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Bioaccumulation and Lethal Body Burden of Four Triorganotin Compounds

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Several triorganotins are used as biocides. Tributyltin (TBT) is used in antifouling paints, triphenyltin (TPT) is used as fungicide, and tri-c-hexyltin (TcHT) as miticide. To our knowledge, tri-n-hexyltin (TnHT) is not applied, but its biocidal properties are being investigated. Considerable amounts of tributyltin are introduced into the environment by its use in antifouling paints. In shallow and poorly flushed marina's, shortly after the introduction of tributyltin containing paints, effects on mussel and oyster cultures have been ascertained (WHO 1990). The environmental effects of the use of tributyltin has been extensively studied, whereas only a few studies have been carried out considering effects of triphenyltin and tri-c-hexyltin in the environment. Monitoring data of organotins in the environment mainly report tributyltin, although in some studies also triphenyltin has been determined. Levels of tributyltin in seawater are determined to reach 6.3 µg/L (Seligman et al. 1989; Tolosa et al. 1992; Stebbing 1990; Waldock et al. 1986; Hall et al. 1988). In marine sediments TBT levels of 9.2 ng/g were found (Tolosa et al. 1992). TPT levels of 0.07 µg/L in seawater and of 4.1 µg/g in marine sediment have been determined (Tolosa et al). In organisms such as mussels *(Mytilus galloprovincialis)* and oysters *(Crassostrea gigas)* 12 µg/g TBT and 1.O µg/g TPT has been determined (Tolosa et al. 1992; Ebdon et al. 1989). Also in freshwater systems TBT and TPT have been detected. TBT levels of 0.4 µg/L in water, 4.7 µg/g in sediment and 8.7 µg/g in the bivalve *(Dreissena polymorpha)* as well as TPT levels of 0.9 µg/g in sediment and 3.3 µg/g in the bivalve were determined (Becker et al. 1992). These values show that elevated levels of organotin are present in the environment.

The present study is a part of a research program studying properties and effects of triorganotins in the aquatic environment. Triphenyltin, tri-c-hexyltin and tri-n-hexyltin were studied since they contain the same number of carbon atoms, whereas the groups attached to tin have a different chemical structure. This might affect physical-chemical properties of the organotins, which possibly is reflected by differences in toxicity, and uptake and elimination rate constants. TBT is included to study the influence of a shorter chain length compared to TnHT, and because of the considerable amount of data available in the literature.

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In other studies, physical chemical parameters of organotin have been studied in relation to their toxicity. Toxicity parameters which have been correlated with molecular parameters include: LC_{50} of adult snail *(Biomomphalaria glabrata)* (Smith et al. 1979), EC_{40} of *Daphnia magna* (Vighi and Calamari 1985), effects on primary production of three axenic algae (Wong et al. 1982), and LC_{50} of the zoeal mud crab *(Rhithropanopeus harrisii* (Laughlin et al 1985, 1984). In the present study, bioaccumulation and lethal body burdens of TBT, TPT, TcHT and TnHT in guppy are compared, and related to their aqueous solubilities.

MATERIALS AND METHODS

Male guppies *(Poecillia reticulata)* were purchased by the local supplier in Utrecht. Their weight was 427 + 97 mg, length 3.7 + 0.3 cm, and fat percentage 3.1 ± 1.1 %. Tri-c-hexyltin chloride and tri-n-hexyltin chloride >98% (determined by thin layer chromatography) were kindly provided by Dr H.A. Meinema, Institute for Applied Chemistry TNO, Utrecht, The Netherlands. Triphenyltin chloride (Merck) was recrystallized from toluene. Tributyltin chloride >95% (Merck) was used without further purification. Triphenyltin chloride, tri-c-hexyltin chloride and tributyltin chloride were dissolved in water with the aid of a generator column (Opperhuizen 1986). Tri-n-hexyltin was dissolved by adding a weighed amount (1.35 µmol) directly into water (5 L), and stirred for 48 hr. The concentration of tri-n-hexyltin in water was measured in triplicate. Appropriate concentrations were obtained by dilution.

