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Toxicity and Accumulation of Tributyltin Chloride on Tilapia

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Acute toxicity (96 h) and bioconcentration experiments of tributyltin chloride (TBT) in tilapia were conducted in an aqueous solution with salinity of 15‰, and a toxicity mechanism has been suggested. The 96-h LC_{50} was 3.80 μg Sn l^{-1}. Bioconcentration factors in different tissues increased in the order muscle < gill < viscera. Studies on the metabolism of TBT showed that it can be easily degraded to DBT (dibutyltin) in these tissues. Further degradation of DBT to MBT (monobutyltin) was much more difficult. A mesocosm was used for the first time to study the toxicity of TBT in tilapia. The result demonstrated that the TBT bioconcentration curve changed with the initial concentrations of TBT but the order of bioconcentration in the tissues did not change. © 1997 John Wiley & Sons, Ltd.

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INTRODUCTION

In recent years, a number of organotin compounds such as tributyltin, triphenyltin and tricyclohexyltin have been more and more widely used as insecticides, fungicides, bactericides and antifouling agents; thus they can be released into the environment through a great variety of entry pathways. TBT, especially, has widespread uses as an effective biocide in marine antifouling paints and coatings. It displays extreme toxicity to aquatic organisms other than the target organisms.[1] The aquatic toxicity of butyltin compounds depends on the degree and nature of alkyl substitution.[2] TBT has the highest biotoxicity, by disturbing the function of mitochondria. The toxicity of dibutyltin is lower than that of TBT, by blocking the absorption of oxygen in the mitochondria, whereas monobutyltin has no obvious toxic effect on mammals. Therefore it was necessary to make a more detailed investigation of aquatic toxicity of these species.

Tributyltin species have been demonstrated to produce sublethal effects on a number of nontarget organisms. These include the development of male characteristics in females; this phenomenon has been observed in numerous species of molluscks[3, 4] and the mud snail.[5] Such effects may occur at a tributyltin concentration of 1 ng l^{-1}. Shell thickening in the oyster, which is significantly apparent at a concentration of 1.6 ng l^{-1} TBT in water, has also been demonstrated to be due to TBT compounds.[6] These effects are assumed to be caused by bioconcentration of relatively low levels of tributyltin in the organisms.

However, much is still unknown about the toxicity of butyltin compounds. For example, in laboratory toxicity studies the experimental concentration is usually much higher than in the real environment, while the experimental space is much smaller. It is not known whether the bioconcentration curves may be different at different concentrations of environmental pollutants. Also, the range of test organisms is not sufficiently complete. Further study is needed for a better understanding of the tributyltin bioconcentration mechanism.

In this work, a classical 96-h acute toxicity experiment was first conducted to determine the safety concentration of TBT in tilapia. Then two

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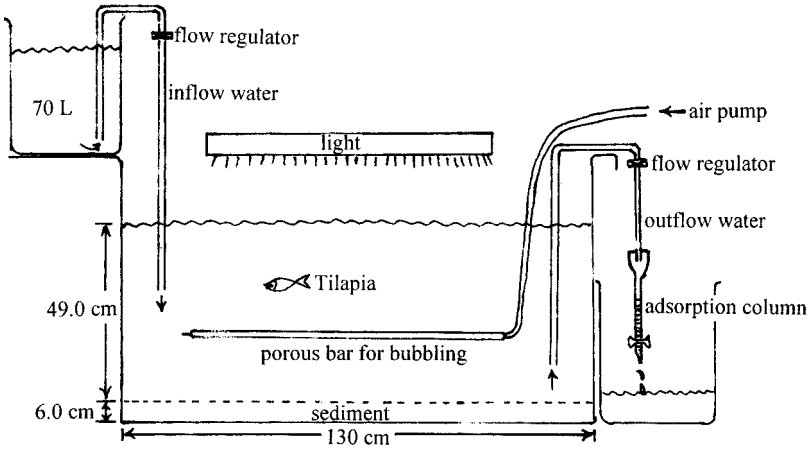

Figure 1 The mesocosm used in the experiment.

different kinds of bioconcentration tests, the microcosm test and the mesocosm test, were conducted. One was carried out with the TBT safety concentration, 500 ng l^{-1}, the other with a TBT concentration of only 45 ng l^{-1}, which is very close to that in the environment. This paper is intended to disclose the mechanism of the butyltin toxic effect.

MATERIALS AND METHODS

Chemicals

Tributyltin and dibutyltin chloride, with a purity of about 96%, were purchased from the American Aldrich Corporation. To diminish the possible effect of degraded contaminants, the TBT and DBT were cleaned by thin-layer chromatography (TLC). Tropolone was purchased from Sigma Corporation and 10% tetramethylammonium hydroxide from Beijing Duli Chemical Corporation, People's Republic of China.

Test fish

Tilapia were purchased from the Hangu Luqian Fish Farm (Tianjin, China). They were 4.41 ± 0.15 cm in body length and weighed 3.64 ± 0.13 g. Butyltin compounds were not detected in the fish before exposure to these chemicals.

