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Review

Biological activity studies on organotin(IV)$^{n+}$ complexes and parent compounds

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Abstract

This review summarized the literature and own data on the parent organotin(IV) compounds and complexes formed with biologically active ligands.

Keywords: Organotin(IV) cations; Complexes; Biologically active ligands; Biological activity; Anticancer activity

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1. Introduction

Organotin(IV) compounds are characterized by the presence of at least one covalent C–Sn bond. The compounds contain tetravalent {Sn} centres and are classified as mono-, di-, tri- and tetraorganotin(IV)s, depending on the number of alkyl (R) or aryl (Ar) moieties. The anions are usually Cl^-, F^-, O^{2-}, OH^-, $–COO^-$ or $–S^-$. It seems that the nature of the anionic group has only secondary importance in biological activity.

Abbreviations: ax, axial position; Bu, *n*-butyl group; cap, *N*-[(S)-3-mercapto-2-methylpropionyl]-L-proline; CPZ, chlorpromazine; DMSA, *m*-2,3-dimercaptosuccinic acid; D, Mössbauer quadrulole splitting; eq, equatorial position; O_h, octahedral; Ph, phenyl group; SP, square-pyramidal; TBP, trigonal bypiramidal; TBTCl, tributyltin(IV) chloride; TEM, transmission electron microscopy; T_h, tetrahedral; TFP, trifluoperazine; TBT, [tributhyltin(IV)]$^+$.

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The rapid rise in the industrial (catalyst in PVC and foam production), agricultural (e.g., as fungicides and acarides, wood and stone preservatives, etc.) and biological applications of organotin(IV) compounds during the last few decades has led to their accumulation in the environment and, consequently, in biological systems.

It is well known that organotin(IV) compounds display strong biological activity [1–5]. Most organotin(IV) compounds are generally very toxic, even at low concentration. The biological activity is essentially determined by the number and nature of the organic groups bound to the central {Sn} atom. The $[R_3Sn(IV)]^+$ and $[Ar_3Sn(IV)]^+$ derivatives exert powerful toxic action on the central nervous system. Within the series of $[R_3Sn(IV)]^+$ compounds, the lower homologues (Me, Et) are the most toxic when administrated orally, and the toxicity diminishes progressively from propyl (Pr) to octyl (Oc), the latter not being toxic at all.

The organization of the paper is as follows: first, we will show the experimental procedures used in biological studies. Because of the diversities of different biological samples, the effects of parent organotin(IV) compounds and complexes will be discussed separately.

2. Experimental procedure of biological studies

The acute effects of organotin(IV) compounds, used as biocides towards a number of organisms, have been extensively investigated. All these works indicate that sublethal effect can have more serious long-term consequences on various processes which can ultimately affect the survival and propagation of the species. Therefore, there is currently considerable interest in understanding the mechanism through which these compounds exert their toxic action on the organisms.

Very few agents break DNA directly, most breaks rather occur as a result of metabolic transformation in cells, DNA recombination, replication, repair or compaction–relaxation during mitosis. However, in addition to affecting the primary structure of DNA, damaging agents induce a complex, prolonged and variable response in cells. The resulting actions of protective mechanisms provide the cell with ways to survive damage. Repair, tolerance, cycle delay, or death via regulated apoptosis or necrosis are the alternatives.

Apoptosis is known as programmed cell death and represents also a control mechanism within the cell that reacts to the changes in its surrounding environment. This active cellular death process is characterized by distinctive morphological changes that include condensation of nuclear chromatin, cell shrinkage, nuclear disintegration, plasma membrane blebbing, and the formation of membrane-bound apoptotic bodies.

In contrast to apoptosis, necrosis is an accidental cell death often caused by toxic agents. Necrosis differs from apoptosis morphologically and biochemically. Necrosis is characterized by swelling and bursting of the cell, leading to release of cytoplasmic materials into the extracellular space, as well as by random cleavage of DNA. Furthermore, plasma membrane integrity, which is conserved during apoptosis, is lost during necrosis. Although toxic agents have the potential to cause necrosis, some of them can interfere with intracellular signalling pathways and induce apoptosis instead of necrosis.

It seems that organotin(IV) compounds exert their toxic effects involving all this processes. The precise balance of these actions and their outcomes may differ radically from one cell type to another and among different organisms.

First of all, biological investigations on organotin(IV) compounds are based on evaluation of cell viability. Cell death could be evaluated by diffusion of Trypan Blue, the most common stain used to distinguish viable cells from non-viable cells. In brief, cells are seeded into a plate in a growth medium, cultures are incubated in appropriate concentrations of the test substances, for appropriate times. After adding the stain, cells are counted under normal light microscopy. The effects of the treatment are quantified as percentage of cell died positive to Trypan Blue dye due to loss of membrane permeability, using untreated cells as control.

Flow cytometry is often chosen as the tool of investigation for its potency, not only for cell viability, but also to probe membrane and chromosomal damage, cell-cycle analysis and morphological alterations.

