OSPAR COMMISSION

Status and Trends in the Levels of Imposex in Marine Gastropods (TBT in Shellfish)

Common Indicator Assessment

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OSPAR's Quality Status Report 2023

Status and Trends in the Levels of Imposex in Marine Gastropods (TBT in Shellfish)

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Contents

Key Message

Following bans on the use of tributyltin and other organotins in antifouling paints there has been a marked improvement in the reproductive condition of marine snails since the Quality Status Report 2010.

Background (brief)

Antifouling paints are widely used on marine vessels and other submerged structures to prevent the growth of unwanted marine organisms on surfaces (**Figure 1**). Since the late 1960s, antifouling/biocide paints containing tributyltin (TBT) have been used to prevent the attachment of undesired algae and other organisms on submerged surfaces. By the mid-1980s, TBT-based antifouling paints used on small craft operating in waters near commercial shellfish beds were identified as the cause of shell chambering and the consequent poor growth in oyster stocks.

TBT is toxic to many marine organisms at very low concentrations and is unequivocally linked to reduced reproductive performance in several snail species (**Figure 2**).
Since the mid-1980s, a range of national and international measures has resulted in the phasing out of paints containing TBT in the OSPAR Maritime Area. In 2008, a global ban on the use of organotins in antifouling paints on all marine boats and ships, regardless of size, came into effect.

Following TBT exposure, the entire female genital system of some marine snail species (gastropods) is conserved, but male sexual organs develop on top of them (Smith, 1981). This is termed 'imposex'. An OSPAR indicator has been developed to measure the intensity of imposex within the OSPAR Maritime Area using the Vas Deferens Sequence (VDS). Although TBT ultimately affects many organisms, marine gastropods such as the dog whelk , are among the most sensitive, making this an ideal bioindicator species for monitoring imposex intensity through VDS. In case of intersex the development of female sexual characteristics can be regressed (e.g., at *Littorina littorea*): The female pallial organs are modified towards male morphology. In higher intersex stages they were supplanted by the corresponding male formation, a prostate gland (Oehlmann, 2002).

OSPAR's Ecological Quality Objective for the North Sea is to reduce the level of occurrence of imposex and intersex in dog whelk and other marine gastropods.

Figure 1: Patch testing antifouling material. © MAPIEM, University of Toulon

Figure 2: Tributyltin affects many organisms, but marine gastropods, such as the dog whelk, *Nucella lapillus* (L.) are among the most sensitive. © Gordon Fletcher

Background (extended)

Organotin compounds are based on tin with hydrocarbon substituents; examples are tributyltin (TBT) and its degradation metabolites dibutyltin (DBT) and monobutyltin (MBT). They were used as insecticides, bactericides, and fungicides since the 1920s, but in the late 1960s it was discovered that they were very efficient at preventing biofouling (growth of organisms) on boat hulls.

Research on the toxicity of TBT in the 1970s focused on lethal, short-term effects, not on sub-lethal, longer-term effects, and thereby failed to connect TBT with the chronic phenomenon of imposex, which had been described in American and English harbours from 1970. Imposex and intersex are known as the condition whereby female marine snails (gastropods) develop male sexual characteristics, with individuals affected to varying degrees. The level of imposex can be less severe, such as development of vas deferens (the male sperm ducts), or more severe, where male reproductive tissue covers the female organs and renders the snail sterile. In for instance *Tritia reticulata*, sterilisation can also happen due to internal modifications of the organs (e.g., vulva, vagina, oviduct) although there is still scarce information in the literature on this.

Research on TBTs were for many years hampered by the inability to estimate the concentrations of TBT in the environment. The link between TBT and imposex/intersex was first discovered in France, where the oyster industry experienced massive mortality of oyster larvae and shell chambering in adults, which consequently resulted in large losses in the oyster industry in the late 1970s. In 1982, France was the first country to regulate TBT by banning the use of TBT paints on small vessels (<25 m).

In the UK, a more voluntary approach was followed, including controls on sales, and issuing guidelines for the use of TBT. The UK also set an environmental target of 20 ng/l TBT in marine waters, which at the time was considered to be sufficiently protective for marine biota. However, during the 1980s it was found that sterilization of female dog whelks occurs at concentrations as low as 3-5 ng/l TBT (Gibbs *et al.*, 1988). In contrast, summertime concentrations of TBT regularly exceeded 100 ng/l. As a result, dog whelks disappeared in many areas as they could no longer reproduce.

Areas such as Arcachon Bay in France did in fact recover. However, the recovery of dog whelk populations was still slower than expected through the 1990s. Studies at oil terminals conducted in 1988 (Bailey and Davies, 1988) had shown that the environment received significant inputs of TBT from TBT-coated oil tankers. Several studies indicated that the shipping industry caused imposex in several snail species also in offshore waters. Moreover, there is evidence that TBT damage is not limited to snails or even molluscs. TBT has also been measured in other non-target species of sea mammals such as bottlenose dolphins and has been linked to mortality events. In the South-North Sea, it has been suggested that the breakdown of the commercial catches of the crustacean *Crangon crangon* in 1990/1991 was caused by endocrine disruption as a consequence of a high body burden of TBT (Parmentier *et al.,* 2019).

By the mid-1990s, it was also realised at the political level that a worldwide phase-out of TBT use on ships should be targeted. This resulted in the International Convention on the Control of Harmful Antifouling Systems on Ships, which was adopted in 2001 and entered into force in 2008. This has been adapted by most coastal countries globally, including all OSPAR countries. As there are still countries where TBT is not banned, TBT is still manufactured in the USA and is available on the market (Uc-Peraza, 2022).

