus grypus*). Of all animals found just a little percentage was identified as grey seal. Experts believe that this difference may be caused by resistance to the virus by the grey seal (www.waddensea-secretariat.org).

Number of Counted Common Seals in the Wadden Sea since 1975

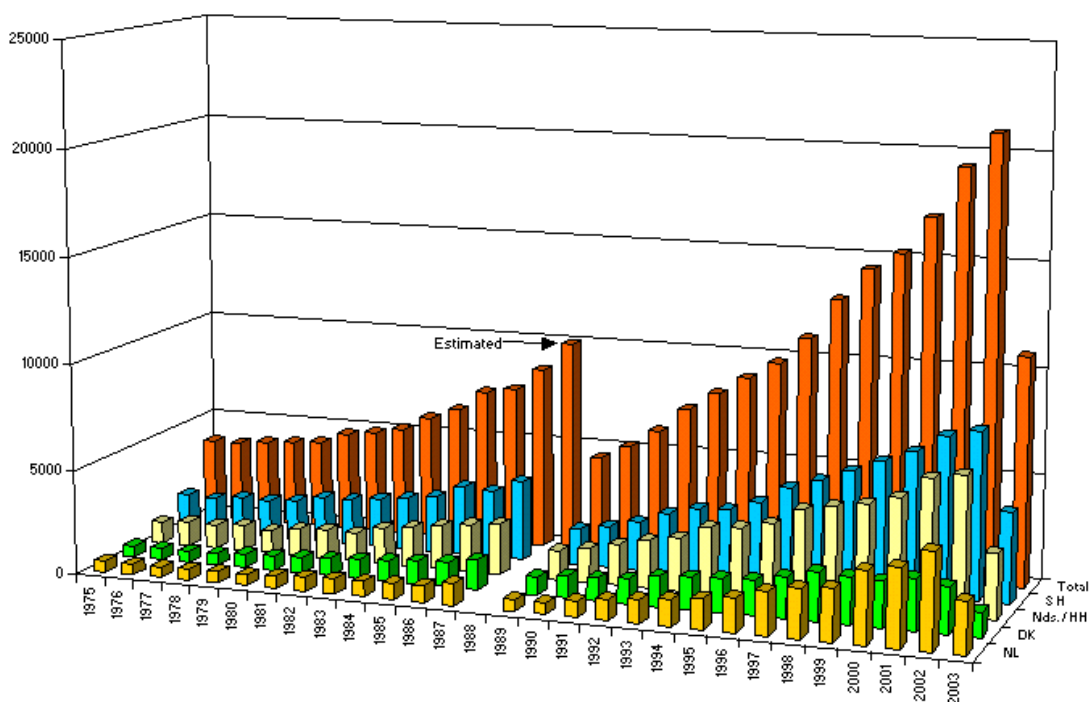


Fig 2: Number of counted harbour seals in the Wadden Sea since 1975
 Source: Common Wadden Sea Secretariat (www.waddensea-secretariat.org)

3.5 Seal platform

To monitor the conditions of the seal population in the Netherlands a platform was established in the autumn of 2002. This platform consist out of a group of experts from the Erasmus University in Rotterdam, the seal rehabilitation centre 'Pieterburen' and Ecomare, Alterra, the Wadden Association and representatives from RIKZ, which is a department of the Ministry of Transport, Public and Water Management. Their main task is describing the seal population. Further more, this group will advice about necessary research and policy decisions. Beside these participants in the platform, it consists out of another member, from the Centre of Bio Ethics and Health Rights of seal day care who is concentrating on the ethics for seal day care (www.waddensea-secretariat.org).

4. Organotin

'Organotin' is a collective name for a group of chemical substances which contain tin, as well as alkyl and phenyl groups. The only known natural organotin is methyltin. All other forms are anthropogenic. In this report tributyltin (TBT) is considered to be the most important. Its decomposition products dibutyltin (DBT) and (monobutyltin) MBT will be discussed as well. Further more triphenyltin (TPT) will be singled out. TBT has been used since the 1970's but in the last decades the harmful effects became obvious. Most famous examples are the imposex effects in whelk (CSTEE, 1999). In order to have a clear overview, the following properties of the compounds will be discussed in this chapter: usage, legislation, decomposability and other important properties there is set up the following chapter. The information will be limited to the compound itself and not to the effects on the environment or organisms. Further more, policy regarding TBT and alternatives for this substance are discussed.

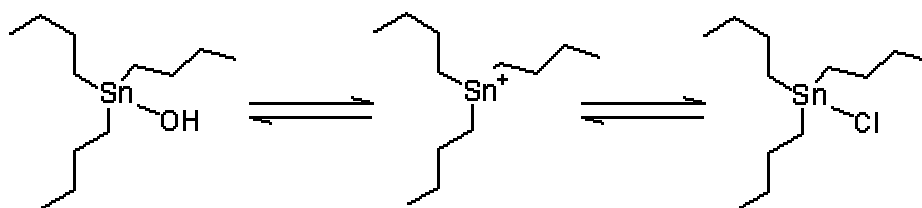
4.1 TBT

TBT is industrially produced from TeBT (tetrabutyltin), which is, in contrast to TBT, a stable molecule. Since 1943 butyltin combinations are widely produced and used (Evers *et al.*, 1995). It has hydrophobic quality and it is thus not very soluble in water and it is therefore mainly found in sediment and suspended matter (sorption). This presence is influenced by many factors. The most important factors are: pH, salinity, organic matter and clay content (Hoch and Schwesig, 2003).

Tributyltin (TBT) is an aggressive biocide and has mainly been used in ocean shipping (Swertz, 1999) as a paint additive (anti-fouling) since the 1970's to prevent fouling (e.g., growth of tubeworms, algae and barnacles) of ship hulls and in nets for fish farming (www.ortepa.org). TBT leaches from the paint and enters the marine environment. Upon till now, it is one of the most cheap and effective ways to prevent the growth of organisms on materials in marine aquatic systems. This organotin compound must be seen as extremely toxic (Rudel *et al.*, 2003), because it disturbs the cell energy metabolism (Fent and Hunn, 1996) (Hunziker *et al.*, 2002).

4.1.1 Chemical properties TBT

Different tributyltin compounds are being used for antifouling purposes. Usually, they are only soluble up to concentrations of tens to hundreds of milligrams per litre, depending on the compound used, salinity and content of particulate matter of the medium. Once dissolved, the tributyltin cation is in equilibrium with the neutral hydroxytributyltin TBTOH, and, in seawater, with the neutral unpolar TBTCI species, which will tend to sorb to particles. Chemical properties are displayed in Table 2.



Tributyltin cation in equilibrium in seawater

Source: <http://eckehaat.uft.uni-bremen.de/~ranke/TBT.html>

Table 2: Chemical and physical properties of TBT

Chemical and Physical properties of Tributyltin (TBT)			
TBT Properties	value(s) or name	influencing factors	References
Formula	C ₁₂ H ₂₈ Sn	*	chemfinder
chemical class	trialkyl organotin compound	*	U.S. National Library of Medicine, 1995
chemical name	Bis(tributyltin)oxide	*	U.S. National Library of Medicine, 1995
Molecular weight	291,04	*	chemfinder
melting point	53 °C	*	U.S. National Library of Medicine, 1995
boiling point	193 °C	*	chemfinder
partition coefficient	5500 ppm in 32% seawater	*	Laughlin, 1986
vapor pressure	0,1 mPa (20 °C)	temperature	U.S. National Library of Medicine, 1995
water solubility	4-6 mg/L (20 °C, pH 7,0 and salinity »0 promille)	temperature and pH	U.S. National Library of Medicine, 1995
		pH and salinity	Evers, 1995
		pH and salinity	Tas, 1993
Log Kow	4.2 (pH>7.4 and salinity 18 ‰)	pH and salinity	Fent, 1996
			Tas, 1993
Log Kp fp/water	3,1 L/Kg (ph:?, salinity: 32 ‰, solid part concentration: 60 mg/L)	pH, salinity, organic matter and solid part concentration	Evers, 1995
Log Kp sed/water	2,7 L/Kg (pH:?, salinity: 18 ‰)	pH and salinity	Tas, 1993 & Fent, 1996
			Tas 1993
			Evers, 1995
Log Kow = Octanol/water coefficient			
Log Kfp = suspended solids/water coefficient			
Log Ksed/water = sediment/water coefficient			

4.1.2 Decomposability

Under environmental conditions, fixation of organotin mainly takes place in the sediment.

Persistence of organotin compounds in the environment is governed by moderate to fast aerobic degradation processes, slow anaerobic biotic degradation, slow abiotic degradation by photolysis, and fast but hard reversible adsorption/desorption processes (Batley, 1996; Maguire, 1996; Berg *et al.*, 2001).

The decomposition of TBT in sediment develops slowly but can vary widely. Under room temperature conditions the TBT concentration in sediment can halve within several months but under anaerobic conditions the half-life can be as much as several years. Metabolites that are produced within this transformation progress are the less toxic DBT, MTB and inorganic tin (Evers *et al.*, 1995).

The environmental half-lives of organotin compounds are in the range of days in the water phase, but are more persistent in anaerobic systems (Batley, 1996; Maguire, 1996).

Animals high in the food chain and sulphate reducing bacteria are able to biodegrade the toxic TBT to less toxic substance (DBT and MBT) (Hoorn, 2003a).

4.1.3 TBT Standards & Policy

Since July 2003, the use of TBT for marine ships larger than 25 meter is prohibited in all member states of the European Union. The usage on ships smaller than 25 m was already prohibited in 1993. DBT and MBT are still legal to stabilize PVC (Hoorn, 2003a). For the year 2008 a total ban for TBT is planned so that the presence in the top layer of paint on ships is prohibited. The International Maritime Organisation (IMO) urges for an administration obligation for ships. Every ship must have this administration to be able to control the regulations. Not all countries are part of this organisation and ships without these papers can be turned down to enter European harbours.

In Table 3, as shown below, the values for maximum acceptable risk and target values are summarised. Also the Tolerable Daily Intake for humans is presented.

Table 3 standards for TBT in saltwater environment and TDI for humans

Standard	Value (in dry matter)	Reference
Max Acceptable Risk dissolved	1 ng/L	Hofstede and Van de Ven, 2001
Max Acceptable Risk SM	0.7 mg/kg	Hofstede and Van de Ven, 2001
Max Acceptable Risk sediment	0.7 mg/kg	Hofstede and Van de Ven, 2001
Threshold dissolved	0.01 ng/L	Hofstede and Van de Ven, 2001
Threshold SM	0.007 mg/kg	Hofstede and Van de Ven, 2001
Threshold sediment	0.007 mg/kg	Hofstede and Van de Ven, 2001
Tolerable daily intake (TDI) for human	0.25 mg per kg body weight	World Health organisation (WHO), 1999

SM: Suspended Matter

4.1.4 Alternatives for antifouling containing organotin

The research on alternatives to TBT for ship-bottom paint may essentially be divided into five main groups. There is active research today in all of these areas (www.bellona.no).

- Copper based anti fouling paints
- Super smooth surfaces to which organisms are unable to attach themselves
- A combination of smooth surfaces and the application of a type of organic biocide with a low leaching rate
- A combination of smooth surfaces and mechanical removal of fouling
- Nature's own fouling inhibitors. There are a number of organisms in the natural world that are hardly affected by fouling, like for example coral.