After purchase, two weeks were taken to make the fish fit a gradual change of salinity from 0 to 15‰. The 15‰ salinity was selected because, in the mesocosm test, the water was sampled from the Haihe estuary, Tianjin Harbor, where the salinity of the water was 15‰. To exclude the

Table 1 Characteristics of water and sediment in the mesocosm test

	Particulate matter (mg l^{-1})	pH	Salinity (‰)	Initial concentration (ng l^{-1}) TBT	DBT	Heavy-metal concentration (mg l^{-1})[a] As	Cd	Zn	Pb	Sn
Water	6.5	8.45	15.55	330	52.7	0.748	0.010	0.210	0.208	10.82

	Organic carbon (%)	Cation-exchange capacity (mg/100 g)	Whole salt (%)	Elemental analysis (%) C	H	N	Heavy-metal concentration (mg/100 g dry seed) As	Cd	Zn	Pb	Sn
Sediment	0.59	13.45	1.04	1.50	0.37	0.00	679.4	11.7	416.8	126.8	652.3

[a] The sediment was transferred from the Haike estuary, which is generally contaminated.

Table 2 The results of acute toxicity tests on TBT in tilapia (20 ± 1 °C)

TBT concentration ($\mu g\ l^{-1}$)	0.00	1.00	1.50	2.24	3.35	5.00
log *c*		0.000	0.175	0.350	0.525	0.700
96-h mortality (%)	0	20	25	30	40	65
Probit of mortality[a]	—	4.16	4.33	4.48	4.75	5.39

[a] See Ref. 9.

impact of salinity, all the experiments were done with a consistent salinity of 15‰.

Test system

The 96-h acute toxicity test was carried out in 40-l white ceramic barrels. TBTCl was spiked into the water at the following concentrations: 0.00, 1.00, 1.50, 2.24, 3.35, 5.00 $\mu g\ l^{-1}$. The temperature of the test water was maintained at 20 ± 1 °C and the salinity at 15‰. Ten acclimatized fish were used in each concentration test. Water was replaced at 24-h intervals; dead fish were recorded and the mortality was calculated. Each experiment was repeated twice.

The microcosm bioconcentration test was carried out in a 120-l glass container under static conditions. During the 50 days of the experiment, half the volume of the water was renewed daily, and the salinity and temperature of the test water were maintained constant as indicated above. The TBT concentration was kept constant at 500 ng l^{-1}. On the fiftieth day, a few test fish were collected. After pretreatment, the fish were observed under an electron microscope. Some control fish were treated simultaneously in the same way as the fish sample. The toxic effect of TBT on different tissues was observed.

Furthermore, a mesocosm of 700 l (Fig. 1) was used to simulate a real estuarine environment, i.e. the Haihe estuary and bioconcentration at very low aqueous concentrations of TBT in the fish was studied. The mesocosm was established as follows. First 700 l of water and 100 l of sediment were transferred from the Haihe estuary. The system was allowed to reach an equilibrium over one week. Then about 300 cultured fish were put in the test system. Another week was allowed to pass before the bioconcentration test began. On the day the test began, some water and sediment variables were tested (Table 1). Then only TBT was added to the system to make an initial TBT concentration of 530 ng l^{-1}. The test lasted 40 days.

Each day, 70 l of the mesocosm water was circulated. An adsorptive agent was used to remove all butyltin compounds from the outflow water. In the inflow water the TBT concentration was always maintained at 200 ng l^{-1} by spiking an amount of TBT into the water.

Analysis

Determination of butyltin compounds in the fish samples was carried out by the following method.[7] Three to four fishes were sampled and 10 ml of 10% tetramethylammonium hydroxide was added to digest the fish sample. The homogenized solution was placed in a 60 °C water bath for 1 h. After being cooled, the homogenized solution was neutralized with concentrated hydrochloric acid to a pH value of about 1; then it was extracted for half an hour with 15 ml of 0.3% tropolone in ethyl acetate and hexane (3:2, v/v) after adding 3.0 g of NaCl. The organic layer was separated and concentrated using a rotary evaporator, then derivatized with PeMgBr (Pe = pentyl). The excess Grignard reagent was destroyed with 0.5 M H_2SO_4. The identification of butyltin derivatives in the

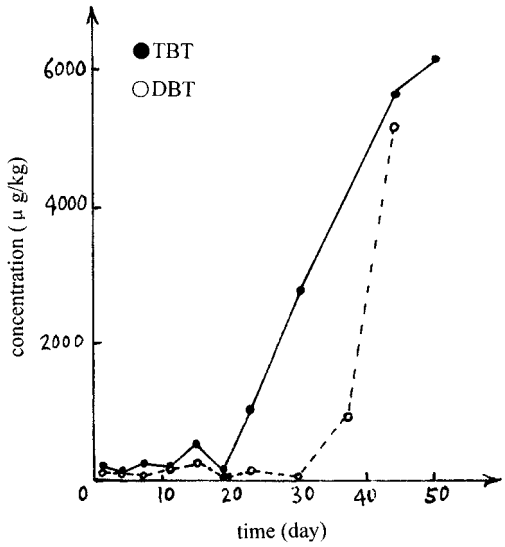

Figure 2 Concentrations of TBT and DBT in the tilapia (20 ± 1 °C) (aqueous TBT concentration: 500 ng l^{-1}).

