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Accumulation and toxic effect of organometallic compounds on algae

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This paper reports toxic effects and bioaccumulation factors of organometallic compounds, mainly organotin species, on algae. We selected two species of microalgae as test algae: one was *Scenedesmus obliquus* as a representative of freshwater algae, the other *Dunaliella salina* and *Dunaliella viridis*, to represent commonly mixed algae which exist abundantly in Tianjin Harbor, People's Republic of China. For comparison, *Chlorella vulgaris* was also used in this study. The toxic effect of ten organometallic compounds on the freshwater alga, *S. obliquus*, was investigated. The ten compounds were (as chlorides) tributyltin (TBT); triphenyltin (TPT); trimethyltin (TMT); dibutyltin (DBT); diphenyltin (DPT); dimethyltin (DMT); trimethyl-lead acetate (TML); dimethylarsine (DMA) and two new mixed-alkyltin pesticides, dicyclohexylmethyltin acetate (Cy_2MTA) and dicyclohexylmethyltin isobutyrate (Cy_2MTB). The order of toxicity of these compounds in freshwater algae, *S. obliquus*, was TBT > TPT > DBT > Cy_2MTA = TML > Cy_2MTB > DPT > TMT > DMA > DMT, according to 96 h EC_{50} values attained. The ten toxicants were divided into three groups according to the sequence of their toxicities: (a) TBT, TPT; (b) DBT, Cy_2MTA, TML, Cy_2MTB; (c) DPT, TMT, DMA, DMT. In each group the EC_{50} values of each compound were quite similar. The difference of EC_{50} values between two vicinal groups was approximately one order of magnitude. The bioconcentration factor (BCF) of TBT and TPT compared with water in the freshwater alga *S. obliquus* was $>3.32\times10^5$ and 1.14×10^5, respectively. The BCF of the marine mixed algae was $>3.48\times10^5$. The marine microalga, *Chlorella vulgaris*, was adaptable to TBT at lower concentration. TBT at high concentration only inhibited the growth of *S. obliquus*, but it could cause chlorosis and disintegration of *D. salina* and *D. viridis*. Resistance to toxicity of algae against TBT appears in order as follows: *C. vulgaris* > S. obliquus > *D. salina* and *D. viridis*. TBT was metabolized by algae to a less toxic product, DBT. The existence of algal cells accelerated the concentration reduction of TBT. The toxic mechanism of TBT was also studied.

Keywords: Organotin, toxicity, algae

INTRODUCTION

Organotin compounds are used in three main ways: as thermal stabilizers for poly(vinyl chloride), as catalysts in the production of polyurethane foams, and as biocides.[1] Their production has risen from under 5000 tons in 1955 to at least 35 000 tons in 1986. Most of these tin compounds are eventually released to the environment. In fact, organotin compounds that are mainly manmade exist ubiquitously in the aquatic environment. The reported maximum concentrations of triorganotin compounds measured in the aquatic environment are 3 ppb and 41 000 ppb in the surface microlayer. The reported highest concentration of dibutyltin found in water is 2600 ppb.[2-5] Detrimental impacts of organotin compounds on the aquatic environment have been found. Thus, during the last 15 years there has been more and more interest in the environmental chemistry of organotin compounds. Their concentration, distribution, pattern of transfer and transformation, toxicology and biological effect in the aquatic environment have been studied. Their bioaccumulation and quantitative structure–activity relationships (QSAR) appear in the literature. The behaviour of organotin compounds is complex.[2,6-15] It is important to evaluate the potential use and environmental risks of organotin compounds, because with their increasing and new uses many questions become more and more complicated. Recently, acts restrictions on the production and use of organotin compounds have been established in several countries. Several monitoring data sets are complete enough to show that the concentrations of tributyltin in

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Table 1. Nutrition media
(a) Aquatic-4 medium

