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Review

Biological impact of organotin compounds on mollusks in marine and freshwater ecosystems

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Abstract—Organotin compounds, like tributyltin (TBT) and triphenyltin (TPT), degrade only slowly in the environment. Today, TBT is known to evoke a variety of effects in animals, even at very low environmental concentrations. In terms of sensitivity, none rivals that of two virilisation phenomena—intersex and imposex—in prosobranch gastropods. The effects of TBT and TPT on various marine and freshwater snails were investigated in newly developed sediment biotests in the laboratory. Most tested species showed very low effect concentrations for both organotin compounds (e.g. the freshwater mudsnail *Potamopyrgus antipodarum* with an EC10 of 0.03 µg TPT as Sn/kg and 0.98 µg TBT as Sn/kg for the reduction of fertility). In order to assess the actual TBT contamination of coastal sediments, extensive surveys were carried out in France and Ireland, indicating that there is still a continuing threat for sensitive marine organisms. To obtain information on the contamination of fluvial and estuarine sediments in Germany, an effect monitoring was conducted using the biotests with the netted whelk *Nassarius reticulatus* and *P. antipodarum*. From our investigations we may conclude that a variety of benthic mollusks is strongly affected by low concentrations of organotin compounds in aquatic ecosystems. Due to their persistence in the environment, a continuing impact on aquatic wildlife has to be expected.

Key words: organotins, tributyltin, triphenyltin, mollusks, snails, imposex, intersex, biotest, biomonitoring, sediment

Introduction

Recently, a variety of xenobiotics in the environment has been shown to induce adverse effects in animals and humans by interfering with endocrine functions at different modes of action. These so-called endocrine disruptors, endocrine modulators or hormon-mimetic substances, have been associated with a decrease in sperm counts in man (Swan et al. 2000), increased frequencies of sex-hormone dependent forms of cancer (breast, testis, prostate etc.), genital abnormalities, premature puberty in females and increased occurence of endometriosis in humans (Gist 1998). The EU commission (1998) defines endocrine disruptors as "exogenic compounds which negatively affect the health of an intact organism or its offspring by interference with its endocrine function". Such endocrine disrupting chemicals (EDCs) may be active already at low concentrations. Among the suspected substances, a number of organotin compounds is listed. Our knowledge about the effects of these substances, especially on invertebrates, is limited. While the effects of tributyltin (TBT) have been studied with growing intensity during the past decade (Oehlmann et al. 1998a) and received widespread attention, triphenyltin (TPT), a chemically rather similar and closely related organotin compound (Fig. 1), has almost totally been neglected in ecotoxicological investigations so far, especially with regard to effects on invertebrates (Oehlmann 2000, Schulte-Oehlmann et al. 2000).

Since the early 1960s, TPT compounds have been utilized as broad-spectrum fungicides in agriculture, mainly as triphenyltinhydroxide [fentinhydroxide] and triphenyltinacetate in Brestan®, Brestanid® (Bayer Crop Science, Frankfurt/Main, Germany) and Du-Ter® (BASF, Limburgerhof, Germany). They combat a range of fungal diseases in various crops, particularly potato blight (*Phytophthora infestans*), leaf spot (*Cercospora beticola*, *Ramularia beticola*) and

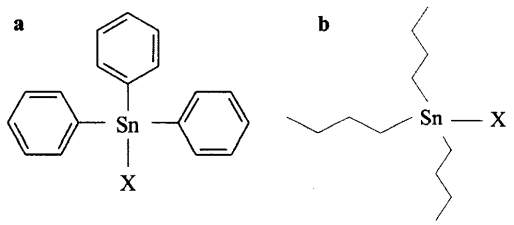

Fig. 1. Chemical structure of **a** triphenyltin and **b** tributyltin. **X** stands for a monovalent anionic ligand (e.g. chloride, hydroxide or acetate).

powdery mildew (*Erysiphe betae*) on celery, peanuts and sugar beet, *Pseudoperonospora humuli* on hop, grey moulds on onions, rice blast, brown rust on beans and coffee leaf rust. Besides, TPT compounds are used in certain antifouling paints on ships, mainly in combination with TBT (Crompton 1998). The annual world production of TPT compounds is unknown. In Germany it is estimated close to 1000 t, while the world consumption of TPT compounds in fungicides is estimated to be several thousand tons per year (Craig 1986). Due to recent studies, the US Environmental Protection Agency stated that TPT may cause endocrine disruption, also in vertebrates (US EPA 1999).

Likewise, since the 1960s, TBT is used as major active biocidal component in antifouling paints on ship hulls, harbour and offshore installations, but also as a biocide in wood preservatives, textiles, dispersion paints, agricultural pesticides and as a by-product of other organotin compounds used as UV stabilizer in plastics. Therefore, most harbour sediments are highly contaminated with organotins. As the disposal of dredged harbour material into the sea is still common practice in many countries, this is likely to result in a non-acceptable contamination and hazard for marine biocoenoses. Also, in freshwater ecosystems along main shipping routes, the occurence of concentrations that might endanger certain species is probable. TBT compounds exhibit the highest toxicity of all organotins (acute toxicity, teratogenicity and immunotoxicity) and have even been characterized as one of the most toxic groups of xenobiotics ever produced and deliberately introduced into the environment. This compound is known to be harmful to many, also "non-target" aquatic organisms, particularly mollusks (Horiguchi et al. 1997). Because TBT tends to accumulate in sediments, which are considered a sink for TBT, they may have become a source, and will continue to have this function in the future. The annual production of tributyltinhydroxide in Germany is estimated to be about 2000 t (70% for antifouling paints, 20% for timber protection, 10% for protection of textile and leather, Fent 1996). The total annual world production of organotins is about 50000 t (Fent 1996). The TPT and TBT compounds are both considered in the Priority Lists of Action of the European Commission (Commission of the European Communities 1999) and of the International Rhine Commission (Vrijhof 1985).

In the early 1980s, the first adverse effects were observed in oysters (*Crassostrea gigas*), e.g. abnormal shell growth and reproductive failure, which led to significant economical damage (Alzieu 1986). Despite the EU-ban of TBT-based antifoulings since 1991 for boats less than 25 m overall length and their complete restriction since January 2003, a broad variety of malformations caused by TBT was and still is found in several groups of animals and plants.

TPT and TBT reveal a substantial bioaccumulation potential with $\log K_{OW}$ values of 4.1 for TPT (Thompson et al. 1985) and 4.4 for TBT (Arnold et al. 1997). TPT accumulates in organisms with bioconcentration factors of 2×10^3 in carp (Tsuda et al. 1987) and 8×10^4 to 4.4×10^5 in crabs (Kannan et al. 1995). Bioconcentration factors for TBT range from 1.5×10^3 to 3×10^5 in marine mussels and oysters (Laughlin and French 1988, Shim et al. 1998) and from 8.3×10^4 to 4.5×10^5 in freshwater bivalves (Becker et al. 1992). High concentrations of TPT and TBT were measured in fish, especially in liver, heart and brain, suggesting that these organotins are able to pass the blood-brain barrier (Harino et al. 2000).

Organotin compounds with small alkyl chains decay generally only at a snail's pace in the environment. According to Federoff et al. (1999), TPT is resistant to photodegradation and hydrolysis, showing half lives of 93 to 111 days in irradiated water samples and 155 days in a dark control. The half-life of TBT in sediments is estimated to be months to several years (Sarradin et al. 1995). Both substances are considered to be rather persistent and accumulate in the sediment of aquatic systems and especially in organisms (Fent 1996, Crompton 1998). Their low solubility in water (1.2 to 8 mg/L, Federoff et al. 1999) and high K_{OC} value (5700 mL/g for TPT and 5500 mL/g for TBT) suggest adsorption onto suspended particles and sediments.

Therefore, the development of whole-sediment biotests, using sediment-dwelling organisms as test species, is essential to obtain information on effects in the sediment compartment, the so-called "memory of the water". This meets an urgent demand, since current analytical detection limits of organotin compounds in water, sediment and organism tissue are exceeding reported effect concentrations. Besides, analytical measurement is rather time-consuming and expensive. Hence, bioindication represents a more economical and relevant method. Recent studies recommend mollusks and particularly snails as the most sensitive organisms among the invertebrates concerning the effects of endocrine disruptors (Matthiessen and Gibbs 1998, deFur et al. 1999).

