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Estimation of the Hemolytic Effects of Various Organotin Compounds by Structure–Activity Relationships

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The hemolytic effects of 27 organotin compounds, which are environmental pollutants, were studied with rabbit erythrocytes. Various EC_{50} values caused by differences in their chemical structures were observed. The hemolytic activities of tri-n-butyltins and triphenyltins were higher than that of sodium-n-dodecyl sulfate, and the hemolysis by tri-n-butyltin chloride proceeded rapidly. Tri-n-butyltin chloride showed the highest hemolytic activity ($EC_{50}=7.48\ \mu M$). Methyltin compounds were less active ($EC_{50}>364\ \mu M$) than any other organotin compound tested. No significant difference in hemolytic activity based on divergences of the anionic functional group which was attached to each triorganotin compound was observed. A structure–activity relationship study was carried out in order to predict EC_{50} values of a series of tested organotin compounds, using various descriptors which represent their physicochemical properties or molecular structures. In the multiparametric regression analysis, the best regression equation ($r=0.854$) for estimation of their hemolytic effects was obtained by adopting the descriptors Index_Value (IV), Mean Information Index (I_D^w) and Molecular Connectivity Index ($^0\chi_p$).

Keywords: organotin compounds; hemolysis; quantitative structure–activity relationship (QSAR); environmental pollution

INTRODUCTION

The industrial usage of organotin compounds has increased and extensive environmental pollution has been caused by these compounds.[1–3] Tri-n-butyltins and triphenyltins, in particular, have been used as biocides, and diorganotin compounds have been utilized as stabilizers of polyvinyl chloride and as industrial catalysts.[2] Because they are environmental pollutants for aquatic biota,[4,5] regulations on industrial uses of tri-n-butyltin compounds have been introduced in many countries. Recently, the toxicities of these compounds for aquatic organisms,[1,2,6,7] for mammals,[2,6,8–10] and for microorganisms[11] were reviewed.

Organotin compounds released into the environment may be converted to other compounds by chemical and biological action. Generally, they would be degraded by dealkylation or dephenylation.[12,13] On the other hand, it is known that methyltin compounds will be produced from inorganic tin(II and IV) in the environment.[1,14,15] Therefore, it is necessary to study the toxicity not only of the organotin compounds which are utilized in industry but also of their environmental metabolites. From this point of view, we have studied the mutagenicity of 14 organotin compounds, which are detected in the environment, and found that several of them are mutagens.[16,17] Moreover, the acute toxicities of 29 organotin compounds on red killifish (*Oryzias latipes*) have been determined, and a good regression equation to predict their LC_{50} value was obtained by application of a quantitative structure–activity relationship (QSAR) method.[18] It has been reported that the QSAR method would be useful for the estimation and assessment of the environmental hazards of chemicals.[19–21] In organotin toxicity studies, many researchers have applied the QSAR method to the prediction of the acute toxicity data (lethal concentration) for various aquatic organisms,[18,22–25] algae[26] and bacteria.[27–29] However, application to biological activity or to biological factors related to toxicity

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Table 1 Organotin compounds tested

No.	Compound	Source[a]	No.	Compound	Source[a]
1	Tetramethyltin	K	15	Bis(tri-n-butyltin)	A
2	Tetra-n-butyltin	M	16	Bis(tri-n-butyltin) oxide	W
3	Tetraphenyltin	N	17	Dimethyltin dichloride	M
4	Trimethyltin chloride	K	18	Di-n-butyltin dichloride	M
5	Triethyltin bromide	A	19	Di-n-butyltin diacetate	K
6	Tri-n-butyltin chloride	K	20	Di-n-butyltin dilaurate	A
7	Tri-n-butyltin fluoride	T	21	Di-n-butyltin maleate	W
8	Tri-n-butyltin hydride	A	22	Di-n-butyltin oxide	M
9	Tri-n-butyltin methoxide	A	23	Diphenyltin dichloride	A
10	Tri-n-butyltin ethoxide	A	24	Methyltin trichloride	A
11	Tri-n-butyltin acetate	M	25	n-Butyltin trichloride	A
12	Triphenyltin chloride	K	26	Mono-n-butyltin oxide	T
13	Triphenyltin hydroxide	T	27	Phenyltin trichloride	A
14	Triphenyltin acetate	W	28	Inorganic tin(II)	H
			29	Inorganic tin(IV)	H

[a] K, Kantoh Chemical Co.; M, Merck; N, Nakarai Tesque Co.; T, Tokyo Kasei Co.; A, Aldrich; W, Wako Pure Chemicals Co.; H, Hayashi Pure Chemical Co.

mechanisms which would be lethal to the tested organisms has not been carried out.

