

## **Coupling marine monitoring and risk assessment by integrating exposure, bioaccumulation and effect studies**

A case study using the contamination of organotin compounds in the Danish marine environment

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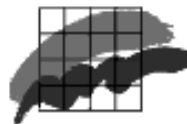
Ph.D. thesis

December 2003

**Coupling marine monitoring and risk assessment by  
integrating exposure, bioaccumulation and effect studies**

A case study using the contamination of organotin compounds  
in the Danish marine environment

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## Abstract

This Ph.D. thesis focuses on the highly toxic organotin compounds, mainly tri-n-butyltin (TBT) but also triphenyltin (TPhT), which have been widely used as antifouling agents in ship paints, and covers several aspects investigated by field studies of spatial distributions, bioaccumulation and ecotoxicological effects in Danish and Greenlandic waters. The amount of field data (from own, national and regional studies and surveys) presented in this thesis has provided an opportunity to integrate actual measured concentrations of contaminants with biological effect studies in a case study that couples marine monitoring and risk assessment for the organotin compounds. Thereby the thesis may also be seen as model for integrated risk assessment of other hazardous substances.

The studies presented in this thesis include analyses of organotin in sediments and marine organisms at various trophic levels to assess the distribution and contamination levels in the Danish and Greenlandic marine environment. The various studies show that in general high levels of organotin contamination occur in the Danish waters. Organotin, especially butyltin compounds, can be detected in all regions in a concentration range, which covers five to six orders of magnitude, from contaminated harbour areas and into coastal waters and the sublittoral parts of the open waters. However, the choice of suitable matrices and bioindicators is important for such a wide-range assessment, because of a high variation in accumulation potential of organotin between different species even at the same trophic level of the food web. In Danish coastal waters TBT and breakdown products were found in all trophic levels of the marine food web, from seaweed and invertebrates to fish, birds and marine mammals. The highest butyltin levels, which can reach several  $\mu\text{g/g}$  ww, are found to accumulate in liver of harbour porpoise, although comparable levels can also be found in molluscs and fish sampled inside contaminated harbour areas.

These studies are supported by biomarker studies of endocrine disruptions in prosobranch gastropods, e.g. imposex and intersex, which specifically can be related to exposure to TBT or TPhT. In addition, a discussion of the risks posed by organotin to organisms at higher trophic levels is included. For instance, marine mammals such as the harbour porpoise may be at risk due to a relatively high intake and accumulation of these substances. But because of the nature of field studies where the influence of confounding factors is difficult to assess, only indications of impairment are provided. The use of biological effect parameters, which can not specifically be linked to exposure to a single type of contaminants, or cause, is also discussed in relation to a study, which examined the impact on larval development in a viviparous fish, the eelpout, in Danish coastal waters. In addition, some other studies of contaminant-induced biological effects, which not specifically can be related to organotin exposure, will also slightly be touched on.

Finally an approach to derive a five-class scheme of assessment criteria of TBT is developed. The assessment criteria have been derived to reflect in the objectives within the OSPAR and EU strategies for priority substances for protection of the marine ecosystems in transitional and open waters. In the development of the five status classes it has been possible to combine the TBT concentration in three matrices, e.g. in seawater, sediment and the bivalve *M. edulis*, with the TBT-specific biomarker responses, e.g. imposex and intersex in five species of prosobranch gastropods. The main advantages with this combined scheme of assessment criteria are that monitoring of TBT levels and TBT-specific biomarker responses can supplement each other in a comprehensive evaluation of the environmental quality in the marine environment over a large scale, both with respect to location and level of TBT contamination. Various kinds of available monitoring and other field data of TBT concentrations and effects can thereby be integrated in the assessment of the TBT contamination in the Danish and neighbouring waters.

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## Sammenfatning

Denne Ph.D. afhandling beskæftiger sig med forskellige miljökemiske og økotoxikologiske aspekter i det marine miljø hovedsageligt med fokus på forureningen af de meget toksiske organotin-forbindelser, især tributyltin (TBT) og triphenyltin (TPhT), der bl.a. har været anvendt som antibegroningsmiddel i skibsmalinger. Afhandlingen omfatter bl.a. forskellige feltundersøgelser af den geografiske udbredelse, bioakkumulering og økotoxikologiske effekter af organotin-forbindelser i danske og grønlandske farvande. Omfanget af de præsenterede data fra egne, nationale og regionale undersøgelser har givet en mulighed for at integrere målte koncentrationer med undersøgelser af biologiske effekter på en måde hvorved marin overvågning og risikovurdering direkte kan kobles. Dermed kan denne afhandling til en vis grad også blive betragtet som modelstudium for en integreret risikovurdering af andre miljøfarlige stoffer end organotin-forbindelser.

De præsenterede undersøgelser inkluderer analyser af koncentrationer af TBT og TPhT og de derfra kommende dealkylerede/dearylerede nedbrydningsprodukter i sedimenter og marine organismer fra forskellige trofiske niveauer for at vurdere udbredelse, fordeling og koncentrationsniveauer i det marine miljø i Danmark og Grønland. De forskellige undersøgelser viser at der generelt forekommer relativt høje koncentrationsniveauer af organotin-forbindelser i det danske havmiljø, hvilket primært må tilskrives den intense skibstrafik i vores farvande. Især butyltin-forbindelser kan findes i alle dele af det marine område i et koncentrationsinterval der dækker fem til seks størrelsesordener fra de stærkt forurenede havne, til de kystnære farvande og ud i de sublittorale dele af de åbne farvande. Valget af passende matricer og bioindikatorer har dog væsentlig betydning for sådan en stor-skala vurdering af forureningsniveauet. Dels på grund af at i havvand og sedimentprøver vil koncentrationen af organotin-forbindelser i de åbne farvande typisk være under den analytiske detektionsgrænse, dels på grund af store variationer i akkumuleringspotentiale af organotin mellem forskellige arter, selv når de forekommer på samme trofiske niveau i den marine fødekæde.

I de kystnære dele af de danske farvande kan TBT og de dertil hørende nedbrydningsprodukter detekteres i organismer på alle trofiske niveauer af fødekæden fra makroalger og invertebrater til fisk, fugle og pattedyr, hvor de højeste niveauer af butyltin, der kan overstige flere  $\mu\text{g/g}$  vådvægt, forekommer i leveren fra den lille tandhval marsvin. Tilsvarende høje niveauer forekommer dog også i mollusker og fisk indsamlet inde i forurenede havneområder.

Disse analytisk-kemiske undersøgelser er suppleret med biomarkør undersøgelser af hormonforstyrrelser i forskellige havsnegle, dvs. imposex og intersex, der specifikt kan relateres til påvirkning af TBT eller TPhT. Derudover tilføjes en diskussion om hvorvidt organotin-forbindelser også udgør en risiko for organismer på højere trofiske niveauer, fx. på grund af immunosuppression. Fx. havpattedyr som marsvin kan være udsat for en vis risiko pga. et

relativt højt indtag og akkumulering af disse forbindelser. Men i feltundersøgelser er indflydelsen af eventuelle influerende faktorer vanskelig at vurdere, hvorved kun indikationer på toksiske effekter af en enkelt faktor kan fremhæves. Brugen af biologiske effektmarkører, der ikke specifikt kan sammenkædes med påvirkning af en enkelt type/gruppe af miljøfarlige stoffer, bliver også diskuteret i relation med en undersøgelse om udvikling af yngel fra den levendefødende fisk, ålekvabben, i forskellige kystnære områder i Danmark. Yderligere vil andre undersøgelser af biologiske effekter, der ligeledes ikke specifikt kan relateres til påvirkningen forårsaget af organotin-forbindelser, blive lettere berørt.

Afslutningsvis er en metode til at definere fem kvalitetsklasser til vurdering af TBT-niveauet i havmiljøet udviklet. Kvalitetsklasserne er udviklet til at afspejle målsætningerne indenfor OSPARs og EUs strategi for prioriterede stoffer mht. beskyttelse af de marine økosystemer. I udviklingen af de fem kvalitetsklasser har det været muligt at kombinere koncentrationsniveauer af TBT i tre matricer, dvs. havvand, sediment og blåmusling, med de TBT-specifikke biomarkører, dvs. imposex og intersex i fem arter af havsnegle. Den primære fordel ved dette kombinerede skema for kvalitetsklasser for TBT er at overvågning af TBT-niveauer og TBT-specifikke effekter kan supplere hinanden i en storskala evaluering af miljøkvaliteten i havmiljøet både mht. geografisk udbredelse og koncentrationsniveauer af TBT. Derved kan en række forskellige typer af data for koncentrationer og effekter af TBT blive integreret i en miljøvurdering af TBT-belastningen i de danske samt i de tilstødende farvande.



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## 1. Introduction

Hazardous substances, such as heavy metals and synthetic organic contaminants, have through the last century been released in large quantities from industrial, agricultural and urban activities and thereby discharged into the marine environment. The great advances made in the field of analytical chemistry have in recent decades provided evidence for a widespread occurrence of many of these contaminants in the environment. Elevated contaminant levels can particularly be found in coastal waters of the industrialised parts of the world, although many persistent contaminants are also transported over long distances, for instance to pristine areas in the Arctic environment. The evidences for a widespread contamination of the environment on earth have been followed by an increasing awareness of the potential long-term toxicological impact of many contaminants on wildlife, ecosystems and humans. Especially the impact of the high levels of organochlorines in the 1960's and 1970's resulted in, among other things, "silent springs" at the lakes of North America and in major population declines of seals and predatory birds in the Baltic region etc. (Colburn et al., 1996). Although legislative actions have reduced the levels of some of the contaminants in the environment, the present levels of many contaminants are still of concern today.

This Ph.D. thesis focuses on the highly toxic organotin compounds, mainly tri-n-butyltin (TBT) but also triphenyltin (TPhT), which have been widely used as antifouling agents in ship paints, and covers several aspects investigated by field studies of spatial distributions, bioaccumulation and ecotoxicological effects in Danish and Greenlandic waters. The amount of field data presented in this thesis has provided an opportunity to integrate actual measured concentrations of contaminants with biological effect studies in a case study that combines marine monitoring and risk assessment for the organotin compounds. Thereby it can also be seen as model for risk assessment of other hazardous substances.

The studies presented in this thesis include analyses of organotin in sediments and marine organisms at various trophic levels to assess the distribution and contamination levels in the Danish and Greenlandic marine environment. These studies are supported by biomarker studies of endocrine disruptions in prosobranch gastropods, e.g. imposex and intersex, which specifically can be related to exposure to TBT or TPhT. In addition, a discussion of the risks posed by organotin to organisms at higher trophic levels is also included. For instance, marine mammals such as the harbour porpoise may be at risk due to a high exposure and accumulation of these substances. However, only some indications of evidence of impairment are provided, because of the nature of field studies where the influence of confounding factors is difficult to assess. The use of biological effect parameters, which can not specifically be linked to exposure to a single type of contaminants or cause will also be discussed in relation to a study, which

examined the impact on larval development in a viviparous fish, the eelpout, in Danish coastal waters.

All of the presented studies can generally be regarded as baseline studies for future assessments of the influence of the coming legislative actions on the TBT contamination in the Danish and Greenlandic waters. Although, the use of TBT and TPhT on ships and other constructions in the marine environment already is restricted in EU including Denmark, TBT and other organotin compounds will continue to be a significant problem for several years to come, because of a high association to particulate material and a high persistence in sediments. Sediments can thereby act as a continuous source of organotins to marine organisms. Additionally, the ban on TBT in ship paint used on larger vessels may not be that effective as ships registered in less restrictive flag nations may still act as a source of TBT to European waters at least until 2008.

The main aims of this thesis are;

1. to examine the spatial distribution of the organotin contamination in the Danish waters using suitable bioindicators with the main focus on subtidal areas.
2. to examine the accumulation of organotin at various trophic level of a marine food web to assess the trophic transfer and identify species, which may be most vulnerable due to a particularly high accumulation potential.
3. to examine the accumulation pattern of organotin in harbour porpoises from Danish waters and to compare with the accumulation of mercury.
4. to examine the organotin contamination in the Greenlandic marine environment including organisms from remote areas.
5. to examine the spatial distribution of impaired development in broods of the eelpout in Danish coastal waters in order to evaluate its potential as a biomarker of contaminant-induced effects.
6. to develop a five-class scheme for environmental assessment criteria combining exposure levels and biological effects of TBT that can be used to classify the contamination levels in Danish waters.

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Five manuscripts (two published, two in press and one in prep.) are included in this thesis:

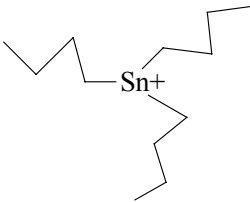
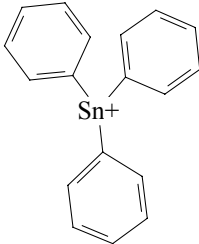
- I. Strand J., Jacobsen J.A., Pedersen B. & Granmo Å. (2003). Organotin compounds in sediment and molluscs from the shipping strait between Sweden and Denmark. *Environ. Poll.* 124: 7-15.
- II. Strand J. & Jacobsen J.A. (in press). Accumulation and trophic transfer of organotin in a marine food web from Danish coastal waters. Accepted by *Sci. Tot. Environ.*
- III. Strand J., Larsen M.M. & Lockyer C. (in press). Accumulation of organotin compounds and mercury in harbour porpoises (*Phocoena phocoena*) from the Danish waters and West Greenland. Accepted by *Sci. Tot. Environ.*
- IV. Strand J. & Asmund G. (2003). Tributyltin accumulation and effects in marine molluscs from West Greenland. *Environ. Poll.* 123: 31-37.
- V. Strand J., Andersen L., Dahllöf I. & Korsgaard B. (in press). Impaired larval development in broods of eelpout (*Zoarces viviparus*) in Danish coastal waters. Accepted by *Fish Physiol. Biochem.*

During this period I have also been involved in other published studies (Strand & Jacobsen, 2002; Beck et al., 2002; Nielsen & Strand, 2002) and in some yet unpublished studies on related subjects. In addition, I have been responsible for the surveys in open waters and as co-ordinator of the coastal programme of TBT-specific biological effects, e.g. imposex and intersex, in marine gastropods in the national monitoring programme in Denmark, NOVA 2003, which have been reported in the National Status Reports for the Marine Environment (Strand, 2001;2003). Many of the results from these studies are also included in the following chapters in the thesis.

### **1.1. Triorganotins - Physical properties, applications, effects and legislation**

Triorganotin compounds, mainly TBT but to some degree also TPhT, have since the early 1960's been widely used as highly effective antifouling agents in marine paints, e.g. for more than three decades. Other applications of (tri-, di- or mono-) butyl- and phenyl-substituted organotin compounds include fungicides, bactericides, preservatives of wood, textiles etc., industrial catalysts and additives in plastic materials including some household products. The release from antifouling paints on ships is estimated to be the predominant source of organotin compounds to the marine environment (Danish EPA, 1999).

Table 1.1. Some physical properties of the triorganotin cations. Data from Fent (1996).

Tri-n-butyltin (TBT)	Triphenyltin (TPhT)
	
M = 290.03 g/mol	M = 350.01 g/mol
pK <sub>a</sub> = 6.5	-
Solubility (aq) = <1 - >200 mg/l *	Solubility (aq) = <1 - 78 mg/l *
log K <sub>OW</sub> = 3 - 4 *	log K <sub>OW</sub> = 3 - 3.5 *

\* depending on what molecules, or ions, the organotin cations are associated with and their concentrations. pH is also an important factor because of the formation of neutral hydroxy complexes.

The negative impact of TBT in the marine environment was first recognised in the 1980's, when cause-effect relationships were established to reduced larva recruitment of and subsequently a near collapse of the oyster production in Archachon Bay in France (Alzieu, 1991), and to the masculinisation of female gastropods, which in some species led to sterility and the disappearance of local populations (Smith, 1981; Bryan et al., 1987). In the 1990's, a high bioaccumulation of organotin in coastal marine mammals was recognised and the potential risks for organisms at higher trophic levels also became of concern (Iwata et al., 1995; Kannan et al., 1998, Tanabe 1999). These studies have been followed by several related studies giving evidence to a widespread TBT contamination world-wide. Controlled laboratory studies have additionally provided evidence that TBT and TPhT have the potential to induce a broad spectrum of toxic effects (Table 1.2).

The increasing awareness of the unexpected effects of organotins on non-target organisms has resulted in enforcement of restrictions on the use of TBT as an antifouling agent on smaller vessels in many countries including Denmark (Table 1.3). Even after national restrictions of the use of TBT on small vessels were introduced in Denmark in 1991, TBT contamination of Danish waters has still been of concern, because of an intense shipping traffic with more than 60,000 larger vessels per year in transit between the North Sea and the Baltic Sea (ADF, 2001). The larger vessels will also be included in the prohibition process in the coming years as a consequence of the ratification of the antifouling-systems convention within the International Maritime Organisation (IMO). The application of TBT containing paints is prohibited from 1. January 2003 and the presence on ship hulls is completely banned from 2008 and at that time ships entering harbours can be controlled in the ratifying countries.

*Table 1.2. Some examples of ecotoxicological effects at the biomolecular, cellular, individual, population and community level, which can be induced by TBT and/or TPhT. Exposure levels and times vary in the studies, but the effects have not been induced at unrealistically high exposure levels.*

Disruption of intracellular energy production by inhibition of ATPase and ion-pump activities (Fent, 1996).
Disruption in detoxification processes of xenobiotics such as inhibition of cyt. P450 activity and thereby the mixed function oxygenase, phase I (Kim et al., 1999; Shim et al., 2003).
Cytotoxicity and immunosuppression such as inhibition of phagocytes, thymocytes and natural killer cells and lysosomal destabilisation in blood and liver cells of invertebrates, fish and mammals. This can result in that the organisms become more susceptible to infectious diseases (Snoiej et al., 1987; Kannan et al., 1998; Cima et al 1998; Grinwis et al., 2000; Whalen et al., 2002).
Endocrine disruptions, for instance inhibition of aromatase activity has been observed in gastropods, bivalves, fish and mammals. Masculinisation of gastropods and fish has been observed. Severe development of imposex and intersex in gastropods can result in sterility and mortality (Mathiessen & Gibbs, 1998; Cooke, 2002; Shimaki et al., 2002).
Abnormal shell growth in bivalves (Alzieu, 1991; Page et al., 1996).
Teratogenic effects resulting in developmental malformations of embryos in bivalves, fish and mammals (Ema et al., 1995; Cima et al., 1996; Fent, 1996).
Reduced fecundity, recruitment and growth of invertebrates and fish (Stenalt et al., 1998; Marin et al., 2000; Davies et al., 1999; Fent, 1996).
Changes in population and community structures of bacteria, algae and invertebrates (Bryan et al., 1986; Alzieu, 1991; Petersen & Gustavson, 1998; Waldock et al., 1999; Dahllöf et al., 2001).

*Table 1.3. Some important years concerning the restrictions on the use of triorganotins.*

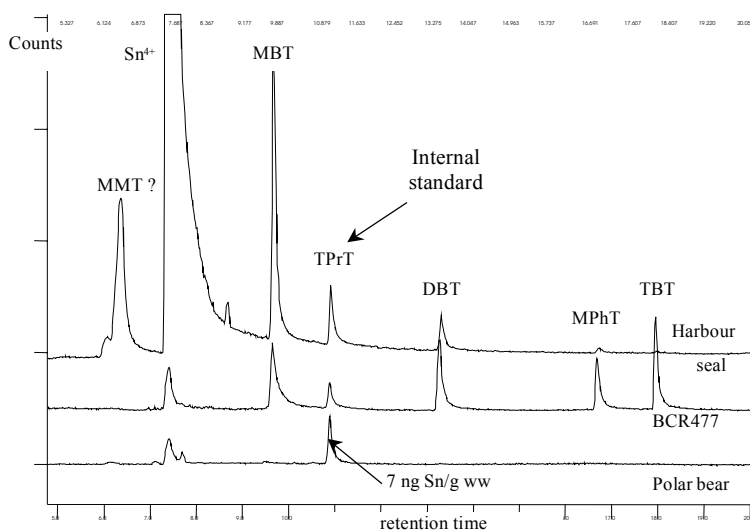
<b>Year</b>	<b>Restrictions</b>
1960's	TBT is introduced as an antifouling agent in the marine environment.
1982	France prohibits the use of TBT as antifouling agent on small vessels with a length <25m.
1987-1991	Many European countries, including Denmark, prohibit the use of TBT as antifouling agents on vessels <25m, stationary marine constructions and fishing gear. Restrictions are also introduced in several over-seas countries such as USA, Canada, Australia and Japan.
1992	The OSPAR declaration. A political commitment to make every endeavour to move towards the target of the cessation of discharges, emissions and losses of hazardous substances to the marine environment by the year 2020. This includes TBT and TPhT.
1992	Denmark stops the import of agricultural fungicides with TPhT as active agent.
1999	The European Union (EU) adapts the directive, which prohibits the use of TBT as antifouling agent on vessels <25m, stationary marine constructions and fishing gear. *
1999	Denmark prohibits the use of TBT as a wood preservative.
1999	The International Maritime Organisation (IMO) adopts deadlines for phase-out of TBT-containing paints.
2003	IMO agrees on the antifouling-systems convention (AFS convention), which bans the use of TBT and TPhT for antifouling on larger vessels. The convention enters into force once 25 countries have ratified it and at least 25% of world tonnage is represented. EU prohibits the application of TBT on ships at European shipyards.
2008	The use of TBT in ship antifouling systems will be completely prohibited on ships. EU and other ratifying countries can enforce restrictions on ships entering their ports if they are painted with TBT- and TPhT-containing paints, if not ban them entering at all.

\* There is no enforcement of restrictions on the use of triorganotins in Greenland, because Greenland is not an EU-member.

## 1.2. The analytical method of organotin

The implementation of a new analytical method to detect the different organotin species has been an important tool in this work. The studies are highly dependent on good quality and sensitive detection of the triorganotins and their primary breakdown products. This has been achieved by using a Gas Chromatograph with a Dual Channel Pulsed Flame Photometric Detector, GC-(DC)-PFPD. The emission of tin atoms is detected very specifically with only minor interference from sulphur and phosphorus containing substances. The method for detection was first used by Jens A. Jacobsen and described in Jacobsen et al. (1997, 2000). Afterwards the clean-up and extraction processes have been modified for analyses of tissue samples. A detailed method description is presented in Manuscript III.

The following organotin (OT) compounds can be detected by this method; tributyltin (TBT), dibutyltin (DBT), monobutyltin (MBT), triphenyltin (TPhT), diphenyltin (DPhT) and monophenyltin (MPhT) using tripropyltin (TPrT) as internal standard. Other, but not identified, tin-containing compounds appeared also in some of the chromatograms for environmental samples, probably reflecting the presence of oxidised metabolites or methylated species.



*Figure 1.1. GC-PFPD chromatograms of ethylated organotin species in tissue samples of polar bear, harbour seal and the certified reference material BCR 477 (mussel tissue). In polar bears a detection limit of butyltin at 0.1 ng Sn/g ww was achieved.*

Analyses of organotins in different kinds of biological matrices, e.g. different organisms and tissues, are included in the studies, but also analyses of sediments have been performed. The species analysed include seaweed, invertebrates, fish, birds and mammals including humans. Certified reference materials of biological tissue or sediments and blanks have been analysed in all studies for the purpose of quality control. In addition, spiking experiments have been performed as part of the Quasimeme development exercises. Quasimeme is a well-established international quality assurance programme for marine measurements (Quasimeme, 2003).

In addition to the organotin analyses, a simultaneous method for detection of other organometallic compounds has been under development during this study. *In situ* derivatisation with NaEt<sub>4</sub>B or NaPr<sub>4</sub>B will not only alkylate tin species in a sample, but also mercury and lead species such as methylmercury, phenylmercury, triethyllead etc. Identification and relatively good detection limits of various organometal species in water samples using GC-MS were achieved (Figure 1.2), but analyses of tissue samples need further development. In a study of harbour porpoises, total mercury (inorganic + organic mercury) was detected using an AAS after reduction of Hg<sup>(2+)</sup> to Hg<sup>(0)</sup> with NaBH<sub>4</sub> (Manuscript III).

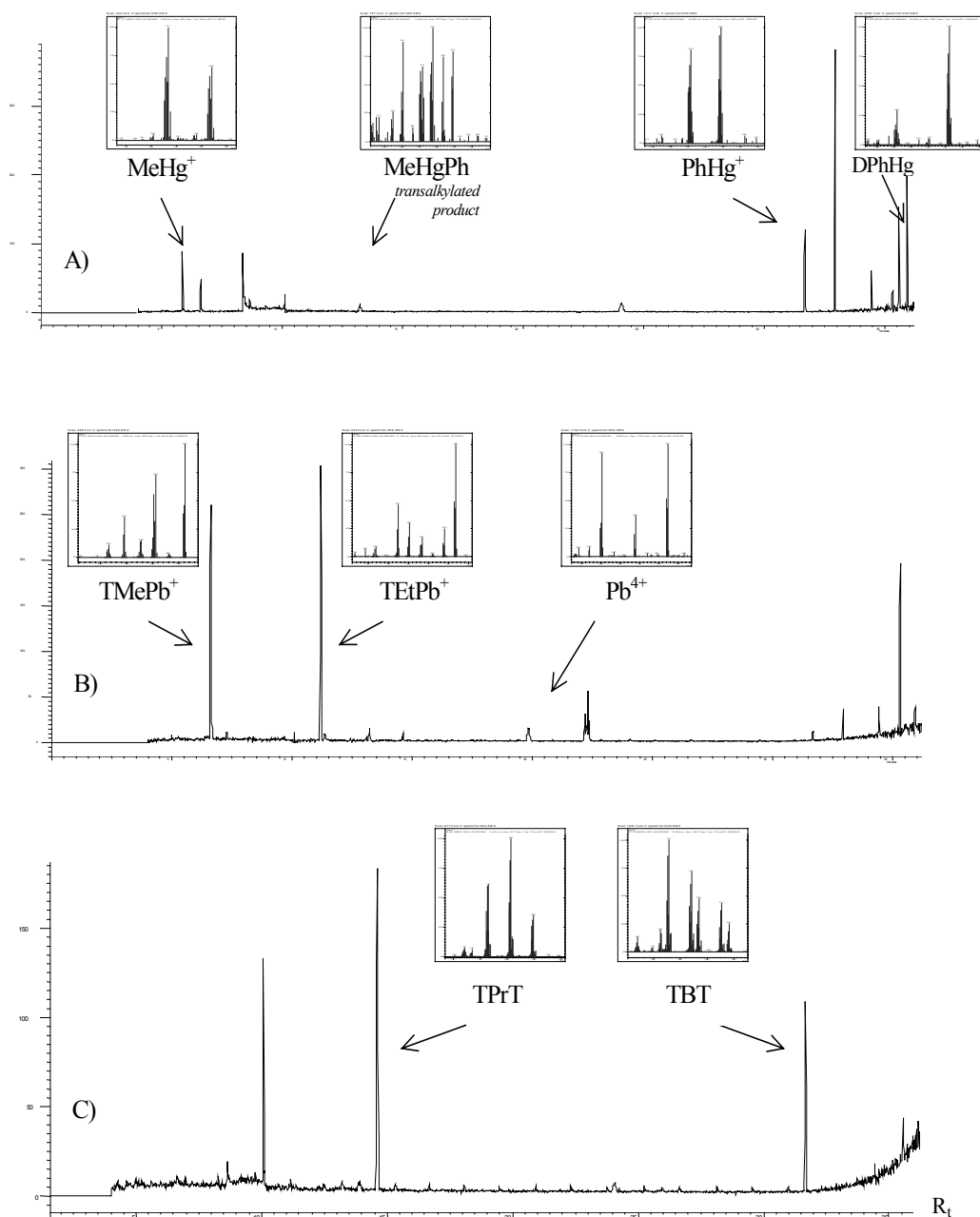


Figure 1.2. GC-MS chromatograms of propylated species of A) organomercury, B) organolead and C) organotin extracted from a 20 ppb spiked water sample.

