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Impact of triphenyltin acetate in microcosms simulating floodplain lakes. II. Comparison of species sensitivity distributions between laboratory and semi-field

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Abstract The study objectives were to shed light on the types of freshwater organism that are sensitive to triphenyltin acetate (TPT) and to compare the laboratory and microcosm sensitivities of the invertebrate community. The responses of a wide array of freshwater taxa (including invertebrates, phytoplankton and macrophytes) from acute laboratory Single Species Tests (SST) were compared with the concentration–response relationships of aquatic populations in two types of freshwater microcosms. Representatives of several taxonomic groups of invertebrates, and several phytoplankton and vascular plant species proved to be sensitive to TPT, illustrating its diverse modes of toxic action. Statistically calculated ecological risk thresholds (HC_5 values) based on 96 h laboratory EC_{50} values for invertebrates were 1.3 μg/l, while these values on the basis of microcosm-Species Sensitivity Distributions (SSD) for invertebrates in sampling weeks 2–8 after TPT treatment ranged from 0.2 to 0.6 μg/l based on nominal peak concentrations. Responses observed in the microcosms did not differ between system types and sampling dates, indicating that ecological threshold levels are not affected by different community structures including taxa sensitive to TPT. The laboratory-derived invertebrate SSD curve was less sensitive than the curves from the microcosms. Possible explanations for the more sensitive field response are delayed effects and/or additional chronic exposure via the food chain in the microcosms.

Keywords Species Sensitivity Distibution · Laboratory vs. Semi-field · Triphenyltin acetate

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Introduction

Relatively few published studies have dealt with ecological risks of fungicides to freshwater communities (Cuppen et al. 2000). Although several studies on the fate and effects of fungicides in aquatic ecosystems have recently been published (Farrel et al. 1998; Van Wijngaarden et al. 1998; Cuppen et al. 2000; Koelmans et al. 2000; Van den Brink et al. 2000, 2002) our knowledge of the ecological impact of fungicides is still limited.

Several of the fungicides studied to date appear to have biocidal properties, and the fact that some of these compounds may also exhibit endocrine-disrupting abilities has certainly drawn attention to this group. An example of this group are the organotins (Schulte-Oehlmann et al. 2000; Tillmann et al. 2001), which are amongst the more frequently studied biocides. The present study deals with the organotin compound triphenyltin acetate (TPT), a fungicide for which little adequate freshwater laboratory toxicity data and no field or semi-field toxicity data has been published.

Organotins, including TPT, are highly toxic to all sorts of aquatic primary producers, invertebartes and vertebrates (Fargasová 1998; Jak et al. 1998; Petersen and Gustavson 2000; Rehage et al. 2002). Organotins have been reported to inhibit mitochondrial oxidative phosphorylation and

consequently energy transfer, Ca^{2+} homeostasis, protein and DNA synthesis in the cell (Chandra et al. 1989; Girard et al. 1997; Tiano et al. 2003), to cause immunosuppression and premature apoptosis (programmed cell death) in both vertebrates and invertebrates (Stridh et al. 1999; Cima et al. 2002), and have photosynthesis inhibiting properties (Mooney and Patching 1995). This variety of fundamental processes are not immediately visible and may take time before effects can be observed. Comparative studies of the relative toxicity of organotin compounds to aquatic organisms like marine crab larvae (*Rhitropanopeus harrisii*) and the freshwater microcrustacean *Daphnia magna* have shown that TPT and TBT (antifouling) are amongst the most toxic of these (Laughlin and Linden 1985; Vighi and Calamari 1985).

The first objective of the present paper was to shed light on the types of freshwater organism that are sensitive to TPT. To this end, the acute effects of TPT on a wide array of freshwater taxa (including invertebrates, phytoplankton, and macrophytes) were tested in a laboratory setting by means of Single Species Tests (SST). Species vary markedly in their sensitivity to environmental contaminants, and this variation can be described by constructing a species sensitivity distribution (SSD). The SSD is a statistical distribution estimated from a sample of toxicity data and visualized as a cumulative distribution function (Posthuma et al. 2002). Species sensitivity distributions are used to calculate the concentration at which a specified proportion of species will be affected, referred to as the hazardous concentration (HC) for p (%) of species (HC_p) (Van Straalen and Denneman 1989). In this way SSDs can be used to assess the potential impact of substances on aquatic ecosystems via direct toxic effects.

The second objective of the present study was to test if aquatic invertebrates measured in the laboratory show the same concentration–response relationships as aquatic invertebrate populations in outdoor microcosms and to address the difficulties involved comparing the two. Since organotin compounds are reported to exert effects via foodchain exposure, is hypothesized that only in the field situation full effects can be observed. When comparing the field with the lab response the first is hypothesized to be more sensitive.

SSDs were constructed for different endpoints (e.g., macrophytes, invertebrates) and for different test systems (e.g., lab, field), compared, and statistically tested for differences. Also SSDs were used to estimate the hazardous concentrations to 5% (HC_5) of the species. This procedure allowed us to evaluate the predictive value of acute laboratory toxicity tests for field effects in the assessment of ecological hazards of a single application of the fungicide TPT in freshwater ecosystems. A detailed description of the semi-field experiment, which studied the ecological impact of a single application of TPT in outdoor microcosms, has been provided in part I (Roessink et al. 2006).

Materials and methods

SST in the laboratory

All tests were performed with Fentin acetate Pestanal (CAS No. 900-95-8; Sigma Aldrich Chemie BV, Zwijndrecht, The Netherlands), which was applied once at the start of each experiment. Nominal concentrations of TPT applied in the different tests are presented in Table 1. In all treatments, 0.04 % (v/v) 96% ethanol was used as a carrier solvent. Except for the algal tests, all experiments were performed with two controls, viz., a solvent control with an equal amount of ethanol (coded: 0^+) and a 'normal' control containing only test medium (coded: 0). For logistic reasons, the algal experiments were only performed with a solvent control. To assess initial exposure concentrations, water samples were taken 1 h after TPT application. In addition, intermediate treatment concentrations were measured at 0, 1, 2, 3, and 4 days after the TPT application in tests with *Endochironomus albipennis* and *Gammarus pulex*, to study the dynamics of this substance during the test. In other invertebrate tests, water samples were only taken on day 0 (1 h after application) and on day 4 (at the end of the test). In the macrophyte tests, which lasted 21 days, TPT concentrations were measured on days 0, 2, 7, 14 and 21. The small test volumes of the algal test flasks did not allow sampling for TPT concentration assessment. Unfortunately, it was impossible to use extra 'fate' flasks, which could be sacrificed for TPT sampling, so TPT exposure concentrations in the algal tests were estimated from measurements in the stock solutions.

For the chemical analysis of TPT, depth-integrated 100-ml water samples were taken out of the test units and stored in a 250 ml flask. To these water samples was added 2 ml buffer solution (pH=5; 120 g HAc+272 g NaAc per liter), 100 µl 2% sodium tetraethyl borate and 20 ml hexane. The water was extracted by shaking for 15 min at 175 rpm. Part of the hexane layer was removed and transferred to a GC vial. Organotin analysis was performed on a GC-MSD in Selective Ion Mode (GC: HP 6890 with auto-injector HP 7683; MSD: HP 5973 Network MSD). The detection limit of TPT in water was 1 µg/l. The recovery of the extraction procedure was tested by spiking blank water samples with a known amount of TPT in ethanol. The recovery was found to be 92.7% (n=4; sd=12.7%); because this was within the measuring error of the GC-MS, no corrections were made for this recovery.

