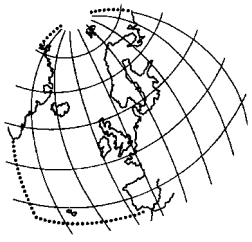
Biodiversity Series

North Sea Pilot Project on Ecological Quality Objectives

Background Document on the Ecological Quality Objective on imposex in dog whelks *Nucella lapillus*



OSPAR Commission
2005

The Convention for the Protection of the Marine Environment of the North-East Atlantic (the "OSPAR Convention") was opened for signature at the Ministerial Meeting of the former Oslo and Paris Commissions in Paris on 22 September 1992. The Convention entered into force on 25 March 1998. It has been ratified by Belgium, Denmark, Finland, France, Germany, Iceland, Ireland, Luxembourg, Netherlands, Norway, Portugal, Sweden, Switzerland and the United Kingdom and approved by the European Community and Spain.

La Convention pour la protection du milieu marin de l'Atlantique du Nord-Est, dite Convention OSPAR, a été ouverte à la signature à la réunion ministérielle des anciennes Commissions d'Oslo et de Paris, à Paris le 22 septembre 1992. La Convention est entrée en vigueur le 25 mars 1998. La Convention a été ratifiée par l'Allemagne, la Belgique, le Danemark, la Finlande, la France, l'Irlande, l'Islande, le Luxembourg, la Norvège, les Pays-Bas, le Portugal, le Royaume-Uni de Grande Bretagne et d'Irlande du Nord, la Suède et la Suisse et approuvée par la Communauté européenne et l'Espagne.

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Contents

1. Background

This Background document aims to explain the thinking behind the ecological quality objective (EcoQO) on imposex in dog whelks.

The Bergen Declaration of the 5th North Sea Conference identified ten issues relating to the ecological quality of the North Sea for the development of ecological quality objectives (EcoQOs). "Benthic communities" is one of these ten issues. Four ecological quality (EcoQ) elements have been identified for this issue, including: *(n) Imposex in dog whelks (Nucella lappillus).*

This is among the ten elements included in the North Sea Pilot Project. An EcoQO was adopted for this element in the Bergen Declaration: "*A low (<2) level of imposex in female dog whelks, as measured by the Vas Deferens Sequence Index*".

This background document was prepared by Belgium and Portugal as input to the review of the advanced EcoQOs under the North Sea Pilot Project.

2. Introduction

The justification for this EcoQO is that the female dog whelk (*Nucella lapillus*) is particularly sensitive to tributyltin (TBT), which has been extensively used as an antifouling treatment on ships. The EcoQO will measure the success of the international agreements to phase out and eventually prohibit the use of TBT in antifouling paints, and the progress of recovery of the marine environment from presence of TBT.

Also other species of gastropods are sensitive to TBT and are being used for monitoring TBT pollution. These gastropods are, or may be used to cover areas where *Nucella* does not occur naturally, or where it has become extinct.

The first adverse effects of TBT have been demonstrated in the 1980's when TBT was linked to shell deformations and effects on the reproduction of the Pacific Oyster (*Crassostrea gigas)* and to the incidence of imposex in dog whelk populations. Imposex in gastropods is the condition where female specimens develop male characteristics that eventually lead to sterilisation and a serious decline of the population. Since its detection, imposex and other effects of TBT have been confirmed particularly in gastropods, but also in other molluscs. However, adverse effects of TBT are not restricted to molluscs alone and have been demonstrated in other invertebrates, such as crustaceans among which caprellid amphipods (Takeuchi *et al*., 2001; Ohji *et al*., 2003). Moreover, the occurrence of TBT in marine mammals is another cause for concern. Recent studies of animals stranded in the UK have shown detectable concentrations of butyl tins in both coastal and pelagic marine mammals, the latter animals feeding over the continental shelf edge and slope, and in the deep oceanic waters of the Atlantic Ocean (Law *et al*., 1998).