Assessment Method

In assessing a suite of marine contaminants both 'relative' and 'absolute' aspects have been analysed:

- 'trend assessment' or spatial distribution assessment to focus on relative differences and changes on spatial and temporal scales – provides information about the rates of change and whether contamination is widespread or confined to specific locations; and
- 'status assessment' of the significance of the (risk of) pollution, defined as the status where chemicals are at a hazardous level, usually requires assessment criteria that take account of the possible severity of the impacts and hence require criteria that take account of the natural conditions (background concentrations) and the contaminants' ecotoxicology. For example, Environmental Assessment Criteria (EAC) are tools in this type of assessment.

In assessing data generated through the Coordinated Environmental Monitoring Programme (CEMP), OSPAR states that the primary assessment value used in the assessment of contaminant concentrations in sediment and biota “corresponds to the achievement, or failure to achieve, statutory targets or policy objectives for contaminants in these matrices” (OSPAR 2009b). These assessment criteria were specifically compiled for the assessment of CEMP data on hazardous substances contributing to the QSR 2010 (OSPAR 2009a,c). Their use was considered an interim solution for the purposes of the QSR 2010 and were to be used until more appropriate assessment methodology could be agreed upon and implemented. These ‘interim’ criteria have also been used in the annual CEMP assessments since 2010, and will continue to be used until OSPAR agrees on the adoption of improved assessment criteria and subject to the conditions set out in the agreement.

OSPAR 2017 Intermediate Assessment (IA) values are not to be considered as equivalent to proposed European Union Marine Strategy Framework Directive (MSFD) criteria threshold values. However, they can be used for the purposes of their MSFD obligations by those Contracting Parties that wish to do so.

Provenance and limitations of BACs

Background assessment concentrations (BACs) have been developed by OSPAR to test whether measured concentrations are near background levels for naturally occurring substances and close to zero for man-made substances, the ultimate aim of the OSPAR Hazardous Substances Strategy 2010-2020. Mean concentrations significantly below the BAC are said to be ‘near background’ (for naturally occurring concentrations). BACs are statistical tools defined in relation to the background concentrations or low concentrations, which enable statistical testing of whether measured concentrations could be considered to be near background levels.

Background concentrations (BC) are assessment tools intended to represent the concentrations of certain hazardous substances that would be expected in the North-East Atlantic if certain industrial developments had not happened. They represent the concentrations of those substances at ‘remote’ sites, or in ‘pristine’ conditions based on contemporary or historical data respectively, in the absence of significant mineralisation and/or oceanographic influences. In this way, they relate to the background values referred to in the OSPAR Hazardous Substances Strategy 2010-2020. BCs for artificial substances should be regarded as zero. It is recognised that natural processes such as geological variability or upwelling of oceanic waters near the coast may lead to significant variations in background concentrations of contaminants, for example trace metals. The natural variability of background concentrations should be taken into account in the interpretation of CEMP data, and local conditions should be taken into account when assessing the significance of any exceedance.

Low concentrations (LC) are values used to assist the derivation of BACs where there have been difficulties in assembling a dataset on concentrations in remote or pristine areas from which to derive background concentrations. LCs were prepared on the basis of datasets from areas that could generally be considered remote, but which could not be guaranteed to be free from influence from long-range atmospheric transport of contaminants. LCs have also been used to assess concentrations in sediments from Spain due to the specific bulk composition of sediments from the coasts of the Iberian Peninsula. It is recognised that natural background concentrations may be lower than LCs and that they may not be directly applicable across the entire OSPAR Maritime Area.
For imposex, BACs are based on the formerly used imposex classes.

Provenance and limitations of EAC

Environmental assessment criteria (EAC) were developed by OSPAR and ICES for assessing the ecological significance of sediment and biota concentrations. Some EACs were specifically compiled for assessing CEMP data on hazardous substances contributing to the QSR 2010 (OSPAR 2009c). The EACs do not represent target values or legal standards under the OSPAR Convention and should not be used as such. The EACs were set such that hazardous substance concentrations in sediment and biota below the EAC should not cause chronic effects in sensitive marine species, nor should concentrations present an unacceptable risk to the environment and its living resources. EAC continue to be developed by OSPAR for use in data assessments.

Countries submit imposex monitoring data to ICES DOME. The imposex assessment presented here was based on data from the ICES database from 1993 to 2020 (with the exception of Portugal, where a separately submitted dataset was used).

Methods for analyses of imposex trends and status

Tributyltin (TBT) is on the OSPAR List of Chemicals for Priority Action (OSPAR, 2004). Monitoring of TBT concentrations in sediment, and its biological effects, are mandatory elements of CEMP (OSPAR, 2010). Thus, TBT differs from the indicators for other compounds, which are only defined as concentrations of the harmful compound in the animal's tissues. Technical Annex 3 to the JAMP Guidelines (OSPAR, 2008a) sets out the guidance for monitoring TBT-specific biological effects (imposex/intersex) in the gastropod species *Nucella lapillus* (dog whelk), *Tritia reticulata* (netted dog whelk, formerly known as *Tritia nitida* or *Nassarius reticulatus*) , *Buccinum undatum* (common whelk) and *Neptunea antiqua* (red whelk). Two time series of *Ocenebra erinaceus* (European sting winkle) were included in the trend assessment, but not in the status assessments (due to the absence of BACs or EACs). The great majority of monitoring stations are of either *Nucella lapillus* (dog whelk) or *Tritia reticulata* (**Table a**). Periodically (typically every 1-4 years), the degree of imposex is measured using the Vas Deferens Sequence (VDS, described below) for a number of snails in each station, which forms the basis for assessment of status and temporal trends (**Table a**). The VDS index (average VDS) is shown for all stations in OSPAR's contaminants assessment tool OHAT (https://dome.ices.dk/ohat/?assessmentperiod=2022).

