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REVIEW

Interactions of organotins with biological systems

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The bonding and structure in the environments of tin atoms in organotin–biological molecules has been investigated by ^{119}Sn Mössbauer spectroscopy, mainly through the rationalization of the nuclear quadrupole splitting parameter by point-charge model approaches.

Organotin moieties R_2Sn^{IV} and R_3Sn^{IV} (R = Me, nBu, Ph) generally assume trigonal-bipyramidal type configurations in membranes of human erythrocytes, when incubated with whole erythrocytes and erythrocyte ghosts at the level of micromolar (μmol dm^{-3}) organotin per mg of membrane protein. Corresponding structures are assumed by Me_2Sn^{IV} and Me_3Sn^{IV} in the cytoplasm. Ethanolic Me_2SnCl_2 yielded distorted *trans*-octahedral species when reacted with ghost cells. These configurations may in principle originate through coordination of the metal by donor nitrogen or oxygen atoms from the cell constituents, such as protein side chains and related component molecules, carbohydrate fragments, and phospholipids, according to data from various model systems. Hydrolyzed species, such as bis(chlorodiorganotin) oxides and triorganotin hydroxides, could also occur for the n-butyltin and phenyltin species.

The moieties Me_2Sn^{IV} and Alk_3Sn^{IV} (Alk = Me, Et, nBu), present as the hydrolysis products $Me_2Sn(OH)_2$ and Alk_3SnOH at physiological pH in the aqueous phase (eventually coordinated by donor atoms from buffers), react with thiol groups of model molecules, as well as of feline and rat hemoglobin, forming tetrahedral or trigonal-bipyramidal tin sites characterized by covalent Sn–S bonds (C_2SnS, C_2SnS_2 and C_3SnS skeletons); tin atoms are eventually further coordinated by nitrogen donors from amino acid fragments or from buffers, as well as by hydroxyl oxygen.

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INTRODUCTION

Organotin(IV) compounds are known to interact with cell membranes and with proteins. The moieties nBu_3Sn^{IV} form spheres connected to the membranes of human erythrocytes,[1,2] possibly consisting of micelles, with the organic radicals interacting with the lipid bilayer. The formation of organotin(IV) micelles has been reported recently.[3]

The neutral species R_3SnX (X = Cl, OH; R = Me, Et, nPr, nBu, Ph) occur in membranes of rat liver mitochondria, of human and ox erythrocytes, as well as in liposomes of lecithin and of bacterial phosphatidylethanolamine.[4–7] The nBu_3SnX derivatives are the most efficient in promoting Cl^-/OH^- anion exchange through the membrane, which is connected to the lipophilicity of the nBu radicals and to the consequent intercalation of nBu_3SnX into the lipid bilayer.[6,7]

The moieties R_nSn^{IV} ($n = 2$ and 3) bind to proteins and glycoproteins of cell membranes, as well as to cellular proteins: for example, (i) Et_3Sn^{IV} to ATPase and hexokinase of trout, feline and human erythrocytes;[8] (ii) nBu_2Sn^{IV} and nBu_3Sn^{IV} to band 3 proteins (e.g. Na^+, K^+-ATPase, acetylcholine esterase) of human erythrocyte membrane;[9] nBu_2Sn^{IV} interacts also with proteins of band 7 and 9,[9] and binding to skeletal muscle membranes occurs;[9] (iii) nBu_3Sn^{IV} to plasma membranes and cellular proteins of murine erythroleukemic cells, inducing denaturations;[10] (iv) Et_3Sn^{IV} and Ph_3Sn^{IV} to the proteolipid fraction from membranes of rat liver mitochondria; Et_3Sn^{IV} to ATPase, and Ph_3Sn^{IV} to proteins with mass 5000–6000.[11,12]

The moieties R_3Sn^{IV} show weak Coulomb interaction with the phosphodiester group of phospholipids: for example, (i) Et_3Sn^{IV} with phospholipids from membranes of rat liver mitochondria;[13] (ii) Me_3Sn^{IV} and Bu_3Sn^{IV} with phos-

phatidylcholine liposomes.[14]

Feline and rat hemoglobin form complexes with R_3Sn^{IV} and Me_2Sn^{IV}.[15–19] Species such as $(Et_3Sn)_2$(hemoglobin tetramer) are formed, characterized by high affinity between tin and hemoglobin.[15,16] The moiety Et_3Sn^{IV} is linked to the α-subunits of the tetramer, through the thiol sulfur from cysteine-13 and the imidazole nitrogen of histidine-113, axially coordinating into a trigonal-bipyramidal type structure.[20] Analogous bonding situations would occur for Me_3Sn^{IV}– and nBu_3Sn^{IV}–hemoglobin.[17] Facial SnC_3 fragments, as well as bonding by two imidazole nitrogen atoms, have been also assumed.[15,21] Me_2Sn^{IV} would be involved in tetrahedral, as well as trigonal-bipyramidal, complexes with 'hemoglobin phases'[18,19] (*vide infra*).

It seems worthy of note that the structural assumptions reported above are generally based on the interpretation of experimental data reflecting bonding and coordination geometries, but are, on the other hand, rather speculative in some other instances. Moreover, information is sometimes missing about the nature of the solutions of organotin compounds employed in the experiments, and the addition of solid organotins to biological systems might have occurred. In addition, organotin hydrolysis processes are often ignored. These circumstances, in conjunction with the different experimental conditions and procedures employed in the individual studies, may originate incongruities and differing interpretations as reported above.

The work reported in the present paper concerns molecular aspects of the *in vitro* interaction of organotin compounds with erythrocytes (representative of simple biological cells and their membranes), as well as with hemoglobin (a model protein). Organotin–native DNA systems[22–26] are discussed in a following paper. Structure and bonding environments of the tin atoms were investigated by ^{119}Sn Mössbauer spectroscopy, selected as a technique which allows the study of the coordination sphere of a metal irrespective of the molecular complexity of the medium.