Compound	Concn ($g\,l^{-1}$)	Compound	Concn ($g\,l^{-1}$)
$(NH_4)_2SO_4$	0.200	$MgSO_4$, $7H_2O$	0.080
$Ca(H_2PO_4)$, $H_2O + 2(CaSO_4$, $H_2O)$	0.03	$NaHCO_3$	0.100
KCl	0.023	$FeCl_3$ (1%)	0.150 ml
Soil-immersed solution	0.5 ml		

(b) Nutrition medium for the mixed algae

Compound	Concn ($g\,l^{-1}$)	Compound	Concn ($g\,l^{-1}$)
NaCl	100.00	$MgCl_2$, $6H_2O$	4.900
KCl	0.680	NaBr	0.080
KH_2PO_4	0.004	Ferric citrate	0.005
$CaCl_2$	1.100	$MgSO_4$, $7H_2O$	6.600
$NaHCO_3$	1.900	$NaNO_3$	0.060
Urea	0.009		

(c) Nutrition medium for *C. vulgaris*

Compound	Concn (ml)	Compound	Concn (ml)
$(NH_4)_2SO_4$ (1%)	3	EDTA (1%)	2
K_2HPO_4 (1%)	1	Seawater	1000

waters have significantly decreased since the regulations went into effect.[15]

In this research, we selected two kinds of organisms as test microalgae: one was *Scenedesmus obliquus* as a representative of freshwater algae, which is of interest in our studies because organotin compounds are found in freshwaters such as municipal wastewaters and rivers, and their toxicity in freshwater organisms has been little reported; the other was *Dunaliella salina* and *Dunaliella viridis* to represent commonly mixed algae which exist abundantly in Tianjin Harbor. In addition, the marine microalga *Chlorella vulgaris* was compared with the mixed algae. Considering the wide use and the high biological activity of triorganotin and diorganotin compounds, we selected six organotin compounds including tributyltin (TBT), triphenyltin (TPT), trimethyltin (TMT), dibutyltin (DBT), diphenyltin (DPT) and dimethyltin (DMT) as chlorides as representatives of organotin compounds and we selected two new mixed-alkyltin pesticides, dicyclohexylmethyltin acetate (Cy_2MTA) and dicyclohexylmethyltin isobutyrate (Cy_2MTB) as representatives of new organotin compounds. This is the first time we report the study of the impact of these two new organotin compounds on organisms. Furthermore, we selected two other organometallic compounds, trimethyl-lead acetate (TML) and dimethylarsine (DMA) for comparison with organotin compounds. We study and discuss their toxicity and accumulation on algae, and compare the results with the data already reported.

MATERIALS AND METHODS

Chemicals

Tri-n-butyltin chloride (TBT), di-n-butyltin chloride (DBT), trimethyltin chloride (TMT), dimethyltin chloride (DMT) and tropolone (2-hydroxy-2,4,6-cycloheptatrien-1-one) were obtained from Aldrich Chemical Co. Triphenyltin chloride (TPT), diphenyltin chloride (DPT) and mixed-alkyltin pesticides were prepared and purified in the laboratory.[16] Trimethyl-lead acetate (TML) and dimethylarsine (DMA) were obtained from Alpha Co. and the Department of Chemistry, Nankai University, People's Republic of China, respectively. All organic solvents were analytically pure. Sulphuric and hydrochloric acids were reagent-grade. Sodium borohydride (97%) was obtained from the Merck-Schuchardt Co.

Stock solutions of the organometallic compounds were made up to 1000 mg l^{-1} with absolute ethanol, and diluted to suitable concentrations for each experiment.

