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Occurrence and Effects of Organotins on Adult Common Whelk (*Buccinum undatum*) (Mollusca, Gastropoda) in Harbours and in a Simulated Dredging Situation

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Transplanted common whelk (*Buccinum undatum*) (Mollusca, Gastropoda) accumulated fairly high levels of organotins (tributyltin (TBT) and triphenyltin (TPT)) during exposure in three harbours with different TBT contamination. This did not though lead to an increase in imposex frequency in the adult females studied. Simulating harbour dredging in an experiment using suspended sediment from one of the harbours only resulted in a low concentration of TBT in the tissues of the common whelk and subsequently no changes in the occurrence and degree of imposex. The common whelk seemed to receive the main part of TBT from the water column and the limited bioaccumulation in the experiment indicates that desorption of TBT from the suspended sediment was slow. After TBT has been totally banned, dredging of contaminated sediments will cause increased exposure of the biota to TBT. Due to slow desorption the increase may however, be slight and temporary to pelagic and epibenthic species unless the settled particles are resuspended.

Keywords: *Buccinum undatum*; organotins; TBT; sediment; dredging; harbours; bioavailability.

Organic tin compounds from antifouling paints have received much attention during the last decade because of their strong effects on marine organisms at low concentrations. This has been particularly evident for gastropod snails and bivalve molluscs (Alzieu, 1991; Bryan and Gibbs, 1991), which show malformations at concentrations as low as 1–2 ng l^{-1}. This is seen in female gastropods as development of male characteristics (penis and vas deferens, termed imposex) and can lead to sterility of the females at higher concentrations.

Despite restrictions on the use of tributyltin (TBT) in many countries in the late 1980s, occurrence of imposex is still extensive in most oceans and has increased in some areas (Minchin *et al.*, 1995). The effects of organotins are mostly seen in areas with extensive shipping activity (harbours, shipyards, shipping lanes; ten Hallers-Tjabbes *et al.*, 1994). Due to the often slow degradation of TBT and its derivatives (dibutyltin (DBT); monobutyltin (MBT)) and their affinity to particulate matter (Langston and Pope, 1995), these are readily accumulated in harbour sediments and may be present there during long time (de Mora *et al.*, 1995). In low temperature regions this may be especially pronounced as the growth and activity of TBT-degrading microorganisms are temperature dependent (Stewart and de Mora, 1990; Stewart and Thompson, 1997). Little is still known of the effects on marine organisms of dredging operations in TBT-contaminated sediments (ten Hallers-Tjabbes *et al.*, 1994).

The aim of the present study was to assess the bioavailability of organotins, in particular TBT, in harbours at northern latitudes and effects due to harbour dredging. We placed adult specimens of the common whelk, *B. undatum* (Mollusca, Gastropoda), in harbours to determine bioaccumulation and development of imposex and used field-contaminated harbour sediment in the laboratory to simulate effects of dredging on bioaccumulation and imposex development in the whelks. The degree of imposex development in this species has earlier been related to field concentrations of TBT (ten Hallers-Tjabbes and van Hattum, 1995), although other

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studies have not shown correlation between imposex and tissue concentrations of butyltins (Nicholson *et al.*, 1998).

Materials and Methods

Field studies

In the field studies adult whelks were transplanted from a clean area to three Icelandic main seaports. All individuals used in the studies were collected in Breidafjordur, western Iceland, far from seaports and major shipping routes (reference area, ≈65°05′N, 22°26′W). Whelks were placed in 3–4 cages in each of the harbours of Reykjavik, Hafnarfjordur (both with intensive shipping and shipyards) and Straumsvik (0–2 large cargo-ships, no shipyard), all in southwestern Iceland (64°09′N, 21°56′W, 64°04′N, 21°57′W and 64°03′N, 22°02′W, respectively), at different seasons. Only active specimens were randomly picked from a large tank and 50 randomly selected specimens were immediately frozen for later analysis of imposex and organotin contamination. The cages were placed on the bottom of the harbours at depths from 2 to 9 m depending upon the tides (tidal range ≈4 m). The whelks were fed with fresh haddock fillets every week or every fortnight and starfish (*Asterias rubens*) having entered the cages, were removed.