In addition, a related sexual development syndrome, intersex, is monitored in *Littorina littorea* (common periwinkle) in seven Danish, Dutch, and German stations on the Wadden Sea coast. No status assessment criteria have been developed for intersex, so only trends are monitored on these stations.

Table a: Number of time series used to assess status and temporal trends of five marine gastropod species.

OSPAR subregion	*Nucella lapillus*	*Tritia reticulata*	*Buccinum undatum*	*Neptunea antiqua*	*Ocenebra erinaceus*
Barents Sea	2	0	0	0	0
Norwegian Trench	3	0	0	0	0
Northern North Sea	26	0	0	0	0
Southern North Sea	3	5	3	0	0
Skagerrak and Kattegat	4	12	3	3	0
Channel	18	0	0	0	0
Irish and Scottish West Coast	10	0	0	0	0
Irish Sea	14	0	0	0	0
Celtic Sea	17	0	0	0	0
Northern Bay of Biscay	10	0	0	0	2
Iberian Sea	17	29	0	0	0
TOTAL	**124**	**46**	**6**	**3**	**2**

Imposex is measured using the Vas Deferens Sequence (VDS), a seven-stage measurement based on the degree of development of a penis and a vas deferens (male primary sex organs) onto females (**Table b**). VDS = 0 indicates normal genitals, VDS = 5, and VDS = 6 indicates that the female is incapable of reproducing.

Table b: Stages of imposex development in females and associated Vas Deferens Sequence (VDS) scores. Note that VDS schemes generally assume that sterility in *Tritia reticulata* is caused by vas deferens proliferation over the vulva - as well described for *Nucella lapillus* - but, in fact, *Tritia* can also get sterilised due to internal modifications of the organs (e.g., vulva, vagina, oviduct) although information is scarce in the literature on the modes of action. Also, a separate index (IMPS) is used to describe imposex in *Buccinum undatum*.

Characteristics of female genitals	Vas deferens Sequence (VDS)
Imposex is not evident	0

Vas deferens development is evident at the site of the vulva	1
A penis primordium is evident behind the right eye tentacle	2
Vas deferens has developed from the base of the penis (inside which a penile duct is already visible) but does not connect with the vulva	3
Vas deferens is completely developed (continuous from the penis to the vulva)	4
Vas deferens tissue proliferates over the vulva opening, rendering the female incapable of breeding	5
Egg capsules that cannot be released by vulva occlusion form a solid mass within the capsule gland	6

Table c: Background Assessment Concentrations (BAC) and Environmental Assessment Criteria (EAC) for the different species.

Measure	Species	Common name	BAC	EAC
VDS	*Nucella lapillus*	Dog whelk	0,3	2,0
VDS	*Neptunea antiqua*	Red whelk	0,3	2,0
VDS	*Tritia reticulata*	Dog whelk		0,3
VDS	*Buccinum undatum*	Common Whelk		0,3

Imposex status

Two assessment criteria are used to assess the status of imposex in snails: BACs and EAC.
Mean imposex values significantly below the BAC are said to be *near background*, and values below the EAC indicate *no chronic effects* of TBT on snails. Both criteria are available for *Nucella lapillus* and *Neptunea antiqua*, while only EAC is available for *Tritia reticulata* and *Buccinum undatum* (**Table c**).

Meta-analysis of imposex status and trends

The meta-analyses summarise status and temporal trends for each assessment area, based on monitoring site-wise estimates. For the **meta**-analysis, a selection of time series are used:

- For trends, baseline stations were excluded, but both representative and impacted sites were included. This is different to the contaminants and reflects the fact that much of the imposex monitoring is focused on harbours (i.e., impacted sites, or sites that were impacted in the past).
- For status, all types of stations were used (baseline, representative, and impacted), as for contaminants.
- Assessment areas with at least three monitoring sites with good geographic spread (the minimum number that can be considered to provide an evidence base at the OSPAR regional level).

Time series of imposex measurements are assessed for status against the EAC if: there are at least three years of data, and there is at least one year with data in the period 2015-2020.

The status of each time series is summarised by two values: (1) the ratio between the estimated mean VDS in the final monitoring year and the EAC (this is modelled on the square root scale to better satisfy the distributional assumptions, but is presented on the original scale for interpretation), and (2) the ratio between the estimated mean VDS in the final monitoring year and the BAC.
Subsequently, these values were summarised by assessment area using meta-analysis. Assessment area status relative to the EAC is assessed by fitting the following linear mixed model by restricted maximum likelihood (McCullagh and Nelder, 1989):

- Response: status (sqrt(mean VDS / EAC));
- Fixed model: OSPAR contaminants assessment area
- Random model: status estimation variation + residual variation.

Imposex temporal trends

The ICES database contains a mixture of time series, some with individual measurements only, some with pooled measurements (i.e., data on several individuals combined into one number), and some with a mixture of individual and pooled measurements. In all cases, fitting a temporal trend requires that there was data for at least one year in the period 2015-2020 (with the exception of a few Portuguese times series, which were treated as a special case).

