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## Accepted Manuscript

A weight-of-evidence approach to assessing the ecological impact of organotin pollution in Dutch marine and brackish waters; combining risk prognosis and field monitoring using common periwinkles (*Littorina littorea*)

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1 **12 February 2008**

2 **A weight-of-evidence approach to assessing the ecological impact of organotin pollution in Dutch**  
3 **marine and brackish waters; combining risk prognosis and field monitoring using common**  
4 **periwinkles (*Littorina littorea*)**

5

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19 **Keywords:** Gastropods, harbours, tributyltin, intersex, risk assessment, sediments, SPM.

20

21

**Abstract**

In the present study an integrated ecological risk assessment based on multiple lines of evidence (LOEs) was evaluated in order to better assess the risk from TBT in Dutch harbours and open coastal waters. On the basis of spatial distributions of measured tributyltin (TBT) concentrations in sediments and suspended matter, predictions of the intersex index (ISI) in *Littorina littorea* and the ecological risk expressed as the Potentially Affected Fraction (PAF) of species were made. The results were compared to actual ISI measurements and presence of *L. littorea* in the field. The PAF calculated on the basis of TBT levels for open coastal waters ranged from 4.2% to 15.3%; for harbours it ranged from 3.5% to 26.9%. Significant intersex levels were observed only in waters where the risk was calculated above 10% PAF. This study suggests that the absence of *L. littorea* from some harbours with high ecological risk values can be explained by high TBT concentrations. A call is made for the use of integrated approaches like weight of evidence (WOE) to help practitioners improve ecological risk assessment.

**1. Introduction**

Tributyltin (TBT) was first used as an antifouling agent in the late 1960s. It proved to be very effective, but also extremely toxic to aquatic life (Champ and Seligman, 1996). TBT effects were first recognized in the marine environment in the 1970s and attracted particular attention in the 1980s, when the effects of TBT started to become apparent in the Netherlands and elsewhere (Marquenie and Vethaak, 1989; Champ and Seligman, 1996). In the 1980s, there was a sharp decline in the population of dog whelk (*Nucella lapillus*) in the Delta area of the south-western Netherlands and in the 1990s the occurrence of imposex was demonstrated among whelks (*Buccinum undatum*) in the same area (Mensink et al., 1996) as well as in the open North Sea (Ten Hallers-Tjabbes et al., 1994).

1 Given the concern about the environmental effects of TBT in the Dutch marine environment a lot of data  
2 has been collected in the past. The available data relate to TBT concentrations in water, sediments and  
3 suspended matter and field measurements of intersex in, and the distribution and abundance of  
4 gastropods. The Chemical Toxicity Test (CTT) has shown TBT to be the most problematic of all the  
5 chemicals tested in toxicological terms in assessing whether dredged material may be relocated in the  
6 marine environment (Schipper and Schout, 2004). Up to now, in assessing the impact of TBT on the  
7 ecosystem, the available information has been evaluated separately. This paper describes the results of  
8 an integrated assessment including all the information collected in recent years. Comparable to the  
9 sediment quality Triad concept, which uses information from the toxicity line, the biological field line and  
10 the chemistry line, this assessment is also based on results of three lines of evidence (LOEs) (Chapman,  
11 1996; Rutgers, 2000). However, instead of using measurements from multiple species in the biological  
12 field line, as normally done in the Triad approach, the assessment in the present study uses information  
13 from one indicator species only. In the present study the LOEs are primarily represented by  
14 measurements of: (i) chemical concentrations (exposure levels), (ii) (eco)toxicological indicators and  
15 results from ecotoxicological laboratory experiments, and (iii) abundance of indicator species *Littorina*  
16 *littorea* in the field. The Potentially Affected Fraction of species (PAF) was used to represent the  
17 ecological risk and quantify the degree of TBT stress on the ecosystem (Aldenberg et al., 2002). All three  
18 LOEs provide indications of the ecological health status related to TBT pollution, though all approaches  
19 based on this level include uncertainties:

- 20 i. TBT pollution is directly chemically measured in samples collected in the field. However, the  
21 relevance of chemical analysis of sediments to the quality of water bodies is not always clear.  
22 Processes such as bioavailability determine the actual relation between chemical concentrations and  
23 possible ecological effects. Ecological risk prognosis, using accepted generic procedures, predicts  
24 the health status related to the stressor under consideration based on the measured or estimated  
25 chemical concentrations assuming equilibrium partitioning and constant bioavailability. The prognosis  
26 will provide the likelihood of effects to occur. Whether or not these effects will actually occur depends  
27 on location specific conditions.
- 28 ii. Biological effect parameters can be used to assess the potential and actual impacts of TBT on  
29 marine coastal life. However, it can be difficult to link biological effects to exposure to a single  
30 contaminant. In this study, the degree of intersex index (ISI) in the common periwinkle, *L. littorea*,  
31 was selected as an indicator of TBT pollution, as this species is the most common prosobranch  
32 species on hard substrates in Dutch coastal waters. Intersex in this species has proven to be  
33 sensitive to the degree of environmental organotin pollution in coastal waters (Oehlmann et al.,  
34 1998). Although periwinkles are not the most sensitive gastropod and ISI is not a rapid response,

1 measuring ISI levels in *L. littorea* in the field is still a useful way of assessing effective levels of TBT  
2 pollution. Several studies have shown that ISI can be used as biological tool to determine the degree  
3 of TBT pollution, which can be expressed as approximate TBT concentrations in seawater (Stroben  
4 et al., 1992; Oehlmann et al., 1998). However, the ISI indicator in periwinkles cannot easily be  
5 translated to a generic health indicator for the water body under consideration.

6 iii. Observations from biological effect monitoring at gastropod level (Ten Hallers-Tjabbes et al., 1994;  
7 Mensink, 1996; De Wolf, 2001) are used to assess effects of the distribution and contamination levels  
8 in the Dutch marine environment. The abundance of *L. littorea* and the far more sensitive dog whelk,  
9 *N. lapillus*, can be used as indicators. TBT contamination has made the dog whelk a rare species in  
10 Dutch coastal waters and it is almost entirely absent from harbours and marinas (Harding et al.,  
11 1992; Van Moorsel, 1996). TBT potentially accumulates in sediments. However, there is no direct link  
12 between sediment concentrations and effects in gastropods, as the preferred habitats of some of the  
13 most common gastropods (e.g. *N. lapillus*, *L. littorea*) are hard substrates, including natural  
14 substrates such as rock and artificial substrates such as harbour walls, breakwaters, etc. (Oehlmann,  
15 2002).

