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Bioconcentration and Bioavailability of Organotin Compounds: Influence of pH and Humic Substances

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Bioconcentration of triphenyltin (TPT) and tributyltin (TBT) was studied in the freshwater organisms *Daphnia magna* (zooplankton), *Chironomus riparius* (sediment organism) and *Thymallus thymallus* (fish yolk-sac larvae). TPT bioconcentration factors (BCFs) at pH 8 were highest for *Thymallus* (2200), followed by *Chironomus* (680) and *Daphnia* (190). The differences could not be fully explained by different total lipid contents. Metabolism and lower bioconcentration were observed for TBT in *Chironomus*. The BCFs of both TBT and TPT were higher at pH 8 than at pH 5, but the difference was much less pronounced than predicted by the octanol–water partition model. This suggests that, besides the hydroxide species (TBTOH and TPTOH), the cations (TBT^+ and TPT^+) are also taken up by the organisms to some extent and that the octanol–water partition model underestimates the uptake of the charged species. Low concentrations of humic substances (HS) led to small reduction in the bioconcentration of TPT in *Daphnia* and *Thymallus*, and a significant reduction occurred at relatively high concentrations of HS (>10 mg C l^{-1}). The results of this study provide an important basis for future investigations aiming at a better understanding of the bioavailability and fate of TBT and TPT in freshwater ecosystems. © **1998 John Wiley & Sons, Ltd.**

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INTRODUCTION

Organotin compounds are among the most hazardous pollutants in aquatic ecosystems.[1] Tributyltin (TBT) and triphenyltin (TPT) are of particular interest because of their widespread use as biocides. TBT is an important biocide in antifouling paints. The ecotoxicological hazards associated with TBT[2–7] have resulted in restrictions on its use in many countries. In spite of these regulations, the release of TBT into aquatic ecosystems persists due to its use in antifouling paints on large vessels, paint removal from pleasure boats, its application in wood preservation and its resuspension from contaminated sediments.[8–11] Although TPT has been used as co-toxicant with TBT in some antifouling paints, the major application of TPT is in agriculture.[12] TPT is used as a fungicide for various crops and enters aquatic ecosystems mainly via leaching and run-off from agricultural fields. Tetrabutyltin (TeBT) occurs as a by-product of the production of other organotin compounds. Although the amounts of TeBT released into aquatic environments are globally of minor importance, some TeBT-contaminated areas have been described.[13] The ecotoxicological consequences of this contamination, however, are not known.

With respect to the ecotoxicology of organotin compounds, many studies have been performed on the contamination of aquatic systems and the toxicity.[1–7,14,15] Knowledge has also been gained on the distribution pattern of organotins in environmental compartments.[12,16–19] However, the long-term ecotoxicological effects of organotin contaminants on the structure and function of aquatic ecosystems are still not well understood, particularly with respect to biomagnification in food webs[12,20–22] and toxicity of contaminated sediments. These hazards are dependent on the bioavailability of the contaminants.

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The bioavailability of environmental chemicals can be studied by assessing the bioconcentration by means of bioaccumulation experiments. Several general factors, such as hydrophobicity, speciation and organism-specific properties, determine the degree of bioconcentration. For neutral organic compounds of medium hydrophobicity and low biotransformation potential, octanol–water partition coefficients (K_{ow}) can serve as an estimate of the maximum possible bioconcentration in organisms.[23–25] For chemicals that can form charged species in water, differential bioconcentration of the charged and uncharged species should be expected. In many cases, however, simple partitioning of the compound between the aqueous phase and the organisms cannot be assumed. Species-specific factors such as uptake efficiency, growth dilution, metabolism and excretion can modify the degree of bioconcentration.

Organotin compounds, in particular TBT and TPT, differ from neutral organic compounds in their properties. The speciation of TBT and TPT shows a strong pH dependence.[26] Both compounds are present as cations at low pH and as hydroxides at higher pH. Although they can also form complexes with other anions, these species are only of minor importance in typical freshwater systems. Therefore, only the hydroxide species (TBTOH/TPTOH) and the cations (TBT^+/TPT^+) have to be considered. The two species, however, exhibit very different partitioning and sorption behavior. The octanol–water distribution ratios (D_{ow}) of TBT and TPT are more than an order of magnitude higher at pH 8 than at pH 3.[26] This is related to the fact that TBTOH and TPTOH, but not TBT^+ and TPT^+, readily partition into the octanol phase.[26] For partitioning and sorption into humic substances (Aldrich HS), both TBT and TPT have a maximum distribution ratio D_{OM} of approximately 140 000 l kg_{OM}^{-1} between pH 5 and 6.[27] At typical ambient conditions (pH 8), the D_{OM} values are considerably lower for both TBT and TPT (10 000 –15 000 l kg_{OM}^{-1}).[27]

In a previous study, TBT bioconcentration in *Daphnia magna* was found to be higher at pH 8 than at pH 6.[28] In the same study, Aldrich humic substance (HS) reduced the bioconcentration of TBT in *Daphnia magna*. In the present work, we have extended our investigations on the bioavailability of organotins to different freshwater organisms and different compounds. The major goals were

(1) to compare the bioconcentration of TPT by three different organisms representing different ecological niches,
(2) to verify whether the pH-dependent speciation of TBT and TPT results in different bioconcentration factors at higher and lower pH, and
(3) to study the effect of relatively low concentrations (< 10 mg l^{-1}) of Aldrich humic substance (HS) on the bioconcentration of TPT.