Whelks were randomly picked from the cages after 4 to 20 weeks and immediately frozen. Prior to analysis the whelks were allowed to thaw slightly. The shell was then broken in a vice and the weight of soft tissues measured. The vagina and the gonads were dissected and weighed. Each individual was then searched for indication of imposex (see Mensink *et al.*, 1997; the scheme is according to suggestions of the OSPAR Training Workshop on TBT Effects, Aberdeen, September 1997), which included the presence of penis and/or vas deferens in the females. The outline of the penis and/or the vas deferens was drawn under camera lucida and the length of the penis calculated from the drawing. All specimens were then immediately frozen for chemical analysis. Feral whelks were captured in traps near Straumsvik harbour and treated in the same way as the whelks placed in cages.

Incubating semipermeable membrane devices (SPMD; Huckins *et al.*, 1996) in cages close to the whelk cages monitored the organotin contamination in the water column of Reykjavik and Straumsvik harbours. The membranes were replaced every fourth week. Temperature was monitored at the same time with continuously measuring loggers.

Laboratory experiment

A continuous flow experiment with seawater was run in the laboratory at the Marine Research Institute (MRI) facilities in Grindavik, southwestern Iceland. Two experimental set-ups were used, one at 2.9 ± 0.5°C and one at 8.0 ± 0.5°C in order to simulate summer and winter conditions. The seawater originated from an inland drilling hole, delivering totally sterile but fully saline seawater from a porous lava-field and unaffected by human activities. The flow of seawater was kept constant by means of a delivery box system (Granmo and Kollberg, 1972). All the glass aquaria contained a layer of 5 cm sediment, either TBT-contaminated harbour sediment or sediment from a clean reference site. Half of the aquaria with contaminated sediment were continuously supplied with suspended contaminated sediment. The stock suspension was on an average stirred for two days and was dosed by a peristaltic pump to the aquaria with a dilution of 1:40 to obtain a turbidity of 10 NTU (nephelometric turbidity units, corresponding to 26 mg l^{-1} sediment dw.).

The contaminated sediment used was collected in Hafnarfjordur harbour which has a considerable shipping activity and shipyards. The sediment was taken with a Shipek sediment sampler from a locality, where large vessels (trawlers, freight ships and oil tankers) frequently appear. Each sample originated from the uppermost 5 cm of the bottom sediment. This part of the harbour had not been dredged for a number of years. Reference sediment was collected far from any harbour on an intertidal flat in Laxarvogur, southwestern Iceland (64°21′N, 21°40′W), where previous investigations showed the absence of imposex in *Nucella lapillus* (Svavarsson and Skarphéðinsdóttir, 1995). At both localities very fine mud was sampled.

All specimens used in the experiment were randomly selected females. In addition 13 specimens from the same population were analysed at the beginning of the experiment for the presence of imposex. All remaining specimens were kept frozen until later analysis. The animals used in the laboratory experiment were allowed to acclimatize in aquaria with 7°C running seawater for > one week prior to the experiment. The whelks in the laboratory were fed weekly or each fortnight. The specimens were examined after 8 weeks (2.9°C) or 12 weeks (2.9°C and 8.0°C).

Chemical analyses

Five specimens were randomly selected from each field study and sampling time or from each aquarium in the laboratory experiment. The material was immediately frozen. Later, after thawing, the samples were homogenized, weighed and freeze-dried. Subsamples were also taken for analysis of the fat content. The samples were analysed for content of TBT and triphenyltin (TPT).

Organotin compounds were extracted in a 50 ml open vessel made of borosilicate glass containing 5 ml of acetic acid, 1 ml of nonane and 3 ml of water with derivatization reagent and internal standard and using a Microdigest model A301 (2.45 GHz, max power 200 W) microwave digester (Prolabo, France) equipped with a TX32 programmer. For atomic emission detection the ethylated species were separated on a HP-1 column

(25 m × 0.32 mm × 0.17 μm) using a HP Model 5890 Series II gas chromatograph (Hewlett-Packard, Avonsdale, PA) equipped with a split/splitless injection port. Detection was achieved with a HP model 7673 automatic sampler. For FPD detection the ethylated species were separated on a HP-1 column (25 m × 0.32 mm × 0.17 μm) using a PE model 1020 gas chromatograph (Perkin–Elmer) equipped with a split/splitless injection port.

Analytical grade chemicals (Merck, Germany) and water deionized and further purified in a Milli-Q system (Millipore, Milford, MA) were used throughout unless otherwise stated. The derivatization reagent was a 1% (w/v) solution of sodium tetraethylborate ($NaBEt_4$, Strem, France); it was prepared daily by dissolving the reagent in water. For organic phase derivatization ethylmagnesium bromide was used.

