TOXICITY OF TRIPHENYL Tin AND TRIBUTYL Tin TO THE FRESHWATER MUDSNAIL
POTAMOPYRGUS ANTIPODARUM IN A NEW SEDIMENT BIOTEST

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(Received 9 January 2002; Accepted 19 June 2002)

Abstract—The effects of two suspected endocrine-disrupting chemicals, the xeno-androgens triphenyltin (TPT) and tributyltin (TBT), were investigated in a new whole-sediment biotest with the freshwater mudsnail Potamopyrgus antipodarum (Gastropoda, Prosobranchia). Artificial sediments were spiked with seven concentrations, ranging from 10 to 500 µg nominal TPT-Sn/kg dry weight and TBT-Sn/kg dry weight, respectively. We analyzed the responses of the test species after two, four, and eight weeks exposure. For both compounds, P. antipodarum exhibited a sharp decline in the number of embryos sheltered in its brood pouch in a time- and concentration-dependent manner in comparison to the control sediment. The number of new, still unshelled embryos turned out to be the most sensitive parameter. The lowest-observed-effect concentration (LOEC) was equivalent to the lowest administered concentration (10 µg/kg of each test compound) for most parameters and thus no no-observed-effect concentration (NOEC) could be established. The calculation of effect concentrations (EC10) resulted in even lower values for both substances (EC10 after eight weeks for unshelled embryos: 0.03 µg TPT-Sn/kg; EC10 after four weeks for unshelled embryos: 0.98 µg TBT-Sn/kg). Our results indicate that P. antipodarum is highly sensitive to both endocrine disruptors TPT and TBT at environmentally relevant concentrations.

Keywords—Triphenyltin Tributyltin Sediment Biotest Potamopyrgus antipodarum

INTRODUCTION

Recently, a variety of xenobiotics in the environment has been shown to induce adverse effects in animals and humans by interfering with their endocrine functions with different modes of action. These so-called endocrine disruptors, endocrine modulators or hormone-mimetic substances, have been associated with a decrease in sperm counts in men [1], increased frequencies of sex hormone-dependent forms of cancer (breast, testis, prostate, and so on), genital abnormalities, premature puberty in females, and increased occurrence of endometriosis in humans [2]. 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 [3] and received widespread attention, triphenyltin (TPT) is 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 [4,5].

Since the early 1960s, TPT compounds have been used as broad-spectrum fungicides in agriculture, mainly as triphenyltinhydroxide (fentinhydroxide) and triphenyltinacetate in Brestan®, Brestanid® (Aventis Crop Science, Frankfurt/Main, Germany), and Du-Te® (BASF, Limburgerhof, Germany), to combat a range of fungal diseases in various crops, particularly potato blight (Phytophthora infestans), leaf spot (Cercospora beticola, Ramularia beticola), and powdery mildew (Erysiphe betae) on celery, peanuts, and sugar beet; Pseudoperonospora humuli on hop; gray molds on onions; rice blast; brown rust on beans; and coffee leaf rust. In addition, TPT compounds are used in certain antifouling paints on ships, mainly in combination with tributyltin [6]. The annual world production of triphenyltin compounds is unknown. In Germany it is estimated close to 1,000 t, while the world consumption of triphenyltin compounds in fungicides is estimated to be several thousand tons per year [7]. Lately, because of recent studies, the U.S. Environmental Protection Agency stated that TPT may cause endocrine disruption also in vertebrates [8] (http://www.epa.gov/REDs/supportdocs/0099hed.pdf).

Likewise, since the 1960s, TBT has been used as a major component in antifouling paints for ships. This compound is known to be harmful to many, also nontarget aquatic organisms, particularly mollusks [9]. Because TBT tends to accumulate in sediments, which are considered to be sink for TBT, they may become a source and still will be in the future. Furthermore, TBT is used in some wood protective coatings in the building industry and may also occur as a by-product of mono- and dibutyltin compounds, which are used as stabilizers for plastics. The annual production of tributyltinhydroxid in Germany is estimated to be about 2,000 t (70% for antifouling paints, 20% for timber protection, 10% for protection of textile and leather [10]). The total annual world production of organotins is about 50,000 tons [10]. The TPT and TBT compounds are both considered in the Priority Lists of Action of the European Commission [11] and of the International Rhine Commission [12].