Table 1. Total surface areas (TSA) and melting point (T_m) of triorganotins.

	TSA [a]) Å^2	T_m [b]) °C
TBT	342.9	liquid at room temperature
TPT	329.6	105.3
TnHT	469 [c])	liquid at room temperature
TcHT	397.5	122.7

[a]) from Laughlin 1984

[b]) measured by Differential Scanning Calorimetry, Thermodynamic Centre, University of Utrecht.

[c]) Calculated from Laughlin, based on 20.9 Å^2 per CH,

To determine organotin bioaccumulation. Fish (19, 20 and 73, respectively) were exposed in 30 L to a mean concentration (± sd) of 1.19 ± 0.76, 1.73 ± 1.21 and 5 ± 0.3 nmol/L for TcHT, TnHT and TPT, respectively. Fish (1) and water (20, 200 and 100 mL, resp) were sampled regularly at the same time. After the period of exposure, the remaining fish were transferred to clean water, which was constantly aerated and filtered with a carbon filter, to remove excreted organotins. The water was changed and the aquarium cleaned when necessary. Fish were fed only during the period of elimination. Fish were killed by immersion in liquid nitrogen and stored at -20°C until analysis. Oxygen concentration was 9.3 ± 1.0 mg/L, pH was 8.3 ± 0.2 and temperature was 19.5 ± 1.3°C.

For the lethal body burden experiments, groups of 4 fish were statically exposed to different concentrations of triorganotin in water. Fish were exposed until death or killed after 14 d. Dead fish were removed from the aquarium and stored at -20°C. The mean (± sd) TcHT concentrations in water were 109 ± 6 nmol/L

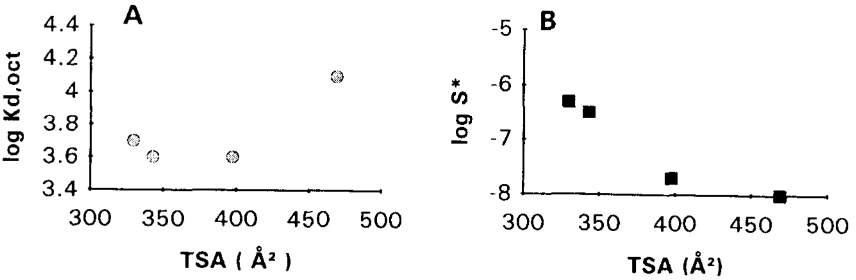

Figure 1. The octan-1 ol/water partition coefficient (Kd,oct) (A) and supercooled liquid solubility (S*) (B) of the four triorganotins versus total surface area (TSA). The supercooled liquid solubility is: log S* = log S + ln 0.01 (T-Tmp); S = solubility (mol/mol), Tmp = melting temperature (Opperhuizen et al 1988)

(in 2 L), 23 ± 3 nmol/L, 1.8 ± 1.5 nmol/L (in 4 L), and 0.99 ± 0.6 nmol/L (in 18 L). Fish were exposed in different volumes of water to avoid excessive use of toxic compound. Volumes of the duplicate water samples were 15, 50, 200 and 200 mL, respectively. To assure detectable amounts hexyltin in the sample, different volumes were taken. The mean TnHT concentrations in water were 54 ± 51 nmol/L (in 1.5 L), 5.4 ± 2.8 nmol/L (in 3 L) and 0.72 ± 0.62 nmol/L (in 10 L). Volumes of the triplicate water samples were 15, 15 and 50 mL, respectively. Water was sampled just before fish were added to the aquaria and after the fish were dead. Fish were not fed. Oxygen concentration ranged between 6.0 and 9.0 mg/L, pH was 7.9 ± 0.6, and temperature 20 ± 1 °C (determined at start and end of the experiment).