Because of these negative effects, the use of TBT has been banned on vessels smaller than 25m. Numerous studies have documented the environmental improvements in estuaries and enclosed waters that followed the return to copper-based antifouling preparations (Law & Evers, 1999). However, attention has focused increasingly on the impact of the continued use of TBT-based paints on larger sea-going vessels. Knowledge of the fate and distribution (and, where possible, effects) of TBT in open sea areas, shipping lanes, and close to ports and harbours frequented by these large vessels, has become the main focus of attention during recent years. Moreover, as degradation of TBT is slow, concentrations of TBT and its degradation products dibutyltin and monobutyltin are often very high in sediments, particularly those in areas frequented by ships, or where inputs from pleasure boats were high (Law & Evers, 1999).

3. Physico-chemical properties and environmental effects of TBT

Organotins is the group name for compounds of mono-, di-, tri-, and tetrabutyltin and triphenyltin. These substances are entirely man-made; there is no known natural source for them. As a result, and not unexpectedly, most organisms have developed little or no resistance or tolerance to them.

Organotins are the most widely used metallo-organic compounds (Fent, 1996). The total world-wide use of organotin compounds has dramatically increased in the last decades from about 5.000 tonnes per year at the beginning of the 1960's to over 60.000 tonnes per year in the mid 1980ies (Morabito *et al*., 1995).

Organotin compounds are potent toxins and this property has lead to their use as an active ingredient in a wide variety of products. Due to their strong toxic effects they have been used in a variety of applications: agricultural pesticides, slimicides in cooling waters, timber preservatives, molluscicides and marine antifouling paints (Aboul Dahab *et al*., 1990; Maguire, 1987). However, TBT based antifouling paints have proven to be of the greatest environmental significance. TBT has been used as a biocide in antifouling paints applied to boats, ships and docks since the late 1950's. By 1985, an estimated 20–30% of vessels world-

wide used TBT -based antifouling paint systems. These paints release highly toxic tributyltins directly into the aquatic environment. These tributyltins are partially adsorbed on suspended solids or bioaccumulated by living organisms.

The environmental persistence and fate of TBT are strictly correlated to the specific characteristics of the aquatic ecosystem such as temperature, salinity, pH, suspended matter, microbial populations, flushing rates etc. Distribution of TBT among the different environmental compartments is regulated by:

a) physical mechanisms (including volatilisation, adsorption, etc),

b) chemical mechanisms (including photochemical reactions) and

c) biological mechanisms (including uptake and transformation).

TBT undergoes degradation processes in the marine environment such as microbial and UV degradation, consisting in a progressive dealkylation down to inorganic tin. As the toxicity of the organotins is maximal for the tri-substituted compounds, the degradation can be considered as a mechanism of detoxification. In fact, elemental tin and its inorganic compounds are practically non-toxic for all living systems. Due to their very low solubility in lipids they are scarcely accumulated by the organisms. Furthermore, at physiological pH the element is not reactive and its oxides are practically insoluble (Morabito *et al*., 1995).

The highest concentrations of TBT are found in sediments from harbours, marinas and shipping channels, as TBT is broken down only very slowly in sediments with a low oxygen content. This is often the case in muddy inshore areas.

The toxicological effects of TBT on molluscs occur at very low concentrations in seawater, below the levels that can be routinely measured by most laboratories. Consequently, the existence of TBT contamination is frequently inferred from biological indicators (i.e. imposex measurements). The general picture of TBT levels in sea water indicates that concentrations offshore are generally lower than the detection limit, whereas much higher values are found in frequently used waterways. This makes tracking TBT in seawater difficult. TBT released to the water will degrade to dibutyltin and monobutyltin. Usually only TBT and total organotin values are reported, which makes it difficult to construct budgets for the fate and distribution of organotin compounds. Toxicological effects due to TBT have been observed at very low concentrations and therefore assessment criteria (ecotoxicological assessment criteria (EACs) – see further) for TBT are very low.