Test algae and media

Test algae were *Scenedesmus obliquus*, mixed algae *Dunaliella salina* and *D. viridis*, and *Chlorella vulgaris*. Aquatic-4 was used as the medium for *S. obliquus*. *D. salina* and *D. viridis*

Table 2. Cell number (×10^4 ml^{-1}) vs time for various concentrations of organometallic compounds, with *S. obliquus*

No	Concn. of TBT (μg l^{-1})	Time (h):					No	Concn. of DBT (μg l^{-1})	Time (h):				
		0	24	48	72	96			0	24	48	72	96
0	0	96	266	340	340	343	0	0	158	290	477	600	595
1	0.5	112	258	290	280	287	1	0.5	157	280	440	591	580
2	1.0	106	244	264	256	265	2	1.0	152	275	410	465	480
3	2.0	111	251	258	249	260	3	5	150	229	390	435	450
4	5.0	113	243	241	245	255	4	10	149	225	375	405	400
5	10	107	226	221	230	235	5	20	146	209	355	430	375
6	15	104	177	171	179	170	6	50	143	195	275	300	295
7	20	106	145	147	112	110	7	100	132	180	222	239	240
8	40	105	135	117	77	81	8	200	130	170	210	199	200
							9	500	126	158	199	200	185

No	Concn. of TPT (μg l^{-1})	Time (h):					No	Concn. of DPT (μg l^{-1})	Time (h):				
		0	24	48	72	96			0	24	48	72	96
0	0	36	122	430	550	575	0	0	33	135	420	460	565
1	0.5	34	105	345	535	550	1	5.0	31	108	390	440	495
2	1.0	31	100	260	445	465	2	10	29	103	350	395	430
3	2.0	31	99	245	385	420	3	50	29	101	280	375	390
4	5.0	30	95	170	360	385	4	100	29	101	245	345	350
5	8.0	30	95	169	320	340	5	200	27	91	235	320	315
6	10	29	94	130	235	255	6	500	23	74	190	265	265
7	20	28	69	77	49	29	7	1000	21	61	146	205	215
8	50	26	38	22	10	7	8	2000	21	44	127	180	170

No	Concn. of TMT (μg l^{-1})	Time (h):					No	Concn. of DMT (μg l^{-1})	Time (h):				
		0	24	48	72	96			0	24	48	72	96
0	0	32	169	290	580	600	0	0	44	150	400	785	785
1	1.0	31	158	270	565	585	1	5.0	42	140	390	750	750
2	5.0	31	156	266	555	565	2	10	41	127	350	650	650
3	10	29	147	253	520	520	3	50	38	125	326	600	615
4	50	29	146	246	500	500	4	100	37	124	300	520	525
5	100	28	142	227	455	450	5	500	36	114	245	490	495
6	200	28	138	208	420	415	6	1000	35	105	131	480	480
7	400	27	131	156	360	340	7	2000	29	107	114	450	445
8	600	21	117	151	315	315	8	3000	29	100	105	365	360
9	800	20	102	142	270	260	9	4000	25	96	100	300	295
10	1000	20	59	60	70	65	10	5000	24	54	93	185	170

Table 2. *Continued.*

No	Concn. of Cy$_2$MTB $(\mu g\, l^{-1})$	Time (h):					No	Concn. of Cy$_2$MTA $(\mu g\, l^{-1})$	Time (h):				
		0	24	48	72	96			0	24	48	72	96
0	0	93	222	385	555	555	0	0	90	225	375	520	705
1	0.5	84	175	320	475	530	1	2.0	79	190	305	450	690
2	1.0	79	162	305	465	520	2	5.0	69	179	280	410	555
3	5.0	75	145	275	450	510	3	10	68	169	260	400	490
4	10	75	137	260	400	440	4	20	65	164	245	375	415
5	20	72	135	245	350	390	5	40	61	153	215	350	370
6	40	70	127	205	320	325	6	60	57	135	150	295	325
7	80	68	122	150	93	106	7	80	56	118	135	150	187
8	100	60	105	80	59	55	8	100	55	103	78	87	86

No	Concn. of TML $(\mu g\, l^{-1})$	Time (h):					No	Concn. of DMA $(\mu g\, l^{-1})$	Time (h):				
		0	24	48	72	96			0	24	48	72	96
0	0	57	144	295	500	515	0	0	42	155	285	440	465
1	1.0	53	128	275	455	465	1	1.0	42	150	270	425	450
2	5.0	53	119	265	425	435	2	5.0	41	135	240	355	440
3	10	52	107	250	295	300	3	10	40	130	230	330	365
4	20	50	100	230	275	275	4	50	39	120	210	315	355
5	50	45	96	165	205	220	5	100	38	105	200	300	345
6	100	45	88	155	195	200	6	200	38	95	180	280	340
7	500	43	79	90	88	85	7	500	37	85	170	190	230
8	1000	42	54	60	55	50	8	1000	30	80	165	175	210