Table 1 Species tested, with their concentration range

#	Species	RFT	SST	Concentration range (µg/l)
1	*Acanthocyclops venustus*	X	X	0, 0$^+$, 1.7, 5, 15, 45, 135
2	*Asellus aquaticus*	X	X	0, 0$^+$, 25, 65, 160, 400, 1000
3	*Bythinia tentaculata*	X	–[a]	0, 0$^+$, 10, 50, 200, 1000
4	*Chaoborus obscuripes*	X	–[a]	0, 0$^+$, 10, 50, 200, 1000
5	*Cloeon dipterum*	X	X	0, 0$^+$, 25, 50, 100, 200, 1000
6	*Daphnia galeata*	X	X	0, 0$^+$, 1.7, 5, 15, 45, 135
7	*Dugesia sp.*	X	X	0, 0$^+$, 2.7, 5.5, 11, 22, 44
8	*Endochironomus albipennis*	X	X	0, 0$^+$, 100, 200, 400, 800, 1600
9	*Erpobdella juv.*	X	X	0, 0$^+$, 4, 10, 25, 60, 150
10	*Gammarus pulex*	X	X	0, 0$^+$, 1.9, 4.8, 12, 30, 75
11	*Glyptotendipes sp.*	X	X	0, 0$^+$, 100, 200, 400, 800, 1600
12	*Lumbriculus variegatus*	X	X	0, 0$^+$, 4, 8, 16, 32, 64
13	*Lymnaea stagnalis*	X	X	0, 0$^+$, 15, 38, 95, 237.5, 594
14	*Physa fontinalis*	X	X	0, 0$^+$, 1.75, 4.4, 11, 28, 69
15	*Planorbis contortis*	X	X	0, 0$^+$, 1.75, 4.4, 11, 28, 69
16	*Polycelis niger/tenuis*	X	X	0, 0$^+$, 2.7, 5.5, 11, 22, 44
17	*Proasellus meridianus/coxalis*	X	X	0, 0$^+$, 2.7, 5.5, 11, 22, 44
18	*Sigara sp.*	X	–[a]	0, 0$^+$, 10, 50, 200, 1000
19	*Sphaerium sp.*	X	–[a]	0, 0$^+$, 10, 50, 200, 1000
20	*Tubifex*	X	X	0, 0$^+$, 4, 8, 16, 32, 64
21	*Desmodesmus subspicatus*	X	X	0$^+$, 0.3, 1, 3, 10, 30, 100
22	*Monoraphidium minutum*	X	X	0$^+$, 0.3, 1, 3, 10, 30, 100
23	*Scenedesmus quadricauda*	X	X	0$^+$, 0.3, 1, 3, 10, 30, 100
24	*Selenastrum capricornutum*	X	X	0$^+$, 0.3, 1, 3, 10, 30, 100
25	*Lemna minor*	–	X	0, 0$^+$, 1, 10, 30, 100, 1000
26	*Lemna trisulca*	–	X	0, 0$^+$, 1, 10, 30, 100, 1000
27	*Elodea nuttallii*	–	X	0, 0$^+$, 1, 10, 30, 100, 1000
28	*Elodea canadensis*	–	X	0, 0$^+$, 1, 10, 30, 100, 1000
29	*Potamogeton crispus*	–	X	0, 0$^+$, 1, 10, 30, 100, 1000
30	*Myriophyllum spicatum*	–	X	0, 0$^+$, 1, 10, 30, 100, 1000
31	*Ceratophyllum demersum*	–	X	0, 0$^+$, 1, 10, 30, 100, 1000
32	*Spirodela polyrhiza*	–	X	0, 0$^+$, 1, 10, 30, 100, 1000

The concentration ranges presented were used in Single Species Tests (SST); when no SST was performed, the range from the Range Finding Test (RFT) is presented. Triphenyltin acetate (TPT) range presented are initial nominal concentrations. 0=control; 0+=solvent control

[a]=No reaction to short-term TPT exposure

Macroinvertebrate SSTs were performed using two replicates per treatment level, and lasted 4 days (96 h). Most tests were performed in 1.8-l glass jars containing 1.5-l filtered (45 µM) nutrient-poor water originating from experimental ditches located at the Sinderhoeve field station (Table 2). Most macroinvertebrate tests involved twenty specimens per jar. The taxa *Endochironomus albipennis*, *Glyptotendipes sp.*, *Lumbriculus variegatus* and *Tubifex sp.*, however, were tested individually in 10-ml glass jars. This was necessary because affected specimens were cannibalized by less affected specimens when tested in the same jar (*Endochironomus* and *Glyptotendipes*) or because all specimens formed a tight ball which prevented accurate observation (*Lumbriculus* and *Tubifex*). The tests were done in a temperature-controlled room (20±2°C) with a 14 h light:10 h dark regime. The test media were not aerated during the tests. Within 4 h of dosing, dissolved oxygen concentrations (YSI model 58) and pH (WTW pH323, equipped with a Sentix pH electrode) were measured in all test units. In addition, DO and pH were measured daily at a fixed time in at least the controls and the treatments with highest concentrations (Table 2). As described in earlier experiments (Roessink et al. 2005) the test medium, obtained from our experimental ditches, had an average Dissolved Organic Carbon (DOC) concentration of 8.8 mg/l. Overnight mortality and excrements sometimes affected water quality parameters (e.g., lower range of DO or pH in *Lymnaea* and *Gammarus* test). However, remaining organisms survived till the end of the test period and did not suggest increased toxicity of TPT under these conditions.

Zooplankton tests used two replicates per treatment level and lasted 4 days (96 h). We used 600-ml glass jars containing 250-ml filtered (45 µm) nutrient-poor water originating from experimental ditches located at the Sinderhoeve field station (Table 2). Other test conditions were similar to the macroinvertebrate tests.

Macrophyte tests were performed in duplicate and lasted 21 days. They were conducted in 1.8-l glass jars containing 1.5-l filtered (45 µm) nutrient-poor water originating from experimental ditches located at the Sinderhoeve field station. In these tests, the water was additionally enriched with the inorganic nutrients N (0.5 mg/l), P (0.075 mg/l) and C (0.08 mg/l), as well as with 0.1 ml/l Tropica Mastergrow (K: 0.79, Mg: 0.39, S: 1.01, B: 0.004, Cu: 0.006 Fe: 0.07, Mn: 0.04 Mo: 0.002

Table 2 Test conditions for selected species in laboratory toxicity experiments with the organotin compound TPT

Species	Taxonomic group	Average size ±sd (mm; n ≥10)	Test unit volume (l)	O_2 (mg/l) (Min–Max)	pH (Min–Max)
Acanthocyclops venustus	Microcrustacean	2.2±0.4		7.8–8.4*	8.1–8.2*
Asellus aquaticus	Macrocrustacean	5.1±1.4	1.8	6.2–8.7	7.9–8.2
Bythinia tentaculata	Mollusc	–[a]	1.8	6.3–9.3	7.4–8.0
Chaoborus obscuripes	Insect	–[a]	1.8	6.1–10.2	7.4–8.0
Cloeon dipterum	Insect	5.5±0.7	1.8	6.3–8.9	7.7–8.2
Daphnia galeata	Microcrustacean	1.8±0.3	0.6	7.1–8.9	7.7–8.2
Dugesia sp.	Turbellarian	–[b]	1.8	8.2–8.9	7.6–8.2
Endochironomus albipennis	Insect	9.2±1.2		8.0–8.7*	7.2–7.6*
Erpobdella juv.	Annelid	11.5±1.7	1.8	4.4–8.8	7.4–8.3
Gammarus pulex	Macrocrustacean	13.0±4.0	1.8	4.7–8.5	5.6–7.9
Glyptotendipes sp.	Insect	11.7±1.9		8.0–8.7*	7.2–7.6*
Lumbriculus variegatus	Annelid	31.4±5.9		8.3–8.4*	7.8–7.9*
Lymnaea stagnalis	Mollusc	26.5±6.4	1.8	0.2–8.3	7.0–7.8
Physa fontinalis	Mollusc	6.5±1.0	1.8	6.1–8.9	7.4–7.9
Planorbis contortis	Mollusc	4.3±0.7	1.8	6.1–8.8	7.8–8.1
Polycelis tenuis/niger	Turbellarian	–[b]	1.8	6.1–9.0	7.8–8.2
Proasellus meridianus/coxalis	Macrocrustacean	5.6±1.7	1.8	4.8–8.7	7.7–8.2
Sigara sp.	Insect	–[a]	1.8	5.6–9.9	7.6–8.1
Sphaerium sp.	Mollusc	8.6±1.4	1.8	6.3–8.7	7.7–8.3
Tubifex sp.	Annelid	7.3±2.4		8.4–8.5*	7.8–7.9*
Desmodesmus subspicatus	Green algae	–[c]	0.1	–[d]	–[d]
Monoraphidium minutum	Green algae	–[c]	0.1	–[d]	–[d]
Scenedesmus quadricauda	Green algae	–[c]	0.1	–[d]	–[d]
Selenastrum capricornutum	Green algae	–[c]	0.1	–[d]	–[d]
Elodea nuttallii	Vascular plant	–[c]	1.8	12.7–16.4	6.9–10.1
Elodea canadensis	Vascular plant	–[c]	1.8	9.6–13.6	7.1–9.3
Lemna minor	Vascular plant	–[c]	1.8	9.9–17.8	6.7–10.0
Lemna trisulca	Vascular plant	–[c]	1.8	9.2–12.1	6.9–9.1
Potamogeton crispus	Vascular plant	–[c]	1.8	11.7–14.8	7.1–9.7
Myriophyllum spicatum	Vascular plant	–[c]	1.8	6.9–19.9	6.6–10.4
Ceratophyllum demersum	Vascular plant	–[c]	1.8	5.1–17	6.6–10.0
Spirodela polyrhiza	Vascular plant	–[c]	1.8	7.6–10.3	6.4–8.7