Materials and Methods

Imposex in the netted whelk *Nassarius reticulatus* and the dogwhelk *Nucella lapillus*

Imposex or pseudohermaphroditism is characterized by the superimposition or additional formation of male reproductive organs, like a penis and/or a vas deferens in females (e.g. in the netted whelk *Nassarius reticulatus* or the dogwhelk *Nucella lapillus*) and is caused by elevated testosterone titres that masculinize TBT-exposed females. This phenomenon is induced by aqueous concentrations as low as 0.5 ng TBT as Sn/L or 10 μg TBT as Sn/kg in sediments, respectively. The ultimate stage of imposex development is a complete sterilization of females, resulting in reproductive

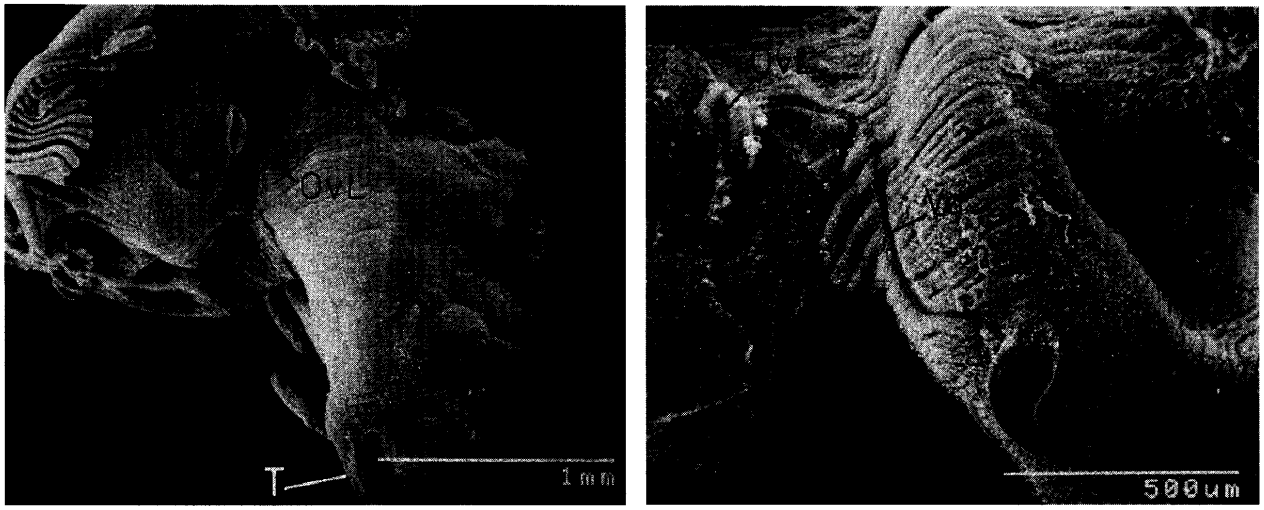

Fig. 2. Imposex in *Hydrobia ulvae*. **a** (left) imposex stage 0, normal female and **b** (right) imposex stage 5, sterilised female. **Kd** capsule gland, **Ovl** vaginal opening, **PP** penis, **R** rectum, **T** tentacle, **Vd** vas deferens.

failure and local extinction of populations (Fig. 2). In very sensitive species, even a protogyne sex change can be induced. The degree of imposex in a population is determined by different biomonitoring indices, which allow an assessment of the degree of TBT contamination in coastal, estuarine and lotic environments at low costs and with high precision. To date, the imposex phenomenon of prosobranchs is not only the best documented example of endocrine disruption in invertebrates, but has also been successfully used as a biological effect monitoring system to determine the degree of environmental TBT pollution (Minchin et al. 1995, Minchin et al. 1996, Oehlmann et al. 1996a, b, Bauer et al. 1997, Minchin et al. 1997, Oehlmann et al. 1998a). This biological marker allows also the assessment of ameliorations of the exposure situation following legislative controls. In most cases, the dogwhelk *N. lapillus* has been used as the sentinel species (e.g. Gibbs et al. 1987, Harding et al. 1992); considerations of additional species such as *Trivia arctica, Nassarius (Hinia) reticulatus, Ocenebra erinacea, Buccinum undatum* or *Neptunea antiqua*, were occasional exceptions in European surveys (e.g. Stroben et al. 1992a-c, Oehlmann et al. 1993, 1996a, b, Ten Hallers-Tjabbes et al. 1994).

Intersex in the periwinkle *Littorina littorea*

Intersex means a modification or supplanting of female by male sexual characteristics (e.g. in the periwinkle *Littorina littorea*), thus a phenotypic disturbance of sex determination between gonad and genital tract. The intersex index (ISI) is calculated as the mean value of intersex stages within a population (ISI=sum of intersex stage values of all females sampled/number of females). Females in the intersex stages 2, 3 and 4 are unable to reproduce due to oviduct malformations or the supplant of female sexual glands by the corresponding male formations (Fig. 3). Females in the intersex stages 0 and 1 are rated as fully capable for normal reproduction although some evidence exists that stage 1 specimens are already characterised by a reduced reproductive success (for details see Bauer et al. 1993, 1995). Therefore, ISI values >1.00 indicate that at least some of the females in the sample are sterilised due to intersex development. The ISI is the equivalent to the VDSI (vas deferens sequence index) in imposex affected prosobranch species such as *Nucella lapillus*.

Nucella lapillus as the established TBT effect monitoring species does not occur in many European coastal regions such as the southern part of the North Sea and the entire Baltic, mostly because of unsuited habitats. In addition, they become extinct near TBT point sources, like harbours and marinas, due to the aforementioned sterilisation of females. The need for a further effect monitoring system, which can be used in those areas, where dogwhelks and other neogastropods are absent, was addressed by Bauer et al. (1993, 1995, 1997) and Oehlmann et al. (1994), who were the first to describe the intersex phenomenon in the periwinkle *Littorina littorea*. Since the first description of intersex in periwinkles, further investigations have been performed in France (Oehlmann et al. 1998b), Ireland (Minchin et al. 1996, 1997) and Germany (Oehlmann et al. 1998b). These analyses have shown that *L. littorea* is well suited for TBT effect monitoring, especially in regions with a relatively high level of contamination. Even in these areas, the species is quite common and can be sampled in sufficient numbers because: a) it is tolerant of high TBT levels, b) it recruits from the plankton and c) it can occur in areas where dogwhelks have become extinct. The intersex phenomenon was considered by OSPAR (Oslo and Paris Commissions) along with the imposex response in the dogwhelk *N. lapillus* for use in biological TBT effect monitoring surveys in the entire convention area. The OSPAR guidelines for the TBT specific biological effect monitoring, using the responses of both

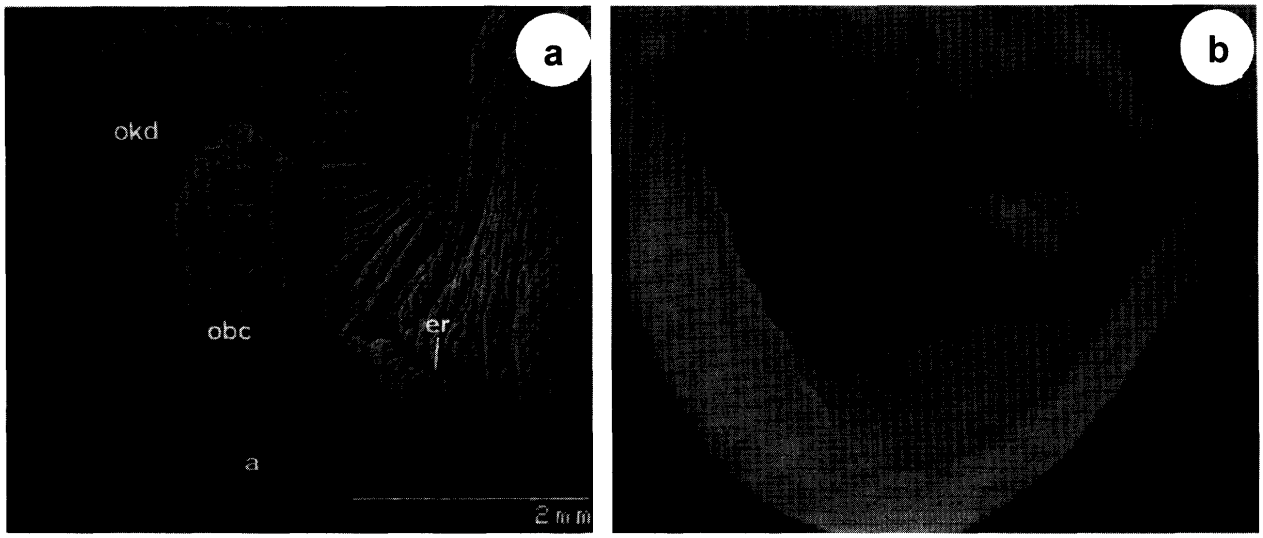

Fig. 3. Intersex in the periwinkle *Littorina littorea*. **a** intersex stage 2, female and **b** same specimen, enlarged view. **a** anus, **er** egg channel, **obc** open bursa copulatrix, **okd** open capsule gland.

prosobranch species, were amended recently (Oslo and Paris Commissions 2001).

Biotest with the netted whelk *Nassarius reticulatus*: imposex

The netted whelk *Nassarius reticulatus* (Neogastropoda, Buccinidae) is a sediment-dwelling scavenger feeding on carrion. It lives burrowed in coastal fine sediments and reaches shell heights of up to 40 mm.

