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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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- 18
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- 20 21

1 Abstract 2

3 In the present study an integrated ecological risk assessment based on multiple lines of evidence (LOEs) 4 was evaluated in order to better assess the risk from TBT in Dutch harbours and open coastal waters. On 5 the basis of spatial distributions of measured tributyltin (TBT) concentrations in sediments and suspended 6 matter, predictions of the intersex index (ISI) in Littorina littorea and the ecological risk expressed as the 7 Potentially Affected Fraction (PAF) of species were made. The results were compared to actual ISI 8 measurements and presence of L. littorea in the field. The PAF calculated on the basis of TBT levels for 9 open coastal waters ranged from 4.2% to 15.3%; for harbours it ranged from 3.5% to 26.9%. Significant 10 intersex levels were observed only in waters where the risk was calculated above 10% PAF. This study 11 suggests that the absence of L. littorea from some harbours with high ecological risk values can be 12 explained by high TBT concentrations. A call is made for the use of integrated approaches like weight of 13 evidence (WOE) to help practitioners improve ecological risk assessment. 14

15 **1. Introduction**

16

17 Tributyltin (TBT) was first used as an antifouling agent in the late 1960s. It proved to be very effective, but 18 also extremely toxic to aquatic life (Champ and Seligman, 1996). TBT effects were first recognized in the 19 marine environment in the 1970s and attracted particular attention in the 1980s, when the effects of TBT 20 started to become apparent in the Netherlands and elsewhere (Marquenie and Vethaak, 1989; Champ 21 and Seligman, 1996). In the 1980s, there was a sharp decline in the population of dog whelk (Nucella 22 lapillus) in the Delta area of the south-western Netherlands and in the 1990s the occurrence of imposex 23 was demonstrated among whelks (Buccinum undatum) in the same area (Mensink et al., 1996) as well as 24 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, 1996; Rutgers, 2000). However, instead of using measurements from multiple species in the biological 11 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 will provide the likelihood of effects to occur. Whether or not these effects will actually occur depends 26 27 on location specific conditions.

ii. Biological effect parameters can be used to assess the potential and actual impacts of TBT on
marine coastal life. However, it can be difficult to link biological effects to exposure to a single
contaminant. In this study, the degree of intersex index (ISI) in the common periwinkle, *L. littorea*,
was selected as an indicator of TBT pollution, as this species is the most common prosobranch
species on hard substrates in Dutch coastal waters. Intersex in this species has proven to be
sensitive to the degree of environmental organotin pollution in coastal waters (Oehlmann et al.,
1998). Although periwinkles are not the most sensitive gastropod and ISI is not a rapid response,

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

substrates such as rock and artificial substrates such as harbour walls, breakwaters, etc. (Oehlmann,2002).

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

23

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

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

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

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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 (foc) 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.

The PAF indicates the likelihood that adverse ecological effects will occur as a result of TBT exposure in a particular area. This value can be interpreted as the probability that a randomly selected exposure concentration will exceed species sensitivity. The probabilistic risk assessment was performed on the clustered data for harbours and for open waters, as well as for each harbour and open water separately. Only water systems for which exposure concentrations were measured at two or more locations were 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 5th and 95th percentile of the exposure

4 concentration distributions were calculated applying Formula 1.

5

6

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

7 where:

8 ISI = calculated ISI level [-]

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

10

11 2.4. Biological effects and abundance surveys

Periwinkles were sampled from 2002 to 2005 at 42 locations along the Dutch coast, representing the main Dutch open waters areas and harbour locations (Figure 1). Grevelingen (D) and Veersemeer (F) lakes were not included. At the sampling locations the presence of *L. littorea* was recorded at every location randomly selected in 5 to 7 areas of 1 m². If present, at least 40 individuals of *L. littorea* with a shell height between 15 and 25 mm (approx. 1.5-year-old new adults) were collected. The occurrence of intersex in the periwinkles was analysed within seven days, in accordance with the methods described by Bauer et al. (1995). ISI is expressed as the average intersex stage within the population.

19 2.5. Overview of chemical and biological datasets

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

24

25 3. Results

- 26
- 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 average concentration was used for further analysis in the risk predictions. Since the concentration 12 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.