We report experimentally determined bioconcentration factors for TPT in *Daphnia magna* (zooplankton), *Chironomus riparius* (sediment organism) and *Thymallus thymallus* (fish yolk-sac larvae). Bioconcentration of TBT, TPT and TeBT in *Chironomus riparius* at pH 8 and pH 5 is compared, using TeBT as a reference compound that may undergo only hydrophobic partitioning. Finally, results on the bioconcentration of TPT in *Daphnia* and *Thymallus* in the presence of Aldrich humic substance (HS) are presented.

MATERIALS AND METHODS

Chemicals

Tributyltin chloride TBTCl (>97%), dibutyltin dichloride $DBTCl_2$ (~97%), triphenyltin chloride TPTCl (>97%) and tetrabutyltin TeBT (~98%) were obtained from Fluka Chemie AG (Buchs, Switzerland). Diphenyltin dichloride $DPTCl_2$ (>98%) and tripropyltin chloride (~98%) were purchased from ABS (Basel, Switzerland). Butyltin trichloride $MBTCl_3$ (~95%), phenyltin trichloride $MPTCl_3$ (~98%) and tripentyltin chloride (~96%) were supplied by Aldrich (Steinheim, Germany). Solutions of ethylated butyl- and phenyltin compounds in methanol (~100 mg Sn l^{-1}) were stored in the dark at +4 °C as primary standards. For external calibration, the primary standards were diluted with hexane. Internal standards were prepared by diluting tripropyltin chloride and tripentyltin chloride with acetone. Stock solutions of tributyltin chloride, triphenyltin chloride and tetrabutyltin in acetone were used to spike experimental media.

Aldrich humic substance (HS) as sodium humate was used without further purification. HS is similar to humic substances in many wetlands.[29] Although HS may not exactly represent the dissolved humic and fulvic acids of many freshwater ecosystems, it

is appropriate for investigations of the general behavior of dissolved humic substances and the results can be compared with those of other studies.[28,30,31,32,33] A HS stock solution for the experiments was obtained by dissolving 2.5 g HS in 5 l of nanopure water, centrifuging at 8000 g for 30 min and filtering through a 0.45 μm filter.

Daphnia experiments

Daphnia magna were cultivated according to the OECD Guideline 202[34] in synthetic medium M7[35] and fed with the green algae *Scenedesmus subspicatus*. The major cations in this medium are Ca^{2+} (2 mM), Mg^{2+} (0.5 mM) and Na^{+} (0.8 mM), while the most important anions are HCO_3^- (0.8 mM), SO_4^{2-} (0.5 mM) and Cl^- (4 mM). The ionic strength of the medium is 13.5 mM. The *Daphnia* were cultured in a 20 ± 1 °C climate chamber with a 16h:8h light:dark cycle. The experiments were conducted under the same conditions using 21±2-day-old *Daphnia* that were not fed during the experiment. Table 1 gives an overview of the experimental conditions. In each test, 80 *Daphnia* were exposed in 1 l glass beakers, containing 800 ml of test solution with TPT. The pH was adjusted to 8.0 at the beginning of the experiment with 0.5 M NaOH, monitored twice a day, and readjusted with 0.5 M NaOH if necessary. Beakers were not aerated. Oxygen saturation was measured at the beginning and end of each experiment and ranged from 78.4 to 86.6%. After 0, 8, 24, 48 and 72 h, 10 individuals were removed. These *Daphnia* were rinsed with nanopure water, dried on cellulose filters, weighed and transferred to glass vials for storage at −20°C until analysis. At 0 h and 72 h, aliquots of experimental medium were taken from each glass beaker, acidified to pH 2 and stored at 4 °C for organotin and dissolved organic carbon (DOC) analyses. In the experiments with humic substance (HS), aliquots of the HS stock solution were added to the experimental medium, resulting in DOC concentrations of 1.1–14.2 mg C l^{-1}. Three replicates were made for every DOC concentration.

Chironomus experiments

Egg masses of the non-biting midge *Chironomus riparius* were received from Springborne Laboratories AG (Horn, Switzerland). The larvae were reared as described by Streloke and Köpp[36] in 10 l all-glass aquaria, containing precleaned and shredded paper towels as substrate and synthetic medium M7[35] as overlying water. The water column was aerated at a rate of 1–2 bubbles per second and the larvae were fed with the fish food Tetramin®. The experiments were conducted in a climate chamber at 20 ± 1 °C with a 16h:8h light:dark cycle. Eighty two-week-old *Chironomus* larvae were exposed in 1 l glass beakers containing 800 ml of M7 medium with TBT, TPT or TeBT (Table 1). The larvae were not fed and the containers were not aerated during the experiments. Oxygen saturation was determined at the beginning and at the end of the experiments. The mean values were $67.1 \pm 5.7\%$ and $64.6 \pm 4.3\%$, respectively. The pH was measured three times a day and adjusted to the desired values of 8.0 and 5.0 with 0.5 M NaOH and 1.0 M HCl, respectively. After 6, 24, 48 and 72 h, samples of 10 *Chironomus* larvae were removed, rinsed, dried on filter paper, weighed into vials and stored at −20 °C for organotin analyses and lipid determination. The TBT and TPT experiments were replicated five times and the TeBT experiment had six replicates.