Studies on the cellular and biochemical activities of organotin compounds are important to elucidate their toxic mechanisms. Triorganotin compounds act as inhibitors of respiration in mitochondria,[9,30] and tri- and di-organotin compounds cause cutaneous toxicity through irritating effects on the skin and eyes in rodents, or inflammation and edema in rats.[9] These toxic actions would appear to be induced through cell membrane effects mediated by these organotin compounds.[10] Particularly, it is known that tri-n-butyltin compounds are membrane toxicants,[10,30] and the hemolytic activity of these compounds has been well investigated with human or mammalian erythrocytes.[30–35] The erythrocyte experimental model system is available for the study of the effects of xenobiotics on cell membranes.[30]

In this study, the hemolytic activities of 27 organotin compounds, which were detected in the environment,[36–38] were investigated with rabbit blood cells, and their characteristics on hemolysis with a series of tested organotin compounds have been revealed. The QSAR method was applied to the prediction of their hemolytic activities with various physicochemical and topological descriptors, and the best regression equation to estimate their hemolytic toxicities was determined. Moreover, the correlation between the EC_{50} values in this hemolysis test and the LC_{50} values for red killifish (*Oryzias latipes*) in a previous report[18] was also compared.

MATERIALS AND METHODS

Chemicals

The 27 organotin compounds tested in this assay are shown in Table 1

All chemical reagents were of the best grades available. Each organotin compound to be tested was dissolved in ethanol and diluted each time to adjust it to the desired concentrations.

Preparation of erythrocytes

The hemolysis test was mainly caried out by the method established by Mino.[39] Blood (10 ml) was collected from white rabbits (body weight *ca* 4 kg). This blood was centrifuged (3000 rpm : 800*g*, 4 °C) for 10 min and plasma was removed. Then 5–10 ml of 0.15 M sodium chloride–0.05 M potassium phosphate buffer was added to the precipitated cells. This resuspension was centrifuged (3000 rpm : 800*g*, 4 °C) and the supernatant was removed. This procedure was repeated once again, after which 0.15 M sodium chloride was added to the washed erythrocytes to achieve a 1 : 9 (v/v) dilution (erythrocytes: 0.15 M sodium chloride solution), and this erythrocyte suspension was used for the hemolysis assay.

Hemolysis test

The standard procedure in this hemolysis assay was as follows: 0.2 ml of 0.05 M potassium phosphate buffer (pH 7.4) was added to 0.25 ml of the

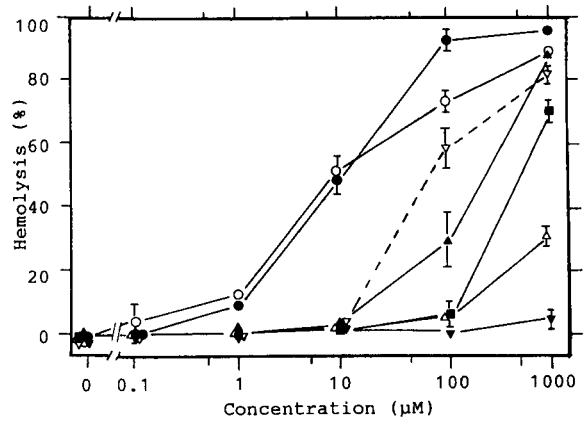

Figure 1 Hemolytic activity of n-butyltin compounds at various concentrations: ■, tetra-n-butyltin; ●, bis(tri-n-butyltin) oxide; ○, tri-n-butyltin chloride; ▲, di-n-butyltin dichloride; △, n-butyltin trichloride; ▼, $SnCl_4$; ▽, SDS.

erythrocyte suspension, and 50 μl of organotin compound in ethanol solution was added. This solution was incubated at 37 °C for 60 min. In the case of negative and positive controls, only 50 μl of ethanol was added to this potassium phosphate buffer–erythrocyte suspension. After reaction, 5 ml of 0.15 M sodium chloride–0.05 M potassium phosphate buffer (pH 7.4) was added to the tested sample and the negative control solution, and centrifuged at 3000 rpm : 800*g* (4 °C, 10 min). In the case of the positive control, 5 ml of distilled water was added and centrifuged. After centrifugation, the absorbance of the supernatant in each sample and the controls was determined at 540 nm and 670 nm. Finally, hemolysis ratio was calculated by Eqn [1]:

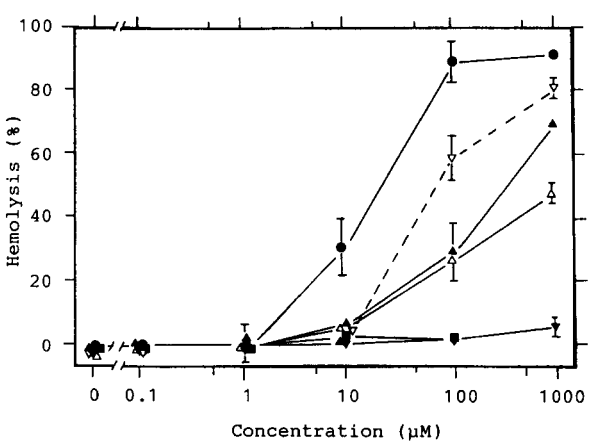

Figure 2 Hemolytic activity of phenyltin compounds at various concentrations: ■, tetraphenyltin; ●, triphenyltin chloride; ▲, diphenyltin dichloride; △, phenyltin trichloride; ▼, $SnCl_4$; ▽, SDS.