4.2 TPT

Triphenyltin (TPT) has also been used as anti-fouling, but its major use was (until 1999) as a fungicide in agriculture, especially in potato cultures (Hoorn, 2003b). It is believed that TPT causes similar effects as TBT. Proven is, that it can cause imposex and that it is highly toxic for fish and bacteria (Kortlandt & Stronkhorst, 1998). Tests show that the input of TPT into the Wadden Sea mainly is derived from the many freshwater canals that discharge into the Wadden Sea (Bellert & Van de Ven, 2003). Like TBT, accumulation mainly

takes place in sediment. Micro-organisms and UV-radiation cause degradation. During this slow process, which is similar to the degradation process of TBT, TPT breaks down to DPT and MPT. The metabolites are, just like the metabolites of TBT, decreasing in toxicity (Hoorn, 2003b). Effects that occur in salty water are higher than the effects of TPT in fresh water at the same concentrations (Crijns *et al.*, 1992). TPT has similar quantities and effects, but the persistence is higher which makes the time for degradation/transformation longer (Kimmel *et al.*, 1997). In the Netherlands all use of TPT in agriculture is prohibited since the first of January 2003.

5. Effects of tin in biota

TBT and its degradation products have been determined in a wide range of marine environmental samples. In many cases, a relationship between levels of environmental contamination and the intensity of shipping traffic can be made (Vos *et al.*, 2003). TBT has been found in the tissues of cetaceans, fish, seals, sea otters and water birds in a wide range of locations around the world (Linley-Adams, 1999). Tissues and sediments sampled from areas with heavy shipping activity show the highest levels of contamination (Vos *et al.*, 2003). Some organisms can reduce the TBT concentrations in their body by biotransformation.

Organisms, which are high in the food chain, can metabolize TBT by the methylate process. Not all organisms are able to degrade this toxic substance with the same ease. Takahashi *et al.* (1999), who analysed the distribution of butyltin compounds in biota sampled near the Japanese Pacific coast, found that TBT was the predominant butyltin compound accumulated. The authors of this research concluded that certain organisms may have less capacity to degrade TBT and therefore may accumulate it at elevated levels. Due to the fact that TPT is a relative new monitoring substance the amount of information available is lower as TBT.

A rough sketch of the differences in concentration between organisms will be presented in the following chapter. Although numerous factors, such as age, health, feeding habits and habitat, may explain the differences in butyltin accumulations among specimen and species (St. Louis *et al.*, 2000), other critical factors that influence the accumulation of butyltins are diet and feeding modes.

5.1 Organotins in invertebrates

TBT is responsible for the disruption of the endocrine system of marine gastropods leading to the development of male sex characteristics in female marine snails. The phenomenon called imposex, where an effect is only expressed in individuals exposed in very early development, typify chemicals that interfere with the endocrine system (Vos *et al.*, 2003). TBT impairs the immune system of some organisms. Shellfish can develop shell malformations after exposure to extremely low levels of TBT in the seawater. Laboratory studies in dogwhelk show that imposex development occurs at levels up to the one billionth of gram per litre. The most sensitive species currently seems to be *Ocenebrina aciculata* with a threshold concentration of 0.1 ng TBT-Sn/l (CSTEE, 1999).

TBT induces imposex by inhibition of aromatase enzyme metabolism of testosterone to 17 β -oestradiol. Aromatase plays a critical role in the conversion of testosterone to estradiol and maintaining the ratio of female hormones to male hormones during sexual differentiation in embryonic development (Vos *et al.*, 2003; CSTEE, 1999).

Although the biotransformation of organotin mainly takes place in organisms high in the food chain Mensink *et al.* (1997) showed that the whelk (*Buccinum undatum*) can biotransform TBT to TPT compared to the mussel (*Mytilus edulis*).

5.2 Organotins in fish

Organotins have been found in many fish species from all over the world (OSPAR, 1999). The toxicity of organotins to fish has been established and there is evidence of their hormone disrupting effect (Morcillo & Porte, 1995). In a report by Fent & Hunn (1999) a relationship was made between TBT, TPT and immunosuppression.

Like in mammals, in fish specific immunological reactions are mediated by B and T lymphocytes (Grinwis *et al.*, 1998). In this study flounder (*Platichthys flesus*) was exposed to TBTO. The total lymphocyte number in the spleen was decreased, and a strong and significant decrease of non-specific cytotoxic cell activity was observed. Also, a significant decrease of the relative thymus volume, but no marked effects on the specific immune system, was noted after exposure to TBTO. A study by RIVO (Leonards, 2002), in which organotin in fishery products were analysed, gave striking results (Fig 3). Firstly, the amount of TPT was higher in comparison with TBT in all fish species examined. This was attributed to the more persistent property of TPT. Secondly the benthic flatfish contained the highest concentration of TPT and the lowest concentration of TBT. The given explanation suggested that habitat preferences, that enlarged contact between organotins in sediment, could enlarge the uptake. Fish can excrete organotins by faeces and urine; also gills can decrease body concentration. Table 4 shows concentration of organotin in fish of the Baltic coast of Poland. This location is seen as a polluted area and this resulted in the concentrations encountered.

CASE STUDY	
Organotins	
In 1990, 58 fish from nine species taken from Gdansk Bay on the Baltic Coast of Poland were sampled and tested for organotin residues.	
Fish species	Total organotins
Flounder	316
Herring	40
Eel	188
Sea Trout	51 (45-57)
Turbot	39
Cod	19 (14-24)
Eelpout	130
Pikeperch	455
Mackerel	27 (23-20)

Table A: Concentrations of organotins (range in brackets) (ng/g wet weight) in fish from the Southern Baltic Sea.

source: Ospar, 1999

Fig 3: Average organotin concentration ($\mu\text{g cation/kg product}$) for different fishery products. No average of fish oil could be given because only 1 sample was available (Leonards, 2002).

Table 4: Concentrations of organotin in fish of the Baltic coast of Poland.

CASE STUDY	
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source: Ospar, 1999

5.3 Organotins in seabirds

Less data exists on organotin residues and their impact in seabirds than for marine mammals. From the limited studies carried out to date, it appears that seabirds such as sea ducks preying on organotin-contaminated marine invertebrates have a greater body burden of organotins, although birds can purge these chemicals by moulting and other shedding of feathers. Even so, high levels of organotins have been found in marine birds in coastal locations. The ecotoxicological significance of seabird exposure to organotins is not known, but laboratory studies have shown toxic effects of TBT in bird embryo: it reduces hatching success and fertility and affects enzyme and hormone activity in adult birds (OSPAR, 1999).

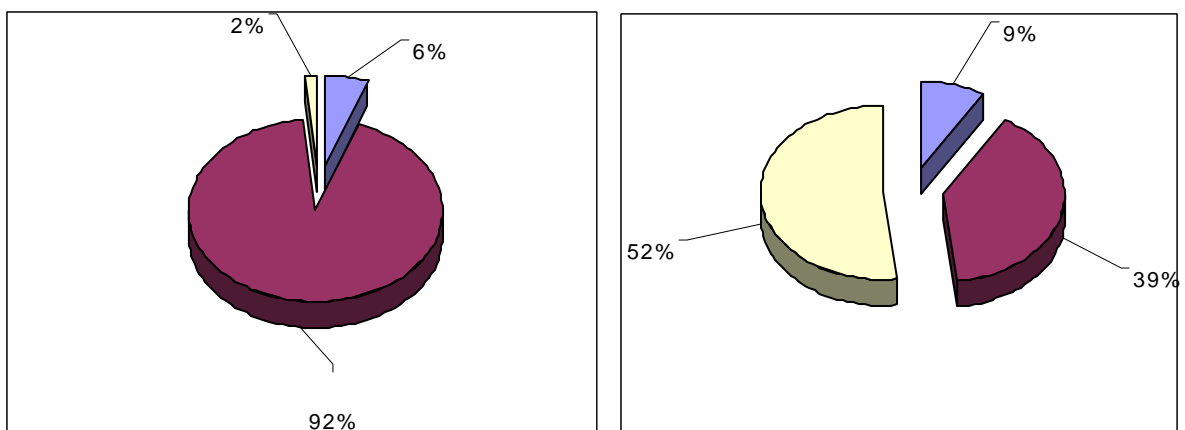


Fig 3. Butyltins and Phenyltins in Eider ducks in the Dutch Wadden Sea. (Source: Werkman *et al.*, 2001).

In 2001, a report was published in which results were presented of the analyses on eider ducks livers (Werkman *et al.*, 2001). 12 (dead) ducks were found in the Dutch Wadden Sea and analysed by the RIKZ. Figure 3 shows the proportions between TBT, TPT and metabolites. Concentrations of all separate samples can be found in Appendix A.

5.4 Organotins in marine mammals

Marine mammals belonging to a range of species have been found to be contaminated with organotin compounds including TBT, DBT and MBT, no matter from where in the world they were taken. The elevated levels of organotins detected in coastal species, and low concentrations found in offshore species, show the high degree of organotin contamination in many coastal waters. Mammals inhabiting waters of developed countries are, in general, found to contain higher concentrations compared with those collected from the waters of developing countries. While some mammals such as the sea lion can degrade organotins from the body, others such as dolphins show increasing biomagnification of organotins, as they grow older.

High doses of organotins have been shown to damage the central nervous system and reproductive mechanisms in mammals. Marine mammals are no different in this respect. TBT is an endocrine disrupting chemical in mammals. It is highly likely that negative effects from exposure to organotins can occur to marine mammals in the wild.

Recent studies conducted by the Dutch Institute for Marine Research (NIOZ) and the Free University of Amsterdam (VU) reveal that sperm whales that live and feed in the deep ocean far from ports and shipping lanes have surprisingly high concentrations of TBT and its breakdown products in their bodies. This indicates that TBT is widely dispersed in the marine environment, including the deep oceans (OSPAR, 1999).

When profiles of TBT metabolites among a number of marine species were compared a difference in efficiency of metabolising and excreting between cetaceans and pinnipeds was discovered. Cetaceans seem to be better able to fulfil this than pinnipeds (Vos *et al.*, 2003).

5.5 Important TBT effects in other organisms

No bioassays in which the effects of organotin on seals are described are found. Concentrations in suspended solids and sediment on itself do not say anything about the effect on an organism which could occur. In order to understand more about the relationship between effects on organism different effects must be known. Especially for the organotin substance the dose-effect relationship differs in large quantities. A doses that is fatal for mollusc, can be harmless for marine mammals. Table 5 will show data from previous studies in different mammals. This will help to estimate a possible effect on seals on specific concentrations.

In a report from the World Health Organisation many different results from previous studies are summarised (WHO, 1999). In this report a LOAEL (Lowest Observed Adverse Effect Level) for mice of 0.1 µg/kg body weight per day was the lowest intake of Tributyltin oxide with negative effects.