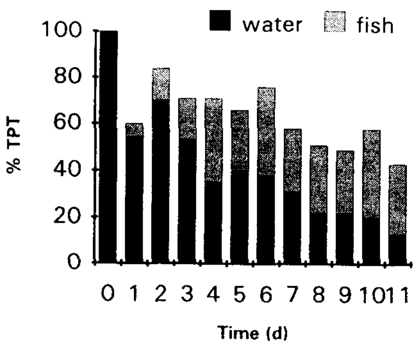

Figure 2. Mass balance of TPT. The water was not renewed, hence for every sample a mass balance was calculated.

Water samples were extracted with 6 mL 2,2,4-trimethylpentane (Baker). The organotins present in 5 mL trimethylpentane were methylated by adding 1 mL methylmagnesium chloride (20% in THF, Merck). After 5 min 2 mL of a solution of ammonium chloride (Merck) in water was added. Organotins in fish were analyzed as previously described (Tas and Opperhuizen 1991 a). A Carlo Erba (HRGC 5300) Mega Series equipped with a Flame Photometric Detector (Carlo Erba, FPD 500) was used to detect the organotins. It contained a 30-m DB 5 column (J & W Scientific), i.d. 320 mm, film thickness 0.25 µm. The organotins were injected on column at 80°C, the column was then heated to 280°C with a rate of 40°C/min. Peaks were recorded and integrated with a Chrompack C-R1A (Shimadzu) integrator. Conditions, standards and recoveries have been reported elsewhere (Tas and Opperhuizen 1991 a).

RESULTS AND DISCUSSION

Tributyltin chloride has the highest aqueous solubility of the four organotins (approximately 62 µmol/L, 20 mg/L). The solubility of triphenyltin chloride is approximately one order of magnitude lower (approximately 5 µmol/L, 1.2 mg/L) than that of tributyltin chloride. This difference in solubility does not correspond with the difference in size of the two molecules (Table 1). Since triphenyltin chloride has a smaller total surface than tributyltin chloride, it is expected that TPT has a higher solubility. However, since triphenyltin is a solid at room temperature, and tributyltin is a liquid, the energy of melting might account for the lower solubility (Opperhuizen et al. 1988).

Table 2. Concentrations in water during lethal body burden experiments, determined before exposure (C_w begin), and after the fish had died (C_w end).

Compound	C_w begin [a]) nmol/L	C_w end [a]) nmol/L	Survival time d
TcHT	107	111	0.18
	26	20	1.5
	3	0.4	11
	0.95	1.0[b])	11
TnHT	84 (42)	7 (5)	2.6
	7 (1)	4 (3)	9
	1.3 (0.3)	0.2 (0.4)	9

[a]) For TcHT duplicate samples were taken, hence no standard deviation could be obtained. The variation between duplicates is negligible, unless otherwise indicated. TnHT was sampled in triplicate, values indicate mean (sd).

[b]) mean of 0.2 and 1.8 nmol/L

The solubilities of the two trihexyltin chlorides are approximately one order of magnitude lower than that of triphenyltin chloride, hence two orders of magnitude lower than that of tributyltin chloride. The solubilities for tri-c-hexyltin and tri-n-hexyltin are determined to be 0.02 ± 0.01 mg/L and 0.04 ± 0.02 mg/L, respectively (Tas 1993). Hence, the different solubilities of the hexyltins and triphenyltin may be explained by structural parameters. Triphenyltin chloride possesses aromatic n-electrons, which can have interaction with water molecules, and may account for a higher solubility than the hexyltins. The trihexyltin compounds are the molecules with the largest TSA of the four organotins (Table I), and they lack n-electron interactions which are present in triphenyltin. When the supercooled liquid solubility (S*) is determined (Opperhuizen et al 1988), a correlation with TSA is observed by visual inspection (Figure 1). However, a correlation of the octan-1-ol/water partition coefficient ($K_{d,oct}$) with TSA is less clear.

