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Total surface areas of Group IVA organometallic compounds: predictors of toxicity to algae and bacteria

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There is a high correlation between molecular surface area (TSA) of triorganotin and triorganolead compounds and their toxicity towards a bacterium (*Escherichia coli*) and an alga (*Selenastrum capricornutum*). Parallel attempts to correlate other Group IVA organometals incorporating silicon or germanium were unsuccessful. It was further demonstrated, however, that a high correlation was obtainable between certain series of compounds with the same organic substituent but different metal centers involving all Group IVA elements. In both instances, the inability to obtain a quantitative structure–activity relationship (QSAR) for all systems studied appears to be a function of the solubility of the compounds. While organotin TSA values have been found to correlate well with their toxicities toward various organisms, this study clearly suggests that this type of QSAR can be readily extended to include other organometal systems, provided that there is no solubility problem and the toxicity is a function of the hydrophobicity of the organometal compounds.

Keywords: Organotin, organolead, organosilicon, organogermanium, toxicity, total surface area (TSA), quantitative structure–activity relationships (QSAR), molecular topology, Group IVA organometals, *Escherichia coli*, *Selenastrum capricornutum*

INTRODUCTION

The industrial production and usage of organotin compounds have increased rapidly in recent years. The world wide production has increased over seven-fold during the last three decades, from 5000 tons in 1955 to over 35000 tons in 1984.[1]

Toxicological activities of organotins have been found to be a function of the organic groups attached to the tin atom as well as of the number of groups involved.[2,3] For example, triphenyltins are commonly employed as agricultural fungicides,[4,5] tricyclohexyltins are known acaricides,[6] while tributyltins are the active agents in antifoulant paints. The biocidal behavior of triorganotin compounds is due to their ability to inhibit mitochondrial oxidative phosphorylation although the exact nature of the binding is not well understood.[8]

There is an increasing concern about the environmental fate of these organotin compounds as well as their degradation products as a result of the increased usage of these biocides. Thus, the development of reliable correlations between the toxicities of organotins and some descriptor or descriptors of the molecule is of interest. This would then allow predictions of toxicities of new or untested compounds and promote more discriminatory usage of these chemicals.

A common technique used for linking structures of molecules with their toxicological activities is Quantitative Structure–Activity Relationship (QSAR) studies. QSAR is a regression equation that relates some measurable biological activity to a physicochemical or biochemical property or properties associated with chemical structure. QSARs have been shown to be useful in assessing environmental impact of hazardous chemicals.[9] The backbone of any QSAR study is the descriptor or predictor. Two of the more common predictors used in QSAR studies are the Hansch and Taft–Hammett parameters.[10,11] At present, emphasis is being placed on the development of descriptors based on molecular structure and an analysis of their correlation by computer. Using this procedure, descriptors can be generated and evaluated to determine whether they

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correlate significantly with the toxicity of the species of interest. One advantage of employing descriptors based upon the molecule's entire geometrical and/or electronic configuration is that no information is lost as can occur in the summation of fragment approach.[12] This holistic approach should distinguish the local atom geometries and their contributions to the overall predictor.

The use of total surface areas (TSAs) as a predictor in QSAR studies for organometallic systems was first effectively employed by Brinckman *et al.* for a series of organoarsenicals and organotins.[13,14] Using TSA as a predictor, Eng *et al.* were able to find a high correlation between TSA values and organotin toxicity towards several distinct types of organotins.[15] In order to assess the possibilities of extending TSAs as a descriptor of toxicities into other Group IVA organometallic systems, which may be of industrial relevance, we have investigated QSARs between the TSA values of a series of triorgano-Group IVA chlorides containing silicon, germanium, lead and tin, and their toxicity toward a bacterium, *Escherichia coli*, and an alga, *Selenastrum capricornutum*.

EXPERIMENTAL

Chemicals

Trimethyl-, triethyl-, tri-n-propyl, tri-n-butyl- and triphenyl-silicon chloride as well as tri-n-butyl- and triphenyl-tin chloride were obtained from Aldrich Chemical Co. Inc. Triethyl-, tri-n-butyl- and triphenol-germanium chloride along with trimethyl- and triethyl-lead chloride were purchased from Organometallics Inc. From Alpha Products Morton Thiokol were purchased trimethyl- and tri-n-propyl-tin chloride as well as triphenyllead chloride. Triethyltin chloride was obtained from Strem Chemicals. The compounds were used without further purification.