EXPERIMENTAL

Organotin–human erythrocyte systems[27]

Erythrocytes were isolated by centrifugation from human blood (obtained from blood banks), washed separately with PBS (NaH_2PO_4 + Na_2HPO_4, 5 mmol dm^{-3}, NaCl 0.15 mol dm^{-3}, pH 8.0), and pelleted by centrifuging at 2500 rpm for 15 min. Pellets (3–8 cm^3) were added with R_nSnCl_{4-n} (R = alkyl) (1 mol dm^{-3} in C_2H_5OH) as well as with aqueous $Me_2Sn(OH)_2$ and Me_3SnOH (each 10–20 mmol dm^{-3}) (final organotin concentration = 5–10 mmol dm^{-3}; C_2H_5OH = 1%), and incubated at 37 °C for 15 min. After washing with PBS, 2 cm^3 of the organotin–erythrocyte suspension was frozen in liquid nitrogen and submitted to Mössbauer spectroscopy, by procedures described elsewhere.[18,27] A further fraction of the erythrocyte–organotin pellets (*vide supra*) was hemolyzed with 5P8 (Na_2HPO_4 + NaH_2PO_4, 5 mmol dm^{-3}, pH 8). The ghost cells were washed with PBS and centrifuged and the supernatant was dried by lyophilization, both being eventually submitted to Mössbauer spectroscopy. The interaction of organotins with erythrocyte membranes was effected by preparing ghost cells through hemolysis of erythrocytes with 5P8 (*vide supra*), and adding organotins in ethanol or aqueous solutions, followed by incubation and centrifugation, with procedures and conditions analogous to those described above.

Organotin–rat hemoglobin complexes[17–19]

Rat erythrocytes were hemolyzed as above, and the concentration of hemoglobin was determined by visible spectrophotometry. The Me_2Sn^{IV} and R_3Sn^{IV} derivatives (5–7 μmol, from aqueous stock solutions containing 5–10 mmol dm^{-3} in

Table 1 The tin environment in organotins as a function of the ^{119}Sn Mössbauer parameter nuclear quadrupole splitting (ΔE)[a]

Species[b]	Structure[c]	ΔE range[d] (mm s^{-1})
R_nSnA_{4-n}	tet	0.00–2.80
R_2SnA_4	oct; *cis*-R_2	1.63–2.34
Alk_2SnA_3	tbp; equatorial Alk	2.85–3.12
Alk_2SnA_4	oct; *trans*-Alk_2	3.64–4.75
Ph_2SnA_3	tbp; equatorial Ph	2.40–3.05
Ph_2SnA_4	oct; *trans*-Ph_2	3.35–4.30
Alk_3SnA_2	tbp; equatorial Alk	3.18–4.57
Ph_3SnA_2	tbp; equatorial Ph	2.73–4.00

[a]A collection of literature data[28,31,32] for the fingerprint assignment of most common geometries.
[b]R = Alk, Ph; n = 1–3; A = ligand donor atom other than C.
[c]Abbreviations: tet = tetrahedral; oct = octahedral; tbp = trigonal-bipyramidal.
[d]Experimental data.

0.2 mol dm^{-3} Hepes buffer, pH 7.4, for R = Me, Et, as well as from stock 0.1 mol dm^{-3} nBu_3SnCl in ethanol) were respectively added to 3.5–4.5 μmol of hemoglobin (the final volume being 5–6 cm^3) and the solution was left to crystallize at 4 °C overnight. The interaction of organotins with crystalline rat hemoglobin was effected by preparing solid samples of hemoglobin (3.5 μmol in 5 cm^3 of H_2O added to 0.5 cm^3 of 0.2 mol dm^{-3} Hepes buffers at pH 7.4, and crystallized on standing at 4 °C for one day), which were suspended in H_2O, added with 7 μmol of the organotin compound, stirred, and stored overnight at 4 °C.

Mössbauer Spectroscopy, and fingerprint and point-charge model treatment of the nuclear quadrupole splitting parameter ΔE, for structural attribution

The apparatus and procedures employed in the determination of ^{119}Sn Mössbauer spectra were as reported earlier.[17,18,25]

The geometry of the environment of the tin atoms was first estimated from the experimental parameters ΔE by fingerprint assignments, e.g. according to the data in Table 1. Detailed information on structure and bonding has been subsequently extracted from calculations of ΔE obtained by the following procedures:

(I) The literal version of the point-charge model (generally concerned with regular structures), where the contributions of *all* (assumed) valence electron pairs to the magnitude of the electric field gradient tensor at the tin nucleus have been taken into account in the calculations.[28]

(II) The point-charge model approach accounting *only* for Sn–C valence electrons in dictating the magnitude of the electric field gradient, with the contributions of bonds between tin and electronegative ligand atoms (Sn–A) being ignored.[29,30]

Calculations according to (I) and (II) have been effected employing partial nuclear quadrupole splitting parameters (p.q.s.) taken from the literature.[18,28,30–32] Model (II) allows the correlation of $\Delta E_{exp,calcd}$ data with distortions from ideal octahedral, trigonal-bipyramidal and tetrahedral structures, and estimates of the order of magnitude of C–Sn–C angles in $R_2SnA_{3,4}$, and C–Sn–A angles in $R_3SnA_{1,2}$.[29,30]

RESULTS AND DISCUSSION

The nature of the reagent organotin species and of the hydrolysis products

Ethanolic solutions of organotins $[R_2SnCl_2(C_2H_5OH)_2$ and $R_3SnCl(C_2H_5OH)]$ show quasi-regular *trans*-R_2 octahedral and trigonal-bipyramidal (equatorial C atoms) configurations respectively, as extracted from experimental ΔE data [Table 2(A)] and point-charge model (II) calculations[26] (see the Experimental section.) Aqueous methyltins (at pH around 4) consist of trigonal-bipyramidal species, $[Me_2Sn(OH)(OH_2)_n]^+$ and $[Me_3Sn(OH_2)_2]^+$ [Table 2(B)] with equatorial carbon and hydroxyl oxygen atoms, according to point-charge model (I) estimates.[17,26] The hydroxides in aqueous media [Table 2(B)] and their adducts with ligand molecules, such as excess Hepes buffer, are generally trigonal-bipyramidal too, except $Me_2Sn(OH)_2$ which is tetrahedral.[17,18]

As reported in the Experimental section, the organotin–human erythrocytes, –erythrocyte ghosts, and –rat hemoglobin systems (as well as related models, *vide infra*) were obtained essentially from aqueous media, eventually buffered at neutral pH.[17–19,27,33] Water-insoluble hydrolyzed organotin derivatives may then form, together with, or in place of, organotins bound to component molecules of biological systems, whose Mössbauer parameters are listed in Table 2(C). Oxides $(R_2SnO)_n$ and stannoxanes $(R_3Sn)_2O$ are tetrahedral solid-state species;[38–41] bis(chlorodiorganotin) oxides $(R_2SnCl)_2O$ are trigonal-bipyramidal ladder- or staircase-type polymers,[36,42–45] and hydroxides (R_3SnOH) are quasi-regular trigonal-bipyramidal polymeric species with axially bridging OH groups.[46,47] Structure and bonding in these molecules, as inferred from Mössbauer parameters and the related point-charge model treatments, often agree remarkably with the correct crystallographic data; this is shown in Fig. 1 for $(R_2SnCl)_2O$ species, which are likely to occur in our systems (*vide infra*).