**Note on units:** The concentration units of organotin used in the different figures and manuscripts may differ. For instance, there is a tradition in the literature of organisms at higher trophic levels that the concentrations are given as organotin cations, but in sediments and invertebrates the concentrations are often normalised to the weight of the tin (Sn) atom. However, all units can be transformed to each other. The conversion factors of TBT, DBT, MBT and TPhT to Sn are 2.44, 1.96, 1.67 and 3.34, respectively. For instance, 1 ng Sn/g (as TBT) = 2.44 ng TBT/g. The following units are used:

- Concentration of single or the total amount of the cations: ng TBT/g, ng BT/g, µg OT/kg etc.
- Concentration of organotin normalised to weight of Sn atoms: ng Sn/g or µg Sn/kg
- Concentration of organotin normalised to wet weight (ww) or the content of dry weight (dw) in the samples. In some occasion also to the content of sediment ignition loss (IL).
- BT = butyltin, single or total amount of species; OT = organotin, butyltin + phenyltin.

### 1.3. General comments on risk assessment based on data from field studies

Environmental risk assessment consists of a framework integrating exposure and effects profiles that can be used to assess the likelihood of contaminants causing adverse effects in ecosystems (TGD, 2002). The following four steps are generally required to evaluate in a retrospective assessment if contaminants are present at a level which can induce adverse effects on biological systems and assess the biological significance of the effects;

1. *Hazard identification*; Often performed by reviewing literature on toxicity test data obtained for both target and non-target organisms. Recently potentially hazardous substances are also identified and classified by QSAR model predictions.
2. *Establishment of dose-response relations*; Determination of the toxic activity of the contaminants.
3. *Exposure assessment*; Quantitative measurements of exposure and bioaccumulation of contaminants to evaluate the exposure level in the environment. Alternatively environmental concentrations can in some cases be predicted by modelling.
4. *Risk Characterization*; Using data from the three preceding steps to provide a prediction of the probability that adverse effects will occur in the ecosystem.

In general the hazard identification, the exposure assessment and effect assessment form a triangular basis for the risk characterisation. But the risk characterisation should also be used to



evaluate the present data material and to suggest where the knowledge of the environmental conditions can be improved through research, monitoring and/or assessments (Figure 1.3).

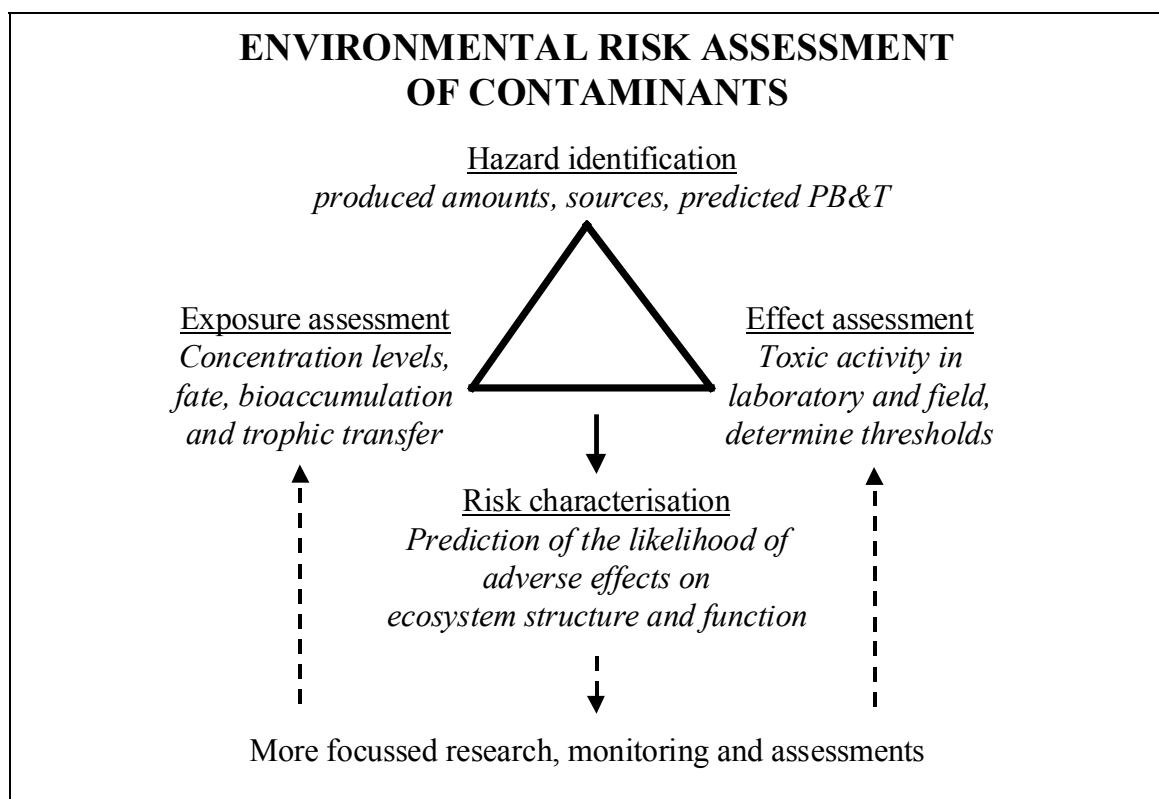


Figure 1.3. Environmental risk assessments of contaminants consists of interactions between hazard identification, exposure assessment, effect assessment and risk characterisation

The hazard identification and establishment of dose-response relations are often based on toxicity tests with survival, individual growth and reproduction as the typical endpoints and used for derivations of sublethal or lethal response concentrations (EC or LC), lowest-observed-effect concentrations (LOEC) and no-observed-effect concentrations (NOEC). Such toxicity tests mainly use single species and exposure to single contaminants performed under controlled laboratory conditions. Such data carry a high degree of uncertainty if directly used in risk assessment of complex field conditions. Species used in toxicity tests may not be representative for the most sensitive species in the ecosystem, and there are difficulties in comparing short-term exposure experiments and the long-term exposures occurring in the environment. Additionally, the endpoints used in toxicity tests are not necessarily the most sensitive endpoints.

To provide a more refined risk assessment for contaminants in a particular area, field data on exposure, bioaccumulation and effects are needed. In many monitoring programmes only water or sediment samples are analysed to assess the level of exposure in an area, even though measuring body burdens in suitable organisms, e.g. bioindicators, showing signs of impact, can

provide much stronger evidence of exposure than environmental concentrations in water or sediment. The accumulation of contaminants in organisms can provide stronger evidence of the actual exposure level than concentrations in water, because body burdens are a time-integrated measure of a chronic exposure. In addition, body burdens account for differences in bioavailability, e.g. the capacity of the organism to accumulate the contaminant from the surroundings and the food. The selection of suitable bioindicators is important for exposure assessment, because species-specific differences in accumulation potential occur even between species in the same taxonomic group and at the same trophic level in the food web. Differences between species in uptake and/or the capabilities to metabolise and eliminate contaminants can affect the accumulation level considerably, see for instance Chapter 2, Manuscript I and II. Other important factors for the selection of bioindicators, are whether the organism is stationary or not, widespread in the area of interest and possible to sample in adequate amounts.

The actual measured contaminant level in an organism also depends on the selection of compartment/organ to be analysed because of large variations in the accumulation levels within the organisms. For instance many halogenated organic compounds accumulate in the lipid rich compartments such as blubber (Tanabe, 2002). In order to compare across organs and species, the concentration is therefore usually normalised to the lipid content. Heavy metals and organometals have a higher affinity to proteins and therefore are more likely to accumulate in protein rich compartments such muscle, liver, kidney and fur or feathers (Tanabe, 1999). Concentrations should therefore be normalised to wet weight or dry weight if being compared. The distribution between mother compounds and their breakdown products may also vary significantly between the compartments within the organism. What compartment to select and whether the mother compounds and the breakdown products are used to describe the exposure level often depends on the scientific tradition, e.g. how the first studies concerning the type of contaminant and group of species were performed.

Regarding organotin bioaccumulation, most studies on molluscs use whole body tissue and often only the accumulation of TBT in the data analyses, whereas the total sum of organotin, e.g. the triorganotins and the breakdown products, in liver tissue is usually used to assess the exposure level in studies concerning organisms at higher trophic levels. The problem with getting adequate amounts of sample material has also influenced what compartments are analysed. Another important limitation in many accumulation studies is that the deposit organs are not necessarily the target organs. Although, liver is one of the main depositional organs of organotin, it does not necessarily imply that potential toxic effects do not occur in other parts of the organism, for instance in the blood, bone marrow, gonads, brain tissue etc. Nor will peaking exposure levels, for instance due to food intake, necessarily be reflected in the depositional organs. Therefore evaluations solely from actual body burdens are not adequate to account for

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potential effects in marine organisms. As a consequence, contaminant residues in prey organisms should also be included in marine risk assessment. It may therefore be difficult to evaluate the risk for adverse effects at an actual concentration measured. Other factors such as variations in exposure and accumulation levels between life stages/age groups, sex and seasons also have to be taken into account.

Risk assessment of contaminants of concern, which are present in a specific area, requires an integration of the established dose-response relationships and the exposure assessment in order to predict the probability that adverse effects will occur in the ecosystem. Adverse effects with biological significance are those that can affect the population and/or community structure or the ecological functions in the ecosystem. Relevant adverse effects thereby include impaired survival, growth or reproduction of a population. Effects on behaviour or avoidance of the habitat should also be considered.

The risk assessment of contaminants includes at present derivations of threshold levels. For instance, for various hazardous substances, including TBT, OSPAR has derived Ecotoxicological Assessment Criteria (EAC), and Environmental Quality Standards (EQS) have been derived in the proposal for the EU Water Frame Directive (WFD). These threshold levels are based on extrapolations of Predicted No Effect Concentrations (PNEC) (OSPAR, 1996; Lepper, 2002). PNEC includes, as a precaution, an application or an uncertainty factor between 10 and 1000 to the NOEC-values (or below EC10) identified in the hazard identification and the following establishment of dose-response relations. The size of the application factor used depends on the confidence with which the PNEC can be derived from available data, e.g. it depends on the quantity and quality of the data material and on the number of trophic levels, taxonomic groups and lifestyles representing various feeding strategies. The uncertainty in extrapolation from laboratory toxicity tests for a limited number of species to the “real” environment is thereby taken into consideration. The most sensitive species should also be protected, and it is therefore predicted that below the EQS adverse effects are unlikely to occur in the marine environment. Alternatively, PNEC can statistically be extrapolated from a species sensitivity distribution (SSD), which can be used to estimate the exposure level that exceeds the critical response for a specified percentages of species, often set to 5% (Lepper, 2002).

One way to provide a risk assessment in practice on the basis of the exposure assessment has been to divide the measured environmental concentration (MEC) by the PNEC-value, referred as a risk quotient (RQ). Normally it is assumed that there is no risk for the ecosystem if  $RQ < 1$ . If  $RQ > 1$  it is recommended to analyse whether further testing/information may lead to a revision of RQ before a final conclusion on the risk is reached (Lepper, 2002).

To validate the risk assessment, studies of the effects in the ecosystems are required (Lam & Gray, 2003). Analyses of population and community structures according to abundance,

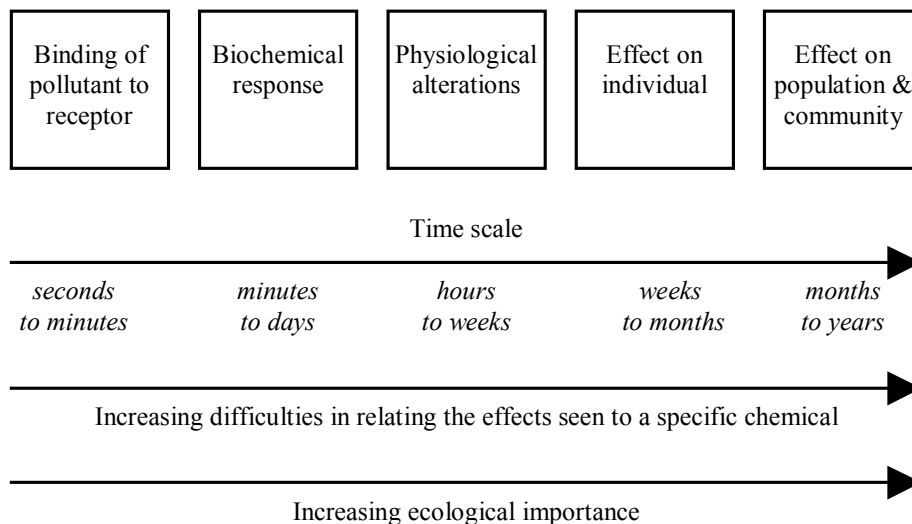
biomass and diversity are often regarded as the optimum risk assessment since it provides a description of the community viability at the field site. However, in field studies the weight of evidence is primarily indicative since cause-effect relationships are based on correlation/regression analyses, and because the possibility of confounding factors is difficult to exclude. There exists several other environmental factors, which may affect individuals and subsequently population and community structure, such as natural variations in the physical conditions, availability of food, inter- and intraspecific competition and predation, which therefore can often hide the influence of contaminant-induced stress. For instance, the degree of eutrophication in an area can be an important factor. Also high temporal variations, e.g. within and between years, in population and community structure can make it difficult to establish cause-effect relationships between contaminant exposure and population effects. More detailed studies on distribution of life stages or age classes within a population can be a more sensitive parameter in the assessment of changes in the populations which may also influence community structure, but such detailed analyses are not always included in these kinds of studies. Studies on the genetic diversity within a population can also provide evidence of contaminant-induced stress since the development of tolerance to contaminant exposure can result in alterations of the genetic variation within a population (Forbes, 1999). In addition, a hidden pressure related to contaminant-induced effects may have the result that populations become more vulnerable/susceptible to the pressure of other stress factors, which may affect the populations adversely and make it more difficult for populations to recover.

Bioassays are alternative methods to analyse if contaminants are present in the environment in a level that can induce adverse effects at population and community structure. In bioassays organisms are exposed to environmental samples under controlled laboratory condition, a method, which are often used in practice to assess the toxicity of environmental sediment and water samples, and various endpoints can be included, from biomarker responses to survival. For instance a sediment bioassay, which measures mortality of benthic amphipods after ten days exposure, is often used to assess the toxicity of dredged materials. However, the results from bioassays may be difficult to extrapolate to the responses in other and more sensitive species and/or relevant endpoints, which can occur in a complex ecosystem.

Another way to provide stronger evidence of the risks of contaminant-induced effects in a specific area is to support the exposure assessment with the use of biomarkers measured in organisms at the site, e.g. natural or transplanted populations (Lam & Gray, 2003). Biomarkers measure molecular, cellular, physiological or behavioural changes at or below the individual level, which are indicative as early warning signals of contaminant exposure and/or impaired biological functions. Biomarkers can be used either as tools to reflect the level of exposure to contaminants in organisms or to diagnose individual health. However, only few cases actually

exist where biomarkers have been used to establish a mechanistic link between exposure, effects revealed in individuals and subsequent changes in populations or community structure. The reasons are partly in the nature of biomarkers as illustrated in Figure 1.4, partly due to limitations in knowledge, so further research is still needed to establish such relationships. In addition, many biomarker responses are not necessarily associated with really harmful effects in the target organism even though some metabolic processes are altered. Therefore some biomarkers are only useful to indicate exposure, whereas other biomarkers indicate biological changes, which the organism might be able to compensate for. Because the weight of evidence in field studies is primarily indicative and because the possibility of various confounding factors, naturally as well as anthropogenic, cause-effect relationships can therefore in many situations be difficult to establish. Subsequently the relevance of biomarkers for environmental risk assessment has been under discussion.

*Figure 1.4. Biomarkers. Linkages between biochemical physiological, individual and population responses to pollutants. Adopted from Peakall (1992).*



However, the use of biomarkers also has advantages, particularly the contaminant-specific biomarkers, where causal relationships between exposure and effects can easily be established. Imposed and intersex in gastropods as a response to TBT (or TPhT) exposure is an excellent example of this, because only triorganotin compounds have been found to induce these phenomena in environmental reasonable concentrations. It is therefore possible to directly relate the exposure to effects, which in some cases can result in impaired reproduction and subsequently in adverse effects at the population level. Such very specific biomarkers can also be used as biological tools to indirectly provide evidence of contaminant exposure without measuring the actual

contaminant levels. But most biomarkers do not respond specifically to an unique type of contaminant, although some biomarkers respond to relatively restricted group of contaminants such as metallothionein for heavy metals, receptor binding assays for dioxin-like compounds, acetylcholinesterase activity for organophosphates and carbamates and vitellogenin for oestrogenic compounds. These biomarkers can only establish changes at the molecular level, of which the biological significance is difficult to evaluate at present.

There also exist other types of biomarkers, which are not induced by a specific group of contaminants. They provide a more general measure of the level of contaminant induced stress. The general stress biomarkes have the advantages that they can give an integrated picture of the total level of induced stress caused by contaminants, accounted as well as unaccounted for. They can thereby act as a kind of screening tool even though causal relationships are difficult to establish, because of difficulties in weighting the significance of the exposure to various contaminants, and because combination effects may occur as a result of exposure to complex mixtures of contaminants. Thus significant results must be followed by more comprehensive studies, where various biological and chemical analyses must supplement each other if the significance of different stress factors is to be evaluated. The significance of natural stress factors such as extreme variations in salinity, oxygen levels or temperature should also be taken into consideration since they also may interfere with most of the responses of general stress biomarkers. The general biomarkers therefore provide weaker indications of exposure and contaminant-induced effects, but it does not necessarily imply that the impact is less significant for populations and/or communities. In addition, populations may become more vulnerable/susceptible to the pressure of other stress factors, which may affect populations adversely and make it more difficult for populations to recover. Some examples of the general stress biomarkers include lysosomal stability, scope for growth, abnormal embryo development, disease development etc.

The following chapters are used as basis for a risk assessment of organotin contamination in the Danish marine environment. Chapters 2, 3 and 4 provide information on exposure levels and some examples of biological effects, which can more or less specifically be related to organotin compounds. In Chapter 5 both the data on exposure and effect levels are used in an integrated risk assessment, which results in a proposal of a five-class scheme for environmental assessment criteria useful for evaluation of the risks of organotin in the Danish marine environment.

## 2. The distribution of organotins in the Danish marine environment

The organotin compounds, mainly TBT, are today widely distributed in the Danish marine environment but with large spatial variations in the contamination levels. TPhT has also been found in some areas, but generally in lower levels than TBT if present at all. The following chapter describes the spatial distribution of organotins in Danish waters, in sediment and by using molluscs as bioindicators. In addition, the accumulation in various marine organisms is included to assess the potential of organotin to be transferred between different trophic levels in a marine food web and to identify species, which may be more vulnerable due to a particularly high accumulation potential.

### 2.1. Spatial distribution of organotins in sediments

In Denmark, most of the studies available today concern TBT contamination of marine sediments. National and regional authorities, such as the Danish EPA, the counties and the port authorities, have performed several studies of TBT contamination in marine sediments in recent years, because of the increasing awareness of the potential toxic impact in marine environment. These studies have been performed as part of monitoring programmes of various hazardous substances or in relation to assessments of the impact of dredging contaminated harbour sediments in coastal waters. Some of the studies have been published (Jacobsen et al., 2000; Pedersen et al., 2001; Jensen 2000;2002; Danish EPA, 2002), but a significant part of the results are unpublished.

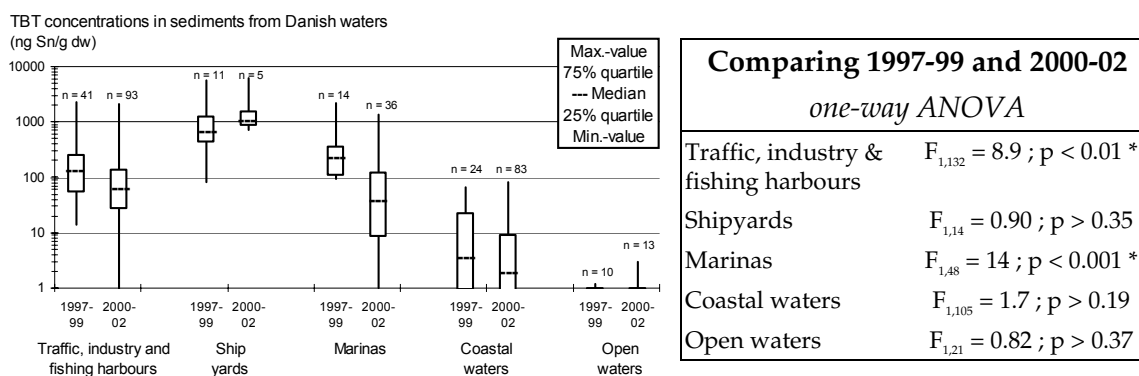


Figure 2.1. TBT concentrations in sediments from Danish waters.

The data material is extracted from various kinds of national as well as regional surveys, published as well as unpublished. *n* is the number of individual data included. Most of the 1997-99 data have also been presented in Jacobsen (2000).

Table 2.1. Statistical comparisons of the TBT concentrations found in sediments in the periods 1997-99 and 2000-02.

The data from commercial harbours and marinas have been log transformed to achieve homogeneity of variances.

Heavily TBT contaminated sediments exist inside harbours and marinas (Figure 2.1.), particularly in harbours with shipyard activities, where the TBT concentration in sediments can exceed 5000 ng Sn/g dw. In most of the analysed sediments the breakdown products DBT and MBT are present at lower concentrations than TBT probably due to the high persistence of sediment-associated TBT. Degradation half-life values of TBT in sediments have been assessed to be in the range of months to decades (Fent, 1996; Dowson et al., 1996). TPhT and its breakdown products have also been found in some harbour sediments, mostly from marinas, but never exceeding 200 ng Sn/g dw.

In sediments sampled in coastal waters outside harbours, the highest TBT concentrations (10 – 100 ng Sn/g dw) generally exist in shallow fiords with larger harbours situated inside, such as Odense fiord, or in narrow shipping channels close to harbours as in Langerak at Ålborg. In sediments from the Belt Sea area and the Sound the TBT concentration seldom exceeds 10 ng Sn/g dw. The spatial distribution of TBT within the regional areas seems to be dependent on diffuse sedimentation processes and on the sediment characteristics. The TBT concentration in sediments from depositional areas for silty and organic material is generally higher than in sandy sediments from erosive areas, resulting in correlations between TBT and the content of organic material as observed in the Sound (Figure 2.2, Manuscript I) and in the Wadden Sea (Jacobsen et al., 2000). There seems to be regional differences in the exact relationship between TBT concentrations and the content of organic material. This may be ascribed to regional differences in sedimentation rates and shipping intensity, but also to differences in salinity and the degree of water exchange with less contaminated adjacent waters.

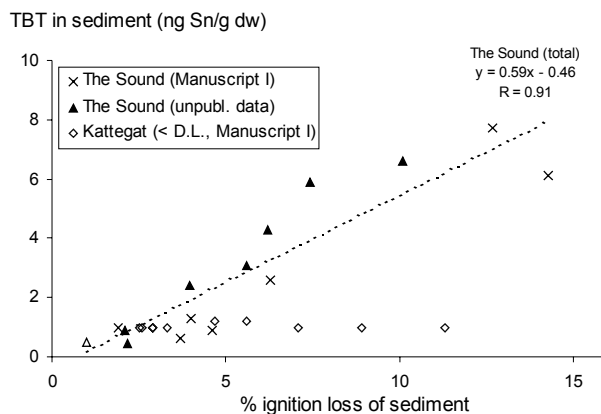


Figure 2.2. TBT concentrations in sediment from the Sound and the Kattegat.

The data from Manuscript I are supplemented with data from Jacobsen (2000) and some unpublished data from the Sound.

The TBT concentration in sediments from more open waters in the Danish parts of the Baltic Sea, the Kattegat, the Skagerrak and the North Sea, is generally below the limit of detection (<0.2 - <2 ng Sn/g dw), even in organic rich sediments sampled near the heavy international shipping routes (Manuscript I; Pedersen et al., 2001).



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It could be expected that the TBT levels in the marine environment have decreased in recent years, because of the restrictions on pleasure boats introduced in 1987 - 1991, larger public awareness and improvements of the TBT-containing paints for the larger vessels. Temporal trend studies have indicated decreasing TBT levels/effects during the 1990's in the proximity of some harbours and marinas (Harding et al., 1997; Miller et al., 1999; Svavarsson, 2000). However, decreasing trends in TBT levels in coastal waters away from harbours are not as evident. For instance, data from the German Specimen Bank cannot provide evidence to decreasing TBT levels in the period 1985 to 1999 in coastal waters of the Baltic Sea (Rüdel et al., 2003).

In Denmark it has not been possible to assess the influence of the restrictive actions on the TBT levels today, since there are only a few TBT measurements from the 1980's and the beginning of 1990's. However, based on the data presented in Figure 2.1, it seems that the TBT concentration in sediments from Danish marinas and commercial harbours, e.g. traffic, industry and fishing harbours, has become significantly lower in the period 2000-2002 compared to 1997-1999 (Table 2.1). A larger proportion of the analysed sediment samples from harbours in 2000-02 have TBT concentrations below 100 ng/g dw and even below 10 ng/g dw. But the data can not directly be compared, because of the inhomogeneity of the sample material due to the differences in sites included and the sampling strategies. These results can therefore only be regarded as an indication of decreasing contamination levels in these kinds of harbours in Denmark.

## **2.2. Spatial distribution of organotin accumulated in molluscs**

Marine organisms are good bioindicators for assessing the spatial distribution of organotins, because of their potential to bioaccumulate organotins. Especially bivalves are suitable bioindicators, because they are stationary and have limited capacity to biotransform TBT (and other contaminants) compared to many other marine organisms (Lee, 1991). However, there can be significant species-specific differences in the accumulation potential of organotins within the molluscs (Manuscript I), as well as between different species at higher trophic levels in the food web (Manuscript II), which must be kept in mind when organotin levels in various marine species are compared.

In the national monitoring programme in Denmark, NOVA 2003, the bivalve, *Mytilus edulis*, has been selected as the key species in the national and regional surveys in the marine environment (Pedersen et al., 2001). This bivalve lives mainly in shallow waters, is widespread and relatively easy to sample. Other species have to be chosen if the contamination level in deeper waters is to be assessed. The deposit-feeding bivalve *Nuculana pernula*, which lives in muddy sediments, was found to be an ideal bioindicator of TBT in such areas (Manuscript I),

because of a particularly high accumulation of TBT compared with other molluscs (Figure 2.3). In addition, the TBT concentrations in *N. pernula* are found to be strongly correlated to the measured TBT concentrations in the sediment. However, compared to *N. pernula* sampled in the field, a relatively low accumulation potential was found in *N. pernula* kept in the laboratory and exposed during one month to sediments spiked with relatively high TBT concentrations (Figure 2.4).