The specimens used for the tests (see Schulte-Oehlmann et al. 2000, Tillmann 2004) were collected in Brittany, France, at Pléneuf Val André in March 1999. The netted whelks were exposed to TBT and TPT via artificial sediments consisting of 90% quartz sand and 10% peat (untreated). For each treatment, 750 g fresh sediment was weighed into an aquarium and covered with artificial sea water. All tests were performed under constant conditions with a temperature of 14±1°C and a light : dark rhythm of 12 : 12 hours.

Adult *N. reticulatus* were exposed to nominal concentrations of 10, 25, 50, 75, 125, 250 and 500 µg TBT-Sn/kg and 50, 125 and 500 µg TPT-Sn/kg dry wt., respectively, for 3 months, including a solvent control (glacial acetic acid; concentration: 5 mg/kg). 30 specimens from each group were analysed at the beginning of the experiment and in monthly intervals.

During the experiments the production of spawning masses with the number of eggs in each of the aquaria and the mortality were recorded twice a week. All specimens were narcotised prior to analysis (7% $MgCl_2$ in distilled water). The individual shell and aperture height were measured to the nearest 0.1 mm before the shell was cracked. The presence, normal appearance and extension to the nearest 0.1 mm of all sex organs was checked, as well as the occurrence of oocytes and sperm in the genital system and of visible excrescences on genital and other organs with a dissection microscope. Additionally, imposex parameters like the VDSI (vas deferens sequence index=mean value of imposex stages in a sample with values from 0 to 4) were calculated (for details see Oehlmann et al. 1991, Stroben et al. 1992b, Schulte-Oehlmann et al. 1995). Furthermore, a histopathological analysis of the gonads was performed for all experimental groups. 6 male and 6 female specimens from each sample were fixed in Carnoy's and Bouin's fluid, respectively, and then preserved in ethanol. After embedding in Paraplast, serial sections (3–7 µm) were made and stained with haemalum-chromotrope, haematoxylin-eosin and periodic-acid-Schiff. The sections were analysed using an image analysis system (Optimas 5.2, Optimas Cooperation coupled with an Olympus microscope BX 50). All specimens found to be afflicted with parasites, mainly trematode larvae, were excluded from the evaluation.

Standard statistical analyses of the results, e.g. analyses of covariance (ANCOVA) and analyses of variance (ANOVA) with multiple comparison of samples according to Tukey (low *n*) or Student-Newman-Keuls (high *n*), EC10 and EC50 calculations (probit analyses, maximum likelihood method), x^2 test, and Weir test for classified values were performed according to Weber (1972) and Lozán (1992), using the computer programme StatEasy for Windows NT.

Biotest with the freshwater mudsnail *Potamopyrgus antipodarum*: embryo production

The freshwater mudsnail *Potamopyrgus antipodarum* (Gastropoda, Prosobranchia, Hydrobiidae) is native to Europe for over 150 years, yet a "newcomer" in European freshwater ecosystems. In the mid 19th century, it was introduced from New Zealand to Europe with ballast water of ships. Since

then, the species in Europe was known as *P. jenkinsi* (Ponder 1988). In contrast to New Zealand populations, European populations consist almost exclusively of females and males are seldomly found (Wallace 1979). *P. antipodarum* is parthenogenetic and ovoviviparous with shell heights reaching up to 6 mm. It inhabits the upper layers of aquatic sediments, feeding on plants and detritus. During dry or cold periods, it lives completely buried in the sediment. *P. antipodarum* lives in freshwater environments, but—being an euryhaline species—also in some regions of the Baltic Sea and in estuaries of the North Sea and Atlantic in co-existence with other hydrobiid (mudsnail) species.

For the experiments (Duft et al. 2003a), we used specimens from the breeding stock of our laboratory which was built up with specimens collected from Gievenbach, a small creek near Ibbenbüren, Germany, in 2000. The snails were kept in 10-L aquaria in artificial freshwater (0.5 g $NaHCO_3$, 5 g $CaCO_3$ and 5 g mineral salt per 10 L demineralised water, Milli Q RG and Milli RO plus, Millipore, Eschborn, Germany) and fed regularly with a mixture of Tetra Phyll® (Tetra, Melle, Germany) and Fish Tamin® (Sera, Heinsberg, Germany), stirred in the medium described above. Additional calcium carbonate was added regularly to improve shell growth.

The experiments were conducted as static systems (without water renewal) in 1-L glass Erlenmeyer flasks. An artificial sediment (95% quartz sand, Quarzwerke Millisil, Frechen, Germany, and 5% beech leaves, collected in the National Park on Rügen Island, Germany, crushed in a coffee grinder MC 23, Siemens, Munich, Germany) was used for the spiking of the test substances. This sediment assured an optimal embryo production of *Potamopyrgus* compared to other artificial and even natural sediments (Duft 2004). The organic carbon content of the artificial sediment was 2.3%, the mean grain size was 180 μm. To each flask, 50 g of artificial sediment (dry weight) was added. For the spiking procedure, 2 ml of the respective concentration of TPT and TBT (dissolved in 100% ethanol) was applied to each treatment and homogenised by stirring. One day evaporation guaranteed a complete removal of the solvent. 1 L of medium was added to the flasks, which were subsequently aerated through glass pipettes (compressed air, 40 A compressor, Die Pumpe, Holm, Germany), enabling manual adjustment of air supply. Equilibration duration was 5 days in darkness. Finally, 80 *Potamopyrgus* individuals were added to each flask.

For tributyltin (TBT chloride, Merck Schuchardt Chemicals, Darmstadt, Germany, >97% purity) and triphenyltin (TPT chloride, Merck Schuchardt Chemicals, Darmstadt, Germany, >98% purity), the following nominal concentrations were applied: 5, 10, 25, 50, 125, 250 and 500 μg Sn/kg. 1 μg TBT-Sn/kg corresponds to 2.44 μg TBT/kg and 1 μg TPT-Sn/kg to 3.24 μg TPT/kg. Additionally, a water control and a solvent control were included in each experiment. At

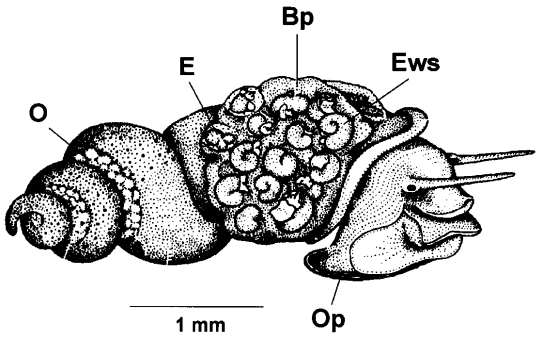

Fig. 4. *Potamopyrgus antipodarum*, female, after removal of the shell (modified after Fretter & Graham, 1994). **O** ovary, **Bp** brood pouch, **E** "new" embryo without shell (unshelled), **Ews** embryo with shell, **Op** operculum.

the end of the experiment, after 8 weeks, the applied nominal concentrations in the sediments were checked analytically, according to the method described by Arnold (1998).

All tests were performed under constant conditions in a climate chamber with a temperature of 15±1°C and a light : dark rhythm of 16 : 8 h. 20 snails were analysed individually after 0, 2, 4 and 8 weeks, respectively. Prior to analysis, the snails were narcotised in $MgCl_2$ (2.5% in distilled water) for 2 h. Shell and aperture heights were measured after which shells were cracked by a small vice and shell parts were removed. The brood pouch was opened carefully and the number of "grown-up" embryos (with shells) and "new" embryos (without shells) were counted using a dissecting microscope (Fig. 4). Additionally, occurrence of egg cells in the oviduct and the maturity of the ovary were noted for each individual. Adult mortality was recorded and dead snails were removed.

All data were analysed statistically using the software package Prism®, Version 2.01 (GraphPad Software, San Diego, CA, USA) for Windows NT. We calculated mean and standard error for each treatment and performed one-way-ANOVA (analyses of variance), followed by Tukey's multiple comparisons of the means to check for differences between the treatments and control. For both substances, non-linear regressions and respective effect concentrations (EC10 and EC50) were calculated using a Weibull model, reparameterized so that the EC50 is one of the fitted parameters (Weltje et al. 2004):

$$Y = \frac{C}{2^{\left[\frac{X}{EC50}\right]^b}} \quad (1)$$

where Y is the number of embryos in percentage of the solvent control, X is the concentration of TPT/TBT (μg-Sn/kg), b is a dimensionless slope parameter, C is the solvent control performance (i.e. a constant, namely 100%) and EC50 the TPT or TBT concentration causing a 50% decline in the number of embryos in comparison to the solvent control sediment (μg-Sn/kg).

Biotest with the freshwater ramshorn snail *Marisa cornuarietis*: imposex, fecundity and gametogenesis

Test species is the gonochoristic freshwater ramshorn snail *Marisa cornuarietis* (Mesogastropoda, Ampullariidae). The specimens for the experiments were imported from Florida (Schulte-Oehlmann et al. 2000) and were exposed to the test compound triphenyltin chloride (Merck, Darmstadt, Germany) via water. These experiments were conducted as 24 h (weekends 48 h) semi-static renewal systems in 60 litre glass aquaria filled with tap water and provided with an Eheim power filter. All tests were performed under constant conditions with a temperature of 22±1°C; the light : dark rhythm was adjusted to 12 : 12 h.