Organotin–erythrocytes and –erythrocyte membranes

The Mössbauer parameters of these systems[27] are summarized in Table 3. The magnitude of the isomer shift (δ) data corresponds to values reported for the individual R_nSn^{IV} moieties.[28–32,34]

Table 2 ^{119}Sn Mössbauer parameters of organotin species interacted with human erythrocytes and erythrocyte ghosts, with rat hemoglobin, and with calf thymus DNA (see Ref. 26), and the parameters of eventual hydrolysis products[a]

Compound or system[b]	δ (mm s^{-1})[c]	ΔE (mm s^{-1})[d]	Ref.
(A) Ethanol solutions[e]			
$Alk_2SnCl_2(C_2H_5OH)_2$[f]	1.35–1.59	3.85–4.05	25, 26
$Ph_2SnCl_2(C_2H_5OH)_2$	1.31	3.58	26
$Alk_3SnCl(C_2H_5OH)$[f]	1.33–1.48	3.31–3.43	26, 17
$Ph_3SnCl(C_2H_5OH)$	1.29	3.07	26
(B) Aqueous solutions[e]			
$[Me_2Sn(OH)(OH_2)_n]^+$	1.20	3.04	25, 26
$Me_2Sn(OH)_2$	0.94	2.24	18
$Me_2Sn(OH)_2$. Hepes	1.11	3.03	18
$[Me_3Sn(OH_2)_2]^+$	1.50	3.87	17
$Me_3Sn(OH)(OH_2)$	1.24	2.80	17
$Me_3Sn(OH)$. Hepes	1.34	3.31	17
$Et_3Sn(OH)(OH_2)$	1.30; 1.33	2.98; 3.02	17, 26
$Et_3Sn(OH)$. Hepes	1.45	3.41	17
(C) Solids[g]			
$(Me_2SnO)_n$	0.91–1.00	1.82–2.07	34, 35
$(Et_2SnO)_n$	1.01–1.05	2.10–2.33	34, 26
$(Et_2SnCl)_2O$	1.34; 1.50	3.34; 3.41	34, 36
$(nBu_2SnO)_n$	0.94–1.15	1.95–2.20	34
$(nBu_2SnCl)_2O$	1.25–1.46	3.20–3.26	34, 36
$(nOct_2SnO)_n$	0.96–1.03	2.00–2.05	34
$(nOct_2SnCl)_2O$	1.42	3.26	26
$(Ph_2SnO)_n$	0.89–0.95	1.73–2.00	34
$(Ph_2SnCl)_2O$	1.26	3.08	36
$(nBu_3Sn)_2O$	1.10–1.29	1.15–2.40	34, 37
nBu_3SnOH	1.37–1.46	2.99–3.24	34, 37
$(nOct_3Sn)_2O$[h]	1.21	1.57	37
$nOct_3SnOH$[h]	1.35	2.95	37
$(Ph_3Sn)_2O$	1.07; 1.08	1.63; 2.15	37, 34
Ph_3SnOH	1.16–1.35	2.68–2.83	34, 37

[a] Measured at liquid-nitrogen temperature.
[b] Hepes is *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; buffer, pH = 7.40. $[Me_2Sn(OH)(OH_2)_n]^+$ and $[Me_3Sn(OH_2)_2]^+$ occur at pH ≈ 4.0, and the hydroxides at pH > 7.0.[26]
[c] Isomer shift with respect to room-temperature $CaSnO_3$; individual values or ranges of values.
[d] Nuclear quadrupole splitting; individual values or ranges of values.
[e] Glassy absorbers, frozen by immersion into liquid nitrogen.[18] Solutions (A) are 0.1 mol dm^{-3}, and (B) are 5–20 mmol dm^{-3}.
[f] Alk = Me, Et, nBu, nOct.
[g] Unless otherwise stated.
[h] Oil.

The ΔE data for systems 1, 2 and 4–9 clearly indicate the occurrence of trigonal-bipyramidal species, whilst system 3 would assume a *trans*-octahedral structure, according to ΔE ranges for fingerprint assignments reported in Table 1. The possible geometries of the environments of the tin atoms are reported in Fig. 2, in conjunction with distortions, as estimated by point-charge model calculations.[27]

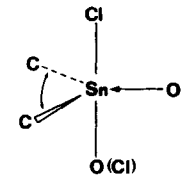


Figure 1 The nature of the environment of tin atoms in bis(chlorodiorganotin) oxides. C–Sn–C angles in $\{[Me_2Sn(NCS)]_2O\}_2$, $[(Me_2SnCl)_2O]_2$, $[(Ph_2SnCl)_2O]_2$, and related species, are in the range 122–141°, as determined by X-ray diffractometry.[36, 42–45] Point-charge model (II) estimates (see the Experimental section of this paper) are as follows [from ΔE_{exp} data in Table 2(C)]: $(Alk_2SnCl)_2O$, C–Sn–C = 125–131°; $(Ph_2SnCl)_2O$, C–Sn–C = 135°.

The molecular nature of the products 1–9, Table 3, is subsequently explored in connection with compounds formed through hydrolytic processes, as well as with organotin–model complexes and/or methyltin–ligands species formed in aqueous solutions.

The occurrence, or the formation, of tetrahedral or quasi-tetrahedral hydrolysis products, such as the water-soluble species $Me_2Sn(OH)_2$ and $Me_3Sn(OH)(OH_2)$, and the solids $(R_2SnO)_n$ and $(R_3Sn)_2O$ (*vide supra*, Table 2(B) and (C)) may be safely excluded by inspection of the ΔE data in Tables 2 and 3; instead, the trigonal-bipyramidal (solid-state) species $(R_2SnCl)_2O$ and R_3SnOH (R = nBu and Ph) could be present in the systems 6, 8, and 7, 9, respectively (Tables 2 and 3; Figs. 1 and 2).