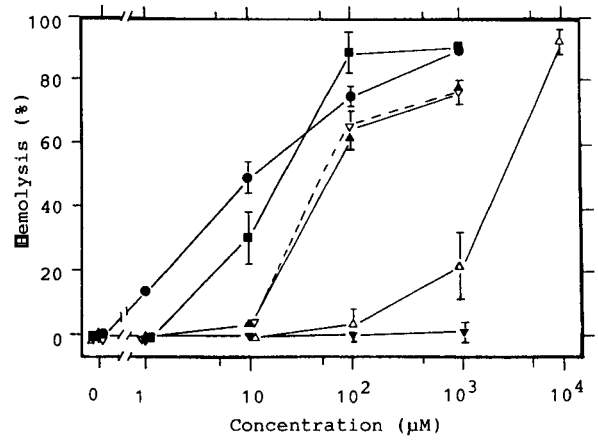

Figure 3 Hemolytic activity of triorganotin compounds at various concentrations: ■, triphenyltin chloride; ●, tri-n-butyltin chloride; ▲, triethyltin bromide; △, trimethyltin chloride; ▼, $SnCl_4$; ▽, SDS.

$$\text{Hemolysis ratio (\%)} = (A_s - A_0)/(A_p - A_0) \times 100 \quad [1]$$

where A_s = absorbance of the tested compound, A_0 = absorbance of negative control, A_p = absorbance of positive control, and in each case A is the difference between the absorbance at the two wavelengths ($A = A_{540} - A_{670}$).

The hemolytic activity of sodium n-dodecyl sulfate (SDS), a surface-active reagent, was always investigated for comparison with those of the tested organotin compounds. Each experiment was carried out four times, and average values and standard deviations were determined. The hemolytic activities of 27 organotin com-

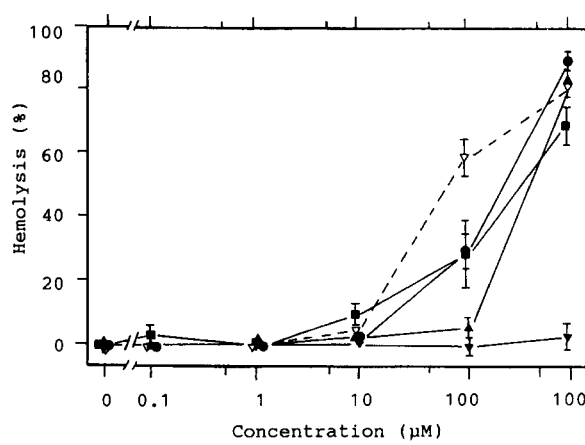

Figure 4 Hemolytic activity of diorganotin compounds at various concentrations: ■, diphenyltin dichloride; ●, di-n-butyltin dichloride; ▲, dimethyltin dichloride; ▼, $SnCl_4$; ▽, SDS.

Table 2 EC_{20} and EC_{50} values of organotin compounds tested for the hemolysis of rabbit blood cells

No.	Compound	EC_{20} (μM)	EC_{50} (μM)	No.	Compound	EC_{20} (μM)	EC_{50} (μM)
1	Tetramethyltin	177	516	**16**	Bis(tri-n-butyltin) oxide	1.75	8.99
2	Tetra-n-butyltin	128	408	**17**	Dimethyltin dichloride	154	364
3	Tetraphenyltin	—[a]	—	**18**	Di-n-butyltin dichloride	51.4	216
4	Trimethyltin chloride	234	1910	**19**	Di-n-butyltin diacetate	47.3	—
5	Triethyltin bromide	15.5	54.19	**20**	Di-n-butyltin dilaurate	18.2	427
6	Tri-n-butyltin chloride	1.60	7.48	**21**	Di-n-butyltin maleate	20.7	375
7	Tri-n-butyltin fluoride	2.19	15.8	**22**	Di-n-butyltin oxide	63.4	—
8	Tri-n-butyltin hydride	12.4	31.8	**23**	Diphenyltin dichloride	57.1	380
9	Tri-n-butyltin methoxide	1.94	17.7	**24**	Methyltin trichloride	400	—
10	Tri-n-butyltin ethoxide	1.20	7.89	**25**	n-Butyltin trichloride	270	—
11	Tri-n-butyltin acetate	3.30	19.5	**26**	Mono-n-butyltin oxide	460	—
12	Triphenyltin chloride	2.77	14.9	**27**	Phenyltin trichloride	19.1	886
13	Triphenyltin hydroxide	1.53	10.5	**28**	Inorganic tin(II)	—	—
14	Triphenyltin acetate	1.84	15.3	**29**	Inorganic tin(IV)	—	—
15	Bis(tri-n-butyltin)	19.5	91.0	**30**	Sodium n-dodecyl sulfate	16.5	73.5

[a] —, EC_{20} or EC_{50} was more than 2 mM, and could not be determined.

pounds were determined at various concentrations (0.1–1000 μM), and EC_{20} and EC_{50} values were calculated. Moreover, differences in the time courses of hemolysis were also studied.