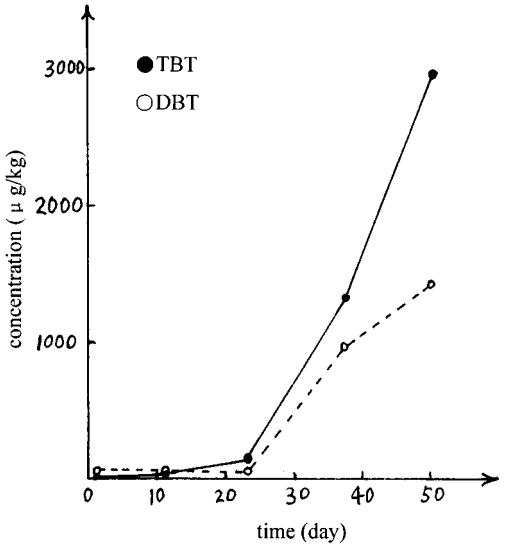

Figure 3 Concentrations of TBT and DBT in the muscle of tilapia (20±1 °C) (aqueous TBT concentration: 500 ng l^{-1}).

organic layer was determined by a GC–AAS combination technique. Determination of butyltin compounds in muscle, gill and viscera was as described above.

The concentrations of butyltins in the test water were determined by the following procedure.[8] A measured volume (5 l) of water was extracted with 200 ml of 0.05% tropolone in benzene after the addition of 100 ml of 48% HBr to adjust the pH value to 1. The organic layer was separated by centrifugation, evaporated to 10 ml

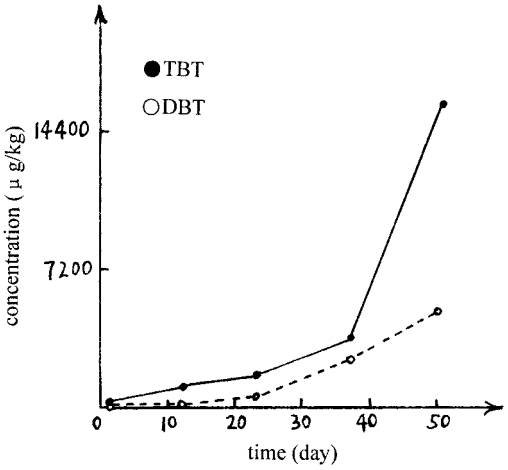

Figure 4 Concentrations of TBT and DBT in the gill of Tilapia (20±1 °C) (aqueous TBT concentration: 500 ng l^{-1}).

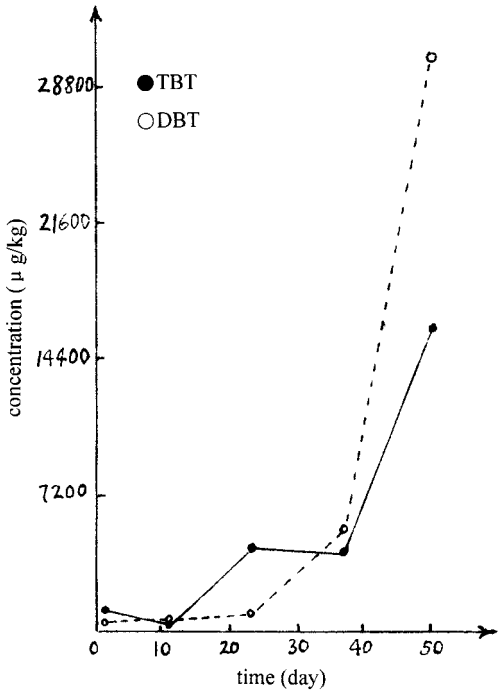

Figure 5 Concentrations of TBT and DBT in viscera of Tilapia (20±1 °C) (aqueous TBT concentration: 500 ng l^{-1}).

and derivatized with PeMgBr, and analyzed by the GC–AAS combination technique.

The above analysis was all carried out using a gas chromatograph equipped with a quartz furnace atomic absorption spectroscopy as detector. The method was described in detail previously[8]. Chromatographic speciation was on a 3 mm (i.d.)*lm(l) glass column packed with 3% OV-225 on Chromosorb W AW-DMCS (80–100 mesh). The detection limits of TBT, DBT and MBT were 0.15 ng, 0.19 ng, 0.25 ng respectively.

Calculation of BCF

(BCF) was calculated by the following equation:

$$\mathrm{BCF}=\frac{\text{concentration of chemical in fish (ng kg}^{-1})}{\text{concentration of chemical in water (ng l)}} \quad [1]$$

In the microcosm bioconcentration test, the TBT concentration in the water remained constant, i.e. at 500 ng l^{-1}, and in the mesocosm test the concentration in the water together with that in the fish was determined.