16 In this study the results of the LOEs are compared in order to establish their similarity and reduce the  
17 uncertainty of the overall assessment. The methodological links between different LOEs must be  
18 evaluated so that the results of monitoring effects on *L. littorea* and risk assessment studies focusing on  
19 the impacts of TBT can be compared. This paper describes a WOE approach (Smith et al., 2002)  
20 focusing on TBT in Dutch harbours and open waters. Focusing on the integration of indicator monitoring  
21 and risk prognosis, the present study applies a more holistic approach to impact assessment (Suter II,  
22 2001).

## 24 **2. Materials and Methods**

### 25 *2.1. Study area*

26 An integrated ecological risk assessment approach including evaluation of three LOEs is applied to the  
27 collected data to assess ecological health status related to TBT pollution in Dutch marine and estuarine

1 waters. Figure 1 shows the harbour locations 1 to 30 and the seven open water areas (A to G) from  
2 which chemical, (eco)toxicological and/or ecological data have been included in this study.

### 3 4 *2.2. Chemistry*

5  
6 The top 5 cm of sediment was sampled using a box core at 120 locations along the Dutch coast between  
7 1998 and 2003. Over this period, 106 locations were sampled twice with an interval of three years. The  
8 locations covered the main open North Sea waters, the Wadden Sea, the Eastern Scheldt and estuarine  
9 areas such as the Western Scheldt and Ems-Dollard (see Figure 1). TBT was also analysed in sediment  
10 from a number of harbour locations from 1999 to 2002 (Table 1).

11 TBT in suspended particulate matter (SPM) was measured four times a year at eight locations in four  
12 different open water areas ( A. Ems-Dollard; B. Wadden Sea; C. North Sea; E. Eastern Scheldt; G.  
13 Western Scheldt, Figure 1) along the Dutch coast between 1998 and 2003, giving information on  
14 fluctuations between and within years. A further 17 locations (data not shown) from the French-Belgian  
15 border to the Ems-Dollard estuary on the Dutch-German border were sampled in 2003. Suspended  
16 matter was sampled by pumping surface water through a flow-through centrifuge lined with Teflon plates  
17 to collect the suspended matter. At harbour locations 23 and 29 (see Figure 1) the seasonal variation of  
18 water-dissolved TBT was sampled from 1999 to 2002.

19 The sediments were freeze-dried and homogenized using a Retsch planetary ball mill. 1.5 ml acetic acid,  
20 15 ml methanol and 7 ml hexane were added to 1 g of sediment. This mixture was stirred for 30 min, after  
21 which 3 ml of 4 M sodium acetate solution (pH=5) was added to obtain optimal ethylation. Then, 4 ml of  
22 5% sodium tetraethyl borate solution was slowly added using a peristaltic pump over 20 min, during  
23 which time the mixture was continuously stirred. The mixture was shaken vigorously and 5 ml 10 M  
24 sodium hydroxide solution added to remove boroxin formed at ethylation. About 5 ml of water was added,  
25 and after shaking and subsequent centrifugation the hexane layer with the ethylated organotin  
26 compounds was collected. This hexane extract was cleaned over a column of 4-gramme aluminium oxide  
27 (Alumina B-super deactivated with 10% water) using hexane as an eluent. 1 ml of iso-octane was added  
28 to the hexane eluate and this extract was then concentrated to a final volume of 0.5 ml using Kuderna-  
29 Danish solvent evaporation. The final extracts were analyzed for ethylated organotin compounds by

1 electron impact (EI) GC-MS using selective ion monitoring. The method uses ethylated Quasimeme  
2 standards and tetrapropyltin as internal standard. Quality assurance recovery control with carried out with  
3 monopropyltin and tripropyltin in each sample and blank control in every analysis batch. Yearly  
4 participation in Quasimeme laboratory proficiency exercises for organotins resulting in satisfactory z-  
5 scores.

### 8 *2.3. Probabilistic risk assessment methods*

9 The measured TBT-Sn concentrations were used as a basis for a probabilistic risk assessment. In those  
10 samples where the fraction organic carbon ( $f_{oc}$ ) was reported, the sediment and suspended matter  
11 concentrations from the field surveys were normalised to water concentrations, applying the formulas in  
12 the European Technical Guidance Document for Risk Assessment (EC, 2003). The ecological risk  
13 expressed as the Potentially Affected Fraction of species (PAF) was calculated in accordance with  
14 Aldenberg et al. (2002). This method can be summarised as follows. The calculated water concentrations  
15 were compiled in a log-normal distribution representing the spatial variation of TBT concentrations in a  
16 specific area. The concentration distribution was then compared to the species sensitivity distribution  
17 (SSD) for TBT. The SSD describes the average sensitivity of species and the variation among species  
18 (Posthuma et al., 2002). The SSD for TBT reported by Lepper (2002) is based on toxicity data for 29  
19 marine and freshwater species representing seven taxonomic groups (algae, annelida, crustacea,  
20 echinodermata, insecta, mollusca, pisces). The SSD for TBT-Sn used in this study is provided in the  
21 supplementary data for this paper.

22 The PAF indicates the likelihood that adverse ecological effects will occur as a result of TBT exposure in  
23 a particular area. This value can be interpreted as the probability that a randomly selected exposure  
24 concentration will exceed species sensitivity. The probabilistic risk assessment was performed on the  
25 clustered data for harbours and for open waters, as well as for each harbour and open water separately.  
26 Only water systems for which exposure concentrations were measured at two or more locations were  
27 selected (all open waters and 19 out of 30 harbours).



1 The likely range of the intersex indicator (ISI) for *L. littorea* was also calculated based on the dose-  
 2 response relationship for *L. littorea* as published by Oehlmann (2002) and the spatial distribution of water  
 3 concentrations. The range of possible ISI values from the 5<sup>th</sup> and 95<sup>th</sup> percentile of the exposure  
 4 concentration distributions were calculated applying Formula 1.

$$6 \quad ISI = \frac{3.35}{1 + \exp\left(\frac{-(PEC_{seawater} - 18.3)}{4.73}\right)} \quad (1)$$

7 where:

8  $ISI$  = calculated ISI level [-]

9  $PEC_{seawater}$  = concentration of TBT-Sn in seawater [ $ng.l^{-1}$ ]

#### 11 2.4. Biological effects and abundance surveys

12 Periwinkles were sampled from 2002 to 2005 at 42 locations along the Dutch coast, representing the  
 13 main Dutch open waters areas and harbour locations (Figure 1). Grevelingen (D) and Veersemeer (F)  
 14 lakes were not included. At the sampling locations the presence of *L. littorea* was recorded at every  
 15 location randomly selected in 5 to 7 areas of 1 m<sup>2</sup>. If present, at least 40 individuals of *L. littorea* with a  
 16 shell height between 15 and 25 mm (approx. 1.5-year-old new adults) were collected. The occurrence of  
 17 intersex in the periwinkles was analysed within seven days, in accordance with the methods described by  
 18 Bauer et al. (1995). ISI is expressed as the average intersex stage within the population.