QSAR study for hemolysis

Molecular weight, log *P* (n-octanol/water partition coefficient), ionization potential, electronegativities and valences, which represent the physicochemical properties of the tested organotin compounds, were applied to this QSAR analysis as descriptors. The log *P* values were obtained from the literature,[18,25] and calculated by the π method[20] if the value was not reported. Molecular Connectivity Indices (χ),[40] Valence Molecular connectivity Indices (χ^v),[40] Wiener number,[41] Information Index (I_D^w),[41–43] Mean Information Index (I_D^w),[41–43] Information Content (IC),[44] Structural Information Content (SIC),[44] Complementary Information Content (CIC)[44] and Index Value (IV)[18] were obtained from their molecular structures by the method described previously.[18]

The correlation between the EC_{50} values obtained and these parameters was analyzed by multiple regression analysis with the stepwise statistical method ($F=2.0$).[19]

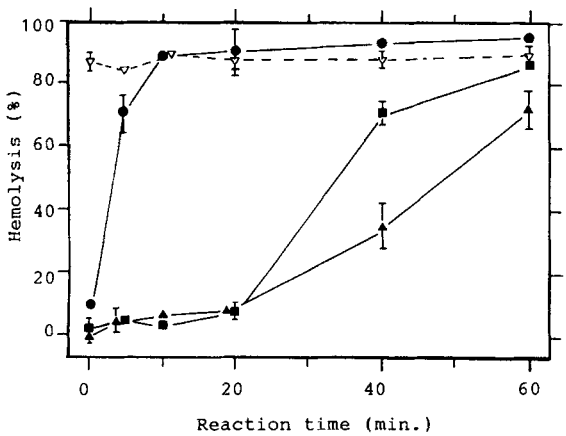

Figure 5 Time course of hemolysis by n-butyltin compounds: ■, 2 mM tetra-n-butyltin; ●, 37 μM tri-n-butyltin chloride; ▲, 1.1 mM di-n-butyltin dichloride; ▽, 0.37 mM SDS.

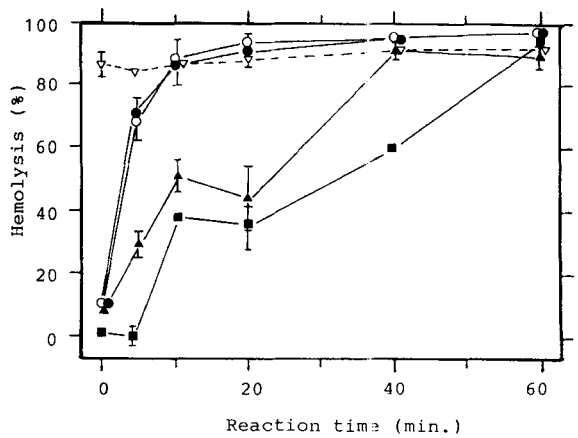

Figure 6 Time course of hemolysis by triorganotin compounds: ○, 45 μM bis(tri-n-butyltin) oxide; ●, 37 μM tri-n-butyltin chloride; ■, 75 μM triphenyltin chloride; ▲, 4.8 mM trimethyltin chloride; ▽, SDS.

Table 3(a) Physicochemical and topological properties of organotin compounds tested and their toxicity data [log(1/EC_{50} (mmol l^{-1})] used for QSAR analysis