The biological molecules providing ligand atoms to tin in the systems 1–9, Table 3 and Fig. 2, could be assumed according to the following (oversimplified) scheme.

(i) In proteins, peptides and amino acids from erythrocyte membranes and cytoplasm, oxygen atoms donate from carboxyl, carbonyl and hydroxide; amino and heterocyclic nitrogen atoms, and thiol sulfur, also donate.
(ii) In oligosaccharides from glycolipids and glycoproteins, alcoholic hydroxyls donate.
(iii) In phospholipids in the lipid bilayers, oxygen atoms of phosphodiester groups donate.

These hypotheses are tested here by taking into account the Mössbauer parameters, as well as representative values of bond angles from

Table 3 ^{119}Sn Mössbauer parameters of organotin(IV)–human erythrocyte systems[a]

System no.	Reacted organotins, and systems[b]	δ (mm s^{-1})[c]	ΔE (mm s^{-1})[c]	Γ_1, Γ_2 (mm s^{-1})[d]
1	$Me_2Sn(OH)_2$–whole erythrocytes, –cytoplasm, and –ghost cells	1.09–1.22	2.96–3.13	0.85–1.27
2	$Me_2SnCl_2(C_2H_5OH)_2$–whole erythrocytes, and –membranes	1.15–1.32	2.75–3.24	0.84–1.85
3	$Me_2SnCl_2(C_2H_5OH)_2$–ghost cells	1.23	3.77	0.90–1.13
4	$Me_3SnOH(OH_2)$–whole erythrocytes, –cytoplasm, and –ghost cells	1.28–1.35	3.20–3.37	0.64–1.10
5	$Me_3SnCl(C_2H_5OH)$–whole erythrocytes, and –ghost cells	1.23–1.35	3.37–3.48	0.73–1.14
6	$nBu_2SnCl_2(C_2H_5OH)_2$–whole erythrocytes, –membranes, and –ghost cells	1.19–1.33	2.94–3.33	0.60–1.21
7	$nBu_3SnCl(C_2H_5OH)$–whole erythrocytes, –membranes, and –ghost cells	1.21–1.37	2.96–3.39	0.80–1.57
8	$Ph_2SnCl_2(C_2H_5OH)_2$–whole erythrocytes, –membranes, and –ghost cells	0.95–1.13	2.47–2.96	0.98–1.16
9	$Ph_3SnCl(C_2H_5OH)$–whole erythrocytes, and –ghost cells	1.12–1.19	2.75–2.83	1.13–1.50

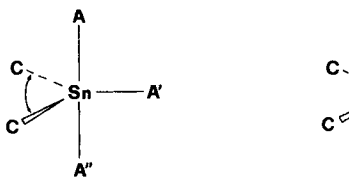
[a] At liquid-nitrogen temperature. Data from Ref. 27

[b] Samples prepared as described in the Experimental section: methyltins from aqueous solutions at physiological pH, $Me_2Sn(OH)_2$ and $Me_3Sn(OH)(OH_2)$, and organotins from ethanol, $R_nSnCl_{4-n}(C_2H_5OH)_m$, reacted with erythrocytes, from which pellets of organotin–cell membrane were subsequently obtained by hemolysis. Ghost cells were prepared by previous hemolysis of erythrocytes, and then reacted with the above solutions of organotins.

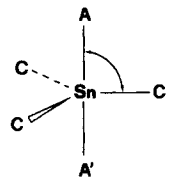
[c] See footnotes c and d to Table 1.

[d] Full width at half-height of the resonant peaks; ranges of 'working' values from computer fitting.

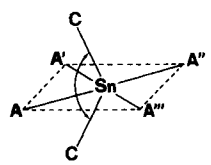
crystallographic studies and the corresponding estimates from the point-charge model treatment of Mössbauer ΔE parameters, for the following classes of model compounds: (i) R_2Sn–dipeptide (Fig. 3); (ii) R_3Sn–acetyldipeptide (Fig. 4); (iii) R_2Sn–carbohydrates and R_2Sn–nucleosides (Fig. 5); (iv) $Me_2Sn(OH)_2$ and $Me_3Sn(OH)(OH_2)$ in aqueous solutions in the presence of excess dimethylphosphinate or cyclic adenosine-3′:5′-phosphate (Fig. 6); (v) R_2Sn– and R_3Sn–phosphate complexes (Fig. 7). These data, when compared with the ΔE parameters and the representative angles reported in Table 3 and Fig. 2, suggest that the organotin–erythrocytes and organotin–erythrocyte membrane systems 1, 2, and 4–7 could consist of five-coordinated



System	Angle C–Sn–C(°)
1	119–123
2	113–127
6	118–129
8	116–131



System	Angle C–Sn–A(°)
4	97–92
5	92–90
7	102–90
9	98–96



System	Angle C–Sn–C(°)
3	152

Figure 2 The structures assigned[27] to the organotin–erythrocytes and related systems through fingerprint criteria (Table 1) concerning experimental values of nuclear quadrupole splitting ΔE (Table 3), and point-charge model calculations of angles according to the procedure (II). A′, A″ and A‴ are electronegative bonding atoms, e.g. O, N.

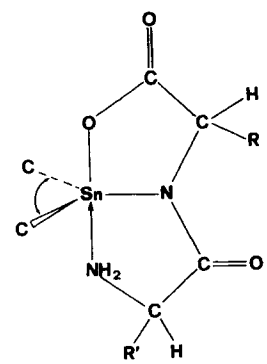


Figure 3 The structure (idealized) of R_2Sn-dipeptide complexes. C–Sn–C angles from X-ray diffractometry[48–53] are as follows: for R = Alk, C–Sn–C = 121.7–131.4°; for R = Ph, C–Sn–C = 117.5°. C–Sn–C angles from ΔE_{exp} data[35,52–55] and point-charge model (II) estimates are: for R = Alk, solid-state and frozen solutions, ΔE_{exp} = 2.46–3.51 mm s^{-1}, C–Sn–C = 104–133°; for R = Ph, solid-state, ΔE_{exp} = 2.21–2.39 mm s^{-1}, C–Sn–C = 108–114°.