#### 19 2.5. Overview of chemical and biological datasets

20  
 21 Table 1 lists the chemical and biological datasets compiled for this study. The number of 'areas' refers to  
 22 the open waters presented in Figure 1. The number of 'locations' refers to different sampled harbours  
 23 within the areas.

### 25 3. Results

#### 27 3.1. Observed and predicted exposure levels

1 The observed concentration ranges of TBT in sediment or SPM in Dutch open waters and 19 Dutch  
2 harbours are shown in Table 2. The highest observed TBT-Sn concentration in Dutch harbours was a  
3 factor 30 higher than the highest TBT-Sn concentrations in open waters. The lowest TBT concentrations  
4 were more or less in the same range. In order to normalise the sediment and suspended matter  
5 concentrations to water concentrations a value for the  $K_{oc}$  (organic carbon partition coefficient) needs to  
6 be estimated. From the distribution of measured sediment and water concentrations in Dutch harbours a  
7 value for the  $K_{oc}$  representative of the Dutch situation was estimated. A value of 5.34 gave the best  
8 correspondence between observed and predicted water concentrations in Dutch waters (More  
9 information on how this value was obtained can be found in the supplementary data). As no temporal  
10 trends were observed in the concentrations of TBT-Sn in either the open waters or the harbours, the  
11 average concentration of TBT-Sn was taken to represent the exposure at a sampling location. This  
12 average concentration was used for further analysis in the risk predictions. Since the concentration  
13 distributions of the normalised sediment samples and SPM samples did not differ significantly (Student's t  
14 test, equal variances,  $p=0.42$ ), the normalised data of the two distributions were pooled. Figure 2  
15 presents the overall exposure concentration distributions for TBT-Sn for 138 open water and 270 harbour  
16 locations respectively.

17 Dedicated exposure concentration distributions were derived for all areas and locations (open waters and  
18 harbours), representing the spatial distribution of water concentrations for each area. In Table 2 the 5<sup>th</sup>  
19 and 95<sup>th</sup> percentile of the distribution represent the range of water concentrations for each area.

20

### 21 3.2. Predicted ecological risk

22 The two distributions presented in Figure 2 were used as input for a probabilistic risk assessment as  
23 described by Aldenberg et al. (2002). This resulted in an expected total ecological risk for open waters of  
24 6.7% and 19.1% for harbours. This implies that, on average, for open waters it is likely that 6.7 % of  
25 species will be negatively affected as a result of exposure to TBT. For the harbours this fraction is 19.1%.  
26 The risk values for the individual open waters and harbours are given in Table 2. Relatively high-risk  
27 values were obtained for open waters in the Western Scheldt (G) and Veersemeer (F), while the risk  
28 values for the Wadden Sea (B) and the Eastern Scheldt (E) were below 5%. For harbours the highest risk

1 value was predicted at Harlingen in the Wadden Sea (location 8). Four harbours, all small harbours in the  
2 Wadden Sea, yielded a risk value equal to or lower than the lowest risk value for the individual open  
3 water bodies (see Table 2). The table presents the observed and predicted concentrations and ISI levels  
4 in *L. littorea*. The ecotoxicological risk presented as PAF can be interpreted as the characterization of the  
5 potential adverse health effects of exposure to environmental hazards. The 5<sup>th</sup> and 95<sup>th</sup> percentiles of the  
6 exposure concentration distribution in the dedicated open water or harbour were used to calculate the  
7 range of ISI values likely to occur in the area. The upper and lower limits of the calculated ISI values are  
8 reported in Table 2.

### 10 3.3 Observed ISI values and species abundance

11 ISI observations are available for the open waters, except for Grevelingen (D) and Veersemeer (F) lakes,  
12 where no ISI sampling was carried out. The observed ISI values for open waters were relatively low. The  
13 highest ISI value was observed in the Western Scheldt. Ten harbours were selected for ISI sampling.  
14 However, since no periwinkles were found in Harlingen (location 8), ISI observations are available for  
15 only 9 harbours. Periwinkles with low abundance were found at the harbours in Delfzijl (location 1), Den  
16 Helder (location 14), IJmuiden (location 15) and Rijnmond (location 18). Much higher ISI levels were  
17 observed in harbours than in open waters. The highest value (3.0) was observed in Den Helder (location  
18 14). The wide range of ISI levels observed in Vlissingen harbour (location 25) indicates that a high  
19 variation in ISI levels can exist within a single harbour.

### 21 3.4. Comparison of the LOE results

22 To check the correspondence between the maximum ISI levels that can be expected in a harbour and the  
23 ecological risk, PAF values have been plotted against the calculated ISI value corresponding to the 95<sup>th</sup>  
24 percentile of the exposure concentration distribution (Figure 3). The 95<sup>th</sup> percentile was chosen because  
25 this value corresponds to the most polluted part of the harbour or water body corresponding to a  
26 maximum ISI value that might be encountered during field monitoring. In ecological risk prognosis a  
27 Potentially Affected Fraction of 5% of species is generally considered acceptable (e.g. Van Straalen and  
28 Denneman, 1989; Aldenberg and Slob, 1993; Newman et al., 2000; Van der Hoeven, 2001; EC, 2003).  
29 This study shows that at this 5% level intersex in *L. littorea* is unlikely to occur in the field. But even at

1 higher risk levels, up to approximately 10%, ISI levels in the field are not expected to exceed the ISI  
2 signalling level of 0.3 (Oehlmann, 2002). This study showed that high ISI levels up to a maximum of 3.35  
3 are expected in the field only when the predicted risk level exceeds 20%. These results indicate that  
4 either the ISI measurements in *L. littorea* are not sensitive enough to assess ecosystem health, or the risk  
5 prognosis is too conservative (predicting risks while there are no actual effects).