For the time series containing individual measurements only, the trend in each is summarised by the estimated odds ratio of the VDS of an individual whelk being above the EAC in a given year relative to the previous year. Values of 1 indicate no trend; i.e., the odds of an individual being above the EAC in a given year are the same as in the year before. Values <1 indicate that the odds of being above the EAC in a given year are lower than in the year before, so there is a decline in the level of imposex. Conversely, values >1 indicate an increase in the level of imposex. Odds ratio, rather than log odds ratio, was used because a few time series with extreme trends have less influence on the odds ratio scale. Time series with individual measurements were assessed for trends if there were at least three years of data (not necessarily consecutive years).

For the time series containing pooled measurements only, a linear trend line was fitted if there were at least four years of data. The trend was then transformed to make comparison among series possible.

For time series that contained a mixture of individual and pooled measurements, the time series was truncated to use the individual-measurement method if there were enough data (i.e., three years); otherwise, a linear trend was fitted.

Regional trends are assessed by fitting the following linear mixed model by restricted maximum likelihood (McCullagh and Nelder, 1989):

- response: trend (odds ratio);
- fixed model: OSPAR contaminants assessment area;
- random model: trend estimation variation + residual variation.

The fixed model means that a separate trend is estimated for each OSPAR contaminants assessment area. The random model has two terms:

- trend estimation variation, i.e., the variation in the trend estimates from the analysis of the original time series, assumed known and fixed; and
- residual variation, i.e., the variation that cannot be explained by any of the fixed effects or the other random effects.

General guidelines for assessment in CEMP

Methods for data screening, treatment of quality assurance information, temporal trend assessment and assessment against criteria used previously by CEMP are described in the CEMP Assessment Manual (OSPAR, 2008b) and in the help files for the contaminants online assessment tool.

Criteria used to assess environmental concentrations of hazardous substances are set out in the OSPAR agreement on CEMP Assessment Criteria for the QSR 2010 (OSPAR, 2009c). The derivation of these criteria for hazardous substances is discussed in a Background Document on CEMP Assessment Criteria for the QSR 2010 (OSPAR 2009b, 2011). These criteria reflect a two-stage process in which data are compared to concentrations yielding limited risk of biological effects (EAC), and then against BCs or zero, expressed as BACs. The latter reflects the OSPAR Hazardous Substances Strategy 2010-2020, in that concentrations should be at or close to background levels for naturally occurring substances (and zero for man-made substances).

Location of monitoring sites

Imposex is currently monitored at more than 200 sites (**Table d** and **Figure 4**) on up to three marine gastropod species.

Table d: Number (No.) of monitoring sites used to assess status and temporal trends in the occurrence of imposex, by OSPAR contaminants assessment area. Regions were excluded from the regional assessment where the number of stations was considered too low.

Region	OSPAR contaminants assessment area	Number of stations, trend		Number of stations, status	
		Total	For regional assessment	Total	For regional assessment
Arctic Waters	Barents Sea[(1)]	1	0	2	0
Greater North Sea	Norwegian Trench[(1)]	3	0	3	0
	Northern North Sea	26	26	26	26
	Southern North Sea	10	10	11	11
	Skagerrak and Kattegat	18	18	19	19
	Channel	18	18	18	18
Celtic Seas	Irish and Scottish West Coast	10	10	10	10
	Irish Sea	14	14	14	14
	Celtic Sea	17	17	17	17
Bay of Biscay and Iberian Coast	Northern Bay of Biscay	12	12	10	10
	Iberian Sea	26	26	34	34
		156	151	164	159

[(1)]The number of stations was considered to be low for regional assessment

Monitoring sites were selected using a range of approaches, although there is an emphasis on monitoring sites which are in, or near, harbours, ports, and marinas.

Methodological improvements

The regional assessment for the QSR 2010 was carried out by means of a simple tabulation of the trend and status at each monitoring site.

For the IA 2017 and the QSR 2023, a meta-analysis was used to synthesise the individual time series results and provide an assessment of status and temporal trends at the assessment area level. Meta-analyses take into account both the estimate of status or trend in each time series and the uncertainty in that estimate, and consequently, provide a more objective regional assessment.

Results (brief)

The intensity of imposex has decreased quite strongly since QSR 2010 (**Figure 3**). In 2008, 81% of sites had imposex levels classified as over EAC (environment assessment criteria), while this number had decreased to 21% in 2020. Presently, 56% of the sites are below safe environmental limits but still above background levels, while 23% of the stations are close to background levels of imposex. Note that this includes impacted stations, i.e., a set of fully representative stations would probably show a lower impact of imposex.

Where sufficient monitoring data is available a decrease in imposex is evident. Despite general downward trends, two subregions, Skagerrak and Kattegat and Iberian Sea, still have many stations above EAC (red) and must be classified as having imposex above EAC (**Figure 4**). Of the remaining subregions, four are classified as above BAC but below EAC (Northern North Sea, Channel, Celtic Sea, Bay of Biscay), while last three subregions are classified as below BAC. Use of different species for monitoring, however, makes it hard to draw safe conclusions about differences among subregions.