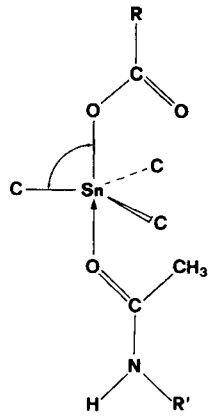


Figure 4 A possible tin environment in complexes R_3Sn–*N*-acetyldipeptide in the solid state; R = Me, Et, nBu, nOct, Cy, Ph; dipeptide = glycylglycine, glycylalanine, glycylvaline.[56]

C–Sn–O angles from ΔE_{exp} data[56] and point-charge model (II) estimates: for R = Alk, $\Delta E_{exp} = 3.29$–$3.66\ mm\ s^{-1}$, C–Sn–O = 95–≈90°; for R = Ph, $\Delta E_{exp} = 3.12$–$3.17\ mm\ s^{-1}$, C–Sn–O ≈ 90°.

Alk_2Sn^{IV} and Alk_3Sn^{IV} moieties bound to electronegative donor atoms, such as oxygen and nitrogen, from protein side chain fragments or from protein constituents (Figs 3 and 4). Moreover, systems 1, 2 and 6 could consist of Alk_2Sn^{IV} moieties bound to hydroxyl oxygen atoms of carbohydrates (Fig. 5), although the molecular structure of these solid-state complexes[57–59] may hardly be assumed to be duplicated in our organotin–erythrocyte systems. Lastly, organotins in systems 1 and 4 could form adducts with phosphodiester fragments (Fig. 6); on the other hand, 2, 3 and 5–9 cannot consist of phosphate complexes such as those in Fig. 7.

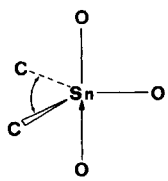


Figure 5 The nature of the environment of tin atoms in derivatives of carbohydrates (five-coordinated tin sites) and of nucleosides. C–Sn–C angles in nBu_2Sn-1,3,2-dioxastannolanes, glucose and mannose derivatives, are in the range 125.8–139°, as determined by X-ray diffractometry[57–59] (C–Sn–C = 137.9° in nBu_2Sn-1,3,2-dioxastannolane, mannose, six-coordinate tin site[58,59]). C–Sn–C angles from ΔE_{exp} data[59–61] and point-charge model (II) estimates for nBu_2Sn-1,3,2-dioxastannolane, glucose and mannose derivatives: $\Delta E_{exp} = 2.38$–$2.78\ mm\ s^{-1}$, C–Sn–C ≈ 100–114°; for Alk_2Sn-nucleosides, $\Delta E_{exp} = 2.97$–$3.24\ mm\ s^{-1}$, C–Sn–C = 119–127°.

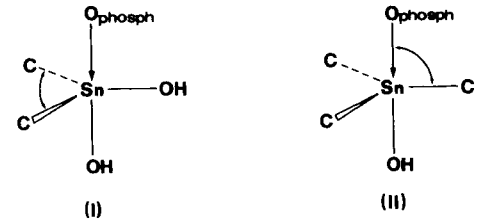


Figure 6 Possible tin environments in $Me_2Sn(OH)_2$ and $Me_3Sn(OH)(OH_2)$ in the presence of excess dimethylphosphinate or cyclic adenosine-3′:5′-phosphate (both characterized by $R_2PO_2^-$ groups), in aqueous solution at physiological pH.[33] O_{phosph} stands for coordinated phosphate oxygen. C–Sn–C and C–Sn–O angles from ΔE_{exp} data[33] and point-charge model (II) estimates: for **I**, [ligand]/[$Me_2Sn(OH)_2$] = 9.97, 8.14; $\Delta E_{exp} = 2.70$, $2.72\ mm\ s^{-1}$; C–Sn–C = 113°;[33] for **II**, [ligand]/[$Me_3Sn(OH)(OH_2)$] = 8.46, 9.85; $\Delta E_{exp} = 3.02$, $3.26\ mm\ s^{-1}$; C–Sn–O = 107°, 105°.[33]

It seems worthwhile to comment here on the ^{119}Sn Mössbauer response from organotin–cell membrane systems and related models reported in the literature. In membranes of rat liver mitochondria, Et_3Sn^{IV} has been assumed to bind to thiol sulfur, and/or to heterocyclic nitrogen from histidine, forming tetrahedral species, or trigonal-bipyramidal species with facial SnC_3 fragments ($\delta = 1.49$, $1.22\ mm\ s^{-1}$; $\Delta E = 2.78$, $1.67\ mm\ s^{-1}$, respectively[11,12]); moreover, a trigonal-bipyramidal species Et_3SnA_2 ($\delta = 1.56\ mm\ s^{-1}$, $\Delta E = 3.44\ mm\ s^{-1}$) would constitute the fraction dissolved into the mitochondrial membrane.[11,12] The latter ΔE parameter corresponds to ΔE data of our systems 4, 5 and 7 (Table 3 and Fig. 2), involving Alk_3Sn^{IV} moieties, where no evidence was obtained for the occurrence of tetrahedral

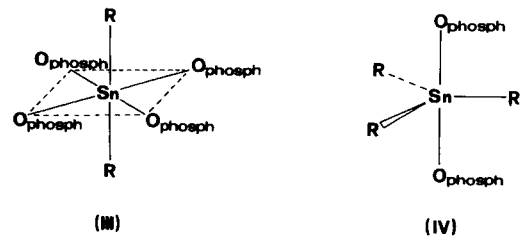


Figure 7 The structures of $R_2Sn[PO_2(XY)]_2$[62–69] and $R_3Sn[PO_2(XY)]$ complexes[63,66–68,70–72].

III: X = Y = H, Cl, OPh, OEt, C_6H_{13}; X = H, Y = Ph; X = OPh, Y = Ph. For R = Alk, $\Delta E_{exp,av} = 4.60\ mm\ s^{-1}$;[63–69] for R = Ph, $\Delta E_{exp,av} = 4.08\ mm\ s^{-1}$.[62,67,68]

IV: X = Y = H, Cl, Ph, OPh, OSn-nBu_3, Me, C_6H_{13}; X = Me, Y = OSn-nBu_3; X = Ph, Y = OSn-nBu_3, OPh, H. For R = Alk, $\Delta E_{exp,ay} = 3.90\ mm\ s^{-1}$;[63,66–68,70–72] for R = Ph, $\Delta E_{exp,av} = 3.49\ mm\ s^{-1}$.[67,68,71]

Table 4 ^{119}Sn Mössbauer parameters of organotin(IV)–rat hemoglobin systems[a]