Individual stock solutions, Bu_3SnCl (TBT) and Pr_3SnCl (TPrT) were prepared in methanol. The internal standard solution was prepared by diluting the Pr_3SnCl stock solution with water to give a concentration of 1 μg/ml. A fish tissue NIES11 from the National Institute of Environmental Studies in Japan with the certified content for TBT and the PACS-1 harbour sediment with certified values for MBT, DBT and TBT from the National Research Council of Canada (NRCC) were used.

Analyses of sediments were performed according to Szpunar *et al.* (1996) and the biological materials were analysed according to the method described in Pereiro *et al.* (1996).

Extracts from the exposed SPMD membranes were made at University of Umeå, Sweden and then analysed in Bordeaux, France. A standard procedure based on Grignard ethylation followed by GC-FPD determination of derivatized extract was used (Dirkx *et al.*, 1994). The 1 ml of organic membrane extract was placed in a 25 ml separatory funnel and 250 μl of 2 M ethylmagnesium bromide solution was added followed by a gentle agitation of the mixture for 2 min. Then the mixture was shaken for 1 min with 15 ml of 0.5 M sulphuric acid to destroy the excess of the Grignard reagent. The aqueous phase was discarded and the organic one rinsed with 5 ml of water. After discarding the aqueous phase the organic layer was analysed by GC-FPD. The precision for butyltin compound analysis is around 10%.

From the determined concentrations of TBT in the SPMDs (ng Sn/g triolein), the mean concentrations of free TBT in the water column were estimated. Then it was assumed that partitioning equilibrium of TBT between the water and triolein had been reached after > 28 days exposure at 3–8°C (J. Huckins, pers. comm.). TBT was assumed to partition in the same way between water and triolein as between water and *n*-octanol (Huckins *et al.*, 1996). A K_{ow} value of 1995 was used for the calculations.

Results

The TBT levels in the SPMDs and estimated concentrations of free TBT in the water column are shown in Table 1. The amounts of TBT and TPT are always expressed as pure tin (TBT–Sn, TPT–Sn).

Imposex was frequently observed in the common whelks from the background population in Breiðafjordur. About 26.4% of the females had imposex and the penises observed were on the average 0.7 mm (±0.6 mm SD; range 0–2.9 mm). The imposex frequency differed somewhat between catches. The penis lengths were, however, considerably less than in the common whelk population of Straumsvik, where the imposex frequency was 81.8% and the penises were 3.8 ± 4.1 mm ($N = 22$, range 0–13.9). Just outside Reykjavik harbour the penises were 6.8 mm (range 0–40.1 mm; most of them (65%) 2–10 mm, autumn 1994; le Roux, pers. comm.).

The females did not show significant increase in the average penis length when individual field experiments were tested, although the penises were somewhat longer at the end of the experiment in some cases (Table 2). When all field experiments were considered there was an indication of increased variation of the penis length within the females, although not being significant (controls vs caged animals, $p = 0.11$), when all gastropods were considered.

The transferred whelks accumulated organotin compounds rapidly and within 55 days of exposure the whelks in Straumsvik harbour (estimated water conc. ≈60 ng TBT/L) had reached fairly similar levels of TBT as the natural population (Table 3). Exposure of similar duration in Reykjavik harbour (estimated water conc. ≈100 ng TBT/L) resulted in approximately 1.6 and 2 times higher values for TBT and TPT, respectively (Table 3), while still longer exposure in Reykjavik harbour resulted in raised level of TPT, but not of TBT. There was generally a high variation in the amount of the organotin compounds, in whelks from the field.

TABLE 1

Measured values of TBT in semipermeable membrane devices (ng Sn/g triolein) and estimated mean concentration of free TBT in the water column. The calculations are based on the assumption that partitioning equilibrium has been reached. Exposure time > 28 days at 3–8°C.

Site	Date	TBT ng Sn/g in SPMD	TBT ng Sn/L in the water column
Reykjavik	August 1996	390	195
Reykjavik	September 1996	21	11
Straumsvik	July 1996	117	59

TABLE 2

Penis lengths of the common whelk (*B. undatum*) during different field experiments.