Table 5: Health effects of triphenyltin compounds (Sekizawa *et al.*, 2002)

Type of test	Organisms (route of exposure, duration of test)	Results/remarks
Single exposure	Rat (oral)	LD ₅₀ 160 mg/kg
Short term	Dog (oral, 52 weeks)	NOAEL: 0.21 mg/kg bw/day, based on relative liver weight decrease at effect levels;
	Rat (dermal, 29 days)	NOAEL: 10 mg/kg bw/day, based on erythema, mortality, lymphocyte decrease at effect levels,
	Rat (inhalation, 4-weeks)	NOAEL: 0.014 mg/m ³ based on IgM (an immunoglobulin species) increase at effect levels
Long-term	Mouse (feeding, 80 weeks)	NOAEL: 0.85-1.36 mg/kg bw/day, based on decreased body weight at effect levels;
Genotoxicity	In vivo/In vitro	Mostly negative
Reproduction	Rat (feeding, two generation)	NOAEL: 0.4 mg/kg bw/day, based on decreased litter size, pup weight, relative spleen/thymus weight in weanlings at effect levels
Teratogenicity	Rabbit (gavage, day 6 to day 18 of gestation)	NOAEL for maternal toxicity: 0.1 mg/kg based on decreased body weight gain
Immunotoxicity *	Rat (feeding, two years)	Immunosuppressive. LOAEL: 0.3 mg/kg bw/day, based on reduced immunoglobulin levels and reduction in white blood cell count
Neurotoxicity	Rat (gavage, 6 weeks)	Toxic at 0.36 mg/kg bw/day in maze learning test

* With tributyltin oxide, when weanling rats fed orally up to 4.5 months, NOAEL was 0.025 mg/kg bw/day, based on the depression of IgE titres and impairment of clearance of injected *Trichinella spiralis* at effect levels

6. Organotin in suspended matter

Taking into account that in the present report a possible relationship between organotin and organisms, in particular seals, is highlighted, the study focused on compartments which have high bioavailability for organisms. As will be discussed in Chapter 8, organotin enters the seals food chain out of the water phase and suspended matter (SM). Therefore, examination of quantities of substance in the compartment sediment was less relevant for this research. This is also true for other forms of exposure. TBT and TPT are hydrophilic substances. Because of this property, they prefer to bind to organic material. Therefore algae that live in the water phase of the Wadden Sea are a highly suitable source of organic matter. In this chapter the concentration of TBT, TPT and its metabolites in suspended matter will be described. Three locations were examined over several years. They are considered to be representative points for the Dutch Wadden Sea. The locations can be found in appendix D. The monitoring of sediment, also performed by RIKZ, is not used because the data range was only available for a short period of time. The regression lines are used to translate the data into a trend. All data is standardised (STD) for 10% organic matter and are measured in the dry matter of the suspended matter. Sampling is performed under conditions in which the water was leaving the Wadden Sea (high-tide to ebb).

6.1 TBT and degradation products in suspended matter

The average concentration of TBT for the 3 locations is around 30 - 40 µg/kg dry weight suspended matter and the metabolites are present in concentrations, which are about 3 times lower than TBT (fig 4 – 6). The graphs show a strong fluctuation between the months and concentration is visible. This unstable factor is caused by the meteorological influence. During the warm months of the Dutch climate the water temperature rises and also the amount of sun radiation increases. Temperature and radiation are the two most important factors that regulate the degradation rate. The warm water makes desorption of TBT from the sediment easier. Also a higher shipping activity will influence the concentrations.

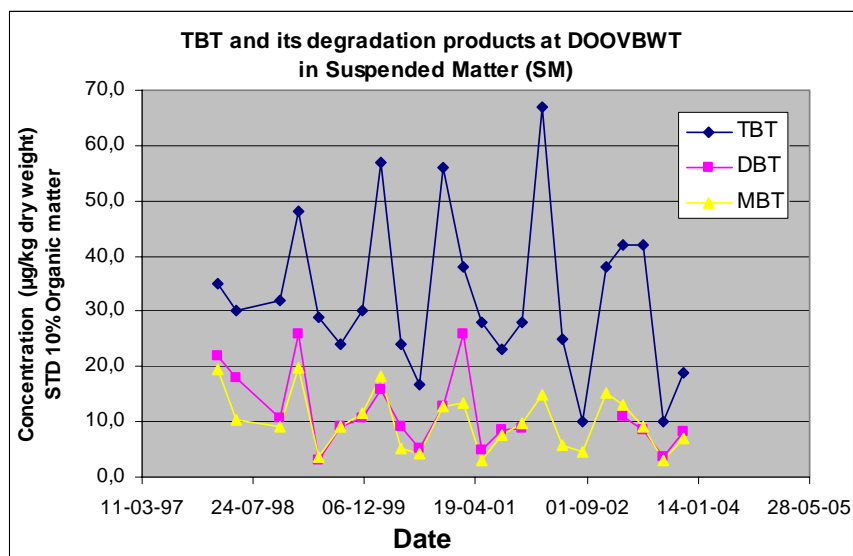


Fig 4: TBT and metabolites at Doove Balg West

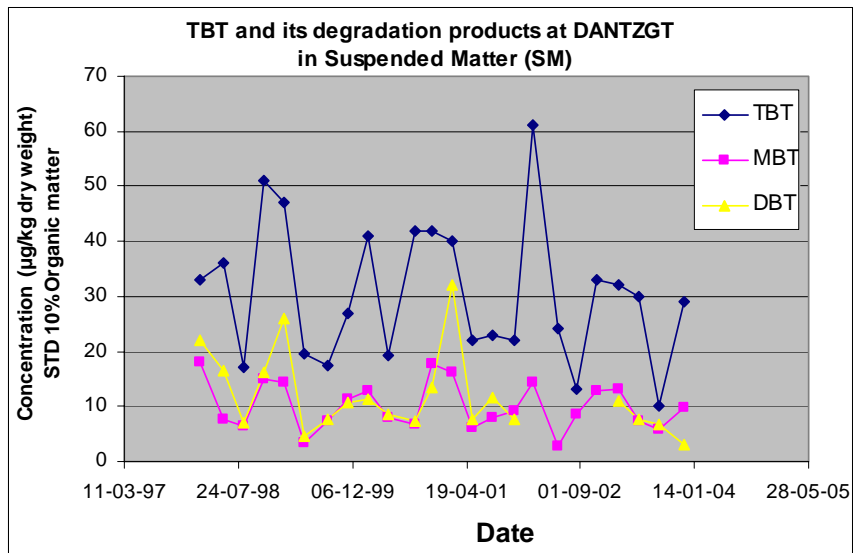


Fig 5: TBT and metabolites at Dantzigat

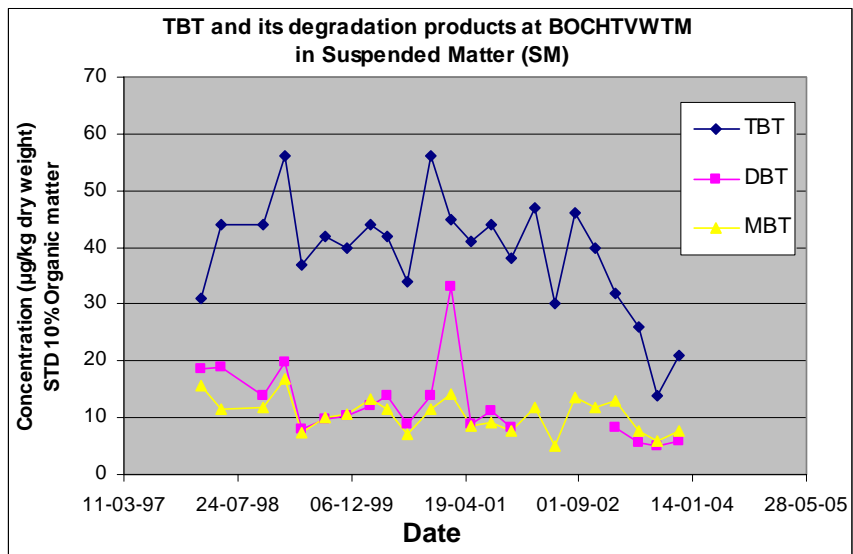


Fig 6: TBT and metabolites at Bocht Van Watum

The high levels of TBT in comparison with its metabolites are caused by the degradation rate of this chemical. TBT, as mentioned in the Chapter 4, is the most complex combination of butyltins and it therefore takes the most time to degrade it.

The reason why samples of the 'BOCHTVWT' location contain a higher concentration of TBT in the floating particles can be attributed on the large amount of shipping in this area (Bellert & Van de Ven, 2003). Furthermore, the outflow of canals from the inland can contribute to a higher concentration.

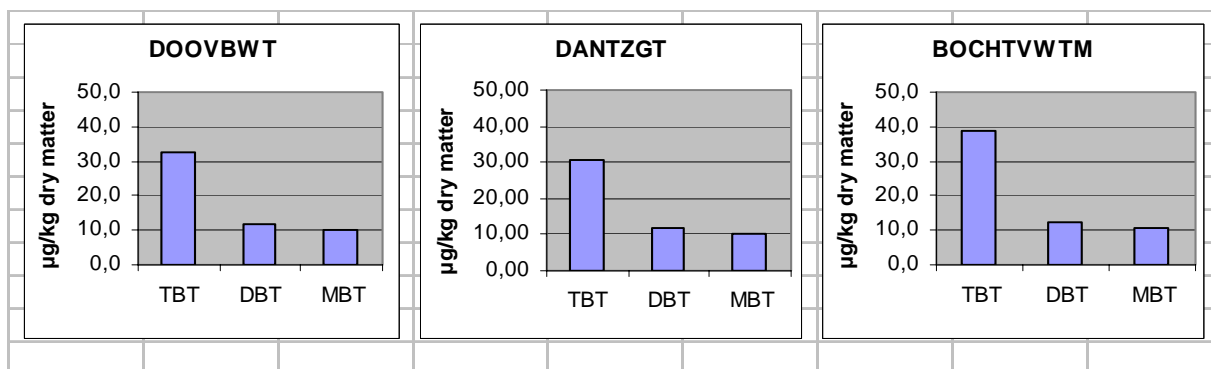


Fig 7: Average concentration TBT and metabolites on 3 locations from the year 1998 till 2003. Average concentration from the year 1998 till 2003 (STD 10% organic matter).

6.2 TPT and degradation products in suspended matter

TPT is an organotin compound that is mainly used as a pesticide in agriculture (potatoes). Only small quantities were applied in antifouling paint for ships. The source is mainly agriculture, which is clearly visible in the graphs below (Fig. 8-10). The average annual concentrations of locations are between 6 - 12 µg/kg dry weight for TPT and in most cases, its metabolites are found in lower concentrations. Bocht van Watum ('BOCHTVWTM') is influenced by canals, because it is close to the river Dollard (Eemskanaal). The high concentrations of TPT in Bocht van Watum can be attributed to this district, which has a high concentration potato crop.

Although less obvious, the same relationship as with TBT between the seasons and the concentration of Phenyl tins is present. In addition to the relationship between concentrations of TPT with temperature and sun radiation the time of the year in which the pesticide is used also influences in this peak.

As with TBT, the high level of TPT in comparison with its metabolites is due to slower degradation rate of this more complex phenyltin.

For DPT and MPT the regression line of the concentration, e.g. DPT in DOOVBWT, is rising. The TPT concentrations decrease, causing an increase in concentrations of its degradation products DPT and MPT.