6 In order to check whether the conservatism in the risk assessment is a result of the estimated  
7 concentrations (chemistry LOE), the calculated ranges of ISI values based on the 5<sup>th</sup> and 95<sup>th</sup> percentile  
8 of TBT concentrations are compared by the observed ISI values in the field (Ecotoxicity LOE). If there is a  
9 good correspondence between calculated and observed ISI, it can be concluded that the concentrations  
10 are a proper basis for the risk calculations as well. Figure 4 presents the comparison between calculated  
11 and observed ISI for open waters (A) and Dutch harbours (B).

12 The observed ISI values were relatively low for open waters, often below the 5<sup>th</sup> percentile of the  
13 predicted ISI range based on the TBT concentrations. Given the TBT concentrations in the Western  
14 Scheldt (G) and Veersemeer (F), predicted ISI values around 1.0 might be expected. Although intersex  
15 was observed at a low level (ISI = 0.15) at one location in the Western Scheldt, the observations never  
16 exceeded the signalling level of 0.3 (Oehlmann, 2002). Nearly all observed ISI levels in harbours fall  
17 within the ISI ranges corresponding to the median and 95<sup>th</sup> percentile of the exposure concentrations.  
18 The ISI levels observed in IJmuiden (location 15), Den Helder (location 14), Vlissingen (location 25) and  
19 Lauwersoog (location 3) corresponded to the upper 25% of the exposure concentrations. At 4 other  
20 harbours – Delfzijl (location 1), Yerseke (location 22), Scheveningen (location 17) and Rijnmond (location  
21 18) – the observed ISI levels corresponded to the upper 50% of the exposure concentrations. Maximum  
22 intersex levels are predicted for three harbours (locations 8, 14 and 18) where no periwinkles were found  
23 at some stations. In IJmuiden (location 15) and Delfzijl (location 1), where periwinkles were in low  
24 abundance, the maximum calculated ISI level is around 1.5.

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#### 4. Discussion

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1 In the present study a weight-of-evidence approach derived on the basis of three LOEs has been  
2 evaluated in order to better assess the risk from TBT in Dutch harbours and open waters. Based on  
3 chemical data, risk values were determined according to Aldenberg et al. (2002) and ISI values were  
4 calculated based on the dose-response curve reported by Oehlmann (2002). Actual ISI observations  
5 were made in the field and the presence of gastropods was recorded. In the overall approach evidence  
6 from the different LOEs was combined.

7 The partition coefficient ( $K_p$ ) was used for the conversion of TBT concentrations in sediment and SPM to  
8 TBT concentrations in water. This  $K_p$  for TBT was calculated by multiplying the organic carbon partition  
9 coefficient ( $K_{oc}$ ) with the measured fraction organic carbon ( $f_{oc}$ ). Consequently, the  $K_{oc}$  value has a strong  
10 impact on the final results of the risk prognosis. Generally, in risk predictions the lowest  $K_{oc}$  value is  
11 applied to calculate concentrations in water (EC, 2003). This results in a worst-case water concentration,  
12 in accordance with the precautionary principle. However, with literature values for the  $K_{oc}$  of TBT ranging  
13 from 3.0 – 6.2 (Lepper, 2002), it is more appropriate to base an assessment on local measured values. In  
14 this study the  $K_{oc}$  value was estimated from measured aqueous and sediment concentrations in 30 Dutch  
15 harbours evenly distributed over the Netherlands. This resulted in an average  $K_{oc}$  value of 5.34 (see also  
16 the supplementary data). This outcome is within the range of reported  $K_{oc}$  values from the literature  
17 (Lepper, 2002).

18 The highest ISI value, measured in the Western Scheldt (G), still fell within the predicted ISI range.  
19 Higher ISI values for samples from the Western Scheldt (G) have, however, been reported by De Wolf et  
20 al. (2001 and 2004), showing a decline from 1.3 in 1998 and 2001 to 0.5 in 2002. This decline was  
21 confirmed by the surveys reported here, where an ISI of 0.15 was found at the same location in 2002,  
22 using only young adults. This is comparable to the ISI value reported by De Wolf et al. (2004), as these  
23 were adults that had been exposed some years before the young adults were exposed. In contrast to the  
24 trend in the ISI, no downward trend in the sediment-derived water concentrations from 1998 to 2003 was  
25 observed in the Western Scheldt (G). We can conclude from the data that observed ISI levels in open  
26 waters are all in the lower range of predicted ISI levels.

#### 27 4.1. Combination of LOE results and WOE

28

1 The results of the risk predictions confirm that *L. littorea* is not the most sensitive species regarding to  
2 TBT pollution (Oehlmann *et al.*, 1998; Ospar, 2003). In the field, significant effects on intersex levels are  
3 expected for this species only when the risk level exceeds 10%, whereas a risk level of 5% is generally  
4 considered acceptable for the protection of ecosystem health (e.g. Van Straalen and Denneman, 1989).  
5 High ISI levels, up to a maximum of 3.35, are expected only when the risk level exceeds 20%. At these  
6 high ISI levels, up to a maximum of 3.35, the population of *L. littorea* is expected to be completely sterile  
7 (Oehlmann, 2002). It may even be argued that a risk level of 5% is far too high for specifically acting  
8 toxicants such as TBT. Even though *L. littorea* is not the most sensitive species to TBT pollution, the only  
9 species that are more sensitive are other species of prosobranch molluscs. Protecting 95% of species in  
10 general would still exterminate the carnivorous neogastropods and also a number of deposit-feeders  
11 such as *Scrobicularia plana*, which can be considered as key species for ecosystem function. Therefore  
12 for specifically acting toxicants even stricter criteria should be argued for. In absence of other indicator  
13 species *L. littorea* can serve as a species for monitoring, however, when observed ISI levels in *L. littorea*  
14 do not exceed natural environmental background levels it is still likely that 10% of other species might be  
15 affected by TBT contamination.

16 Both calculated ISI and PAF values are based on the spatial distribution of concentrations. This  
17 distribution is derived from TBT concentrations in sediment and SPM. One complicating factor lies in the  
18 fact that, in most water systems, periwinkles do not live near the sampled sediment (which is also used to  
19 derive the water concentrations), but considerably higher up, on the hard substrates where the macro  
20 algae they feed on grow. There might therefore be no direct relationship between TBT in sediment in  
21 harbours and ISI values in periwinkles. Instead, the periwinkles are probably predominantly exposed to  
22 TBT dissolved in the water and adsorbed to SPM circulating in the water. The fair correspondence  
23 between observed and calculated ISI values for *L. littorea* as presented in this study shows that sediment  
24 and SPM concentrations, combined with a realistic partition coefficient ( $K_{oc}$ ), can be usefully applied to  
25 estimate effective concentrations in the water column.