Mass balances of the lethal body burden experiments, calculated at the end of the period of exposure, are variable. The decline in aqueous concentration (Table 2), could not be explained by uptake in fish. The mass balances of the uptake experiments with the trihexyltins are poor. After 4 d exposure only 26% of the tri-c-hexyltin and 9% of the tri-n-hexyltin were recovered from the system. Hexyltin adsorption to the glass aquarium walls may explain the loss of organotin, but this was not analytically verified. The amounts of TPT recovered in water and fish are shown in Figure 2. The concentrations of triorganotins in fish (C_f, nmol/g) are presented in Figure 3. The uptake and elimination rate constants (k_1 and k_2 in mL/g.d and d^{-1}, respectively) are calculated from these data by using one fish-compartment, first order kinetics [Branson et al. 19751:

$$C_f = \frac{k_1}{k_2} C_w \left(1 - e^{k_2 t} \right) \tag{1}$$

in which C_w is concentration in water (nmol/mL) and t is time (d). Since, in the present study, the aqueous concentrations are not constant, the uptake rate constants are calculated with a computer programm specially derived for these kind of circumstances (Gobas et al 1993). The rate constants for the two hexyltins and triphenyltin, as well as previously obtained rate constants for tributyltin and triphenyltin in guppy (Tas et al 1990; Tas 1993) are listed in Table 3. The uptake rate constants of the four organotins are comparable, and range between 50 and 320 mL/g.d. Uptake rate constant of organotins are considerably lower than those of many chlorinated aromatic hydrocarbons, such as polychlorinated benzenes, and -biphenyls (Opperhuizen and Sijm 1990). These narcotic compounds have uptake rate constants of approximately 1000 for small fish (< 5 g). Polarity and ionisation of organotins may be an explanation for the difference in uptake rate constants between aromatic hydrocarbons and organotins.

The elimination rate constants of the four organotins differ substantially. During the 143-d period of elimination the TPT concentration in fish declined to approximately 0.3 µg/g (Figure 3). TPT shows a single logarithmic elimination, with an elimination rate constant of 0.014 d^{-1}. For TnHT and TcHT no elimination is observed during the experimental period (Figure 3). There is a correlation between the aqueous solubility and the elimination rate constants. TPT, which has a higher aqueous solubility, is also eliminated faster than TcHT and TnHT. And TBT, which has the highest solubility of the four triorganotins, also has the highest elimination rate constant. However, the elimination of TBT shows more phases of elimination, so that a single elimination rate constant cannot be obtained. This may be a consequence of the short period of accumulation (15 min) compared to the period of uptake of the other triorganotins (4-30 d) (Tas 1993). Another explanation may be that the elimination of TBT is partly due to biotransformation of TBT. It is unlikely that the other organotins are significantly biotransformed, since no compound is lost during elimination. For triphenyltin, the elimination was studied using cold triphenyltin chloride (TPTC) and radiolabelled tripheyltin hydroxide (^{14}C-TPTH) (Tas et al 1989). To obtain a reliable elimination constant, the elimination was studied during 143 d (three half-lives). The elimination rate constant obtained during 30 d of elimination was 0.014 d^{-1}, which is identical to the k_2 of cold TPTC obtained in the present study (0.041 d^{-1}). The disadvantage of radiolabelled compound is that the mother compound plus the metabolites present in the fish are analyzed. If the TPT is metabolized, the k_2 determined with cold test material should be higher than with radiolabelled compound. Hence an identical elimination rate constant for cold and radiolabelled compounds gives an indication that biotransformation is slow or absent.