Harbour	Experiment	Time	Exposed Weeks	All females			Only females with penis		
				N	Average (mm)	SD	N	Average (mm)	SD
Reykjavik	1	Nov.–Jan.	0	25	0.09	0.31	2	1.11	0.08
			4	23	0.25	0.50	7	0.82	0.60
			8	22	0.72	1.02	10	1.58	0.96
	2	Jan.–May	0	13	0.16	0.25	8	0.27	0.27
			13	22	0.49	0.80	10	1.09	0.88
			17	35	0.78	1.95	14	1.94	2.73
	3	May–Sept.	0	50	0.20	0.50	11	0.91	0.72
			8	26	0.18	0.39	9	0.52	0.53
			16	26	0.31	0.55	10	0.81	0.62
Hafnarfjordur		Nov.–April	0	25	0.09	0.31	2	1.11	0.08
			11	13	0.33	0.68	3	1.43	0.64
			16	12	0.60	0.85	5	1.43	0.77
			20	20	0.22	0.46	7	0.62	0.62
Straumsvik		May–June	0	20	0.09	0.98	1	(1.8)	–
			8	19	0.59	1.16	6	1.89	1.41

TABLE 3

Levels of organotins (ng Sn/g dw.) in transferred gastropod snails caged in Straumsvik, Reykjavik and Hafnarfjordur harbours, in the feral population and in animals from a laboratory experiment. TBT = tributyltin, TPT = triphenyltin. 95% confidence limits indicated.

Source	Compound	Straumsvik	Reykjavik	Hafnarfjordur	Laboratory experiment
Background transferred snails	TBT	7±7	6±6	4±1	4±1
	TPT	8±14	20±4	–	–
Feral population	TBT	14±17	–	–	–
	TPT	31±28	–	–	–
55–57 days of exposure	TBT	17±14	27±16	–	<5 (90 days)
	TPT	18±2	36±23	–	–
112 days of exposure	TBT	–	27±48	24±15	–
	TPT	–	97±77	–	–

The level of TBT in the water column of Hafnarfjordur harbour has been estimated from the levels in the whelks incubated there and a BCF value (280) obtained from the concentrations in animals and SPMDs from Reykjavik and Straumsvik. The value was estimated to 90 ng TBT/L in Hafnarfjordur.

The sediment that was collected in Hafnarfjordur harbour and was used in the laboratory study contained 120 ± 6 (95% CI) ng TBT–Sn/g dw. There was only a slight accumulation of TBT in the whelks during the three months period; i.e., in no treatment the TBT level was above the detection limit of 5 ng TBT/g dry weight in the whelks. From the tissue concentration and the BCF value given above the level of dissolved TBT in the experiment is estimated to be <20 ng l^{-1}. There were further no changes in the average penis length or in the frequency of imposex among the whelks.

Discussion

Imposex in gastropods is presumably the best biological indicator of TBT pollution in marine waters. Numerous previous studies on many species and in many areas have shown that near harbours and in areas with important shipping routes the frequency of imposex is high (ten Hallers-Tjabbes *et al.*, 1994; Svavarsson and Skarphéðinsdóttir, 1995; Huet *et al.*, 1996; Davies *et al.*, 1998). After the ban of the use of TBT-based antifouling paints on smaller vessels (<25 m) in many countries, a decline has been observed in the frequency of imposex in the intertidal gastropod *N. lapillus* at locations far from harbours (Minchin *et al.*, 1995; Evans *et al.*, 1996) and even at locations near large and small harbours (Svavarsson, 2000).

For some years it has been known that the common whelk can develop imposex (Brick and Bolte, 1994). This has been shown to be an important indicator species for TBT contamination in offshore waters and in muddy near-shore habitats (ten Hallers-Tjabbes *et al.*, 1994; Ide *et al.*, 1997), though its value has recently been questioned (Nicholson *et al.*, 1998). The common whelk is a long-lived animal (over 10 years, ten Hallers-Tjabbes and Boon, 1995), involving that the effects seen may be due to contamination long time ago.

Despite a prolonged exposure to harbour water with high concentrations of TBT the penis sizes increased only slightly in the adult female gastropods, and there was no correlation of penis length with the exposure time of the gastropods. Mensink *et al.* (1997) did not observe development of imposex in their experiments with adult common whelks. The adults thus seem to be rather resistant to TBT contamination in terms of development of imposex. In this respect the common whelk seems to be more resistant than the dogwhelk (*N. lapillus*), of which the penis length increased significantly within a few weeks in populations having already a small penis, with populations from different areas responding similarly (Gibbs *et al.*, 1991). Laboratory experiments indicate that juvenile female gastropods are more sensitive to TBT contamination than adults and Mensink *et al.* (1996a) found imposex to develop in more than 80% of the juvenile common whelks after 10 months of exposure to TBT.