RESULTS AND DISCUSSION

Acute toxicity test of TBT on tilapia

A 96-h acute toxicity test of TBT on the fish was conducted; the results are listed in Table 2. The following equation expresses the linear relationship between the logarithm of concentration ($x = \log_{10} c$) and the probit of mortality (y):[9]

$$y = 4.05 + 1.65x \quad [2]$$

Suppose $y = 5$; the LC_{50} and a 95% credible limit can be calculated:

$$LC_{50} = 3.80\ \mu g\ l^{-1}$$

$$5\%\ \text{credible limit: } 2.57–5.62\ \mu g\ l^{-1}$$

Therefore the safety concentration of TBT in tilapia is $\frac{1}{10} \times LC_{50} = 0.38\ \mu g\ l^{-1} = 380\ ng\ l^{-1}$.

Based on the result, a TBT concentration of $500\ ng\ l^{-1}$, which was a little higher than the safety concentration, was chosen in the microcosm bioconcentration test to make the analysis easier, especially for TBT and its degradation products DBT and MBT.

The microcosm bioconcentration test of TBT on tilapia

Tilapia were exposed to water containing $500\ ng\ l^{-1}$ of TBT for 50 days. Figures 2–5 show the bioconcentration of TBT and its metabolites in the whole fish, muscle, gill and viscera of tilapia. During the 50 days of the experiment, enrichment of TBT in the whole fish did not reach a plateau, but at the end of the test it increased more slowly (Fig. 2). The order of enrichment for the experiment was viscera->gill>muscle. TBT can be metabolized to DBT in the fish, which is most obvious in viscera (Fig. 5). Only a very small amount of DBT can be further metabolized to MBT. When the experiment had proceeded for 50 days, rather high concentrations of DBT was detected in viscera, gill and muscle. The enrichment order of DBT was the same as that of TBT. This characteristic was due to their lipophilic nature, which caused butyltin compounds to be easily enriched in tissues where more lipid existed. Tsuda *et al*. also demonstrated that in carp the enrichment of TBT in viscera such as kidney, liver and bladder was higher than that in muscle.[10]

TBT degradation in different tissues varied remarkably. It was much the most easily degraded to DBT and a small amount of MBT in viscera. At the end of the test, DBT concentration in viscera was nearly two times higher than that of TBT; although TBT could also be degraded in the gill and muscle, the amount of degradation was much smaller than that in viscera. Thus DBT concentrations in gill and muscle were always smaller than TBT concentrations.

Effect of TBT on liver and brain of tilapia in the microcosm test

By the end of the bioconcentration test, namely on the 50th day of the test, the effect of TBT on the cells and mitochondria in liver, and on the cerebral nerves, blood vessels and mitochondria in the brain, were studied under a electron microscope.

The electron micrographs of the hepatocytes in the control group showed normal morphology with a homogeneous cytoplasm and abundant organelles including mitochondria and ribosomes. The intact outer and inner membranes of the mitochondria and structural integrity of the ridge existed in most of the cells observed. The endoplasmic reticulum appeared normal; the ribosomes of rough endoplasmic reticulum adhered tightly to the outer membranes (see Figs 6 and 7). Significant changes in the hepatocytes were observed in exposed groups. The nucleus was condensed and its envelope was not clear.

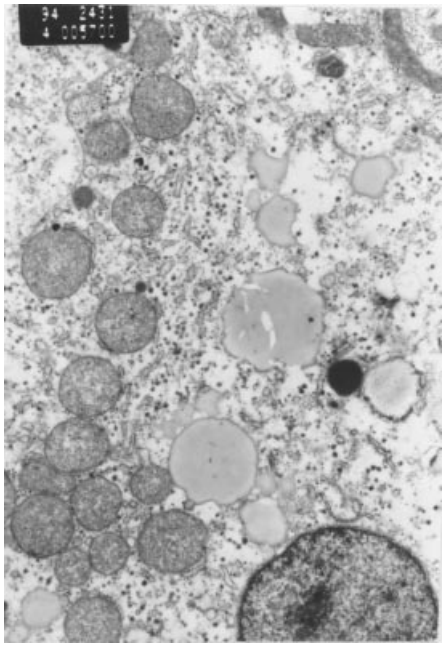

Figure 6 Hepatocytes in control fish (×5700).

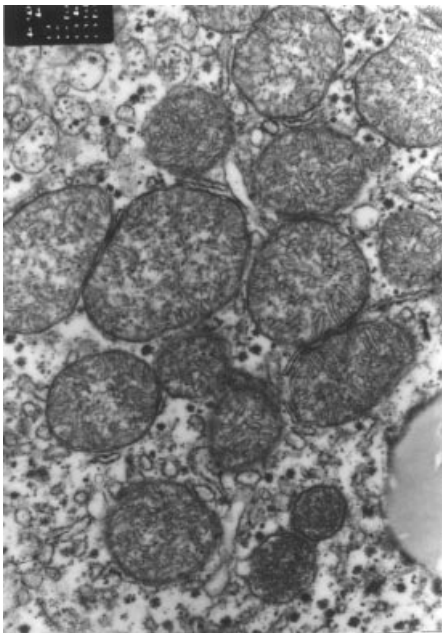

Figure 7 Hepatocytes in control fish ($\times$11 000).