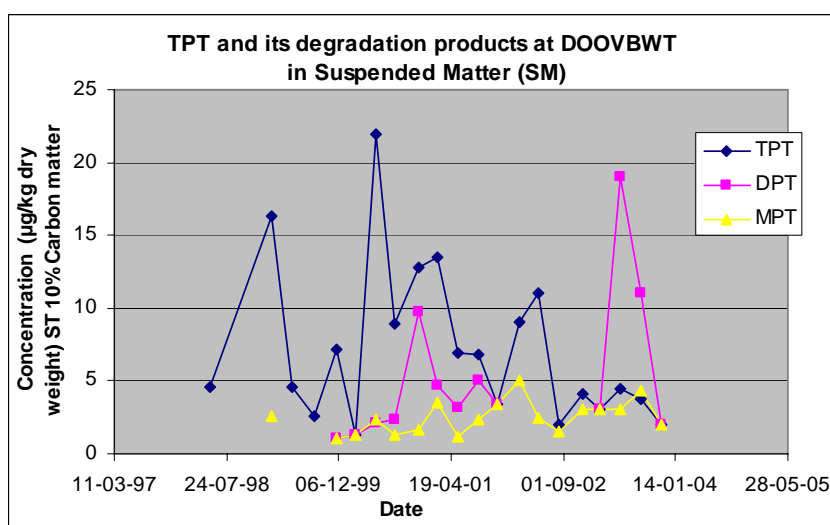


Fig 8: TPT and metabolites at Doove Balg West

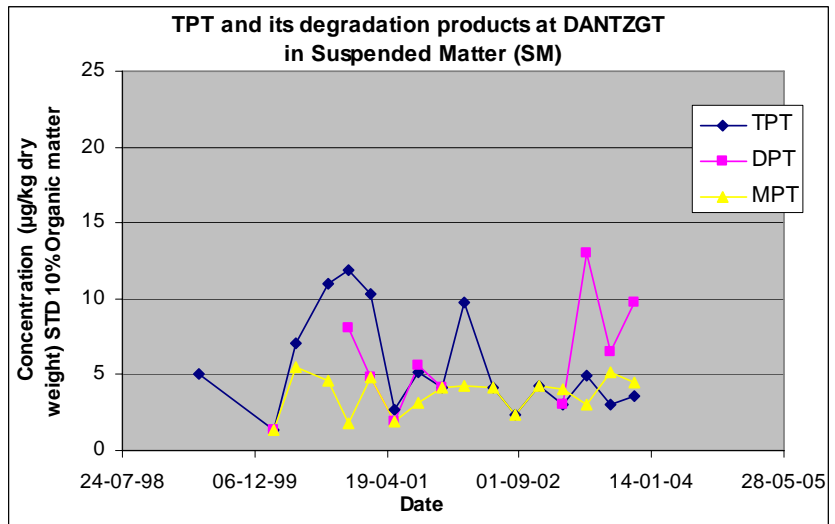


Fig 9: TPT and metabolites at Dantzigat

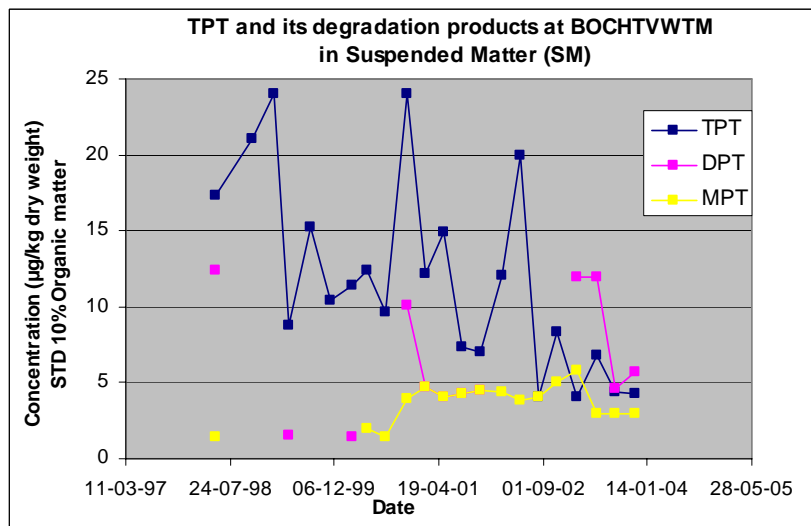


Fig 10 TPT and metabolites at Bocht Van Watum

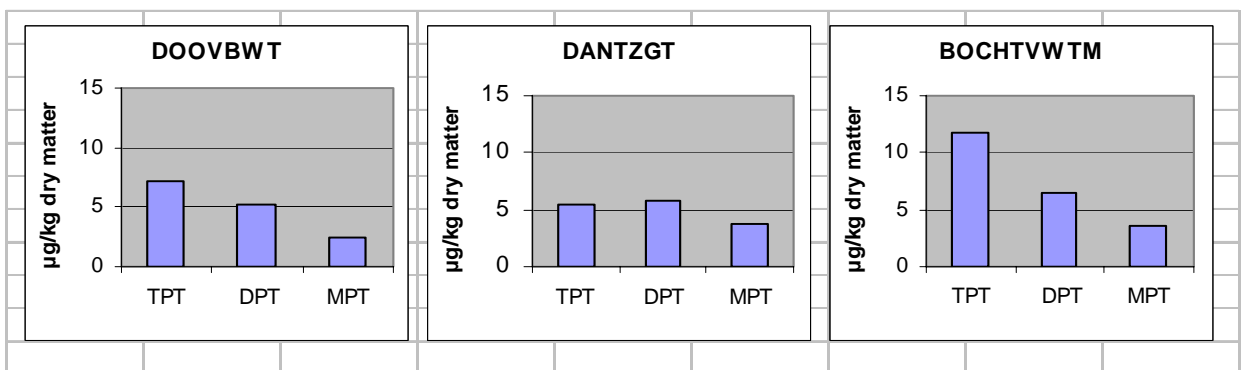


Fig 11: Average concentration TPT and metabolites on 3 locations from the year 1998 till 2003 (STD 10% organic matter). Locations on a map in appendix D

However this data should be approached with a certain amount of caution because the number of samples is lower or the line up in time doesn't fit.

Sampling is performed under conditions in which the water was leaving the Wadden Sea (high-tide to ebb). All data is standardised (STD) for 10% organic matter and are measured in the dry matter of the suspended matter.

6.3 Overall conclusion

All efforts of several organisations for a strict policy seem to have its effect on the concentration in the Wadden Sea. For as well as TBT as TPT the concentrations have been decreasing the last couple of years. Never the less, the concentrations in the Wadden Sea are far above the Max. Acceptable Risc (TBT: 0.7 µg/kg, TPT: 1 µg/kg) (Hofstede & Van de Ven, 2001), with the average concentrations of TBT and TPT being up to 56 and 12 times higher, respectively. When comparing the results of the present study with thos of study published in 2003, the concentrations are relatively low (Bellert & Van de Ven, 2003). This could be attributed to the chosen sampled locations. The three representatives in this research are less influenced by the output of rivers and harbours. This means more diluted samples.

7. Organotin in seals

The accumulation and concentrations of organotin in biota differ all over the world (Linley-Adams, 1999). In 2002, the epidemic, that started in Denmark and slowly moved towards the Dutch Wadden Sea, caused death to a lot of seals. After research, a virus named Phocine distemper (pdv-virus) appeared to be the cause of death (CSTEE, 1999). Samples from dead seals were collected and stored. The unusual large amount of seal material made it possible to do a lot of research. Alterra (Alterra-Marine and Coastal Zone Research) offered 30 liver samples from seals found in the district of the Dutch Wadden Sea for chemical analyses. Also data such as location where seal was found, length, weight, and estimated age of each examined seal was available. In order to understand more of the organotin characteristics and the metabolism ability of the harbour seal the RIKZ Laboratory in Haren examined all 30 seal livers for TBT, TPT and metabolites.

In the following chapter the data will be visualized and explained. The reason why liver tissue was studied is because earlier studies concluded that butyltins mostly accumulates in the liver of marine mammals (Vos *et al.*, 2003).

7.1 TBT in analysed seals

The TBT concentrations in harbour seal liver varied between <1 and $38 \mu\text{g}/\text{kg}$ and had an average of $10 \mu\text{g}/\text{kg}$. Noted that most analysed samples were under the detection limit ($1 \mu\text{g}/\text{kg}$). All concentrations of TBT and metabolites can be found in Appendix C.

Figure 12 shows the proportion of the main component TBT and its metabolites DBT and MBT. With 3%, TBT is clearly the lowest compound present. The fact that there are mainly metabolites of TBT found in seal liver can be explained by a number of factors, such as lower concentrations in the environment, metabolism of fish and more. It is believed this is not the reason based on the concentrations in fish (Chapter 5.2) and available TBT concentration in suspended material (Chapter 6). The concentration didn't obviously decline the last couple of years and therefore it is not conceivable that this is the cause.

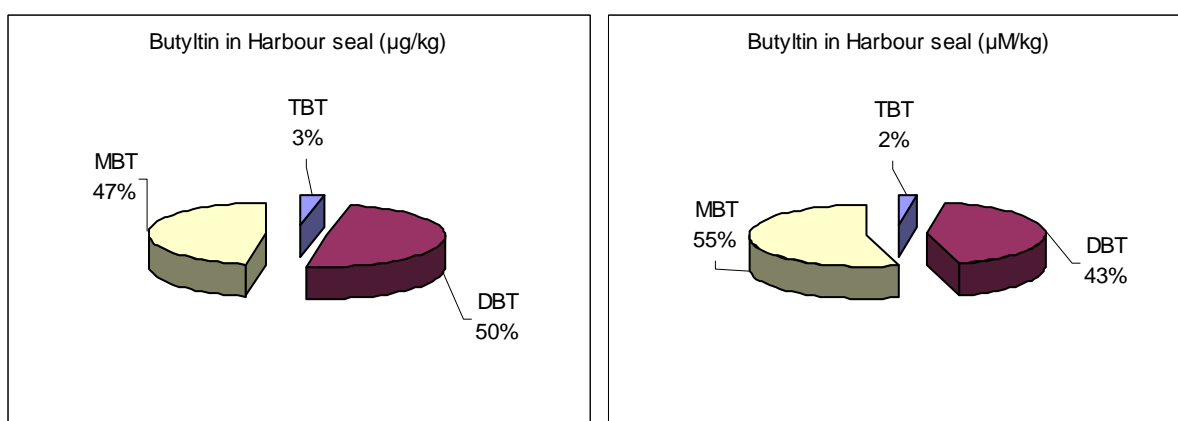


Fig 12: The percentages are the average of 30 samples for each substance measured in seal livers. Average concentrations for TBT, DBT and MBT in liver tissue respectively are 4, 53 and 51 $\mu\text{g}/\text{kg}$ dry weight. Average concentrations for TBT, DBT and MBT in liver tissue respectively are 0.01, 0.23 and 0.29 $\mu\text{M}/\text{kg}$ dry weight.

Fish as a metabolizer is the second option. Fish may be able to transform most of the TBT and form metabolites. Seals eat this fish and consequently will receive more metabolites than TBT. Again this can be rejected because previous studies tells us that organisms that are high in the food chain are better able to transform TBT to less toxic metabolites (Evers *et al.*, 1995).

A study by RIVO proves as well that fish in the North Sea (feeding area of seal) contains more TBT and TPT compared to metabolites (Leonards, 2002). Seals are capable of biotransformation of TBT. It suggests that the harbour seal, in the Dutch Wadden Sea, is able to degrade TBT with more ease as DBT and MBT. The study conducted in the spotted dolphin, from the coast of Taiwan, had similar result. In this study MBT was also the significant dominant substance (Liu *et al.*, 2003). Because of Molecular weight differences the percentages can differ between TBT and metabolites.