Adult *M. cornuarietis* were exposed to nominal aqueous concentrations of 75, 150, 250 and 500 ng TPT-Sn/L for 4 months, including a solvent control (ethanol; concentration: 12.5 µg/L). 30 specimens from each group were analysed at the beginning of the experiment and in monthly intervals. Additionally, a complete life-cycle test was planned with an exposure *ex ovo* over a period of 12 months until the hatched F1 specimens were one year old (compare Oehlmann et al. 2000). However, due to the severe effects of the test compound on the reproductive performance of *Marisa*, this was not possible.

The nominal TPT concentrations were checked analytically, using the GC-MS method described by Kalbfus et al. (1996). The measured concentrations in the exposure groups ranged from 57.8 to 94.3% of the nominal concentrations. During the experiments, the production of spawning masses, the number of eggs in each of the aquaria and the mortality were recorded in daily intervals.

All specimens were narcotised prior to analysis (2.5% $MgCl_2$ in distilled water). The individual shell and aperture height were measured to the nearest 0.1 mm before the shell was cracked. The presence, normal appearance and extension to the nearest 0.1 mm of all sex organs was checked, as well as the occurrence of oocytes and sperm in the genital system and of visible excrescences on genital and other organs with a dissection microscope. Additionally, imposex parameters like the VDSI (with values from 0 to 3) were calculated (for details see Oehlmann et al. 1991, Stroben et al. 1992a, b, Schulte-Oehlmann et al. 1995). Furthermore, a histopathological analysis of the gonads of 6 male and 6 female specimens was performed for the control and 500 ng TPT-Sn/L groups. Samples were fixed in Carnoy's and Bouin's fluid, respectively, and then preserved in ethanol. After embedding in Paraplast, serial sections (5–7 µm) were made and stained with haemalum-chromotrope. The sections were analysed using an image analysis system (Optimas 5.2, Optimas Cooperation, coupled with an Olympus microscope BX 50). All specimens found to be afflicted with parasites, mainly trematode larvae, were excluded from the evaluation.

Standard statistical analyses of the results, e.g. analyses of covariance (ANCOVA) and analyses of variance (ANOVA) with multiple comparison of samples according to Tukey (low *n*) or Student-Newman-Keuls (high *n*), EC10 and EC50 calculations (probit analyses, maximum likelihood method), x^2 test, and Weir test for classified values were performed according to Weber (1972) and Lozán (1992), using the computer programme StatEasy for Windows NT.

Results

Laboratory experiments with *Nassarius reticulatus*: imposex

The concentration-response relationship between TBT concentrations in spiked artificial sediments and the increase of the VDSI within one month of exposure is shown in Fig. 5a. This graph shows that a maximum effect is reached if the VDSI increases by 1.0 during the test duration. The LOEC for the increase of the VDSI, thus imposex development, in the experiments is equivalent to the lowest administered concentration of 10 µg TBT-Sn/kg. The results for the netted whelk *Nassarius reticulatus* are mostly in line with findings for *Nucella lapillus* (results not shown).

While exposure to TBT via sediments led to the expected statistically significant (Weir test for classified values, $p<0.05$) time- and concentration-dependent increase of imposex intensities (Fig. 5a), TPT exhibited no comparable androgenic activity in the applied nominal concentration range of 50 to 500 µg TPT-Sn/kg dry wt. The VDSI values in all TPT test groups and in the control remained on the same level with no indication for any differences between the groups or within a group during the three months experiment (ANOVA, $p>0.05$).

However, the gonads of *N. reticulatus* revealed marked disturbances in differentiation and maturation processes in both sexes. In 20% of the females exposed to 50 µg TPT-Sn/kg for 1 and 2 months and in 83% after 3 months, the follicles remained at a low maturation level. They were predominantly filled with oogonia, incorporation of vitelline was absent. At the concentration of 125 µg TPT-Sn/kg, 60% of the females exhibited gonads without follicle maturation after 1 month, 100% after 2, and 50% after 3 months, respectively. Females exposed to 500 µg TPT-Sn/kg showed inhibition of maturation of follicles in 40% after 1, 100% after 2, and in 83% after 3 months (Fig. 5b). This phenomenon was accompanied by an increasing rate of atresia but a low degree of resorption. The presence of "nurse cells" was lower than in specimens of the control group. The follicle epithelium appeared translucent with small droplets of fat vacuoles after 2 months. Abundant yellow lipopigment, autochrome, insoluble in organic solvents and PAS positive, was present in the follicle epithelium in the control group and filled the lumina of follicles and the oviduct to a high degree in all exposed

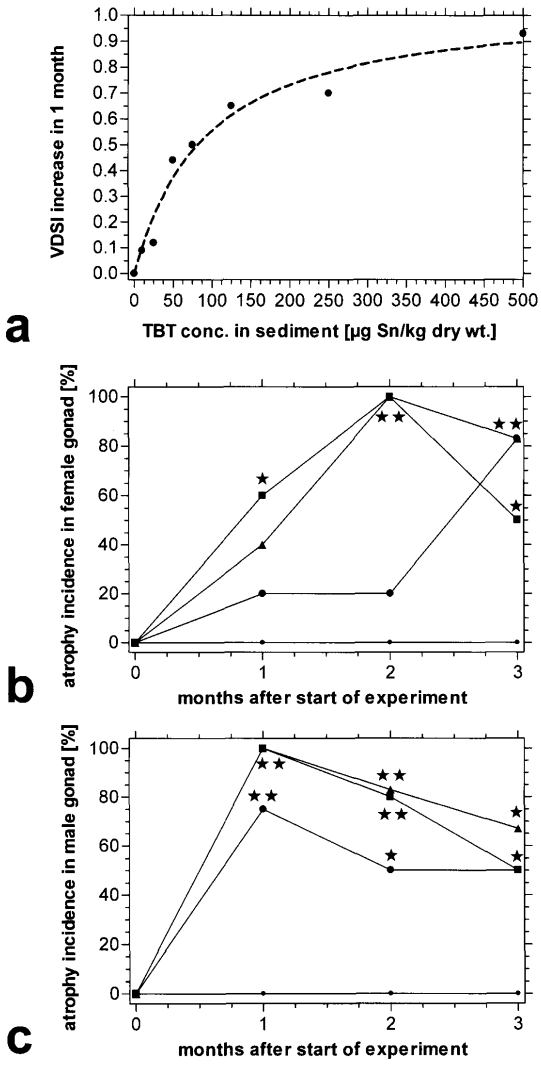

Fig. 5. Effects of **a** tributyltin (TBT) concentrations in the sediment (µg TBT-Sn/kg) on imposex development (increase of the VDSI vas deferens sequence index) in the netted whelk *Nassarius reticulatus* after 4 weeks of exposure (n=30, r^2=0.98, p<0.0005) and **b** triphenyltin concentrations in the sediment (µg TPT-Sn/kg) on incidences of atrophy in female and **c** male gonads of *Nassarius reticulatus*. Concentrations (dry wt. basis): (·) solvent control, (●) 50 µg as Sn/kg, (▲) 125 µg as Sn/kg, (■) 500 µg as Sn/kg. Asterisks denote statistical significant differences to control (* p<0.05, ** p<0.01).

specimens. At concentrations of 125 and 500 µg TPT-Sn/kg the oviduct was clogged with this lipopigment.

The gonads of male *Nassarius* in the control group were predominantly composed of primary and secondary spermatogonia and to a lower degree with spermatids after 1 month. All stages from primary spermatogonia to ripe sperm could be found after 2 months. In the 50 µg TPT-Sn/kg exposure group, 75% of the males showed an atrophy in the form of a "Sertoli only" change after 1 month, and 50% after 2 and 3 months. A similar situation was present in animals exposed to 125 and 500 µg TPT-Sn/kg, whereas the highest degree of testis atrophy occurred after 1 month and dropped thereafter down to 80 and 50% (Fig. 5c). Seminiferous tubules with