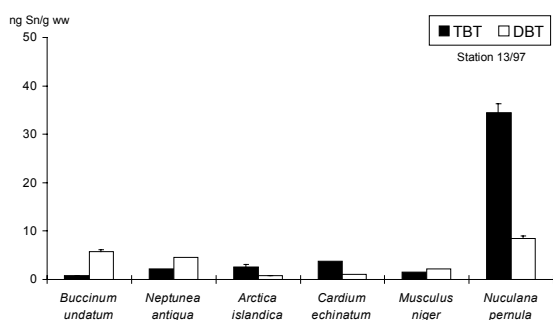


Figure 2.3. TBT and DBT concentrations in six different species of molluscs with different feeding strategies, two scavenging gastropods, three suspension- and one deposit-feeding bivalves, respectively. They were all sampled at the same station in the northern part of the Sound. Data from Manuscript I.

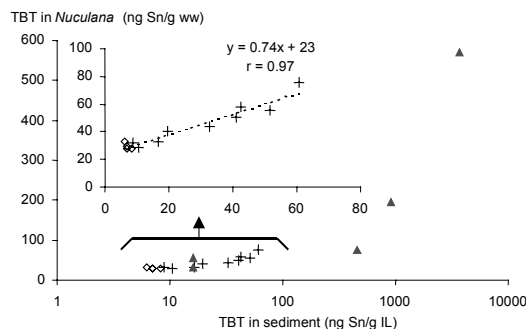


Figure 2.4. TBT accumulation in *Nuculana pernula* as a function of the TBT in the sediment normalised to the content of organic matter (as ignition loss, IL). Field data (+,◇) from Manuscript I compared with results from a laboratory experiment (Δ) (Strand, unpubl.).

Prosobranch gastropods, such as *Buccinum undatum*, are also suitable bioindicators (Strand & Jacobsen, 2002), especially, since accumulation studies thereby can be combined with TBT specific effect studies (see Paragraph 3.1). However, DBT is often the main contributor to the total body burden of butyltin in gastropods in contrast to in bivalves where TBT is the dominating organotin compound (see Figure 2.3).

Only few studies have in recent years been performed on the concentration of total butyltin in molluscs and other organisms sampled inside Danish harbours, but in such areas the concentration in species like *M. edulis* and *L. littorea* can exceed 500 ng Sn/g ww (Jensen, 2000; Jensen 2002). Earlier studies from the 1980'ties have even found TBT concentrations exceeding 1000 µg Sn/g ww in molluscs from Danish harbours (Jensen & Zoulian, 1989; Kure & Depledge, 1994).

In shallow fiords with a major harbour situated inside, like Odense fiord and Naksov fiord, the total butyltin concentration in molluscs is generally in the range of 30 - 200 ng Sn/g ww. In the molluscs (except *N. pernula*) sampled in the Belt Sea and the Sound the butyltin concentration is the range 5 - 40 ng Sn/g ww, and in the Kattegat and the Skagerrak the butyltin concentration in molluscs is even lower, mainly in the range 2 - 7 ng Sn/g ww but always above the limit of

detection (Manuscript I; Manuscript II; Strand & Jacobsen, 2002; Pedersen et al., 2001; Strand, unpubl.). Similar concentration levels as in the Kattegat can be found in molluscs living in shallow fiords with only minor ship traffic, for instance Ringkøbing fiord. The concentrations found in *M. edulis* from the Wadden Sea are within the range found in the Belt Sea and the Sound (Figure 2.5) and similar levels have been found in *M. edulis* from Polish coastal waters (Albalat et al., 2002).

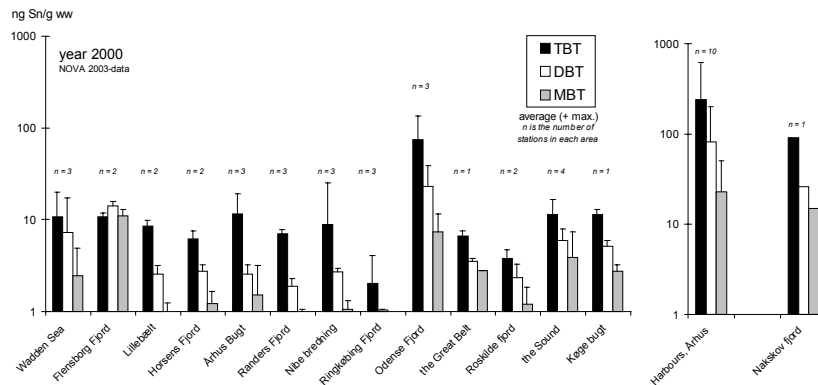


Figure 2.5. The levels of TBT and DBT in the bivalve, *Mytilus edulis*, sampled in various coastal areas of the Danish waters.

Data from the national monitoring programme NOVA 2003 (Pedersen et al., 2001), harbours at Århus (Jensen, 2002) and Nakskov fiord (Strand, unpubl.).

Because of the high TBT concentrations accumulated in *N. pernula* compared to other molluscs, this species is very suitable as a bioindicator in less contaminated areas, such as in the subtidal regions of the Kattegat. By using this species a clear picture of a gradually decreasing TBT contamination was found from south to north, e.g. from the mid-part of the Sound, through the Kattegat and into the Skagerrak (Figure 2.6). *N. pernula* was even found to be useful as a bioindicator of TBT exposure in deep parts of the west Greenlandic waters (see also Chapter 4).

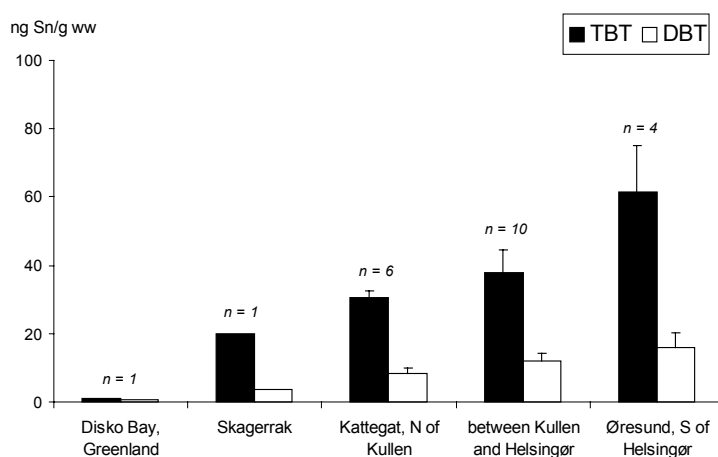


Figure 2.6. The levels of TBT and DBT in the deposit-feeding bivalve, *Nuculana pernula*, sampled in four regions of the Danish waters and at West Greenland.

Data from Manuscript I and IV.

The accumulation of TPhT in molluscs indicates that the contamination level is significantly lower than of TBT since TPhT has been detected only in very few of the analysed bivalves and almost only in bivalves sampled inside harbour areas (Jensen, 2000; 2002). However, based on the accumulation of TPhT in gastropods there is evidence of a more widespread occurrence of TPhT in Danish waters (Manuscript II; Jensen, 2000; 2002). The difference in TPhT accumulation between bivalves and gastropods may be due to species-specific differences in uptake and elimination processes, however, another explanation may be that TPhT, rather than TBT, is passed on from the sediment to organisms living in or near to the sediment (Manuscript II).

In conclusion, the spatial distribution of the TBT contamination in Danish waters using molluscs shows a similar, but more detailed distribution pattern than observed for the TBT levels in sediments, especially in coastal and subtidal areas away from harbour areas (see Paragraph 2.1).

### **2.3. Bioaccumulation and trophic transfer of organotin in a marine food web**

Other organisms than molluscs are also exposed to organotin compounds in the Danish waters, and TBT, TPhT and/or their breakdown products can be detected in organisms at all trophic levels of the marine food web from seaweed, to invertebrates, fish, birds and mammals (Figure 2.7). A high variance in accumulation levels of organotin was found between and within various species and between species at the same trophic level. The highest concentrations of TBT and its breakdown products were found in livers of harbour porpoises, where the concentrations were in the range between 130 and 2290 ng Sn/g ww. Butyltin levels exceeding 200 ng Sn/g ww were also found in liver of flounder and eider duck. The lowest butyltin concentrations were found in seaweed and in mute swan, which is a plant feeding bird.

TPhT or its degradation products were also detected in most of the species with the highest concentrations in flounder, cod and great black-backed gull, where the phenyltin concentrations were in the range between 10 and 75 ng Sn/g ww (Figure 2.7).

The high interspecies variance in the distribution pattern between the triorganotins and their breakdown products probably reflects species-specific differences in exposure routes and the capabilities to metabolise and eliminate the organotin compounds. For instance, TBT contributes significantly to the butyltin concentration in liver of harbour porpoise, but not in liver of the fish-eating birds and harbour seal, where the breakdown products, DBT and MBT are the dominating compounds. The high metabolic capacity for persistent organic contaminants in birds and seals in comparison to cetaceans has also been suggested in other studies (Tanabe, 2002). A similar accumulation pattern to birds and seals has also been found

in a study on liver of humans, where only DBT and MBT were present in the 18 males analysed, in a range between 0.6 and 18 ng Sn/g ww (Nielsen & Strand, 2002). However, marine food may not be the only source of exposure to butyltin in humans since DBT and MBT also are used in some household products (Paragraph 1.1).

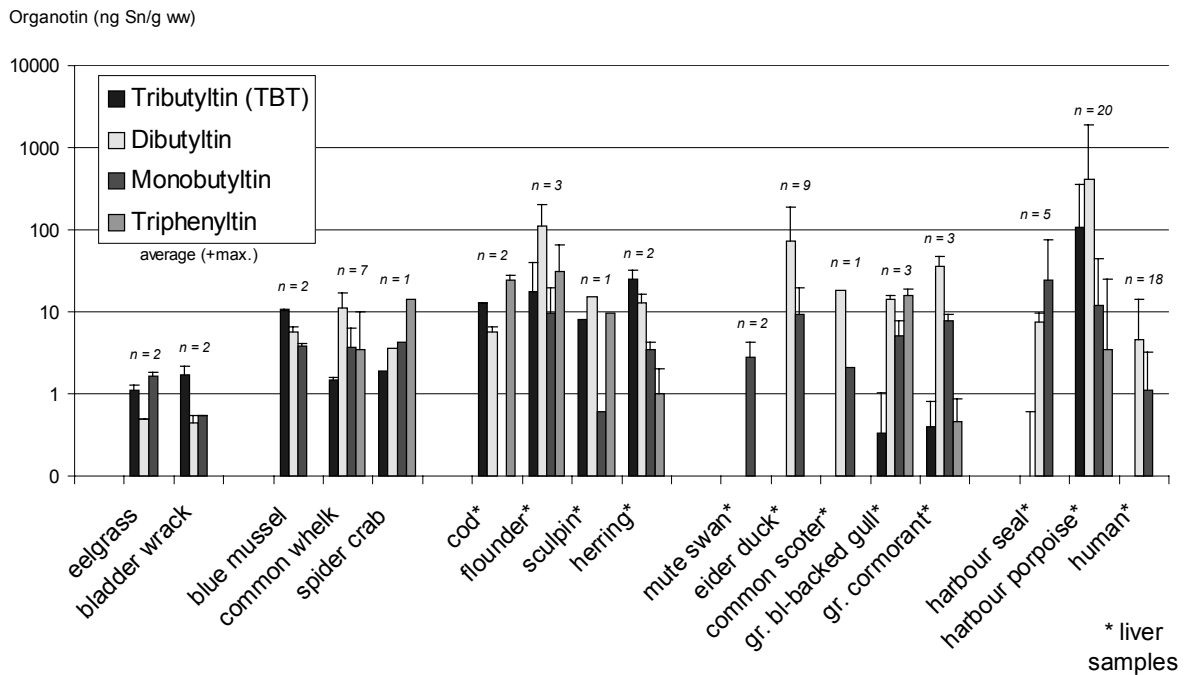


Figure 2.7. Residues of organotin compounds in various marine organisms inclusive humans from the Inner Danish waters. Data from Manuscript II and III, except the data on humans, which is from Nielsen & Strand (2002).

To understand the trophic transfer of organotin in the examined marine food web in more detail, biomagnification factors (BMF) describing the ratio between the concentrations in predator and prey were calculated and by definition BMF values above one for the compounds indicate biomagnification (Table 2.2). Strong indications of biomagnification of butyltin was found especially for harbour porpoise with an average BMF = 4.4 which varied from 1.1 to 18.7 between individuals. Some degree of biomagnification of butyltin was also demonstrated in fish and diving ducks, but to a lesser extent than in harbour porpoise. In contrast, low average BMF values of 0.2, 0.5 - 0.7 and 0.2 were estimated for mute swan, fish-eating birds and harbour seals, respectively. This reflects large differences in trophic transfer and the potential for biomagnification of butyltin even between species at the same trophic level as illustrated for harbour seals and harbour porpoises, both mammals predated on fish.

Table 2.2. Biomagnification factors (BMF) estimated from average concentrations of total body burdens of butyltin (TBT+DBT+MBT) in seaweed and organisms at different trophic levels. Liver contents are transformed to whole body burdens by using following conversion factors; *L* is the average values of the fraction the liver weight contributes to the total body weight. *P* is literature values from studies with related species of the amount of butyltin retained in the liver relative to the total body burden. In seaweed and invertebrates the butyltin concentration in the whole body has been analysed and *L* and *P* values are therefore not relevant. From Manuscript II

Consumer	L (%)	P (%)	$C_{w.b.}$ ng Sn g <sup>-1</sup> ww	Food	L (%)	P (%)	$C_{w.b.}$ ng Sn g <sup>-1</sup> ww	BMF <sub>Butyltin</sub> average (range)
swan	2.3	10	0.6	seaweed	-	-	3.0	~ 0.2 (0.1 - 0.3)
whelk, crab	-	-	15	mussel	-	-	17	~ 0.9 (0.2 - 1.9)
fish <sup>b</sup>	2.8	10	20	invertebrate <sup>a</sup>	-	-	16	~ 1.2 (0.3 - 4.7)
diving duck <sup>c</sup>	2.4	10	18	invertebrate <sup>a</sup>	-	-	16	~ 1.2 (0.2 - 3.1)
fish-eating bird <sup>d</sup>	3.3	10	10	invertebrate <sup>a</sup>	-	-	16	~ 0.7 (0.4 - 1.2)
fish-eating bird <sup>d</sup>	3.3	10	10	fish <sup>b</sup>	2.8	10	20	~ 0.5 (0.3 - 0.9)
seal	3.5	40	2.8	fish <sup>b</sup>	2.8	10	20	~ 0.2 (0.1 - 0.4)
porpoise	3.2	20	86	fish <sup>b</sup>	2.8	10	20	~ 4.4 (1.1 - 18.7)

The average concentration in the whole body,  $C_{w.b.}$ , in organisms at the different trophic level are calculated as an average of the following species; a) invertebrates: mussel, whelk and crab, b) fish: flounder, cod, herring and sculpin, c) diving ducks: eider duck and common scoter, d) fish-eating birds: great black-backed gull and great cormorant.

It has to be noted that the performed estimations of BMF include several simplifications such as assuming identical distribution and metabolism of organotins in related species. It is further assumed that the organisms used are good representatives for the actual food preferred both according to species and size/age of the species. For instance, in the estimations of BMF for harbour porpoises from the Inner Danish waters all available data were included, e.g. new-born and younger as well as older individuals. Since butyltin, like mercury, tends to accumulate with age and size (Figure 2.8a,b) this may have resulted in an underestimation of BMF for harbour porpoise. It can also be questioned how relevant the calculations of BMF-values are for the carnivore invertebrates and fish, because food is not necessarily the dominant exposure route in invertebrates and fish, where the uptake from water and sediment also can contribute considerably to the accumulation of organotin.

In contrast to the trends observed in the spatial distribution of TBT in sediments and molluscs (Paragraph 2.1, 2.2 and 3.1), a regional difference in butyltin concentrations in harbour porpoises from the Inner Danish waters and the Danish part of the eastern North Sea/Skagerrak region could not be established, and even after the influence of age was removed as confounding factor (One-way ANOVA:  $P > 0.17$ ) (Manuscript III). This might indicate comparable levels of butyltin exposure to harbour porpoises in both regions. The lack of regional differences in harbour porpoises might also be due to migration of the harbour

porpoises from the eastern North Sea and Skagerrak into more TBT polluted areas, for instance the Inner Danish waters or the southern North Sea inclusive of the Wadden Sea and the German Bight.

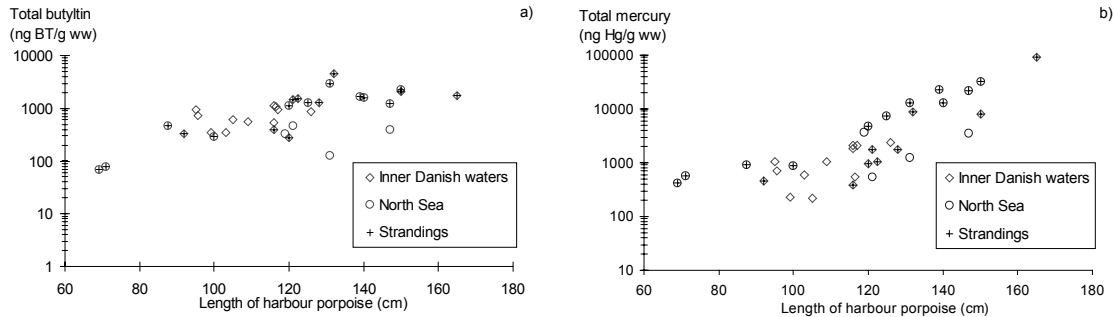


Figure 2.8. The concentration of a) butyltin and b) mercury in the liver in relation to the length of by-caught or stranded harbour porpoises from the Inner Danish waters and the Danish North Sea (LS regression:  $P_{BT} < 10^{-4}$ ,  $P_{Hg} < 10^{-8}$ ). Data from Manuscript III.

The butyltin concentrations in the Inner Danish waters as well as in the Danish part of the eastern North Sea are higher than found in previous studies of harbour porpoises from the coasts of England and Wales, the Black Sea and the Baltic Sea (Table 2.3). The levels found in harbour porpoises from the Danish waters are in the same range as the highest levels found in coastal dolphins from Japan, the USA and the Mediterranean (Tanabe, 1999). This suggests that TBT contamination in the Danish waters is significant, also compared to other areas worldwide.

Table 2.3. Comparison of different regional studies on butyltin residues in liver of harbour porpoises (*Phocoena phocoena*). From Manuscript III.

Region	n	Total butyltin (ng BT/g ww)	
		mean (range)	References
Inner Danish waters	20	1092 (285 – 4605)	Manuscript III
Danish North Sea	15	951 (68 – 2905)	Manuscript III
West Greenland	3	12 (2 – 18)	Manuscript III
English North Sea and Irish Sea	29	214 (22 – 640)	Law et al., 1998
Polish Baltic Sea	2 <sup>a</sup>	23 (18 – 27)	Kannan & Falandysz, 1997
Turkish Black Sea	27	156 (89 – 219)	Madhusree et al., 1997

<sup>a</sup> new-born specimens.

However, harbours must still be regarded as the real hot-spot areas as described for the sediments and molluscs (Paragraph 2.1 & 2.2), and very high butyltin levels can also be found in fish caught in Danish harbours. For instance the butyltin concentration in liver of flounder from Copenhagen harbour can exceed 2500 ng Sn/g ww (Figure 2.9). Further, the butyltin

levels accumulated in liver of Danish harbour porpoises are comparable or even higher than in sediment, molluscs and fish from the most contaminated harbours (Figure 2.9).

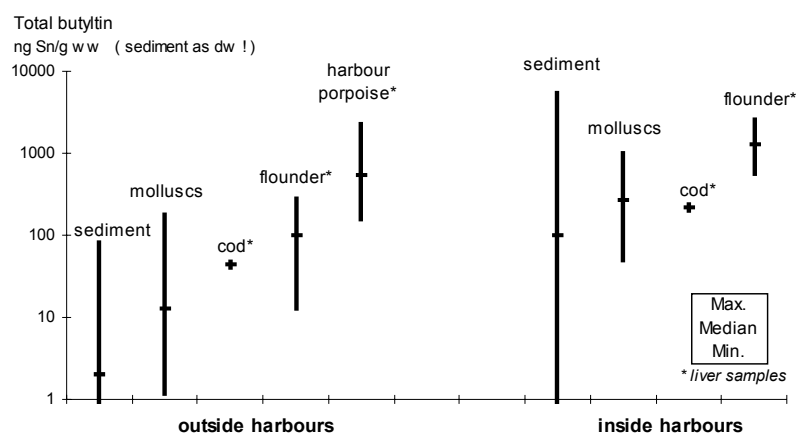


Figure 2.9. Comparison of the range of butyltin concentrations found in sediment, molluscs, fish and harbour porpoise from inside and outside harbour areas. The data is extracted from Paragraph 2.1, 2.2 and 2.3 except the data on harbour fish (Strand & Jacobsen, 2000).

#### 2.4. Estimated concentrations of TBT in Danish marine waters

Water samples is generally not regarded as the most suitable matrix to analyse if the levels of persistent organic contaminants in the marine environment are to be assessed. This is due to the generally low levels, which is often close to or below the detection limit, and high temporal fluctuations in the concentration levels usually occur (Phillips & Rainbow, 1993).

Table 2.5. Estimated TBT concentration in seawater from different types of areas in the Danish waters based on the TBT concentrations measured in the bivalve *Mytilus edulis*. A dry weight content of 15% in *M. edulis* has been assumed.

Type of area	TBT conc. in <i>M. edulis</i> (ng Sn/g ww)	Estimated TBT conc. in seawater (ng Sn/l)
The Kattegat, the Skagerrak and the Danish North Sea	no data	-
Coastal waters in the Belt Sea, the Sound and the Wadden Sea	5 - 15	0.3 – 0.9
Shallow fiords with major harbours situated inside Harbours	10 - 100 50 - 1000	0.6 – 6 3 – 60

Another method to assess the concentration level in water is to use bioindicators, with a validated bioconcentration factor (BCF), for instance the bivalve *Mytilus edulis*, since the bioaccumulation can be regarded as an integrated measure of the exposure level over a longer time period. However, BCF is based on the assumption of equilibrium, e.g. steady-state



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conditions, between the concentration in the water and concentration accumulated in the organism. Another assumption is that BCF is constant at all exposure levels, although BCF generally decreases at increasing exposure levels, and that BCF is not affected temporally or spatially by for instance physical or physiological factors. Based on various bioaccumulation studies in the literature, BCF of TBT in *M. edulis* is estimated to be 116000 l/kg dw as the geometric mean (OSPAR, 1996). By using the knowledge of TBT concentration measured in molluscs from Danish waters (see Paragraph 2.2), estimates of the average TBT concentration in seawater can be made (Table 2.5).

The estimated TBT concentrations are in good agreement with the few TBT analyses made of seawater from the Danish marine environment in the period 1996 – 2003, where TBT concentrations between <0.5 and 4 ng Sn/l have been detected outside harbours and between 4 and 125 ng Sn/l inside harbours (Jacobsen, 2000; Strand, unpubl.). An earlier study has also reported TBT concentrations of 11 – 14 ng Sn/l (measured as total extractable tin) in the Kattegat in 1992 (Mortensen et al., 1993).

### 3. Studies of some potential TBT-induced effects in the Danish waters

The widespread and relatively high organotin contamination in the Danish marine environment provides a good opportunity for supporting exposure assessment with biological effect studies. Studies on imposex and intersex in prosobranch gastropods are especially useful, because of the specificity in the cause-effect relationship. That the relatively high organotin levels in Danish waters may also pose a threat to other taxonomic groups is here discussed in relation to immunotoxicity in marine mammals, impaired development and reproduction fish and mortality of a benthic amphipod. The difficulties in establishing the same specific causality for other organisms, as in the imposex and intersex studies on gastropods, does however not necessarily imply that organotin does not pose a threat to other marine organisms inhabiting Danish waters. As listed in Table 1.3 exposure to organotin can result in different kinds of ecotoxicological effects.

#### 3.1. Imposex and intersex in prosobranch gastropods

Imposex and intersex in gonochoristic prosobranch gastropods refer to two types of morphological alterations caused by a masculinisation of females. Imposex means that the females develop male sexual characters, e.g. a vas deferens and/or imposition of a penis, in addition to the normal female sexual characters (Smith, 1971). In the development of intersex, an important female sexual character, e.g. the pallial oviduct, is altered to be similar to a male prostate gland (Bauer et al., 1995). The females, in some species, become sterile in the severe developmental stages of imposex and intersex, e.g. the imposex stages 5 or 6 according to the vas deferens sequence index (VDSI) classification (Stroben et al., 1992) and in the intersex stages 2, 3 or 4 according to the intersex stage index (ISI) classification (Bauer et al., 1995). Imposex has been observed in more than 100 species world-wide, while intersex has only been found in a single species, *Littorina littorea*. The imposex and intersex phenomena are induced very specifically by TBT and /or TPhT and are a result of elevated testosterone levels due to interference with the endocrine system in the female gastropod. TBT concentrations as low as <1 to >15 ng TBT/l can induce imposex or intersex (Mathiessen & Gibbs, 1998). The phenomena are irreversible by nature and already induced in the immature life stages, although imposex can in some species also be induced in adult females transplanted to contaminated conditions (Davies et al., 1997; Beck et al., 2002). However, the irreversible nature of imposex may not necessarily generate a measurable relationship between imposex development and actual TBT concentrations. While TBT concentrations are contemporary and dynamic, because of the capability of the gastropods to biotransform and eliminate TBT, and because of resting periods in feeding uptake, the imposex intensity will represent a level of TBT-exposure with some years delay.

Even though some studies claim that other environmental pollutants or other stress factors also can induce imposex development in gastropods (Nias, 1997; Evans, 2002), there is in my judgement no adequate evidence for such relationships yet. Because of the specificity and sensitivity, imposex and intersex are very suitable biomarkers for exposure and effects of TBT, and therefore useful in monitoring and risk assessment. Since 1998 imposex and intersex have been included as biomarkers in the national monitoring programme in Denmark, NOVA. Four key species of gastropods have been selected, *Buccinum undatum*, *Neptunea antiqua*, *Hinia reticulata* and *Littorina littorea*, but imposex development has also been found in other Danish species (Table 3.1). *Nucella lapillus*, which is the key species in most monitoring programmes in the North Sea region, has not been selected since it does not occur in the Kattegat and other parts of the Inner Danish waters. In Denmark *N. lapillus* can today only be found in isolated populations on the west coast of Denmark. In NOVA, I have been in charge for the studies on *B. undatum* and *N. antiqua*, sampled at depths from 15 to 100 m in the open waters, whereas the regional amts are responsible for most studies on *H. reticulata* in shallow coastal waters and *L. littorea* from the tidal zone (Strand, 2001; 2003).