Organotin compounds with small alkyl chains decay generally only at a snail’s pace in the environment. According to Fedoroff et al. [13] (http://www.epa.gov/oppsrrd1/REDS/
supportdocs/0099efed.pdf), TPT is resistant to photodegradation and hydrolysis, showing half-lives of 93 to 111 d in irradiated water samples and 155 d in dark control. The half-life of TBT in sediments is estimated to be months to several years [14]. Both substances are considered to be rather persistent and accumulate in the sediment of aquatic systems and especially in organisms [6,10]. Their low solubility in water (1.2–8 mg/L [13]) and high $K_{ow}$ value (5,700 ml/g for TPT and 5,500 ml/g for TBT) suggest adsorption onto suspended particles and sediments. These organotin compounds reveal a substantial biocumulation potential with log $K_{ow}$ values of 4.1 for TPT [15] and 4.4 for TBT [16]. They accumulate in sediments with bioconcentration factors of $2 \times 10^3$ to $4.4 \times 10^5$ in crabs [18] and $8 \times 10^3$ to $4.4 \times 10^5$ in crabs [18] for TPT and bioconcentration factors between $1.5 \times 10^3$ up to $3 \times 10^5$ for TBT in marine mussels and oysters [19,20] and in freshwater bivalves between $8.3 \times 10^3$ up to $4.5 \times 10^5$ [21]. High concentrations of TPT and TBT were measured in fish, especially in liver, heart, and brain, suggesting that those organotins are able to pass the blood–brain barrier [22].

Therefore, the development of a whole-sediment biotest, especially for the freshwater environment, using a sediment-dwelling organism as test species, is essential to obtain information on effects in the sediment compartment, the so-called memory of the water. Recent studies recommend mollusks and particularly snails as most sensitive organisms concerning effects of endocrine disrupters in invertebrates [23,24].

**MATERIALS AND METHODS**

Our test species, 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 *Potamopyrgus jenkinsi* [25]. In contrast to New Zealand populations, European populations consist almost exclusively of females, and males are seldom found [26]. *Potamopyrgus 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. *Potamopyrgus antipodarum* lives in freshwater environments but—being a euryhaline species—also in some regions of the Baltic Sea and in estuaries of the North Sea and Atlantic in coexistence with other hydrobiid (mudsnail) species.

For all experiments, 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 an artificial freshwater (0.5 g NaHCO$_3$, 5 g CaCO$_3$, and 5 g mineral salt per 10 L demineralized 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 previously. 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 ensured optimal embryo production of *Potamopyrgus* compared to other artificial and even natural sediments (M. Duft, International Graduate School Zittau, Zittau, Germany, unpublished results). The organic carbon content of the artificial sediment was 2.3%, and the mean grain size was 180 µm. To each flask, 50 g of artificial sediment (dry wt) were added. For the spiking procedure, 2 ml of the respective concentration of TPT and TBT (dissolved in 100% ethanol) were applied to each treatment and homogenized by stirring. One-day evaporation guaranteed removal of the solvent. One liter 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 d 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, >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 control and a solvent control was included in each experiment. At the end of the experiment, after eight weeks, the applied nominal concentrations in the sediments were checked analytically according to Arnold [27]. For the analysis, approximately 2 g of the respective freeze-dried sediment samples were extracted with 3% acetic acid in methanol with five static cycles of 5 min at 100°C and 1,500 psi using an accelerated solvent extractor (ASE 200, Dionex, Sunnyvale, CA, USA). The extracted organotin compounds were derivatized with 4% NaBEt$_4$ in H$_2$O, concentrated into cyclohexane by liquid–liquid extraction, and subsequently analyzed with gas chromatography tandem mass spectrometry (GC: CP 3800, MS: Saturn 2000, Varian, Walnut Creek, CA, USA). Monohexyltinrichloride and tripropyltinchloride solutions (in methanol) served as internal standards. For the calibration, seven concentrations of each compound (monophenyltin [MPT], diphenyltin [DPT], triphenyltin [TPT] and monobutyltin [MBT], dibutyltin [DBT], tributyltin [TBT]) corresponding to concentrations between 4 and 200 µg Sn/kg were analyzed. The PACS-2 reference material (National Research Council Canada, Ottawa, ON) was analyzed to check extraction efficiency, showing recoveries of 95 to 100%. The detection limit for the analyses was approximately 2 µg Sn/kg for each compound. Results are shown in Table 1.