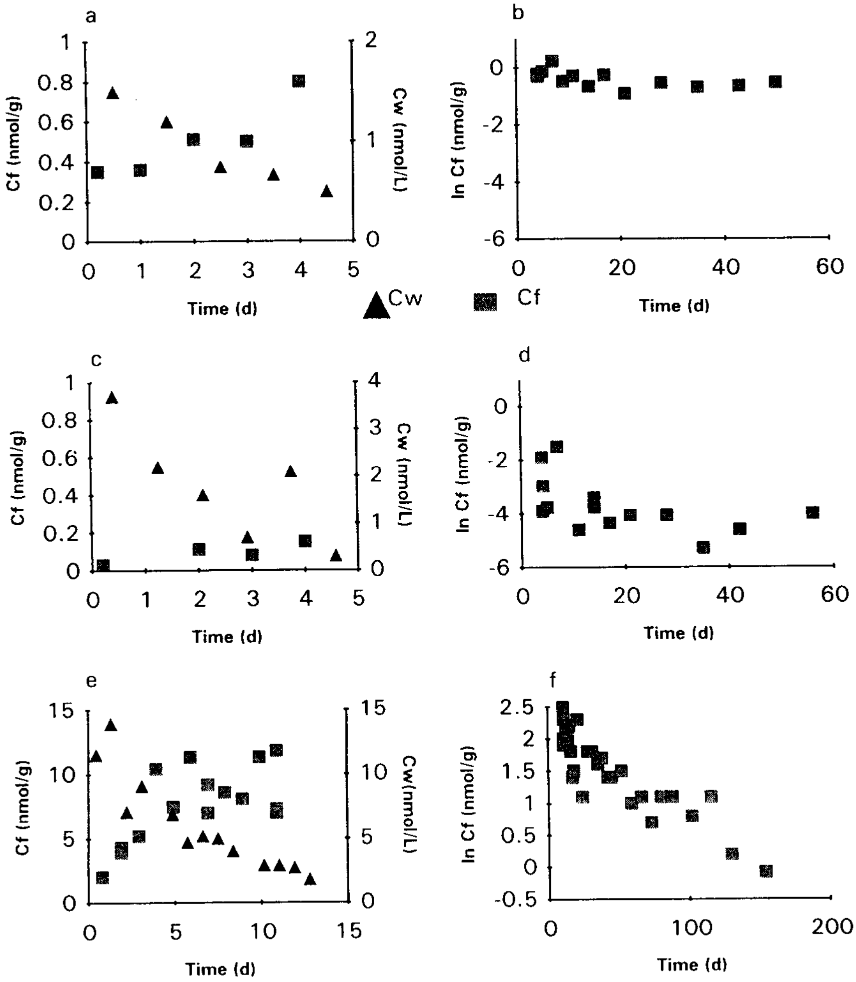

Figure 3. Uptake and elimination of tri-c-hexyltin (a and b), tri-n-hexyltin (c and d), and triphenyltin (e and f). Each data point represents one fish or water sample.

Therefore, the elimination of TPT is proposed to be predominantly physico-chemical. The amounts of ^{14}C-TPTH in fish and water are calculated assuming that the determined label was present as the parent compound. Negligible biotransformation of triphenyltin is in agreement with results of other studies. By RF Lee (Skidaway Institute of Oceanography, University System of Georgia, Savannah, Georgia, personal communication) the biotransformation of ^{14}C-TPTH in the crab hepatopancreas was found to be very slow. Tsuda et al. (1988) reported that biotransformation of TPTC in gold fish is slow: after 14 days of exposure 97.5% of the organotin is present as TPTC and 2.5% as diphenyltin, while no monophenyltin was detected. Comparable degradation of TPT however, can also take place during the extraction and clean-up procedure that was used. It was found that due to the derivatization of TPTH approximately 1% di- and monophenyltin occurred (Tas and Opperhuizen 1991 a).

Table 3. Uptake and elimination rate constants (k_1 and k_2, respectively) for four triorganotins

Compound	C_w nmol/L	expos. period d	k_1 mL/g.d	elim. period d	k_2 d^{-1}
TcHT[a])	1.2 (0.8)	4	320 (80)	63	no correlation
TnHT[a])	1.7 (1.2)	4	50 (12)	56	no correlation
TPT[a])	5 (0.3)	11	140 (30)	154	0.014 (0.001)
TPT[b])	11 (0.2)	30	41 (2)	53	0.014 (0.002)
TBT[c])	4400-29	0.7h-5d	80 (15)	23 [d])	[e])

[a]) This study [b]) data from Tas et al 1990 [c]) data from Tas 1993

[d])The uptake rate constant and the elimination rate constant were determined in separate experiments.

[e]) Since different phases in the elimination could be distinguished, no unique elimination rate constant can be given. The different phases have half-lives of 0.1, 2.3 and 69 days.