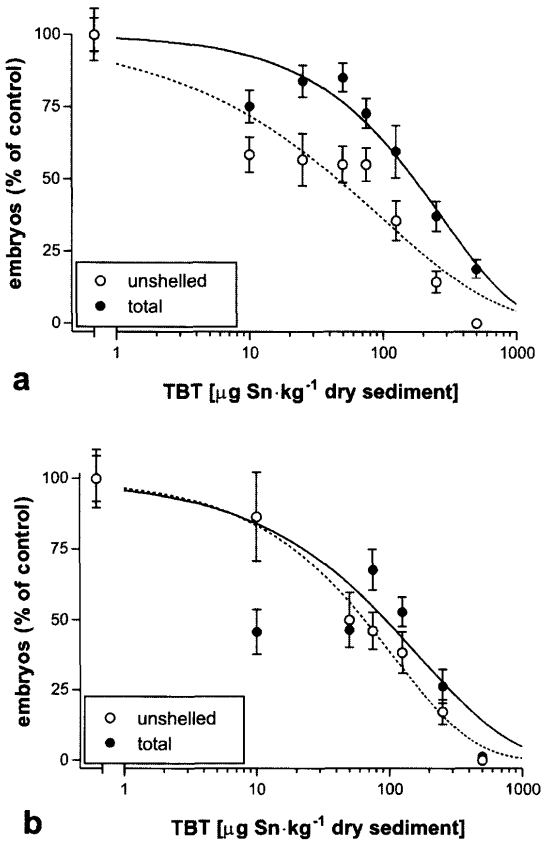

Fig. 6. Effects of tributyltin (TBT) concentrations in the sediment (µg TBT-Sn/kg) on the embryo production (without shell and total) of *Potamopyrgus antipodarum* in % of the solvent control (mean±standard error of the mean, SEM, n=20) **a** after 4 weeks of exposure, **b** after 8 weeks of exposure. Regression lines (Eqn. 1) are added—solid line for the total embryo number (4 weeks r^2=0.46, 8 weeks r^2=0.64), dotted line for the unshelled embryo number (4 weeks r^2=0.64, 8 weeks r^2=0.91).

"Sertoli only" changes and a maturation depletion contained degenerated spermatogonia and spermatids in the lumen. In the 125 µg TPT-Sn/kg exposure group, massive degeneration of all stages of germ cells, necrosis and shrinking of tubules were present.

Laboratory experiments with *Potamopyrgus antipodarum*: embryo production

The exposure to both test substances TPT and TBT resulted in a marked decrease in the number of embryos in the brood pouch of *Potamopyrgus antipodarum*.

For TBT, the effect was evident already after 4 weeks: the number of unshelled embryos decreased continuously and significantly in all tested concentrations of TBT and reached 0 at the highest applied concentration of 500 µg TBT-Sn/kg (Fig. 6a). In this treatment, we also found the highest mortality (35%, LC50 542 µg TBT-Sn/kg). The corresponding LOEC for the embryo production was again the lowest applied concentration of 10 µg TBT-Sn/kg and the calculated EC10 yielded a concentration of 0.98 µg TBT-Sn/kg (EC50 45.8 µg TBT-Sn/kg). Furthermore, the total em-

bryo number confirmed this tendency (Fig. 6a). The exposure groups of all tested concentrations, except for 25 and 50 μg TBT-Sn/kg, differed significantly from the solvent control sediment (ANOVA, $p<0.05$). The treatments with the lowest concentration of 10 μg/kg were more affected than the mid-range applications, whereas in the treatments with the highest concentrations embryo numbers were completely inhibited. A calculation of the EC10 resulted in a value of 10.6 μg TBT-Sn/kg (EC50 173 μg/kg).

After 8 weeks, the calculated EC50 for the production of new, unshelled embryos was 64.0 μg TBT-Sn/kg, the respective EC10 2.98 μg TBT-Sn/kg. Except for the lowest concentration, all treatments showed a significant decrease in embryo production (ANOVA, $p<0.01$, Fig. 6b). The embryo number in the highest concentration of 500 μg TBT-Sn/kg could not be assessed as in this treatment mortality was 100%. The calculated LC50 was 431 μg TBT-Sn/kg, hence lower than after 4 weeks exposure. A typical concentration-response curve was noticed, whereas for the total embryo production another response was observed: there was a decrease of embryos at the lowest and also at the highest applied concentrations. The total number of embryos after 8 weeks comprises "grown-up" embryos (with a shell) that have grown in the presence of TBT, thus describing a mid-scale effect. Compared to the results after 4 weeks, we see that now the lower concentrations also affect the total embryo number. Again, most treatments (except for 75 μg TBT-Sn/kg) differed significantly from the solvent control treatment (ANOVA, $p<0.001$).

Also for TPT, a decline of embryos was observed after 4 weeks of exposure (Fig. 7a). The production of "new", unshelled embryos was significantly inhibited (ANOVA, $p<0.001$) especially at lower concentrations (10, 25, 50 and 75 μg-Sn/kg) resulting in a kind of u-shaped curve which may indicate the disturbance of an endocrine function. For the total number of embryos, a similar tendency can be seen, yet the percentage of embryos compared to the solvent control was higher than for the unshelled embryos (Fig. 7a). All exposure groups, except for the higher concentrations of 125, 250 and 500 μg TPT-Sn/kg, differed significantly from the solvent control (ANOVA, $p<0.05$). The number of shelled embryos after 4 weeks was not affected (ANOVA, $p<0.05$) and is therefore not shown in the figures. Likewise, after an exposure of 2 weeks only, no effects were observed.

After 8 weeks, the decline of the embryo production was most conspicuous (Fig. 7b): the number of unshelled embryos was significantly lower in all tested concentrations of TPT compared to the solvent control treatment (ANOVA, $p<0.001$), hence the LOEC was 10 μg TPT-Sn/kg. The calculation of effect concentrations yielded even lower values: the calculated EC50 turned out to be 0.74 μg TPT-Sn/kg, the EC10 was 0.03 μg TPT-Sn/kg. TPT inhibited the embryo production down to 25% and less in most exposure groups. A

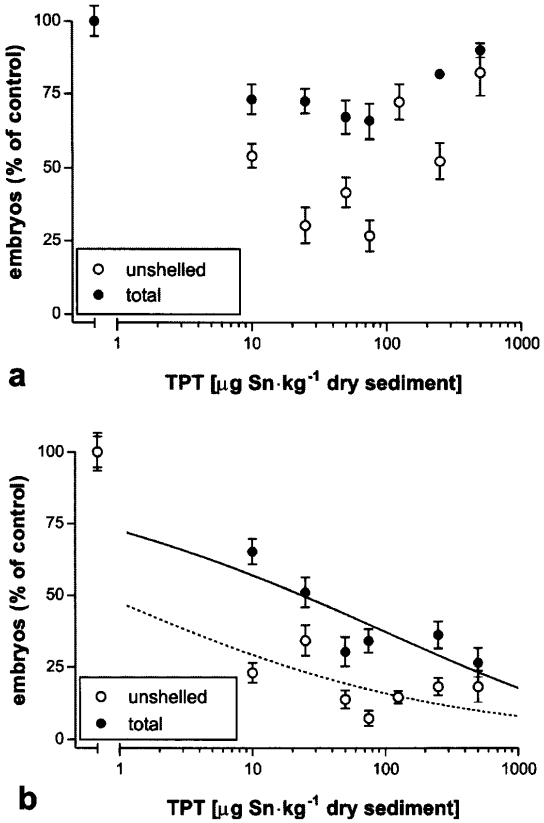

Fig. 7. Effects of triphenyltin (TPT) concentrations in the sediment (μg TPT-Sn/kg) on the embryo production (without shell and total) of *Potamopyrgus antipodarum* in % of the solvent control (mean±standard error of the mean, SEM, $n=20$) **a** after 4 weeks of exposure, **b** after 8 weeks of exposure. Regression lines (Eqn. 1) are added—solid line for the total embryo number ($r^2=0.51$), dotted line for the unshelled embryo number ($r^2=0.67$).

sharp and significant (ANOVA, $p<0.01$) decrease was also noted in the total number of embryos (Fig. 7b), the corresponding EC50 was 23.6 μg TPT-Sn/kg. The solvent, ethanol, had no effect on embryo production (ANOVA, $p>0.05$). The embryo number in the solvent control did not change during the exposure periods of 0, 2, 4 and 8 weeks (mean unshelled 6.31±0.46, mean total 11.50±0.74; ANOVA, $p>0.05$). Only after the exposure period of 2 weeks, a slight increase in the total embryo number was observed (14.32±2.77). No significant mortality occurred during the experiments with TPT and therefore, no LC values could be determined.

Laboratory experiments with *Marisa cornuarietis*: imposex, fecundity and gametogenesis

The normal morphological and histological structure of the male and female genital system of the ramshorn snail and of pathomorphological changes during imposex development following TBT exposure is well documented by Schulte-Oehlmann et al. (1995). In *Marisa cornuarietis*, exposure to TPT resulted in a time- and concentration-dependent enhancement of the imposex intensities. Not only the VDSI in

females increased (Fig. 8a), but also the length of the female penis, the mean length of the penis sheath and the extension of the female penis pouch (data not shown).