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. Twenty snails were analyzed individually after zero, two, four, and eight weeks, respectively. Prior to analysis, the snails were narcotized in MgCl$_2$ (2.5% in distilled water) for 2 h. Shell and aperture heights were measured,
Table 1. Nominal concentrations (treatments) applied to the test vessels (in μg TPT-Sn/kg or TBT-Sn/kg, respectively) and analytically measured concentrations (in μg-Sn-kg dry wt) of mono- (MPT), di- (DPT), and triphenyltin (TPT) and mono- (MBT), di- (DBT), and tributyltin (TBT) in the spiked sediment and the solvent control (SC) after eight weeks of exposure, absolute and in percentage of nominal concentration (recovery)

<table>
<thead>
<tr>
<th>Compound</th>
<th>SC</th>
<th>10</th>
<th>25</th>
<th>50</th>
<th>75</th>
<th>125</th>
<th>250</th>
<th>500</th>
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<td>% Recovery</td>
<td>—</td>
<td>135</td>
<td>105</td>
<td>82</td>
<td>78</td>
<td>90</td>
<td>85</td>
<td>82</td>
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RESULTS

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

Effects of TPT

For TPT, a decline of embryos was observed already after four weeks of exposure (Fig. 3a). The production of new,
Table 2. Comparison of effect concentrations (EC10 and EC50, with 95% confidence interval [CI]) for triphenyl- (TPT) and tributyltin (TBT) in μg TBT/TPT-Sn/kg for the analyzed endpoints: new embryos without shell and total embryo numbers after four and eight weeks

<table>
<thead>
<tr>
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<th>Embryos without shell</th>
<th>Total embryos</th>
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<tr>
<td></td>
<td>Four weeks</td>
<td>Eight weeks</td>
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<tr>
<td>TPT</td>
<td>EC10</td>
<td>NC&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(95% CI)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>(0–1.80)</td>
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<tr>
<td>TBT</td>
<td>EC10</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>(95% CI)</td>
<td>(15.4–76.2)</td>
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<tr>
<td></td>
<td>EC50</td>
<td>45.8</td>
</tr>
<tr>
<td></td>
<td>(95% CI)</td>
<td>(23.6–38.7)</td>
</tr>
</tbody>
</table>

<sup>a</sup> NC = not calculable.  
<sup>b</sup> CI = confidence interval.

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 that 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. 3a). 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 shedded embryos after four weeks was not affected (ANOVA, \( p < 0.05 \)) and is therefore not shown in the figures. Likewise, after an exposure of two weeks only, no effects were observed.

After eight weeks, the decline of the embryo production was most conspicuous (Fig. 3b): The number of unshelled embryos was significantly lower in all tested concentrations of TPT compared to the solvent control sediment (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, and the EC10 was 0.03 μg TPT-Sn/kg (Table 2). The TPT inhibited the embryo production down to 25% and less in most exposure groups. A sharp and significant (ANOVA, \( p < 0.01 \)) decrease was also noted in the total number of embryos (Fig. 3b), and the corresponding EC50 was 23.6 μg TPT-Sn/kg (Table 2). The solvent, ethanol, had no effect on embryo production (ANOVA, \( p > 0.05 \)). The embryo number in the solvent control was again the lowest applied concentration (10 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 midrange applications, whereas the treatments with the highest concentrations exhibited embryo numbers near zero. A calculation of the EC10 resulted in a value of 10.6 μg TBT-Sn/kg (EC50 173 μg/kg; Table 2).

After eight weeks, the calculated EC50 for the production of new, unshelled embryos was 64.0 μg TBT-Sn/kg, and the respective EC10 was 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. 5b). 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 four weeks exposure (Fig. 6b). A typical concentration–response curve was noticed, whereas for the total embryo production another response was observed: a decrease of embryos at the lowest as well as the highest applied concentrations. The total number of embryos after eight weeks comprises grown-up embryos (with a shell) that have grown in the presence of TBT, thus describing a midscale effect. Compared to the results after four 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 sediment (ANOVA, \( p < 0.001 \)).

Table 2 gives a comparison of calculated effect concentrations of most of the investigated parameters in the test. It is 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 midrange applications, whereas the treatments with the highest concentrations exhibited embryo numbers near zero. A calculation of the EC10 resulted in a value of 10.6 μg TBT-Sn/kg (EC50 173 μg/kg; Table 2).

Fig. 4. Development of embryo number in the brood pouch of Potamopyrgus antipodarum in solvent control sediment during the course of the triphenyltin experiment (open symbols represent unshelled, filled symbols represent total embryo number, mean ± standard error of the mean, \( n = 20 \)). The dotted lines denote a linear regression; the slope is not significantly different from zero.
Sediment toxicity of TPT and TBT to *Potamopyrgus antipodarum*

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**Fig. 5.** Effects of tributyltin (TBT) concentrations in the sediment (µg TBT-Sn/kg) on the embryo production (without shell and total) of *Potamopyrgus antipodarum* in percentage of the solvent control (mean ± standard error of the mean [SEM], n = 20). (a) After four weeks of exposure, (b) after eight weeks of exposure. Regression lines (Eqn. 1) are added—solid line for the total embryo number (four weeks $r^2 = 0.46$, eight weeks $r^2 = 0.64$), dotted line for the unshelled embryo number (four weeks $r^2 = 0.64$, eight weeks $r^2 = 0.91$).