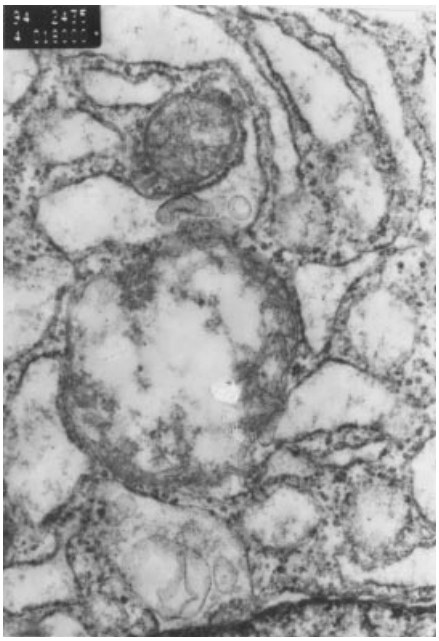

Figure 9 Hepatocytes in exposed fish ($\times$18 000).

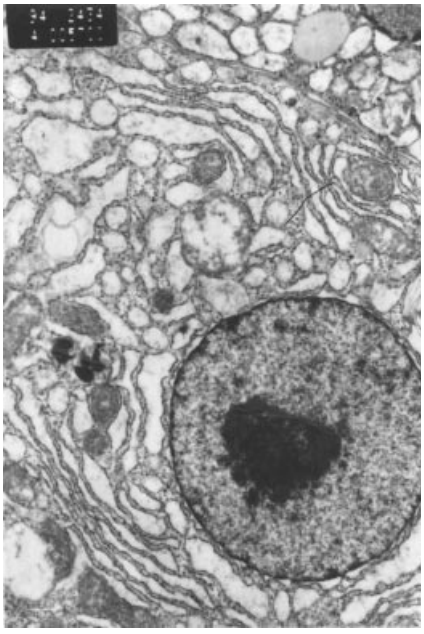

Figure 8 Hepatocytes in exposed fish ($\times$5700).

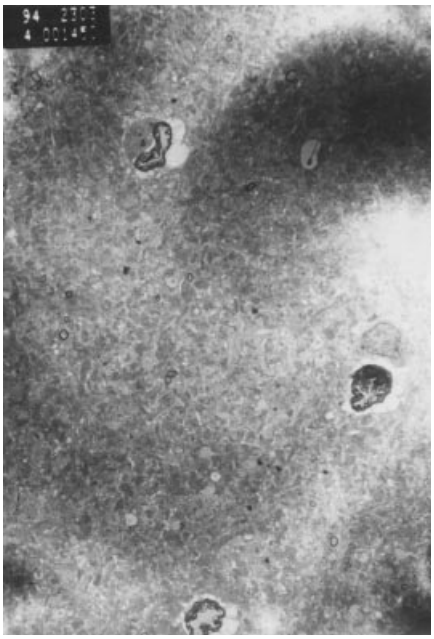

Figure 10 Cerebral vascular system in exposed fish ($\times$1450).

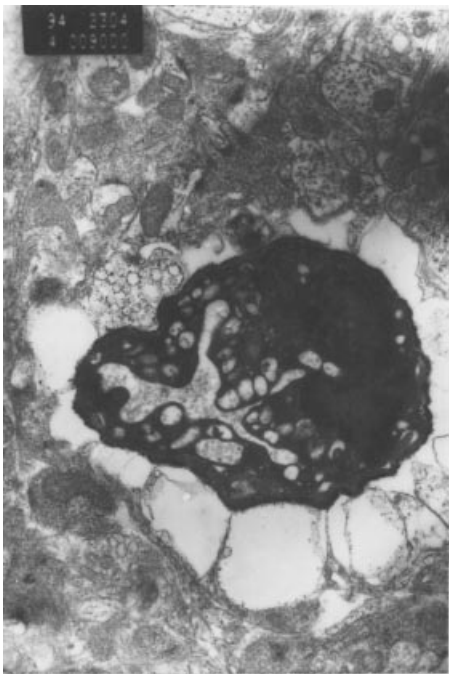

Figure 11 Cerebral vascular system in exposed fish ($\times$9000).