Stock solutions of the organometal compounds were made up in spectrophotometric-grade methanol obtained from Fisher Scientific Inc. at compound concentrations greater than 1000 ng μl^{-1} (ppm) when solubility permitted. Solubility problems were encountered for triphenyllead and triethyllead chlorides. Stock solution for these compounds were made up to 500 ng μl^{-1} and 218 ng μl^{-1} respectively.

The distilled water employed during this study was further purified using a Culligan exchange resin system to obtain 13–15 Ω cm resistivity. The buffer used in study of the algae was a 0.065 mol dm^{-3} $NaHCO_3$–0.035 mol dm^{-3} $KHCO_3$ buffer.

Instrumentation

The turbidity measurements were recorded with a Klett–Summerson photoelectric colorimeter using a blue filter (No. 42). The volume of oxygen liberated in the algae study was measured using a Gilson single valve differential respirometer. Cells were centrifuged at 13800 *g* with a Sorvall SS-4 Manual Superspeed Centrifuge. The numbers of cells in suspensions were determined with a Bausch & Lomb phase-contrast flat field microscope using a Petroff–Hausser counter. The bacteria were incubated in a New Brunswick Scientific Gyrotory water bath shaker.

Microorganisms and culture conditions

E. coli strain DH5alpha was a gift from Mr Sam Woo of the National Institute of Standards and Technology, and was grown in 250-cm^3 Erlenmeyer flasks at 37°C containing M9 salts medium. The M9 salts medium consisted of 100 cm^3 M9 salts stock solution, 20 cm^3 glucose (20% w/v), 10 cm^3 0.1 mol dm^{-3} $MgSO_4$, 10 cm^3 0.01 mol dm^{-3} $CaCl_2$ and diluted to 1 dm^3 with deionized water. The M9 salts stock solution consisted of 60 g Na_2HPO_4, 30 g anhydrous KH_2PO_4, 5 g NaCl, 10 g NH_4Cl and 1 dm^3 of deionized water. The bacterial culture was shaken at 180 rpm for approximately 20–24 h to obtain logarithmic-phase cells as the inoculum, for toxicity tests.

S. capricornutum 22662 was obtained from the American Type Culture Collection, Rockville, MD, USA. The algae were grown routinely in Gorham's medium[16] under fluorescent lights. The inoculum for toxicity tests was prepared by diluting a 1:30 culture in fresh Gorham's medium in a screw-top test-tube equipped with an inlet and an outlet port. Water-saturated air was bubbled through the test-tube while being placed 1 in (2.5 cm) from the fluorescent light source. The active cells were harvested after 72 h, washed

twice with 40 cm^3 of 0.065 mol cm^{-3} $NaHCO_3$/0.035 mol cm^{-3} $KHCO_3$ buffer and then resuspended in 40 cm^3 of buffer.

Toxicity studies

E. coli

The inoculum was added to a nephelo culture flask (Wheaton Brand) containing 25 cm^3 of M9 salts medium to obtain a turbidity reading of 10 Klett units. Appropriate amounts of an organometal solution was then added to the flasks in order to obtain a range of concentrations. Methanol was added to the control flask and to the test flasks so that the total volume of methanol and organometal solutions was 200 μl. Turbidity readings were then taken periodically with incubation time.

S. capricornutum

Oxygen evolution was measured using a Gilson differential respirometer equipped with incandescent lamps (Gilson Medical Electronics, Madison, WI, USA). Reaction flasks equipped with two side-arms were used. In one side-arm was placed 2 cm^3 of bicarbonate ($NaHCO_3$/$KHCO_3$) buffer and the other side-arm contained the buffer to which was added the appropriate amounts of organometal solution and methanol to obtain a range of concentrations. The control flask contained 30 μl of methanol and no toxicant. A portion (1 cm^3) of alga inoculum was placed in the bottom of the flask. The flasks were then lowered into an illuminated constant-temperature water bath (28°C) and held there for 10 min to achieve thermal equilibrium, after which the flasks were tilted so that the organometal solution and algae could mix. The flasks were shaken at 120 rpm. The volume of oxygen liberated by the algae was measured periodically.

To obtain the LC_{50} values (the concentration at which 50% of the species are inhibited), growth (*E. coli*) or oxygen evolution (*S. capricornutum*) rates were determined at each of several organometal concentrations. A plot of the rate versus their concentrations then yielded the LC_{50} values.