Table 3.1. *Imposex or intersex has been observed in the following ten species of prosobranch gastropods in Danish waters (Strand, unpubl.).*

<i>Neptunea antiqua</i>	<i>Aporrhais pespelicani</i>	
<i>Buccinum undatum</i>	<i>Colus gracilis</i>	<u>NOT observed in:</u>
<i>Hinia reticulata</i>	<i>Hydrobia ulvae</i>	<i>Colus jeffrisianus</i>
<i>Hinia pygmae</i>	<i>Rissoa violacea</i>	<i>Littorina saxatilis</i>
<i>Nucella lapillus</i> *	<i>Littorina littorina</i> *	<i>Theodoxus fluviatilis</i>

\* sterile females observed.

If imposex or intersex studies also should be performed in the eastern or northern Baltic Sea, other species, which inhabit this region have to be chosen. *Hydrobia ulvae* seems to be the only species, which is a suitable bioindicator since other Baltic prosobranch gastropods do not seem to have the potential to develop these phenomena. For instance, imposex development was only found in *H. ulvae* in a small study of the sexual characters in six species of prosobranch gastropods sampled in Finnish coastal waters at Helsinki (Strand, unpubl.). A similar conclusion was made in the study in the southern Baltic Sea by Schulte-Oehlmann et al. (1998). The different gastropod species do not have the same potential to develop imposex, e.g. they are not all equally sensitive to TBT, see for instance Figure 3.1. Some muricid neogastropods, like *N. lapillus*, are generally regarded as belonging to the more sensitive gastropod species (Stroben et al., 1995), but also the buccinid neogastropod *N. antiqua* seems to be one of the more sensitive species (Strand & Jacobsen, 2002). However, the muricids have additionally the potential to develop sterile imposex stages due to a blockage of the vulva with the vas deferens,

which thereby makes the release of egg capsules impossible (vdsi = 5 or 6). Such sterile stages do not develop in many other prosobranch species including the buccinids *B. undatum*, *N. antiqua*, and *H. reticulata* (maximum vdsi stages are 4 or 4+), although further alterations, for instance coiling of the female oviduct toward a male seminal vesicle, have been observed (Barreiro et al., 2001; Strand & Jacobsen, 2002).

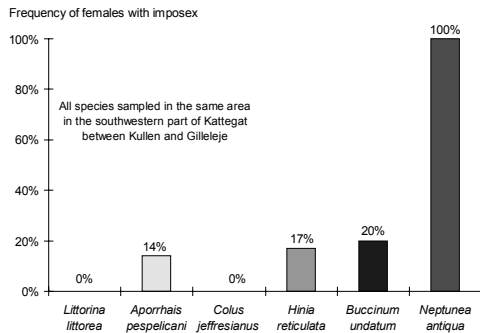


Figure 3.1. The frequencies of females with imposex or intersex in 6 species of prosobranch gastropods, all sampled in the same area in the southwestern part of the Kattegat. A high variance in imposex development occurs even between species from the same trophic level and taxonomic group such as *Colus*, *Hinia*, *Buccinum* and *Neptunea*, which all are carnivore neogastropods belonging to the Buccinidae family (Strand, unpubl.).

The studies performed in NOVA have shown that imposex and intersex are widespread phenomena in all regions of the Danish waters, especially in the most sensitive species. For instance, almost all *N. antiqua* in the Inner Danish waters have developed imposex and generally in highly developed stages, VDSI = 2.6 - 4.0, but significant imposex levels are also present in the open waters of the Skagerrak and the North Sea, VDSI = 0.7 - 1.8 (Figure 3.2). In comparison, less frequent and less developed stages are present in *B. undatum* in the Inner Danish waters, VDSI = 0.1 - 1.6, and in the Skagerrak and the North Sea, VDSI = 0.1 - 0.7. The highest imposex levels in *B. undatum* occur in the Belt Sea (Figure 3.2). The overall imposex levels have been fairly constant during the period from 1998 to 2002, but time trends have not been statistically tested yet.

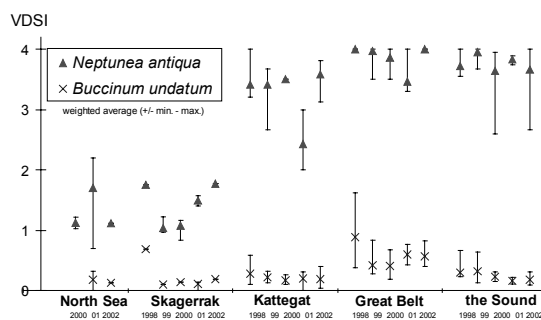


Figure 3.2. Imposex in *Neptunea antiqua* and *Buccinum undatum* from five Danish water regions in the period 1998 to 2002 (NOVA). From Strand (2001;2003).

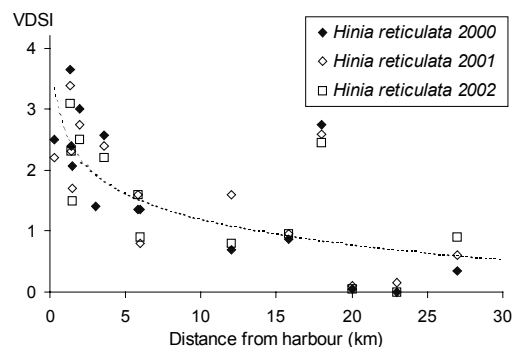


Figure 3.3. Imposex in *Hinia reticulata* from 13 stations in four coastal areas visited each year in the period 2000 to 2002 by Århus, North Jutland and Vejle amts (NOVA). From Strand (2001;2003).

In the coastal waters, decreasing gradients of imposex in *H. reticulata* are evident away from TBT contaminated harbour areas (Figure 3.3). High imposex levels, VDSI > 2, occur usually within a distance of 3 km from the harbours. At two stations in the Kattegat, imposex is almost absent in *H. reticulata* (VDSI = 0.0 - 0.2).

Regarding intersex, high intersex levels in *L. littorea*, ISI > 1.2, occur inside the majority of Danish harbours and marinas, and sterile females therefore often dominate in such areas (Figure 3.4). This strongly supports the significance of the TBT contamination in harbour areas since intersex is not induced as easily as imposex in the majority of the neogastropods. Outside harbours elevated intersex levels, ISI = >0.3, are often found in the vicinity of harbours, but only seldom can sterile females be found.

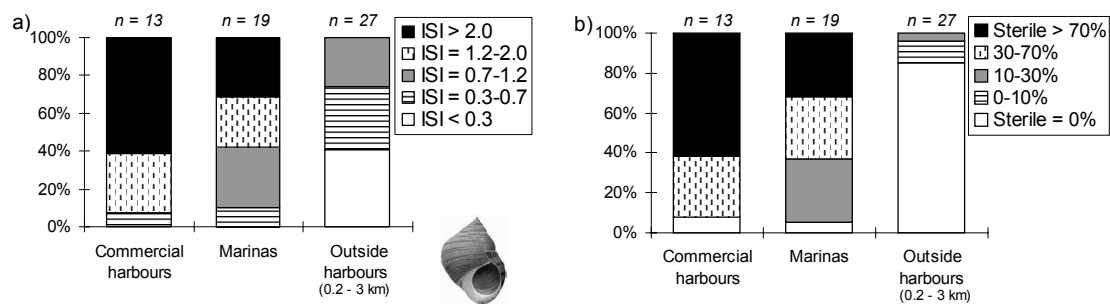


Figure 3.4. The distribution of intersex severity in *Littorina littorea* as a) ISI and b) % sterile females in 13 Danish harbours and 19 marinas compared to 27 stations outside harbours. From Strand (2003).

Sterile females also occur in some populations of *N. lapillus* from the west coast of Jutland, sampled just outside harbour areas, VDSI = 4 – 5. Less severe imposex stages in *N. lapillus* occur away from harbours, VDSI = 2 – 3 (Harding et al., 1999; Strand, unpubl.), which at present reflects a kind of “background level” in the Danish part of the North Sea and the Skagerrak. Compared to this, only few incidences of imposex if any (0 - ~30%, e.g. VDSI < 0.3) occur in populations of *N. lapillus*, which lives in reference areas in the North Atlantic, e.g. in Iceland, Norway, Canada and UK (Svavarsson & Skarhedinsdottir 1995, Berge et al., 1997, Prouse & Ellis 1997, Evans et al., 1998). By using the Danish data from the North Sea and the Skagerrak, it seems that *N. lapillus* and *N. antiqua* have approximately the same level of sensitivity to TBT, because VDSI = 1 – 2 in *N. antiqua* from the stations within 10 km from the coast line (Figure 3.2). However, comparisons of tidal and subtidal species should be treated with some caution. Tidal species are primarily exposed to the contamination in the upper water bodies including the microsurface layer, whereas subtidal species are rather exposed to deeper water as well as the sediment.

Spatial trends in the imposex and intersex levels in prosobranch gastropods living in the Danish coastal as well as in the open waters reflect almost the same trends as the spatial distributions of

the measured TBT concentrations in sediment and biota, which were presented in Chapter 2. However, the use of these biomarkers seems to provide an even more detailed picture of the spatial distribution than the chemical measurements. For instance, imposex can be used to distinguish between areas, in coastal as well as in open waters, where the TBT concentration in sediment often is below the detection limit. The potential of imposex to reflect gradients in TBT contamination at low TBT levels is illustrated in the correlations between VDSI in the subtidal buccinids and the actual measured body burdens of butyltin (Figure 3.5), or the TBT concentration in the sediment from the same areas where the buccinids are sampled (Figure 3.6). Even TBT concentrations in sediment below the limit of detection are found to induce elevated levels of imposex (VDSI > 2) in *N. antiqua*, while TBT concentrations at 10 - 20 ng Sn/g dw in sediment were found to induce elevated imposex levels (VDSI > 2) in *H. reticulata* and probably also in *B. undatum* (Figure 3.6). In comparison, exposure for one month to sediment with a TBT concentration of 25 ng Sn/g dw induced increased imposex development in a sediment bioassay with *H. reticulata* (Oehlmann et al., 2000).

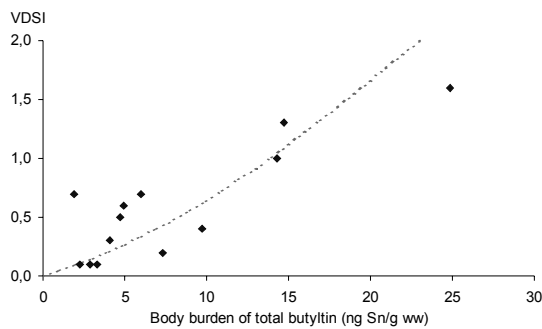


Figure 3.5. The body burden of total butyltin is significantly correlated with VDSI in *Buccinum undatum* (Spearman rank correlation;  $r_s = 0.664$ ,  $p < 0.05$ ). From Strand & Jacobsen (2002).

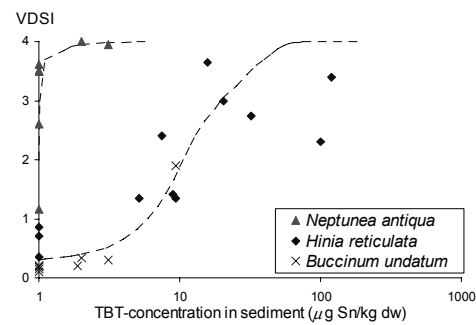


Figure 3.6. Imposex versus TBT concentration in sediment from Danish waters. VDSI in *Hinia reticulata* is significantly correlated with the TBT concentration (Spearman rank correlation;  $r_s = 0.664$ ,  $p < 0.01$ ). Areas, where the concentration was below the limit of detection, are marked as 1 ng/g dw in the diagram. The lines represent sigmoid fits by eye. From Strand (2001).

Studies on gastropod population structure in relation to imposex or intersex development have not been performed systematically in Danish waters, although the presence of immature specimens indicates a continuing recruitment, impaired or not. In England, significant changes in population structure have been demonstrated in *L. littorea* from TBT contaminated harbour areas (Mathiessen et al., 1995), and in *N. lapillus* populations, where a significant proportion of the females were sterile as a consequence of imposex (Bryan et al., 1987; Spence et al., 1990;

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Birchenough et al., 2002). Especially gastropods with non-pelagic larval stages, such as *N. lapillus*, are vulnerable, because recolonisation of an area therefore is expected to occur slowly. In the Skagerrak and the Kattegat there are some indications of serious declines in several marine gastropod populations during the 20th century. It cannot be excluded that widespread TBT contamination might have contributed to the population declines of some species by affecting reproduction and recruitment. However, the changes in gastropod populations may also be due to other environmental changes of habitats such as increasing prevalence of hypoxia caused by eutrophication. For instance, 52 species of marine gastropods are today red listed in Sweden (SSIC, 2003). Nine species are listed as endangered or critically endangered and eleven species are listed as vulnerable. In addition, 32 species are listed with an unknown status, because the data are insufficient to classify the species into the red list categories. There exists no red list information on the status of marine gastropods from the Danish and Norwegian parts of the Kattegat and the Skagerrak. However, there are also some evidences of population declines in the Danish waters such as declines of *H. reticulata* and *B. undatum* between the beginning and the end of the 20<sup>th</sup> century in the Inner Danish waters. For instance, these species are almost absent today in the Sound between Funen and Langeland, where they previously were relatively abundant (Glob, 2002). Another example is the relative high abundance of empty shells of *H. reticulata*, which can be found buried in the sediment from the Inner part of Odense Fiord (Strand, unpubl.). *H. reticulata* has not been found at all inside Odense Fiord in the recent decades (MADS database, unpubl.).

Another example is the muricid neogastropod *Trophon truncatus*, which in the 19<sup>th</sup> century was relatively abundant at some sites in the southern part of the Kattegat, the Belt Sea and the Sound (Petersen, 1888). Today this species may be extinct from the Danish waters. To my knowledge *T. truncatus* has not been found at all in the second half of the 20<sup>th</sup> century, for instance in the intensive national and regional monitoring of benthic fauna (MADS database, unpubl.). Only shells of *T. truncatus* from the first half of the 20<sup>th</sup> century are stored in the extensive shell collection at the Zoological Museum in Copenhagen (Strand, unpubl.). Concerning *N. lapillus*, this species was present at Frederikshavn in the northern part of the Kattegat in the beginning of the 20<sup>th</sup> century (Ris, 1930). Today, *N. lapillus* cannot be found at this site (Strand, unpubl.) suggesting that it may also have disappeared from this area.

There are also evidence for decreases in several marine prosobranch gastropod populations in the inner part of the German Bight in the southern North Sea (Nehring, 2000), but for most species the declines seem to be ascribed to other factors than TBT. Only a severe decline in the population of *N. lapillus* at Helgoland can be directly attributed to TBT. However, it cannot be excluded that the significant decreases in some of the other gastropod populations, including *H. reticulata* and *N. antiqua*, may have been caused by TBT (Nehring, 2000).

### **3.2. Risk of organotin to organisms at higher trophic levels**

Several types of contaminants can affect the immune and endocrine systems of vertebrate organisms that may lead to increased susceptibility to infectious diseases or impairment of reproduction (Ross, 2000). While many factors, both intrinsic and extrinsic, can affect the immune system of vertebrates, hazardous substances are not likely to be the trigger in the outbreak of epidemic diseases, which in recent years have resulted in for instance mass mortality in Danish waters among harbour seals (*Phoca vitulina*) in 1988 and 2002 (Heide Jørgensen et al., 1992; Dietz et al., 2003) and eider ducks (*Somateria mollissima*) in 1996 and 2001 (Christensen et al., 1997; SNS, 2002). However, contaminants may still pose a “hidden” threat as they are suspected to increase the susceptibility to such diseases. Top predators in the food web, like marine mammals, may be particularly vulnerable, because they are exposed to and accumulate high levels of many persistent contaminants from their food.

Most studies focus on the associations between effects and exposure to organochlorines like PCBs and dioxin or to heavy metals like mercury (Ross, 2000), but increasing attention is also given to other “novel” hazardous substances like brominated and fluorinated organic compounds (de Boer et al., 1998; Giesy & Kannan, 2001) and organotin compounds. Butyltin and phenyltin compounds have been related to immunotoxicity in for instance fish (O’Halloran et al., 1998; Grinwis et al., 2000) and mammals (Snoeijs et al., 1987; Kannan et al., 1997; 1998; Whalen et al., 2002). The risk of endocrine disruptions caused by organotin compounds has also to be considered based on studies on fish (Shimasaki et al., 2002) and mammals (Omura, 2001; Cooke, 2002).

Nevertheless, it has been evaluated within the framework of marine risk assessment affiliated to the Water Frame Directive (WFD) within the European Union (EU) that the primary threat of the triorganotins appears to be for organisms like molluscs at the lower trophic levels of the food web (Lepper, 2002). Even though the lack of appropriate avian and mammalian oral toxicity data is recognised, it is also stated unlikely that poisoning will occur in organisms at the higher end of the trophic scale, because TBT will be metabolised quickly into less toxic di- and monobutyltin compounds in vertebrate organisms.

In contrast, a study focused on human risk assessment has suggested that fish as a food source in some regions can contribute with tri- as well as di-substituted organotin compounds to an extent that health risks should be considered (Belfroid et al., 2000), because the effect levels of tri- and di-substituted organotins in some toxicological studies are comparable and even might be combined. From such a perspective, the breakdown products of TBT and TPhT may also pose a threat to organisms at higher trophic levels especially as they, in contrast to humans, are feeding entirely on marine food sources.



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The risks of secondary poisoning of top predators are usually assessed based on oral intake (Lepper, 2002). The dietary exposure to butyltin in marine mammals inhabiting Danish waters can be estimated to about 1.6  $\mu\text{g Sn/kg bw/day}$  by using the average body burden in fish of 20 ng Sn/g ww (estimated in Table 2.4 in Chapter 2) and by assuming a daily consumption of 4 kg fish/day and a body weight (bw) of 50 kg. This is 16 times higher than the tolerable daily intake (TDI) of 0.10  $\mu\text{g Sn/kg bw/day}$  proposed for human exposure to TBT and DBT (Belfroid et al., 2000), derived from immunological studies with rodents. However, even though environmental and human risk assessment can not be compared directly, as human risk assessment has individuals as the protection target, and environmental risk assessment has populations and communities as the protection target, it indicates that the marine mammals inhabiting Danish waters can be at risk of organotin-induced immunotoxicity. To what extent is as yet undetermined.

The risk of exposure to organotin for organisms at higher trophic levels is discussed in this thesis in relation to studies on two species of marine mammals, harbour porpoises (*Phocoena phocoena*) and grey seals (*Halichoerus gryphus*), inhabiting Nordic waters.

Because of the very high organotin concentrations found in harbour porpoises compared to in other marine organisms from Danish waters (Figure 2.7; Manuscript II), a more extended study was made that focused on this small cetacean (Figure 2.8a; Manuscript III). The study on harbour porpoises from Danish waters showed that stranded harbour porpoises generally had accumulated higher levels of both butyltin and mercury in the liver than by-caught porpoises, even after age was removed as a confounding factor (Figure 3.7).

The higher butyltin and mercury concentrations found in stranded than in by-caught porpoises may indicate that a high accumulation of these contaminants might be related to the “cause of death”. However, a more conclusive relationship can not be established because of the lack of extensive post-mortem examinations and the small size of the data set. Two other studies, supported by post-mortem examinations (Jepson et al., 1999, Bennett et al., 2001), have also found a higher accumulation of contaminants, e.g. PCB and mercury, in stranded than in by-caught harbour porpoises from the North Sea. A study by Siebert et al. (1999) also found associations between mercury levels and severity of certain pathological lesions in harbour porpoises from the Baltic Sea and the North Sea, but was unable to establish differences between groups of stranded and by-caught harbour porpoises. This suggests that these and/or co-varying contaminants such as butyltin (see Figure 2.8a,b) might influence the health and viability of harbour porpoises.

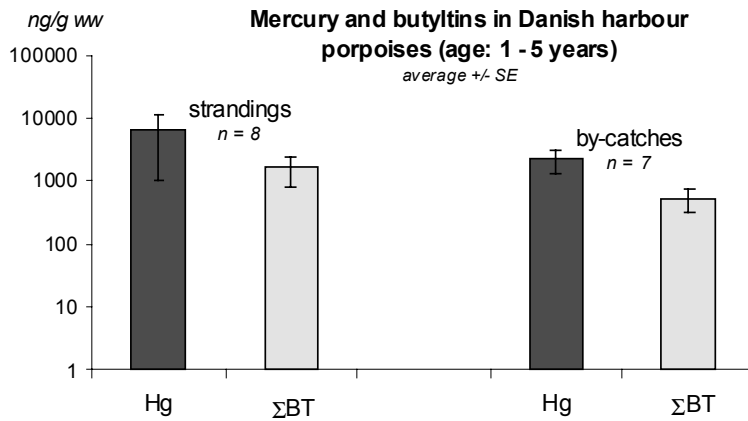


Figure 3.7. The mean concentration of butyltin, but not mercury, is significantly higher in the group of stranded than in the by-caught harbour porpoises with age between 1 and 5 years.

(One-way ANOVA,  $^{BT}F_{1,13} = 8.2, p < 0.02$ ;  $^{Hg}F_{1,13} = 1.6, p > 0.22$ ).

From Manuscript III.

It may be argued that particularly harbour porpoises are at higher risk of secondary poisoning than other marine mammals, due to a generally higher accumulation potential of contaminants including organotin. On the other hand, because these species predate on almost the same food source as for instance seals, it can be argued that exposure to organotin is at the same level. The primary deposit organs are not necessarily the target organs in which the toxic effects first will occur. For instance *in vitro* studies on the immunotoxicity of organotins have shown that short-term exposure can lead to irreversible inhibition of natural killer cells from mammalian blood samples (Whalen et al., 2002). Therefore peak levels of organotin exposure, for instance due to intake of food, should be considered. Evaluations solely from actual body burdens are not adequate to account for potential effects in marine organisms.

A study on organotin accumulation in marine mammals was also done on Baltic grey seals (Strand & Roos, unpubl.). The study of accumulation of butyltin and phenyltin compounds in grey seals from the Swedish east coast was performed as part of a larger screening survey of several other persistent contaminants. The study was initiated after pathological examinations of Baltic grey seals (Bergman, 1999) had revealed that more than 50% of immature grey seals aged between 1 and 3 years are suffocating from a serious intestine sore disease, e.g. colonic ulcers, and that this disease was more frequent in the 1990's than in the previous decade (Table 3.2.). Colonic ulcers can cause acute mortality, and are estimated to be the second most frequent cause of death among immature Baltic grey seals, just after drowning in fishing gear.

Table 3.2. Health conditions in Baltic grey seals from the Swedish east coast during two decades. Data from Bergman (1999).

Diseases in grey seals	1977 – 1986	1987 - 1996
Colonic ulcers in immatures	15 %	53 %
<i>Baltic seal disease syndrome</i>		
Occlusions in adult females	42%	11%
Pregnancy of females (aug-feb)	9%	60%

It has been suggested that the findings indicate that the food consumed by the Baltic seals may contain “new” or increased amounts of unidentified toxic factors, which affect their immune system (Bergman, 1999) and hazardous substances like organotin compounds or brominated flame retardants should be considered (Roos, 2000).

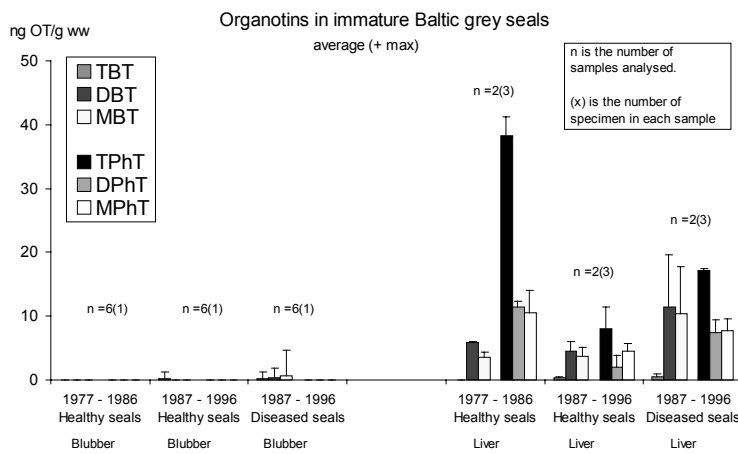


Figure 3.8. Butyltin and phenyltin compounds in blubber and liver from immature grey seals with and without severe colonic ulcers during two decades (Strand & Roos, unpubl.). The three groups of immature grey seals refer to the same groups as in Table 3.2.

The results showed that butyltin as well as phenyltin compounds had accumulated in all samples of grey seal liver from both decades, while only small residues of butyltin, and not phenyltin, could be detected in five of 18 analysed blubber samples, and only in the seals from the period 1987-1996 (Figure 3.8). The breakdown products, DBt and MBt, were the dominant butyltin compounds, whereas TPhT was the main contributor to phenyltin in the livers. The phenyltin concentration was highest in the seals from 1977-1986, while butyltin was somewhat higher in 1987-1996 than in the previous decade. The concentrations of butyltin as well as phenyltin were higher in the diseased than in the healthy seals from 1987-1996. Because only two pooled liver samples (consisting of three specimens each) from each group were analysed, it has not been possible to test these differences statistically. The extremely low organotin levels in the blubber did not provide a possibility for statistical comparisons of the groups even though each group consisted of eight samples of individual specimens. The presence of organotin in the grey seals in this study therefore only provides evidence for exposure to organotin.

On the other hand, a Dutch semi-field study in the 1990's found higher immuno-suppression in seals fed on fish from the Baltic Sea than from a relatively uncontaminated site in the North Atlantic. Similar results were found in rats exposed to the same fish (Van Loveren et al., 2000). In the 1980's a similar kind of semi-field study showed impaired reproduction in seals fed on fish from the Baltic Sea compared to those fed on fish from the Atlantic (Reijnders, 1986). This suggests that the exposure levels of contaminants, based on organochlorine levels, to seals and other marine mammals inhabiting the Baltic Sea (including Danish waters) can be a real threat to their immune system. This also might have consequences at the population level like the

contaminant-induced impaired reproduction in Baltic seals had in the 1970's and 1980's. Baltic grey seals in this period were affected with a high prevalence of the so-called Baltic seal disease syndrome resulting in decreased fecundity, reproductive impairment and sterility (Table 3.2), which were attributed to with the high PCB levels.

The presented results on organotin exposure and accumulation in relation to marine mammals suggest that organotin might also be an important immunotoxic factor. Organotin compounds should therefore also be integrated when assessing the risk of hazardous substances on the health and viability of organisms at higher trophic levels, fish and birds included, inhabiting Danish waters.

### **3.3. The eelpout (*Zoarces viviparus*), a bioindicator useful for integrated studies**

Another example of contaminant-induced biological effects, which like immunosuppression not specifically can be related to single group of hazardous substances are impaired fish reproduction including developmental effects including teratogenesis in fish embryo. The early life stages of fish are typically more susceptible to environmental contaminants than other life stages (McKim, 1977) and developmental effects in fish embryos and larvae can therefore be regarded as a sensitive general stress biomarker and not as a contaminant-specific biomarker for TBT. Although TBT has the potential to induce such kind of effects, most attention has been on other substances like organochlorines, pesticides, PAH, and heavy metals (Weis & Weis, 1989; Bodammer, 1993). In addition, natural stress factors such as extreme variations in oxygen levels, salinity and temperature have also been associated with increased prevalence of malformations and mortality in free-living fish embryos (Bodammer, 1993). In field studies causal relationships between a concrete exposure and teratogenic effects are therefore difficult to establish because of lack of specificity in contaminant response and because combined effects due to mixtures of various contaminants and other stress factors can occur.