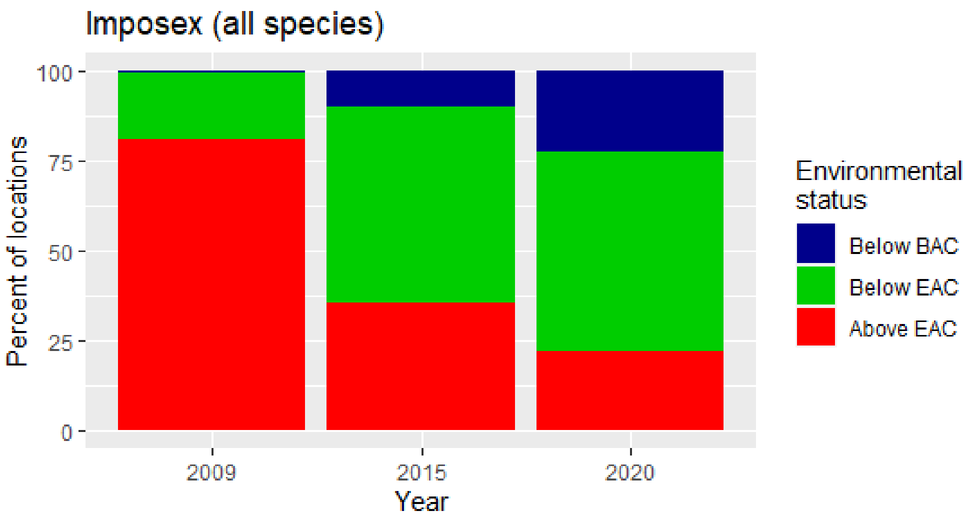

Figure 3: Percentage of stations classified as "background level" (below BAC), between BAC and EAC (denoted "below EAC") and above EAC. BAC = background assessment concentrations, EAC = environmental assessment criterion. Percentages are within each year.

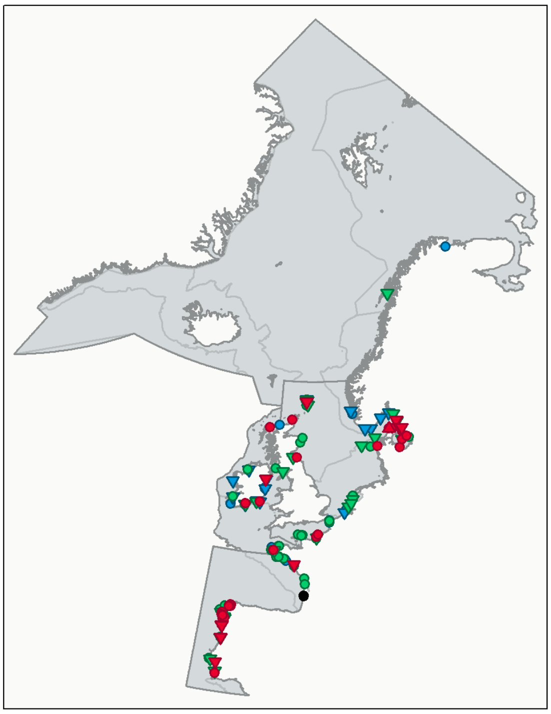

Figure 4: Status and trends of imposex on each monitoring site. Colours indicate the status: blue = not over background (imposex < BAC); green = over background level, but below Environmental Assessment Criteria (EAC); red = over EAC; black = no EAC or BAC. The shape indicate time trends of imposex (downwards pointing triangle = decreased level of imposex, circle = no changes, upwards pointing triangle = increased level of imposex). OSPAR contaminants assessment areas are shown by grey lines and OSPAR internal boundaries are shown in black lines. Available via: [ODIMS]

Results (extended)

In the QSR 2010, imposex levels were still substantially higher than at present, although the large majority of imposex time series showed a decreasing trend in the period 1997-2008. This indicates that TBT levels in the environment were still high after TBT and other organotins had been in widespread use since the 1960s. While imposex was linked with TBT already in 1981, a general ban on all vessels and offshore installations was not put in place until 2008. Thus, in 2008, as much as 81% of sites still had imposex levels classified as over EAC , in other words outside safe environmental limits (**Figure a** and **Table e**).

In 2020, the percentage of sites classified as over EAC has decreased to 22%. 56% of the sites are below safe environmental limits but still above background levels. The remaining 22% of the stations are below BAC , i.e., close to background levels of imposex; this percentage was only 0,5% in 2008 (**Table e**). A meta-analysis of imposex levels by subregion shows that all subregions can be classified as below safe environmental limits, with the exception of subregions Skagerrak and Kattegat and Iberian Sea. It should be mentioned that these two areas are also the areas with highest proportion of stations with the species *Tritia reticulata*, which may affect the status assessment (see Methods).

Table e: Percentage of stations classified as below BAC, between BAC and EAC (denoted "below EAC") and above EAC. BAC = background assessment concentrations , EAC = environmental assessment criterion. Percentages are within each year (the number of stations is given in parentheses).

Status	**2009**	**2015**	**2020**
Below BAC	0,5% (1)	9,7% (32)	22,6% (61)
Below EAC	18,4% (35)	54,7% (181)	55,6% (151)
Above EAC	81,1% (154)	35,6% (118)	21,9% (59)

The decline in imposex since 2009 can be seen in all the main OSPAR Regions with enough data, i.e., Region II-IV (**Table f**). For Region I, there are not enough stations to conclude that there has been an improvement. Region IV, which had the highest percentage of stations over EAC in 2009 (95,5%), still has the highest percentage of stations over EAC (41,1%).

Table f: Percentage of imposex stations classified according to BAC and EAC (see Figure a) for every Region and by year (in parenthesis: number of stations).