**Fig. 6.** Effects of tributyltin (TBT) concentrations in the sediment (µg TBT-Sn/kg) on survival of adult *Potamopyrgus antipodarum* in percentage and lethal concentration for 50% of the test animals (LC50). (a) After four weeks of exposure, (b) after eight weeks of exposure. Regression lines (Eqn. 1) are added (four weeks $r^2 = 0.99$, eight weeks $r^2 = 0.98$).

obvious that the number of embryos without shell was the most sensitive parameter for both tested compounds. The number of embryos with shell, which is reflecting effects on already developed embryos, was also affected, being visible after eight weeks.

The chemical analysis of phenyltin (butyltin) compounds in the spiked sediments after the total exposure period of eight weeks showed recoveries of 43% (for nominal 50 µg/kg) to 170% (for nominal 10 µg/kg) for TPT and recoveries of 78% (for nominal 75 µg/kg) to 135% (for nominal 10 µg/kg) for TBT. In Table 1, the analytically determined concentrations of TPT and TBT, plus their degradation products DPT, MPT, DBT, and MBT, are given, as it is probable that the applied chemicals are being decomposed during the exposure time of eight weeks. While for TBT most of the nominally applied contents were still present as TBT and only a small amount has been degraded into MBT and DBT, the results for TPT are different. After eight weeks, TPT has obviously degraded into MPT and DPT, and with few exceptions (75 and 125 µg/kg), TPT was not detected in the samples.

**DISCUSSION**

The results of the analytical determination of the remaining phenyltin/butyltin concentrations in the sediment after eight weeks showed that the total concentration of phenyltin/butyltin with few exceptions (50 and 250 µg TBT-Sn/kg) remained approximately the same. While only little of the TBT content in the sediments was degraded into MBT and DBT during the course of the experiment, the composition in the TPT experimental groups changed, and part of the applied TPT has transformed into the degradation products MPT and DPT. It remains speculative whether the shown effects were caused by TPT and TBT during the first stages of the experiments or additionally by degradation products (MPT, DPT; MBT, DBT) during the course of the experiment or by a combination of both. Nevertheless, it is obvious that the total phenyl/butyltin content of our sediments remained approximately constant during 56 d in aerated sediments at standard light regime. The degradation to mono- or diphenyl/butyltin could be due to light, oxygen, or microbial activity [10,13] but also to metabolism and subsequent elimination by the snails [29].

For the development of a biotest, it is necessary to use an artificial sediment in order to facilitate comparison with other substance tests and to standardize the test conditions. Furthermore, the availability of unpolluted natural sediment is limited. However, the use of artificial sediments has several drawbacks, as they are generally not aged and may display a different behavior than natural sediments in terms of surface adsorption and bioavailability of test substances. As bioavailability is a function of the chemical composition of the sediment and its abiotic parameters, the organic carbon content and grain size of our artificial sediment was deliberately adjusted to those found in natural sediments. Nevertheless, the bioavailability of TPT and TBT might have been highest at
the start of the experiment in our artificial sediments and probably decreased during the course of the experiment. However, this might have been compensated by degradation of the substances.

Effects of TPT

Our results are in good accordance with other studies showing severe adverse effects of TPT on various organisms. The freshwater ramshorn snail *Marisa cornuarietis* exhibited imposex in the presence of TPT (LOEC 75 ng TPT-Sn/L, EC10 49 ng TPT-Sn/L) with a comparable intensity as for TBT, whereas for other marine gastropods (*Nucella lapillus* and *Hinia reticulata*), no imposex development was observed [9,30]. Furthermore, fecundity of *M. cornuarietis* was reduced, and evidence for impairment of spermatogenesis and oogenesis as well as for a carcinogenic potential of TPT in the marine species was found. It is difficult to compare our results of exposure to TPT via sediment with other results, as few studies exist 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 [31]), below 68 ng TPT-Sn/L for water fleas (NOEC reproduction [13]), and 34 ng TPT-Sn/L for marine invertebrates (LOEC inhibition of arm regeneration in *Ophioderma* [32]). Impospx was developed after injection of TPT in the rock shell *Thais clavigera* at 0.1 μg/kg body weight [9]. For freshwater vertebrates, the following effect concentrations were found: 0.5 μg TPTAc/L (LOEC) delayed hatching in the zebra fish *Danio rerio* [33] and 0.23 μg TPTOH/L (LOEC) reduced growth in the fathead minnow *Pimephales promelas* [34].