		parameters										
No.	Compound	MW[a]	logP[b]	IP[c]	EN[d]	No. of atoms	W[e]	$^{1}P_{D}^{w}$[f]	$\overline{I}_{D}^{w}$[g]	IC[h]	CIC[i]	SIC[j]
1	Tetramethyltin	178.8	−2.19	215.5	37.0	17	16	52.0	3.25	1.09	3.00	0.27
2	Tetra-n-butyltin	347.2	3.90	677.0	117.4	53	560	3867.7	6.91	1.25	4.47	0.22
3	Tetraphenyltin	427.1	4.39	549.6	103.8	45	1248	10 078.3	8.08	1.47	4.02	0.27
4	Trimethyltin chloride	199.3	−2.30	176.5	31.2	14	16	52.0	3.25	1.09	2.71	0.29
5	Triethyltin bromide	285.8	−1.80	290.7	51.1	23	64	299.2	4.68	1.33	3.20	0.29
6	Tri-n-butyltin chloride	325.5	2.60	522.6	91.5	41	343	2163.8	6.31	1.26	4.09	0.24
7	Tri-n-butyltin fluoride	309.0	2.03	527.0	92.5	41	343	2163.8	6.31	1.26	4.09	0.24
8	Tri-n-butyltin hydride	291.1	1.89	523.2	90.6	41	300	1822.8	6.08	1.26	4.09	0.24
9	Tri-n-butyltin methoxide	321.1	1.78	575.2	100.8	45	400	2611.0	6.53	1.48	4.01	0.27
10	Tri-n-butyltin ethoxide	335.1	2.18	613.7	107.5	48	472	3175.0	6.73	1.49	4.09	0.27
11	Tri-n-butyltin acetate	349.1	1.16	600.1	106.8	47	546	3773.9	6.91	1.65	3.91	0.30
12	Triphenyltin chloride	385.5	2.65	427.0	81.3	35	730	5399.5	7.40	1.49	3.64	0.29
13	Triphenyltin hydroxide	367.0	1.27	441.2	83.9	36	730	5399.5	7.40	1.78	3.39	0.35
14	Triphenyltin acetate	409.1	1.30	504.6	96.6	41	1026	8031.5	7.83	2.18	3.18	0.41
15	Bis(tri-n-butyltin)	580.1	—[n]	1019.1	177.0	80	1549	12 687.1	8.19	1.28	5.04	0.20
16	Bis(tri-n-butyltin) oxide	596.1	2.29	1032.7	180.5	81	1804	14 955.0	8.29	1.36	4.98	0.22
17	Dimethyltin dichloride	219.7	−3.10	137.5	25.4	11	16	52.0	3.25	1.10	2.36	0.32
18	Di-n-butyltin dichloride	303.8	1.49	368.2	65.6	29	180	1002.3	5.57	1.13	3.23	0.23
19	Di-n-butyltin diacetate	351.0	−1.21	523.2	96.2	41	532	3680.5	6.92	1.95	3.40	0.37
20	Di-n-butyltin dilaurate	631.6	—	1292.3	230.2	101	5892	53 634.4	9.10	1.60	5.04	0.24
21	Di-n-butyltin maleate	351.0	—	523.2	96.2	41	532	3680.5	6.92	3.06	1.69	0.64
22	Di-n-butyltin oxide	248.9	—	355.8	63.1	28	149	784.1	5.26	1.50	3.31	0.31
23	Diphenyltin dichloride	343.8	1.40	304.5	58.8	25	352	2295.9	6.52	1.51	3.13	0.33
24	Methyltin trichloride	240.1	−3.10	98.4	19.6	8	16	52.0	3.25	0.81	2.19	0.27
25	n-Butyltin trichloride	208.8	0.35	213.8	39.7	17	71	328.0	4.62	1.16	2.93	0.28
26	Mono-n-butyltin oxide	302.2	—	215.6	39.8	17	52	218.2	4.20	2.13	1.96	0.52
27	Phenyltin trichloride	282.2	1.15	181.9	36.3	15	114	607.8	5.33	1.53	2.37	0.39

RESULTS

Hemolysis test

The results on the hemolytic activity of a series of n-butyltin chlorides and bis(tri-n-butyltin)oxide (TBTO) are shown in Fig. 1. The hemolytic activities of TBTO and tri-n-butyltin chloride were higher than that of SDS at all the concentrations tested (0.1–1000 μM). A more intensive hemolysis by TBTO than by tri-n-butyltin chloride was observed in the range from 100 to 1000 μM.

Di-n-butyltin chloride and tetra-n-butyltin showed similar hemolytic activity profiles. n-Butyltin trichloride showed the lowest hemolytic effect among the n-butyltin compounds, and its hemolytic activity at 1000 μM was 31% of that of tri-n-butyltin chloride.

Hemolysis activities of phenyltin chlorides are summarized in Fig. 2. The highest hemolytic effect was observed with triphenyltin chloride, and its hemolytic activity was higher than that of SDS. The hemolytic activity of diphenyltin dichloride was higher than that of phenyltin trichloride only at concentrations above 100 μM. Tetraphenyltin hemolysis could not be determined at concentrations higher than 0.1 mM, because of its lower solubility, and weaker hemolytic activity was observed.

Hemolytic effects were also studied with various tri- and di-organotin compounds as shown in Figs 3 and 4, respectively. On the triorganotin compounds, trimethyltin chloride showed the weakest hemolytic activity. A pronounced difference in hemolytic activity could not be found among the other triorganotin compounds. In Fig. 4, similar hemolytic activities were observed in the diorganotin compounds tested; dimethyltin

Table 3(b)