Concerning TBT specifically, laboratory studies have shown that the potential toxic modes of TBT in fish include reduced hatching success of eggs and reduced survival of pelagic fish embryos and larvae. However, at relatively high exposure levels of TBT between 1,500 and 20,000 ng Sn/l (Fent, 1996; Bentivegna & Piatkowski, 1998; Granmo et al., 2002), but this also indicates a differentiated sensitivity between the examined fish species, although life stages used and/or exposure time differ between the studies. Some of the studies have also found that TBT caused deformations of the embryo, which could result in different types of gross abnormalities such as yolk sac defects and bend or spiral shapes of the spinal axis (Fent, 1996; Bentivegna & Piatkowski, 1998). Compared with estimated TBT concentrations in water from type-specific areas in Danish waters (Paragraph 2.4) pelagic fish embryo seems to be rather insensitive and may only be at risk of TBT in extreme polluted harbour areas. However, these

laboratory studies did not take TBT exposure of the parent fish and the transfer from mother to eggs into account even though this is an important factor for the development of teratogenic effects (Weis & Weis, 1989). In field-based studies the exposure and bioaccumulation of contaminants in adult fish should also be considered, for instance in viviparous fish where the embryos and larvae might be at higher risk of contaminants due to the possibility of maternal-fetal transfer (see Figure 3.9). It can therefore not be excluded that TBT can be an important stress factor for the reproductive success in fish, not only inside some harbours areas, but also in some Danish coastal waters.

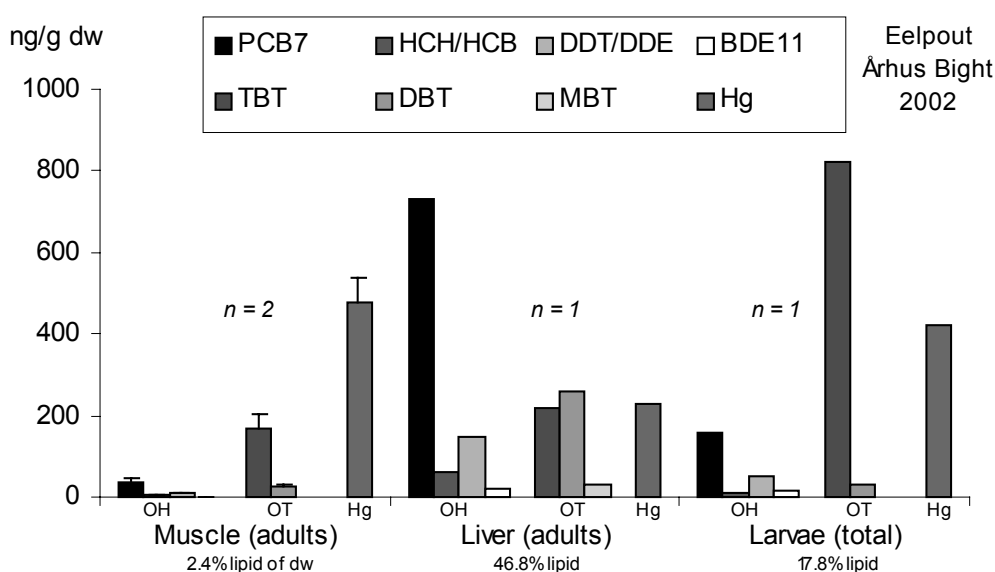


Figure 3.9. Residues of organohalogenic (OH) and organotin (OT) compounds in pooled samples of muscle, liver and larvae of the viviparous eelpout from Århus 2002 (St.8). Data from Jensen & Strand (unpubl.).

To examine the potential of developmental effects of larvae in broods of the viviparous eelpout (*Zoarces viviparus*) as a biomarker for contaminant-induced stress, a study was performed in ten different areas of Danish coastal waters known to be more or less contaminated due to effluents from cities, industry and harbour activities (Manuscript IV). This study aimed on to examine eelpouts from rather type-specific areas and not only focusing on levels of TBT contamination in the respective areas, since the levels of many anthropogenic substances often will co-vary between more and less contaminated areas. The ten sampling areas could be characterized into two categories as described in Table 3.3.

Table 3.3. Type-specific descriptions of the ten sampling areas for studies of eelpouts in Danish coastal waters 2001 and 2002. From Manuscript V.

Type	Description of areas	St. No.:	Location (year)
I	Coastal areas including shallow fiords with effluents from larger cities, industry and/or harbours. These areas are considered to be moderate or in some situations as relatively highly contaminated areas.	St. 3: St. 4: St. 5a,b: St. 6a,b: St. 7: St. 8: St. 10:	Nakskov (2002) Frederiksværk (2002) Roskilde (2001,2002) Lindø (2001,2002) Odense (2002) Århus (2002) Ålborg (2001)
II	Coastal areas at some distance to larger cities and industry and with a high potential for water exchange with adjacent waters. These areas are considered to be less contaminated and are therefore regarded as reference areas in this study.	St. 1: St. 2: St. 9:	Fakse (2002) Nivå (2001) Egense (2001)

The examination of eelpouts showed a relatively high variation in the proportion of broods, which had elevated levels of deformed larvae between the different sampling areas, and also that the deformations were represented by various types of gross abnormalities. These deformations could be categorized in to six different morphological characteristics (B – G) as described in Figure 3.10. Deformations like spirality or other defects of the spinal axis, cranio-facial defects, eye lesions or entirely loss of eyes were dominating in all areas, but also other deformations such as yolk sac or intestine defects and siamese twins were found. In addition, the distribution pattern between the different types of deformations varied between some of the areas. For instance at St. 4, eye defects and absent eyes (Type E) was the predominating type of deformation, whereas at the other stations deformations like bent shape and spirality of the spinal axis (Types C and D) were more frequent (Figure 3.10).

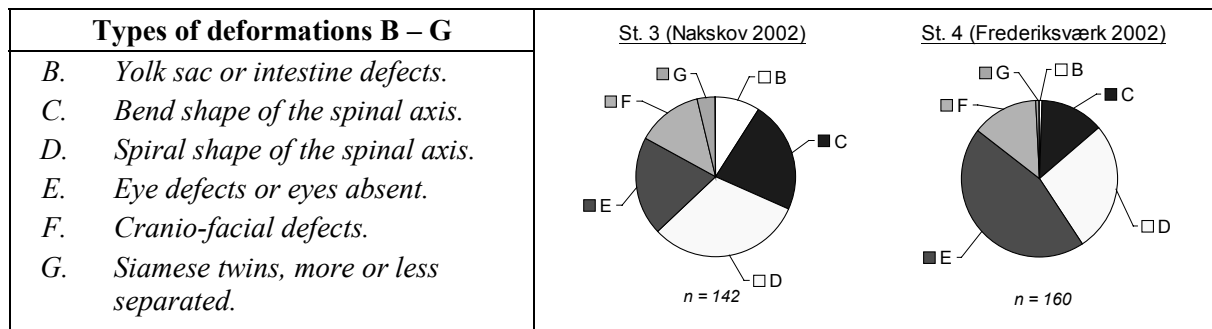


Figure 3.10. Distribution pattern of deformed larvae (type B – G) in broods from two highly affected areas, St.3 and St.4. n is the total number of deformed larvae found. Data from Manuscript V.

In all of the three areas (st. 1, St. 2 and St. 9), which were considered as less contaminated reference areas only a small proportion, if any (0% - 3%), of the pregnant eelpout had elevated

levels of deformed larvae (e.g. >5%) in the broods indicating a very low natural occurrence of deformed larvae in broods of the eelpout (Figure 3.11). Swedish studies have also found similar low levels of deformations in broods of the eelpout sampled in reference areas of the Skagerrak and the Baltic Sea (Vetemaa et al., 1997; Sandström 2000).

In comparison, increased proportions of the pregnant eelpout (6 – 53%) that had elevated levels of deformed larvae (e.g. >5%) in the broods were found in seven of the eight areas, which were considered to be at least moderately contaminated with effluents from larger cities and industry compared to reference areas (Figure 3.12). The highest levels were found in sampling areas situated in the inner parts of shallow fiords with larger cities, industry and harbours situated inside, such as at St. 3: Nakskov (32%), St. 5: Roskilde (5.7% - 20.5%), St. 4: Frederiksværk (53.1%) and St. 7: Odense (50%). This indicates that the larval development is impaired by site-specific conditions, which occur particularly in the inner parts of fiord areas where contaminants are most likely to accumulate. Similar observations have been made in a Swedish study (Vetemaa et al., 1997).

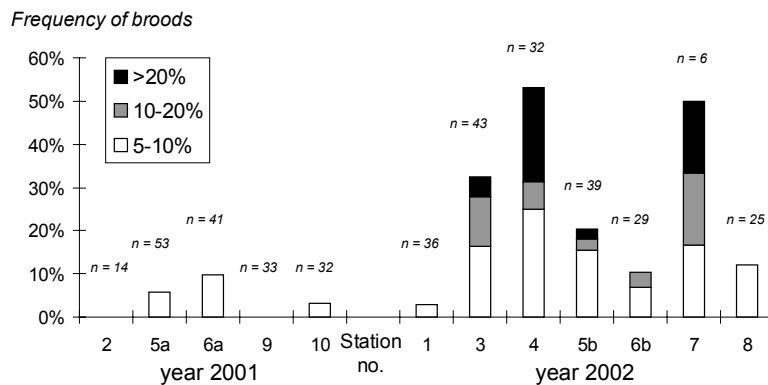


Figure 3.11. The proportion of the examined broods with elevated levels (5-10%, 10-20% and >20%) of deformed larvae (type B – G) in the ten sampling areas in 2001 and 2002. n is the number of examined broods. From Manuscript V.

In addition, the variations observed in the distribution of different types of deformations may suggest that it can be associated to differences in pollution patterns between the areas. It seems therefore reasonable to suggest that these developmental effects in eelpout larvae are caused by contaminant-induced stress.

Another indication of that contaminants could be present in levels, that can affect the fish, was provided by physiological examinations of the adult eelpouts. The liver-somatic index (LSI) of adult males was found to be significantly higher at some of the same areas where increased prevalence of deformations occurred in the broods, e.g. at St. 3, St. 5 and St. 7 (Figure 3.12). Several studies has suggested that there can be a causal relationship between liver enlargement and exposure to contaminants like PAH and organochlorines but the reason for this is not fully understood (van der Oost et al., 2003). For instance a study on the eelpout had shown that

increased LSI can be induced as a response to 7 - 14 days exposure to PAH (Celander et al., 1994). However, other factors such as differences in food quality and intake may also influence the nutrition status and thereby LSI and conclusions from comparisons of geographical separated populations must therefore be treated with some caution. However, the significantly enlarged liver in eelpouts, which were sampled in areas with elevated levels of deformations in the broods, supports that contaminants are present at a level, which can affect the development of the fish, although increased LSI was not found at St. 4, which was the station with the highest levels of deformations in the broods. The fact that also the distribution pattern between different types of deformations at St. 4, differed from St. 3, St. 5 and St. 7 could suggest that other contaminants/factors are important at St. 4 compared to the other areas.

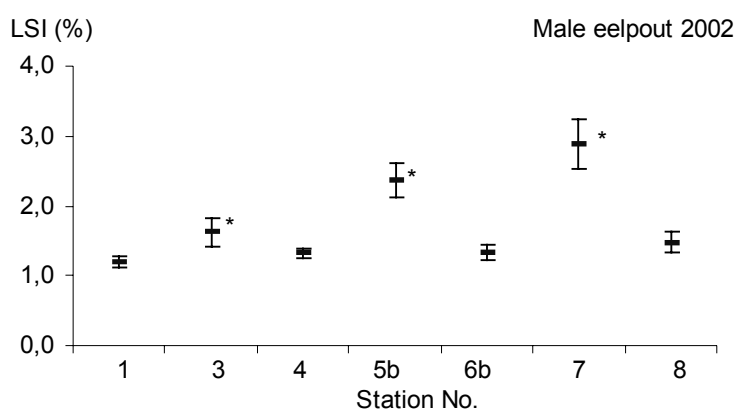


Figure 3.12. Liver-Somatic Index (LSI) in male eelpouts sampled at seven stations in 2002 (average  $\pm$  SE).

\* marks if LSI is statistic higher than at other stations. In female eelpouts LSI was also significantly increased at station 5 and 7.

Data from Manuscript V.

In addition to LSI, ten male eelpouts from the ten sampling areas were also examined for vitellogenin induction as biomarker for estrogenic substances, but none of the males showed any signs vitellogenin induction in the plasma (Manuscript V). This suggests that the eelpouts not are exposed to estrogenic substances in biological active levels in these areas, although further biochemical analyses are still in progress (Andersen et al., in prep.).

To support the hypotheses that the embryo development is affected by contaminants in some of the areas, data on contaminant levels in sediment (Pedersen et al., 2001; unpubl. data) from the respective sampling areas were used test such a relationship. Because the biological significance of the single substances is not possible to evaluate, an integrated non-parametric rank was made to describe the combined contamination level, where the different substances were weighted equally (Table 3.4). This ranking of the areas must only be regarded as a rough characterization of the contamination level in the areas. The direct comparison of sediment data must also be treated with some caution since the data are not corrected for relevant normalisation parameters, since the relevant normalisation parameters are not available from all



areas. However, the data still provide some indications of the relative contamination levels in the respective areas.

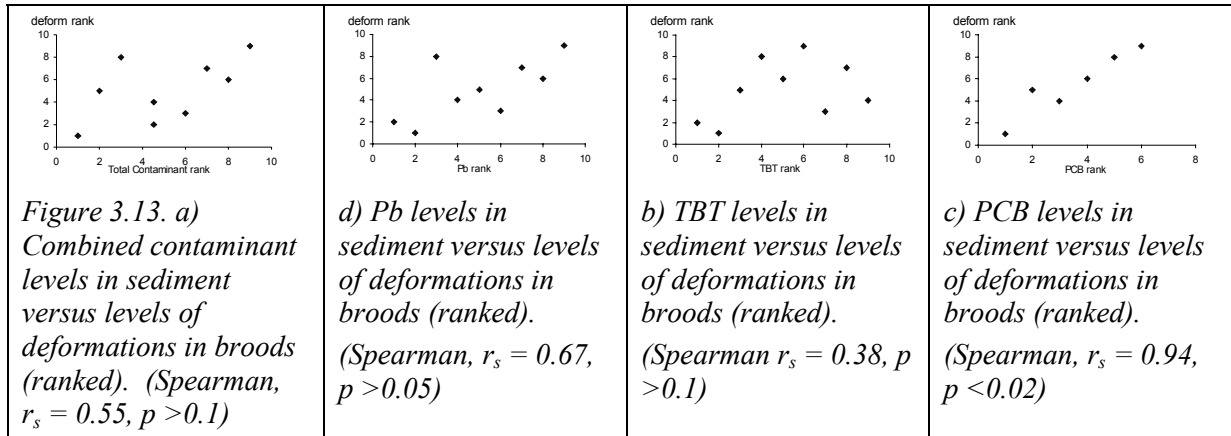
*Table 3.4. Non-parametric ranking (low:1 – high:10) of the contaminant levels in sediment of Hg (24 – 174 ng/g dw), Cd (60 – 1250 ng/g dw), Pb (5500 – 47000 ng/g dw), TBT (<0.5 – 54 ng Sn/g dw), PCB<sub>7</sub> (1.2 – 14.4 ng/g dw), DDT/DDE (0.1 – 5.1 ng/g dw) and PAH<sub>15</sub> (118 – 2302 ng/g dw) compared with the ranking of the proportion of eelpouts with elevated levels of deformations in the broods from the same areas. Contaminant data from Pedersen et al. (2001) and unpubl. data from the regional amts.*

Area	Hg rank	Cd rank	Pb rank	TBT rank	PCB <sub>7</sub> rank	DDT/E rank	PAH <sub>15</sub> rank	Combined contaminant rank *	Type of area	Deform rank
Fakse	8	5	1	1	<i>n.d.a</i>	<i>n.d.a</i>	7	4.5	II	2
Nivå	1	1	2	2	1	1	1	1	II	1
Nakskov	2	6	7	8	<i>n.d.a</i>	<i>n.d.a</i>	8	7	I	7
Frederiksværk	7	8	9	6	6	5	4	9	I	9
Roskilde	9	7	8	5	4	6	5	8	I	6
Lindø	6	4	4	9	3	3	2	4.5	I	4
Odense	5	2	3	4	5	4	6	3	I	8
Århus	3	3	5	3	2	2	3	2	I	5
Ålborg	4	<i>n.d.a</i>	6	7	<i>n.d.a</i>	<i>n.d.a</i>	<i>n.d.a</i>	6	I	3
Egense	<i>n.d.a.</i>	<i>n.d.a</i>	<i>n.d.a</i>	<i>n.d.a</i>	<i>n.d.a</i>	<i>n.d.a</i>	<i>n.d.a</i>	-	II	(1)

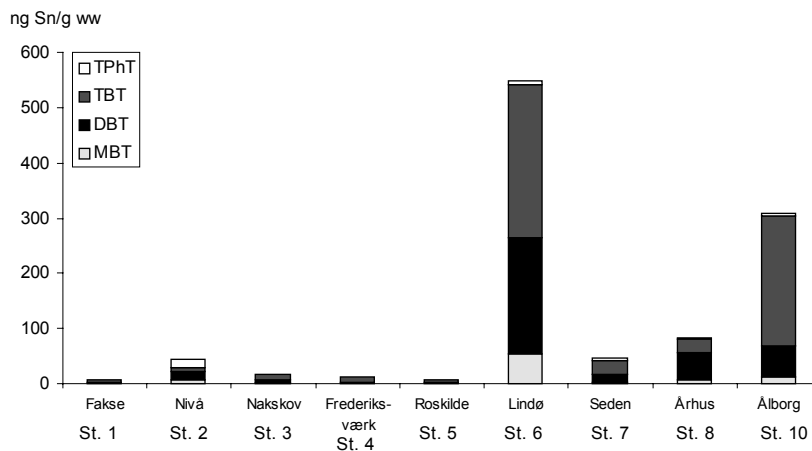
*n.d.a.: no data available. \* Ranking of the sum of rank of each substance, which are weighted equally.*

When comparing the integrated ranking of contaminant level in the areas in Table 3.4 there seems to be a good consistency with the categorization of the contaminant levels in the areas based on sources and geographical conditions (Table 3.3). However, in the area at St.7 (Odense) and St. 8 (Århus) and relatively low levels of contaminants (combined rank of 2 and 3 respectively) were present in the sediment, even though these areas are situated in coastal waters just outside a harbour to a large city, why they were expected to be a relatively highly contaminated areas (e.g. type I). A reason may be that the sediment samples included in the analysis in Table 3.4 are not that representative for the areas.

When comparing the levels of the specific contaminants in sediment, PCB was found to be the only substance, which was significantly associated with the proportion of broods with elevated levels deformations (Figure 3.13a-d), suggesting that PCB can be the most important factor. But this analysis is attached which a high degree of uncertainty since the weight of evidence for a single stress factor only is relatively weak.



In addition, TBT does not seem to be an important factor for the development of deformed larvae in the broods, because of the lack of relationship between deformations and butyltin burdens in the liver of the female eelpouts. The highest butyltin levels of 543 and 304 ng Sn/g ww) was found at St. 6 and St.10, respectively (Figure 3.14), both areas where low levels of deformed larvae occurred in the broods. In comparison, only between 9 and 42 ng Sn/g ww was found at St. 3, 4, 5 and 7, where relatively high levels of deformations occurred in the broods.



*Figure 3.14. Organotin in pooled samples of liver from female eelpouts from nine of ten sampling areas. (Strand, unpubl.)*

The studies of the eelpout broods from the ten sampling areas showed that also late dead larvae, which had died without visible gross abnormalitie, often occurred in addition to deformed larvae. In some broods it was evident that several larvae had died almost at the same time since they were similar in length. This occurred particularly in two of the sampling areas, at Lindø 2001 and at Århus 2002 (Figure 3.15). By analysing the regional monitoring data of oxygen profiles from the different areas in August – December (MADS database, 2003), it could be established that both these areas were affected by oxygen depletion events prior the sampling time (Figure 3.16a,b).

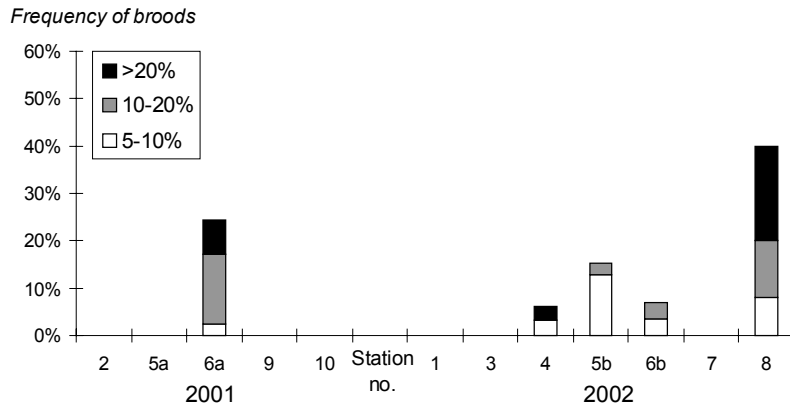


Figure 3.15. The proportion of examined broods with elevated levels (5-10%, 10-20% and >20%) of late dead larvae without visible deformations (type A) in the ten sampling areas in 2001 and 2002.

Data from Manuscript V.

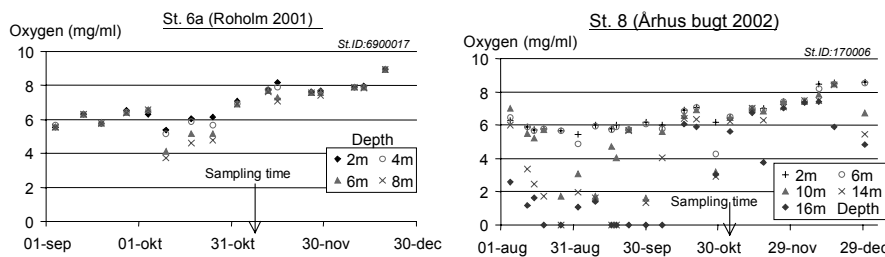


Table 3.16a,b. Regional monitoring data of oxygen levels at Lindø 2001 (St. 6a) and Århus 2002 (St. 8), which were affected by oxygen depletion prior to the sampling (measured with CTD). Data from MADS database, 2003)

A Swedish study has made a similar observation in a fiord on the Swedish westcoast after a prolonged time with eastern winds and warm temperatures (Veteema, 1999). In this study the elevated levels of late dead larvae were related to the temperature, since no information of oxygen conditions were provided, but such weather conditions could very likely also have caused oxygen depletion in that area. In a minor pilot experiment, where pregnant females were exposed to oxygen depletion for 1 hour over two periods, it was shown that oxygen depletion can cause acute death of eelpout larvae (Manuscript V). The acute larvae mortality occurred without killing any pregnant females. There were 30 - 95% dead larvae in nine of ten broods compared to 0 - 10% in the ten females, which were treated as controls. These preliminary experiments indicate that larvae are more vulnerable to oxygen deficiency than adult fish, and that the impact of the larvae seems possible to differentiate from other deformities. If so, developmental effects in broods in the eelpout can be used as a retrospective biological tool to integrate monitoring on the impact of hazardous substances and eutrophication on fish reproduction in the marine environment.

### 3.4. Toxicity of TBT in a sediment bioassay with *Corophium volutator*

Sediment bioassays are in several countries (NL, UK, USA etc.) used to evaluate the toxicity of dredged materials including harbour sediments, which are going to be discharged in marine coastal waters. This method is often in practise included in the assessment of contaminant-

levels in the dredged material to evaluate if it poses a risk, apart from the physical disturbance due to the disposal activities, for benthic fauna populations at the dumpsite. Sediment bioassays are generally used as a supplement to the chemical analyses of concentration levels of hazardous substances, because the bioassays are expected to reflect the toxicity and bioavailability of the sum of toxic substances, identified as well as unidentified. A widely used sediment bioassay is with the benthic amphipod *Corophium volutator*, where the mortality is observed after a ten day exposure to the sediment (ISO, 2001). *C. volutator* is an abundant amphipod in tidal and shallow water sediments in the Danish marine environment, where it is as an important prey item in the food web.

Table 3.5. Toxicity classification based on sediment bioassay with *Corophium volutator* as used in assessment of dredged materials in the Netherlands (Stronkhorst, 1998).

Species	Parameter	Non-toxic	Toxic	Highly toxic
<i>C. volutator</i>	% mortality	<20%	>20% - <50%	≥50%

The Netherlands, among other countries, has based assessment criteria of dredged materials on this kind of *Corophium* bioassay (Table 3.5.), e.g. if there is more than 50% mortality of *C. volutator*, the dredged material is classified as highly toxic, and it is considered to pose a too high a risk to be discharged in coastal waters. The sediment is classified as non-toxic if less than 20% die. Results in between these values require some supplementary chemical or ecotoxicological tests before the toxicity can be assessed (Stronkhorst, 1998).

To evaluate the significance of TBT contamination in this type of risk assessment of marine sediments, a small study (Strand, unpubl.) was made to assess the toxicity of sediments with known TBT concentrations from Danish waters, e.g. sediments sampled inside and outside harbours areas. In addition, two types of sediments, with a high and a low content of organic matter, from relatively uncontaminated areas were spiked with TBT at various concentrations. The bioassays were performed according to the international standard guideline (ISO, 2001) using immature *C. volutator* (length 3 - 4 mm) sampled in Roskilde fiord 2000.

The bioassays with the TBT-spiked sediments revealed that only relatively high TBT concentrations caused increased mortality of *C. volutator*;  $LC_{50} = 570$  ng Sn/g dw and  $LC_{50} = 1030$  ng Sn/g dw were found in two sediments with a low and high content of organic matter, respectively (Figure 3.17). The higher  $LC_{50}$ -value in the sediment with a high content of organic matter may reflect a reduced toxic activity of TBT due to a reduced bioavailability of TBT associated with organic matter (Meador, 2000). At higher TBT concentrations, e.g. 1725 and 2650 ng Sn/g dw, respectively, all specimens had died within the ten day exposure period.

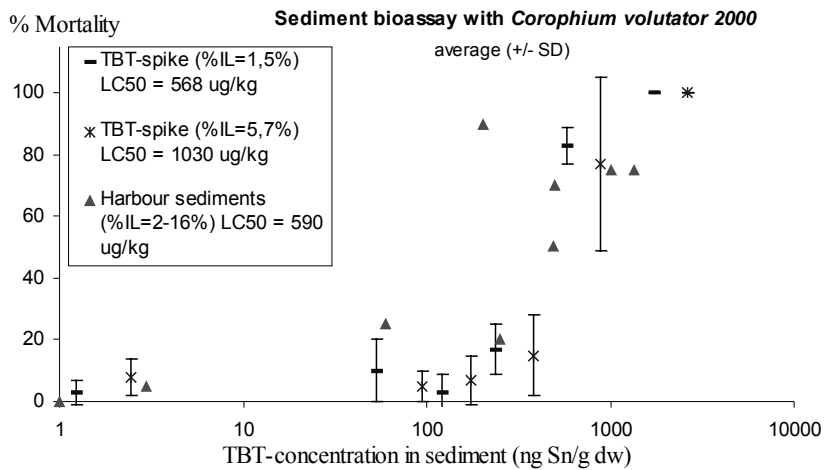


Figure 3.17. Mortality of *Corophium volutator* in relation to TBT concentrations in sediment.