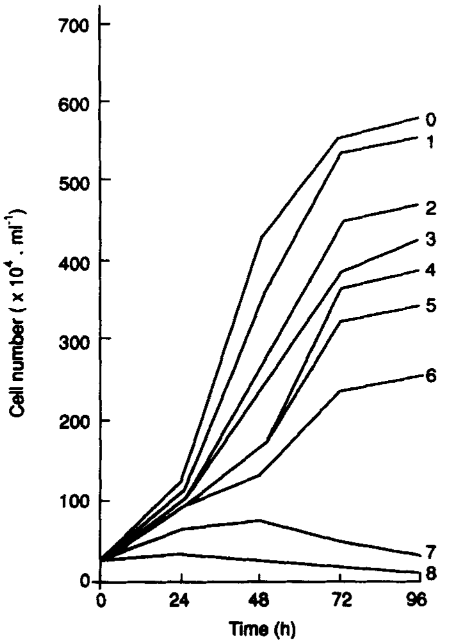

Figure 1. Inhibition growth (curve) of *S. obliquus* by TPT (data given in Table 2).

nutrition medium was used for the mixed algae and *C. vulgaris* nutrition medium was used for the alga (*C. vulgaris*).[17, 18] These media are described in Table 1.

Methods in this study were described by Rand and Petrocellic.[19] After inoculation, each culture was incubated in 100 ml of nutrition medium at (25 ± 1) °C under conditions of 12 h of light (4000 lx) and 12 h of darkness. When the cultures reached the logarithmic phase of growth (about four days), they were spiked with TBT at different concentrations. Every day at regular time intervals samples were withdrawn for determination. Cells of algae were counted via microscope examination. The shapes of algae were also observed.

Accumulation of TBT and TPT by algae

The experimental method adopted was described by Maguire *et al.*[20] Initial concentration was 1 ppb and 3 ppb, respectively under light radiation (4000 lx). After seven days, the algae were homogenized with a homogenizer. The upper clean layer was enriched with mercapto cotton, followed by determination of TBT with GC FPD.[21]

Table 3. 96-h EC_{50} and relative toxicity of organotin compounds for growth of *S. obliquus*

Compd	TBT	DBT	TPT	DPT	TMT	DMT	Cy_2MTB	Cy_2MTA	TML	DMA
EC_{50} ($\mu g\,l^{-1}$)	3.4	16.7	5.6	256.3	388.9	1118.4	33.2	24.3	24.3	822.2
Relative toxicity	328.9	67.0	199.7	4.4	2.9	1	33.7	46.0	46.0	1.4

Instrumentation

Algal cells were centrifuged at 15 000 rpm with an automatic refrigerating centrifuge. The numbers of algal cells in suspension were determined with a Dual-View microscope using a cell-counter. Samples were measured at regular time intervals. Butyltins were measured after hydride generation using a 3700-Model chromatograph (Varian–Beijing Analytical Factory) equipped with a flame photometric detector (FPD) with a 600 nm interference filter. Phenyltins were determined by a Waters HPLC with a variable-wavelength UV detector.

GC conditions

The packing column was a 2 mm i.d. × 2 m glass column filled with stationary phase, which was 3% OV-101 coated on Chromosorb W HP (80–100 mesh).

The column temperature programme was as follows. The initial column temperature was 40 °C, held for 2 min then heated to 170 °C at 32 °C min^{-1} and kept isothermal at 170 °C for 7 min. FPD was operated at a temperature of 220 °C. The injector temperature was set at 200 °C. Pure nitrogen (99.99%) served as carrier gas at a flow rate of 45 $cm^3\,min^{-1}$. Hydrogen and air flow rates were 60 and 80 $cm^3\,min^{-1}$, respectively.