O_2 and pH ranges are given for the control treatments only

a=Sizes were not measured in the 'range finding' test, b=Tricladida could not be conserved for measurements, c=not selected on size, but on biovolume (2×106 μm^3/ml) in the case of algae or wet weight (approximately 2 g) in the case of macrophytes, d=Small size of test unit did not allow for pH and O_2 measurements, *=Only measured in stock solutions, test units too small for probe

and Zn: 0.002 (W/W%)). These amounts of inorganic nutrients (N, P and K), inorganic C and trace elements were added twice a week. Other test conditions were similar to those of the invertebrate testing, with the only difference that extra illumination was provided to ensure good macrophyte growth. The macrophytes were illuminated with Philips HPI-T, 400 W lamps at 223-$\mu mol/m^2/s$ at the water surface using a 14 h light:10 h dark regime. DO and pH measurements took place in all treatments on days 1, 6, 8, 13, 15, 20 after application. Degrading biomass resulted in lower DO and pH levels in higher treatments but there was no indication that sensitivity of the macrophytes to TPT was affected.

Algal tests were performed in 100-ml cellulose-plug capped Erlenmeyer flasks with 50 ml fresh medium (Baer and Goulden 1998) and an initial algal density of $2*10^6$ μm^3/ml (on a biovolume basis using a Coulter Multisizer II electronic particle counter). Three replicates per treatment were used and the test was run for 4 days (96 h). Test units were constantly illuminated by cool-fluorescent white tubes producing 100 $\mu mol/m^2/s$ at the water surface (Osram L 36W/21-840, OSRAM Nederland BV, Alphen a/d Rijn, The Netherlands), at a temperature of 20±1°C. Phytoplankton taxa originated from algal stock cultures that have been maintained for years at the laboratory of the Department of Aquatic Ecology and Water Quality Management (Wageningen University) by regular (every 2–4 weeks) inoculation of existing stock material in fresh autoclaved medium.

Endpoints

In the invertebrate tests, sub-lethal (behavioural and immobility) and lethal effects were monitored. Since mortality is the ultimate phase of immobility, scores for mortality and immobility were summated into one logistic regression analysis of EC_x-values (Effect Concentration

where $x\%$ of the population is affected). For all invertebrates, effects were scored as mortality when no response of any kind was observed for about 10 s under a stereomicroscope after repeated tactile stimulation of the organism's body. A behavioural effect was scored when invertebrate behaviour in treated systems deviated from controls.

The measurement endpoints in the macrophyte tests were biomass, which was converted to relative growth (using the biomass at the start of the test), and photosystem II efficiency (Φ_{PSII}), an endpoint frequently used in phytoplankton testing (Fairchild et al. 1998; Juneau et al. 2002; Lürling and Verschoor 2003) which can also be used in macrophyte testing (Snel et al. 1998). Φ_{PSII} is a measure of the efficiency of the photosystem II electron flow, measured as chlorophyll fluorecence, and was sampled non-destructively by means of a mini-PAM photosynthesis yield analyzer (WALZ, Germany). Since the structure of the aquatic plants prevented the use of the 'leaf clip' supplied with this analyzer, the diode of the mini-PAM was fixed at 3 mm from the macrophyte by means of an adjustable stand. Every plant was sampled three times, with two-minute intervals between measurements. These three samples were pooled and the average was used for further analysis. Φ_{PSII} was sampled on days 0, 2, 7, 9, 14, 16 and 21, while biomass was sampled at the beginning and end of the experiment. The biomass at the start of the experiment was estimated by weighing three extra portions.

The measurement endpoint for algae was Φ_{PSII}. A PHYTO-PAM phytoplankton analyzer (WALZ, Germany) was used to measure photosynthetic activity (Φ_{PSII}) every day; this was converted to chlorophyll-*a* content of the algae (Lürling and Verschoor 2003).

Field experiment

In 2001, an outdoor microcosms experiment with TPT was performed at the Sinderhoeve experimental field station at Renkum, The Netherlands, using a total of 20 concrete cosms (length 140 cm, width 120 cm, and height 80 cm) with a water column of approximately 50 cm and a sediment layer of approximately 10 cm. DOC, suspended solids, and chlorophyll-*a* concentrations in the water phase were 8.8 mg/l, 4.9 mg/l and 58.5 μg/l, respectively, as determined in earlier experiments by Roessink et al. (2005). The microcosm experiment aimed to compare the ecological impact of a single application of TPT between test systems with clean and systems with polluted sediments derived from river floodplain lakes. The polluted sediment contained higher levels of nutrients, metals, PAH, PCB, and organic carbon.

The experiment used a regression design with five duplicate concentrations of TPT (controls, 1, 10, 30 and 100 μg/l) per sediment type. TPT (Fentin acetate Pestanal; Sigma Aldrich Chemie BV, Zwijndrecht, The Netherlands) was applied once with ethanol as the carrier solvent. Control test systems were not dosed with TPT but received an equal amount of ethanol. Responses of populations of macroinvertebrates, zooplankton, phytoplankton, and macrophytes were studied at several time intervals after TPT application. Since no major differences in community responses between systems was observed (Roessink et al. 2006)only the clean sediment systems are used for the comparison with the response in the laboratory. For a detailed description of the design and results of the microcosm experiment, see Roessink et al. (2006).

Data analysis

The threshold level for P was 0.05 for all statistical analyses. Logistic regression was used to calculate the laboratory EC_{50} values for algae and macrophytes according to the following formula after the model describing hormesis by Van Ewijk and Hoekstra (1993):

$$y = \frac{k[1 + f * \exp(\log x)]}{1 + [1 + 2 * f * \exp(a)] * \exp[b(\log x - a)]} \quad (1)$$

where y=expected number/biomass/relative growth, a=ln(EC_{50}), b=slope parameter, k=maximal growth (upper limit), f=hormesis, x=concentration.

In the case of living biomass as endpoint, the 100% effect was set at a biomass of 0 g. In the case of relative growth, the 100% effect was set at a growth of 0 g per 3 or 4 days for algae and 0 g per 21 days for macrophytes. This meant that, based on the same data, EC_{50} values for biomass and relative growth could differ substantially (for a visual representation see Van den Brink et al. 1997).

Logistic regression of the invertebrate data of the laboratory SST was performed using the following general logistic model:

$$y = \frac{(1 - c)}{1 + \exp[b * \ln(x - a)]} \quad (2)$$

where y=expected affected fraction, a=ln(EC_{50}), b=slope parameter, c=fraction of affected individuals in controls.