Topological calculations

The TSA values were calculated using the SAVOL program[17] employing conventional bond distances, bond angles and van der Waals radii obtained from the literature. The details of our procedures have been previously described.[12–14]

Table 1 Toxicity (LC_{50}) of *E. coli* and *S. capricornutum* and total surface areas (TSA) values for triorgano-Group IVA chlorides

Compound	Toxicity, Lc_{50} (ln μmol dm^{-3})		TSA ($Å^2$)
	E. coli	*S. capricornutum*	
Me_3SiCl	>9.00	4.70	139.41
Et_3SiCl	8.07	>5.76	202.61
n-Pr_3SiCl	>8.34	>6.16	270.23
n-BuSiCl	>7.77	>1.75	33.83
Ph_3SiCl	>0.31	>1.75	306.95
Et_3GeCl	6.71	1.77	213.36
n-Bu_3GeCl	>6.50	3.38	348.64
Ph_3GeCl	>3.38	>4.13	321.84
Me_3SnCl	5.82	3.11	150.12
Et_3SnCl	2.40	1.60	212.80
n-Pr_3SnCl	1.12	0.73	280.53
n-Bu_3SnCl	0.28	−0.65	348.42
Ph_3SnCl	1.05	−0.52	330.66
Me_3PbCl	1.94	2.31	161.36
Et_3PbCl	0.51	1.11	228.53
Ph_3PbCl	−1.29	−1.22	344.39

RESULTS AND DISCUSSION

E. coli

Tabulated in Table 1 are the LC_{50} values from the *E. coli* and *S. capricornutum* studies for each organometal with its TSA value. For some of the organosilicon and germanium compounds, the solubility limit was reached before inhibition of growth by 50% of the control could be obtained. Thus, the LC_{50} values were unobtainable for Me_3SiCl, n-Pr_3SiCl, n-Bu_3SiCl, Ph_3SiCl, n-Bu_3GeCl and Ph_3GeCl.

The toxicity results verses TSA values are plotted in Fig. 1 for the organotin and organolead compounds. As can be seen from this figure, there is a high correlation between the toxicity of the individual series and their TSA values. The attempt to correlate the entire set of data with TSA was not possible, as is evident from the low correlation coefficient ($r^2 = 0.61$). However, when the TSA values for the ethyl substituents were plotted against their respective toxicity (Fig. 2), there is a high correlation ($r^2 = 0.92$) for the four compounds, indicating that there is a relationship between compounds with the same alkyl substituent but different metal centers. These results are consistent with prior evidence for correlating TSA with toxic effects on varied micro and macro cells (13–15).

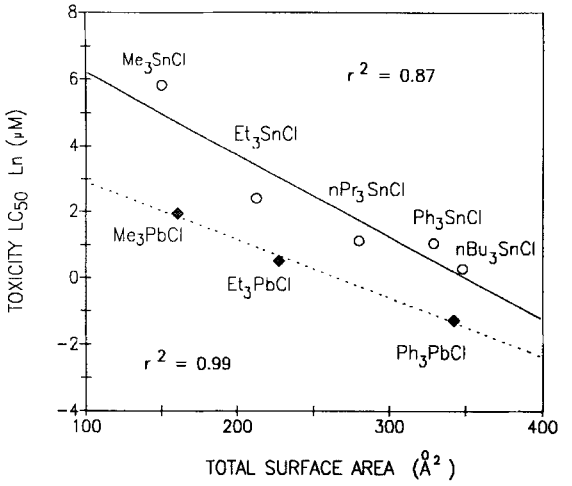

Figure 1 A plot of the logarithm of the toxicity for *E. coli* as indicated by LC_{50} values versus computed molecular total surface areas (TSA) for the triorganotin and triorganolead compounds.

S. capricornutum

As with *E. coli*, there was difficulty in obtaining some of the toxicity data needed to achieve 50% inhibition due to low solubilities of Et_3SiCl, n-Pr_3SiCl, n-Bu_3SiCl and Ph_3GeCl. From this and the previous studies on *E. coli*, it appears that organosilicon and organogermanium compounds are less toxic than the corresponding compounds of higher members of Group IVA.