26 With this relationship between predicted ISI in periwinkles and the PAF value, the ISI indicator might  
27 potentially not only serve as an indicator of TBT pollution but also to represent generic ecological health  
28 status. However, this relationship is useful only if many ISI measurements are available for an area,

1 covering the whole distribution of possible ISI values. The wide range of ISI levels observed in Vlissingen  
2 harbour (location 25) indicates that a high variation in ISI levels can exist within a single harbour.

3 For open waters, only two measured ISI values fell within the predicted ISI ranges (North Sea (C)) and  
4 Western Scheldt (G), while the rest of the observations were below the calculated ranges. This is  
5 presumably a consequence of the asymptotic minimum of 0.07 in Formula 1. Differences in local  $K_{oc}$   
6 values may have caused the predicted ISI levels in open waters to exceed the observed ISI values. In  
7 this study the  $K_{oc}$  is based on measured concentrations in sediment and water from harbours. This is in  
8 line with the fact that the correspondence in harbours is much better. It might be possible to derive a  
9 dedicated  $K_{oc}$  for open waters from the comparison of water and sediment concentrations in open waters,  
10 potentially improving the correspondence between predicted and measured ISI levels for these water  
11 systems. However, such data are not available. The possible over-estimation of water concentrations in  
12 open waters would also imply that the ecological risk values presented in this study have been over-  
13 estimated for open waters.

14 There is good correspondence between observed ISI levels and the predicted ISI ranges for each  
15 harbour. Since almost all observed ISI levels fell within the ranges of predicted ISI levels in open waters,  
16 it can be concluded that the spatial distribution of TBT concentrations is a good basis for determining  
17 predicted ISI levels, and therefore ecological risk.

18 Periwinkles were found in low abundance at several harbour locations: Delfzijl (location 1); Harlingen  
19 (location 8); Den Helder (location 14); IJmuiden (location 15); and Rijnmond (location 18). It is unclear  
20 from the observations alone whether this was caused by high levels of TBT or other stress factors (e.g.  
21 salinity, excessive sand transport, etc.). The WOE approach can help to indicate the likelihood of TBT  
22 causing this absence. High risk values ( $PAF > 20\%$ ) and maximum ISI levels have been predicted for  
23 Harlingen (location 8), Den Helder (location 14) and Rijnmond (location 18), so it is not unlikely that high  
24 TBT pollution caused the absence of periwinkles from these harbours.

25 Based on the evaluation of TBT effects on intersex development in the periwinkle *L. littorea*, and  
26 considering ecological health status as a criterion for decision-making, With reference to Table 3, LOEs  
27 were derived for *L. littorea*, representing the species used in the OSPAR monitoring guideline. The  
28 relationships between LOEs obtained from comparison of observed effects in the field aid the

1 environmental assessment process. Table 3 brings together the objectives of the ecological health  
2 strategy for TBT substances and existing action by water managers.

3 As gastropods are the most sensitive species to TBT, few of these species are likely to be present in a  
4 TBT-contaminated harbour environment. The consequences of the absence of this group for the  
5 functioning of the system should be evaluated. Probabilistic risk assessment based on chemical data  
6 might provide a generic ecosystem health indicator related to TBT exposure to improve our  
7 understanding of the impact of organotin pollution. The results of such an assessment should be  
8 validated in the field, in order to reveal the actual field status. Given the relationships presented,  
9 observations with ISI as a specific indicator could serve to validate the generic assessment. Inspection of  
10 location-specific conditions alongside generic risk prognosis improves the assessment of impacts related  
11 to TBT exposure. This study provides evidence of the poor ecological health status of some Dutch  
12 harbours as a result of TBT pollution and promotes the use of integrated approaches like the WOE to  
13 help practitioners improve ecological impact assessment.

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## 16 **5. Conclusion**

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18 In this study an integrated ecological risk assessment including three LOEs has been applied to assess  
19 the risk from TBT pollution in coastal and harbour environments. Only two measured ISI values for  
20 coastal environments fell within the predicted ISI ranges (North Sea and Western Scheldt), while the rest  
21 of the observations were below the calculated ranges. In harbours there is good correspondence  
22 between observed ISI levels and the predicted ISI ranges for each harbour. This study provides evidence  
23 of the poor health status of some Dutch harbours as a result of TBT pollution and promotes the use of  
24 integrated approaches like the WOE to help practitioners improve ecological impact assessment.



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## 8 **Appendix A. Supplementary data**