Fish larvae experiments

Fertilized eggs of graylings, *Thymallus thymallus*, from the River Rhine were transported to the laboratory and adapted to aerated groundwater in a climate chamber at 15 ± 1 °C in the dark for three days. The groundwater had a total hardness of 6.8 meq l^{-1} ($Ca^{2+} + Mg^{2+}$), an alkalinity of 5.8 meq l^{-1} and an ionic strength of 14.9 mM. DOC, organotins, nitrite and ammonia were not present at detectable levels. Experiments were performed with freshly hatched yolk-sac larvae in the same climate chamber, in the dark. In 10 l all-glass aquaria, 250 larvae were exposed for up to 168 h in 3 l of groundwater with TPT (Table 1). The larval density was 1.3 – 2.1 g l^{-1}. Aquaria were aerated, and O_2 and pH determined daily. The average pH was 8.3 ± 0.1 and all measured oxygen saturation values were $\geq$ 97%. No food was provided. The experimental water was changed every 48 h, and 250 ml aliquots were removed at the beginning of the experiment and before the water was renewed. Corresponding water samples were pooled, acidified to pH 2 and stored at 4 °C for determination of DOC and organotins. The experimental set-up included one test with TPT and no humic substance (HS), and tests with TPT plus 1.0, 1.7, 4.3 and 8.8 mg Cl^{-1} HS. After seven different time points, 20–30 fish larvae were removed, rinsed, dried on aluminum foil, weighed and stored at −20 °C until

Table 1 Experimental conditions[a]

Organism	Compound	Exposure concentration, c_w (μg l^{-1})	pH	Temperature (°C)	Wet wt (mg)	Dry weight (% wet wt)	Lipid content (% wet wt)	Control mortality (%)
Daphnia	TPT	8.0 ± 1.4 (15)	8.0	20 ± 1	2.1 ± 0.4 (60)	7.1 ± 0.5 (14)	0.3 ± 0.1 (14)	20 ± 11 (15)
Chironomus	TPT	4.3 ± 0.5 (5)	5.0	20 ± 1	5.5 ± 1.1 (40)	12.0 ± 1.0 (7)	0.6 ± 0.1 (7)	3.8 ± 3.5 (5)
Chironomus	TPT	4.8 ± 0.4 (5)	8.0	20 ± 1	—	—	—	—
Chironomus	TBT	7.1 ± 0.8 (5)	5.0	20 ± 1	4.4 ± 0.8 (40)	—	—	9.0 ± 5.0 (5)
Chironomus	TBT	6.5 ± 0.9 (5)	8.0	20 ± 1	—	—	—	—
Chironomus	TeBT	1.6 ± 0.3 (6)	5.0	20 ± 1	4.5 ± 0.5 (40)	—	—	4.9 ± 2.7 (6)
Chironomus	TeBT	1.7 ± 0.6 (6)	8.0	20 ± 1	—	—	—	—
Thymallus	TPT	3.2 ± 0.4 (10)	8.3	15 ± 1	18.9 ± 0.9 (40)	21.1 ± 4.0 (12)	3.1 ± 0.5 (12)	4 (1)

[a] Data are given as mean ± SD. Numbers in parentheses indicate the number of independent measurements. The exposure concentration (c_w) refers to the geometric mean of the concentration at the beginning of the experiment and after 72 h (*Daphnia*, *Chironomus*) or 48 h (*Thymallus*), respectively. —, Not determined. With the exception of tetrabutyltin, all exposure concentrations refer to the respective butyltin and phenyltin chlorides. The factors for converting the reported concentrations into μg Sn l^{-1} are 0.42, 0.39, 0.36, 0.34, 0.39, 0.35, and 0.31 for MBT, DBT, TBT, TeBT, MPT, DPT and TPT, respectively.

analysis. The experimental conditions are summarized in Table 1.

Organotin analysis

Butyltins (MBT, DBT, TBT, TeBT) and phenyltins (MPT, DPT, TPT) in water and biological samples were determined according to methods described elsewhere,[18,37] with slight modifications. The acidified water samples (pH 2) were transferred into 50 ml volumetric flasks, spiked with the internal standards tripropyltin and tripentyltin chloride, and extracted with two portions of 0.25% tropolone solution in pentane–diethyl ether (2 : 3) and one portion of pentane. The combined organic phases were dried on anhydrous $CaCl_2$, concentrated to 1 ml under a gentle nitrogen stream and ethylated with 2M ethylmagnesium bromide in tetrahydrofuran. After 10 min, the excess of Grignard reagent was hydrolyzed by dropwise addition of 1 M HCl. The top layer was removed and concentrated under nitrogen to 2 ml for GC analysis. Biological samples were acidified to pH 2 with HCl, homogenized and spiked with tripropyltin and tripentyltin chlorides. The extraction and derivatization procedure was the same as for water samples, but the extracts were purified by adsorption chromatography on silica gel (0.5 g of silica gel in a Pasteur pipet) and subsequent elution with hexane. The hexane extracts were analyzed using a Carlo Erba HRGC 5160 gas chromatograph with split/splitless injector, a flame photometric detector (SSD 250) and a DB-5 column (J&W) with i.d. 0.32 mm and a 0.25 μm film. Recovery rates refering to tripropyltin and tripentyltin chloride were routinely assessed in every sample and ranged from 51 to 81% and from 55 to 69% in water and biological samples, respectively. The relatively low overall recovery rates are mainly due to the fact that the whole analytical procedure was covered by this internal standardization method. The reported recoveries include reduced extraction efficiency in water samples with humic substances as well as slight evaporation losses in the concentration steps and sorption to silica gel (phenyltin compounds in biological samples). The detection limits for butyltin and phenyltin compounds ranged from 0.09 to 0.35 μg l^{-1} in water samples and from 24 to 139 ng g^{-1} in biota. With the exception of tetrabutyltin, all results in this paper refer to the respective butyltin and phenyltin chlorides.