The toxicity results for the algae studies with the organolead and organotin toxicants are plotted and shown in Fig. 3. As is evident from the

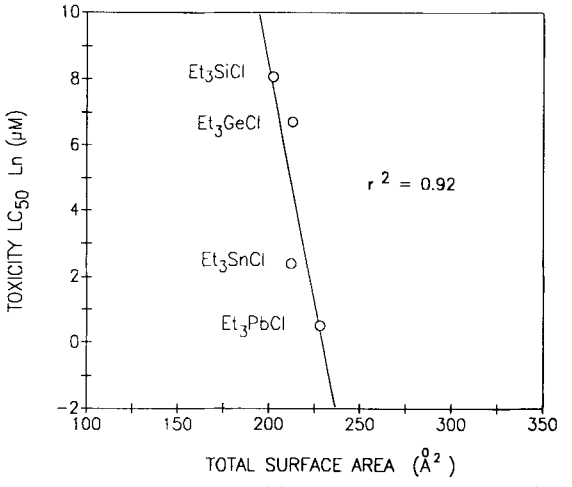

Figure 2 A plot of the logarithm of the toxicity for *E. coli* as indicated by LC_{50} values versus computed molecular total surface areas (TSA) for the Et_3MCl compounds where M = Si, Ge, Sn and Pb.

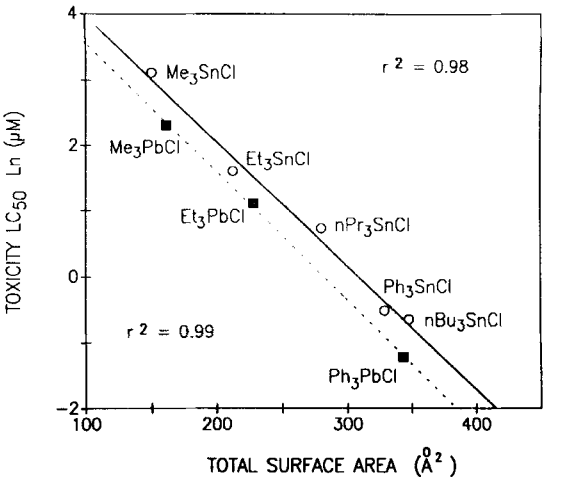

Figure 3 A plot of the logarithm of the toxicity for *S. capricornutum* as indicated by LC_{50} values versus computed molecular total surface areas (TSA) for the triorganotin and triorganolead compounds.

plot, there is a high correlation for both families. Unlike the case for *E. coli*, these two families can be intercorrelated, as is evident from the high correlation coefficient ($r^2 = 0.96$). Furthermore, for the *S. capricornutum* study, it was possible to intercorrelate the family of compounds having the methyl or ethyl group as the substituent. The correlation coefficients are 0.96 and 0.92, respectively. These results are shown in Fig. 4. Again, this indicates that there is a relationship between compounds with the same alkyl group but different metal centers.

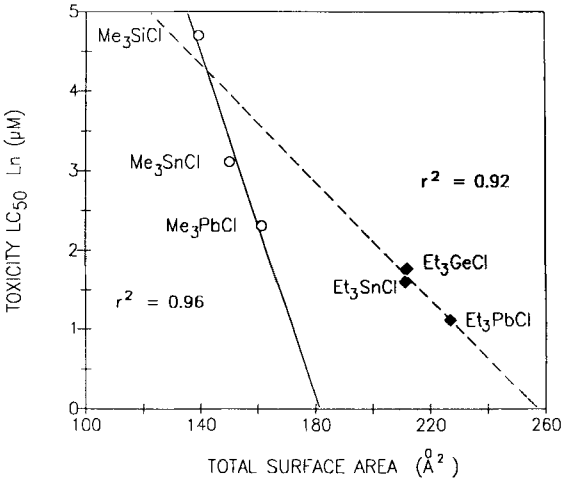

Figure 4 A plot of the logarithm of the toxicity for *S.capricornutum* as indicated by LC_{50} values versus computed molecular total surface areas (TSA) for the various trimethyl- and triethyl-substituted Group IVA metals.

These two studies demonstrate that TSA values can be used as a predictor of toxicity for the two micro-organisms tested. Whether compounds with different metal centers can be intercorrelated is highly dependent on the solubility of the compounds. Although the present study has been limited to Group IVA, it has been shown that the method can be expanded to other hydrophobic species.[15] Consequently, predictive QSAR studies of analogous carboligated metal centers with the same or different groups are possible.

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Note Certain commercial products or equipment are mentioned in order to describe adequately experimental procedures. In no case does such identification imply endorsement by the National Institute of Standards and Technology, nor does it imply that the material is necessarily the best available for the purpose.

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