9 Supplementary data associated with this article can be found, in the online version, at.....  
10

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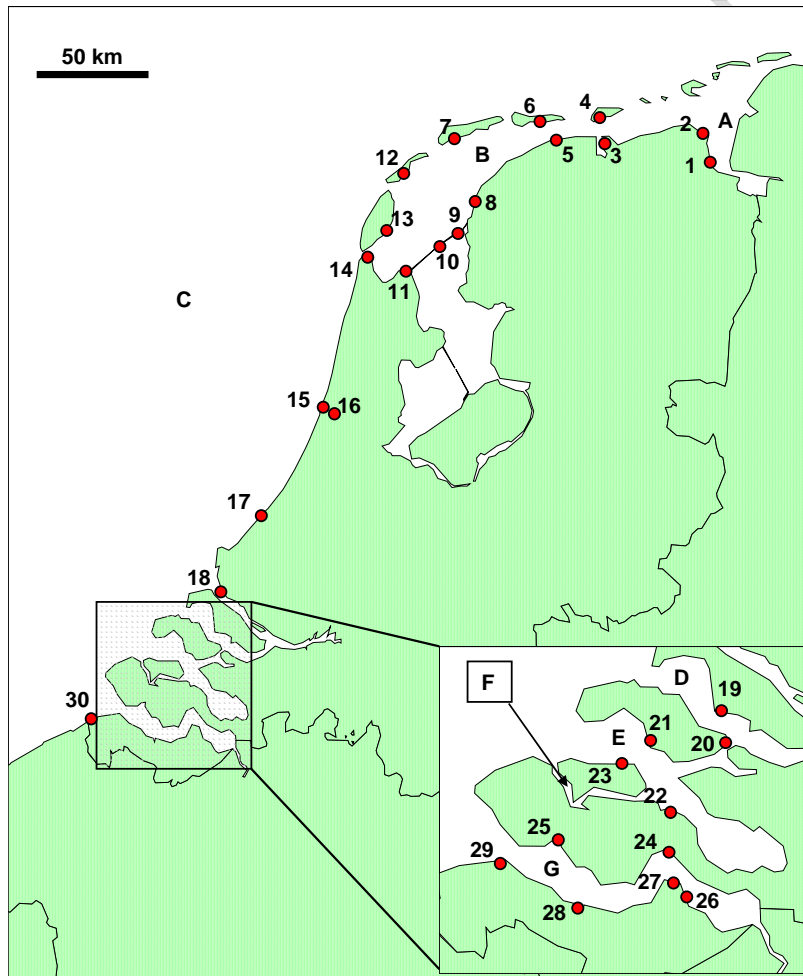
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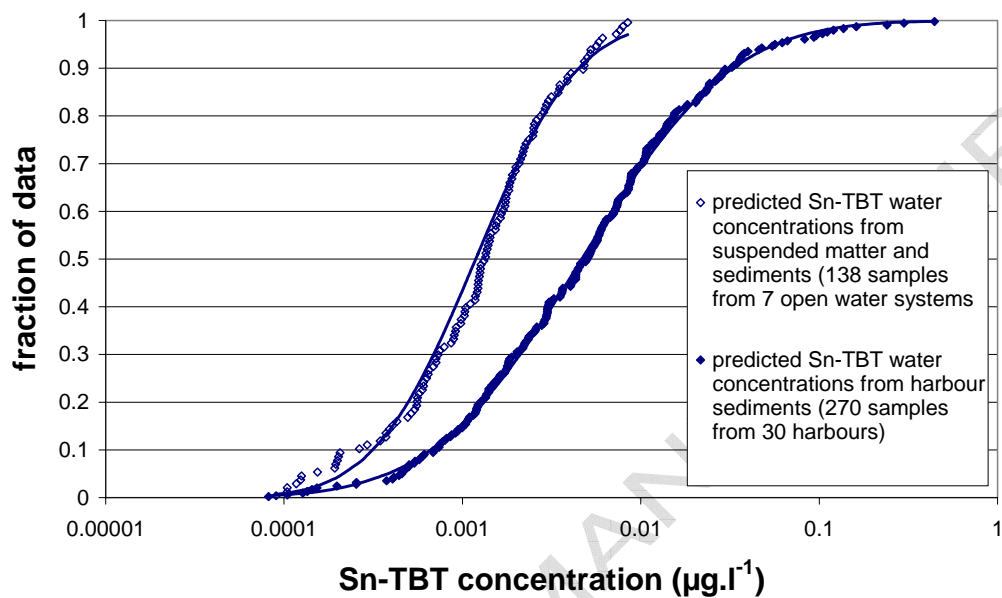
- 1 *Figure 1* Map showing sampled harbours (1-30) and open waters (A-G) along the Dutch coast.
- 2 1. Delfzijl; 2. Emshaven, 3. Lauwersoog; 4. Schiermonnikoog; 5. Holwerd; 6. Ameland; 7. Terschelling;
- 3 8. Harlingen; 9. Kornwederzand; 10. Breezand; 11. Den Oever;
- 4 12. Vlieland; 13. Texel; 14. Den Helder; 15. IJmuiden; 16. Noordzeekanaal;
- 5 17. Scheveningen; 18. Rijnmond; 19. Herkingen; 20. Bruinisse; 21. Zierikzee;
- 6 22. Yerseke; 23. Colijnsplaat; 24. Hansweert; 25. Vlissingen; 26. Walsoorden;
- 7 27. Perkpolder; 28. Terneuzen; 29. Breskens; 30. Appenzak; A. Ems-Dollard;
- 8 B. Wadden Sea; C. North Sea; D. Grevelingen; E. Eastern Scheldt; F. Veersemeer;
- 9 G. Western Scheldt.

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- 1 *Figure 2 Exposure distributions for all sample locations in Dutch open/estuarine/marine waters*  
2 *and harbours.*



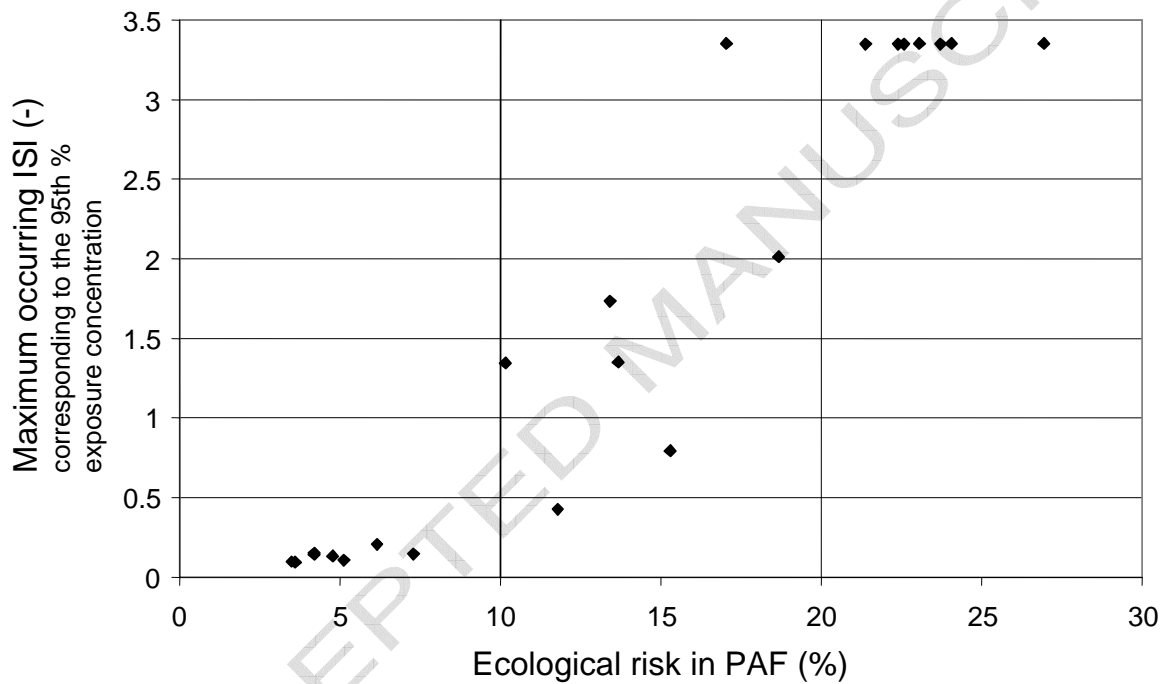
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2 *Figure 3 Correspondence between ecological risk expressed as the Potentially Affected Fraction*  
3 *of species (PAF) and the calculated ISI level corresponding to the 95<sup>th</sup> percentile exposure concentration*  
4 *in Dutch harbours.*