DOC analyses

Water samples were membrane-filtered (0.45 μm) and DOC was determined by high-temperature combustion followed by IR detection of CO_2 using a Shimadzu TOC-5000A total organic carbon analyzer. In the controls without HS, DOC concentrations of 1.5 mg C l^{-1} (medium M7) and 1.7 mg C l^{-1} (groundwater) were detected at the end of the experiments. These residues were subtracted from the measured DOC, as they originate partly from the acetone in the spiked stock solutions and partly from exudates from the test organisms.

Determination of total lipid content

The total lipid content in *Daphnia*, *Chironomus* and *Thymallus* samples was determined following the sulfophosphovanillin method of Barnes and Blackstock[38] with specific adaptations for small organisms.[39] The lipids were extracted from the lyophilized samples with hexane–isopropanol (3:2) and hydrolyzed with sulfuric acid. After reaction with vanillin in concentrated orthophosphoric acid for 90 min, the absorbance at 528 nm was measured with a Hitachi U-1000 UV/Vis spectrophotometer. The calibration standards were obtained by treating triolein standards in the same way as the samples.

Data analysis

The organotin uptake curves were fitted through the experimental data points with the integrated equation of the one-box model.[40,41,42] The model yielded the uptake rate constant k_1 and the elimination rate constant k_2 as fitted parameters. Individual bioconcentration factors were calculated for each replicate and the differences between the different pH values and the different concentrations of humic substance were tested for statistical significance with a Student *t*-test (two-sided, significance level $P = 0.05$).

The following definitions and equations were used:

One-box model, integrated form (Eqn [1])

$$\frac{c_b}{c_w} = \frac{k_1}{k_2}(1 - e^{-k_2 t}) \qquad [1]$$

where k_1 = uptake rate constant (ml g^{-1} h^{-1}); k_2 = elimination rate constant (ml g^{-1} h^{-1}); c_b = concentration in biota (ng g^{-1}); c_w = exposure concen-

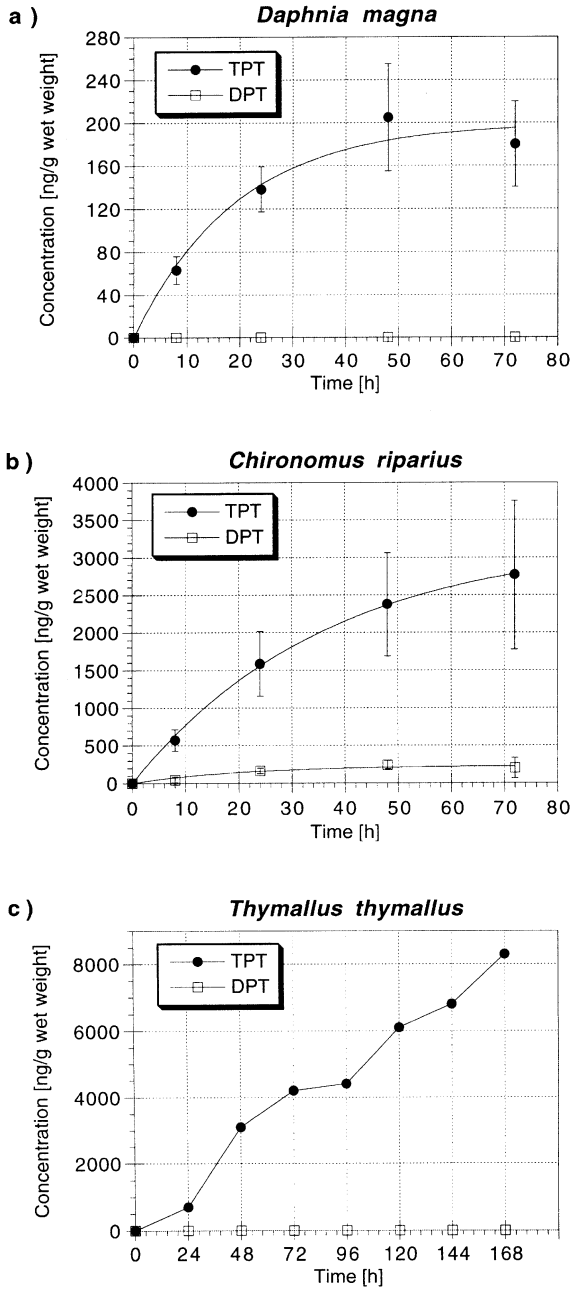

Figure 1 Bioconcentration of TPT in (a) *Daphnia magna* ($n = 3$, 30 *Daphnia*), (b) *Chironomus riparius* ($n = 5$, 50 *Chironomus*) and (c) *Thymallus thymallus* ($n = 1$, 10 *Thymallus*) at pH 8.0 and 8.3, respectively. Data are given as mean ± SD except for *Thymallus* (only one experiment). Also shown is diphenyltin (DPT) as a possible metabolite. Monophenyltin (MPT) was not detectable in any of the organisms.

tration (ng g^{-1} or ng ml^{-1}), calculated as the geometric mean of the concentration at the beginning of the experiments and the concentration after 48 h (*Thymallus*) or 72 h (*Daphnia*, *Chironomus*).

BCF (bioconcentration factor at the end of the experiment; Eqn [2])

$$\mathrm{BCF} = \frac{c_\mathrm{b}}{c_\mathrm{w}} \qquad [2]$$

All BCF values reported in this study were experimentally determined and refer to the end of the experiments, during which a steady state was not necessarily achieved.