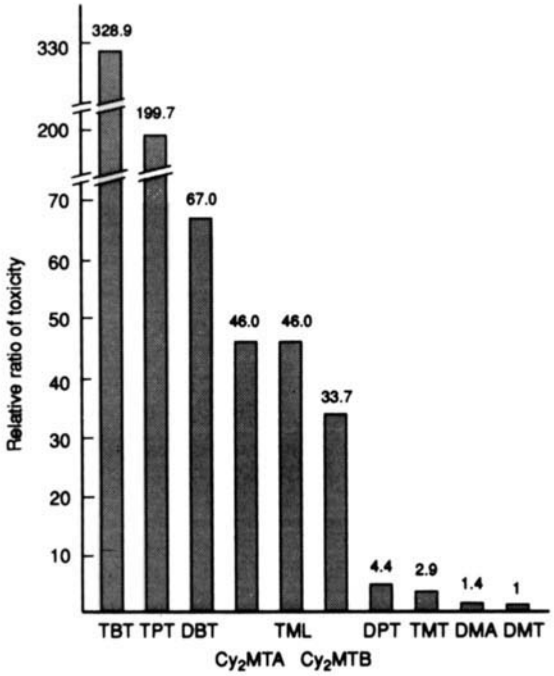

Figure 2. Comparison of the EC_{50} (96 h) for various organometallic compounds

HPLC conditions

Column 10 μm C_{18} (4 mm i.d. × 30 cm stainless steel tube)
Mobile phase, Methanol/NH_4Ac buffer solution (60:40, pH = 3.5)
Flow rate 1 $cm^3\,min^{-1}$
Injection volume 10 μl
Detection UV (254 nm)
Ls-5 Luminescence spectrophotometer (Perkin–Elmer Co.)
Scanning range 600–750 nm
Maximum emission wavelength 668 nm
Maximum excitation wavelength 331 nm
F/S 0.5;
Slit 5/5.

RESULTS AND DISCUSSION

Growth curves

Data collected for the growth curves are shown in Table 2. Figure 1 is representative of the growth curves: it illustrates that under concentration gradients established in this experiment TPT has various effects on the growth of *S. obliquus*. As the TPT concentration increases, its inhibition of the growth of *S. obliquus* tends to increase. The other nine compounds have the same influence upon *S. obliquus*.

Table 4. 96-h EC_{50} of TBT on growth of algae

	TBT-Du[a]	TBT-Sc[b]	TBT-Ch[c]
96 h EC_{50} ($\mu g\,l^{-1}$)	0.68	3.4	44.1

[a] Du, mixed algae, *D. salina* and *D. viridis*. [b] Sc, *S. obliquus*. [c] Ch, *C. vulgaris*.

Table 5. Accumulation of organotin compounds in algae over seven days[a]

	Initial concn ($\mu g\ l^{-1}$)	Algal burden ($\mu g\ g^{-1}$ fresh weight)	Residue in supernatant ($\mu g\ l^{-1}$)	BCF ($\times 10^5$)
TBT–Sc	1.0	16.6	Not detected	>3.32
Control	1.0	—	0.84	—
TPT–Sc	3.0	114	1.0	1.14
Control	3.0	—	2.74	—
TBT–Du	1.0	17.4	Not detected	>3.48
Control	1.0	—	0.84	—

[a] Detection limit for organotins in supernatant is 0.05 $\mu g\ l^{-1}$; each BCF value is an average of duplicate experiments.

EC_{50} values were calculated from the diagram of I vs $\log C$, where I is the inhibition rate of growth. It can be calculated from Eqn [1]:

$$I = ((A - B)/A) \times 100\% \qquad [1]$$

where A equals the 96-h cell number (control) and B equals the 96-h cell number (test).