For the VDSI, the differences between the groups exposed to the three highest nominal TPT concentrations (150, 250, 500 ng TPT-Sn/L) and the control were statistically significant during the entire experiment including the first month, but with the exception of month 2 for the 150 ng TPT-Sn/L group (Weir test for classified values, $p<0.05$). At a concentration of 75 ng TPT-Sn/L, the increase of the VDSI compared to the control was not significant until the fourth month of exposure. During the entire test, the values for the control group were not statistically significant different from each other (ANOVA, $p>0.05$). For the VDSI, an EC10 value of 12.3 ng TPT as Sn/L was calculated. The statistical analysis of the development of the mean female penis sheath length revealed comparable results as indicated for the VDSI: the differences to the control were significant throughout the experiment for 500 ng TPT-Sn/L, for 250 ng TPT-Sn/L from the second, for 150 ng TPT-Sn/L from the third and for 75 ng TPT-Sn/L not before the last month of the test (ANOVA with multiple comparison of samples according to Student-Newman-Keuls, $p<0.05$).

Originally, it was planned to expose *Marisa* for five months to TPT, but due to an unexpected high mortality in all groups—except for the solvent control—the experiment had to be aborted after four months and after three months in the 500 ng TPT-Sn/L group.

TPT affected not only the female specimens by the development of imposex, but had furthermore an adverse impact on the extension of the pallial sex organs in males (prostate gland, penis, penis sheath, penis pouch; results not shown). The reduction of the mean penis size by up to 25% compared to the control was only statistically significant for the two highest exposure groups (250, 500 ng TPT-Sn/L) throughout the experiment (ANOVA with multiple comparison of samples according to Student-Newman-Keuls, $p<0.05$).

Another effect of the test compound was a negative impact on the fecundity, measured as the numbers of produced spawning masses (Fig. 8b) and eggs. For the two lowest nominal concentrations (75 and 150 ng TPT-Sn/L), a marked decrease of both parameters was observed, and in the 250 and 500 ng TPT-Sn/L exposure groups, a complete cessation of spawning occurred. An ANCOVA analysis, with multiple comparison of samples according to Student-Newman-Keuls, revealed that the spawning mass and egg production in all TPT treated groups was significantly lower than in the control group ($p<0.05$). The *Marisa* females in the control group produced a total number of 53 spawning masses containing 2850 eggs while the corresponding numbers were 47% and 53% lower in the 75 ng TPT-Sn/L and 93% and 98% lower in the 150 ng TPT-Sn/L group (values for spawning masses and eggs, respectively). The EC10 value for fecundity was 5.6 ng TPT as Sn/L and the snails were unable to spawn at concentrations above 163 ng TPT as Sn/L. The reduced fertility or complete lack of eggs in the experimental groups was the reason why an originally planned complete life-cycle test with *M. cornuarietis* could not be performed.

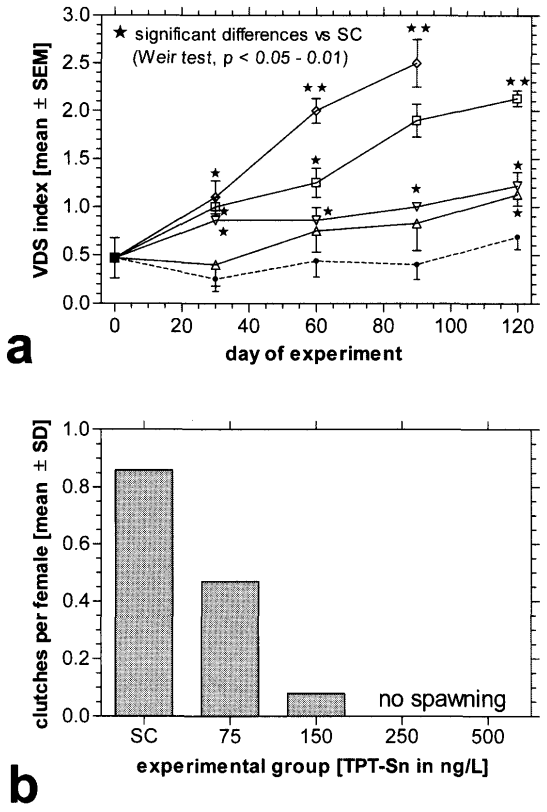

Fig. 8. Effects of triphenyltin (TPT) concentrations on the freshwater ramshorn snail *Marisa cornuarietis*. **a** Imposex development (VDSI vas deferens sequence index) and **b** spawning mass per female, after four months of exposure to the following concentrations (via water): (·) solvent control, (△) 75 ng as Sn/L, (▽) 150 ng as Sn/L, (□) 250 ng as Sn/L, (◇) 500 ng as Sn/L.

As the sex ratios were slightly different in the test groups, the fecundity parameters were also analysed per female for the experiment. Those females which received TPT via ambient water produced 14% (75 ng TPT-Sn/L), 91% (150 ng TPT-Sn/L), and 100% (250, 500 ng TPT-Sn/L) fewer spawning masses (Fig. 8b) and 24% (75 ng TPT-Sn/L), 96% (150 ng TPT-Sn/L), and 100% (250, 500 ng TPT-Sn/L) fewer eggs than the control females. Furthermore, the mean number of eggs per spawning mass was statistically significantly lowered by the test compound (ANOVA with multiple comparison of samples according to Tukey).

Interestingly, the histopathological analyses of the gonads showed no evidence for any disturbances of oogenesis in the TPT treated *Marisa* specimens compared to the control, but there were marked differences for the males. As stated above (in Materials and methods), six males and females from the control group and the highest TPT concentra-

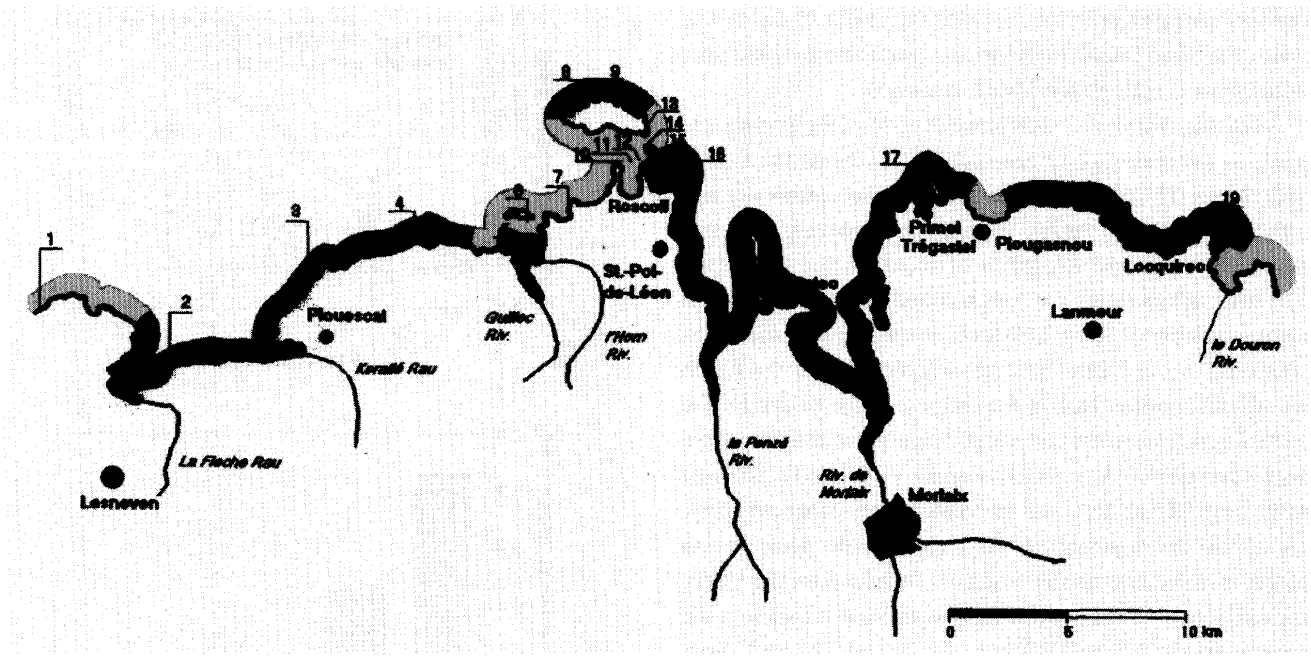

Fig. 9. Biomonitoring with the dogwhelk *Nucella lapillus* in Brittany, Northern France. Imposex intensities. Greyscales are equivalent to the following colours mentioned in the text: light for yellow (VDSI=4.0), medium for green (VDSI<4.0) and dark for red (VDSI>4.0).

tion (500 ng TPT-Sn/L) were analysed histopathologically in monthly intervals. One month after the start of the experiments, no statistically significant differences in the incidence or intensity of spermatogenesis disturbances could be detected. After 2 and 3 months however, all analysed males from the TPT-treated group exhibited not only a severe disruption of germ cell formation with a 100% incidence of the effect, which was statistically significant compared to the control (x^2 test; second month: $p<0.05$; third month: $p<0.001$), but also regarding the intensity of the disturbances. These males were found to be sterile with an azoospermia spermatogenesis index=2.0 according to Schulte-Oehlmann et al. (2000). The histopathological results indicated that the TPT induced reduction of the fecundity during the experiment might be due to an impairment of spermatogenesis.