The effects of TBT have been well-documented since the 1980's, when TBT was linked to shell deformations and effects on the reproduction of the Pacific Oyster (*Crassostrea gigas)* (Alzieu *et al*.,1986; 1980) and to the incidence of imposex in dog whelk (*Nucella lapillus*) populations (Bryan *et al*., 1986). *Nucella lapillus* is a neogastropod which does not have a planktonic stage in its life cycle and occurs on hard substrates, predominantly in the intertidal zone. Female individuals develop non-functional male characteristics, such as a penis and a vas deferens. The phenomenon was called *imposex* and defined as a superimposition of male characteristics on to […] females resulting in pseudohermaphroditism (Smith, 1971). This phenomenon is fully developed at ambient TBT concentrations of 1–2 ng l^{-1}, and females are fully sterilised at concentrations above 5 ng l^{-1} (Gibbs & Bryan, 1996; Matthiessen & Gibbs, 1998). Sterilisation in the later stages of imposex is mainly the result of vas deferens formation, not of penis development. Affected populations decline or may become locally extinct. The dog whelk has proven to be a highly sensitive bioindicator of organotin contamination. Besides *Nucella lapillus,* other gastropods, in particular neogastropoda such as red whelk (*Neptunea antiqua*), common whelk (*Buccinum undatum*) and netted dog whelk (*Nassarius reticulatus),* proved to be very vulnerable to the effects of TBT. As such, these species may be useful as bioindicators in the absence of *Nucella lapillus*.

Besides causing imposex, TBT may produce another masculinising condition, called *intersex*. This phenomenon, defined as a change in the female palial oviduct towards a male morphological structure, was first described in the periwinkle *Littorina littorea* (Bauer *et al.,* 1995). Intersex is expressed at higher concentrations of TBT (10 ng l^{-1}). This also leads to sterility. Therefore also intersex in *Littorina littorea* could be used as an alternative biomonitor for TBT pollution. However *Littorina littorea* populations are less likely to become locally extinct because *L. littorea* is less sensitive to TBT, and has – contrary to *Nucella lapillus* - a planktonic development stage.

For the expression and intensity of the imposex and intersex phenomenons, different anatomical characteristics can be used, such as the Vas Deferens Sequence Index (VDSI) (Gibbs *et al.,* 1987) in females or the Intersex Index (ISI) (Bauer *et al.,* 1995).

4. Assessment and monitoring

TBT is part of the group of organic tin compounds included on the OSPAR List of Chemicals for Priority Action (OSPAR agreement 2004-12). According to the 2003 Strategy for a Joint Assessment and Monitoring Programme (JAMP) (OSPAR agreement 2003-22), the following issues need to be addressed for these *priority chemicals*:

- What are the concentrations in the marine environment, and the effects, of the substances on the OSPAR List of Chemicals for Priority Action? Are they at, or approaching, background levels for naturally occurring substances and close to zero for man-made substances?
- For the individual OSPAR chemicals for priority action, what are the sources, what are the levels of discharges, emissions and losses, and what are the pathways to the marine environment? Are the discharges, emissions and losses from sources of these substances to the marine environment continuously decreasing, and are they moving towards the target of cessation by 2020?
- Are there any problems emerging related to the presence of hazardous substances in the marine environment? In particular, are any unintended/unacceptable biological responses, or unintended/unacceptable levels of such responses, being caused by exposure to hazardous substances?

In addition, the Strategy provides a time schedule for tools that need to be developed, the information that needs to be collected, and the assessments that need to be produced. The latter includes *an initial assessment by 2005 of biological effects of hazardous substances in the maritime area* (JAMP Assessment HA-2), and *a more elaborated assessment by 2009 of biological effects of hazardous substances in the maritime area* (JAMP Assessment HA-4).

The biological effect assessment is one of the assessments that contribute to the *general assessment by 2009 of the development in the quality status of the maritime area in relation to hazardous substances* (JAMP Assessment HA-6).

Since 2003 monitoring of organotins in sediments and TBT-specific biological effects have been included as mandatory components of the OSPAR Co-ordinated Environmental Monitoring Programme (CEMP) (OSPAR agreement 2005-5). The CEMP can be described as that part of monitoring within the JAMP where the national contributions overlap and are co-ordinated.

The following table provides an overview of the monitoring Contracting Parties already have in place (table 4.9. from ICES, 2004b – WGBEC 2004).