Experimental data were substituted into Eqn [1] and I values were obtained. I was plotted against the concentration of organometallic compounds and an inhibition growth curve was attained. In this diagram, let I be 50%; a line is then drawn to intercept with the inhibition growth curve. From the interception point a perpendicular line to the x-axis is drawn; the interception point equals the corresponding concentration, which is the EC_{50} value.

According to the 96-h EC_{50} values attained, the order of toxicity of the ten organometallic compounds in the freshwater alga *S. obliquus* is TBT > TPT > DBT > Cy_2MTA = TML > Cy_2MTB > DPT > TMT > DMA > DMT (Table 3; Figure 2). The ten toxicants were divided into three groups according to the sequence of their toxicities: (a) TBT, TPT; (b) DBT, Cy_2MTA, TML, Cy_2MTB; (c) DPT, TMT, DMA, DMT. In each group the EC_{50} value of each compound is comparatively similar. The difference of the EC_{50} values between two vicinal groups is approximately one order of magnitude. Triorganio-substituted compounds have higher toxicity than the diorganio-substituted compounds (TBT > DBT; TPT > DPT; TMT > DMT). The most toxic species for *S. obliquus* is TBT among the six common organotin compounds, the second being TPT. Both compounds have a very low EC_{50} value (at the ppb level). The marine microalga *Chlorella vulgaris* is adaptable to TBT at lower concentrations. TBT at high concentration only inhibited the growth of *S. obliquus*, but it could cause chlorosis and disintegration of *D. salina* and *D. viridis*. Resistance to toxicity of algae against TBT appears in order as follows: *C. vulgaris* > *S. obliquus* > *D. salina* and *D. viridis* (see Table 4).

Table 6. Comparison of disappearance between TBT and TPT under the condition with *S. obliquus*[a]

	Total (μg)	Total after 7 days ST (μg)	Concentration reduction (%)
Control (TBT)	2.0	1.68	16.0
TBT-Sc	2.0	<0.31	>84.5
Control (TPT)	6.0	5.48	8.7
TPT-Sc	6.0	3.45	22.5

[a] In 2 l nutrition medium under conditions of 12 h of light (4000 lx) and 12 h of darkness.

Comparing the data listed in Table 3 with those for other algae given in the literature, the sequence of EC_{50} values is consistent.[6, 22–24]

TBT is mainly used as the toxic component in antifouling paints. It slowly leaches into natural waters. TPT is widely used as a fungicide and pesticide which enters water bodies through drainage and run-off. Both are very effective biocidal agents, but they can also induce deleterious effects on non-target organisms at very low levels, thus having an influence on some coastal fisheries. After a ban of TBT use on small boats, the potential adverse impact on the environment decreased.[15, 25, 26]

Pesticidal activity

Mixed trialkyltin derivatives have wide-ranging effect as pesticides. The results by bioassay are that all these compounds have very high activities as acaricides and fungicides. The mixed trialkyltin derivatives overcome the drawback of pesticides with the same alkyl groups which only have toxic

Table 7. Relationship between $\log EC_{50}$ (lognM) and 'X^v', TSA and 'X^r'

Compound	lognM	'X^v'	r[a]	TSA[b]	r	'X^r'	r
TBT	1.456	16.0389	−0.9953 ($n=3$)	331.5	−0.8337 ($n=3$)	6.8405	−0.7870 ($n=3$)
TPT	2.674	14.5653		322.2		7.0323	
TMT	3.515	13.8648		143.4		2.3115	
DBT	2.148	15.7631	−0.9995 ($n=3$)	263.7	−0.7957 ($n=3$)	4.9456	−0.7449 ($n=3$)
DPT	3.334	14.7811		257.5		5.0735	
DMT	3.974	14.3136		138.3		1.9263	
Trisubstituted and disubstituted-tin chloride ($n=6$)		−0.8915		−0.8297		−0.7932	

[a] r = correlation coefficient. [b] TSA = total surface area.

effects on one species of organism (for example either on bacteria or acarids, but not on both). Manufacture of the new organotin compounds provides extensive uses as pesticides.[16] This research selected two new mixed-alkyltin pesticides, dicyclohexylmethyltin acetate (Cy_2MTA) and dicyclohexylmethyltin isobutyrate (Cy_2MTB) as representatives of new organotin compounds. The toxicity of these two organotin pesticides on algae is reported for the first time. The result shows that they are less toxic than DBT for *S. obliquus*. Further investigation of their degradation products is needed. We can estimate that their toxicity should be lower than that of their parent compounds, because the triorganosubstituted tin compounds are still the most toxic forms.