Biomonitoring with *Nucella lapillus* and *Littorina littorea* in Germany, France and Ireland

Since 1988, imposex intensities in populations of the dogwhelk *Nucella lapillus* and 10 other species were analysed at more than 200 stations along the French, Irish and German coastline (see Minchin et al. 1995, Minchin et al. 1996, Oehlmann et al. 1996b, c, Bauer et al. 1997, Minchin et al. 1997, Oehlmann et al. 1998b). As an example of these surveys, Fig. 9 shows the level of analysed imposex intensities in *N. lapillus*, represented by different colours, at the coast of northern Brittany in France in the surroundings of Roscoff and Morlaix. The red coloured areas denote VDSI values above 4.0, which means that sterilised females occur and hence, that the population is endangered. In the yellow coloured areas VDSI values are exactly 4.0 and in the green areas values lie below this critical level. Even without knowing the coast of northern Brittany, the hot spots of pollution can be identified quite easily: the Bay of Carantec with its ports for ferry boats and ocean-going vessels and the small fishing harbour at Mogueriec.

In Northern Brittany, the investigated sites have been monitored since 1989. Again, the biomonitor was the dogwhelk *Nucella lapillus*, and the imposex index was the biomarker. Four sites in this region were monitored: Roscoff harbour, the Ile verte, Beg an Fry and Méan Mélen. Since about 1991, a slightly decreasing VDSI was observed, which is nevertheless still very high at Roscoff harbour and the Ile verte (results not shown). The site of Beg an Fry showed highly decreasing imposex intensities, also Méan Mélen, which has always been considered as a reference site.

Another biological effect monitoring with the dogwhelk *Nucella lapillus* on the effects of TBT was carried out in Ireland in 1993. Fig. 10 shows the respective VDSI in different categories of sampling sites: reference sites, salmon farms, marinas and shipping ports. Highest imposex intensities were found for shipping ports. These values are also above the threshold value for the induction of sterility in this snail and hence, at these sites, populations of *Nucella* are highly endangered. Reference sites showed a VDSI of about 1, but salmon farms and marinas also indicated high VDSI values. The imposex intensities are already near the sterility threshold, but considering the respective standard deviation, a serious threat for *Nucella* populations at these sites has to be expected.

An additional biological effect monitoring on the effects

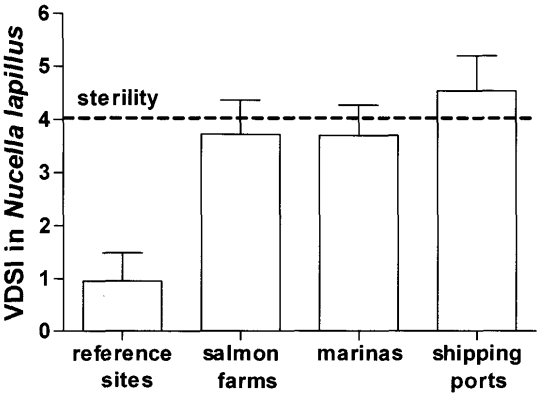

Fig. 10. Biomonitoring with the dogwhelk *Nucella lapillus* in Ireland. Imposex intensities (mean with standard deviation).

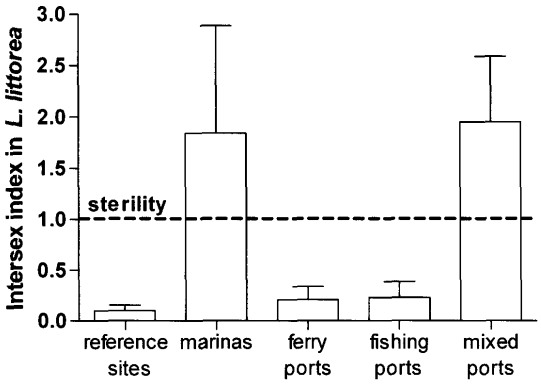

Fig. 11. Biomonitoring with the periwinkle *Littorina littorea* in Germany. Intersex intensities (mean with standard deviation).

of TBT was carried out in Germany in 1994/1995. Hereby, the intersex index in the periwinkle *Littorina littorea* was investigated. Fig. 11 shows the respective ISI of different sampling sites which were comprised into the following categories: reference sites, marinas, ferry ports, fishing ports and mixed ports.

Highest intersex intensities were found for marinas and mixed ports. These values are also well above the threshold value for the induction of sterility in this snail. At these sites, populations of *Littorina* are highly endangered. Reference site values were near 0, and ferry and fishing ports also showed low intersex intensities.

Biomonitoring with *Nassarius reticulatus* in Germany

During the last years, a biomonitoring with *Nassarius reticulatus* was conducted on sediments of the rivers Rhine, Main, Neisse, Weser and Elbe in Germany (Schulte-Oehlmann et al. 2001, Tillmann 2004). As an example, the Elbe results are shown here. Along the extension of the Elbe in Germany, 29 sampling sites have been analysed. Site 1 is situated directly at the border between the Czech Republic and Germany, site 29 in the Elbe estuary and already influenced by the North Sea.

Fig. 12 denotes the VDSI increase, hence the androgenic

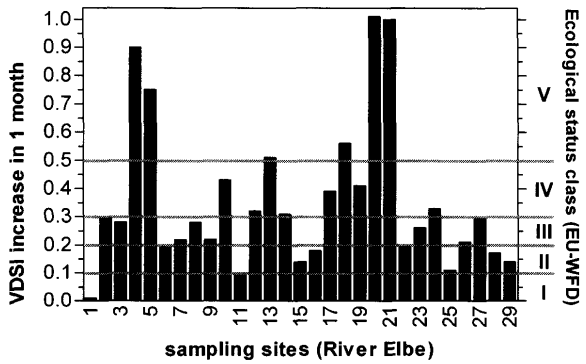

Fig. 12. Biomonitoring with the netted whelk *Nassarius reticulatus* in the river Elbe, Germany. Imposex development (increase of the VDSI vas deferens sequence index after 4 weeks exposure). Ecological status classes I-V according to the European Water Framework Directive.

activity, and also the respective ecological status class of the sampling sites according to the new European Water Framework Directive. Obviously, there is only one sediment (site 1) which is categorized into class 1 and even in class 2, there are only 8 sediments. Two hot spots can be identified: the most drastic effects—considering that the maximum possible VDSI increase is 1.0 in 1 month—were found at station 4 and around station 20. At the first highly polluted point, number 4, the river Mulde is joining the Elbe. In former times, before the German reunification, the Mulde received waste water from the greatest organotin producing plant in the former German Democratic Republic. The influence of this plant is still present today and can be found more than 50 km downstream the Elbe. The second hot spot is the Hamburg harbour, but even between the Mulde and Hamburg local point sources are visible.

Interestingly, the androgenic response in eight of the tested 29 sediments was so marked that it could not be explained by the analytically determined residues of TBT compounds in the sediments, so that additional and up to now unidentified androgenic compounds might have been present, contributing to the observed effects.

Biomonitoring with *Potamopyrgus antipodarum* in Germany

The same river system was also tested with the new sediment biotest with the mudsnail *Potamopyrgus antipodarum* (Duft 2004, Schulte-Oehlmann et al. 2001). With this test, a total of 51 sampling sites were investigated. Fig. 13 shows the inhibition of the total embryo number in % of the control and also denotes the ecological status classes I to V of the stations, according to the new European Water Framework Directive. Obviously, there are only two sediments in class 1 and in class 2, there are only 10 sediments. The highest effects were found around the region of Magdeburg, an area characterised by heavy industrial activity, and further downstream in the Hamburg harbour area.

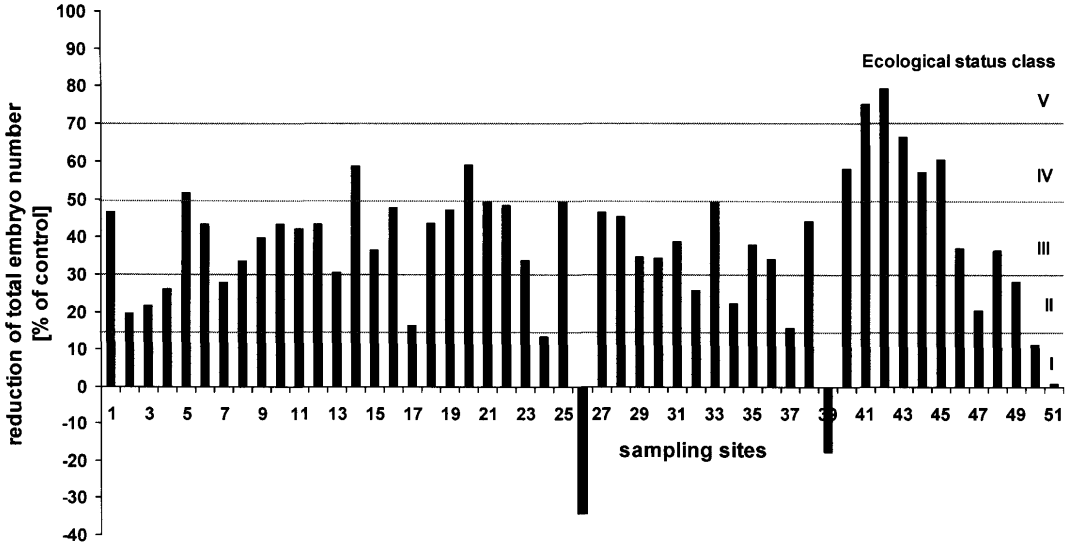

Fig. 13. Biomonitoring with the freshwater mudsnail *Potamopyrgus antipodarum* in the river Elbe, Germany. Inhibition of the embryo production after 4 weeks exposure in comparison to the control sediment. Ecological status classes I-V according to the European Water Framework Directive.