Fish exposed to the highest concentrations of TcHT and TnHT died, and they survived the lowest concentrations. The time of death increased with decreasing aqueous concentration, whereas the concentrations in fish remained constant. The TcHT concentrations in fish which were killed after 11 d were 4.9 ± 2.8 and 2.6 ± 0.3 nmol/g for the concentration of 1.8 and 1.0 nmol/L, respectively. Hence they were below the lethal body burden determined in the higher exposure concentrations. However, for TnHT the concentration in the fish killed after 9 days were 1.0 ± 1.0 and 0.4 ± 0.5 nmol/g for the concentrations of 5.4 and 0.7 nmol/L, respectively. Hence for TnHT the concentrations in surviving fish are very close or similar to the lethal body burden determined in the exposure concentration of 46 nmol/L. For comparison, the previously obtained lethal body burdens of triphenyltin en tributyltin in guppy (Tas et al 1991; Tas 1993) are also included (Figure 4). The lethal body burden of TcHT, TPT and TBT are comparable, whereas the lethal body burden of TnHT is lower. The aqueous solubility of the two hexyltins is much lower than those of TBT and TPT. Hence, lethal body burdens of TPT and TBT could be determined at much higher aqueous concentrations. For TBT a decreasing trend in lethal body burden with decreasing aqueous TBT concentration has been observed (Tas 1993). This is explained by adsorption of a certain amount of the compound to the exterior of the fish, this amount being higher when higher exposure concentrations have been used. It may be possible that this will occur for the TnHT and the TcHT as well. However, this cannot be verified since the aqueous solubilities of the latter compounds are too low to determine the lethal body burden at the same concentration as TBT. The lethal body burden of TPT has only been determined at relatively high aqueous concentrations compared to the other organotins. It will be interesting to determine the lethal body burden of TPT at lower aqueous concentrations, comparable to the TBT concentrations, to check whether a decrease in lethal body burden will occur. The solubility of TBT in water is higher than the highest concentration tested. When fish are exposed to a very high TBT concentration, a very short time of death is obtained. Fish exposed to a concentration obtained directly by the generator column (approx 20 mg/L), die within 2 min.

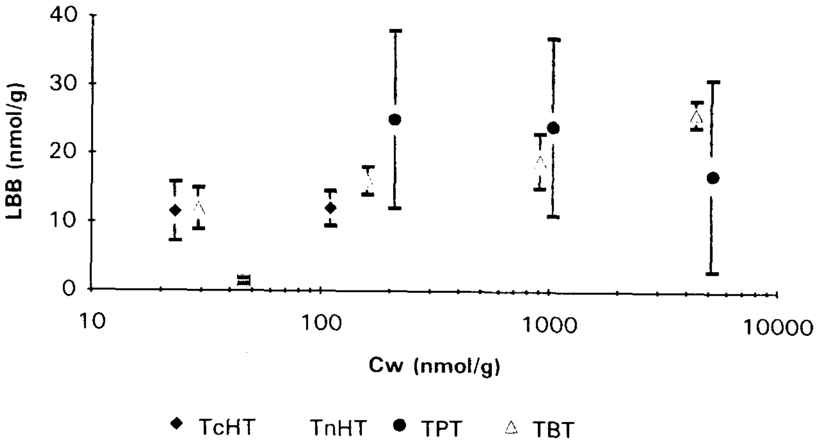

Figure 4. Lethal body burdens of tributyltin (TBT), triphenyltin (TPT), tri-c-hexyltin (TcHT) and tri-n-hexyltin (TnHT), versus exposure concentration. Bars indicate standard errors, and each data point represents the mean of 4-8 fish.

The present study shows that estimated uptake rate constants for TcHT and TnHT are comparable to those of TPT and TBT, while the elimination of the former compounds is much slower. The lethal body burden for TnHT is lower. Differences of the toxicokinetic behavior of the hexyltins can be explained by differences in physicochemical properties, such as aqueous solubility.

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