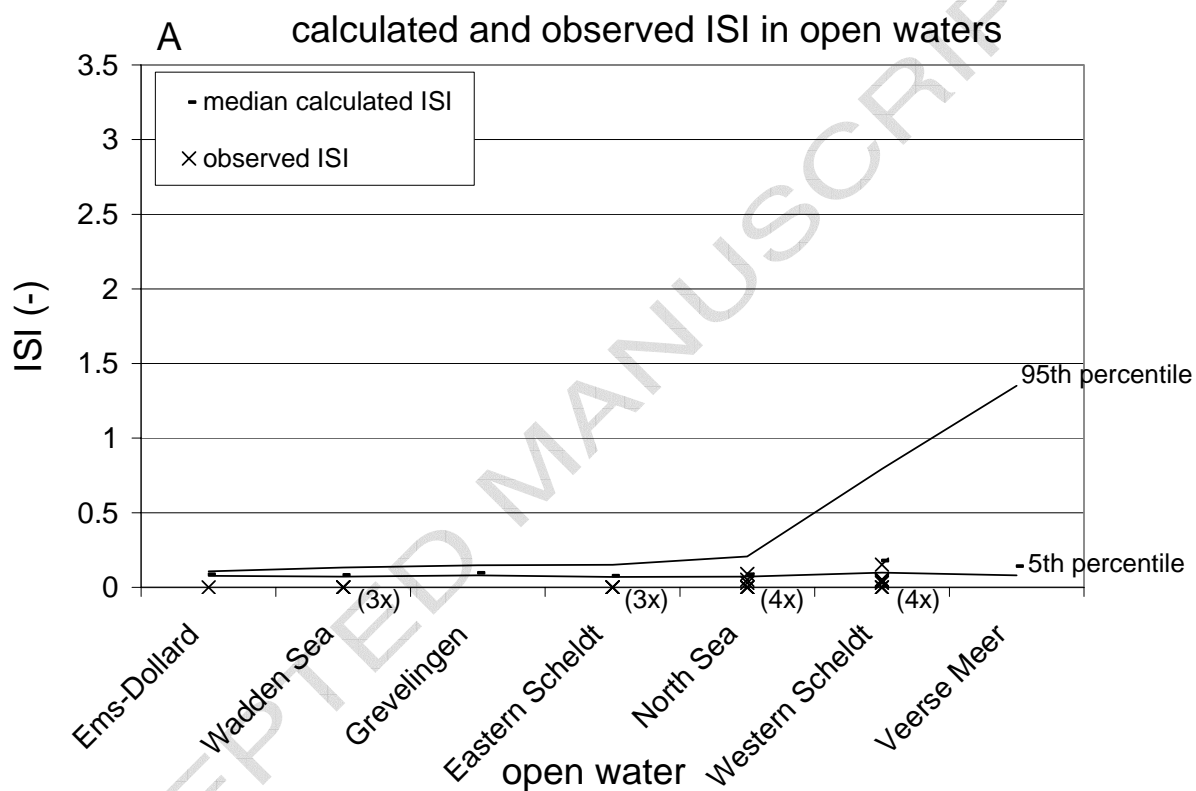
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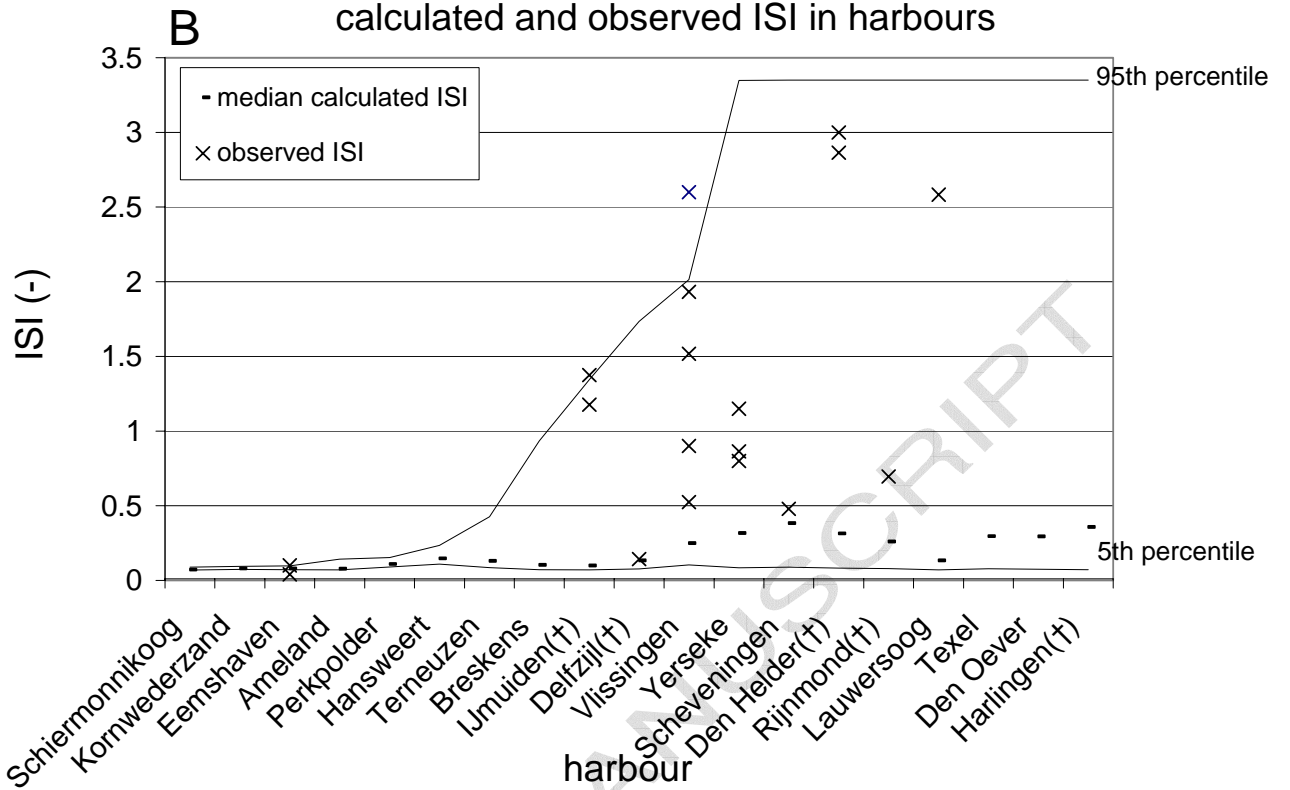
1 *Figure 4. Comparison of the observed ISI values and the predicted ISI ranges based on the*  
 2 *concentration distribution in the corresponding open waters (A) and harbours (B). The calculated ISI*  
 3 *values corresponding to the median and the 5th and 95th percentile of the exposure concentration*  
 4 *distribution are also shown. At some sampling locations in harbours periwinkles were rare, while at*  
 5 *other locations(†) no periwinkles were observed.*