Triphenyltin (TPT) was observed in the tissues of the gastropods from Straumsvik and Reykjavik harbours, and was particularly high in the gastropods in Reykjavik harbour. TPT is not considered to promote imposex in gastropods (see Bryan *et al.*, 1988). TPT has been observed in the tissues of other gastropod species in or near harbours or shipping lanes (Horiguchi *et al.*, 1994; Ruiz *et al.*, 1998) and also in the common whelk (Mensink *et al.*, 1996b). Sources of TPT may be either antifouling paints, agriculture fungicides (Stäb *et al.*, 1995; Ruiz *et al.*, 1998) or house paints. The source of triphenyltin in the Icelandic harbours is evidently antifouling paint. In Straumsvik cove there is only a small harbour for large vessels and an aluminium factory and no other industry or agricultural run-off. The three studied harbours are not located in estuaries, involving that all input comes from the harbour activity itself.

As the TBT level in the whelks caged for 55 days was about the same as in the natural population in Straumsvik, it is concluded that the TBT distribution between the animals and the water had reached an approximate steady state. This conclusion is further supported by the results from Reykjavik harbour, where the TBT level was similar in animals caged for 57 and 112 days. Uptake of TBT is mainly from the water in the dogwhelk *N. lapillus* (Bryan *et al.*, 1989) and is presumably so also in the common whelk. This assumption is supported by the fact that TBT reached about the same level in the caged whelks receiving low-contaminated food from an external source as in the wild ones catching their own food. Even if the lowest temperature used in the laboratory experiment (3°C) was lower than in the field study (3–8°C) it is probable that bioaccumulation in the laboratory was as close to steady state as in the field because of the longer exposure period in the laboratory (90 compared to 57 days).

Assuming that the bioaccumulation of TBT had reached steady state both in the animals in the experiment and in the ones caged in Hafnarfjordur, and as uptake from food seems to be unimportant, it is possible to compare TBT exposure via the outer body surfaces in the two cases. From the concentration of 24.3 ± 10 ng/g dw. in the caged whelks and < 5 ng g^{-1} in the laboratory whelks it is seen that the former ones were at least five times more contaminated by TBT even if both groups of animals were exposed to sediment from the same environment. Since our study indicates that bioaccumulation of TBT depends mainly on uptake from water it is concluded that the concentration of dissolved TBT in Hafnarfjordur was at least five times higher than in the laboratory study. As the dilution water used in the laboratory was free of TBT the desorption rate from the contaminated sediment should determine the concentration of dissolved TBT. As this was low compared to the field conditions it is concluded that desorption was slow. This is contrary to the results of Langston and Pope (1995) who observed that TBT was rapidly desorbed from sediment particles. However, in the mentioned case TBT had been in contact with the sediment particles for only 24 h, which is a very short period of time compared to the field-contaminated sediment and this difference is important for the desorption rate. Thus it has been shown for hydrophobic organic pollutants that the fraction of readily desorbed compound, the so-called rapidly reversible pool, is decreasing with prolonged contact time between the micropollutant and the sediment particles (Landrum *et al.*, 1989). This phenomenon is especially evident for porous particles that usually have a high organic content. As the sediment used in the present study had an organic carbon content of 1.76% and as the inorganic particles were of volcanic origin with a porous structure, slow desorption is expected and was also indicated by the results. Also the low temperature in the present laboratory test should contribute to a slow desorption. As TBT also behaves like a metal ion other factors may also affect sorption.

The laboratory test may be considered to simulate a dredging scenario where the load of TBT in the water column has decreased to a low level due to a total ban of TBT but where the sediment is still heavily contaminated. Under such conditions desorption of TBT from the released contaminated particles is probable but may be slow as in the experiment. Unless the settled contaminated particles are resuspended the increase of dissolved TBT in the water column may be only temporary. To the benthic infauna a prolonged increase of exposure is however anticipated. Rapidly declining TBT levels in common mussels near a Swedish marina after the ban of TBT on smaller boats indicated that there was limited leaching from the contaminated sediment (I. Björklund, pers. comm.). The reverse state seemed to exist in the Eastern Scheldt where a high level of TBT was maintained in the water column in spite of the partial ban of TBT (Mensink *et al.*, 1996a).

A dredging operation in the three studied Icelandic harbours under the existing conditions may contrary to the above-mentioned scenario lead to a transient decrease of dissolved TBT in the water column. Such an effect is possible as long as TBT is in common use, if non-contaminated sediment from pre-industrial time is mobilized and makes possible adsorption of TBT to the clean suspended particles. On the other hand, if the dredged material is disposed at a site with a lower level of TBT, an increased exposure of the biota to TBT at this site is expected. ten Hallers-Tjabbes and van Hattum (1995) have also demonstrated this.

The low water temperatures in subarctic regions slow down diffusion, sorption and degradation of TBT and this should be considered when assessing the environmental effects of dredging.

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