System no.	Organotin compounds reacted with hemoglobin[b]	r[c]	δ (mm s^{-1})[d]	ΔE (mm s^{-1})[d]	Γ_1, Γ_2 (mm s^{-1})[d]
10	$Me_2Sn(OH)_2$. Hepes	1.81; 1.18	1.19; 1.24	2.23; 2.30	0.84–1.32
11	$Me_2Sn(OH)(GlyGly)$. Hepes[e]	1.76; 1.40	1.29; 1.28	2.23; 2.10	0.97–1.53
12	$Me_2Sn(OH)[SCH_2CH(NH_3^+)COO^-]$	1.83; 1.44	1.16; 1.33	2.19; 2.39	0.93; 1.12
13	$[Me_2Sn(SCH_2CH_2SO_3)_2]^{2-}$[f]	1.05–2.07	1.33–1.35	1.58–1.71	0.57–1.06
14	$Me_2Sn[SCH_2CH(NH_3^+)COO^-]_2$	1.45	1.27	2.21	0.83; 0.96
15	$Me_2Sn[SC(CH_3)_2CH(NH_3^+)COO^-]_2$	0.97	1.23	2.48	0.70; 0.83
16	$Me_3Sn(OH)$. Hepes	1.90; 2.00	1.25; 1.32	1.53; 1.57	0.70–1.03
17	$Et_3Sn(OH)$. Hepes[g]	1.83–1.95	1.38–1.48	1.61–1.78	0.74–0.88
18	$nBu_3Sn(OH)$	0.73–2.35	1.45–1.50	1.77–1.94	0.63–1.03

[a] At liquid-nitrogen temperature. Data from Refs 15, 17–19.

[b] Samples prepared as described in the Experimental section; see Table 2(B) for the parameters of the reactant species in Hepes buffer. Nos 10–12, 14, 15 refer to samples obtained by co-crystallization of the organotin compounds and hemoglobin, while Nos 13, 16–18 concern samples from both co-crystallization and diffusion of aqueous organotins into crystalline hemoglobin (see the Experimental section).

[c] r = [Sn]/[hemoglobin tetramer]; estimated by visible spectrophotometry analysis of pellets for R_nSn^{IV} and hemoglobin contents for Et_3Sn^{IV}– and Bu_3Sn^{IV}–hemoglobin.[17]

[d] See footnotes c, d to Table 1, and d to Table 2.

[e] Glygly = glycylglycinate.

[f] Sodium and guanidinium salts.

[g] Includes the data for a sample $(Et_3Sn)_2$(feline hemoglobin).[15]

species, and consequently for binding to thiol groups, which instead generally occurs in rat hemoglobin derivatives (*vide infra*). Moreover, Ph_3Sn^{IV} in cell walls of *Ceratocystis ulmi* yields[73] $\delta = 1.23$ mm s^{-1}, $\Delta E = 3.06$–3.13 mm s^{-1}, which suggests the occurrence of trigonal-bipyramidal species Ph_3SnA_2 (Table 1) analogously to our Ph_3Sn^{IV} system 9 (Table 3 and Fig. 2).

In the study by Farrow and Dawson[11] referred to above, the binding sites for Et_3Sn^{IV} in rat liver mitochondria were investigated at different triethyltin incubation concentrations in the range 8.9–35.7 nmol (mg of protein)$^{-1}$;[11] the increase of the amount of Et_3Sn^{IV} induces a parallel increase of the species Et_3SnA_2.[11] In *Ceratocystis ulmi*, for example, 12.9 mmol of Ph_3SnCl is added to 5×10^{-6} cells (5 cm^3 of packed cells).[73] In our present study we have employed systems consisting of 0.7–7.6 μmol of organotin per mg protein or, for example, 30 μmol of organotin per 3 cm^3 of packed erythrocytes or ghosts, in the incubations; these ratios are intermediate between those referred to above for mitochondria and fungi, and correspond to the conditions employed in the study of the interaction of Me_3Pb^{IV} with glutathione in human erythrocytes (1 μmol of organolead per 0.4 cm^3 of packed cells[74]).

It is concluded that our systems, generally involving trigonal-bipyramidal organotin complexes with erythrocyte membranes and cytoplasm, correspond to Farrow's species dissolved in the mitochondrial membrane; these systems may be consistent with the simultaneous presence of a series of species, originated by bonding to tin of the biological donors in Figs 3–6, as well as by micelles intercalated into lipid bilayers and formed for example by trigonal-bipyramidal hydrolysis products of organotins, or by other types of coordination. The assumption of multiple tin sites is supported by the magnitude of the linewidths, Γ, of the Mössbauer resonant signals, which often exceed 1 mm s^{-1}, in a special way for the Me_2Sn^{IV} systems 2 and 3 (which is consistent for example with the formation of both trigonal-bipyramidal and octahedral species reported in Fig. 2), as well as for the nBu_3Sn^{IV} and Ph_3Sn^{IV} species 7 and 9 (Table 3). It therefore seems to us that the important conclusion reached in the present investigation consists in the general occurrence of five-coordination in our erythrocyte systems. Further work is clearly needed in order possibly to identify individual binding sites, concerning specific ligand atoms from the biological constituents. The possible bonding by thiol sulfur

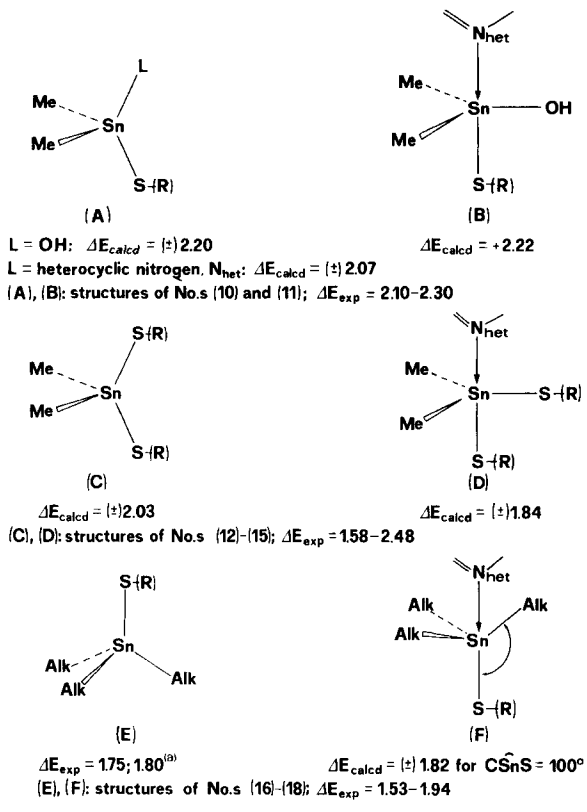


Figure 8 The structures assigned[17–19] to the organotin–rat hemoglobin systems 10–18 through correlation of the experimental ΔE values (Table 4) with point-charge model calculations, ΔE_{calcd}, mm s^{-1},[17–19] effected according to the literal version [(I); Experimental section, this paper]. Heterocyclic nitrogen atoms, from histidine side chains for example, are indicated N_{het}, and thiol groups S(R). Structures A–E are regular; (F) is distorted at the C–Sn–C angles.
[a] Ref. 17.