7.1.1 Weight related to TBT and metabolites

Previous studies show a relation between the weight of bottlenose dolphin (*Tursiops truncatus*) and accumulated concentration of butyltins (Liu *et al.*, 2003; Hattum *et al.*, 2002). This pattern has been attributed to the balance between the uptake and excretion processes of butyltins (Liu *et al.*, 2003). This relation is also examined for seals. Regression lines shows a trend for the relation between bodyweight and concentration (Fig. 13).

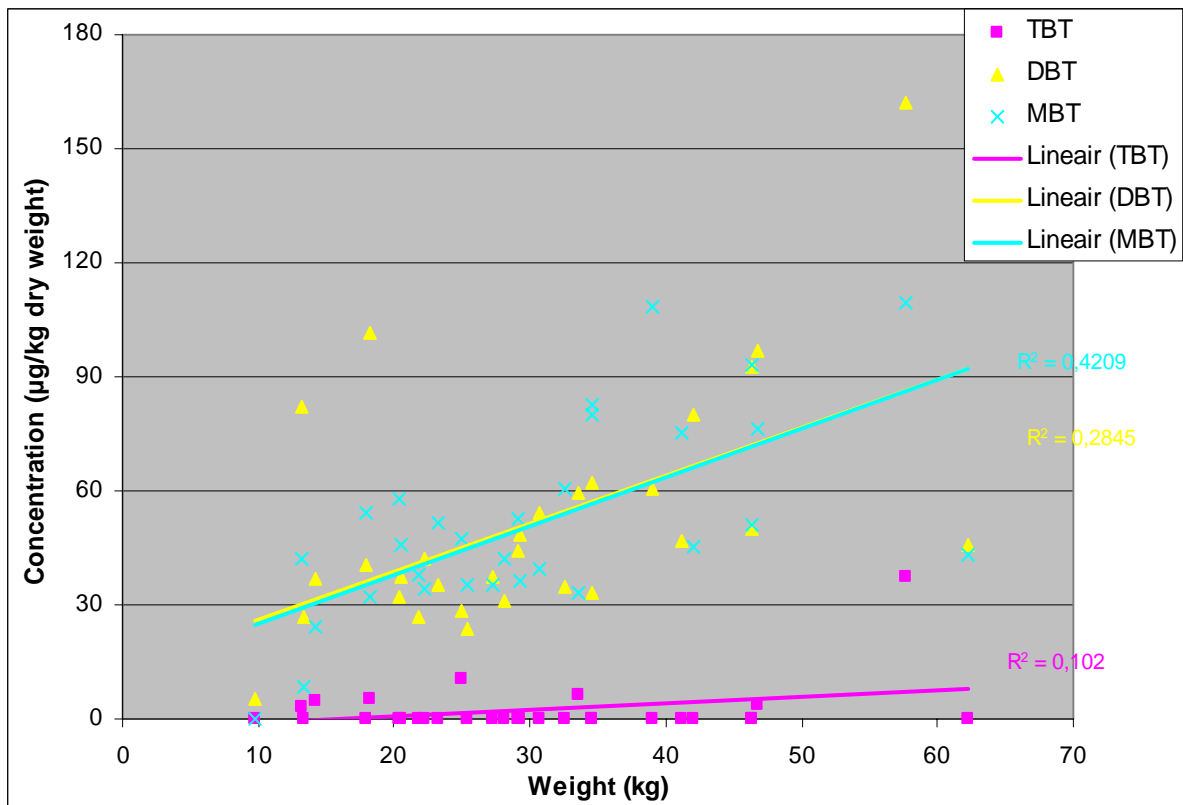


Fig 13: Butyltins measured in seal livers compared to seals weight. Linear lines show a trend. When highest concentration in TBT series in not taken into account a R² is given of 0.0239

In the figure 13 a graph is plotted for weight and concentration butyltins per kg body weight. The result is a line that shows the increase of butyltins. Remarkable is that accumulation is shown for the metabolites but not for TBT during growth of a harbour seal. The bioaccumulation of TBT seems to be

minimal. Ratio differences show that the results are well founded. The accumulation of TBT is significantly different (lower) as compared to its metabolites DBT and MBT. The difference may be explained by the fact that seals have less ability to biotransform the smaller butyl groups (DBT & MBT). Again this shows that less TBT is accumulated, possibly due to biotransformation, than its metabolites.

7.1.2 Age related

Comparable with the previous plotted graph there is detected a significant (>5%) relation between age and BT concentrations. Unfortunately no exact age, from teeth analyses, were available. The age is estimated and categorized in three levels: young (< 1 year), semi-adult (1 – 3 years) and adult (> 3 years).

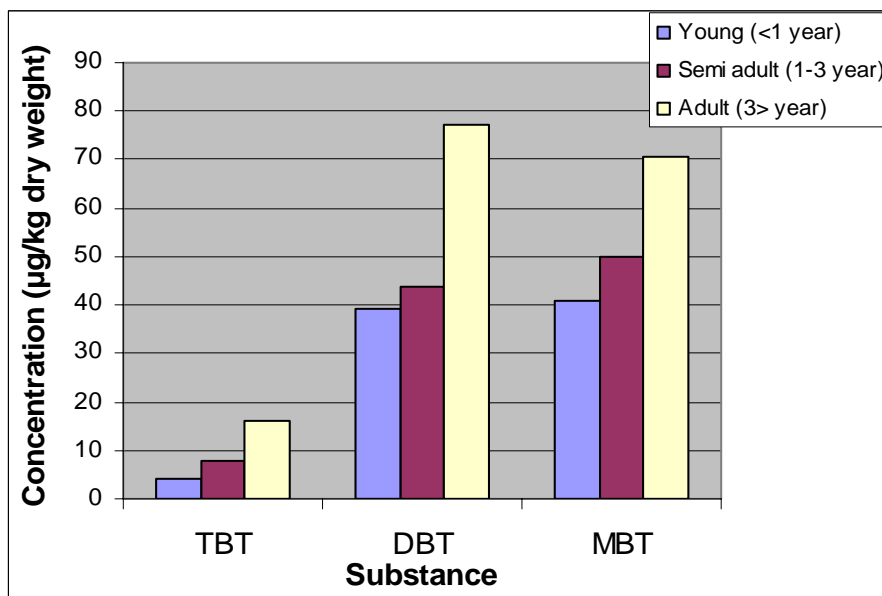


Fig 14: Butyltin measured in seal livers in age groups

The category 'Young' exhibits for all substances the lowest level of organotins. Hattum *et al.* (2002) studied concentrations in a foetus and concluded that there was no indication for transplacental transfer of organotins. Organotin tends to be less dependent of lipid affinity for the distribution when compared to PCB's and DDT (Sekizawa *et al.*, 2002). This can be the reason why butyltins are transferred less to the pup by the mother's milk. Though, some organotins are transferred. The metabolistic systems are supposed to be underdeveloped in a seal pup. The only way of intake for a seal pup is the mother's milk. This takes place only during the first 6 weeks after birth. The increase of concentration can be explained by growth in body length and weight (Liu *et al.*, 2003; Hattum *et al.*, 2002).

7.2 TPT in analysed seals

In case of phenyltins the results of analyses show a dominant presence of TPT (66%). Although chemical quantities and effects of TBT and TPT are similar, this result was expected. Earlier results of studies suggest that TPT is more persistent and less degradable by organisms (Sekizawa *et al.*, 2002). A study of RIVO found TPT as dominant compound in both flat and pelagic fish and crustaceans (Leonards, 2002). Examined eider ducks in the Dutch Wadden Sea don't showed similar results (Werkman *et al.*, 2001) (Chapter 5.3). The fact

that TPT concentrations in suspended particulate matter has been decreasing the last years does not explain the high concentration of TPT in seals.

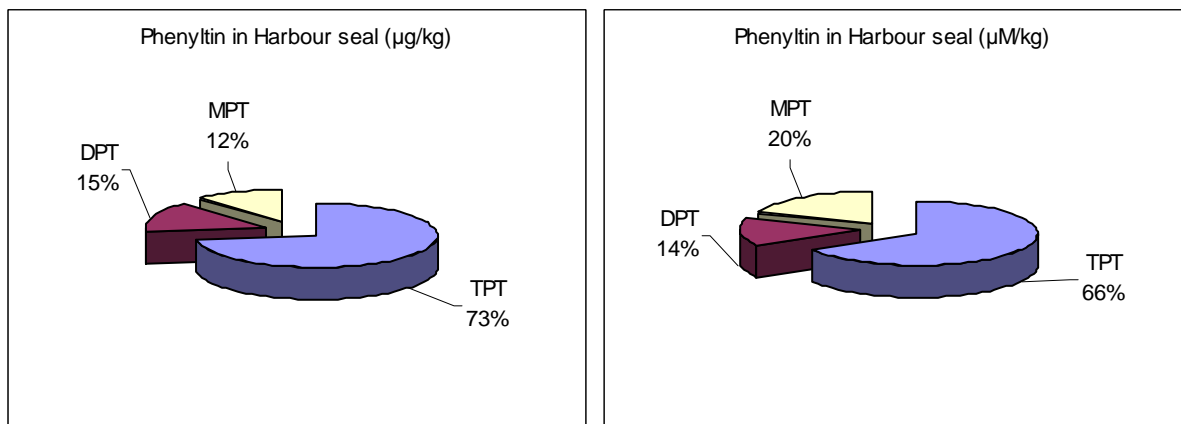


Fig 15: The percentages are the average of 30 samples for each substance measured in seal livers. Average concentrations for TPT, DPT and MPT in liver tissue respectively are 44, 9 and 7 µg/kg dry weight. Average concentrations for TPT, DPT and MPT in liver tissue respectively are 0.13, 0.03 and 0.04 µM/kg dry weight.

7.2.1 Weight related to TPT and metabolites

In contrast to TBT, figure 16 shows that TPT bioaccumulates strongest as compared to lower compounds. The accumulation of TPT shows a relation between increasing bodyweight and concentration of the primary component TPT. Bioaccumulation seems to be present in larger extent in comparison with TBT. Metabolites of TPT seem to be less persistent and are transformed with relative ease. Difference in numbers of correlation (R^2) show that the plotted lines fit the data well.