TBT-spiked sediments compared with "real" sediments.

10 days exposure period of 20 immature *C. volutator* per sample (Strand, unpubl.).

Compared with the TBT concentration in the non-spiked sediments, it seemed that TBT could be the main contributor to the toxic activity in most of these sediments. Four harbour sediments with TBT concentrations between 490 and 1350 ng Sn/g dw could be classified as highly toxic with a mortality between 50 to 75% (Figure 3.17). The mortality in only one of seven harbour sediments was higher than TBT could account for (90% mortality and TBT concentration at 205 ng Sn/g dw). The sediment toxicity of this sample may be ascribed to other types of contaminants like high levels of for instance PAH, heavy metals or organochlorines, which can occur in some Danish harbour areas (Danish EPA, 2001).

Another study on *C. volutator* exposed to TBT spiked sediments reported that highly toxic effects occurred at even higher TBT concentrations,  $LC_{50} = 1890 - 2530$  ng Sn/g dw (Stronkhorst et al., 1999). However, the effect levels in the two studies can be difficult to compare directly, since the bioavailability is influenced by the sediment characteristics and since immature specimens were used in the study reported here and adults in the Dutch study by Stronkhorst et al. (1999).

The toxicity of TBT contaminated sediments in the bioassay demonstrates that TBT is a significant risk for *C. volutator*, but direct TBT-associated mortality will only occur in sediments from the highly contaminated harbour areas. However, mortality as an endpoint is quite an insensitive parameter compared to other less adverse effects, such as reduced growth and fecundity. In addition, *C. volutator* can probably not be regarded as one of the most TBT sensitive species compared to for example molluscs. Sediment bioassays with more sensitive endpoints or species, for instance growth inhibition of sediment-dwelling invertebrates (Meador, 2000), avoidance (Bat et al., 1998) or imposex development or reduced fecundity in gastropods (Oehlmann, 2000; Duft et al., 2002), seem therefore to be more appropriate if the risk of TBT contaminated sediments for the ecosystem should be assessed. An assessment of dredged materials based solely on results of the *Corophium* bioassay can therefore not be

recommended if the risk of adverse effects caused by long-term exposure of TBT to the benthic community at the dumpsite and adjacent areas also should be taken into account. Cut-off values based on chemical analyses of TBT concentrations and/or more sensitive bioassays are recommended. Further, dilution effects of the sediment due to the physical conditions at the dumpsite and cost-benefit analyses, and not only ecotoxicological criteria, are also parameters likely to be taken into account in the development of assessment criteria and management for disposal of dredged materials (Danish EPA, in press).

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#### 4. TBT in the Arctic marine environment

The Arctic environment is generally considered to be one of the most pristine regions on the Earth since the Arctic is populated by few people, has little industrial activity and is remote from the industrialised parts of the world. The Arctic can therefore be regarded as relatively unaffected by human activity.

However, various kinds of persistent organic pollutants (POPs) are present throughout the Arctic environment, and elevated levels have been detected in Arctic wildlife and humans, which is why their potential toxic effects give rise to concern (AMAP, 2002). The presence of POPs in the Arctic can mostly be ascribed to long range transport from mid-latitude industrial and agricultural areas even though human activities may contribute locally to elevated contamination levels, for instance due to mining and traffic activities, dump sites, waste combustion and city effluents (AMAP, 2002). In addition, since the degradation of POPs is slow, especially in cold climates, sediments in the Arctic have potential for acting as long term reservoirs.

TBT has been recognised to belong to the group of POPs (AMAP, 2002), and one aim within this thesis was also to study the TBT distribution in the Greenlandic environment. A survey along the coastline of West Greenland showed that elevated TBT levels are very local and seem only to occur in relation to harbour areas (Manuscript IV), where the TBT contamination level was found to be related to the intensity of shipping activities (Figure 4.1). Consequently, TBT-induced effects expressed as imposex in the prosobranch neogastropods *Buccinum finmarchianum* and *B. undatum* occurred only inside or very close to harbours (Figure 4.2; Manuscript IV). No imposex was found in neogastropods sampled outside the West Greenlandic harbours in this study, which supports the earlier findings that the development of imposex does not occur naturally and therefore most likely is an anthropogenic induced phenomenon in neogastropods. However, in a recent study (Strand et al., 2003) at Thule Air Base, an area in Northwest Greenland locally contaminated with various types of organic pollutants, a more widespread occurrence of imposex in gastropods, mainly *B. finmarchianum*, was found (Figure 4.2). The highest incidence of imposex, e.g. in 77% of the *Buccinum* females, occurred at station PM6, where imposex also was found in one of seven females of *Colus sabini*. Compared to chemical analyses, PM6 was the only station at Thule where detectable concentrations of butyltins were found in whelks and bivalves with body burdens of 0.8 and 1.5 ng Sn/g ww, respectively. Imposex was also present at seven of the other stations (Figure 4.2), where butyltin not could be detected (<0.5 ng Sn/g ww), indicating that imposex in the Arctic *Buccinum* neogastropods is a more sensitive biomarker of TBT than the detection limits of the analytical chemistry can achieve.

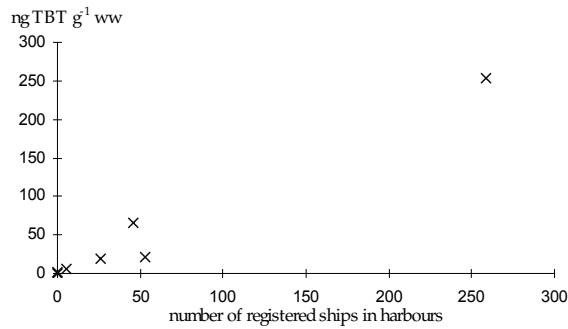


Figure 4.1. The TBT-concentration in the bivalve, *Mytilus edulis*, is correlated to the number of registered ships in the Greenlandic harbours (Spearman rank,  $r_s = 0.95$ ,  $p < 0.02$ ). From Manuscript IV.

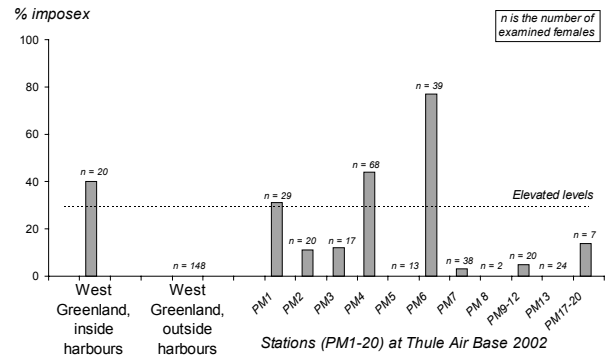


Figure 4.2. The frequency of imposex in female *Buccinum* sp. sampled inside and outside West Greenlandic harbours and at eleven stations at Thule Air Base in Northwest Greenland. Data from Manuscript IV and Strand et al. (2003).

Within this thesis it has been possible to demonstrate that TBT and its metabolites are accumulated in marine organisms living in the open waters of the Arctic. By choosing suitable bioindicators with a high accumulation potential like the deposit-feeding bivalve, *Nuculana pernula*, (Figure 2.6) or small cetaceans like harbour porpoise and pilot whale (Figure 4.3), it can be estimated that the TBT contamination outside harbour areas is 20 to >500 times lower in the Arctic marine environment than in Danish waters (Manuscript II, III & IV). A pilot study on five specimens of polar bears sampled in East Greenland 2001 showed that no butyltin residues were present in the livers (<0.3 ng BT/g ww) (Figure 4.3). These results indicate either a low accumulation potential in polar bear compared to cetaceans and/or an extremely low exposure level probably due to that they are feeding mainly on seal blubber, where only small organotin residues are deposited (see Figure 3.8), and/or that the shipping traffic is very scarce in the east Greenlandic region of the Arctic.

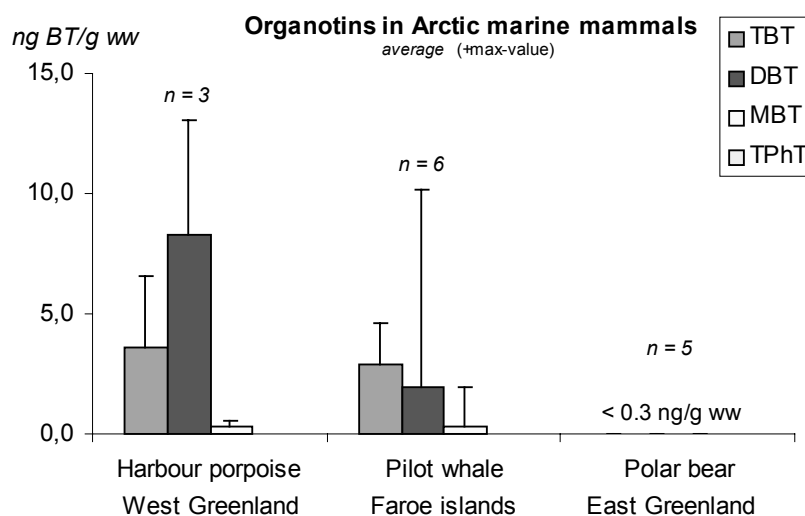


Figure 4.3. Organotin concentrations in livers from Arctic marine mammals. The concentrations in the three species can not directly be compared, because they are sampled in different regions of the Arctic.  $n$  is the number of specimens analysed. Data from Manuscript III, Strand & Nielsen (unpubl.) and Strand & Sonne-Hansen (unpubl.).



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The presence of TBT in organisms living in open marine waters in the Arctic can probably be ascribed to diffuse sources rather than to outflows from the local harbour areas. The following three transport routes may also be possible sources of organotin compounds in the Arctic marine environment;

- Shipping traffic in open waters such as cross-Atlantic shipping routes and ships with destinations in harbours situated in the Arctic. Such traffic will result in a deposition of organotin compounds in the open waters.
- Long range transport with ocean currents from contaminated coastal waters in America, Europe and Asia. The role of ocean currents is probably more important for contaminant levels than previously thought. Water-soluble chemicals may reach the Arctic environment primarily via ocean currents (AMAP, 2002).
- Long range atmospheric transport of volatile organotin compounds evaporated from coastal waters in the industrialised regions. The presence of volatile methylated butyltin compounds, like tributylmethyltin, in European coastal sediments and waters has been demonstrated. Contaminated sediments can therefore be regarded as a continuous source of volatile organotin compounds to water and atmosphere with estimated export fluxes from estuarine sediments of  $4 - 400 \text{ nmol m}^{-2} \text{ y}^{-1}$  (Tessier et al., 2002). In consequence, this can lead to an atmospheric export from contaminated to adjacent areas. Potentially, this may also include long range transport to remote areas in the Arctic environment since the transport mechanism may be similar to the processes assumed for other volatile and semi-volatile contaminants such as mercury or organochlorines (AMAP, 2002).

In conclusion, due to the presence of elevated TBT levels in harbour areas, TBT may only pose a threat to some local Arctic wildlife populations such as molluscs. TBT is also distributed more widely in the Arctic marine environment but at levels, which currently do not seem to pose a risk to resident species.

## **5. Combining chemical and biological elements in a five-class scheme of assessment criteria for TBT**

In this chapter, a five-class scheme of assessment criteria for TBT based on the objectives in the strategies for priority hazardous substances within OSPAR and the EU Water Frame Directive (WFD) will be developed.

The derived assessment criteria are intended to combine chemical and ecotoxicological derived quality standards for TBT concentrations in seawater, sediments and mussels with TBT-specific biological effect parameters, e.g. imposex and intersex in gastropods, into an integrated scheme useful for evaluating the risk of impact of the TBT contamination in the marine environment with special attention to the Danish waters, e.g. coastal as well as open sea areas. Thereby various types of data available can be used in an intergrated assessment of the TBT contamination in the Danish waters. The data presented in the previous chapters and the papers I - V are used to verify the consistency between the different parameters included in the developed five-class scheme of assessment criteria for TBT.

### **5.1. Current quality standards for TBT in the marine environment**

The triorganotins, TBT and TPhT, have for more than a decade been identified as highly toxic and persistent substances, which can induce adverse effects in the ecosystem. They have therefore been adopted to the priority lists of hazardous substances by OSPAR, HELCOM and the WFD. The overall objectives in the strategies for priority hazardous substances within OSPAR, HELCOM and WFD are to reach concentrations “near zero” for anthropogenic substances in the marine environment to achieve “high status” which implies undisturbed environmental conditions. In practice, this means that the detection limit may serve as a borderline quality standard in order to prevent pollution of the open sea. However, ecotoxicological criteria have also to be taken into account when assessing the significance of contaminant levels in the environment. The general principles for the derivation of ecotoxicological threshold levels are described in Paragraph 1.3.

Invertebrates, especially molluscs and crustaceans, have been identified to be at risk from TBT, since concentrations in the range of 1 - 10 ng TBT/l can induce adverse effects on growth or reproduction (Table 5.1). Subsequently, these kinds of data have provided the basis for the derivation of the ecotoxicological threshold levels of TBT.

Table 5.1. Some examples of toxicity data for invertebrates exposed to TBT in seawater relevant for the development of assessment criteria. These studies has also been included in the deviation of the chemical quality standards for TBT, e.g. EQS, MAC-QS (Lepper, 2002) and EAC (OSPAR, 1996), respectively.

Species	Endpoint	TBT conc. (ng TBT/l)	Reference
Bivalve <i>Crassostrea gigas</i>	LOEC reduced growth	5	Nell & Chrojka, 1992
Bivalve <i>Mytilus edulis</i>	LOEC reduced growth	3	Stenalt et al., 1998
Gastropod <i>Buccinum undatum</i>	LOEC reduced growth	8	Mensink et al., 2002
Gastropod <i>Nucella lapillus</i>	NOEC VDSI ~3, imposex	<1.2 (detec. limit)	Bryan et al., 1987
Gastropod <i>Nucella lapillus</i>	LOEC VDSI >4, sterile females	~5 (detec. limit)	Stroben et al., 1995
Copepod <i>Arcasia tonsa</i>	LOEC reduced growth	3	Kusk et al., 1998
Copepod <i>Arcasia tonsa</i>	LC50 mortality	15	Kusk et al., 1998

LOEC (Lowest-observed effect concentrations), VDSI (Vas deferens sequence index)

OSPAR has estimated NOEC-values of 0.4 and 0.5 ng TBT/l based on results from studies on imposex development in the gastropod *Nucella lapillus* and growth inhibition in different species of molluscs, respectively (OSPAR, 1996). The identified NOEC-values were used by OSPAR to propose an Ecotoxicological Assessment Criterion (EAC) of 0.01 - 0.1 ng TBT/l. Likewise, in the WFD an Environmental Quality Standard (EQS) for TBT at 0.1 ng TBT/l has been proposed also based on PNEC-values estimated from NOEC ~ 1 ng TBT/l for imposex in *N. lapillus* and with application of factor ten as precaution (see Paragraph 1.3). EQS is in the WFD referring to an annual average concentration in seawater (Lepper, 2002). Subsequently, it is predicted that below the EQS adverse effects due to long-term exposure to TBT are unlikely to occur in the marine ecosystem. In addition, the PNEC value has been verified by the 5-percentile cut-off value calculated by using statistical extrapolation in a species sensitivity distribution (SSD) resulting in a  $PNEC_{SSD} = 0.18$  ng TBT/l after using 5 as application factor. This value is not regarded as significantly different from the PNEC derived by the assessment factor method. It is therefore deemed reasonable to use the  $PNEC = 0.1$  ng TBT/l (Lepper, 2002). The derived EQS is, however, regarded as provisional due to a lack of appropriate toxicity data on secondary poisoning of mammals and birds (Lepper, 2002).

In addition, in the WFD a so-called Maximum Admissible Concentration Quality Standard (MAC-QS) for TBT of 1.5 ng TBT/l has been derived (Lepper, 2002). MAC-QS refers to “short-term transient exposure” and is intended not to be exceeded at any time. MAC-QS is derived on the basis of the lowest acute toxicity test available and  $LC_{50} = 15$  ng/l for the

crustacean *Acartia tonsa* (Table 5.1) has been accepted as the relevant value (Lepper, 2002). Sublethal endpoints such as growth inhibition have not been considered to be a relevant short-term adverse effect, but MAC-QS = 1.5 ng TBT/l is derived after an uncertainty factor of 10 is applied. Below MAC-QS adverse effects due to short-term exposure are predicted to be unlikely in the marine ecosystem.

EAC- or EQS-values have, to my knowledge, not been derived for TPhT within the frameworks of OSPAR or WFD.

## 5.2. Combining chemical and biological quality elements in a five-class scheme of assessment criteria for synthetic priority substances

In the quality objectives within OSPAR and the WFD both the chemical and biological status of the environment should be assessed by using both physico-chemical and biological quality elements. The definitions of the physico-chemical and biological quality elements in the WFD are listed in Table 5.2 and Table 5.3, respectively. The assessment shall identify if the chemical and biological status is high, good, moderate, poor or bad as the worst case, and the member states shall achieve compliance with any standards and objectives (e.g. achieve “good status”) at the latest of 15 years after the date of entry into force of the Directive (EC, 2000) in 2007.

The WFD physico-chemical and biological quality standards are intended to protect the structure and function of ecosystems in transitional, coastal and territorial marine waters (and freshwater) from any significant alterations by the impact of hazardous substances. According to current scientific knowledge, the objective of maintaining ecosystem function can be best achieved by protecting the community structure, i.e. species diversity, abundance and seasonal dynamics. Thus, not only toxic effects or adverse effects on growth or reproduction should be considered when assessing possible impacts on community structure by a chemical, but all relevant effects on the population dynamics and abundance of species. Effects on behaviour or avoidance of the habitat should for instance also be accounted for. It is generally accepted that community structure is preserved by protecting the most sensitive species known and by accounting for additional uncertainties due to limitations of the data available (Lepper, 2002).

Table 5.2. Physico-chemical quality elements for specific synthetic pollutants. Definitions from WFD annex V to the directive 2000/60/EC (EC, 2000).

High status	Good status	Moderate status
Concentrations close to zero and at least below the limits of detection of the most advanced analytical techniques in general use.	Concentrations not in excess of the standards set in accordance with the procedure in the Technical Guidance Document (TGD) for WFD, e.g. concentrations below EQS.	Conditions consistent with the achievement of the values specified for the biological quality elements.

Table 5.3. *Biological quality elements for benthic invertebrate fauna as an example of a relevant biological community. Definitions from WFD annex V to the directive 2000/60/EC (EC, 2000).*

<b>High status</b>	<b>Good status</b>	<b>Moderate status</b>	<b>Poor status</b>	<b>Bad status</b>
The level of diversity and abundance of invertebrate taxa is within the range normally associated with undisturbed conditions. All the disturbance-sensitive taxa associated with undisturbed conditions are present.	The level of diversity and abundance of invertebrate taxa is slightly outside the range associated with the type-specific conditions. Most of the sensitive taxa of the type-specific communities are present.	The level of diversity and abundance of invertebrate taxa is moderately outside the range associated with type-specific conditions. Taxa indicative of pollution are present. Many of the sensitive taxa of the type-specific communities are absent.	The relevant biological communities deviate substantially from those normally associated with undisturbed conditions.	Large portions of the relevant biological communities normally associated with undisturbed conditions are absent.

However, there appears to be a disagreement between the protective targets of the chemical and biological quality objectives in the WFD to achieve “good status” (Table 5.2; Table 5.3). In the physico-chemical quality for “good status” EQS aims at protecting the whole ecosystem from any impact of priority substance (part of the rationale behind the method for setting EQS with application factors), whereas in the biological quality element a slight impact on diversity and abundance of for instance benthic invertebrate fauna are accepted as “good status”. The reason for these differences could reflect different traditions of risk assessment, which probably originate from the different types of data material assessed. The physico-chemical quality elements are derived from ecotoxicological studies performed mainly under controlled laboratory conditions, whereas the biological quality elements are derived from field-based interpretations of benthic communities. Generally when assessing the biological quality in the environment, for instance in relation to eutrophication problems, the natural variability of the biological elements, which includes spatial and temporal variations in populations and communities, has to be accounted for. Slight changes in the ecosystems are therefore considered as acceptable in the biological quality elements, probably because it is difficult to distinguish between natural variations and variations, which arise from the impact of anthropogenic factors. Another reason may also be that minor changes in populations and communities due to for instance eutrophication problems are considered in some degree to be reversible.

In the ecotoxicological risk assessment of priority substances by the use of EQS means that significant alterations by the impact from hazardous substances are not tolerated on population level where the whole ecosystem should be protected. This includes that uncertainty in the data

material available also is accounted for as a precaution to protect the most sensitive species. Another reason for that impact is not tolerated at population level is probably that the nature of this problem is considered as less reversible. If persistent contaminants appear in the environment, they will often remain there for decades and thereby continuously pose a threat. Therefore the acceptance of “a slight impact on diversity and abundance” as defined in the biological quality elements to achieve “good status” will not be suitable to assess the impact of hazardous substances caused by long-term exposure, since at the time when hazardous substances have caused chronic effects on populations, the changes can be considered as almost irreversible. It could therefore be questioned if the descriptions of the biological quality elements are operative at present. Therefore the definition of “good status” will in the following assessment be based on the ecotoxicological derived chemical quality elements (Table 5.2) and be achieved, if the concentration in the environment does not exceed the EQS derived in the WFD (Lepper, 2002). This is also in line with the WFD as it is stated “where more than one of the objectives relates to a given body of water, the most stringent shall apply” (EC, 2000). Based on the discussion above, the following approach based on the principles described in Table 5.4 are suggested for derivation of a five-class scheme for assessment criteria for synthetic priority substances, which take into account both the objectives for the chemical and the biological quality elements within the WFD and OSPAR. The main objective of this approach is to harmonize the protection targets in the assessment criteria for the physico-chemical and biological quality elements, which, I think, will be most beneficial for combined monitoring and assessment programmes. The status classes I, II and III will refer to the chemical quality standards defined in the WFD, e.g. near zero concentration, EQS and MAC-QS, and the status classes IV and V are derived on basis of the LC<sub>50</sub>-value used in extrapolation of the MAC-QS-value.

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*Table 5.4. Suggestion to an approach for the derivation a five-class scheme of assessment criteria for synthetic priority substances, which combines physico-chemical, ecotoxicological and biological elements.*

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**Status class I: High status**

Concentration of synthetic priority substances is close to zero and at least below the limits of detection of the most advanced analytical techniques in general in use. This reflects the objectives in the WFD and OSPAR strategy of hazardous substances for protection of the open sea.

An assessment of the achievement of the objective also requires that no biological effects at the individual as well as population level, which can be related specifically to exposure to the priority substances, can be detected. E.g. the response must not be significantly different from the natural background level.

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**Status class II: Good status**

Concentration of priority substances is not in excess of the chemical quality standards, e.g. below the ecological quality standard ( $< \text{EQS} = < \text{PNEC}$ ).

Adverse effects in the most sensitive species caused by long-term exposure are assessed to be unlikely to occur.

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**Status class III: Moderate status**

Concentration of priority substances is not in excess of the lowest-observed-effect concentration and/or the so-called maximum admissible concentration quality standard ( $< \text{LOEC}$  or  $< 1/10 * \text{LC}_{50} = < \text{MAC-QS}$ ).

Moderate deviations of biological communities may occur, because there is a risk of adverse effects caused by long-term exposure in the most sensitive species. However, adverse effects in the most sensitive species caused to short-term exposure are assessed to be unlikely to occur in the marine ecosystem.

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**Status class IV: Poor status**

Concentration of priority substances is not in excess of  $\text{LC}_{50}$ -value derived for the most sensitive species ( $< \text{LC}_{50}$ ).

Substantial deviations of biological communities can occur, because there is evidence of adverse effects caused by long-term exposure. In addition, there is a risk of adverse effects caused by short-term exposure in the most sensitive species.

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**Status class V: Bad status**

Concentration of priority substances is in excess of  $\text{LC}_{50}$ -value derived for the most sensitive species ( $> \text{LC}_{50}$ ).

Severe alterations of biological communities occur due to adverse effects caused by long-term exposure in the more and less sensitive species. In addition, there is a risk of adverse effects caused by short-term exposure in both the more and less sensitive species.

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**NOTE.** *All thresholds refer in this scheme to mean concentrations and not exposure level, which can cause acute effects in short-term episodic events. Addressing acute effects by mean concentrations as in status class III, IV and V may therefore not be that consistent, but the definition of the status classes should rather be seen as a potential tool useful for the interpretation of monitoring data and the development of monitoring strategies.*

### 5.3. Derivation of a five-class scheme of assessment criteria of TBT in seawater, mussel and sediment

On basis of the definitions of the five status classes in the assessment criteria presented in Table 5.4 and the values derived for EQS/EAC and MAC-QS for TBT in the WFD (Lepper, 2002) and by OSPAR (1996), presented in Paragraph 5.1 the following five status classes according to ambient TBT concentrations can be suggested (Table 5.5).

Table 5.5. Five-class scheme for assessment criteria for TBT concentrations in seawater.

Status class	I	II	III	IV	V
TBT conc. (aq)	close to zero	< 0.1 (ng TBT/l)	0.1 – < 1.5 (ng TBT/l)	1.5 – 15 (ng TBT/l)	> 15 (ng TBT/l)
Thresholds used	OSPAR objective	< EQS	EQS – < MAC-QS	MAC-QS – < LC <sub>50</sub>	> LC <sub>50</sub>

However, most data available on TBT concentrations in from Danish waters is not measured in seawater but in sediments and the bivalve *Mytilus edulis* (Paragraph 2). It will therefore be most relevant to include such data in the derivation of assessment criteria for TBT. It is possible to extrapolate to nominal TBT concentrations in biota and sediment from the derived ambient TBT concentrations in seawater by using the equilibrium partitioning principle and derived partition coefficients for TBT, e.g. the bioconcentration factor (BCF) in biota and the equilibrium partitioning coefficient in sediment ( $K_p$ ).

For the bivalve *M. edulis*, a geometric mean of BCF for TBT has been estimated to be 116000 l/kg dw (OSPAR, 1996), although a great uncertainty is related to this value. In literature the variance in value covers one – two orders of magnitude and the BCF generally decrease with increasing exposure concentrations. The five status classes for TBT concentrations in seawater in Table 5.5 can thereby be transformed to the corresponding five status classes for TBT concentrations in *M. edulis*, when the average dry weight content at 15% of the wet weight in *M. edulis* analysed in NOVA 2003 (MADS database, unpubl.) is used (Table 5.6). OSPAR has also derived a similar EAC-value of 0.8 ng Sn/g ww for *M. edulis* (OSPAR, 1996).

Table 5.6. Five-class scheme for assessment criteria for TBT concentrations in the bivalve *Mytilus edulis* derived on basis of BCF = 116000 l/kg dw.