Country/area	Programme type	Number of stations	Frequency	Number of years	Species
BE	monitoring	3	annual	1	*Littorina littorea*
DK	monitoring	15	every 2 yrs	5	*Nassarius reticulatus*
DK	monitoring	14	every 2 yrs	6	*Buccinum* and/or *Neptunea*
DK	monitoring	12	every 2 yrs	5	*Littorina littorea*
FR	monitoring	108	annual	2	*Nucella lapillus*
IS	monitoring		every 5 yrs	10	*Nucella lapillus*
NL	monitoring	6	new	new	*Littorina littorea*
NO	monitoring	15	every 4 yrs	12	*Nucella lapillus*
SE	monitoring	5	new	new	*Nassarius reticulatus*
UK	monitoring (hotspot)	8	every 5 yrs	15	*Nucella lapillus*
UK	monitoring (offshore)	21	new	new	*Buccinum undatum*
UK	monitoring (spatial)	82	every 5 yrs	10	*Nucella lapillus*

5. Background Concentrations (BCs) and Environmental Assessment Criteria (EAC)

Monitoring data *per se,* of e.g. hazardous substances in the marine environment, has little importance if one cannot interpret, or assess, their significance to man or to the environment. Therefore, a set of criteria or a reference scale is needed to describe the significance of the data. Two criteria have been developed in OSPAR to support the assessment process, namely *background concentrations* (BCs) and *environmental assessment criteria* (EAC)[1].

In general, for substances that are only man-made, one can expect a zero background level in the environment. However, due to their persistence and long-range transport, many substances are now found all over the world.

EACs are concentrations above which there is concern that negative effects might be observed in marine organisms. These have been set using all available information on the toxicity of the substances. Where this is inadequate, additional safety factors have been included.

Recently OSPAR BCs and EACs were reviewed (OSPAR/ICES, 2004). BCs (formerly BRCs) have been set at zero for man-made substances, including TBT. The proposed EACs are given in the table below.

Medium	Concentration / content	Status*
Water (µg l^{-1})	0,0001	Firm
Sediment (mg kg^{-1} dw)	0,00001	Provisional
Fish (mg kg^{-1} lw)	-	
Mussel (mg kg^{-1} dw)	0,0024	Firm
* These assessment criteria have no legal significance and should only be used for the preliminary assessment of JAMP chemical monitoring data with the aim of identifying potential areas of concern. When applied, an indication should be given as to whether the EAC was firm or provisional.		

Note that according to the agreement adopted by OSPAR in 1997 on EACs (OSPAR agreement 1997-15), EACs:

- should not be used as firm standards;
- do not take into account specific long-term biological effects such as … reproductive disruption due to hormone imbalance;
- need to be refined and updated at periodic intervals;
- should be further derived taking biological effect monitoring data into account.

6. Assessment criteria for the biological effects of organotin

The biological effect assessment criteria for TBT were discussed during a 2-day workshop (6-7 November 2003, The Hague, The Netherlands; see ASMO 2004). Assessment criteria were derived for the Vas Deferens Sequence Index (VDSI) in *Nucella lapillus*, representing the most sensitive species known. Considering the absence of populations of *N. lapillus* in some coastal areas, other species should be used for monitoring the effects of TBT. The criteria for *Nucella* were presented alongside equivalent measures of TBT-specific biological effects for other gastropods i.e. VDSI for *Nassarius reticulatus* and *Neptunea antiqua,* Intersex State Index (ISI) for *Littorina littorea* and Penis Classification Index (PCI) for *Buccinum undatum*. The effects of TBT on different species were compared using sympatric populations in the field (Stroben *et al*., 1995; Oehlmann, 2002). The proposed criteria enable the consideration of the likely effects on *N. lapillus* based on effects in other species and allow the adoption of a consistent approach over the whole OSPAR maritime area. Six assessment classes were defined for the various gastropods considered during the workshop. The assessment criteria developed through this workshop were adopted by OSPAR 2004 as provisional JAMP assessment criteria for TBT (OSPAR agreement 2004-15). An overview of the assessment classes is given in the table below:

[1] ASMO 2004 agreed that the terms Background Concentration (BC) and Environmental Assessment Criteria (EAC) should replace the formerly used terms Background/Reference Concentration and Ecotoxicological Assessment Criteria, which were used in OSPAR agreements 1997-14 and 1997-15 on assessment criteria.