The logistic regression of the invertebrate data obtained from the outdoor microcosms used the same general logistic model described in Eq. 1, although without the possibility of hormesis. In this case the k parameter stands for the expected number in the control microcosms. The models (Eq. 1 and 2) were programmed in GenStat for Windows, 6th edition (Payne 2002). A Poisson distribution of the abundance data was assumed.

SSD analyses were performed according to (Aldenberg and Jaworska 2000) by the ETX-2000 computer program (Van Vlaardingen and Traas 2002). This spreadsheet program calculates the HC_5 (Hazardous Concentration for 5% of the species) and the 90% confidence limits.

The model assumes a log-normal distribution of toxicity data, thus:

$$f(x) = \frac{1}{\sqrt{2\pi\sigma^2}} * \exp\left(\frac{-0.5 * (x-\mu)^2}{\sigma^2}\right) \quad (3)$$

where x= ln (EC_{50}), μ=median EC_{50}, σ=standard deviation of ln (EC_{50}).

The SSD was defined as the cumulative density function of toxicity data as follows:

$$F(x) = \int_{\infty}^{x} f(x)ds \quad (4)$$

Tests for log-normality were performed by means of the Anderson–Darling goodness-of-fit test, a standard statistic output of the ETX version 1.403 computer program. Normality of toxicity data was assumed when P was ≥0.05 (Aldenberg et al. 2002). In accordance with Schroer et al. (2004), a two-sample F-test was used to assess significant differences in the variances of SSDs. T-tests were used to determine significant differences in SSDs. Both tests were performed for 'full' curve comparison.

No observed effect concentrations (NOECs) were calculated at parameter or taxon level using the Williams test (ANOVA) (Williams 1972). This test assumes that the mean response of the variable is a monotonic function of the treatment, thus leading to the expectation of increasing effects with increasing dose. The analyses were performed with the Community Analysis computer program (Hommen et al. 1994), resulting in a summary of NOECs for each sampling day for the data analyzed.

Results

SST in the laboratory

Exposure concentrations

One hour after application, mean measured concentrations (standard deviation in parenthesis) in the water of the test systems ranged from 94% (±15), 98% (±5), to 97% (±8) of the intended nominal concentrations, for invertebrates, macrophytes and phytoplankton, respectively. Since the mean measured concentrations are well in agreement with the intended exposure concentrations, calculated toxicity values in the present paper are based on nominal concentrations.

Table 3 shows that in test systems with small taxa, exposure concentrations were rather stable (approximately 82% of initial concentration remained at the end of the test). In contrast, test systems with relatively large taxa (*Lymnaea stagnalis*, *Lumbriculus variegatus*) showed a faster decline of TPT in the water phase (down to 20.9% of initial dosage for *Lymnaea*).

In the tests with relatively small floating (*Lemna minor* and *Spirodela polyrhiza*) or submerged (*Lemna trisulca*) macrophytes, the decrease of TPT in the water phase was slower than in tests with relatively large plants. This faster decline in tests systems with relatively large plants can be explained by the relatively large macrophyte surfaces to which the substance can be sorbed. In the tests with *Ceratophyllum demersum*, *Elodea nuttallii*, *Lemna minor*, and *Myriophyllum spicatum*, no TPT could be retrieved from the water phase at 14 and 21 days after application. No periphyton growth was observed in these test systems either.

Toxicity

In total, 32 different taxa were tested in the laboratory, 27 of which were used for the estimation of an appropriate

Table 3 Percentages of TPT in the water phase, relative to the initial test concentrations (as tested in the stock solutions), during the SST

Taxon	Fraction (%) of compound after				
	1 h	24 h	48 h	36 h	96 h
Gammarus pulex	90.2	85.5	83.5	78.7	81.4
Endochironomus albipennis	97.2	94.8	99.8	97.8	110.2
Cloeon dipterum	88.1				101.7
*Lymnaea stagnalis**	117.0				20.9
Physa fontinalis	131.8				86.7
Planorbis contortis	137.6				105.6
*Lumbriculus variegatus**	92.5				24.1
Tubifex sp.	111.0				89.2
Polycelis niger/tenuis	88.6				104.1
Dugesia sp.	89.0				93.0
Average	104.3				81.7

*Tests are more uncertain due to loading issues

EC_x value (Tables 1, 2). The tests performed with *Bythinia tentaculata*, *Sphaerium sp*., *Sigara sp.*, and *Chaoborus obscuripes* could not be used for EC_x calculations. *Bythinia tentaculata* and *Sphaerium sp*. showed behavioral avoidance to TPT exposure by closing their opercula and/or shells. When placed in clean test medium after the range finding test, *Bythinia* and *Sphaerium* resumed their normal mode of action (moving through the jar/filtering activities). For *Sigara sp.* and *Chaoborus obscuripes* no apparent treatment-related response was observed during the 96 h test period.

The use of ethanol as a carrier solvent had no adverse effects in the invertebrate tests; only in the test with *Endochironomus albipennis* was there a slight difference in behavior between control and solvent control. In the macrophyte SST, the only difference observed between control and solvent control was for *Elodea nuttallii* and *Potamogeton crispus*. In this case, the solvent control was used in further analysis, while in all other cases controls and solvent controls were pooled into one control treatment for EC_x estimations.

Looking at the range of sensitivities of the invertebrates tested; the most sensitive taxa included turbellarians, annelids, gastropods, micro-crustaceans, and *Gammarus pulex*, while Insecta and Isopoda were less sensitive (Table 4). For all toxicity data presented here, survival of organisms in controls was more than 80% and all estimated EC_x values of sensitive taxa fell within the range of the tested TPT concentrations (Tables 1, 4). Of the invertebrates tested, the copepod *Acanthocyclops venustus* was the most sensitive species tested, with a 96 h EC_{50} of 1 µg/l (Table 4). The least sensitive invertebrate taxon for which an EC_{50} value was estimated was *Glyptotendipes* (96 h EC_{50}=205 µg/l). On average, the difference between EC_{10} and EC_{50} values of invertebrates was a factor of 2 to 3. The difference between EC_x and LC_x values was, on average,

Table 4 Results of short-term laboratory Single Species Tests (SST) of the toxicity of the fungicide TriPhenylTin-Acetate to aquatic invertebrates