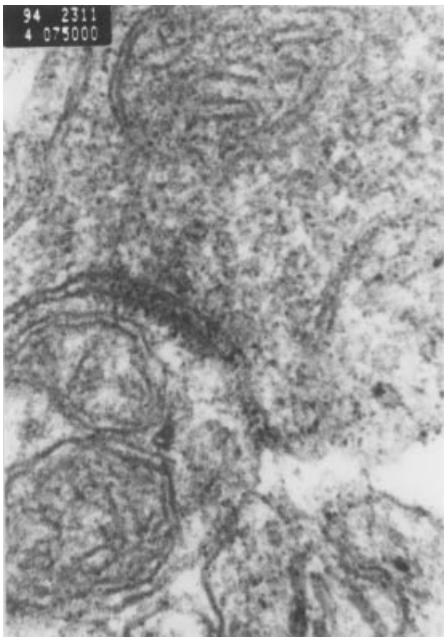

Figure 13 Cerebral nerve in exposed fish ($\times$75 000).

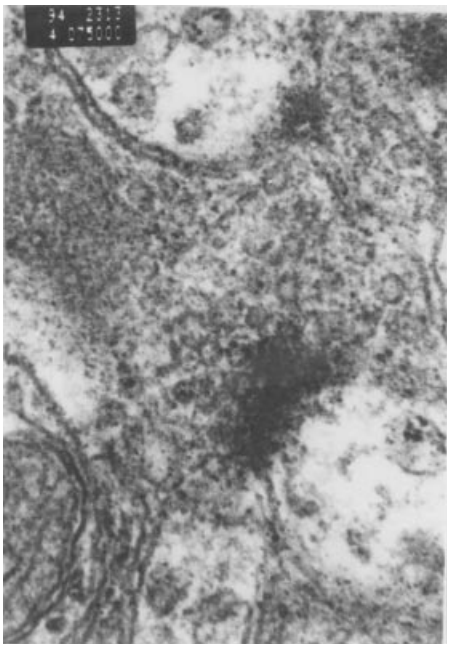

Figure 12 Cerebral nerve in exposed fish ($\times$75 000).

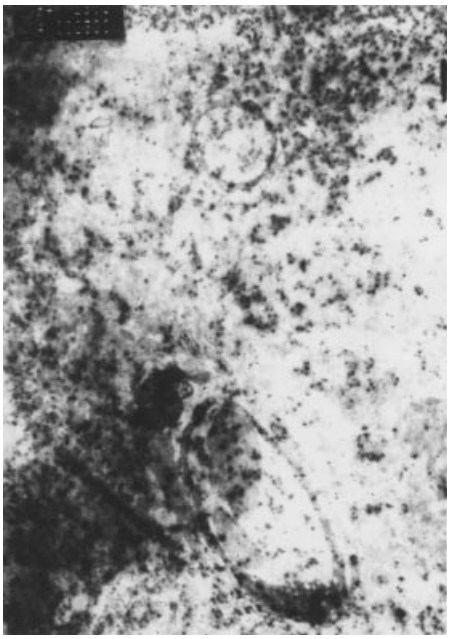

Figure 14 Cerebral vascular system in control fish ($\times$2300).

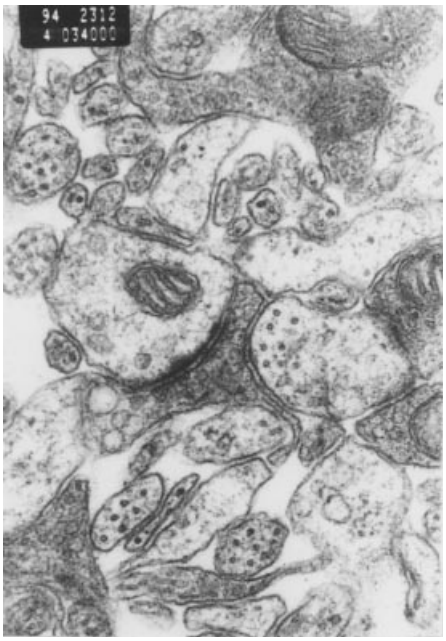

Figure 15 Cerebral nerve in control fish (×34 000).

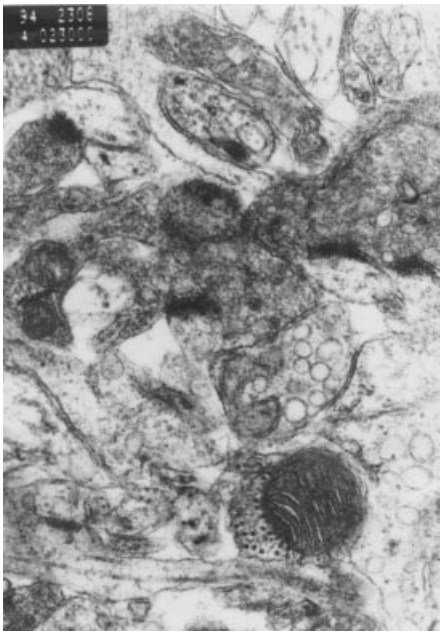

Figure 16 Cerebral nerve in control fish (×23 000).