No.	Compound	parameter									
		$^{0}\chi_{p}$[k]	$^{1}\chi_{p}$	$^{2}\chi_{p}$	$^{3}\chi_{p}$	$^{0}\chi_{p}^{v}$[l]	$^{1}\chi_{p}^{v}$	$^{2}\chi_{p}^{v}$	$^{3}\chi_{p}^{v}$	IV[m]	log(1/EC$_{50}$)
1	Tetramethyltin	4.50	2.00	3.00	0.00	7.35	13.42	20.12	0.00	2	0.29
2	Tetra-n-butyltin	12.99	8.24	5.91	4.24	15.84	16.31	20.18	20.39	2	0.39
3	Tetraphenyltin	16.95	14.31	10.34	9.79	16.90	14.42	17.09	18.52	2	—
4	Trimethyltin chloride	4.50	2.00	3.00	0.00	7.49	13.86	21.47	0.00	3	−0.28
5	Triethyltin bromide	6.62	3.68	2.87	2.56	10.44	16.35	26.12	24.04	3	1.26
6	Tri-n-butyltin chloride	10.86	6.68	5.12	3.40	13.85	16.03	20.69	17.44	3	2.13
7	Tri-n-butyltin fluoride	10.86	6.68	5.12	3.40	13.10	13.50	15.31	13.63	3	1.80
8	Tri-n-butyltin hydride	9.94	6.35	4.29	2.90	13.24	13.34	14.18	13.38	3	1.50
9	Tri-n-butyltin methoxide	11.57	7.24	5.16	3.93	14.13	14.01	16.09	16.69	3	1.75
10	Tri-n-butyltin ethoxide	12.28	7.74	5.56	3.96	14.83	14.60	16.78	16.81	3	2.10
11	Tri-n-butyltin acetate	13.15	8.10	6.41	4.02	15.03	14.51	16.70	16.20	3	1.71
12	Triphenyltin chloride	13.84	11.23	8.37	7.52	14.65	14.61	17.26	17.57	3	1.83
13	Triphenyltin hydroxide	13.84	9.82	8.37	7.46	13.96	12.13	13.81	13.58	3	1.98
14	Triphenyltin acetate	16.12	11.23	9.72	7.86	15.83	13.04	14.79	15.35	3	1.82
15	Bis(tri-n-butyltin)	19.73	12.61	9.18	7.19	25.44	35.70	72.98	107.84	3	1.04
16	Bis(tri-n-butyltin) oxide	20.44	13.07	9.80	7.11	25.84	27.20	35.65	47.06	3	2.05
17	Dimethyltin dichloride	4.50	2.00	3.00	0.00	7.62	11.77	22.88	0.00	2	0.44
18	Di-n-butyltin dichloride	8.74	5.12	4.37	2.41	11.86	15.76	21.81	13.06	2	0.67
19	Di-n-butyltin diacetate	13.31	7.95	6.90	3.80	14.23	12.71	13.52	12.61	2	—
20	Di-n-butyltin dilaurate	27.45	18.03	13.56	9.29	28.37	22.83	20.44	17.18	2	0.37
21	Di-n-butyltin maleate	13.31	8.12	6.90	3.80	14.23	12.62	13.52	12.61	2	0.43
22	Di-n-butyltin oxide	7.82	4.81	3.39	2.10	10.00	4.33	8.67	6.82	2	—
23	Diphenyltin dichloride	10.73	7.21	6.49	5.32	12.40	14.76	18.79	14.17	2	0.42
24	Methyltin trichloride	4.50	2.00	3.00	0.00	7.76	14.76	24.35	0.00	1	—
25	n-Butyltin trichloride	6.62	3.56	3.66	1.28	9.88	15.48	23.54	7.24	1	—
26	Mono-n-butyltin oxide	5.70	3.27	2.54	1.14	7.33	6.95	5.17	2.97	1	—
27	Phenyltin trichloride	7.61	4.61	4.70	2.83	10.14	14.97	21.66	8.31	1	0.05

[a] Molecular weight. [b] n-Octanol/water partition coefficient. [c] Ionization potential. [d] Electronegativity. [e] Wiener number. [f] Information index. [g] Mean Information Index. [h] Information content. [i] Complementary information content. [j] Structural information content, [k] $^{n}\chi_{p}$ are Connectivity indices. [l] $^{n}\chi_{p}^{v}$ are Valence connectivity indices. [m] Index value. [n] Not determined.

dichloride did not show specific activity in this case.

The effects of the anionic functional group attached to organotin compounds were investigated with both tri-n-butyltins and triphenyltins. A significant difference in the effect of hemolysis by various anionic functional groups was not observed in either case (details are available from the authors if required).

The EC_{20} and EC_{50} values for the hemolysis of the 27 organotin compounds tested are summarized in Table 2. Generally, mono-organotin compounds showed lower hemolytic activity than the other organotin compounds, and EC_{50} values could be determined only for phenyltin trichloride. The hemolysis of tri-n-butyltin chloride had been investigated with human erythrocytes.[30–35] In those studies, hemolysis was induced at 5–10 μM concentrations, but it did not occur at concentrations below 1 μM of tri-n-butyltin chloride.[33] However, 1 μM of tri-n-butyltin chloride induced hemolysis in our experiment with rabbit erythrocytes. This suggested that rabbit erythrocytes would be more sensitive to tri-n-butyltin exposure.

Time courses of hemolysis by n-butyltin compounds are shown in Fig. 5 and those for triorganotin compounds are shown in Fig. 6. In these results, the hemolysis of tri-n-butyltin compounds proceeded rapidly, and hemolyses over 80% in both tri-n-butyltin chloride and TBTO were observed after 10 min. However, the hemolytic rates of the other organotin compounds increased gradually.

Table 4 Linear correlations of $\log(1/EC_{50})$ with physicochemical and topological parameters

Independent variables, X	Equation $\log(1/EC_{50}) = a(X) + b$		Statistics	
	a	b	n	r[a]
M.W.[b]	0.003	0.147	21	0.377
log P[c]	0.214	1.001	18	0.516
IP[d]	0.001	0.470	21	0.414
EN[e]	0.008	0.432	21	0.423
No. of atoms	0.017	0.454	21	0.418
W[f]	0.000	0.913	21	0.251
$\overline{I_D^w}$[g]	0.000	0.965	21	0.232
I_D^w[h]	0.270	−0.554	21	0.525
IC[i]	0.880	−0.113	21	0.285
CIC[j]	0.349	−0.147	21	0.352
SIC[k]	−0.932	1.395	21	−0.074
$^0\chi_p$[l]	0.076	0.254	21	0.442
$^1\chi_p$	0.115	0.288	21	0.483
$^2\chi_p$	0.131	0.346	21	−0.074
$^3\chi_p$	0.171	0.441	21	0.517
$^0\chi_p^v$[m]	0.066	0.206	21	0.404
$^1\chi_p^v$	0.008	0.996	21	0.059
$^2\chi_p^v$	−0.003	1.204	21	−0.055
$^3\chi_p^v$	0.008	0.986	21	0.218
IV[n]	0.990	−1.415	21	0.749