RESULTS AND DISCUSSION

Bioconcentration at pH 8

The concentrations of organotins in the exposure waters decreased in all the experiments. The TPT concentration decreased in the experiments with *Daphnia* from 8.6 ± 1.7 to 7.4 ± 1.6 μg l^{-1}, with *Chironomus* from 6.3 ± 0.4 to 3.1 ± 0.6 μg l^{-1} and with *Thymallus* from 4.2 ± 0.2 to 2.4 ± 0.5 μg l^{-1}. In the TBT and TeBT experiments with *Chironomus*, the concentrations declined from 10.0 ± 1.0 to 4.7 ± 0.8 μg l^{-1} and from 3.1 ± 0.5 to 0.9 ± 0.4 μg, l^{-1}, respectively. Taking into account the organotin concentrations in water and biota, no quantitative mass balance was achieved. Losses may have been caused mainly by absorption to the glass walls. The analyses of water samples from control experiments showed, however, that the decrease in the concentrations occurred in a time-dependent, non-linear fashion (data not shown). Thus, the geometric mean of the measured initial and final concentrations gives realistic values for the average exposure concentrations (Table 1).

The uptake of TPT was species-dependent. Considerable differences occurred among *Daphnia* (pH 8.0), *Chironomus* (pH 8.0) and *Thymallus* (pH 8.3), as shown in Fig. 1. A steady state was reached in the experiment with *Daphnia*, whereas bioconcentration in *Chironomus* was approaching a plateau after 72 h, but did not reach a steady state. In *Thymallus* larvae, high uptake persisted, even after 168 h. The bioconcentration factors (BCF) at the end of the exposure periods were 190 ± 50 for *Daphnia*, 680 ± 200 for *Chironomus* and 2200 for *Thymallus*. Of the major metabolites of TPT, only diphenyltin (DPT), but not monophenyltin (MPT)

Table 2 Toxicokinetic parameters and experimentally determined bioconcentration factors for TPT, TBT and TeBT[a]

Organism	Compound	Number of replicates	Uptake rate constant, k_1 (ml g^{-1} h^{-1})	Elimination rate constant, k_2 (ml g^{-1}, h^{-1})	BCF at end of experiment, c_b/c_w	Duration of experiment (h)
Daphnia	TPT	3	9.7 ± 2.2	0.049 ± 0.019	190 ± 50	72
Chironomus	TPT	5	19.9 ± 4.0	0.025 ± 0.009	680 ± 200	72
Chironomus	TBT	5	28.6 ± 8.9	0.102 ± 0.039	310 ± 100	72
Chironomus	TeBT	6	17.1 ± 9.5	0.002 ± 0.019	1200 ± 300	72
Thymallus	TPT	1	15.1	0.002	2200	168

[a] Data are given as mean ± SD. The *Daphnia* and *Chironomus* experiments were performed at pH 8.0, the *Thymallus* experiment at pH 8.3. The ionic strength in the experimental water was 13.5 mM for the *Daphnia* and *Chironomus* experiments, and 14.9 mM for the *Thymallus* experiment. A steady state was achieved for TPT in *Daphnia* and for TBT in *Chironomus*. In the TPT experiment with *Chironomus*, bioconcentration was approaching a plateau after 72 h, but did not reach a steady state. In the TeBT experiment with *Chironomus* and in the TPT experiment with *Thymallus*, considerable uptake persisted at the end of the experiment.

could be detected in the organisms. DPT did not increase significantly in either the organisms or the exposure waters during the experiments. For all species the mortality in the exposure waters did not differ significantly from the control mortality (Table 1). The experimentally determined BCFs and toxicokinetic parameters for *Daphnia* and *Chironomus* at pH 8.0 and and for *Thymallus* at pH 8.3 are listed in Table 2.

Since *Daphnia*, *Chironomus* and *Thymallus* are organisms with different physiological and ecological characteristics, the differences between the BCFs are not surprising. Part of the differences can be explained by the different lipid contents (Table 1). On a wet weight basis, the total lipid contents for *Daphnia*, *Chironomus* and *Thymallus* are 0.3, 0.6 and 3.1%, respectively i.e. they are in a ratio of 1:2:10. The ratio of the BCFs determined in *Daphnia*, *Chironomus* and *Thymallus* is 1:3.6:11.6. It should be noted that in *Chironomus* and *Thymallus* a steady state was not reached. Field studies with various vertebrates reported that the distribution pattern of TBT and TPT in the different organs was not related to the lipid content of the corresponding tissues.[12,20] It can therefore be assumed that species-specific factors other than lipid content, such as uptake mechanism, toxicokinetics and other factors account for the differences among species.

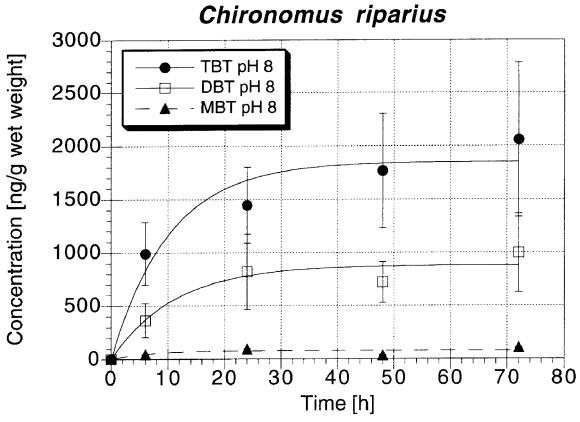

Figure 2 Time course of tissue TBT, DBT and MBT concentrations in *Chironomus* larvae. Exposure concentration was 6.5 µg TBT l^{-1} at pH 8.0. Data are given as mean ± SD of 50 *Chironomus* ($n = 5$). The increasing DBT concentrations indicate metabolism of TBT.