The most sensitive parameter in our 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 [35]. 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, Rotenburg, Germany, 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). The highest concentrations of TPT in freshwater sediments were 309 μg TPT-Sn/kg in Lake Geneva sediments [21]. In sewage sludge in Switzerland, concentrations up to 3,400 μg TPT/kg (dry wt) were found [36]: 1,860 μg TPT-Sn/kg was the maximum concentration measured in marine sediments [21]. In marine harbors, areas characterized by intensive shipping activity, even higher concentrations were measured, the highest being 5,500 μg/kg (dry wt) in the Antwerp harbor [37]. Predicted environmental concentrations, derived from tissue residue measurements of TPT in biota and the known bioconcentration factors, range from 46 to 216 ng TPT-Sn/L for freshwater and 9.2 to 56 ng TPT-Sn/L for marine and coastal ecosystems, respectively [5]. No data are available of TPT concentrations in small surface waters from regions characterized by intensive agriculture, where it is probable that high TPT concentrations occur.

It is apparent that the LOEC and EC10 values for the decrease of embryo production in *P. antipodarum* (like in other mollusks [30]) are considerably lower than the actual and predicted environmental concentrations, indicating that the freshwater mudsnail and further prosobranch populations are endangered by the extensive utilization 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. The permission of TPT as fentin hydroxide was phased out in 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 withdrew this decision and thereby reapproved the application of TPT as fungicide in Brestan (Aventis CropScience, Hattersheim, Germany). 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, a risk assessment of TPT is currently ongoing. The outcome of this assessment and the decision of this committee, with the United Kingdom as the responsible state, will determine the further use of TPT in Europe.

Effects of TBT

Analogous to TPT, it is difficult to relate our effects to other studies, as experiments in sediments have rarely been performed [38]. This deficit is most obvious for freshwater sediments and freshwater organisms. For marine organisms, sediment concentrations of 100 to 1,000 μg/kg had severe effects on polychaetes [38] and clams [39]. A study on the effects of TBT on *P. 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 four months [29].

Similar to TPT, the most sensitive parameter in our experiments was the number of unshelled embryos. The EC10 for this endpoint after exposure of four weeks was 0.98 μg TBT-Sn/kg, equivalent to 2.39 TBT/kg. The effect concentration after eight 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 eight weeks than after four weeks, which is probably due to the embryos present in the brood pouch at the beginning of the experiment that have grown up in the presence of the toxicant. The Netherlands proposed a limit value for freshwater sediment (dredged material) of 0.6 μg/kg [40]. 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 five times lower than the proposed limit value of the ARGE Elbe (Arbeitsgemeinschaft für die Reinhaltung der Elbe) [41] 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 International Maritime Organization (London, UK) 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 3,920 μg-Sn/kg in harbor sediments in 1997 [41]. In Swiss marina sediments, up to 838 μg TBT-Sn/kg were measured [39], and in some German marinas, contents between 54,000 and 340,000 μg TBT-Sn/kg were found [41].
CONCLUSION

Evaluating the results for both tested substances TPT and TBT, we can conclude that the chronic effects on *P. antipodarum* and their intensity are comparable and in good accordance with the experiments of Horiguchi et al. [9] and Schulte-Oehlmann et al. [30]. The reproductive performance is more affected in the presence of TPT, where we find sharper declines of the embryo numbers and lower effect concentrations (EC10 and EC50) than under TBT exposure. Acute toxicity occurs at an exposure to TBT but not to TPT.

Our results show that *P. antipodarum* is highly sensitive to the organotin compounds TPT and TBT in the sediment in environmentally relevant concentrations. Finally, it can be concluded that the sediment biotest with *P. antipodarum* represents a promising system for the identification of endocrine-disrupting substances and, as shown in recent studies [42], offers the more general possibility as a tool for the assessment of sediment quality.

Acknowledgement—The authors are grateful to S. Ziebart, U. Schneidner, and M. Goth for technical and snail assistance in the laboratory; L. Weltje and two anonymous reviewers for helpful comments; and A. Laufer and J. van Doornmalen for the TBT and TPT analyses. Parts of the research were funded by the German Federal Environmental Agency.

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