Species	*x*	EC_x(µg/l)		LC_x(µg/l)	
		48 h	96 h	48 h	96 h
Acanthocyclops venustus	10	2.7 (1.8–4.0)	0.1 (0.0–1.5)	2.9 (1.9–4.5)	0.1 (0.0–0.9)
	50	5.8 (4.7–7.1)	0.5 (0.1–2.2)	6.9 (5.5–8.8)	0.8 (0.3–2.0)
Lumbriculus variegatus	10	4.1 (2.6–6.7)	3.5 (2.1–5.8)	21.4 (*)	13.3 (12.2–14.5)
	50	8.8 (6.7–11.5)	6.3 (4.8–8.3)	22.6 (*)	14.8 (13.7–15.9)
Physa fontinalis	10	5.8 (3.7–8.2)	4.2 (2.8–6.1)	17.2 (4.3–69.1)	10.6 (9.7–11.5)
	50	9.3 (7.6–11.5)	7.1 (5.6–9.1)	96.3 (36.3–255.0)	11.8 (10.9–12.8)
Dugesia sp.	10	2.7 (1.5–5.0)	2.9 (1.7–5.0)	24.9 (18.7–33.1)	19.0 (18.0–20.0)
	50	9.8 (7.2–13.2)	6.1 (4.6–8.0)	35.3 (29.9–41.6)	20.9 (19.9–22.0)
Polycelis niger/tenuis	10	3.1 (1.7–5.6)	3.4 (1.9–5.9)	42.4 (39.2–45.8)	20.8 (*)
	50	10.6 (7.9–14.2)	6.6 (5.0–8.8)	46.9 (43.1–51.0)	23.2 (*)
Tubifex	10	2.4 (0.6–9.2)	2.3 (0.5–9.3)	13.1 (7.7–22.1)	9.2 (5.5–15.4)
	50	14.2 (8.0–25.3)	10.7 (5.5–21.1)	27.0 (20.5–35.5)	12.9 (10.0–16.7)
Planorbis contortis	10	5.7 (3.0–10.9)	3.5 (2.2–5.5)	–[a]	–[a]
	50	14.7 (10.6–20.3)	6.6 (5.0–8.6)	–[a]	–[a]
Daphnia galeata	10	7.3 (4.9–10.8)	5.4 (3.4–8.5)	28.2 (14.1–56.5)	13.1 (*)
	50	16.1 (13.1–19.9)	8.4 (6.8–10.4)	41.9 (35.8–49.1)	16.0 (*)
Gammarus pulex	10	5.6 (2.4–13.6)	4.5 (2.1–9.8)	18.5 (4.6–74.1)	11.6 (5.7–23.7)
	50	18.5 (12.3–27.9)	8.9 (6.1–12.7)	104.4 (39.4–276.5)	12.6 (7.0–22.9)
Lymnaea stagnalis	10	10.0 (5.3–18.6)	9.7 (*)	263.5 (124.0–559.9)	85.8 (*)
	50	24.9 (18.3–34.0)	11.8 (*)	906.9 (387.0–2125.7)	92.1 (*)
Erpobdella juv.	10	15.3 (*)	9.6 (6.1–15.0)	50.5 (47.1–54.1)	23.8 (*)
	50	25.9 (*)	17.1 (13.2–22.1)	56.6 (53.2–60.3)	27.1 (*)
Cloeon dipterum	10	34.7 (19.2–63.0)	12.3 (4.9–31.2)	251.8 (173.6–365.2)	39.8 (*)
	50	120.9 (89.6–163.1)	63.0 (42.3–93.8)	442.5 (327.4–598.1)	168.9 (*)
Proasellus meridianus/coxalis	10	37.0 (19.3–71.1)	32.4 (17.1–61.2)	137.4 (74.7–253.1)	39.1 (21.4–71.6)
	50	139.0 (97.8–197.4)	90.9 (65.7–125.8)	558.5 (364.7–855.4)	138.5 (99.1–193.6)
Asellus aquaticus	10	78.3 (36.7–167.3)	26.0 (8.7–77.8)	72.8 (33.9–156.4)	72.8 (33.9–156.4)
	50	212.8 (146.3–309.5)	95.6 (56.6–161.3)	271.3 (184.1–399.7)	271.3 (184.1–399.7)
Endochironomus albipennis	10	343.0 (162.5–724.0)	181.9 (170.0–194.6)	306.8 (163.9–574.0)	179.2 (112.5–285.3)

All tests were performed at 20±2°C; the test medium was filtered water from the 'Sinderhoeve' experimental field station. Calculated EC_x values are plotted with their 95% confidence limits between brackets

(*)=Standard error of parameters not available due to singularity in regression model

[a]=Since the response in SST did not allow clear discrimination between sublethal and lethal effects, no LC could be calculated

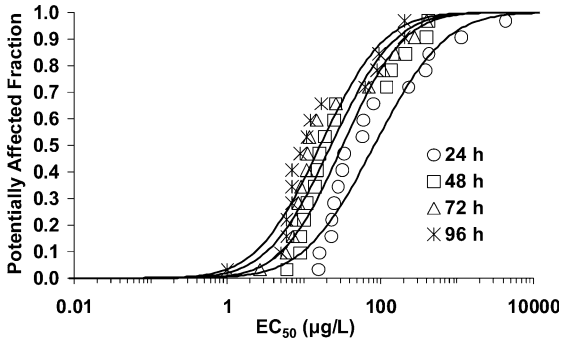

Fig. 1 Species sensitivity distribution (SSD) curves calculated from the estimated EC50 values of the invertebrate laboratory Single Species Tests (SST) at 24, 48, 72 and 96 h after TPT application

approximately a factor of 4. Increasing sensitivity (lower EC_{50} values) with increasing exposure time was observed for all invertebrate taxa (Table 4).

Figure 1 shows the four Species Sensitivity Distributions (SSD) constructed with the invertebrate EC_{50} values at 24, 48, 72, and 96 h. The Anderson–Darling test revealed that both of the curves were not accepted at the 0.05 but only at the 0.025 level. Acceptance at this lower level is not indicating that all the data are not log-normal but that upper values in the SSD seem to deviate from log-normality (e.g., EC_{50} values of *Asellus*, *Proasellus*, *Endochironomus*, and *Glyptotendipes*).

The median EC_{50} (or location parameter μ; see Eq. 3) of the species tested decreases as exposure time increases. Indicating that the average sensitivity increased over time. This phenomenon is also reflected in other percentiles of the SSD, such as the HC_5. For respectively 24, 48, 72, and 96 h median HC_5 values (with 90% lower and upper limit) of 5.0 (1.2–12.6), 2.9 (0.8–6.3), 1.8 (0.5–4.1), and 1.3 (0.4–3.0) µg/l were found. HC_5 values decreased with increasing exposure time. Statistical evaluation of the SSD curves (Fig. 1) shows that only the 24 h curve differs significantly from the other curves ($P < 0.01$).

Based on Φ_{PSII}, the algae we tested were more sensitive than the vascular plants, and with an EC_{50} of 5.6 µg/l, the green alga *Selenastrum capricornutum* was the most sensitive plant species tested (Tables 5, 6). Only for *Potamogeton crispus*, *Myriophyllum spicatum*, and *Elodea nuttallii* hormesis played a significant role in terms of relative growth based on biomass. Comparison of toxicity values based on Φ_{PSII} for days 2, 7, and 21 shows that the values were lowest on day 7 and had the smallest 95% confidence interval. *Myriophyllum spicatum* could not be analyzed by the mini-PAM because its leaf structure was too fine and delicate. The comparison for the vascular plants also shows that for most of the species tested, toxicity values based on relative growth (21 days) were lower than those based on PSII (7 days), except for *Potamogeton crispus* and *Lemna minor*. *Spirodela polyrhiza* (EC50=4.6 µg/l based on relative growth) was the most sensitive macrophyte species tested (Table 6). The average toxicity ratio for algae tested after 72 and 96 h [EC_{50}-72 h/EC_{50}-96 h] was 1, indicating that, in contrast to the invertebrates we tested, algae did reach the incipient value within 3 days.

Comparison of sensitivities of 96 h invertebrate EC_{50} and primary producer toxicity data (Φ_{PSII} for both algae and macrophytes; relative growth for macrophytes only) showed that toxicity of TPT is in the same range (Fig. 2). Accompanying HC_5 values with lower and upper limit in parenthesis are 1.3 (0.4–3.0), 1.9 (0.4–4.9), and 4.2 (1.0–9.3), respectively. All curves partly overlap and although the lower parts of the curves seem to differentiate,

Table 5 Results of short-term laboratory Single Species Tests (SST) of the toxicity of the fungicide TriPhenylTin-Ac to several phytoplankton species

Species		X	Time after application (h)		
			48	72	96
Selenastrum capricornutum	EC_x (µg/l)	10	5.5 (4.8–6.3)	2.6 (1.9–3.6)	3.2 (2.7–3.7)
		50	58.0 (51.4–65.5)	8.8 (7.0–11.0)	5.6 (4.9–6.4)
	NOEC (µg/l)		3.0	3.0	3.0
Desmodesmus subspicatus	EC_x (µg/l)	10	15.9 (8.9–28.5)	10.1 (8.4–12.3)	11.1 (9.9–12.5)
		50	101.9 (84.9–122.4)	23.0 (20.1–26.3)	18.1 (16.5–19.9)
	NOEC (µg/l)		10.0	10.0	10.0
Monoraphidium minutum	EC_x (µg/l)	10	39.2 (17.5–87.5)	14.3 (11.7–17.4)	2.5 (1.1–6.1)
		50	187.7 (104.7–336.5)	51.5 (40.5–65.4)	15.8 (10.6–23.7)
	NOEC (µg/l)		10.0	10.0	10.0
Scenedesmus quadricauda	EC_x (µg/l)	10	54.6 (35.1–84.8)	7.2 (2.9–17.9)	17.0 (12.9–22.6)
		50	352.9 (133.6–931.7)	29.1 (19.4–43.6)	36.0 (30.8–42.1)
	NOEC (µg/l)		30.0	3.0	3.0