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

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21 3.2. Predicted ecological risk

The two distributions presented in Figure 2 were used as input for a probabilistic risk assessment as described by Aldenberg et al. (2002). This resulted in an expected total ecological risk for open waters of 6.7% and 19.1% for harbours. This implies that, on average, for open waters it is likely that 6.7 % of species will be negatively affected as a result of exposure to TBT. For the harbours this fraction is 19.1%. The risk values for the individual open waters and harbours are given in Table 2. Relatively high-risk values were obtained for open waters in the Western Scheldt (G) and Veersemeer (F), while the risk 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 5th and 95th 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.

9

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.

20

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 95th percentile of the exposure concentration distribution (Figure 3). The 95th percentile was chosen because 24 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

higher risk levels, up to approximately 10%, ISI levels in the field are not expected to exceed the ISI
signalling level of 0.3 (Oehlmann, 2002). This study showed that high ISI levels up to a maximum of 3.35
are expected in the field only when the predicted risk level exceeds 20%. These results indicate that
either the ISI measurements in *L. littorea* are not sensitive enough to assess ecosystem health, or the risk
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 5th and 95th 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).

The observed ISI values were relatively low for open waters, often below the 5th percentile of the 12 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 within the ISI ranges corresponding to the median and 95th percentile of the exposure concentrations. 17 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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29 4. Discussion

In the present study a weight-of-evidence approach derived on the basis of three LOEs has been evaluated in order to better assess the risk from TBT in Dutch harbours and open waters. Based on chemical data, risk values were determined according to Aldenberg et al. (2002) and ISI values were calculated based on the dose-response curve reported by Oehlmann (2002). Actual ISI observations were made in the field and the presence of gastropods was recorded. In the overall approach evidence 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 Koc 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 Koc 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 Koc value was estimated from measured aqueous and sediment concentrations in 30 Dutch 15 harbours evenly distributed over the Netherlands. This resulted in an average Koc 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).

The highest ISI value, measured in the Western Scheldt (G), still fell within the predicted ISI range. 18 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

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.

Both calculated ISI and PAF values are based on the spatial distribution of concentrations. This 16 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.

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

covering the whole distribution of possible ISI values. The wide range of ISI levels observed in Vlissingen
 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.

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

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

Based on the evaluation of TBT effects on intersex development in the periwinkle *L. littorea*, and considering ecological health status as a criterion for decision-making, With reference to Table 3, LOEs were derived for *L. littorea*, representing the species used in the OSPAR monitoring guideline. The 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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In this study an integrated ecological risk assessment including three LOEs has been applied to assess the risk from TBT pollution in coastal and harbour environments. Only two measured ISI values for coastal environments fell within the predicted ISI ranges (North Sea and Western Scheldt), while the rest of the observations were below the calculated ranges. In harbours there is good correspondence between observed ISI levels and the predicted ISI ranges for each harbour. This study provides evidence of the poor health status of some Dutch harbours as a result of TBT pollution and promotes the use of integrated approaches like the WOE to help practitioners improve ecological impact assessment.

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7	
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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- 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. Appelzak; 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 95th percentile exposure concentration



4 in Dutch harbours.

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.*
- 6





Dataset	Area type	Medium	Time range	# areas	# locations
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

Chemical and biological datasets used in this study Table 1

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

Area	# locations	Observed	Calculated	Calcul	Calculated	Observed ISI
		concentration	concentration	ated	predicted ISI range	(min – max)*
		range in	range in water	Ecolo	(based on	
		sediments and	Sn-TBT ng.l ⁻¹	gical	5 – 95 %tile	
		SPM	(5 - 95 %tile)	Risk	concentrations)	
		Sn-TBT µg.kg ⁻¹		as		\langle
		dw		PAF	0	
		(min – max)		(%)		
	I	(Open waters			
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)
All open waters	138	-	-	6.72	-	-
			Harbours			
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 [†]	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 [†]	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 [†]	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 [†]	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 [†]	15	0.5 – 1700	0.27 – 255.4	26.9	0.07 - 3.35	-
All harbours	270	-	2	19.1	-	-

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

2 [†]No periwinkles were present at any sampling stations.

3 n.d. = location not sampled

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1	Table 3 Combinations o	f LOE results and likely	v ecological health status in	n relation to TBT pollution.
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LOE – Chemistry Predicted risk; Predicted ISI	LOE – Toxicology Observed ISI	LOE – Ecology Presence of gastropods at all locations	Likely ecological health status in relation to TBT	Action
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	ţ	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
- 3

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