Status	2009	2015	2020
Region I			
Below BAC		100% (2)	50% (3)
Below EAC	50% (1)		50% (3)
Above EAC	50% (1)		
Region II			
Below BAC	0,7% (1)	15,3% (21)	27,4% (26)
Below EAC	14,3% (20)	52,6% (72)	49,5% (47)
Above EAC	85% (119)	32,1% (44)	23,2% (22)
Region III			
Below BAC		4,9% (7)	28,1% (27)
Below EAC	50% (13)	73,2% (104)	64,6% (47)
Above EAC	50% (13)	28,1% (31)	7,3% (7)
Region IV			
Below BAC		4,0% (2)	6,8% (5)
Below EAC	4,5% (1)	10,0% (5)	52,1% (38)
Above EAC	95,5% (21)	86,0% (43)	41,1% (30)

On the subregion level, only two subregions, Skagerrak and Kattegat and the Iberian Sea, are classified as above EAC (**Figure a**). Of the remaining subregions, four are classified as above BAC but below EAC, while three subregions are classified as below BAC. For the remaining subregions there are not enough data to warrant a metanalysis.

Of the nine OSPAR subregions with enough data, imposex levels have decreased significantly in all subregions (**Figure b**). The decrease appears to have been highest on the Scottish and Irish West Coast and the Celtic Sea, and lowest in Skagerrak and Kattegat and the Iberian Sea.

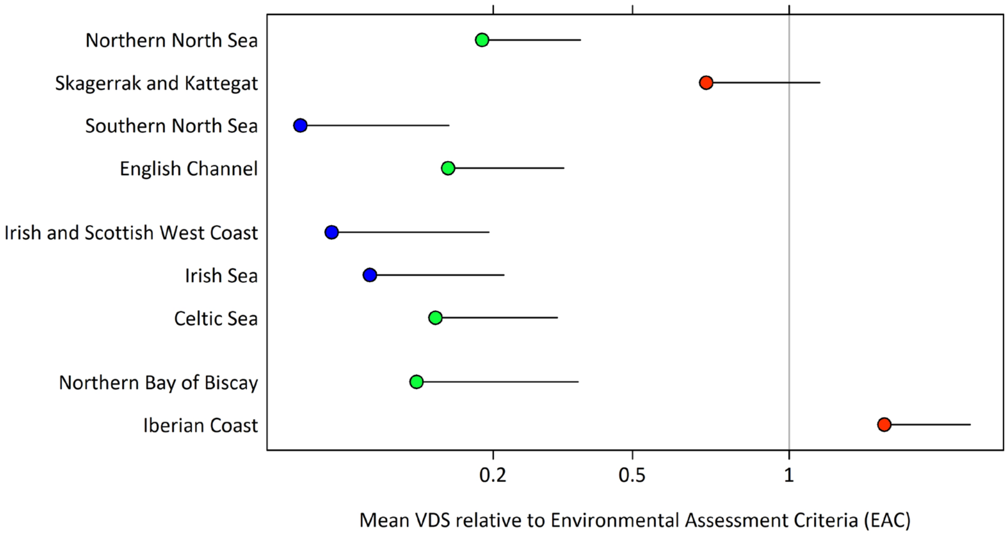

Figure a: Status on subregional level, with colour corresponding to the classification of each subregion. The status has been measured in the most recent year in the period 2015-2020. The x-axis is the VDS divided by the EAC for the given species; i.e., the x axis represents the relative VDS level after adjusting for different sensitivities among species. Subregions are classified depending on the upper confidence interval (the right end of each line) of the relative VDS level.

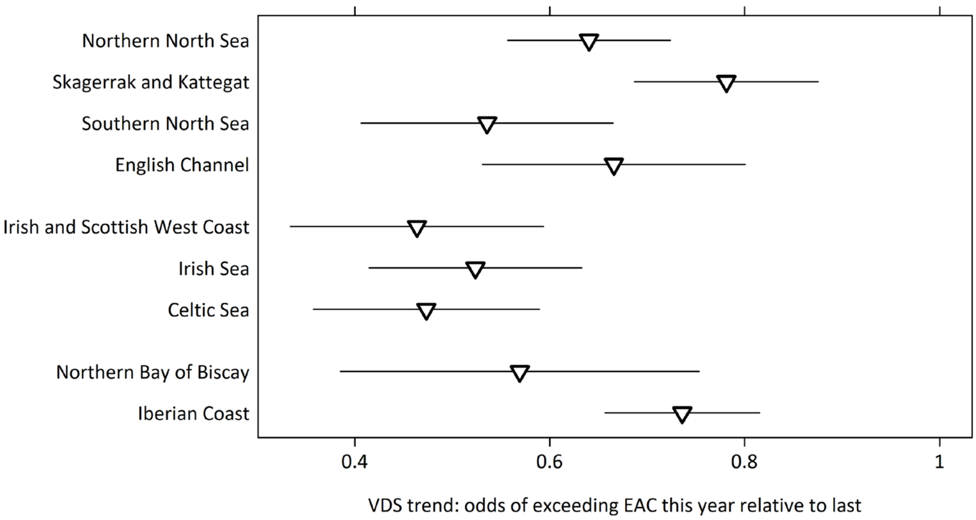

Figure b: Estimated time trends (with uncertainty) by subregion. Numbers below 1 indicate a decreasing occurrence of imposex. When both the point and the line denoting uncertainty are not overlapping with the dotted vertical line, we can conclude that the decline in imposex is statistically significant.