All test were performed at 20±2°C; the test medium was filtered water from the 'Sinderhoeve' experimental field station. Calculated EC_x values are plotted with their 95% confidence limits between brackets. The endpoint was photosystem efficiency (PSII) and was measured at 24, 48, 72, and 96 h. However, no EC_x values could be calculated at 24 h after application

Table 6 Results of short-term laboratory Single Species Tests (SST) of the toxicity of the fungicide TriPhenylTin-Ac to several macrophyte species

Species	x	EC_x (µg/l)			Relative growth 21 days
		PSII 2 days	PSII 7 days	PSII 21 days	
Spirodela polyrhiza	10	386.2 (234.0–637.3)	5.6 (2.3–13.3)	28.9 (26.5–31.5)	0.1 (0.0–3.9)
	50	$5.6*10^3$ ($2.6*10^3$–$1.2*10^4$)	29.0 (18.3–45.8)	33.1 (30.3–36.3)	4.6 (0.7–29.5)
Potamogeton crispus [a]	10	9.0 (3.2–25.6)	5.6 (2.3–13.3)	–	23.8 (18.8–30.1)
	50	127.9 (78.5–208.2)	29.0 (18.3–45.8)	–	38.8 (31.0–48.4)
Lemna trisulca	10	21.9 (13.3–35.8)	9.9 (5.4–18.1)	11.2 (6.3–19.7)	1.8 (0.2–15.4)
	50	122.5 (93.9–159.9)	69.5 (51.1–94.6)	36.1 (27.5–47.5)	64.5 (25.6–162.6)
Ceratophyllum demersum	10	62.2 (38.8–99.7)	1.6 (0.0–82.4)	48.1 (0.9–2548.2)	0.4 (0.0–17.6)
	50	240.6 (184.9–313.1)	92.5 (18.1–473.4)	1357.3 (327.5–$5.6*10^3$)	12.9 (2.0–82.8)
Elodea nuttallii [a]	10	6.1 (1.1–34.0)	34.8 (11.0–109.9)	79.9 (x–x)	1.8 (1.1–3.0)
	50	59.4 (25.7–137.1)	101.9 (63.8–162.9)	97.7 (x–x)	11.8 (7.4–18.8)
Lemna minor	10	$9.1*10^2$ ($2.1*10^2$–$3.9*10^3$)	104.8 (93.0–118.2)	96.7 (x–x)	180.0 (x–x)
	50	$6.4*10^4$ ($1.0*10^2$–$4.0*10^7$)	138.9 (60.1–321.4)	130.4 (x–x)	198.9 (x–x)
Elodea canadensis	10	5.1 (1.9–13.8)	2.1 (0.2–23.9)	1.8 (0.0–214.9)	1.5 (0.1–29.7)
	50	197.8 (132.6–295.1)	176.6 (69.1–451.7)	44.5 (4.8–413.8)	23.4 (8.5–64.5)
Myriophyllum spicatum	10	NA	NA	NA	32.3 (18.7–55.6)
	50	NA	NA	NA	73.4 (44.9–200.0)

All tests were performed at 20±2°C; the test medium was filtered water from the 'Sinderhoeve' experimental field station. Calculated EC_x values are plotted with their 95% confidence limits between brackets. The endpoint was photosystem efficiency (PSII) and relative growth. *x–x*=No convergence for model, NA=not applicable

[a]=EC_x-calculation not with pooled control and solvent control but with solvent controls only

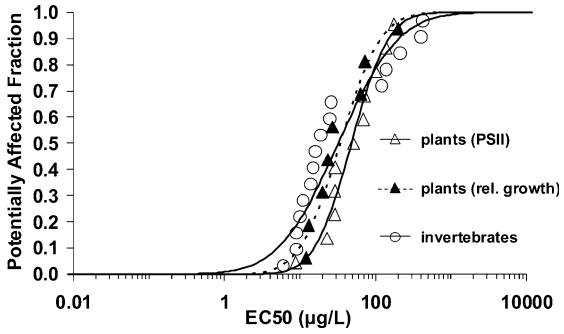

Fig. 2 SSD of 28 indigenous freshwater taxa for TPT tested in the laboratory. EC50 values were estimated using 48 h response data for invertebrates (○) and Φ_{PSII} (Δ) and relative growth (▲; dotted line) responses for phytoplankton and macrophytes (Tables 4, 5)

90% confidence intervals of HC_5 values overlap and statistical testing did not reveal any significant differences ($P>0.05$).

Microcosm semi-field experiment

In the present paper we focuss on the comparison of the results of the laboratory SSD with that of the microcosms constructed with clean sediment. However, we also present the summary data for the microcosms with polluted sediment. Figures 3a–c show the SSD curves constructed from the EC_{50} values (based on intended nominal concentrations) of the free-living invertebrate populations for weeks 2, 4, and 8 after application, together with the curve obtained from the 96 h invertebrate laboratory data (Table 4). We considered only the EC_x values of those taxa that had a mean abundance of 4 or higher on the artificial substrates of control microcosms. Calculated EC_x values for low-abundance taxa (≤3 per test system) were considered uncertain and therefore not representative. Statistical testing reveals significant differences ($P<0.01$) between the curves indicating a higher sensitivity of invertebrates in the microcosms compared to the lab (Fig. 3). Overall, the HC_5 calculated from invertebrate toxicity data for microcosms (based on nominal peak concentrations) was a factor of 2–4 lower than the HC_5 calculated from laboratory invertebrate EC_{50}-96 h toxicity data. While the microcosm HC_5 values between test systems constructed with clean and polluted sediment were very similar (Table 7).

Discussion

Laboratory responses

In our laboratory experiments, we observed that representatives of several taxonomic groups of freshwater invertebrates, as well as several phytoplankton and vascular plant species, showed a clear response to a single application of TPT at treatment levels higher than 1 µg/l (Tables 4, 5). On average, EC_x values were a factor of 4 lower than LC_x values and this difference decreased (to a factor of 3) as exposure time increased, indicating that

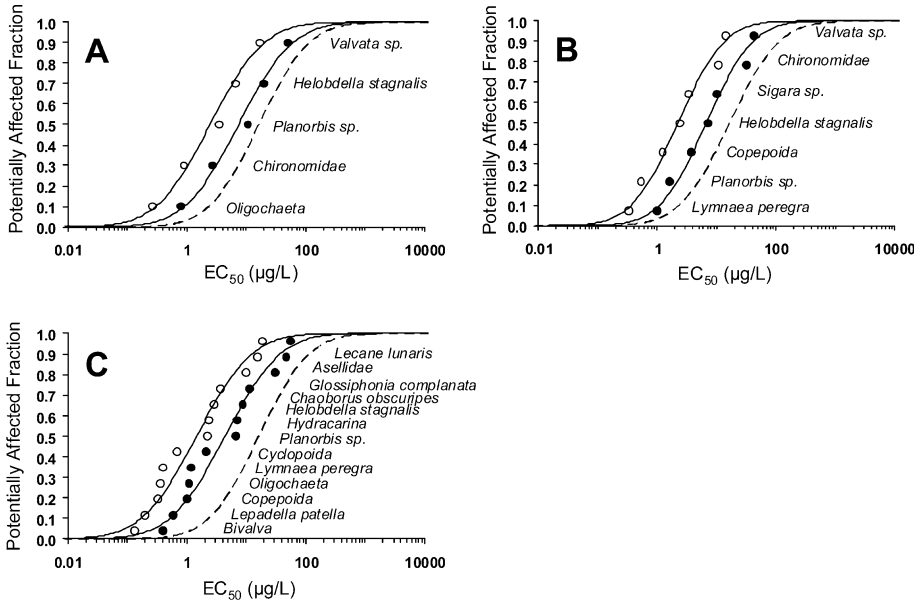

Fig. 3 SSD curves of invertebrates after treatment with the fungicide TPT in outdoor cosms (constructed with clean sediment) based on initial nominal concentrations (●) and based on 21-days time weighted average concentrations (○). Panels a–c present the SSD curves at 2 (A), 4 (B), and 8 (C) weeks after application of TPT. The dashed line represents the 96 h-SSD curve of the invertebrates tested in the laboratory SST