Status class	I	II	III	IV	V
TBT conc. in <i>M. edulis</i>	close to zero	< 0.8 (ng Sn/g ww)	0.8 – < 12 (ng Sn/g ww)	12 – 120 (ng Sn/g ww)	> 120 (ng Sn/g ww)

Notice that ng TBT/l in seawater is transformed to ng Sn/g ww in mussel.



Since there can be a high interspecies variance in bioaccumulation potential of TBT in bivalves (see Paragraph 2.2), the five status classes defined according to TBT concentrations in *M. edulis* cannot be used directly to evaluate the TBT contamination in other bivalve species. If other species are to be used, interspecies comparisons of accumulated body burdens of TBT in the *M. edulis* and the other relevant bivalve species should be performed to estimate a transformation factor, which can be applied to the TBT concentrations derived in Table 5.6.

For sediment, the assessment criteria for TBT can be derived by using the equilibrium partitioning coefficient for TBT in sediment,  $K_p$ .  $K_p$  has been estimated to be 400 l/kg dw for sediment when 1% carbon in the sediment is assumed (OSPAR, 1996). A similar  $K_p$  (= 320 l/kg dw) can be derived from the review by Meador (2000). The five status classes for TBT in seawater in Table 5.5 can thereby be transformed to the corresponding five status classes for TBT in sediment (Table 5.7).

*Table 5.7. Assessment criteria of estimated TBT concentrations in sediment derived on basis of  $K_p = 400$  l/kg dw.*

Status class	I	II	III	IV	V
TBT conc. in sediment	close to zero	<0.02 (ng Sn/g dw)	0.02 - < 0.3 (ng Sn/g dw)	0.3 - 3 (ng Sn/g dw)	> 3 (ng Sn/g dw)

*Notice that ng TBT/l in seawater is transformed to ng Sn/g dw in sediment.*

Comparing the TBT levels in sediment in the respective status classes with the literature on sediment toxicity of TBT contaminated sediments, especially the TBT concentrations are too low in the status class IV to cause short-term adverse effects, and in status class V to cause significant mortality in the most sensitive species as defined in Table 5.4. Comparison of the levels of TBT in *M. edulis* and in sediment from various types of areas in Danish waters, described in Chapters 2, also suggest that the estimated TBT concentrations in sediment are far too low compared to the corresponding status classes for TBT concentrations in *M. edulis*. An explanation for these deviations is probably that the  $K_p$  used in the calculation of TBT concentration in sediment, is rather describing the equilibrium coefficient between sediment particles and porewater than the equilibrium coefficient between the sediment and the upper water bodies. In the field it is unlikely that TBT in overlying water would come to equilibrium with sediment-bound TBT, because the overlying water has such a large volume that it acts as an infinite sink (Meador, 2000), but also because the upper and lower water bodies can be totally separated due to stratification by pycnoclines. Using the equilibrium partitioning approach, e.g.  $K_p$ , does therefore not seem appropriate. However, OSPAR and in the WFD the provisional EAC and EQS for sediment have been derived by the equilibrium partitioning

approach achieving EQS = 0.004 ng Sn/g dw (Lepper, 2002) and EAC = 0.002 - 0.02 ng Sn/g dw (OSPAR, 1996).

Another approach to derive assessment criteria for TBT levels in sediment is to use the chemical and ecotoxicological approaches of the five status classes directly as defined in Table 5.4, where the biological endpoints to be included are based on toxicity tests with sediment-dwelling organisms exposed to TBT contaminated sediments. In this way the derivation of assessment criteria for TBT in sediment is based on the same principles as in the derivation of assessment criteria for TBT concentrations in seawater (Table 5.5). The derivation of the relevant TBT concentrations in sediment in the respective five status classes are done by using the same endpoints and application factors as defined in Table 5.4.

A few relevant endpoints to be included in such a kind of derivation of status classes for TBT contaminated sediments are listed in Table 5.8, but these examples cannot be seen as an adequate literature study on sediment toxicity, which is needed to make a more comprehensive hazard assessment.

Table 5.8. Some examples of toxicity data for sediment-dwelling organisms exposed to TBT contaminated sediments.

Species	Endpoint	TBT conc. (ng Sn/g dw)	Reference
Gastropod <i>Potamopyrgus antipodum</i>	LOEC reduced fecundity	10	Duft et al. (2003)
Bivalve <i>Scrobicularia plana</i>	LOEC avoidance (immatures)	300	Ruiz et al. (1994)
Polychaete <i>Armandia brevis</i>	LOEC growth inhibition	42	Meador (2000)
Amphipod <i>Eohaustorius washingtonianus</i>	LOEC growth inhibition	12 (predicted)	Meador (2000)
Amphipod <i>Eohaustorius washingtonianus</i>	LC <sub>50</sub> mortality	200	Meador (2000)
Amphipod <i>Corophium volutator</i>	LC <sub>50</sub> mortality (adults)	1890 - 2530	Stronkhorst et al. (1999)
Amphipod <i>Corophium volutator</i>	LC <sub>50</sub> mortality (immatures)	590 - 1030	Strand (unpubl.)

Based on the effect levels in the toxicity tests with sediment-dwelling organisms presented in Table 5.8, the TBT concentrations in sediment relevant for the derivation of the assessment criteria can be estimated (Table 5.9).

Table 5.9. Ecotoxicological threshold levels of TBT in sediment to be included in the derivation of assessment criteria.

Thresholds	Derivation	Used values	TBT-concentration
EQS <sup>(a)</sup>	= 1/10 * LOEC * 1/2	= 1/10* 10 ng Sn/g dw * 1/2	= 0.5 ng Sn/g dw
MAC-QS	= 1/10 * LC <sub>50</sub>	= 1/10 * 200 ng Sn/g dw	= 20 ng Sn/g dw
LC <sub>50</sub>	= 1 * LC <sub>50</sub>	= 1 * 200 ng Sn/g dw	= 200 ng Sn/g dw

<sup>(a)</sup> Conversion of LOEC \* 1/2 ≈ NOEC, if LOEC >10% and <20% effects (Lepper, 2002).

Subsequently, the five status classes in the assessment criteria for TBT in sediment (Table 5.10) can be derived based on the ecotoxicological threshold levels of TBT estimated in Table 5.9.

Table 5.10. Assessment criteria for TBT contaminated sediment derived from the ecotoxicological data in Table 5.8 & 5.9.

Status class	I	II	III	IV	V
TBT conc. in sediment	close to zero	< 0.5 (ng Sn/g dw)	0.5 - < 20 (ng Sn/g dw)	20 - < 200 (ng Sn/g dw)	> 200 (ng Sn/g dw)

The TBT concentrations in the five status classes in Table 5.10 are about two orders of magnitudes higher than the TBT concentrations calculated from equilibrium partitioning approach (Table 5.7). Comparing TBT concentrations in sediments and *M. edulis* in various types of areas in Danish waters (Chapter 2), the assessment criteria for TBT concentrations in sediment derived from the ecotoxicological approach are to a higher degree to be in accordance with the corresponding classification of status classes for TBT concentrations in *M. edulis* (Table 5.6).

However, it has to be recognised that the status classes only have been derived from a limited number of sediment toxicity studies. Another limitation in the derivation of sediment criteria is that the respective TBT concentrations included are based on dry weight content and not normalised to the content of organic matter in the sediment.

Effect levels (based on dry weight content) derived from different studies may be difficult to compare, because sediment characteristics, including the content of organic matter, affect the bioavailability and thereby the toxic activity of TBT in sediment. It has therefore been recommended in comparative studies to normalise the TBT concentrations to a content of 1% TOC in the sediment (Meador, 2000). The suggested five-class scheme for assessment criteria for TBT in sediment in Table 5.10 should therefore be considered as provisional.

It is noteworthy, that mortality as the endpoint in the bioassay with *Corophium volutator*, which is commonly used in assessment of dredged materials, seems only adequate to evaluate if the

sediment can be classified beyond status class V since  $LC_{50}$  lies between 590 and 2530 ng Sn/g dw in this kind of bioassay (Table 5.9).

#### **5.4. Integrating levels of exposure and biomarker responses in the derivation of a five-class scheme of assessment criteria for TBT**

Because of the sensitivity, specificity and causality in dose-response relationships, imposex and intersex can be used as biomarkers to assess the level of TBT contamination and impact on reproduction in marine populations of prosobranch gastropods. The vas deferens sequence index (VDSI) and the intersex stages index (ISI), which describe the severity of these phenomena, can be used to evaluate the effect level in gastropod populations. In addition, several studies have proved that VDSI and ISI, in for instance field populations of *Nucella lapillus* and *Littorina littorea*, respectively, can be used as biological tools to determine the degree of environmental organotin, especially TBT, pollution in the marine environment, which can be expressed as approximate TBT concentrations in seawater (for instance Bryan et al., 1987; Stroben et al., 1992; Huet et al., 1995; Stroben et al., 1995; Oehlmann et al., 1998). Therefore OSPAR has recommended that imposex and intersex should be included in the Joint Assessment and Monitoring Programmes (JAMP) in the participating countries, e.g. including Denmark. *N. lapillus* and *L. littorea* have been identified as the key species but in areas where these species do not occur, as in some estuarine and subtidal areas, other species like *Hinia reticulata*, *Buccinum undatum* and *Neptunea antiqua* have been identified as suitable alternatives (OSPAR, 2003).

Recently there has within the framework of OSPAR been discussions on whether, and how, imposex and intersex can be integrated in a five-step scheme for assessment criteria for biological effects of TBT, which can reflect environmentally relevant concentrations and effects of TBT (Oehlmann 2002; Strand, 2003). It has been debated whether these assessment criteria should be harmonized with the objectives related to the chemical quality elements for priority substances defined for protection of the whole ecosystem (Table 5.2), or whether the assessment criteria more generally should describe the degree of impact on the viability of the populations of the specific gastropod species according to the biological quality elements defined for benthic fauna (Table 5.3). A central question for the development of the assessment criteria is how to take the biological significance of the TBT exposure into consideration and relate it to biomarker responses such as imposex and intersex, which measure effects at the individual level and not necessarily at the population level. A proposal for assessment criteria for biological effects of TBT (Oehlmann, 2002), which was presented to OSPAR in 2002, focused on the derivation of assessment criteria from the objectives defined in the biological quality elements for benthic invertebrate fauna (Table 5.3) rather than the principles behind the physico-chemical

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quality elements. Thereby the levels of concern, e.g. status class III, IV and V, were defined to describe the level of impaired reproduction as a consequence of sterile females, which can be directly related to imposex or intersex development in the respective gastropod populations (Table 5.11).

Because this proposal included *N. lapillus*, which is considered to represent the most TBT-sensitive group of prosobranch gastropod species, the protection of other less sensitive gastropod populations should thereby also have been taken in to account. However, this implies that only evidence of sterility in female gastropods in field populations due to imposex or intersex are valid endpoints in the derivation of the assessment criteria. Thereby the ambient TBT concentrations in seawater associated to the respective status classes in the proposal by Oehlmann (2002) (Table 5.11) are derived as a secondary achievement from the imposex data and are not reflecting the ecotoxicological derived criteria for acceptable TBT concentrations. For instance, the TBT concentration to achieve “good status“ in the assessment criteria is  $< 5$  ng TBT/l, which is 50 times higher than EQS = 0.1 ng/l derived for TBT in seawater. It is even higher than MAC-QS = 1.5 ng TBT/l, which should not be exceeded at any time. In that context it should be recognised that severity of imposex/intersex in gastropod populations probably is reflecting average concentrations of TBT (with some years delay) rather than occasionally peaking levels in the environment. Thereby the proposal by Oehlmann (2002) based on biological effects of TBT (Table 5.11) differs significantly in protection level compared to the protection levels defined in the approach for derivation of assessment criteria for priority substances in general. The general outline of risk assessment of the impact of hazardous substances includes an acceptance of a degree of uncertainty as precaution due to limited data available of the relationships between exposure levels and adverse effects in various taxa of the ecosystem (Lepper, 2002). The approach in the proposal by Oehlmann (2002) has its advantage if these assessment criteria only shall be used in assessing the capability of female gastropods to reproduce in field populations of gastropods. However, it is thereby neglecting the risk that other adverse effects, such as reduced growth and fecundity, in the most sensitive taxa in the marine ecosystem may occur.

The consequence is that the physico-chemical assessment criteria, which are set to protect the whole ecosystem, can classify TBT to be an environmental problem in an area, whereas the assessment criteria for biological effects of TBT in gastropods do not. This inconsistency can provide causes for confusion in the interpretation of the risk of the TBT contamination in the ecosystem. It would therefore be an advantage if the two kinds of assessment criteria for TBT could be harmonized. It would be unwise to develop several assessment criteria for the same chemical using various matrices and endpoints, if the status classes based on these criteria can not be linked to each other, especially when they have potential to do so.

Table 5.11. A proposal by Oehlmann (2002) of assessment criteria for biological effects of TBT in sympatrically living populations of *Nucella lapillus* and *Littorina littorea*.

Ecological status class	ISI or VDSI value	Description	TBT conc. ng TBT/l
<b>I – II</b> <b>(high – good)</b>	ISI < 0.30  VDSI < 4	The effects on <i>Littorina littorea</i> specimens are low and reflect a condition with little signs or low levels of anthropogenic distortion. Sterile females may occur as isolated cases in populations of <i>L. littorea</i> , but no restrictions of the reproductive capability on the population level are assessable. No adverse effects of TBT in sympatrically living <i>N. lapillus</i> at the population level.	< 5
<b>III</b> <b>(moderate)</b>	ISI: 0.30 - < 0.70  VDSI: 4.00 - < 5.00	The effects on <i>Littorina littorea</i> indicate moderate and significant levels of anthropogenic distortion. Reproduction in <i>Littorina</i> populations with only little signs of impairment. However, reproduction is significantly affected in more sensitive taxa of the coastal ecosystem such as <i>Nucella lapillus</i> (percentage of up to 100% sterile females) or other imposex-affected prosobranchs.	~ 5 - 30
<b>IV</b> <b>(poor)</b>	ISI: 0.70 - < 1.20  VDSI ≥ 5.00	The effects on <i>Littorina littorea</i> indicate major alterations and substantial deviations of relevant biological communities from those under undisturbed conditions. Even reproduction of populations of <i>L. littorea</i> is negatively affected with an incidence of up to 30% sterile females. <i>Nucella</i> populations are unable to reproduce (100% sterile females).	> 30 – 40
<b>V</b> <b>(bad)</b>	ISI: ≥ 1.20  VDSI: (expired)	The effects on <i>Littorina littorea</i> indicate severe alterations and the absence of large portions of relevant biological communities, which are normally associated with undisturbed conditions. In most cases, more than 50% of the females in periwinkle populations are sterile. Imposex affected species such as <i>Nucella lapillus</i> and <i>Ocenebrina aciculata</i> have expired.	> 40

To harmonize the chemical assessment criteria for TBT with the biological effects of TBT, an alternative proposal for assessment criteria for biological effects of TBT has therefore been suggested in this thesis. Thereby the protection levels in the assessment criteria will to a higher degree reflect the protection levels in the assessment criteria for priority substances in general as defined in the WFD. In the derivation of the five status classes, imposex in *Nucella lapillus* as a representative of the most sensitive species, have merely been used as a biological tool in the

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association to the respective five status classes defined in Table 5.4. Thereby the assessment criteria are guided by exposure levels of TBT concentrations in seawater as derived in Table 5.5. Thereby the degree of imposex development, defined by VDSI, should not only be seen as a direct evidence of impaired reproduction due to sterility in specific gastropod populations, but rather as tools/measurements useful for protection of the whole ecosystem. The causality in the derived assessment criteria are confirmed in the definitions of status classes IV and V in Table 5.4, which thereby also will reflect the development of sterility and the disappearance of sensitive gastropod populations, respectively.

Status class I, which is associated with biological effects of TBT close to zero on individuals as well as at the population level, is associated with VDSI-values  $< 0.30$  in *N. lapillus* since this species is regarded to belong to the group of gastropods, which has the highest likelihood to develop imposex. Based on statistical analysis it can be shown that a frequency of  $\sim 30\%$  females with imposex (VDSI  $\approx 0.3$ ) is significantly different from zero, when one misjudgement of the presence of imposex in 20 females, is accepted ( $G_{adj} = 4.4$ ,  $p < 0.05$ ,  $2 \times 2$  contingency table). Zero levels of imposex in gastropods from areas with zero concentrations of TBT are based on the following two arguments;

- 1) Imposex development in gastropods is a recent phenomenon, which has been introduced within the last four decades. No imposex development in neogastropods has been found in the literature or in examinations of conserved specimens stored in collections at natural museums, which can provide clear evidence of significant levels of imposex before TBT was introduced as an anti-fouling agent in the marine environment.
- 2) Imposex in gastropods in the marine environment is most likely induced only by TBT and/or TPhT. There is no adequate evidence that other factors in the environment can induce imposex.
- 3) An acceptance of a frequency of  $\sim 30\%$  imposex open for the opportunity that other environmental factors to may also have a minor potential to induce imposex, although evidence of such relationships to my knowledge not yet has been established.

A practical problem with status class II is that the EQS-value = 0.1 ng TBT/l is below the limit of detection with most analytical methods available. It is therefore difficult to establish an experimental or field-derived dose-response relationship at this low level of TBT. However, it can be argued by extrapolation with a sigmoid function for the dose-response relationships derived for less TBT sensitive species that EQS  $< 0.1$  ng/l to some degree is associated with imposex development in populations of *N. lapillus* corresponding to VDSI = 0.30 -  $< 2.0$ . 90 – 100% of females in a gastropod population have developed imposex at VDSI = 2 but generally in less severe stages.

The assessment criteria in Table 5.12 are a modified version of the proposed assessment criteria by Oehlmann (2002), so that the five-class scheme now is focused on reflecting the chemical

quality elements in Table 5.2 and the suggested definitions for assessment criteria for priority substances presented in Table 5.4. The main modification is that the ranges of nominal TBT concentrations in the five status classes have been changed to fulfil the objectives in Table 5.4 and the respective status classes are thereby defined to be associated with the assessment criteria derived for TBT in seawater in Table 5.5.

Status class I is thereby defined so that the TBT concentration and effects, even at the individual level, should be close to zero rather than a direct lack of impaired reproduction, e.g. sterility, in sensitive gastropod populations. In addition, status classes III and IV in the proposal by Oehlmann (2002) are now combined to one status class, e.g. status class IV, now associated with TBT concentrations higher than MAC-QS and below  $LC_{50}$  for the most sensitive species, rather than the proportion of sterile females in the respective gastropod populations. Status class III has been redefined so that  $VDSI >4 - >5$  is associated with TBT-concentrations below MAC-QS. The derivation of the five status classes in Table 5.12 has also been slightly changed compared to a previous proposal on assessment criteria for biological effects of TBT by the author (Strand, 2003).

The ranges of nominal TBT concentrations in the respective status classes have been slightly changed to fulfil the objectives in Table 5.4, which results in that the defined VDSI- and ISI-ranges may underestimate the ambient TBT-concentrations in sea water by a factor 2-3 compared to the approximate relationships established in the proposal by Oehlmann (2002). However, this deviation can be regarded as a minor, but acceptable, uncertainty introduced in the relationship between TBT exposure and the severity of imposex or intersex, since the status classes are defined on basis of differences in TBT concentrations, which are in order of magnitudes.



Table 5.12. Suggestion to assessment criteria for biological effects of TBT, which combine assessment criteria for TBT in seawater and TBT-specific biological effects (Modified from Oehlmann, 2002). Development of imposex (as VDSI) and intersex (as ISI) in the two gastropod species *Nucella lapillus* and *Littorina littorea* are included in this table. *N. lapillus* represents the most sensitive gastropod species in the ecosystem. The TBT concentrations given in the brackets (< x) reflect the TBT concentrations, which have been associated with the higher end of the respective VDSI and ISI ranges in the proposal by Oehlmann (2002).

Status class	TBT conc. (ng TBT/l)	VDSI or ISI	Description of effects in sympatrically populations of <i>Nucella lapillus</i> and <i>Littorina littorea</i> compared to the general biological interpretations, which can derived from Table 5.4.
I	close to zero	VDSI < 0.30  ISI < 0.30	The level of imposex in <i>Nucella lapillus</i> is close to zero (0 - ~30% of females have imposex) indicating exposure to TBT concentrations close to zero, which reflects the objectives in the WFD and OSPAR strategy of hazardous substances for protection of the open waters.  No significant intersex development in <i>Littorina littorea</i> specimens, which indicates effects caused by TBT exposure.  <i>Interpretation:</i> No significant biological effects at the individual as well as population level (includes biomarker responses) can be related to TBT exposure.
II	< 0.1 (EQS)	VDSI 0.30 - < 2.0  ISI < 0.30	The level of imposex in <i>Nucella lapillus</i> (~30 - 100 % of the females have imposex) indicates exposure to TBT concentrations below the EQS derived for TBT.  No significant intersex development in <i>Littorina littorea</i> specimens, which indicates effects caused by TBT exposure.  <i>Interpretation:</i> Adverse effects, such as reduced growth and recruitment, in the more sensitive taxa of the ecosystem caused to long-term TBT exposure, are unlikely to occur.
III	> 0.1 (EQS) - < 1.5 (MAC-QS)  (< 5)	VDSI 2.0 - 4.0  ISI < 0.30	The level of imposex in <i>Nucella lapillus</i> (~90 - 100 % of the females have imposex) indicates exposure of TBT concentrations higher than the EQS but below the MAC-QS derived for TBT.  No significant intersex development in <i>Littorina littorea</i> specimens, which indicates effects caused by TBT exposure.  <i>Interpretation:</i> There is a risk of adverse effects caused by long-term TBT exposure in the more sensitive taxa of the ecosystem. However, adverse effects caused to short-term exposure are unlikely to occur.
IV	1.5 (MAC-QS) - < 15 (LC <sub>50</sub> )  (~5 - < 40)	VDSI > 4.0 - > 5.0  ISI 0.30 - < 1.2	The reproductive capacity is significantly affected in <i>Nucella lapillus</i> . <i>Nucella</i> populations are in some situations unable to reproduce with up to 100% sterile females.  The reproduction in <i>Littorina</i> populations may have signs of impairment. In some situations can up to 30% of females be sterile.  <i>Interpretation:</i> There is evidence of adverse effects in the more sensitive taxa of the ecosystem caused by long-term TBT exposure, because of the presence of sterile females in gastropod populations. In addition, there is a risk of adverse effects in the more sensitive taxa caused by short-term exposure.
V	> 15 (LC <sub>50</sub> )  (> 40)	VDSI -  ISI ≥ 1.2	The imposex-affected populations of <i>Nucella lapillus</i> have disappeared.  In most cases, more than 50% of the females in <i>Littorina</i> populations are sterile.  <i>Interpretation:</i> There is evidence of disappearance of the more sensitive taxa of the ecosystem. In addition, there is a risk of adverse effects in the less sensitive species caused by long- and short-term TBT exposure.

When accepting the principles of extrapolating ambient TBT concentrations to the degree of imposex/intersex in the derivation of status classes I – V as suggested in Table 5.12, it is consequently also possible to include imposex development in other relevant gastropod species, because of the causality between exposure and response levels resulting in that interspecies correlations in VDSI can be established. The buccinid gastropods, *Hinia reticulata*, *Buccinum undatum* and *Neptunea antiqua*, which are relevant bioindicators in Danish waters, can be included in the assessment criteria. However, interspecies comparisons of tidal and subtidal species should be treated with some caution because tidal species are primarily exposed to the contamination in the upper water bodies, while subtidal species are rather exposed to bottom water as well as the sediment.

Comparative studies on co-existing populations of *H. reticulata* and *N. lapillus* populations have shown that *H. reticulata* is less sensitive to TBT than *N. lapillus* (Stroben et al., 1992; Huet et al., 1995; Barroso et al., 2000). The findings suggest that VDSI ~ 2 in *H. reticulata* corresponds to VDSI ~ 4 in *N. lapillus*. In addition, VDSI = 4 in *H. reticulata* corresponds to VDSI = >4 - >5 in *N. lapillus*, where reproduction is significantly affected and in some situations the gastropods are entirely absent. At VDSI <2 in *N. lapillus*, only insignificant levels of imposex are generally developed in *H. reticulata*.

According to the comparison between *H. reticulata* and *B. undatum*, it has been suggested that the TBT sensitivity in the two species is almost at the same level (Stroben et al., 1995; Gibbs et al., 1997). The Danish monitoring data support this similarity in likelihood to develop imposex (Figure 3.1; 3.2; 3.3; 3.4). However, it has to be recognised that there is some degree of uncertainty coupled to the use of *B. undatum*, because correlations of VDSI in this species with other relevant species have not yet been established.

Based on the Danish monitoring data and other studies (Poloczanska & Ansell, 1999; ten Hallers Tjabbes et al., 2003), it can be concluded that *N. antiqua* has a higher likelihood to develop imposex than *B. undatum* and *H. reticulata* (Figure 3.1, 3.2, 3.3 & 3.4), and that the likelihood is almost comparable with *N. lapillus* (Paragraph 3.1). Therefore VDSI in *N. lapillus* and *N. antiqua* are set to be identical in the status classes I, II and III. *N. antiqua* can not be used to classify status classes IV and V, because the maximum value of VDSI is 4 in this species. However, it has to be recognised that there is some degree of uncertainty coupled to the use of *N. antiqua*, because correlations of VDSI in this species with other relevant species have not yet been established.

The assessment criteria based on the assumptions above can be derived for *H. reticulata*, *B. undatum* and *N. antiqua* (Table 5.13) by comparing with the imposex (and intersex) levels in *N. lapillus* and *L. littorea*. This includes an extrapolation from non-sterile imposex stages in the buccinid species to situations where sterile females occur in populations of *N. lapillus* and/or *L. littorea* in the status classes IV. Because VDSI = 4 is the maximum degree of imposex severity,

which can be developed according to the VDSI classification, the buccinid species do not seem appropriate to use in the assessment of highly TBT affected areas to be included in status class V.

Table 5.13. Suggestion to assessment criteria for biological effects of TBT using the development of imposex (as VDSI) in the three buccinid gastropod species *Hinia reticulata*, *Buccinum undatum* and *Neptunea antiqua*.

Status class	I	II	III	IV	V
VDSI in <i>H. reticulata</i>	< 0.3		0.3 – < 2	2 – 4	(4+)
VDSI in <i>B. undatum</i>	< 0.3		0.3 – < 2	2 – 4	(4+)
VDSI in <i>N. antiqua</i>	< 0.3	0.3 – < 2	2 – 4	(4+)	(4+)

The various parameters used in the derivation of status classes I - V in Table 5.5, 5.6, 5.10, 5.12 and 5.13 are all combined in a united scheme for assessment criteria for TBT (Table 5.14).

Table 5.14. Assessment criteria for TBT combining ambient TBT concentrations in water, mussel (*Mytilus edulis*) and sediment and TBT-specific biological effects, e.g imposex (as VDSI) and intersex (as ISI), in five species of prosobranch gastropods (*Nucella lapillus*, *Littorina littorea*, *Hinia reticulata*, *Buccinum undatum* and *Neptunea antiqua*).