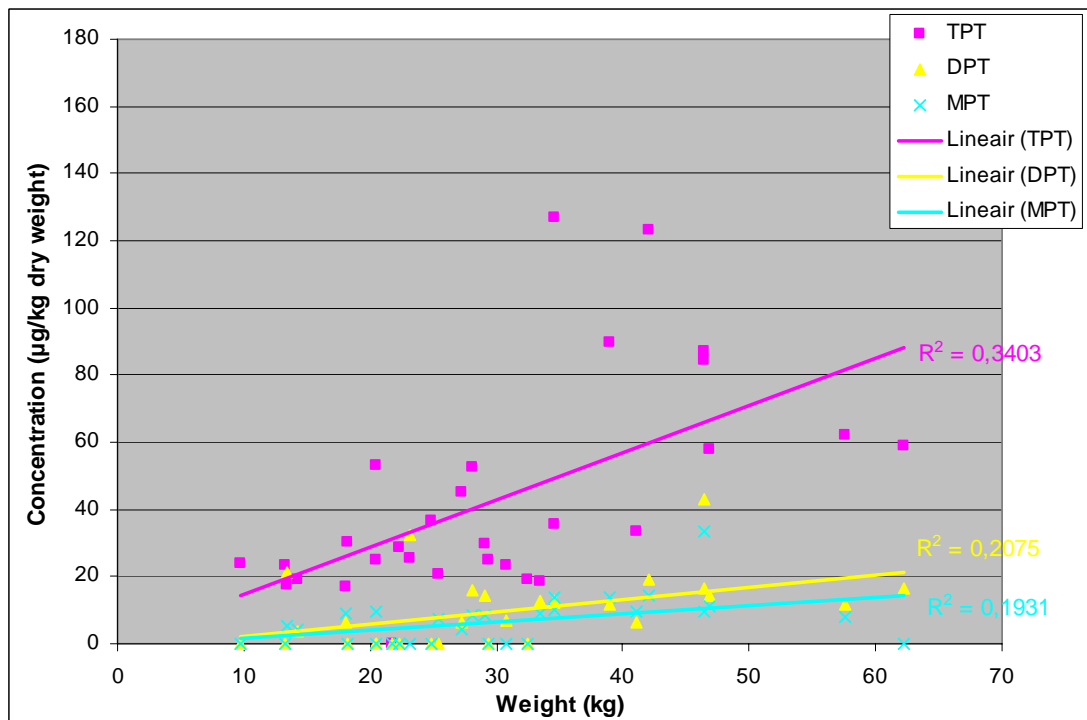


Fig: 16 Phenyltins measured in seal livers compared to seals weight. Linear lines show a trend

7.2.2 Age related

Again TPT is present in dominant amounts. Mothers milk transfer can cause high concentrations in the group of 'Young'. No data of exact age was available which made the transfer quantification only an estimate. Uncertainty can be explained by this.

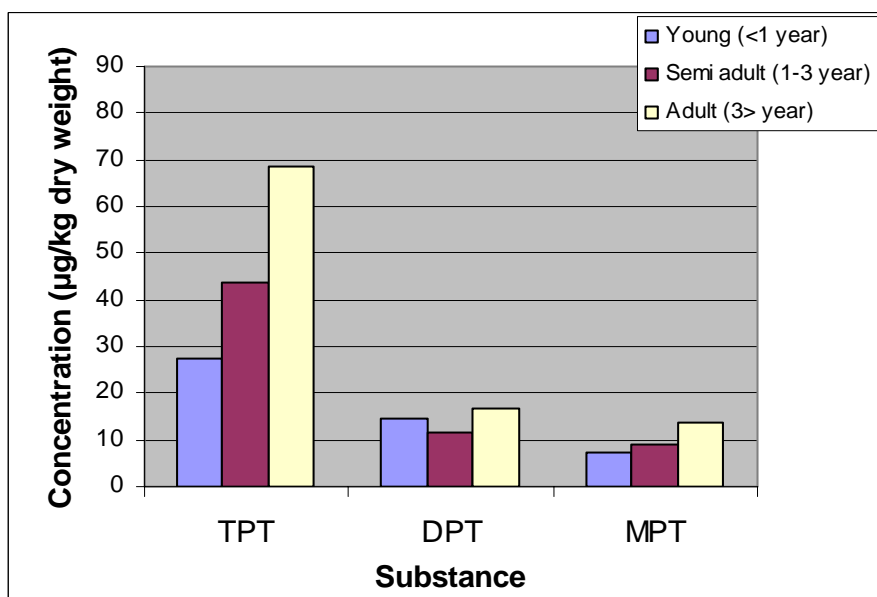


Fig 17: Phenyltin in age groups measured in seal livers

7.3 Gender related

Weaners are considered more vulnerable for chemical effects. Examples of transport of toxic substances from mother to pups by mother milk are PAHs and PCBs. When an organism is not able to transform a substance bioaccumulation can occur. Concentration differences between male and female can be distinguished. Mother seals feed their pups with milk during the first 6 weeks. This milk contains a lot of fat in which certain pollutants can be transported. There was no significant difference found between gender and concentration for TBT and TPT. This could be explained by the fact that organotin is less lipophilic and therefore less likely to be transported by fat in milk. Sekizawa *et al.* (2002) has suggested this appearance as well.

7.4 Compared data

With the available data more relations are tried to find. No significant results are detected when the following parameters are compared with the concentrations of organotin components in the analysed livers of harbour seals from the Dutch Wadden Sea:

- Date found;
- Location found.

8. TBT and TFT in the food chain of marine mammals

Because TBT and TPT are considered to be toxic (Evers *et al.*, 1995) and the toxic effects differ from its form (Linley-Adams, 1999). Although the majority of accumulation of chemicals like TBT and TPT will typically be found in those organisms that are at the top of the food chain, e.g. marine mammals (Hayteas and Duffield, 2000), it is important to understand how contaminants first enter and then accumulate through the food web. Aquatic organisms lower in the food chain are directly exposed to contaminants in water and sediment. The knowledge of exposure to organotins is largely based on analyses of waters and tissues of various organisms at local, regional and global scales. Organotin behaves as a substance that is able to accumulate in a food chain (Fig 18).

TBT and TPT are only slightly soluble in water and easily adsorbed to particulate matter in the aquatic environment (see chapter 4). Hence they are accumulated in sediment where they are relatively persistent and are taken up by the benthic organisms such as clams. Clams can be eaten by benthic fish, such as flat fish. These fish can be eaten by seals.

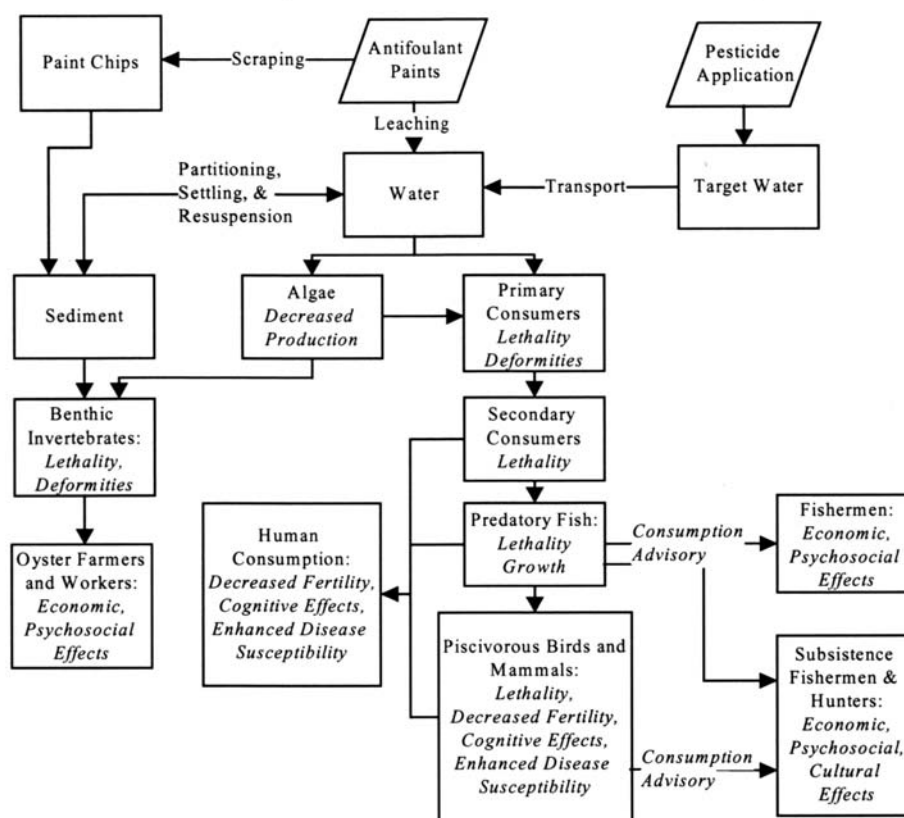


Fig 18: A conceptual model for exposure and effects of humans and marine mammals to tributyltin and triphenyltin compounds (Sekizawa *et al.*, 2002)

Another form of additions after exposure of TBT and TPT to an aquatic system takes place in suspended matter. Heavy metals and organic pollutants, like TBT and TPT, are absorbed by plankton at the base of the webs and biomagnificated to significant concentrations at higher thropic levels (Bard, 1999). Hargrave *et al.* (2000) indicate that planctonic primary producers take up chemicals directly from the water through bioconcentration. From primary consumers they pass on to secondary consumers. Fish eats these secondary consumers. TBT and TPT are accumulated in fish and other aquatic organisms with bioconcentration factors of 10^2 - 10^4 (Sekizawa *et al.*, 2002). Since fish is the food of the seal, this route represents the most important common way of TBT and TPT exposure for marine mammals (Linley-Adams, 1999). In figure 18 a conceptual model for exposure and effects of humans and marine mammals to TBT and TPT compounds is shown (Sekizawa *et al.*, 2002). The italic words indicate the possible effects of TBT and TPT in the parts of the food chain in question. Noted that organotin can be biotransformed by seals (See chapter 7).

9. Estimated daily intake of organotins for harbour seal

To predict possible negative effects of organotin for the seal in the Dutch Wadden Sea an estimation of the daily intake must be made. In this chapter an estimation will be made of the daily intake of organotins for seals in the Dutch Wadden Sea. A rough estimation which is based on the following 1) average seal 2) no consideration of consumption patterns 3) Organotin is only taken up by fish consumption 4) only adult animals 5) weight of an average adult seal is 35 kg.

9.1 Calculation of daily intake

For the estimation the harbour seal consumed 8.5 kilo of fish. A possible concentration difference in fish between seasons was not taken into account. Assumed was that all fish eaten by the seal had its original habitat in the North Sea. The harbour seal eats mainly benthic fish, often in combination with pelagic fish. In the following calculations the highest concentrations in fish were used; 10 µg/kg TBT in pelagic fish and 18 µg/kg TPT in benthic fish contributed to this calculation (Leonards, 2002).

Estimated daily intake of organotin for harbor seals in the Dutch Wadden Sea

Fish consumption is 8.5 kg per day

Weight adult seal is 35 kg

daily intake		daily intake	
TBT	$8.5 \cdot 10 = 85 \text{ µg/day}$	TPT	$8.5 \cdot 18 = 153 \text{ µg/day}$
DBT	$8.5 \cdot 2.5 = 22 \text{ µg/day}$	DPT	$8.5 \cdot 2 = 17 \text{ µg/day}$
MBT	$8.5 \cdot 1.0 = 8.5 \text{ µg/day}$	MPT	$8.5 \cdot 1 = 8.5 \text{ µg/day}$
total butyltin	116 µg/day	total phenyltin	179 µg/day
Total intake of organotin:		$116 + 179 = 295$	
		$295 / 35 = 8 \text{ µg/kg bw/day}$	

Table 6: Daily intake of organotin calculation for the Harbour seals in the Dutch Wadden Sea. Highest concentrations in fish are used.

Error is caused by: low average seal weight, metabolites are in calculation, all contaminants are added up and large fish consumption each day.