Table 7 Calculated ecological risks thresholds (HC_5 values, with 90% lower and upper limit) in both types of outdoor microcosms based on initial peak concentrations and 21-days time weighted average concentrations (TWA)

		Week 2	Week 4	Week 8
Clean sediment	*n*	5	7	13
	Peak (µg/l)	0.4 (0.0–2.0)	0.6 (0.1–1.8)	0.3 (0.1–0.7)
	21-days TWA (µg/l)	0.1 (0.0–0.7)	0.2 (0.0–0.6)	0.1 (0.0–0.2)
Polluted sediment	*n*	11	16	9
	Peak (µg/l)	0.3 (0.1–0.6)	0.6 (0.2–1.2)	0.2 (0.0–0.6)
	21-days TWA (µg/l)	0.1 (0.0–0.2)	0.2 (0.1–0.5)	0.1 (0.0–0.2)

n=Number of taxa used in calculation

TPT is a compound with a relatively 'slow' mode of action. The average EC_{50}-48 h:EC_{50}-96 h ratio was 2, against a ratio of 4 when calculated with LC_{50} values. This ratio was considerably higher for several individual taxa. The copepod *Acanthocyclops venustus* had an EC_{50}-48 h/EC_{50}-96 h ratio of 6, while *Physa fontinalis*, *Gammarus pulex* and *Lymnaea stagnalis* had LC_{50}-48 h/LC_{50}-96 h ratios of 8–10. This indicates that at the frequently used time interval for acute effects (48 h), the incipient value for acute toxicity of TPT may not have been reached (Fig. 1 and Table 4).

Several factors seem to govern TPT toxicity in the organisms tested. In particular, organism morphology is a factor, since soft-bodied taxa (e.g. triclad an annelid worms) are more susceptible to TPT than taxa with 'harder' bodies (such as *Endochironomus albipennis* and *Glyptotendipes sp.*, with more closed and chitin-based structures; see Table 4). A faster decline of TPT concentrations was indeed observed in the water phase of the test systems with relatively large soft-bodied taxa (down to 20.9% of the initial dosage for *Lymnaea;* see Table 3). The EC_x-values estimated from the *Lymnaea* and *Lumbriculus* test are more uncertain due to possible loading issues. The decline in TPT concentrations could be test volume related and it is uncertain if the use of a larger volume would also show such a decline. Such a decline in a larger test volume would indicate that a greater amount of TPT is sorbed to the organisms, enlarging exposure and therefore likely to cause a more sensitive response (lower EC_x-values). Neither *Lymnaea stagnalis* or *Lumbriculus variegatus* are the most sensitive species and ommitting them from the SSD hardly affected the HC_x-values.

Another important factor seems to be the size of the organism. Among the crustaceans, the most sensitive taxa were the zooplankters *Acanthocyclops* and *Daphnia*. These smaller organisms possess a larger surface: volume ratio for TPT uptake. It has frequently been reported in the literature that smaller and younger life stages of organisms are more susceptible to toxicants (Hutchinson et al. 1998). Based on Φ_{PSII}, smaller phytoplankton species are, on average, more sensitive than larger vascular plants. Establishing these 'rules of thumb' for vascular plants is more difficult because of the large differences in growth form between species (Table 6).

The 'Φ_{PSII}' endpoint shows a distinct treatment-related response from 48 h onwards, especially for submerged macrophytes, indicating that TPT did indeed inhibit photosynthesis, ultimately resulting in decreased relative growth (Table 5, 6). Except for *Ceratophyllum demersum* where periphyton growth resulted in Φ_{PSII} recovery, while the estimated EC_{50} values for relative growth (based on biomass) contradict this. When PSII artifacts are omitted macrophyte sensitivity does not differ from invertebrate sensitivity ($P>0.05$).

The use of ethanol as carrier solvent resulted in large effects on dissolved oxygen in the outdoor experiment thus posing an extra stress on the systems (Roessink et al. 2006). However, to keep conditions similar enabling comparison between field and laboratory, ethanol was also used in the latter set-up. In contrast to the outdoor situation, due to the lack of sediment, and consequently of microbial biomass in the laboratory SST, ethanol had only minor effects on the response of the taxa tested (Roessink et al. 2006).

Comparison with literature data

Published literature data on TPT toxicity (48, 72, or 96 h) to aquatic taxa is presented in Table 8 (Fargasová 1998; De Zwart 2002). This data relates both to triphenyltin acetate and triphenyltin hydroxide. We have pooled the data for these two compounds because the acetate is rapidly hydrolyzed to hydroxide (Eng et al. 1996; Nguyen et al. 2000), so we assume that triphenyltin acetate exposure can also be classified as triphenyltin hydroxide exposure.

The literature data allow acute:chronic ratios of 22 and 17 to be derived for *Daphnia magna* and *Pimephales promelas*, respectively. This indicates that effects in a long-term study of invertebrates and fish to TPT may be considerably greater than the effects in a short-term study. However, due to differences in exposure time, the slow time-to-event, and kinetics issues related with this type of compound it is hard to distinguish if the differences in effects between short and long-term studies are related to time of exposure or latency of effects.

An SSD analysis with the invertebrate data from the literature (see Table 8) resulted in an acute HC_5 value of 0.8 (0.0–4.6) μg/l. This value is somewhat lower than, but not significantly different from the acute HC_5 value of 2.9 (0.8–6.3) μg/l that we calculated from our 48 h EC_{50} values for invertebrates (Table 5). Combining the available literature data with the data from our study results in the SSD presented in Fig. 4. We used EC_{50} values based on the Φ_{PSII} response after 72 h and 7 days for green algae and vascular plants, respectively, to construct the curve for the primary producers (Tables 5, 6). The curves for plants, invertebrates, and vertebrates are located close together in the graph and sometimes partially overlap. The corresponding acute HC_5 values and 90% lower and upper limit are 1.8 (0.7–4.0), 10.7 (3.9–19.1), and 11.9 (2.4–24.3) μg/l for the invertebrate, vertebrate, and primary producer curves, respectively. Although HC_5 values seem to differ,

Table 8 Toxicity values of triphenyltin acetate (TPT-Ac) and triphenyltin hydroxide (TPT-OH) obtained from the open literature for several taxa originating from and tested in brackish (MX), fresh (FW) and salt (SW) water (De Zwart, 2002)