Status class	I	II	III	IV	V
TBT conc. (aq) in seawater	close to zero	< 0.1 (ng TBT/l)	0.1 - < 1.5 (ng TBT/l)	1.5 - 15 (ng TBT/l)	> 15 (ng TBT/l)
TBT conc. in <i>M. edulis</i>	close to zero	< 0.8 (ng Sn/g ww)	0.8 - < 12 (ng Sn/g ww)	12 - 120 (ng Sn/g ww)	> 120 (ng Sn/g ww)
TBT conc. in sediment	close to zero	< 0.5 (ng Sn/g dw)	0.5 - < 20 (ng Sn/g dw)	20 - < 200 (ng Sn/g dw)	> 200 (ng Sn/g dw)
VDSI in <i>N. lapillus</i>	< 0.3	0.3 - < 2	2 - 4	> 4 – > 5	Disappeared
ISI in <i>L. littorea</i>	< 0.3			0.3 - 1.2	> 1.2
VDSI in <i>N. antiqua</i>	< 0.3	0.3 - < 2	2 - 4	(4+)	(4+)
VDSI in <i>B. undatum</i>	< 0.3		0.3 - < 2	2 - 4	(4+)
VDSI in <i>H. reticulata</i>	< 0.3		0.3 - < 2	2 - 4	(4+)
Risk Quotient, RQ (aq)	< 0.1	0.1 - < 1	1 - < 15	15 - 150	> 150

The main advantages with this combined scheme of assessment criteria are that monitoring of TBT levels and TBT-specific biomarker responses can supplement each other in a comprehensive evaluation of the environmental quality in the marine environment over a large scale, both with respect to location and level of TBT contamination. Various kinds of available monitoring and other field data of TBT concentrations and effects can thereby be used in an integrated evaluation of the TBT contamination in the marine environment. What parameters to be preferred depends on for instance if the TBT levels are high enough to be detected by the chemical analytical methods or what relevant bioindicators are present in the areas of concern. For instance, *M. edulis* can be a suitable bioindicator in areas, where the TBT concentration is below the limit of detection in water and sediment. Likewise, imposex as a biomarker in the most sensitive gastropod species can be used in to assess the TBT levels in areas, where even TBT concentration in *M. edulis* is below the limit of detection. In addition, by including TBT concentrations in sediments and *M. edulis* and biomarker responses in gastropod species in the assessment criteria, it also possible to assess the long-term level of TBT contamination in an area, since they can be regarded to give a more time-integrated measure of the TBT exposure. In practice, because the comprehensive scheme for assessment criteria for TBT, which includes nominal TBT concentrations in various matrices, e.g. water, sediment and a mussel, combined with the levels of TBT-specific effects in five gastropod species, is rather complex there may become disagreements depending on which parameters are used in the assessment of a specific area. Especially in situations where the TBT levels and/or effects in an area are close to the borderline between two status classes. Various factors, which occur in a complex ecosystem, can affect the direct relationships between the different parameters. Therefore in some situations there may occur some variation in the classification depending on what parameters are used in the evaluation of TBT contamination in an area. For instance, because of the irreversible nature of imposex and intersex, they may not generate a direct relationship to the actual measured levels of TBT exposure. While actual TBT concentrations are contemporary and dynamic, the severity of imposex may represent a level of TBT-exposure with some years delay. Also the assessment based on the chemical measurements may depend on what matrices, that are used. If the evaluation of the level of TBT contamination in an area is based solely on measured TBT concentrations in water and/or sediments, it may be more difficult to evaluate according to the respective status classes. TBT concentrations in water may to a higher degree fluctuate temporally, and TBT concentrations in sediments depend on sediment characteristics and due to inhomogeneity in the sediment the TBT concentrations can fluctuate spatially, even within restricted areas. Evaluations of a more general level of TBT contamination in an area will often be better done if based on measured TBT concentrations in *M. edulis* and/or the TBT-specific biological effect parameters, because of higher consistency in concentration/effect

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levels over time and space. Also the high interspecies variability in TBT sensitivity between the different gastropods affects the usefulness of the single species as bioindicators to evaluate the TBT contamination level in an area. However, in the next chapter the consistency in the combined assessment criteria will be evaluated and discussed in relation to the data from Danish waters, which were presented in the previous chapters 2, 3 and 4.

## 6. Discussion

The wide range of data presented on concentration levels of TBT and other organotin compounds, both regarding spatial distribution and bioaccumulation in various organisms, is used as a basis for a risk assessment covering the organotin contamination in the Danish marine environment.

The different studies show that generally high levels of organotin contamination occur in the Danish waters. Organotin, especially butyltin compounds, can be detected in all regions in a concentration range, which covers five to six orders of magnitude, from contaminated harbour areas, into coastal waters and the sublittoral parts of the open waters and further to the North Atlantic and the Arctic (Chapter 2 and 4). However, the choice of suitable matrices and bioindicators is important for such a wide-range assessment. Especially the use of bioindicators is influenced by a high variation in accumulation potential of organotin between different species even at the same trophic level of the food web.

In addition, different studies, which potentially can associate organotin exposure and biological effects, have been included (Chapter 3). The risk assessment is therefore not solely based on measured organotin levels, which are compared with extrapolated threshold levels derived from toxicity tests performed in laboratory. Especially the studies of the presence of endocrine disruptions in prosobranch gastropods, e.g. the highly TBT-specific and sensitive biomarker responses imposex and intersex, provide evidence of the high ecotoxicological potential of TBT since these abnormal phenomena are widespread in all regions of the Danish waters. But how widespread depends on the specific species examined, because some gastropod species have a higher likelihood to develop imposex/intersex than others do.

In the development of the five-class scheme of assessment criteria for TBT presented in Table 5.14, it has been possible to combine the TBT concentration in three matrices, e.g. in seawater, sediment and the bivalve *M. edulis*, with the TBT-specific biomarker responses, e.g. imposex and intersex in five species of prosobranch gastropods. The main advantages with this combined scheme of assessment criteria are that monitoring of TBT levels and TBT-specific biomarker responses can supplement each other in a comprehensive evaluation of the environmental quality in the marine environment over a large scale, both regarding location and level of TBT contamination. Various kinds of available monitoring and other field data of TBT concentrations and effects can thereby be integrated in the assessment of the TBT contamination in the Danish marine environment.

To verify the relationships between the different parameters in the combined chemical and biological assessment criteria for TBT in Table 5.14, the different parameters are used to evaluate the significance of TBT contamination in different types of inter- and intraregional

areas in Danish waters (Table 6.1). The detailed knowledge of relevant concentration and effect levels, presented in Chapter 2, 3 and 4, has provided a good basis to categorize areas in the Inner Danish waters, the North Sea and the North Atlantic with respect to levels of TBT concentration and effects. Thus the consistency in the interparametrical relationships can be compared (Table 6.1).

*Table 6.1. Different types of inter- and intraregional areas in Danish waters evaluated according to the suggested five-class scheme of assessment criteria for TBT using data on TBT levels and effects in the Danish marine environment.*

	<b>Reference areas in the Atlantic and the Arctic</b>	<b>The Skagerrak &amp; the Eastern North Sea</b>	<b>The Kattegat</b>	<b>The Belt Sea, the Sound &amp; the Wadden Sea</b>	<b>Shallow fiords with harbours inside</b>	<b>Close to contam. harbours or inside less contam. harbours</b>	<b>Inside contam. harbours</b>
TBT conc. in seawater *	n.d.a.	n.d.a.	n.d.a.	III	III, IV	IV	IV, V
TBT conc. in <i>M. edulis</i>	I, I-II	n.d.a.	n.d.a.	III	III, IV	IV	IV, V
TBT conc. in sediment	I-II	I-II	I-II	I-II, III	III, IV	III, IV	IV, V
VDSI in <i>N. lapillus</i>	I	II, III	n.d.a.	n.d.a.	n.d.a.	IV	(V ?)
ISI in <i>L. littorea</i>	n.d.a.	n.d.a.	I-III	I-III, IV	I-III, IV	I-III, IV	IV, V
VDSI in <i>N. antiqua</i>	n.d.a.	II	III	III, IV	n.d.a.	n.d.a.	n.d.a.
VDSI in <i>B. undatum</i>	I-II	I-II	I-II, III	I-II, III	n.d.a.	n.d.a.	n.d.a.
VDSI in <i>H. reticulata</i>	n.d.a.	n.d.a.	I-II	III	IV	IV	n.d.a.

*n.d.a.: no data. \* TBT concentrations in seawater are based on measured and estimated data. I-II: For TBT in sediment and M.edulis and for VDSI in B. undatum and H. reticulata, there cannot be distinguished between status classes I and II. I-III: For ISI in L. littorea, there cannot be distinguished between status class I, II and III. (V?): For N. lapillus there is only indications that they have disappeared from some Danish harbour areas since they cannot be found inside many harbour basins at the Danish west coast.*

When comparing the various parameters there generally seems to be a high consistency between the status classes connected to the different types of inter- and intraregional areas in Danish waters, from less to severely contaminated areas. Especially the consistency for areas, which are classified as status class III and IV, verifies the integration of measured TBT concentrations and imposex/intersex as biomarker responses in the derived assessment criteria. In general, the TBT contamination in most areas of the Inner Danish waters can be classified as status classes

III or IV suggesting that the TBT levels in the Danish marine environment are problematic as there is a risk and in some areas even evidence that adverse effects occur in the ecosystem. Status classes I and II, i.e. below the level of concern, are typically only achieved in the open waters of the North Atlantic, the North Sea and the Skagerrak, although for some parameters also in the Kattegat.

From Table 6.1 it can also be evaluated which parameters are most suitable to use in TBT assessment in the different areas. For instance, the interspecies variance in likelihood to develop imposex or intersex affects the potential of the different species as bioindicators in more or less TBT contaminated areas. Imposex in the muricid and buccinid neogastropods has, because of a high TBT sensitivity, a higher potential in assessments of coastal and open waters, whereas intersex in *L. littorina* primarily can be used in assessments in areas in the proximity to point sources, e.g. mainly inside harbours. *L. littorea* is typically too insensitive to show significant effects outside most harbours and this species is therefore not that suitable in assessment of areas classified as status classes I, II and III. Only imposex in *N. lapillus* and *N. antiqua* has the potential to be used in areas, which can be classified as status classes I and II. Concerning the chemical measurement, TBT concentrations in *M. edulis* have the potential to be used in areas classified as status classes II to V as status class I (and often also II) are associated with TBT concentrations below the currently achievable analytical detection limits. The extensive data material on TBT concentrations in sediments can typically only be used to classify areas as status classes III, IV and V since both status class I and II also are associated with TBT concentrations below the currently achievable analytical detection limits.

Compared with the proposed assessment criteria for biological effects of TBT by Oehlmann (2002) where sterile gastropods have to be present in areas classified as status class III (Table 5.11), it can be concluded that the association between the chemical and biological parameters would not have been that consistent as achieved in Table 6.1. Which type of areas to be classified as status class II, III and IV will depend on the parameters used. Using the assessment criteria proposed by Oehlmann (2002), the biological parameters would typically all classify the Belt Sea, the Sound and the Wadden Sea as status class II, although the chemical assessment criteria for TBT in water, sediment and mussel would classify these areas as status class III. Status class III would in the proposal by Oehlmann (2002) first be achieved in shallow fiords with a major harbour inside or in close proximity to contaminated harbours, although the chemical/ecotoxicological-derived assessment criteria have indicated that not only TBT effects caused by long-term exposure occur, but that there also is a risk of acute TBT effects caused by short-term exposure. The interpretation of the protection levels defined for the chemical/ecotoxicological and biological quality elements respectively is the main difference, as discussed in Chapter 5.



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The combined assessment criteria derived in this thesis harmonize the chemical and biological assessment criteria. A generally high consistency is thereby reached between the chemical assessment criteria for TBT in seawater, sediment and mussel and the assessment criteria for biological effects of TBT derived from the imposex and intersex development in five species of prosobranch gastropods.

Another important point is, if consequently the Belt Sea and other parts of the Inner Danish waters are classified as status class III, that the risk of other possible adverse effects of TBT (see Table 5.4), also can be regarded as being taken into consideration when the protection level is defined. The effects do not have to be as evident as the impaired reproduction caused by sterility in gastropod populations.

For instance, the high exposure and accumulation of organotin in marine mammals inhabiting the Inner Danish waters can also to some degree be regarded as included in status class III, although only weak indications of adverse effects exist, since no substantial field evidence of immunosuppression and higher susceptibility to diseases due to TBT exposure have been provided (Paragraph 2.3 and 3.2). Likewise the risk that the significant declines in some gastropods populations in the Danish and the Swedish coastal waters (Paragraph 3.1) may be related to the widespread TBT contamination, can to some degree be regarded as being taken into consideration by the classification as status class III.

The status class III thereby acts as a kind of “status class of precaution”, where the risk of adverse effects of TBT in various taxa in the marine ecosystem (including a degree of uncertainty because of limitations in knowledge) has been taken into account in the definition of protection level, e.g. the border between status class II and III. The derived assessment criteria is therefore in agreement with the objectives in the strategies within OSPAR and the WFD for protection of the marine ecosystems, where adverse effects in sensitive populations caused by exposure to priority hazardous substances will not be tolerated.

In traditional risk assessment of contaminants, the data from the exposure assessment is mainly compared with laboratory derived data from toxicity tests to provide a prediction of the risk of adverse effects in the ecosystem structure and function. This includes also the use of an application factor such as 10, as a precaution in the derivation of quality standards from NOEC-values to account for the uncertainties in the extrapolation of the toxic potential of TBT from available data to situations in the field, where the whole ecosystem should be protected (Paragraph 1.3). The general idea is that the risk assessment should finally be evaluated by studies of population and community structure or function in the areas of concern. However, alterations at the population level in a complex ecosystem can often be difficult to elucidate as specific contaminant-induced effects have to be distinguished from effects of confounding factors including natural variations, spatially as well as temporally, and combination effects of

the mixture of contaminants present in the environment. But such difficulties do not necessarily imply that the contaminant-induced effects are of insignificant relevance for the ecosystem. In addition, relevant field studies of populations and communities can easily get extensive and thereby also often expensive to perform. If first field evidence of population declines or alteration of communities has to be provided, there is a high risk that such ecological damage is extensive and will be present for several years to come. This is due to the persistent nature of many pollutants and the time-lag from evidence to possible effects of relevant political and management decisions of restrictive actions. Consequently, it is in practise a major challenge to develop an approach, which as a precaution can account for the uncertainty in the cause-effect relationship present in field studies into environmental risk assessment in general. The difficult task in the field application of risk assessment is especially to evaluate areas, where the contamination level or rather the biological impact in an area of concern has to be categorized as status class II or III. E.g. whether potential adverse effects, such as reduced growth, reproduction etc., caused by long-term contaminant exposure are important for the ecosystem. It has therefore in several years been discussed whether biomarkers can be integrated in the framework of environmental risk assessment or not, because biomarkers reflect exposure of contaminants or diagnose health at the individual level, which in many situations can be most difficult to associate with adverse effects at the population or community level. It has generally been accepted that the primarily role of biomarkers is to be used as early warning signals or for mechanistic studies of cause-effect relationships, and significant results provide a basis for initiation of more integrated studies of exposure, bioaccumulation and effects on individuals and populations.

In field studies biomarkers are mainly used as a biological interpretation/indication/evidence of the presence of biological active levels of contaminants and they are recommended for spatial and temporal monitoring of the environmental quality - and they have their primary force here. However, I suggest on the basis of the data presented in this thesis that biological effects at the individual level, e.g. biomarker responses, in some situations have the potential to be integrated in the environmental risk assessment of threats to the ecosystem. Particularly in situations in which substantial data are provided to support that there is an evident risk, for instance if exposure levels are higher than derived EQS-values in the WFD. However, it has to be accepted that the potential risk of contaminant-induced effects at the population level also have to be accounted for as precaution in the risk assessment of field situations even though there may be some uncertainty in the establishment of an specific cause-effect relationship. One has to keep in mind that the organism and endpoints studied also often are selected for more practical reasons. The chosen bioindicators represent therefore seldom the most sensitive

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species in the ecosystem. In addition, there have been several examples that the scientific knowledge at a present time does not take all relevant effects into account.

If the risk of adverse effects also must be taken into account, some biomarkers have the potential as tools to assess whether areas of concern should be classified as status class III as defined in the suggested assessment criteria of hazardous substances in general as presented in Table 5.4. E.g. where status class III is regarded as a “status class of precaution”, which reflects a potential risk of effects caused by long-term exposure of contaminants in the most sensitive species. In this way the biomarkers are still recognised to be early warning signals in case that adverse effects may occur. However, biomarkers will also have the potential to be used in assessment of areas classified as status class IV or V. E.g. when the risk characterization has identified that there is a risk of contaminant-induced adverse effects caused by long-term exposure in both more and less sensitive species or even acute effects in the most sensitive species. Interspecies comparisons of biomarker responses between more and less sensitive species will be a great advantage in such an approach.

To fulfil this approach it would be ideal to have a limited set of biomarkers using relevant and sensitive bioindicators and endpoints, which could be used as a supplement to the chemical analyses in the exposure assessment. This battery should ideally include contaminant-specific biomarkers, which can indicate the level exposure and assessing the toxic impact of all classes of relevant contaminants as well as non-specific biomarkers, which can combine the pressure of a wide range of contaminants and thereby assess the general health condition of the organisms in the ecosystem.

From my point of view, some examples of biomarker responses can with advantage be integrated as biological tools in the assessment of the environmental quality in a specific area of concern. Not only some of the biomarkers, which measure effects on individuals for instance such as impaired reproduction caused by sterility, can be integrated, but also in some cases biomarkers, which give evidence of molecular, cellular or physiological alterations e.g. effects on the sub-individual level (see also Figure 1.4). But of course effects on individuals can more easily be associated with biological significant endpoints for specific organisms examined than responses at the sub-individual level.

Therefore it has to be distinguished between at least three different kinds of approaches for biomarkers since integration of biomarker studies into monitoring and risk assessment probably will require different strategies as suggested below;

- I. Contaminant-specific biomarkers, where the majority is measuring alterations at the sub-individual level such as imposex and intersex development, AChE activity, DNA-adducts and vitellogenin. However, as an exception imposex and intersex can also provide evidence of effects at the individual level, e.g. impaired reproduction caused by

sterility in some species. These types of biomarkers give evidence to exposure of biological active levels of specific groups of contaminants. They can potentially be integrated into risk assessment by two different approaches. Firstly, if correlative dose-response relationships have been established they can be used to reflect contamination level in relation to defined threshold levels, and they will thereby act as a direct supplement to chemical analyses. Secondly, the specific biomarker responses can be used as evidence of a potential risk if they, although in other studies, have been associated with adverse effects such as impairment of growth, reproduction, behaviour, functions etc. This will correspond to a classification of an area of concern as status class III or even IV.

- II. General biomarkers, which measure effects on individuals such as scope for growth, impaired reproduction etc. These biomarkers measure the total pressure of various stress factors. They have therefore the potential to integrate the impact of various factors, and not contaminants specifically. This is also the overall objective in assessment of the ecological quality status in an area of concern. The assessment does not necessarily need to discriminate between the impact of the single factors. If the general biomarker responses, although in other studies at controlled conditions or by ecosystem modelling, have been associated with effects on population or community level, they can be used as an evidence of a potential risk of adverse effects, which correspond to a status class III classification of an area of concern.
- III. General biomarkers, which measure alterations at the sub-individual level such as lysosomal (de)stability, heat shock proteins etc. These biomarkers are probably most difficult to integrate into a risk assessment. However if it is possible to associate these biomarker responses with adverse effects such as impairment of growth, reproduction, behaviour, functions etc., they can be used to provide some indications of potential risks. At present it does not seem possible to clarify whether these indications will be strong enough to classify an area of concern as status class III or not.

Although I have argued for an approach in which some biomarkers have the potential to be integrated in risk assessment, their limitations must of course still be recognized since the protection of the ecosystem structure and function, and not the individuals, is the overall objective in environmental risk assessment.

At present, TBT is maybe the most obvious example, which illustrates that risk assessment of a specific hazardous substance closely can integrate exposure assessment and biomarker responses in the derivation and verification of consistent assessment criteria. It will be more difficult to establish the same causality for other biomarkers, although they may be relevant if

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threats are to be recognised before irreversible changes occur in the marine ecosystem. Biomarkers, which respond more generally to contaminant-induced stress are probably much more difficult to include directly in such an integrated approach because of the lack of specificity to a single stress factor, although they to a higher degree may reflect effects of biological significance. However, when the overall objective is to assess the ecological quality of the entire ecosystem in area of concern, the assessment does not necessarily need to discriminate between the impact of the single factors. It is actually the sum of all stress factors, which is the important issue.

The presence of developmental defects in fish embryo and larvae, e.g. impaired reproduction, is an example of a general biomarker, which measure effects at the individual level (see Paragraph 3.3). It can thereby provide evidence if significant biological effects occur in an area of concern. However, it can not at present be excluded that other environmental factors than contaminants contribute to the observed effects. It requires further research developments. Therefore more substantial evidence of the impact at population and community level, for instance by ecosystem modelling, is required than in the approach for contaminant-specific biomarkers, because it can not be excluded that the nature of the problem is less persistent, compared with the impact of persistent pollutants. How evident and specific the cause-effect relationship between contaminant exposure and effects has to be, before it is accepted as a persistent problem, is however open for discussion.

Finally, how the assessment of the marine ecological quality and ecosystem health will be performed in the future, especially with the implementation of the EU Water Framework Directive in mind, will probably influence the general strategies for biological effect monitoring and how it will be integrated in assessment strategies of hazardous substances. If only evidence of effect at population or community levels will be accepted as significant endpoints in future environmental risk assessment, it will provide a lower protection level than in the suggested approach in this thesis, where the biological elements is more harmonised with the physico-chemical/ecotoxicological-derived assessment criteria (quality standards) for hazardous substances. The suggested approach includes as a precaution that the risk of adverse effects in the most sensitive taxa of the ecosystem also has to be taken into account when the protection level is defined. This is due to the fact that adverse effects in the ecosystem will not be tolerated, although the risk of adverse effects in the most sensitive taxa of the ecosystem only can be regarded as as a hypothetical endpoint.

Integration of biomarkers has the potential to provide an approach, which can take such a risk into account. Biomarkers will consequently become of higher relevance and should be further integrated in future monitoring and assessment of hazardous substances in the marine environment.

Alternatively the main focus should continue to be at chemical analyses of hazardous substances in combination with analyses of population and community structures with the huge limitations they have in field studies and biomarkers should primarily be used as mechanistic describing parameters if alterations of populations are observed. This will probably give the result that combined studies of exposure and effects almost only will be a relevant issue in the proximity of highly contaminated areas, where the causality between exposure, effects on individuals and alterations of populations has a chance to be established. Consequently monitoring and assessments of hazardous substances in coastal and open parts of the Danish waters will primarily be a relevant issue in relation to temporal trend analyses and human risk assessment thereby neglecting that there also is a risk of adverse effects in the marine ecosystem caused by long-term exposure in these areas.

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## 7. Conclusions

The wide range of data presented on concentration levels of TBT and other organotin compounds give evidence to a widespread contamination of the Danish marine environment.

Especially, butyltin compounds can be detected in all regions in a concentration range, which covers five to six orders of magnitude, from contaminated harbour areas, into coastal waters and the sublittoral parts of the open sea. The general high levels of organotin contamination in the Danish waters are caused by the widespread use of TBT in ship paint since the Danish waters are highly trafficked.

In Danish coastal waters TBT and breakdown products were found in all trophic levels of the marine food web, from seaweed and invertebrates to fish, birds and marine mammals. The highest butyltin levels, which can reach several  $\mu\text{g/g}$  ww, are found to accumulate in liver of harbour porpoise, although comparable levels can also be found in molluscs and fish sampled inside contaminated harbour areas.

In addition, biological effect studies, particularly the surveys of TBT-specific biological effects in prosobranch gastropods e.g. imposex and intersex development, have provided evidence of the high ecotoxicological potential of TBT since these abnormal phenomena are widespread in all regions of the Danish waters. But how widespread depends on the specific gastropod species examined, because some species have a higher likelihood to develop imposex/intersex than other do. Evidence of impaired reproduction caused by the presence of sterile gastropod females in local population, can only be found inside or in the proximity of contaminated harbours, although there are also some indications of more widespread population declines of gastropods in the Danish-Swedish coastal waters during the last century. The contribution of the relative high TBT levels to such population declines is however only hypothetical.

Whether organotin also poses a threat to other taxa in the marine ecosystem is similarly very difficult to evaluate, because of the lack of contaminant-specific responses, but such relationships can at present not be excluded. Particularly marine mammals may be at risk since the exposure of TBT is much higher than TDI derived for protection of humans, which in harbour porpoises also results in a very high deposition of organotin. Organotin is suspected to induce immunosuppression in marine mammals thereby making them more susceptible to diseases. A minor indication of such a relationship was found in one study since stranded harbour porpoises have accumulated higher levels than specimens caught by fishermen.

Another study on larval development in broods of a viviparous fish, the eelpout, did not show any association between organotin accumulation and impaired reproduction, indicating that fish reproduction is not that sensitive to TBT. However, the spatial differences in the frequencies of teratogenic effects suggest that other groups of contaminants locally in Danish coastal waters

affect reproduction in fish. Indications of impaired reproduction caused by oxygen depletion events were additionally found suggesting that the eelpout is a promising bioindicator, which can combine monitoring of effects caused by hazardous substances and eutrophication derived problems.

Finally, based on an evaluation using the developed five-class scheme of assessment criteria for TBT, which integrates exposure, bioaccumulation and biomarker studies, it can be concluded that the levels of TBT not only pose a threat to the marine ecosystem in the most contaminated areas in Denmark, e.g. inside or in the proximity of harbours where the effects are most evident, but in the entire region of the Inner Danish waters.

In comparison, the TBT contamination in West Greenland is only of concern inside harbour areas. However, using cetaceans as bioindicators, e.g. as a kind of effective “sampling devices” of TBT, it has been possible to demonstrate that TBT also occurs in the open sea of the North Atlantic and the Arctic although in concentrations, which are orders of magnitude lower than in the Danish waters.

The approach for derivation of these assessment criteria for TBT may provide a solution to how exposure, bioaccumulation and effects studies can be combined and integrated into risk assessment of hazardous substances in general.



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## I - V: Manuscripts for publication in international scientific journals

Five manuscripts (two published, two in press and one in prep.) are included in this thesis:

- I. Strand J., Jacobsen J.A., Pedersen B. & Granmo Å. (2003). Organotin compounds in sediment and molluscs from the shipping strait between Sweden and Denmark. *Environ. Poll.* 124: 7-15.
- II. Strand J. & Jacobsen J.A. (in press). Accumulation and trophic transfer of organotin in a marine food web from Danish coastal waters. Accepted by *Sci. Tot. Environ.*
- III. Strand J., Larsen M.M. & Lockyer C. (in press). Accumulation of organotin compounds and mercury in harbour porpoises (*Phocoena phocoena*) from the Danish waters and West Greenland. Accepted by *Sci. Tot. Environ.*
- IV. Strand J. & Asmund G. (2003). Tributyltin accumulation and effects in marine molluscs from West Greenland. *Environ. Poll.* 123: 31-37.
- V. Strand J., Andersen L., Dahllöf I. & Korsgaard B. (in press). Impaired larval development in broods of eelpout (*Zoarces viviparus*) in Danish coastal waters. Accepted by *Fish Physiol. Biochem.*