Interestingly, there are two sites where a significant increase of the embryo number was observed. This might be due to estrogenic compounds in the sediment.

It has to be added that not only organotin compounds may be responsible for the observed effects, but also other substances with a general reproductive toxicity, like heavy metals or PAHs—the observed effect is so to say a "net effect".

Discussion

Effects of triphenyltin

The reported results are in good accordance with other studies showing severe adverse effects of TPT on various organisms. It is difficult to compare the results of exposure to TPT via sediment with other results, as there are few studies in the literature. Some experiments were performed with water exposure, mostly using standard test organisms. The lowest reported effect concentrations are 200 ng TPT-Sn/L for algae (EC50 growth inhibition, Walsh et al. 1985), <68 ng TPT-Sn/L for water fleas (no observed effect concentration (NOEC) reproduction, Federoff et al. 1999) and 34 ng TPT-Sn/L for marine invertebrates (LOEC inhibition of arm regeneration in *Ophioderma*, Walsh et al. 1986). Imposex was developed after injection of TPT in the rock shell *Thais clavigera* at 0.1 μg/kg body weight (Horiguchi et al. 1997). For freshwater vertebrates, the following effect concentrations were found: 0.5 μg TPTAc/L (LOEC) delayed hatching in the zebra fish *Danio rerio* (Strmac and Braunbeck 1999) and 0.23 μg TPTOH/L (LOEC) reduced growth in the fathead minnow *Pimephales promelas* (Jarvinen et al. 1988).

The most sensitive parameter in our sediment experiments with *P. antipodarum* was the number of unshelled embryos. The EC10 for this endpoint was 0.03 μg TPT-Sn/kg, equivalent to 0.09 μg TPT/kg. This value (with no safety factor for risk assessment included) is already more than 70 times lower than the limit value for freshwater sediments, as proposed by the Netherlands in 2000 (Crommentuijn et al. 2000). For sediments, limit values of 6.4 μg/kg for freshwater and 1 μg/kg for marine ecosystems were proposed. In addition, they suggested a limit value of 5 ng/L for freshwater and 0.78 ng/L for seawater.

Compared to these values, actual environmental TPT concentrations in German river sediments are well above our EC50 and range from 55 μg/kg (river Neckar, 1988) to several hundreds of μg/kg: 112 μg/kg in the river Weser and 220 μg/kg in the rivers Elbe and Rhine (1988). Highest concentrations of TPT in freshwater sediments were 309 μg TPT-Sn/kg in Lake Geneva sediments (Becker et al. 1992). In sewage sludge in Switzerland, concentrations of up to 3400 μg TPT/kg (dry weight) were found (Becker-van Slooten and Tarradellas 1995). 1860 μg TPT-Sn/kg was the maximum concentration measured in marine sediments (Becker et al. 1992). In marine harbour sediments, areas characterised by intensive shipping activity, even higher concentrations were measured, the highest being 5500 μg TPT/kg (dry weight) in the Antwerp harbour (Ceulemans et al. 1998). Predicted environmental concentrations (PEC), derived from measurements of TPT residues in biota and the known bioconcentration factors, range from 46 to 216 ng TPT-Sn/L for freshwater and from 9.2 to 56 ng TPT-Sn/L for marine and coastal ecosystems, respectively (Oehlmann 2000). There are no data available of TPT concentrations in small surface waters from regions characterised by intensive agriculture, where it is probable that high TPT concentrations occur.

It is apparent that the LOEC and EC10 values for most investigated parameters in the various snail species are considerably lower than the actual and predicted environmental concentrations, indicating that these and other prosobranch populations are endangered by the extensive utilisation of TPT compounds as broad-spectrum agricultural fungicides. In 2000, the issue of TPT as antifouling paint on ships was prohibited, yet only for ships below 25 m length. TPT, as fentin hydroxide, was phased out since 1991, whereas the production and use of fentin acetate was still allowed. In Germany, the permission was revoked in March 2000. In spring 2001, the Federal Biological Research Centre for Agriculture and Forestry (BBA) withdrew this decision and thereby reapproved the application of TPT as fungicide in Brestan®. In August 2001, this decision was again revoked, which subsequently led to a phasing out of the use of Brestan® in Germany. However, on the level of the European Union there is currently a risk assessment of TPT going on. The outcome of this assessment and the decision of this committee, with the United Kingdom as responsible state, will determine the further use of TPT in Europe.

Effects of tributyltin

Analogous to TPT, it is difficult to relate our work to other studies, as experiments in sediments have rarely been performed (Meador 2000). This deficit is most obvious for freshwater sediments and freshwater organisms. For marine organisms, sediment concentrations of 100 to 1000 µg/kg had severe effects on polychaetes (Meador 2000) and clams (Fent and Hunn 1995). A study on the effects of TBT on *Potamopyrgus antipodarum* during water exposure also showed decreases in the number of embryos and in the extension of the brood pouch for concentrations of 50, 100 and 200 ng-Sn/L, while 400 ng-Sn/L resulted in acute toxicity after 4 months (Schulte-Oehlmann 1997).

Similar to TPT, the most sensitive parameter in our experiments was the number of unshelled embryos. The EC10 for this endpoint after 4 weeks exposure was 0.98 µg TBT-Sn/kg, equivalent to 2.39 µg TBT/kg. The effect concentration after 8 weeks exposure was slightly higher (2.98 µg TBT-Sn/kg) which was unexpected. However, for the total number of embryos, the effect concentrations were lower after 8 weeks than after 4 weeks, which is probably due to the fact that the embryos present in the brood pouch at the beginning of the experiment have grown up in the presence of TBT. The Netherlands proposed a limit value for freshwater sediment (dredged material) of 0.6 µg/kg (Krebs and Nehring 1997). Our measured effect concentration for the most sensitive parameter is already in the range of this limit value (with no safety factor for risk assessment included) and more than 5 times lower than the proposed limit value of the ARGE Elbe (Arbeitsgemeinschaft für die Reinhaltung der Elbe 1999) of 5 µg/kg.

In most European countries, the use of TBT is restricted for paints on small boats (below 25 m) and in autumn 2001, the IMO (International Maritime Organization) has proposed a total ban of TBT-based antifouling paints by January 2003 and their presence on ship hulls by January 2008. However, TBT is still ubiquitous: actual environmental concentrations in the German river Elbe range from 21 to over 200 µg TBT-Sn/kg with peaks of 3920 µg-Sn/kg in harbour sediments in 1997 (Arbeitsgemeinschaft für die Reinhaltung der Elbe 1999). In Swiss marina sediments, up to 838 µg TBT-Sn/kg were measured (Fent and Hunn 1995) and in some German marinas, contents between 54000 and 340000 µg TBT-Sn/kg were found (Arbeitsgemeinschaft für die Reinhaltung der Elbe 1999). Recently, in marine harbour sediments in Fiji, maximum concentrations of 360000 µg TBT/kg (i.e. 360 mg TBT/kg) were measured (Maata and Koshy 2001).

Conclusion

The cases of TBT and TPT and their effects in freshwater and marine mollusks provide a number of important insights, which are valuable for the endocrine disruption issue in general. First, it demonstrates that such endocrine disrupting chemicals may impact different levels of biological integration from molecules to communities, affecting also the viability of populations in the field. Second, it indicates that androgenic compounds can have considerable deleterious impact on wildlife and finally, it provides evidence that vertebrate-type steroids play an important functional role in prosobranchs.

From our investigations, we learned that a variety of benthic mollusks is strongly affected by low environmentally relevant concentrations of organotin compounds in aquatic systems, including sediments. The tested snail species provide striking examples for endocrine-mediated effects of environmental chemicals in laboratory experiments. Furthermore, extensive surveys of coastal, estuarine and freshwater sediments in France, Germany and Ireland indicate that there is still a threat for sensitive marine organisms—despite the ban on TBT. Finally, due to the persistence of organotin compounds in the environment, a continuing negative impact on aquatic wildlife has to be expected.

It can be concluded that the developed sediment biotests represent promising systems for the identification of endocrine disrupting substances and, as shown in recent studies (Duft et al. 2002, Duft et al. 2003a, b, Duft 2004, Tillmann 2004), offer the more general possibility as a tool for the assessment of sediment quality.

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