Assessment class **Criterion**	***Nucella*** **VDSI**	***Littorina*** **ISI**	***Nassarius*** **VDSI**	***Buccinum*** **PCI**	***Neptunea*** **VDSI**
A Level of imposex is close to zero	<0,3	<0,3	<0,3	<0,3	<0,3
B Level of imposex (~30-~100% of the females have imposex) indicates exposure to TBT concentrations below the EAC derived for TBT	0,3 - <2,0	<0,3	<0,3	<0,3	0,3 - <2,0
C Level of imposex indicates exposure to TBT concentrations higher than the EAC derived for TBT	2,0 - <4,0	<0,3 - <0,7	0,3 – 4,0	0,3 – 4,0	2,0 – 4,0
D Reproductive capacity in the gastropod populations is affected as a result of the presence of sterile females, but some reproductively capable females remain	4,0 – 5,0	0,7-2,0	May occur beyond 4,0	May occur beyond 4,0	May occur beyond 4,0
E Populations are unable to reproduce. The majority, if not all females within the population have been sterilised	5,0 – 6,0	>2,0			
F Populations are absent/expired	-				

7. ICES assessment of the EcoQO

ICES assessed the EcoQ element and the EcoQO. The technical evaluation by ICES is summarised below (adapted from ICES, 2004a).

		Comments	
1	**Issue**	6. Benthic communities	
2	**Element**	(n) Imposex in dog whelks *Nucella lapillus*	
3	**ICES criteria**		
	Relatively easy to understand by non-scientists and those who will decide on their use	Usually	Dog whelks are very sensitive to TBT. A number of scientific reports documenting this are available.
	Sensitive to a manageable human activity	Usually	Several documented cases of a recovery in dog whelk populations after the decrease in the use of TBT.
	Relatively tightly linked in time to that activity	Usually	Detection of change after a decrease in the use of TBT should be less than 10 years.
	Easily and accurately measured, with a low error rate	Usually	There is a standard method (VDSI).
	Responsive primarily to a human activity, with low responsiveness to other causes of change	Usually	There is a clear cause-effect relationship between the presence of TBT and imposex in dog whelks.
	Measurable over a large proportion of the area to which the EcoQ metric is to apply	Usually or occasionally	Dog whelks are widely distributed in the North Sea area, but only on rocky substrates and predominantly intertidally.
	Based on an existing body or time series of data to allow a realistic setting of objectives	Usually	Data exist from “pristine areas” where TBT concentrations are zero or almost zero.

4	**Ecological relevance/basis for the metric**	The cause-effect relationship between the presence of TBT and imposex in dog whelks is clear and direct. The toxicological effects of TBT on gastropods occur at very low concentrations in seawater, below the levels that can be routinely measured by most laboratories.	
5	**Current and historic levels (including geographic areas)**	The historical level of TBT is zero, with a corresponding VDSI<0,3. At present, elevated levels occur in many coastal areas. Trends are now decreasing due to regulations on TBT use.	
6	**Reference level**	Reference level for TBT concentration (and imposex) is zero (VDSI<0,3).	
7	**Limit point**	VDSI > 5, which means that dog whelks cannot reproduce.	
8	**Time frames**	*Detection of change*	Less than ten years.
		Use in advice	
9	**Advice on EcoQO options (scenarios)**		
10	**Monitoring regimes**	Monitoring should be focused on areas where the risk of high TBT concentrations is evident (harbours, etc.).	
11	**Management measures to achieve EcoQO**	There is an IMO resolution banning the presence of TBT on ships' hulls from 2008 onwards. Dumping of dredge spoil from harbours should be avoided in cases where these contain high amounts of TBT. Spoil materials should be assessed for TBT.	