9.2 Daily intake compared to effect level for rats

The harbour seal from the Dutch Wadden Sea has a very developed transformation system. This system transforms toxic compounds to less toxic components. This means that the risk for effects may be decreased when an organism can transform chemical compounds. For TBT and TPT the degradation products are considered to be less toxic. In Table 5 is seen that the 'no observed adverse effect level' (NOAEL) for rats is as low as 0.025 mg/kg body weight per day for baby rats. The rodent immune system appears to be the most sensitive to TBT and its metabolites during the early stages of development (prenatal and postnatal) when compared with adults (Vos *et al.*, 2003). The reason why rats are chosen as animals with comparable effects have the following reasons:

- Rats are world wide used as test animals.
- Rats are mammals.
- Rats have lowest NOAEL in comparison with other organisms found in Literature.
- In a report of WWF effects of organotin in rats is not seen as different in respect to marine mammals (Lindsley-Adams, 1999).

In a book written by Vos *et al.* (2003) a study by DeLong & Rice (1997) is described in which is noted that 1.0 mg/kg oral intake (ppm) inhibited the Ah enzyme activity. This concentration is also higher than the daily intake of organotin for seals in the Dutch Wadden Sea. The studies suggest that the present uptake of TBT by seals may not be high enough to cause immuno suppression.

10. Important facts found in Literature

The most important findings of literature studies are listed in a following part.

- Organotins are substances that are able to bioaccumulate in a food chain. Mainly for animals that stand low in this food chain (e.g. primary producers) biotransformation to less toxic metabolites of organotins is considered hard.
- In general can be said that animals high in the food chain perform best in biotransformation of organotins.
- Organotin accumulates more in liver than other organs and fat (Vos *et al.*, 2003).
- The uptake of organotins for seals is mainly from the fish that is eaten (Linley-Adams, 1999).
- No indication of organotin transfer by placenta has been found (Hattum *et al.*, 2002).
- TBT and TPT are similar in characteristics and effects. The difference between TPT and TBT can be found in the persistency. The ease of degradation for as well as in sediment as in organisms seems to be less for TPT. This is funded by the results of the concentrations in the Harbour Seal.
- DBT and MBT are considered less toxic in comparison with TBT (Evers *et al.*, 1995).
- Butyltins concentrations appear to be higher when body length and weight is increasing (Liu *et al.*, 2003).
- TBT and TPT cause severe hormone disrupting effects in mice and rats (Zijlstra *et al.*, 2002).
- Imposex occurs in the dog whelk already at 1 ng TBT/l (CSTEE, 1999).
- In fish immunosuppressive effects can occur due to exposing to organotin (Fent & Hunn, 1999)
- Cetaceans are not as efficient in metabolizing and excreting TBT as Pinnipeds (Vos *et al.*, 2003).
- The quantity of butyltin accumulation depends on age, health, feeding habits, habitat and diet (St. Louis *et al.*, 2000).
- Sorption to sediment is present in a slightly higher amount for TBT as for TPT (Rudel *et al.*, 2003).
- TBT in water phase is absorbed to sediment for 90%.
- In comparison with other parts in the world the concentration in fish from the North Sea is not extremely high (OSPAR, 1999).
- Fish is able to excrete organotin by gills, faces and urine (OSPAR, 1999)
- B and T cells mediate the immunological reactions in fish and mammals (Grinwis *et al.*, 1998).
- Thymus volume increase can be noted as a possible reaction of immuno suppression (Vos *et al.*, 2003).

11. Discussion

During the development of this report the analyses in seal livers and suspended matter are worked out. It became obvious that the results on itself don't show a direct relation between organotin and the negative effects for seals. Never the less many conclusions can be drawn. To answer the subquestions, as described in the introduction (Chapter 1), information of different backgrounds is used. The answers of these subquestions form an answer of to the main question (as written below). To be able to answer the subquestions some discussion did arise. These explain uncertainties or assumptions that have been made in order to establish a conclusion.

Main question

Is a sufficient amount of information present to conclude a relation between organotins and immuno suppression for seals in the Dutch Wadden Sea?

Discussion points

- For the concentration of organotin in the Wadden Sea, an analysis of RIKZ in the particular suspended matter is used. In the food chain (Chapter 8) of the seal can be seen that the organotin enters the seal by the consumption of fish. Subsequently the data of organotin of fish from the North Sea is used for the calculation. Seals take their food mainly from the North Sea therefore the fish that is eaten in the Wadden Sea is neglected.
- All results of the analyses in harbours seal livers are used. No selection for excluding highest or lowest data was used. Possible peaks can influence the average.
- The process of biotransformation was considered to reduce toxicity by less toxic metabolites. The substances itself are considered toxic but also negative effects can occur in the transformation process. These effects are not expected and therefore not taken into account.
- Concentrations in liver tissue are not standardised for fat. Fat concentration can differ between animals and this can effect the concentration. The concentration is determined by the total weight of the seal liver material.
- Tributyltinoxide is not assumed to be more toxic than TBT or TPT. No research has been found in which Tributyltinoxide (TBTO) is considered more toxic. The effect concentration of TBTO is used because it is the lowest concentration found in which negative effects occur.
- No difference for excretion ability of seals of TBT and TPT is used. TBT and TPT are not assumed to be excreted with different speed.
- No increasing concentrations, which occur during body weight decrease, are taken into account. During body weight decrease a seal can have higher concentrations organotin. The weight is expected to be normal during measurements.
- For seals the NOAEL of rats and mice is used to estimate an effect of organotin (Linley-Adams, 1999). In the report by Linley-Adams the effects on mammals as rats was assumed not to be different for sea mammals like the harbour seal.
- To be able to make a more accurate calculation of organotin uptake into the food choice of the harbour seal a more thorough study needs to be undertaken. At the moment NIOZ is going to study this more

closely. For concentrations in fish a wider range should be examined for a better estimate of the food choice and location of feeding from the Harbour seal. This decreases the amount of uncertainties (See advisory).

- A NOAEL contains a large cover area to neglect possible uncertainties. This cover area shuts out possible uncertainties so that the value can be used in a safe way in other circumstances.
- In handling the data of liver analyses the concentration can better be rendered in nmol/kg dry weight instead of µg/kg dry weight because the molecule weight can differ between primary component and metabolites. In consultation with an expert the data was rendered in µg/kg dry weight because results are easier to compare with earlier research.

12. Conclusion

Conclusions that can be drawn from this research can be separated in two parts. In the first part conclusions will be enumerated which are related to the analysed liver samples. In the second part the conclusions of the suspended matter samples are handled. Because some fragmental questions have an overlap in explanation the conclusions are not answered separated but are divided in the analyses and literature study instead of separate questions.

Organotin is detected in all analysed livers from harbour seals in the Dutch Wadden Sea.

TPT is the most dominant substance of the phenyltins in the liver samples. Concentration in samples varied between <5 and 127 µg/kg dry liver weight and contained an average of 44 µg/kg dry liver weight (Chapter 7). For TBT this is the other way around, here the metabolites DBT and MBT are present in higher amounts as the primary substance TBT. TPT seems to be more persistent in liver tissue from harbour seals compared to TBT. TBT is hardly accumulating in the harbour seal because of their capability to biotransform TBT with relative ease. Bioaccumulation is more applicable for TPT than for TBT in the Harbour Seal. For TPT the relation between weight and concentration can be found.

Organotin is detected in all suspended matter samples of the Dutch Wadden Sea.

In suspended matter TBT and TPT are present in higher amounts compared to their metabolites (Chapter 6). For as well as TBT as TPT the concentrations are higher as the Maximum Acceptable Risk. TBT is 56 times too high and TPT is 12 times too high.

A calculation (Chapter 9) estimated an average daily intake of 8 µg/kg bodyweight for harbour seals in the Dutch Wadden Sea.

13. Overall conclusion

In this section the results of the report will be summarised and an answer will be given of the main question which provocation of this report. The main question will be repeated followed by the conclusion.

Main question

Is a sufficient amount of information present to conclude a relation between organotins and immuno suppression for seals in the Dutch Wadden Sea?

The diverse ecosystem of the Wadden Sea makes it possible for many animals to find a suitable habitat. These animals are from many different trophic levels and have different reactions to habitat changes, pollution and disturbance. During literature study it is found that negative effects of organotin for Whelks already occur at a billionth of gram (ng) per litre. The metabolistic system and the way of uptake are examples of parameters that must be taken into account for a doses-effect relation in fish and marine mammals. For the 3 examined locations in the Dutch Wadden Sea TPT and TBT are the dominant substances in suspended matter compared to its metabolites. The concentrations in suspended matter in the Dutch Wadden Sea seem to be high when compared to the threshold. The threshold is established for the whole ecosystem. When looking at the effects on one organism, in this case the Harbour seal, this value is not the best way for comparison. Levels of e.g. effects in gastropods are also taken into account for the threshold but are not related to the Harbour Seal.

No direct relation can be determined between particulate suspended matter and the intake of the harbour seal. Because the harbour seal is a mammal, uptake by gills or skin can be neglected. The uptake is restricted mainly by the food choice of the seal. The present concentrations of organotin in fish from the North Sea, the main food of the harbour seal, made it possible to estimate a daily intake of organotin for the harbour seal.

No research was found in which a direct relation between organotin concentration and immuno suppression was executed. In order to predict a possible effect for the seals, the daily intake is compared to doses-effect relations of other organisms. Experiments in rats and mice are best suitable to be used as comparable data because these animals are also mammals and are worldwide used as test organisms. A 'no observed adverse effect level' (NOAEL) for rats is determined at 25 µg/kg bw/day. This is 3 times higher than the estimated daily intake (8 µg/kg bw/day) of seals in this report. The levels of organotins that are present in harbour seal in the Dutch Wadden Sea in combination with the ability of biotransformation makes the possibility of effects small. TBT is thought to have less possibilities for negative effects on harbour seal, than TPT because the results of the liver analyses show that biotransformation of TBT is faster than TPT. Although the possibility of immunosuppressive effects in harbour seals is thought to be low, for the current concentration in fish from the Wadden Sea, clear evidence is not given. Due to the legislation, which prohibits the use of TBT and TPT in the future, no increase of compounds, and so effects, in the future is expected.

14. Advisory

Research in sea mammals is hard to execute because of the large amount of parameters. E.g. habitat can influence the results. A more reliable conclusion of effects can be obtained by more research in the food chain of the harbour seal. More information of concentrations in fish must be collected to have better statistical foundations. How many fish is eaten, from what fish specie and in what month are examples of parameters that can influence the daily intake. Example: The concentrations organotin in suspended matter in the Wadden Sea fluctuate between seasons. Is the uptake of organotin for fish in the peak months higher and is this directly transferred to seals? Is this during the period that the seal eats more to build up fat reserves? Does the concentration organotin in fish differ in North Sea and Wadden Sea?

15. Warranty of quality

A warranty of quality is established by a content and research check by experts. These experts have an environmental science and/or ecotoxicologist background.

- C.L.M. van de Ven, RIKZ-Haren.
- J.F. Bakker, RIKZ-Haren.
- M.J. Van den Heuvel-Greve, RIKZ-Den Haag.
- A.D. Vethaak, RIKZ-Den Haag.
- J. Åkerman, RIKZ-Haren.

Explanation of words

Bioconcentration:

The accumulation of chemicals in tissues of a fish or other organism to levels greater than the surrounding medium (<http://www.biomed.cas.cz/mbu/biotrans/biotrans.html>).

Bioconcentration factors:

Indicating the factors that the organisms are bioconcentrating chemical contaminants higher than ambient water concentration (Hall, 2002).

Biotransformation:

Conversion of a substance into other compounds by organisms; includes biodegradation (<http://www.biomed.cas.cz/mbu/biotrans/biotrans.html>).