The ratio of VDS to EAC showed a large spread within two subregions, Iberian Sea and the Skagerrak and Kattegat. An important cause for this spread appears to be that some sites monitor *Nucella lapillus* (EAC = 2), while others monitor *Tritia reticulata* (EAC = 0,3), with the higher (poorer) estimates of status being those for *Tritia reticulata* (i.e., with the lower EAC). In principle, this should not matter as the different EAC thresholds for each species should compensate for the differences in sensitivity among species. For instance, reasons for the high sensitivity of *Nucella lapillus* may be that it is predatory and thereby affected by bioconcentration through the food chain, and also it occupies the rocky shore where it is highly exposed to the contaminant micro-layer. However, within each region, it is striking that the VDS/EAC ratio tends to be much higher for *Tritia reticulata* and *Neptunea antiqua* than for *Nucella lapillus* (**Figure c**). This may be interpreted as an indication that the difference in EAC thresholds may overcompensate for the differences in sensitivity among species. However, this interpretation may be an oversimplification due to inherent differences between the two species. First, the two species occupy different sites: *Nucella lapillus* is mostly found on the open coast, while *Tritia reticulata* is found in more sheltered sites, including near harbours, which often are hotspots for TBT. Secondly, they occupy different habitats: *Nucella lapillus* lives on rocky shores, while *Tritia reticulata* lives within the sediment. In a TBT-declining situation, the decrease in TBT concentration in the water column can be expected to be faster than its decrease in sediments, since the sediment acts as a sink for TBT. In line with this, Laranjeiro *et al.*, (2018) observed that VDSI levels in *N. lapillus* start to decrease before *Tritia reticulata* on the Portuguese coast. Thus, the "correct" difference in EAC thresholds between the species may be different in the declining phase of the TBT problem. This issue has not been further addressed.

Thus, the differences among Regions should be treated with some care, as they may differ in the species used, as well as the exact choice of sample locations within each country. For instance, the high relative VDS values for the Skagerrak and Kattegat and Iberian Sea may be to some extent linked to the low fraction of *Nucella lapillus* in these two subregions. In most subregions, most or all stations monitor *Nucella lapillus*, which is more sensitive to TBT than other species used, but in Skagerrak and Kattegat and Iberian Sea, less than 40% of the stations are *Nucella lapillus* stations.

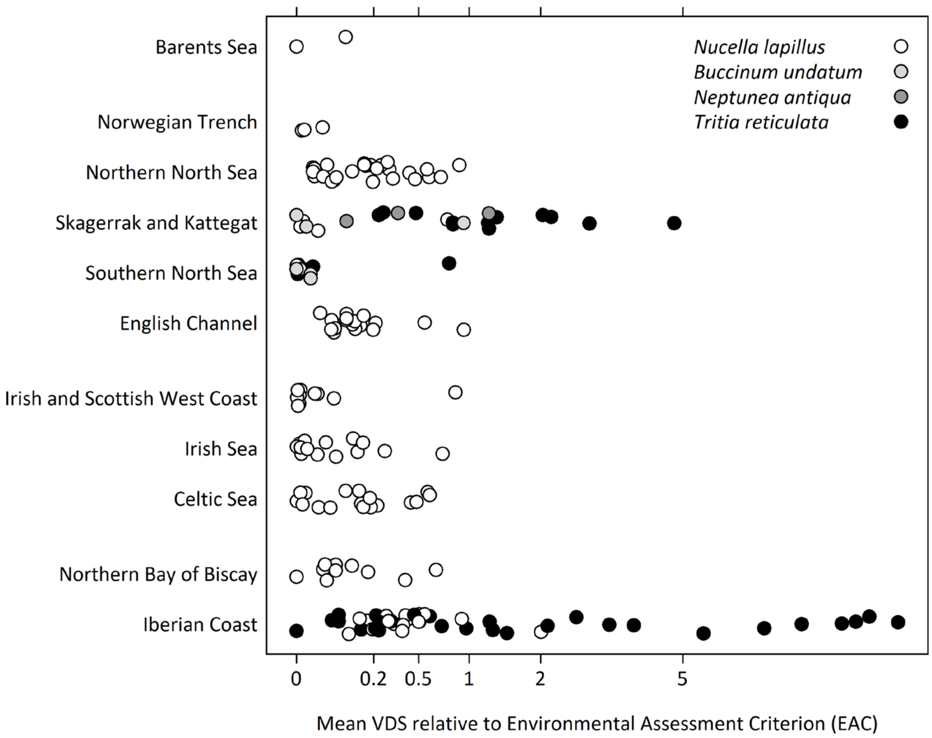

Figure c: VDS relative to the assessment criteria shown for each station. Thus values to the left of 0 are under EAC, while values to the right of 0 are above EAC. Colours indicate the species used. The values are for the last year for which data exists in the period 2015 to 2020.

Conclusion (brief)

TBT was gradually restricted from the 1980s, and finally became banned worldwide for all types of boats in 2008. The big decrease in imposex since the QSR 2010 is a clear sign that the banning of TBT has had a big effect. However, about three quarters of the sampling sites still have a higher than normal frequency of imposex, and two subregions are classified as above the EAC, i.e., outside safe environmental limits. There still also exists many local TBT hotspots that are not routinely monitored, and the long-term effects of TBT on the marine ecosystem and on higher trophic levels is uncertain. Therefore, the legacy effects of the past usage of TBT are still visible.

Conclusion (extended)

While the deliterious effects of TBT became known around 1980, it was not until 2008 (i.e. approximately 30 years later) that TBT became illegal to use in the large majority of coastal nations (and some countries still have not ratified the ban). The ban has clearly had a large effect, as shown by a big decrease in imposex since the QSR 2010. Also, many positive recoveries of local gastropod populations that were formerly decimated by sterility due to imposex, have been documented.