Compound	Species	Taxonomic group	Water	Test duration (h)	EC50 (μg/l)	NOEC (μg/l)
TPT-Ac	*Skeletonema costatum*	Diatom	MX	72	0.7	
TPT-Ac	*Thalassiosira guillardii*	Diatom	SW	72	1.1	
TPT-Ac	*Thalassiosira pseudonana*	Diatom	SW	72	1.5	
TPT-Ac	*Tubifex tubifex*	Annelid	FW	96	1.9	
TPT-Ac	*Ceriodaphnia dubia*	Microcrustacean	FW	48	11.1	
TPT-Ac	*Daphnia magna**	Microcrustacean	FW	504	0.8	
TPT-OH	*Daphnia magna*	Microcrustacean	FW	48	16.7	
TPT-OH	*Daphnia pulex*	Microcrustacean	FW	48	14.7	
TPT-OH	*Gammarus fasciatus*	Macrocrustacean	FW	96	66.0	
TPT-OH	*Chironomus plumosus*	Insect	FW	96	0.3	
TPT-OH	*Chironomus riparius*	Insect	FW	48	50.0	
TPT-OH	*Cipangopaludina malleata*	Mollusc	FW	48	720.0	
TPT-OH	*Indoplanorbis exustus*	Mollusc	FW	48	840.0	
TPT-OH	*Physella acuta*	Mollusc	FW	48	300.0	
TPT-OH	*Semisulcospira libertina*	Mollusc	FW	48	550.0	
TPT-OH	*Carassius auratus*	Fish	FW	96	62.0	
TPT-OH	*Lepomis macrochirus*	Fish	MX	96	23.0	
TPT-OH	*Oncorhynchus mykiss*	Fish	FW	48	32.6	
TPT-OH	*Oryzias latipes*	Fish	MX	48	69.9	
TPT-OH	*Pimephales promelas*	Fish	FW	96	20.0	
TPT-OH	*Pimephales promelas**	Fish	FW	720		1.2
TPT-OH	*Rasbora heteromorpha*	Fish	FW	48	96.1	

*=Chronic exposure toxicity value, acute: chronic ratio>10

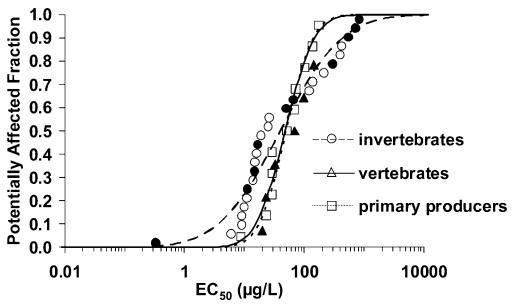

Fig. 4 The analysis of species sensitivity of TPT constructed with combined lab toxicity data from the literature (Fargasová 1998; De Zwart 2002) and the present study (Tables 4–7). The curve for primary producers (□; dotted line) was constructed with the EC50 values based on 72 h Φ_{PSII} for green algae and 7 days values for vascular plants. Curves for invertebrates (○; dashed line) and vertebrates (Δ; solid line) were constructed using 48 h EC50 values. Data points for TPT are transparent while points for TPT-OH are plotted in black

their confidence limits overlap and no significant differences between the curves were found. This shows once again that TPT is a compound that targets a broad spectrum of taxa in a relatively small toxicity range. Figure 4 also shows that triphenyltin acetate (transparent) and triphenyltin hydroxide (black) data points are mixed throughout the invertebrate curve, indicating that there is no great difference in toxicity and justifying the lumping of these two particular compounds. We also constructed a single SSD based on all acute toxicity data available of which the corresponding HC_5 value was 3.1 (1.5–5.3) µg/l.

Comparing laboratory and field responses

There were several sensitive populations in the microcosms, including representatives of Annelida, Mollusca, Crustacea, Insecta, and Rotifera (e.g. *Keratella*, *Lecane*, *and Lepadella*). These observations are in line with the results of our laboratory SST with TPT (for further details on the outdoor microcosm study see part I, Roessink et al. (2006)).

The hazardous concentration for 5% of the invertebrate species (HC_5) calculated on the basis of laboratory data (EC_{50}-96 h) was 1.3 µg/l, while invertebrate HC_5 values (based on initial nominal concentrations) calculated on the basis of SSD curves derived from the clean microcosms in sampling weeks 2–8 ranged from 0.3 to 0.6 µg/l. A perhaps more realistic manner of calculating EC_{50} values is not using the initial nominal (peak) concentrations but using a more chronic exposure e.g., the 21-days time weighted average (TWA) as stated in part I (Roessink et al. 2006). The TWA is approximately a factor 3 lower than the initial nominal concentration and results in a more sensitive response (Fig. 3) with HC_5 values ranging from 0.1–0.2 µg/l. In all cases, invertebrate populations in the cosms responded significantly more sensitive than invertebrate species in the laboratory (Figure 3 and Table 4). Possible explanations for the more sensitive field response immediately address the difficulties involved when comparing lab and field studies and comprise differences in sampling techniques, latency of effects, and/or additional chronic exposure via the food chain in the microcosms.

Invertebrate field sampling occurred by means of the artificial substrate technique which monitors the activity of macroinvertebrates, rather than their total numbers. A small decline in activity/movement could have a larger effect on the recolonisation of the substrate (and thus recovered numbers) than on the behavior observed in the laboratory, perhaps explaining the difference in response. Differences in sampling techniques are even larger concerning phytoplankton and vascular plants were a lab vs. field comparison between different endpoints (viz., Φ_{PSII} and relative growth in the lab versus abundance and percentage coverage in the field) has to be made. Observed differences in sensitivity may reflect an effect of the different types of endpoint measured. In addition, phytoplankton responses in the outdoor test systems and the laboratory are difficult to compare, because the dose–response relationship in the outdoor test systems can easily be obscured by inter-species relationships (e.g., grazing, predation and competition).

Also, TPT disappears quickly from the water phase and can sorb to other compartments (e.g., sediment, macrophytes) (Looser et al. 2000). It has been reported that TPT is transferred through the food web (Stäb et al. 1996; Traas et al. 1996) and low concentrations could mediate effects through bioaccumulation, resulting in a more sensitive response than in the laboratory.

In the outdoor cosms, invertebrates may have suffered long-term exposure to TPT via the water and/or food, causing the incipient value to be reached and maximum effects to be expressed. In contrast, exposure in the laboratory was mainly via water, and the exposure time (96 h) may also have been too short to allow this maximum effect to be expressed.

In conclusion, in the long-term the invertebrate populations in the microcosms indeed showed greater sensitivity than the invertebrate species tested in short-term lab tests. Not only was the time to event (effect expression) not reached in the lab (maximal duration 4 days) for several species in contrast to the field situation, but also differences in exposure regime (maximal 4 days in the lab versus minimal two weeks in the field) and measurement endpoints between the laboratory and microcosms were of influence. The populations in the microcosms suffered long-term exposure due to TPT uptake via water and food,

while only short-term responses to TPT exposure (via water) were monitored in the laboratory.

Other studies comparing lab and field responses of invertebrate populations to the insecticides chlorpyrifos, ensdosulfan, and lambda-cyhalothrin found very similar lab and field SSDs (Van den Brink et al. 2002; Hose and Van den Brink 2004; Schroer et al. 2004; Maltby et al. 2005). These relatively non-persistent insecticides, however, only need a short time to express their toxic effects, while exposure via the food chain plays a minor role.

Risk assessment of TPT

HC5 values based on concentration-response relationships observed in the outdoor microcosms did not differ between sampling dates (week 2, 4, and 8) and type of test system (constructed with clean or polluted sediment) suggesting that in the present study spatio-temporal differences in community structure did not affect the sensitivity indicated by the SSD (Table 7). The analysis of species sensitivity of TPT indicates that a very broad spectrum of aquatic taxa is affected and there does not appear to be a great difference in sensitivity between aquatic primary producers, invertebrates, and vertebrates. This suggests that every aquatic community can be expected to include taxa sensitive to TPT. The physiological processes of organisms impacted by TPT are basal and take time to get expressed in the endpoints measured, except for unicellular phytoplankton. This delay in time of onset of effects in invertebrate populations is one of the reasons why the sensitivities we observed in the laboratory were lower than those in the microcosms. In addition, the phenomenon that the compound dissipates relatively fast from the water and accumulates in organic matter and the upper sediment layers may result in a chronic long-term exposure regime in the field (Looser et al. 2000; Roessink et al. 2006). The present study clearly shows that, for this compound that accumulates in the foodchain, data from conventional acute laboratory single species tests with invertebrates cannot be simply used to assess the risk to the aquatic community exposed to a similar concentration regime (single application) as simulated in our microcosm experiment without appropriate considerations for exposure and/or endpoints affected.

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