Accumulation in algae

The results of accumulation experiments are shown in Table 5. Bioconcentration factor (BCF) can be calculated from Eqn [2]:

$$\mathrm{BCF} = \frac{\text{concn of TBT in algae } [\mu\mathrm{g\ g}^{-1}]}{\text{concn of TBT in supernatant medium } [\mu\mathrm{g\ kg}^{-1}]} \qquad [2]$$

It can be seen from Table 5 that the BCF of algae for TBT and TPT is comparatively high, and it is greater than the order of magnitude of accumulation reported in the literature. The difference between this work and the studies already reported is related to the different experimental conditions. In this research the concentration of organotins in the culture was lower, which is more similar to the environment. The BCF value is not only related to the species; it might also be due to the lower aqueous concentration.

The concentration of organotin compounds in the surface microlayer is much higher than that in the subsurface water;[27] a large amount of algae floated on the surface of the water, so the toxicant would give more adverse effects on the aquatic biota through accumulation of algae. TBT and TPT at low concentration not only have an effect on a single-species aquatic biota, but they also affect the whole aquatic ecosystem, even the terrestrial ecosystem. Therefore accumulation of organotin compounds by algae should attract much interest.

The mechanism of TBT toxicity for algae is likely to be that the pathways of certain biosynthesis are blocked, e.g. inhibition of photosynthesis, chlorosis of chlorophyll, cytochrome oxidase and glucose-6-phosphate dehydrogenase are affected.[28] We investigated the relationship between TBT concentration and chlorophyll content of algae (*D. salina* and *D. viridis*) using a luminescence spectrophotometer, and the result obtained indicated that chlorophyll content was significantly affected by TBT when this compound was at very low levels. At a TBT concentration of 1 ppb, chlorophyll content was significantly lower than the control. The colour of the algal cell was changed from green to brown and it then disintegrated.

Structure–activity relationship

In the presence of algae (*S. obliquus*), the decrease in concentration of TBT and TPT was higher than 84.50% and 22.5%, respectively (see Table 6). This indicated that TBT was more easily accumulated than TPT by *S. obliquus*, which was probably related to the steric effect of the molecule. TPT occupies more space than TBT.

Therefore TBT permeated the cell membrane more easily than TPT, which made the reduction of TBT concentration take place more rapidly in the aqueous solution.

In this study a good linear correlation of $r \geqslant 0.9953$ was found between $\log(EC_{50})$ (or log nM) and $'X^{v}$ (valence molecular connectivity index) for trisubstituted tin chloride and disubstituted tin chloride, respectively. In addition, there is poor linear correlation between log nM, total surface area (TSA) and $'X^{r}$ (correlated-radius molecular connectivity index) for these six organotin compounds. The above Quantitative Structure–Activity Relationship (QSAR) achieved is based on data obtained which are given in Table 7.

CONCLUSIONS

Today the study of organotin ecotoxicology is of great concern. In order to understand better the effect of organotin compounds upon organisms and their impact on the environment, it is necessary to investigate the risk and to achieve a reasonable use of organotin compounds. Partition coefficient (K_{ow}) of organotins is not the only controlling factor of accumulation for algae, bivalves, fish, etc. Laughlin *et al.*[29] deduced that the chemical binding of TBT with the biomolecule could be the main controlling factor. On this basis we are carrying out a further study of the mechanism using the Mössbauer method. Most recently Barbieri has presented a review on the interactions of organotins with biological systems using Mössbauer spectroscopy.[12]

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