However, although the big picture is a positive one, one should still remember the following:

- Two subregions (Skagerrak and Kattegat, and the Iberian Sea) are classified as being generally above the EAC, i.e., outside safe environmental limits
- Overall, about three quarters of the sampling sites still have a higher than normal frequency of imposex
- While the general coastal environment may be of good status, local hotspots with high TBT concentrations remain. For instance, while routinely monitored stations may have no or very few snails with signs of imposex (**Figure a**), there still exist hotspots with high TBT concentrations in the marine sediment, typically close to shipyards and busy harbours (reviewed by Beyer *et al.* 2022).
- The combined effect of TBT and other contaminants on higher trophic levels (e.g., marine mammals) is poorly studied

Knowledge Gaps (brief)

Although TBT and imposex has largely decreased, more "hotspot" locations may need to be monitored. Assessment of TBT contamination through monitoring of imposex present difficulties due to confounding factors such as species and phsiological effects (Dumptons syndrome). Studies indicate that TBT also affects non-mollusks (such as crustaceans and mammals), but extent of its deliterious effects are unclear. The anti-fouling paints that has replaced TBT also contain toxic metals (copper and tin), and it should be investigated whether current usage of these substitutes are sustainable.

Knowledge Gaps (extended)

Given that similar decreasing levels and downward trends are being observed for imposex in different regions, including Ireland, Iceland, Norway and Portugal (Wilson *et al.* 2015; Guðmundsdóttir *et al.* 2011; Schøyen *et al.* 2019), it is clear that legislative prohibitions on the phase out of TBT have been effective and can make a difference to the impact on organisms. While there is general consensus of improvement of environmental status (and the potential for reduced monitoring effort), some “hotspot” or locations may still have imposex levels that are not decreasing or that still exceed EAC thresholds (Beyer *et al.* 2022). The possible ecological impacts in biota have been investigated only to a limited degree. It is suggested that monitoring efforts could primarily focus on such locations as part of wider spatial and investigative studies (e.g. incorporating other matrices).

The usage of several species for monitoring represents a confounding factor that is difficult to deal with, and it is uncertain whether the present species-specific assessment criteria (EACs) result in an index of TBT contamination that is homogenous across species (**Figure d**). Literature additionally suggests the potential for a confounding factor in imposex measurements that should be considered in TBT specific effect monitoring programmes, i.e. Dumptons syndrome, which has been shown to reduce the sterilising effect of TBT in dogwhelks (Quintela *et al*. 2002). Additionally, a strong case has been made that the retinoid X receptor (RXR) and its natural ligand, 9-cis-retinoic acid, play major roles in the development of imposex caused by organotin compounds, but there is still a major question as to why only one group of mollusks and not other invertebrates are affected (Sumpter and Johnson, 2005). In addition research towards the potential for other (or synergistic) contaminants potential to induce the imposition of male characteristics on female gastropods merits inclusion in future research in this area.

Tributyltin has been shown to have significant effects on the fitness of organisms other than mollusks, including shrimp (Parmentier *et al.*, 2019) and sea mammals (Zhang *et al*., 2022 and references therein). The extent to which non-mollusk species are affected by legacy and ongoing TBT contamination is poorly known. Also, the combined toxicity effects of TBT in combination with other inorganic/organic pollutants is poorly known.

In European waters, TBT has almost universally been replaced by paints containing other tin substances and/or copper, typically in self-polishing paints that slowly release these toxic metals into the environment. Several studies have indicated that such paints increase the concentrations of dissolved or nano-sized particles of these metals (Adeleye *et al.* 2016 and references therein). Lagerström *et al.* (2020) show that the use of antifouling paints leads to exceedance of both EQS and PNEC values, and argue that current risk assessment procedures may mean that the environmental risks are underestimated as they fail to take into account that changes in the speciation of dissolved Cu and Zn may increase the proportion of metals that may be considered bioavailable. Thus, there may be good reasons to further investigate whether the current use of TBT substitutes are ecologically sustainable.

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Assessment Metadata

Field	Data Type	
Assessment type	List	Indicator assessment
Summary Results (template Addendum 1)	URL	https://odims.ospar.org/en/submissions/ospar_tbt_biota_msfd_2022_06
SDG Indicator	List	14.1 By 2025, prevent and significantly reduce marine pollution of all kinds, in particular from land-based activities, including marine debris and nutrient pollution
Thematic Activity	List	Hazardous Substances
Relevant OSPAR Documentation	Text	OSPAR Agreement 2004-12 List of Chemicals for Priority Action Revised 2013 OSPAR Agreement 2008-09 JAMP Guidelines for Contaminant-Specific Biological Effects OSPAR Publication 2008-379 Coordinated Environmental Monitoring Programme Assessment Manual for contaminants in sediment and biota OSPAR Publication 2009-390 CEMP assessment report: 2008/2009 Assessment of trends and concentrations of selected hazardous substances in sediments and biota.

Field	Data Type	
		OSPAR Publication 2009-461 Background Document on Assessment Criteria used for assessing CEMP Monitoring Data for the Concentrations of Hazardous Substances in Marine Sediments and Biota in the Context of QSR 2010 OSPAR Agreement 2009-02 on CEMP Assessment Criteria for the QSR 2010 OSPAR Agreement 2010- 01 OSPAR Coordinated Environmental Monitoring Programme (CEMP) (amended in 2011, 2012, 2013) OSPAR Publication 2011-535 Background document on organic tin compounds
Date of publication	Date	2022-06-30
Conditions applying to access and use	URL	https://oap.ospar.org/en/data-policy/
Data Snapshot	URL	https://doi.org/10.17895/ices.data.21229139
Data Snapshot	URL	https://doi.org/10.17895/ices.data.18601820
Data Results	Zip File	https://odims.ospar.org/en/submissions/ospar_tbt_biota_results_2022_06/
Data Source	URL	https://dome.ices.dk/ohat/?assessmentperiod=2022

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