Figure 2 shows the bioconcentration of TBT in *C. riparius* at pH 8.0. A steady state was reached within the 72 h exposure period. For TBT, a BCF of 310 ± 100 was determined at the end of the experiment. In contrast to the experiment with TPT, significant concentrations of metabolites were observed. Dibutyltin (DBT) in the tissues increased during the experiment and reached a steady state (Fig. 2). After 72 h, the concentrations of the DBT tissue residues were about 50% of those of the TBT tissue residues. Monobutyltin (MBT) occurred only in low concentrations. The concentrations of DBT and MBT in the exposure waters remained constant over time at levels of 0.38 ± 0.09 µg l^{-1} and 0.12 ± 0.03 µg l^{-1}, respectively, indicating that DBT was not released by the organisms. However, the DBT tissue concentrations leveled off quickly (Fig. 2) suggesting that either TBT metabolism slowed down after about 24 h or the DBT produced was metabolized further.

Based on the log K_{ow} values for TPT and TBT at pH 8 (3.5 and 4.1, respectively), a lower BCF would be expected for TPT than for TBT. The TPT and TBT bioconcentration factors determined in *Chironomus* contrast sharply with the predictions of the octanol–water model. The BCF for TBT (310)

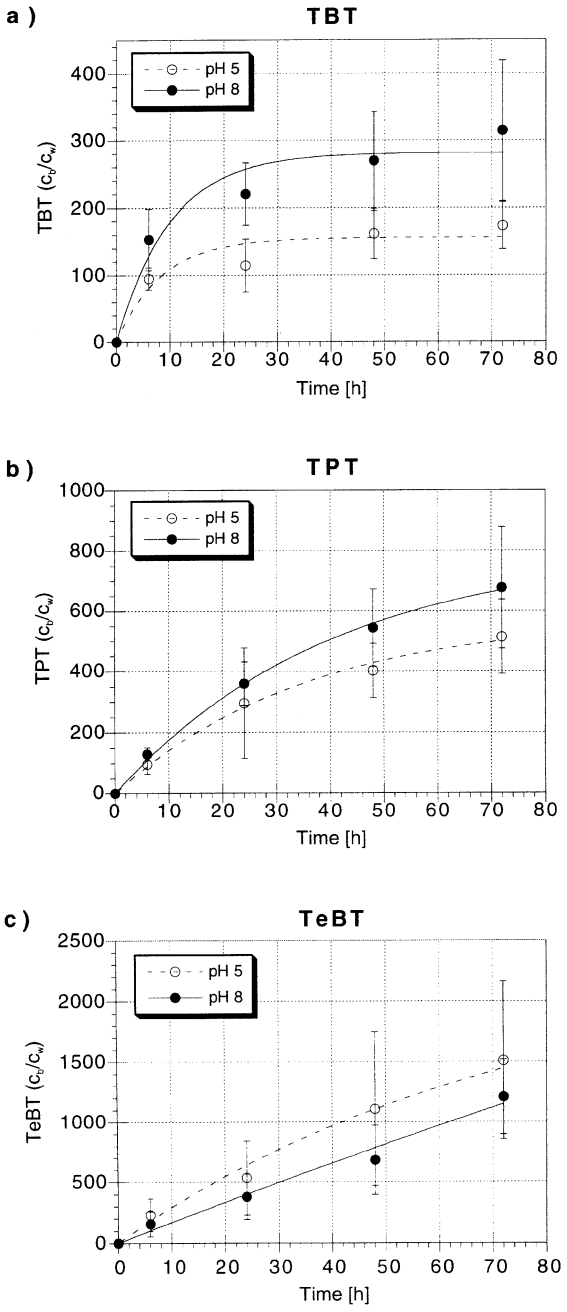

Figure 3 Bioconcentration of (a) TBT, (b) TPT and (c) TeBT in *Chironomus* larvae at pH 8 and pH 5. TBT and TPT data are given as mean ± SD of 50 *Chironomus* ($n = 5$), TeBT data as mean ± SD of 60 *Chironomus* ($n = 6$).

is lower by a factor of more than two than the BCF for TPT (680). This result is related to TBT metabolism in *Chironomus*, whereas no metabolism of TPT could be observed. The presence of an efficient TBT metabolism in *Chironomus* is supported by the fact that the DBT tissue residues increased significantly over time, reaching high levels, while the concentrations of MBT and DBT in the exposure waters remained constant at a low level. Furthermore, achievement of a steady state within three days is at least partly the result of TBT metabolism, which is indicated by the relatively high elimination rate constant k_2 of 0.10 ± 0.04 h^{-1} (Table 2). A short time until achievement of a steady state (seven days) has also been found for the uptake of TBT by *Hyalella azteca*,[43] but concentrations of DBT and MBT were not reported. Stäb *et al.*[12] found high concentrations of MBT and DBT in chironomids and *Gammarus* and pointed out the possibility of relatively high TBT degradation rates in these organisms. Our study demonstrates TBT metabolism in *Chironomus riparius*, which is of importance for the fate of TBT in the food webs of freshwater lakes and rivers, as various fish species are predators of chironomids.

A much higher bioconcentration of tetrabutyltin (TeBT) than TPT or TBT was observed in *Chironomus*. The experimentally determined BCF at pH 8.0 was 1200 ± 300 after 72 h (Table 2). No other butyltin compounds occurred at detectable levels in *Chironomus* larvae in this experiment. Metabolism was therefore not present, or only of minor importance. To our knowledge, no octanol–water partition coefficient (K_{ow}) for TeBT has been reported so far, but it is expected to be higher than the K_{ow} of TPTOH or TBTOH.