would be investigated at the level of nanomolar organotin per mg of membrane protein, according to studies on mitochondrial membranes.[11]

Organotin–rat hemoglobin

The Mössbauer parameters[17–19] are summarized in Table 4. Isomer shifts (δ) are typically in the ranges usually shown by dialkyltin and trialkyltin derivatives.[28–32,34] The linewidths (Γ) are generally less than 1 mm s^{-1}, consistent with the occurrence of single tin sites. Fingerprint criteria for ΔE data (Table 1) indicate tetrahedral tin environments for all the hemoglobin derivatives 10–18. It follows that the trigonal-bipyramidal organotins $Me_2Sn(OH)_2$.Hepes, $Alk_3Sn(OH)(L)$, nBu_3SnOH, and related species [Table 2(B)] react fully with the protein.

Due to the non-zero value of the partial nuclear quadrupole splitting of thiol sulfur,[18] point-charge model calculations on the structures related to organotin–feline hemoglobin and rat hemoglobin complexes (characterized by the occurrence of Sn–S bonds; see Introduction) have been made by the literal version (I), where all bonds to tin are accounted for (see the Experimental section of this paper). The point-charge model structures thus obtained, yielding ΔE_{calcd} values in agreement with the ΔE_{exp} data (Table 4), are reported in Fig. 8. The related data for model systems in solution phases and in the solid state are referred to in Figs 9 and 10.

The reaction of rat hemoglblobin with Me_2Sn^{IV}, as well as of its complexes with electronegative donors such as the peptide nitrogen atoms of glycylglycine,[18,35] in aqueous systems at physiological pH (systems 10 and 11, Table 4), could yield complexes with tin environments as in **A** or **B**, Fig. 8.[18]

The possible tetrahedral environments (**A**) are in line with assumptions on R_3Sn^{IV}–hemoglobin in solution.[15,86] Bonding by both thiol sulfur and heterocyclic nitrogen (**B**) corresponds to the structural assumptions for $(Et_3Sn)_2$(hemoglobin tetramer), the axial location of thiol sulfur being dictated by conformational constraints.[20] In fact, model aqueous systems with $Me_2Sn[SCH_2CH(NH_2)COO]$, $Me_2Sn[SC(CH_3)_2CH(NH_2)COO]$ and $Me_2Sn(SCH_2CH_2SO_3)$ clearly assume structure **G**, (Fig. 9) with equatorial thiol sulfur and axial hydroxyl and amino nitrogen (the latter being the more electronegative bonding atoms), according to the Muetterties rule on five-coordinated configurations.[87]

Mono- and bis-thiolate derivatives of Me_2Sn^{IV} (12–15, Table 4) react with rat hemoglblobin, apparently occurring as bisthiolates in the hemoglobin 'phase', with the tetrahedral or trigonal-bypiramidal configurations **C** or **D** (Fig. 8).[18,19] Bisthiolates in the hemoglobin systems 13–15 have been assumed as tetrahedral species **C**, linked to the protein by Coulomb and hydrogen bonds formed by the sulfonate and amino-acid residues,[19] analogous to literature reports.[88–90] In fact the model aqueous systems $Me_2Sn[SCH_2CH(NH_3^+)COO^-]_2$, $Me_2Sn[SC(CH_3)_2CH(NH_3^+)COO^-]_2$ and $[Me_2Sn(SCH_2CH_2SO_3)_2]^{2-}$ would either assume the trigonal-bipyramidal structure **H** (Fig. 9) or the tetrahedral structure **J** (Fig. 10) in Hepes buffer, whilst $[R_2Sn(SCH_2CH_2SO_3)_2]^{2-}$ appear to adopt the tetrahedral form **J** (Fig. 10) in the solid state (R = Me, Ph) and in aqueous

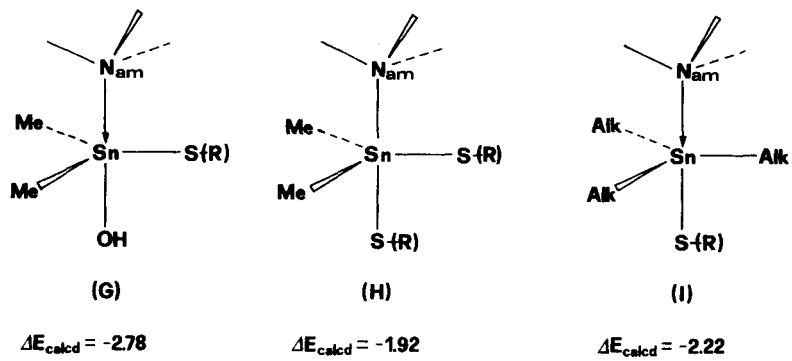


Figure 9 The (regular) trigonal-bipyramidal structures assigned to model systems Me_2Sn^{IV}–monothiolates and Me_2Sn^{IV}–dithiolates, and Alk_3Sn^{IV}–thiolates (Alk = Me, Et) in aqueous solution at physiological pH, optionally in the presence of Hepes buffer (coordinating through amino nitrogen atoms, N_{am}). ΔE_{calcd} values (mm s^{-1}) have been estimated by the literal version of the point-charge model, (I). Model systems, and related ΔE_{exp} parameters, are as follows:

G: Me_2Sn^{IV}–cysteine, Me_2Sn^{IV}–2-mercaptoethanesulfonate, Me_2Sn^{IV}–penicillamine, 1:1, in Hepes; $\Delta E_{exp} = 2.63–2.91$ mm s^{-1}.[18,75,76]

G: Me_2Sn^{IV}–cysteine, 1:1, in H_2O, pH = 7.5–9.5; $\Delta E_{exp,av} = 2.57$ mm s^{-1}.[77] The axial amino nitrogen atom is from the amino group of cysteine.[77]

H: Me_2Sn^{IV}-cysteine, Me_2Sn^{IV}–2-mercaptoethanesulfonate, Me_2Sn^{IV}-penicillamine, 1:2–1:4, in Hepes; $\Delta E_{exp} = 1.97–2.63$ mm s^{-1}.[18,75,78] Tetrahedral structures (J) (Fig. 10) have been also advanced.[75,78]

I: Me_3Sn^{IV}–cysteine and Et_3Sn^{IV}–cysteine, 1:1, in Hepes; $\Delta E_{exp} = 2.44$, 2.59 mm s^{-1}.[17] These values are essentially unchanged for molar ratios 1:2–1:3.[17]

solution.[18,75,78] It is worthy of note that the saturated complexes are characterized by the C_2SnS_2 skeleton[18,75,78] (**H**) (Fig. 9), there being no evidence for further tin coordination by thiol sulfur.