8. Measures and actions taken to prevent the input of TBT in the marine environment

In 1990 the use of TBT on vessels smaller than 25 meters was banned. In many areas the effects of historical inputs are still visible (OSPAR Commission, 2000a). Within the IMO, a mechanism for a general ban on the use of organotin compounds in antifouling paints has been decided. On 5 October 2001 at the end of a five-day Diplomatic Conference held at IMO headquarters in London, the draft *International Convention on the Control of Harmful Anti-fouling Systems on Ships* (*AFS Convention*) prepared by the MEPC, was adopted. As recommended by MEPC 46, the AFS Convention ensures a ban on the application of TBT based anti-fouling paints by 1 January 2003 and a ban on the presence of TBT on ships' hulls by 1 January 2008. The Convention will also establish a mechanism to prevent the potential future use of other harmful substances in anti-fouling systems. Given the serious adverse effects of TBT on organisms, the effective implementation of this measure is thus immediately required.

After the outcome of the AFS Convention, the European Commission issued a Community Regulation, (Regulation (EC) No 782/2003) which forbids, in accordance with the AFS Convention, the application by 1 July 2003 of organotins in anti-fouling systems, to all ships flying flags of the EU. *Active* (on the exterior) organotin on ships sailing to or from Community ports is prohibited from 1 January 2008 onwards, irrespective of the entry into force of the Convention.

In order to assess the effectiveness of applicable OSPAR measures on organic tin compounds used as antifoulings, and to enable a verification of the need for further measures within the OSPAR framework, the implementation reporting on PARCOM Recommendations 87/1 and 88/1 was carried out in 1999. The results of the implementation reporting were published on the OSPAR website. The main conclusions of the report concerned the need for further work on the application on sea-going vessels, quality standards for marine waters and discharges from shipyards. In the overview assessment report it was concluded that reporting on the implementation of both PARCOM Recommendation 87/1 and PARCOM Recommendation 88/1 is valuable in the future to obtain information on the progress of:

- the further reduction of (the use of) organotin antifoulings, especially with regard to application on sea-going vessels;
- the development of measures to reduce organotin compounds reaching the aquatic environment through docking activities;

- the adoption of quality standards on organotin compounds for sea water;
- implementation by those Contracting Parties that have not reported until now.

The following are alternatives to the use of organic tin compounds as anti-foulants, which are either currently in use or in development: the use of copper compounds, metal-free chemicals (e.g. triazine compounds), products which are based on silicone to prevent organisms sticking to ships' hulls, biological biocides, electrochemical methods and the improvement of mechanical cleaning methods (foils) (OSPAR Commission, 2000b).

Given the fact that measures are being taken, this EcoQ will provide a basis for monitoring the level of TBT in the environment after the implementation these measures.

9. Monitoring requirements

For implementing this EcoQO, monitoring is required. For this monitoring, sample sizes should be at least 20 females per site. As monitoring of TBT specific biological effects is already a mandatory component of the CEMP, it should therefore be possible to determine whether or not the objective is met from reports on the existing monitoring (not necessarily using dog whelk). In countries that already fulfilling the commitment to the CEMP (i.e. Denmark, France, Iceland, Norway, Sweden and the UK according to ICES, 2004b) there should be no additional cost for implementing the monitoring required for this EcoQO. The monitoring of intersex in *Littorina sp.* (ICES, 2004b) is not useful for this EcoQ element, because of the lower sensitivity for TBT of this species.

Achieving the OSPAR EAC will also meet the definition of 'good ecological status' in the *physico-chemical quality elements for specific synthetic pollutants* of the Water Framework Directive (EC).

10. Recommendations

Other species of gastropod than *Nucella lapillus* are useful, and some are being used already to monitor the effects of TBT in the environment. Also, *Nucella lapillus* does not occur everywhere (eg. soft bottom substrates, deeper water), or has become scarce or extinct in some areas. It may take a long time for *Nucella* to recolonise areas where it formerly occurred, due to the absence of a pelagic larval stage. Therefore we recommend taking up the monitoring of other gastropods, and thus change the wording of the EcoQ element into:

> "Imposex in dog whelks *Nucella lapillus* or other selected gastropods",

Given the fact that not all gastropods are equally sensitive, it is recommended to set the EcoQO as:

> "the level of imposex in selected gastropods that indicates exposure to TBT concentrations below the EAC derived for TBT (assessment class B or A)".

For *Nucella* this would be a VDSI <2.

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