Influence of pH

Figure 3(a) shows the bioconcentration of TBT in *Chironomus* larvae at pH 8 and pH 5. A steady state was achieved within the exposure period. The BCF values determined after 72 h were 310 ± 100 (pH 8) and 170 ± 30 (pH 5). The difference between these BCFs is statistically significant (*t*-test, two-sided, $P = 0.045$, $n = 5$). As found at pH 8 (Fig. 2), the DBT residues in the larvae also increased at pH 5, to a level approximately 50% that of the TBT residues, indicating metabolism of TBT. These findings are consistent with the relatively high elimination rate constants k_2 of 0.10 ± 0.04 h^{-1} (pH 8) and 0.12 ± 0.04 h^{-1} (pH 5) compared with the k_2 values in the TPT experiment (Table 2). The bioconcentration of TPT was also higher at pH 8 than at pH 5 (Fig. 3b), but the difference was less pronounced than for TBT and was not statistically significant (*t*-test, two-sided, $P = 0.17$, $n = 5$). At

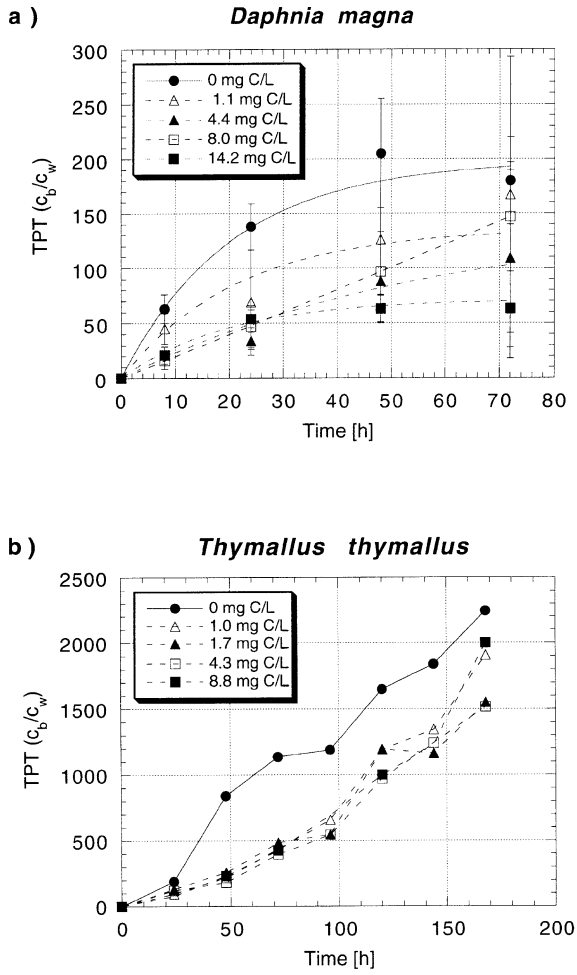

Figure 4 Bioconcentration of TPT at different concentrations of humic substances (DOC in mg C l^{-1} in (a) *Daphnia* and (b) *Thymallus* larvae, at pH 8.0 and pH 8.3, respectively. Data are given as mean ± SD of 30 *Daphnia* ($n = 3$) and values for 10 *Thymallus* larvae ($n = 1$), respectively.

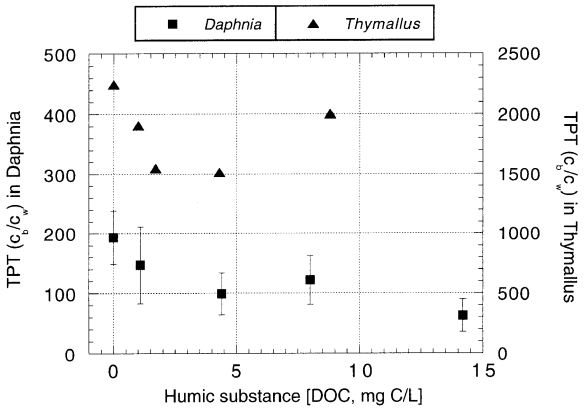

Figure 5 Influence of different concentrations of humic substances (DOC in mg C l^{-1} on the bioconcentration of TPT in (a) *Daphnia* and (b) *Thymallus* larvae. Data refer to the mean bioconcentration factors (c_b/c_w) at 48 and 72 h in *Daphnia* and at the end of the experiment (168 h) in *Thymallus* larvae.

the end of the experiment, the BCFs were 680 ± 200 (pH 8) and 510 ± 120 (pH 5).

The higher BCF of TBT at pH 8 than at pH 5 may be explained by a higher uptake of the uncharged hydroxide species as compared with the cation. At pH 8 the hydroxide complex TBTOH predominates. The pK_a value of TBT is 6.25.[26] Based on this pK_a, the fraction of TBTOH drops below 10% at pH 5, and the cation TBT^+ becomes the predominant species. TPT has a pK_a of 5.2,[26] which results in approximately 40% TPTOH and 60% TPT^+ at pH 5. The weaker effect of the pH on the bioconcentration of TPT, compared with TBT, is consistent with the higher fraction of the hydroxide species at pH 5. This indicates that the hydroxide species TBTOH and TPTOH are the predominant forms taken up by the organism.