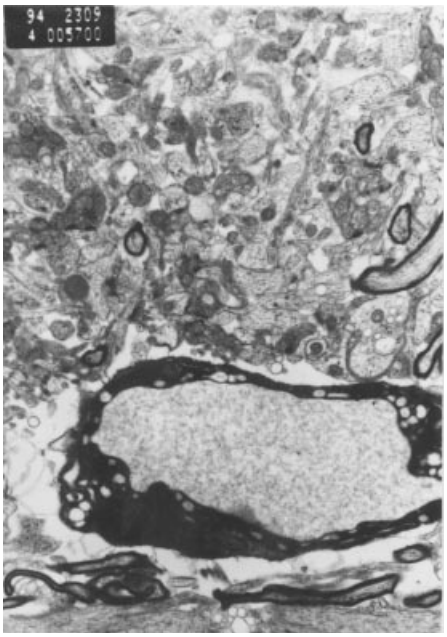

Figure 17 Cerebral nerve in control fish (×5700).

There existed fewer organelles in cytoplasm, swollen endoplasmic reticulum and degranulated ribosomes. Mitochondria were destroyed with the appearance of vacuoles and the disappearance of the ridge (see Figs 8 and 9).

The results of the electron microscope study of tilapia indicated that TBT caused the cerebral vascular system to shrink, producing an internal concavity. There was a little non-homogeneous body fluid inside the lumina. Mitochondria in neuron synapses were abnormal; cerebral cells

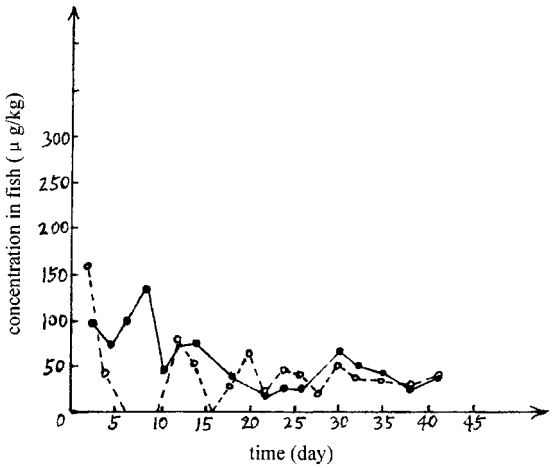

Figure 18 Concentrations of butyltins in biota in the mesocosm (20±1 °C): ●, TBT; ○, DBT.

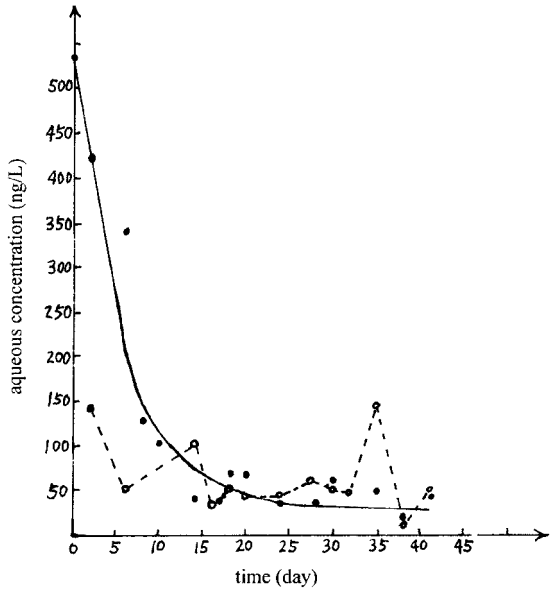

Figure 19 Aqueous concentrations of butyltins in the mesocosm (20±1 °C): ●, TBT; ○, DBT.

were deficient in synaptic vesicles. The synaptic clefts were confused and enlarged with a high electron density, which might lead to neural transmission across synapses (see Figs 10–13). These changes were not found in the cerebral mitochondria in the control group (see Figs 14–17).

During the test, exposed tilapia were found to act slowly and to breath rapidly. These phenomena were consistent with the results obtained with the electron microscope. Due to the TBT toxicity, the blood vessels became deformed and thinner, thus causing the fish to breathe rapidly, while the blockage of the transmission nerves made the fish act slowly.

The mesocosm bioconcentration test on TBT in tilapia

In the mesocosm the relative changes of TBT in the fish and the three tissues are shown in Fig. 18 and Table 6. In general, the butyltin concentrations in fish did not increase continuously but

Table 3 BCF data for the microcosm test (20±1 °C)

Day	1	4	7	11	15	19	23	30	37	44	50
TBT (μg kg^{-1})	198	114	225	192	536	114	1976	2796	1233	5658	6162
BCF	395	228	450	385	1070	228	3950	5590	2470	11 300	12 300

Table 4 BCF data for the mesocosm test (20±1 °C)

Day	2	4	6	8	10	12	14	16	18	20
TBT (μg kg^{-1})	91.6	68.6	96.8	130.7	40.2	73.1	74.4	9.6	33.5	62.4
BCF	217	—[a]	286	1024	400	—	2214	307	505	973
Day	22	24	26	28	30	32	35	38	41	
TBT (μg kg^{-1})	13.3	22.1	22.9	16.7	65.3	48.9	39.7	22.0	37.2	
BCF	—	639	—	493	1140	935	794	1517	838	

[a] —, Some BCF data are unavailable due to absence of aquatic concentration data for that day.