Triorganotin species are likely to yield the environement **E** (Fig. 8) in hemoglobin complexes, as viewed from the magnitude of the parameters ΔE of 16–18 (Table 4); this assumption is in line with literature reports.[15,86] The latter structure is typically assumed by a series of thiolates R_3SnSR in the solid state and in solution phases[17,75,79–83] (**K**, Fig. 10). The assumption of trigonal-bipyramidal configurations implies a severe distortion from the ideal geometry in order to account for the ΔE_{exp} data (**F**, Fig. 8);[17] in fact, the experimental and calculated parameters ΔE for regular trigonal-bipyramidal model systems in solution and in the solid state (**I** and **L**, Figs 9 and 10) are inconsistent with ΔE data for the hemoglobin complexes. It is noteworthy that **I** and **L** are saturated complexes, occurring in excess of both thiol sulfur and nitrogen ligands.[17,84,85]

Axial coordination by two heterocyclic nitrogen atoms in R_3Sn–hemoglobin is excluded by point-charge model calculations, the related parameter being[91] $\Delta E_{calcd} = -3.25$ mm s^{-1}; the possible occurrence of facial as well as meridional fragments SnC_3[91] is ruled out by the value of the asymmetry parameter $\eta = 0.00$ determined from the Mössbauer–Zeeman spectra of the $(Et_3Sn)_2$(hemoglobin tetramer).[17]

CONCLUSIONS

The water soluble organotin(IV) species $Me_2Sn(OH)_2$, $Me_3Sn(OH)(OH_2)$ and $Et_3Sn(OH)(OH_2)$, formed at physiological pH, react with thiol groups of biological molecules, yielding products characterized by covalent Sn–S bonds, with stoichiometries corresponding to $[R_2Sn(SR')]^+$, $R_2Sn(SR')_2$ and R_3SnSR'. Ligand molecules with electronegative donor atoms, such as amino nitrogen and phosphate oxygen, appear to produce labile adducts with, for example, $Me_2Sn(OH)_2$ and $Me_3Sn(OH)(OH_2)$, in aqueous solution at pH ≈ 7, for large ligand-to-metal molar ratios.

These assumptions are in line with potentiometric studies on hydrolysis and stability constants of methyltin(IV) moieties.[92,93] Consequently, one is tempted to assume that water-soluble organotin moieties preferentially occur in biological systems in the form of the thiol species

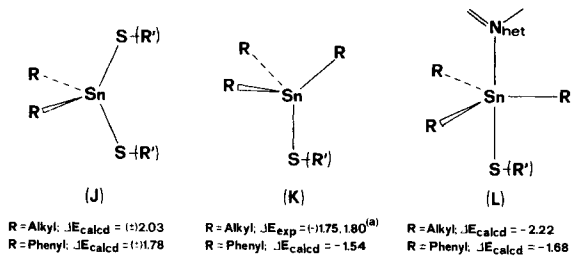


Figure 10 The (regular) tetrahedral and trigonal-bipyramidal structures attributed to model systems R_2Sn^{IV}–dithiolates and R_3Sn^{IV}–thiolates, R = Alk, Ph, in the solid state as well as in solution phase (aqueous or organic). The literal version of the point-charge model, (I), has been employed for the estimate of ΔE_{calcd} in mm s^{-1}. The model systems, and the respective parameters, are as follows.

J: $R_2Sn[S(CH_2)_2SO_3^-]_2$, sodium and guanidinium salts. Solid-state: R = Me, ΔE_{exp} = 1.66, 1.68 mm s^{-1};[75] R = Ph, ΔE_{exp} = 1.44, 1.67 mm s^{-1}.[75] Aqueous solution, pH = 6.6–7.4; R = Me, ΔE_{exp} = 1.84 mm s^{-1}.[75]

K: $R_3Sn(SR')$. Solid-state: R = Alk, ΔE_{exp} = 1.40–1.76 mm s^{-1};[75,79–82] R = Ph, ΔE_{exp} = 1.20–1.42 mm s^{-1}[17,80,82,83] [SR' = SCH_2COO^-, $S(CH_2)_{1,2}CH(NHR'')COO^-$, glutathione, $S(CH_2)_2SO_3^-$]. Aqueous solution, pH = 7.4: R = Me, ΔE_{exp} = 1.42 mm s^{-1}[75] [SR' = $S(CH_2)_2SO_3^-$]. Benzene solution, R = Alk, ΔE = 1.97, 2.06 mm s^{-1}; R = Ph, ΔE = 1.37, 1.46 mm s^{-1}[17] [SR' = SC_6H_5, 8-thioquinoline].

L: $R_3Sn(SR')$ + ligand in benzene, and $R_3Sn(SR')$ in pyridine. R = Alk, ΔE = 2.62–2.78 mm s^{-1};[17,84] R = Ph, ΔE = 2.34–2.54 mm s^{-1}[17,84,85] (SR' = SC_6H_5, 4-thiopyridine; ligand = *N*-methylimidazole).

[a] Ref. 17.

referred to above. The biological activity of organotins would then be ascribed to the molecules above, or to the original hydrolysis products, in the present context. In fact, effects due to organotin thiolates have been reported earlier.[76,77,94,95] It seems somewhat unrealistic to transfer totally into biological environments results concerning chemical bonding and reactivity in aqueous solutions. Moreover, the possible correlation with real biological processes of our *in vitro* interactions by ethanolic organotin chlorides with erythrocytes and native DNA has still to be investigated, and the biological effects possibly induced by water-insoluble (e.g. micellar) hydrolyzed organotins have still to be determined.

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