Although the uptake of TBT and TPT by *Chironomus* larvae was higher at pH 8 than at pH 5, the difference is less pronounced than the octanol–water model would predict. For TBT, the overall octanol–water distribution ratios (log D_{ow}) are 4.1 and 2.9 at pH 8 and pH 5, respectively.[26] Based on these results, the accumulation of TBT would be expected to be more than tenfold lower at pH 5 than at pH 8. The experimentally determined (steady-state) BCFs differed by a factor of only 1.8. Clearly, the octanol–water partition model underestimates the bioconcentration of TBT at pH 5. The discrepancy may be related to different behavior of the TBT^+ cations in biological membranes than in octanol–water systems. It has been shown for substituted phenolic compounds that charged species partition to a higher extent into liposomes than the octanol–water distribution ratios (log D_{ow}) predict.[44] It should be investigated whether or not triorganotin cations behave in a similar fashion to charged species of other hydrophobic ionizable compounds. A further explanation for the discrepancy with the octanol–water model may be pH differences at the uptake membranes relative to the pH of the ambient water. Although the mechanism remains to be elucidated, the results indicate that *Chironomus* larvae take up the cations TBT^+ and TPT^+ more efficiently than the octanol–water partition model suggests.

In the experiment with TeBT (Fig. 3c), the *Chironomus* larvae showed a slightly higher BCF at pH 5 (1500 ± 650) than at pH 8 (1200 ± 310), but the difference was not significant (*t*-test, two-sided, $P = 0.39$, $n = 6$). In contrast to TBT and TPT, TeBT does not dissociate in water. Therefore, no pH dependence of the TeBT speciation was expected. The similar BCFs for TeBT at pH 8 and pH 5 thus indicate that the H^+ concentration itself has no significant influence on the bioconcentration at these pHs. It can be concluded, therefore, that the differences in the bioconcentration of TBT and TPT at pH 8 and pH 5 are related to the chemical speciation rather than to pH-induced alterations of uptake membranes.

Influence of humic substance

Figure 4(a) shows the bioconcentration of TPT in *Daphnia magna* in the presence of Aldrich humic substance (HS). The HS concentrations ranged from 0 to 14.2 mg C l^{-1} and the pH was 8.0. For *Daphnia* in exposure waters without HS, a BCF of 190 ± 50 was determined. The BCFs for experiments with 1.1, 4.4, 8.0 and 14.2 mg C l^{-1} were 150 ± 60, 100 ± 40, 120 ± 40 and 60 ± 30, respectively. The average mortality under TPT exposure ($33 \pm 12\%$, $n = 15$) was not significantly different from the control mortality ($20 \pm 11\%$, $n = 15$). A significant decrease in the BCF was only observable for an HS concentration of 14.2 mg C l^{-1} (t-test, two-sided, $P = 0.03$, $n = 3$). Lower DOC concentrations did not produce significant effects. A very similar picture was obtained in experiments with fish yolk-sac larvae, *Thymallus thymallus*, at pH 8.3 (Fig. 4b). The BCFs at the end of the experiments were 2240 (0 mg C l^{-1}), 1900 (1 mg C l^{-1}), 1550 (1.7 mg C l^{-1}), 1520 (4.3 mg C l^{-1}) and 2000 (8.8 mg C l^{-1}). Although there is a trend towards lower BCFs in the presence of HS, the differences between the determined BCFs are modest and may in part be attributed to the natural variability of the fish yolk-sac larvae. Mean mortality in all tests with TPT ($3.5 \pm 3.1\%$, $n = 5$) was not significantly different from the control (4%). It should be noted that a steady state was not reached within the 168 h experimental period.

A variety of studies have shown that the bioavailability of hydrophobic organic chemicals is reduced in the presence of humic substances.[30–33,45] Similar effects were shown for TBT in a previous study with *Daphnia* and *Thymallus*.[28] It can generally be expected that high concentrations of humic substances reduce the bioavailability of organotins. Concentrations higher than 10 mg C l^{-1} are, however, not very representative for many natural freshwater systems. Since the D_{OM} value of TPT is approximately 15 000 l kg_{OM}^{-1} for Aldrich humic substance (HS) at pH 8,[27] a concentration of 10 mg C l^{-1} is expected to reduce the free TPT concentration by a factor of 1.3. Lower HS concentrations will result in a minor reduction of the bioavailable TPT fraction. The results of the experiments with *Daphnia* and *Thymallus* lead to the hypothesis that relatively high HS concentrations (>10 mg C l^{-1}) are necessary to generate pronounced effects on the bioavailability of TPT to freshwater organisms (Fig. 5).

CONCLUSIONS

This study shows differences in the bioaccumulation of TPT in three different freshwater species representing different ecological niches. Total lipid content explains a part of the differences, but other species-specific factors such as toxicokinetics influence the balance between uptake and elimination. Rapid achievement of a steady state makes *Chironomus riparius* a promising species for monitoring TBT and TPT availability in contaminated harbor sediments. Furthermore, *Chironomus* is a key organism in benthic communities. Thus, the presence of an efficient TBT metabolism in *Chironomus* larvae can influence the distribution pattern of butyltin compounds in the food webs of freshwater lakes. If additional organisms at low trophic levels can metabolize TBT more easily than TPT, a lower biomagnification potential can be expected for TBT than for TPT.

Both the pH and humic substances influence the bioconcentration of organotins in freshwater organisms. The bioconcentration of TBT and TPT was found to be higher at the ambient pH of surface waters than at low pH. The hydroxide species TBTOH and TPTOH are taken up to a higher degree than the corresponding cations. The cations, however, are more readily taken up than the octanol–water model would suggest. Humic substances (HS) reduce the bioavailability of TBT and TPT, but only relatively high concentrations of HS lead to a substantial reduction. In contaminated harbor sediments, however, the bioavailability of TBT or TPT may be reduced by high organic matter contents.

Further experiments with *Chironomus riparius*

are needed to answer the open questions concerning organotin mass balances and to provide quantitative toxicokinetic data. Future research should focus on the bioavailability and toxicity of TBT and TPT in sediments.

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