[a] Correlation coefficient. [b] Molecular weight. [c] n-Octanol/water partition coefficient. [d] Ionization potential. [e] Electronegativity. [f] Wiener number. [g] Information Index. [h] Mean Information Index. [i] Information Content. [j] Structural Information Content. [k] Complementary Information Content. [l] $^n\chi_p$ are Connectivity Indices. [m] $^n\chi_p^v$ are Valence Connectivity Indices. [n] Index Value.

QSAR study of the hemolysis

All descriptors used for this QSAR study are summarized in Table 3.

Log $(1/EC_{50})$ was adopted as an indication of hemolytic toxicity. Linear correlations were analyzed between each eigenvalue of the tested organotin compound as an independent variable and $\log(1/EC_{50})$ value as the dependent variable. In this regression analysis, the parameters which represent physicochemical properties of organotin compounds (e.g. molecular weight, log P) were not suitable as descriptors to estimate their toxicity because of their lower correlation coefficients, as shown in Table 4.

Luedke *et al.*[29] reported that molecular volume was a good descriptor for prediction of toxicity (LC_{50}) of five triorganotin compounds on *Ankistrodesmus falcatus* and LC_{50} of four diorganotin compounds on *Selenastrum caprlcornutum*. However, in our analysis, good correlation of physicochemical parameters with hemolytic activities of the 27 organotin compounds tested was not obtained, and log P showed the best correlation among these physicochemical parameters. The correlation coefficient of log P was only 0.516. The Index Value (IV) (monoorganotin, 1; diorganotin, 2; triorganotin, 3; tetraorganotin, 2), which was the parameter created to estimate the acute toxicity on red killifish (*Oryzias latipes*) in a previous study,[18] showed the best correlation with $\log(1/EC_{50})$, $r = 0.749$. When a multiple regression analysis ($F = 2.0$)[19] was carried out, a good rgression formula (Eqn. [2]) to predict their hemolytic activities was obtained.

$$\log(1/EC_{50}) = 0.891(IV) + 0.589(\overline{I_D^w}) - 0.145(^0\chi_p) - 3.51 \quad (r = 0.854) \quad [2]$$

The relations between determined and estimated $\log(1/EC_{50})$ values showed reliable correlation, as shown in Fig. 7.

By this regression analysis, it became apparent that the hemolysis of the organotin compounds tested could be estimated with some topological parameters expressed by their chemical structures.

Furthermore, the relationship between EC_{50} values in this hemolysis test and LC_{50} values on red killifish (*Oryzias latipes*)[18] (as shown in Table 5) was analyzed, and a regression equation (Eqn [3]) was obtained.

$$\log(1/EC_{50}) = 0.617 \log(1/LC_{50}) - 0.558 \quad (r = 0.932) \quad [3]$$

A very good correlation was found, as shown in Fig. 8, this result suggests that the hemolytic activity caused by organotin compounds may closely relate to the lethal factor for red killifish.

DISCUSSION

Differences of hemolytic intensities among the tested organotin compounds would be caused by the discrepancy of the action to membranes originating from their biochemical characteristics. Cellular toxicity mechanisms of several organotin compounds (e.g. trimethyltin, triethyltin, tri-n-

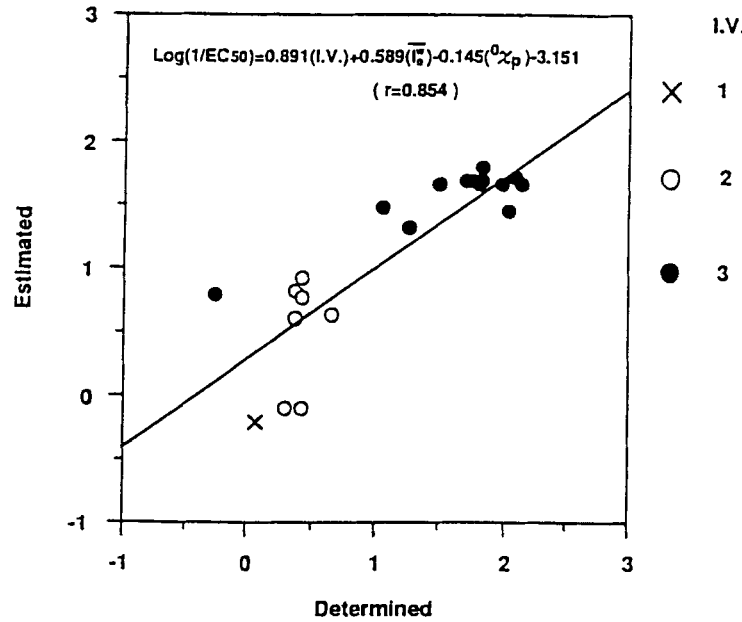

Figure 7 Relationship between determined and estimated $\log(1/LC_{50})$ on hemolysis of 27 organotin compounds.

butyltin, di-n-butyltin) have been studied, and it is known, for example, that triorganotin compounds induce swelling, discharge of external chloride/internal hydroxide gradient, and inhibition of the ATP synthase system in mitochondria.[10] These actions of organotin compounds are signifcant since they may affect cell membranes, especially *in vivo*.