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1 *Table 1 Chemical and biological datasets used in this study*

<b>Dataset</b>	<b>Area type</b>	<b>Medium</b>	<b>Time range</b>	<b># areas</b>	<b># locations</b>
TBT concentrations	Open water	Suspended matter	1998-2003	5	25
TBT concentrations	Open water	Sediment	1998-2003	7	123
TBT concentrations	Harbour	Suspended matter	1999-2002	4	8
TBT concentrations	Harbour	Water	1999-2002	2	6
TBT concentrations	Harbour	Sediment	1999-2002	31	270
ISI values and abundance	Open water	Periwinkle	2002-2005	5	24
ISI values and abundance	Harbour	Periwinkle	2002-2005	9	18

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10 *Table 2 Observed and predicted concentrations, ecological risk values and observed and predicted ISI*11 *levels in different Dutch open waters.*

Area	# locations	Observed concentration range in sediments and SPM Sn-TBT $\mu\text{g}\cdot\text{kg}^{-1}\text{dw}$ (min – max)	Calculated concentration range in water Sn-TBT $\text{ng}\cdot\text{l}^{-1}$ (5 - 95 %tile)	Calculated predicted ISI range (based on 5 – 95 %tile concentrations)	Observed ISI (min – max)*	
<b>Open waters</b>						
Ems-Dollard	10	5.1 – 23.0	0.59 – 2.20	5.13	0.08 - 0.11	0.00
Grevelingen Lake	5	7.0 – 42.7	0.79 – 3.72	7.29	0.08 - 0.15	n.d.
North Sea	49	0.2 – 52.5	0.23 – 5.45	6.16	0.07 - 0.21	0.00 – 0.09
Eastern Scheldt	15	0.2 – 24.4	0.10 – 3.87	4.2	0.07 - 0.15	0.00 (3x)
Veersemeer	6	0.2 – 97.9	0.76 – 16.4	13.7	0.08 - 1.35	n.d.
Western Scheldt	13	7.4 – 160.7	1.73 – 12.8	15.3	0.10 - 0.80	0.00 – 0.15
Wadden Sea	40	0.2 – 36.6	0.25 – 3.23	4.78	0.07 - 0.13	0.00 (3x)
<b>All open waters</b>	<b>138</b>	-	-	<b>6.72</b>	-	-
<b>Harbours</b>						
Schiermonnikoog	2	0.5 – 0.5	0.04 – 1.22	1.56	0.07 – 0.09	n.d.
Emshaven	5	1.8 – 12	0.29 – 1.71	3.50	0.07 - 0.10	0.04 – 0.10
Kornwederzand	3	4.8 – 15	0.37 – 1.55	3.61	0.07 - 0.09	n.d.
Ameland	4	0.5 – 25	0.12 – 3.60	4.21	0.07 - 0.14	n.d.
Perkpolder	2	3.9 – 24	1.36 – 3.92	8.92	0.09 – 0.15	n.d.
IJmuiden <sup>†</sup>	32	0.5 – 980	0.20 – 16.4	10.2	0.07 - 1.35	1.18 – 1.38
Breskens	2	0.5 – 13	0.29 – 13.8	10.2	0.07 – 0.93	n.d.

Terneuzen	6	4.3 – 24	1.06 – 9.19	11.8	0.09 - 0.43	n.d.
Hansweert	2	19 - 37	2.27 – 6.07	12.6	0.11 – 0.23	n.d.
Delfzijl <sup>†</sup>	11	6.8 – 240	0.57 – 18.6	13.4	0.08 - 1.73	0.14
Lauwersoog	6	0.5 – 210	0.15 – 70.3	17.0	0.07 - 3.35	2.58
Vlissingen	7	3.4 – 180	2.01 – 20.2	18.7	0.10 - 2.01	0.52 – 2.60
Rijnmond <sup>†</sup>	124	0.5 – 5700	0.70 – 61.9	21.4	0.08 - 3.35	0.70
Yerseke	3	16 – 250	1.04 – 56.1	22.4	0.08 - 3.35	0.80 – 1.15
Den Helder <sup>†</sup>	12	3.2 – 460	0.93 – 61.6	22.6	0.08 - 3.35	2.86 – 3.00
Texel	7	4.1 – 190	0.61 – 86.8	23.1	0.08 - 3.35	n.d.
Scheveningen	8	7 – 370	1.21 – 61.3	23.7	0.09 - 3.35	0.48
Den Oever	5	4.2 – 110	0.41 – 127.0	24.0	0.07 - 3.35	n.d.
Harlingen <sup>†</sup>	15	0.5 – 1700	0.27 – 255.4	26.9	0.07 - 3.35	-
<b>All harbours</b>	<b>270</b>	-		<b>19.1</b>	-	-

1 \* Where no range was measured, single values are reported in the table.

2 <sup>†</sup> No periwinkles were present at any sampling stations.

3 n.d. = location not sampled

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1 Table 3 Combinations of LOE results and likely ecological health status in relation to TBT pollution.

<b>LOE – Chemistry</b> <i>Predicted risk; Predicted ISI</i>	<b>LOE – Toxicology</b> <i>Observed ISI</i>	<b>LOE – Ecology</b> <i>Presence of gastropods at all locations</i>	<b>Likely ecological health status in relation to TBT</b>	<b>Action</b>
PAF < 10%; ISI < 0.3	ISI < 0.3	+	Good	No action
PAF < 10%; ISI < 0.3	ISI < 0.3	-	Good	Other stressors present?
PAF > 10%; ISI > 0.3	ISI < 0.3	+ or -	Probably poor	Extend monitoring
PAF > 10%; ISI > 0.3	ISI > 0.3	+	Poor	Mitigating measures and monitoring of recovery
PAF > 10%; ISI > 0.3	ISI > 0.3	-	Poor	Mitigating measures and monitoring of recovery

2 LOE = Integrated ecological risk assessment is based on multiple Lines Of Evidence

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