B and T cells:

Two major populations of lymphocytes play an important role in all immune functions.

- B lymphocytes (humoral immunity) are white blood cells that secrete antibodies. B lymphocytes are formed by bone marrow stem cells and migrate into the circulation and lymphoid tissue. If bacteria or viruses invade the body, they give off a specific chemical, an antigen. This antigen binds to the B cells and stimulate the B cells to produce antibodies to the specific virus /bacteria or toxin (www.soton.ac.uk/~gk/scifi/blymphocytes.htm).
- T lymphocytes: (cell-mediated immunity)
Cytotoxic T cells help rid the body of cells that have been infected by viruses as well as cells that have been transformed by cancer. They are also responsible for the rejection of tissue and organ grafts (press2.nci.nih.gov/sciencebehind/immune/immune14.htm).

Endocrine:

Disruption of the hormone level in organisms.

Food chain:

A food chain is a linear arrangement of feeding relationships that trace energy flow. That is, a food web starts with an autotroph population (the producers), and then a heterotroph population (the primary consumers) consumes some of the autotrophs, and the sequence continues in a chain, with one or more additional heterotroph populations (secondary consumers, tertiary consumers, etc.) included.

Immunosuppression:

Lowering the body's normal immune response to invasion by foreign substances (<http://www.websters-online-dictionary.org/>).

Imposex:

Development of male sexual characteristics in females.

LOAEL:

Lowest Observed Adverse Effect level.

Metabolites:

Produced products during the biotransformation or degradation process. E.g.; TBT forms DBT.

NOAEL:

No Observed Adverse Effect Level.

Organotin:

An organic molecule on which a tin atom is attached.

Primary consumer

A primary consumer is an herbivore.

TBT: TriButylTin

DBT: DiButylTin

MBT: MonoButylTin

TPT: TriPhenylTin

DPT: DiPhenylTin

MPT: MonoPhenylTin

Secondary consumer

A secondary consumer is a carnivore that consumes herbivores.

Trophic level

A trophic level is a numerical level within a food chain or food web where an organism typically feeds or is consumed.

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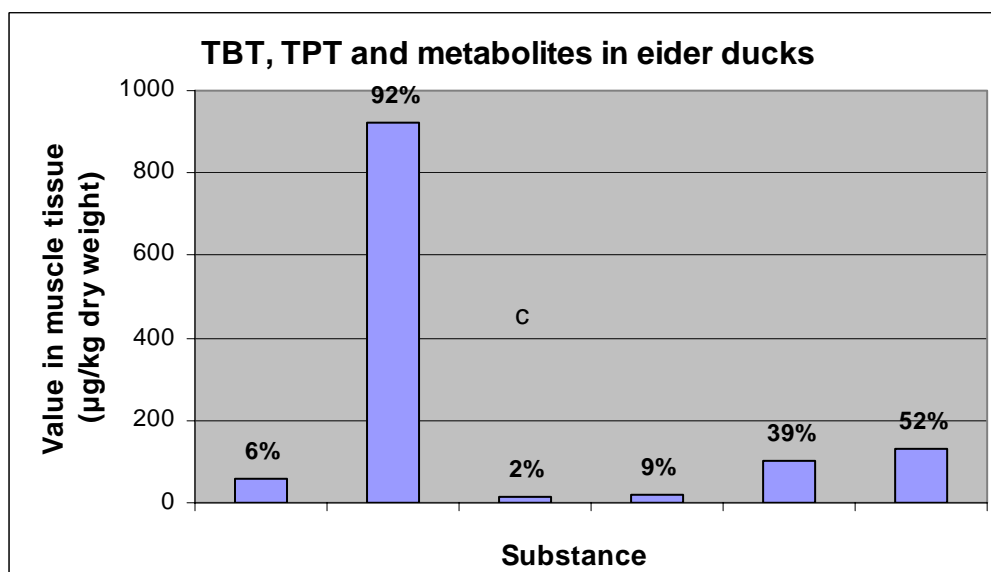
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Zeehondencrèche Pieterburen : <http://www.zeehondencreche.nl/> (Last visit 04-10-04)

Appendix A: Concentrations organotins in Eider ducks

Sample code	MBT	DBT	TBT	MPhT	DPhT	TPhT
SM-1	81	2090	17	22	132	159
SM-2	44	781	9	48	345	237
SM-3	31	443	5	<1	21	37
SM-4	115	2859	48	5	65	163
SM-5	101	985	17	17	79	87
SM-6	71	547	<1	1	23	71
SM-7	60	904	10	12	28	67
SM-8	24	160	<1	56	159	318
SM-9	42	891	13	13	89	66
SM-10	26	475	11	19	147	228
SM-11	39	409	9	36	98	91
SM-12	42	544	14	11	26	76
Average	56	924	15	22	101	133

MBT: monobutyltin, DBT: dibutyltin, TBT: tributyltin, MPhT: monophenyltin, DPhT: diphenyltin, TPhT: triphenyltin



Expressed in µg butyl/phenyltin-ion / kg dry weight. Percentage is taken of total butyltins or phenyltins.

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Appendix B: Extra information about analysed seals

ID	loc	nr	AD	categ. A many samples, B&C some samples taken	Date	location	ageE	sex	length standard (LS)	length total (TL)	length reduced (RL)	girth (AG)	weight
1	NH	3		B	21-jul-02	Landsend Sloephelling	j	m	93	109	63	64	20,4
2	NH	9		A	8-aug-02	Huisduinen	j	f	102	116	70	69	23,2
3	NH	11		B	22-aug-02	Den Helder	j	m	118	131	75	80	28,1
4	NH	14		B	22-aug-02	Hippolytushoef	j	f	116	127	79	72	29,3
5	NH	27		C	1-sep-02	Den Helder dijk	A	m	144	164	94	89	46,4
6	TX	12		A	18-jul-02	Paal 26	j	f	100	113	70	70	22,3
7	TX	14	B	A	19-jul-02	Paal 15	a	m	132	149	80	89	57,6
8	TX	17		C	21-jul-02	Schorren	j	m	73	86	49	50	9,7
9	TX	22		A	26-jul-02	Paal 18	a	m	150	175	95	100	62,2
10	TX	36		A	4-aug-02	Paal 26	s	f	97	110	61	62	18,2
11	TX	44		A	7-aug-02	Vliestroom	s	f	110	123	69	76	24,9
12	TX	46		A	7-aug-02	Noorderhaaks	s	m	102	117	67	67	21,8
13	TX	47		B	7-aug-02	Noorderhaaks	s	m	122	138	80	80	29,1
14	TX	48		A	8-aug-02	Paal 31	s	f	108	122	70	74	25,4
15	TX	57		B	10-aug-02	Paal 16	s	m	100	112	83	74	27,3
16	TX	59		B	11-aug-02	in zee? Phoca	s	f	99	111	65	66	20,5
17	TX	71		A	15-aug-02	Vuurtoren Paal 30	j	m	120	140	77	79	32,5
18	TX	86		B	22-aug-02	Paal 24	a	m	138	155	88	80	46,8
19	TX	114	A	C	28-aug-02	Paal 33	j	m	85	99	48	55	13,4
20	TX	118		C	29-aug-02	Paal 18	s	f	124	139	84	79	34,6
21	TX	124		B	31-aug-02	Paal 26	j	f	85	97	56	52	13,2
22	TX	143		B	3-sep-02	Monument	a	m	139	153	96	94	46,4
23	TX	147		B	4-sep-02	Schorren Zuid	j	f	130	145	81	86	30,7
24	TX	152	B	B	4-sep-02	Noorderhaaks	S	f	121	132	82	80	34,6
25	TX	153		B	4-sep-02	Noorderhaaks	a	f	133	150	86	88	41,1
26	TX	187		A	13-sep-02	Hors	a	f	134	150	86	69	39
27	TX	190		C	15-sep-02	Cocksdoorp	a	f	127	135	98	75	33,5
28	TX	202		B	20-sep-02	Paal 26	a	m	128	145	88	72	42,1
29	TX	216		A	24-sep-02	Surfstrand	j	f	100	113	65	60	18
30	TX	220		A	24-sep-02	Paal 25 Slufter	j	f	86	98	55	59	14,2

Appendix C: Results of seal livers analyses

labnummer	sub	lokatie	code	TBT (ug/kg)	DBT (ug/kg)	MBT (ug/kg)	TPT (ug/kg)	DPT (ug/kg)	MPT (ug/kg)
10039399	WZ	LANDSEND SI	NH3	1	32	58	53	< 5	10
10039400	WZ	HUISDUINEN	NH09	1	35	51	25	32	< 5
10039401	WZ	DEN HELDER	NH11	1	31	42	53	16	9
10039402	WZ	HIPPO.HOEF	NH14	1	49	36	25	5	5
10039403	WZ	DEN HELDER	NH27	1	93	93	87	43	34
10039404	WZ	PAAL 26	TX12	1	42	34	29	5	5
10039405	WZ	PAAL 15	TX14B	38	162	110	62	12	8
10039406	WZ	SCHORREN	TX17	1	6	1	24	5	5
10039407	WZ	PAAL 18	TX22	1	46	43	59	16	5
10039408	WZ	PAAL 26	TX36	6	101	32	30	5	5
10039409	WZ	VLIESTROOM	TX44	10	29	47	36	3	3
10039410	WZ	NOORDERHAAN	TX46	1	27	38	< 5	5	5
10039411	WZ	NOORDERHAAN	TX47	1	44	52	30	15	8
10039412	WZ	PAAL 31	TX48	1	24	35	21	3	7
10039413	WZ	PAAL 16	TX57	1	38	35	45	7	5
10039414	WZ	ONBEKEND	TX59	1	37	46	25	3	3
10039415	WZ	VUURTOREN P	TX71	1	35	61	19	3	3
10039416	WZ	PAAL 24	TX86	4	97	76	58	15	11
10039417	WZ	PAAL 33	TX114A	1	27	8	17	21	5
10039418	WZ	PAAL 18	TX118	1	62	82	127	13	14
10039419	WZ	PAAL 26	TX124	3	82	42	23	3	3
10039420	WZ	MONUMENT	TX143	1	50	51	84	17	10
10039421	WZ	SCHORRENZUID	TX147	1	54	39	23	7	3
10039422	WZ	NOORDERHAAN	TX152B	1	33	80	35	12	10
10039423	WZ	NOORDERHAAN	TX153	1	47	75	34	6	10
10039424	WZ	HORS	TX187	1	61	108	90	12	14
10039425	WZ	COCKSDORP	TX190	6	59	33	19	13	9
10039426	WZ	PAAL 26	TX202	1	80	45	123	19	14
10039427	WZ	SURFSTRAND	TX216	1	41	54	17	6	9
10039428	WZ	PAAL25 SLUFT	TX220	5	37	24	19	4	4
				TBT	DBT	MBT	TPT	DPT	MPT
Average (µg/kg)				4	53	51	44	9	7
Average (µM/kg)				0,01	0,23	0,29	0,13	0,03	0,04

Note: When concentration is coloured the detection limit is reached. E.g. concentration of 1 is in real time <1.

For a concentration in µM/kg the concentration in µg/kg should be divided by the molecular weight. The table below shows the molecular weight of each molecule.

Molecule	Moleculair weight	Molecule	Moleculair weight
TBT	291	TPT	350
DBT	234	DPT	273
MBT	177	MPT	196