In our experiments, the hemolytic activity of bis(tri-n-butyltin) oxide (TBTO) was slightly higher than that of tri-n-butyltin chloride. It is thought that this result is due to an increase of tri-n-butyltin formation as a hydrolysis product from TBTO in the reaction shown in Eqn [4]).

$$(Bu_3Sn)_2O + H_2O \rightarrow 2Bu_3SnOH \qquad [4]$$

Laughlin *et al.*[45] reported that hydrolysis of TBTO in aqueous solution was confirmed by NMR. In this TBTO result, the hemolysis would

Table 5 Acute toxicity [$\log(1/LC_{50}$ mmol l^{-1}) values] of organotin compounds on *Oryzias latipes*

No.	Compound	$\log(1/LC_{50})$	No.	Chemicals	$\log(1/LC_{50})$
1	Tetramethyltin	1.44	**15**	Bis(tri-n-butyltin)	3.30
2	Tetra-n-butyltin	1.82	**16**	Bis(tri-n-butyltin) oxide	3.92
3	Tetraphenyltin	2.03	**17**	Dimethyltin dichloride	1.56
4	Trimethyltin chloride	1.55	**18**	Di-n-butyltin dichloride	1.72
5	Triethyltin bromide	2.58	**19**	Di-n-butyltin diacetate	1.97
6	Tri-n-butyltin chloride	3.96	**20**	Di-n-butyltin dilaurate	2.49
7	Tri-n-butyltin fluoride	3.70	**21**	Di-n-butyltin maleate	1.42
8	Tri-n-butyltin hydride	—	**22**	Di-n-butyltin oxide	2.47
9	Tri-n-butyltin methoxide	4.15	**23**	Diphenyltin dichloride	1.05
10	Tri-n-butyltin ethoxide	4.13	**24**	Methyltin trichloride	—[a]
11	Tri-n-butyltin acetate	3.65	**25**	n-Butyltin trichloride	0.87
12	Triphenyltin chloride	3.78	**26**	Mono-n-butyltin oxide	0.58
13	Triphenyltin hydroxide	3.75	**27**	Phenyltin trichloride	0.44
14	Triphenyltin acetate	3.74			

[a]—Not tested.

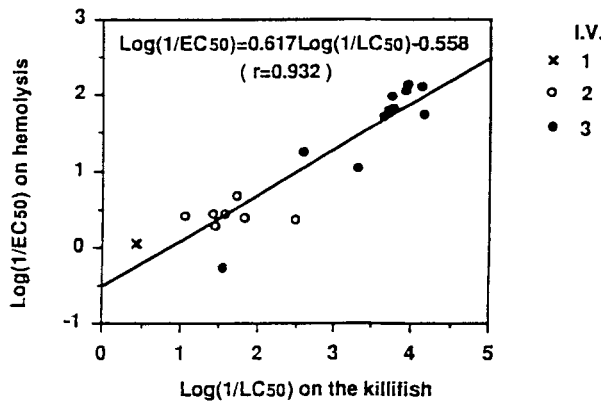

Figure 8 Relationship between $\log(1/LC_{50})$ on acute toxicity for red killifish, *Oryzias latipes*, and $\log(1/EC_{50})$ on hemolysis.

be induced by tributyltin cation (Bu_3Sn^+) rather than by TBTO itself. Furthermore, in triorganotin compounds, any significant difference of hemolytic activity due to various anionic functional groups was not observed. Accordingly, it is speculated that dissociated or equated Bu_3Sn^+ or Ph_3Sn^+ may play an important role in the induction of hemolysis.

Gray *et al.*[30] proposed that tri-n-butyltin-mediated erythrocyte membrane lysis may occur through lipid peroxidation in the membrane by a tri-n-butylstannyl peroxy free radical. Rapid hemolysis of tri-n-butyltin chloride and TBTO in our assay may be induced through radical reactions.

In a time course study, the hemolysis of diorganotin compounds proceeded very slowly compared with those of tri-n-butyltin compounds, so the mechanisms of hemolysis may differ from each other. The effect on the ordering of cell membranes related to inhibition of phospholipid metabolism by di-n-butyltin was also reported.[46]

More research on membrane toxicity will be required to reveal the divergences of hemolytic intensities of various organotin compounds. However, in our study, we determined the intensities of hemolytic activity among various organotin compounds which exist in the environment and may be hazardous to human health, and these hemolytic activities were estimated by a regression equation obtained by our QSAR study with topological parameters which are capable of calculation from the chemical structures.

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