Table 5 Concentrations of butyltins in three tissues from tilapia in the microcosm test (20±1 °C)

	Muscle		Gill		Viscera	
Day of sampling	37	50	37	50	37	50
TBT (μg kg^{-1})	1335	2952	3657	15 940	4140	16 010
DBT (μg kg^{-1})	981	1440	2547	5106	5421	30 160
DBT/TBT	0.73	0.48	0.70	0.32	1.31	1.82

Table 6 Concentrations of butyltins in three tissues from tilapia in the mesocosm test (20 ± 1 °C)

	Muscle		Gill		Viscera	
Day of sampling	16	32	16	32	16	32
TBT ($\mu g\ kg^{-1}$)	20.1	28.7	188.3	83.0	45.4	139.7
DBT ($\mu g\ kg^{-1}$)	n.d.[a]	40.0	n.d.	67.5	n.d.	186.1
DBT/TBT	—	1.39	—	0.81	—	1.33

[a] n.d., not detected.

changed with those in water (Figs 18 and 19). When the aqueous concentration of TBT reached a steady state at 30–40 days, the concentration in both the fish and the sediment also reached a steady state, the average values being 30 $\mu g\ kg^{-1}$ and 8.2 $\mu g\ kg^{-1}$ (wet sediment) respectively.

The enrichment of TBT in tilapia is very different when the initial concentrations are different (Figs 2 and 18). In the mesocosm test, when the system reached a steady state the aqueous concentration was about only 45 $ng\ l^{-1}$, which was nearly 10 times smaller than the safe concentration. In this case, the fish could maintain its normal physiological function, and metabolize TBT effectively, thus reducing the toxicity. When the bioconcentration rate and degradation rate of the fish were equal, the concentrations of TBT in the fish remained constant. However, in the microcosm test, the aqueous concentration was maintained at 500 $ng\ l^{-1}$, which was very close to the safe concentration, and was much higher than that in the mesocosm test. Therefore, many tissues in the fish such as liver cells, nerves etc. were seriously damaged. The fish could not metabolize TBT normally and TBT was accumulated in the fish continuously till the concentration was as high as 6 $mg\ kg^{-1}$ (Fig. 2). Tables 3 and 4 show that both experiments had gradually increased BCF values as the test period progressed. However, in the mesocosm experiment, after an initial increase, the BCF values fluctuated around a mean value. It is apparent that under low pollutant concentrations the system can adjust itself to a new equilibrium. A phenomenon that is very common in the real environment thus can be easily explained by the mesocosm test.

After 16 and 32 days in the mesocosm test, the concentrations in muscle, gill and viscera were measured. Table 6 shows that when the system reached a steady state, relatively small amounts of butyltins were found in muscle compared with gill and viscera, which is consistent with the results obtained from the microcosm bioconcentration test. Both TBT and DBT were also detected in these tissues. However, the DBT/TBT ratio was quite different for the two experiments. This could be related to a more efficient dealkylation of TBT in the mesocosm experiment or a different DBT/TBT ratio in water; in the microcosm experiment this ratio was always low, because of the constant high concentration of TBT that was maintained, while in the mesocosm experiment this ratio was approximately 1 : 1 after 10 days or so. Since the ratios in viscera for both experiments are consistent in spite of the different DBT/TBT ratios in water, it can be concluded that most of the DBT in the viscera came from TBT degradation in the tissue and it was not concentrated from the water directly. In the mesocosm experiment, dealkylation played a most important role in the transport and transformation of TBT. From the mass-balance equations, it can be calculated that when the mesocosm reached a steady state 34% of the TBT was degraded to DBT and MBT daily. The high degradation rate indicates that TBT is fairly rapidly degraded in estuarine waters (at least at 20 °C).

The results of the experiments reveal that when the initial concentrations of the toxicant is different, the bioconcentration phenomena are also different. Therefore it is very important that one should be very careful when choosing the experimental concentration.

CONCLUSION

TBT has a very high biotoxicity to tilapia, with a 96-h LC_{50} value of 3.8 $\mu g\ l^{-1}$. It tends to accumulate more in organs where more lipid exists. The order of bioconcentration factors is muscle < gill < viscera. Tilapia can degrade TBT to less toxic DBT and a very small amount of MBT. It is most remarkable to note that in tilapia the enrichment of butyltins at a concentration of 45 $ng\ l^{-1}$ is very different from that at 500 $ng\ l^{-1}$. In the former case, i.e. in the mesocosm test, the butyltin concentration in the fish body remains constant, while in latter case it does not. It is clear that the enrichment of TBT in biota is related to the concentration of the toxicant. Studies also demonstrate that the results

of a mesocosm test which is more similar to the real environment are very different from that of the usual bioconcentration test.

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