The DNA content of each cell in any living organism is generally highly uniform. In the resting phase of the cell-cycle, a human somatic cell contains approximately 6–7 pg of DNA (diploid DNA content). This stage is occupied by non-cycling cells (G0 phase) as well as those recovered from the previous division or prepared for the next cycle (G1 phase). When beginning the process of replication, a cell enters DNA synthesis (S phase). As DNA synthesis proceeds, cellular DNA content progressively increases until replication is complete and the cell enters the G2 phase with a DNA content twice that of G1 (tetraploid). After repairing DNA damage and organizing the chromosomes, the cell enters mitosis (M phase) dividing into two diploid daughter cells and completing the cycle.

The DNA flow histograms represent *snapshots* of the distribution of nuclei in the various phases of the cell-cycle at the time of fixation.

Because organotin(IV) toxicity in cell cultures evolves over 24–48 h, these are, usually, chosen incubation times at which cells are harvested. By comparing the normal cell-cycle DNA content distribution of appropriate cell lines with that of cells incubated with 10^{-5} M concentration of the various potential toxicants for 24 h, it is possible to remark effects on the DNA content distributions. The appearance of distinct subpopulations, characterized by reduction of PI-fluorescence (DNA content) and forward angle (1–19°) light scatter (cell size), is indicative of apoptotic cells.

Another morphological assay apoptosis is Acridine Orange, nuclear staining that revealed chromatin condensation by observing under light and fluorescent microscope. Also Annexin V–FLUOS staining labels cells and allows to see morphological features of apoptosis. In fact one of the early events of the apoptotic process involves the translocation of phosphatidylserine on the surface of cell membranes; Annexin V binding and propidium iodide (PI) uptake reveal various cellular states. After treatment with organotin(IV) compound substances, the cells could be categorized into populations; vital cells (Annexin V^-/P^-), early apoptotic cells (Annexin V^+/P^-), late apoptotic cells (Annexin V^+/P^+), and necrotic cells (Annexin V^-/P^+). Cells are observed with a fluorescent microscope and it is possible to observe translocation of phosphatidylserine (PS) from the inner side of the plasma membrane to the outer one and to see a green stain for Annexin V–FLUOS bound to PS and a red stain for propidium iodide.

The terminal dUTP nick end labelling assay, (TUNEL reaction) and electrophoretic analysis of DNA/organotin(IV) mixtures allow to investigate DNA fragmentation.

Two main apoptotic pathways have been identified in mammalian cells: the extrinsic pathway that is activated by the binding of ligands to cell-surface death receptors [6], and the intrinsic pathway that involves the mitochondrial release of cytochrome *c* [7]. The activation of extrinsic and intrinsic apoptotic pathways promotes the cleavage into the active form of the pro-caspase-8 and pro-caspase-9 respectively, that mainly determines the activation of effector caspase-3 [8]. The intrinsic pathway is the main apoptotic pathway activated by chemotherapeutic drugs, while the cytotoxic drug-induced activation of the extrinsic pathway is a more controversial issue [9].

Active effector caspases, such as caspase-3, mediate the cleavage of an overlapping set of protein substrates, resulting in the morphological features of apoptosis and the demise of the cell. Therefore, it could be crucial to determine if organotin(IV) compounds could induce the activation of caspases. The proteolytic processing of caspase-9 and -3 could be examined by Western blotting experiments using appropriate antibodies (anticaspase), to look over if compounds induce apoptosis via the mitochondrial pathway.

Developmental systems represent a suitable field of study because they share certain fundamental features that includes: (1) storage and transfer of developmental information; (2) molecular, cell or organism growth; (3) morphogenesis; (4) differentiation, the emergence of a functionally specialised state.

The genotoxicity studies of organotin(IV) compounds and mutagenicity test are widely developed and used mainly in aquatic developing embryos such as *Truncatella subcylindrica* (Mollusca, Mesogastropoda), *Anilocra physodes* (Crustacea, Isopoda), *Aphanius fasciatus*, *Rutilus rubilio* (Pisces, Teleost) and *Paracentrotus Lividus* (Echinodermata). At the ultrastructural level, biological investigations include the observation by transmission microscopy (TEM) of fertilized eggs and embryos at different development stages. Specimens in the TEM are examined by passing the electron beam through them, revealing structural lesions: breakages, bridging, irregular outline and light areas after staining. As the electron beam passes through the specimen, some electrons are scattered while the remainder are focused by the objective lens either onto a phosphorescent screen or photographic film to form an image. Unfocussed electrons are blocked out by the objective aperture, resulting in an enhancement of the image contrast. Contrast in the TEM depends on the atomic number of the atoms in the specimen; the higher the atomic number, the more electrons are scattered and the greater the contrast. Biological molecules are composed of atoms of very low atomic number (C, N, H, P and P). Thin sections of biological material are made visible by selective staining.

It is possible to observe effects of organotin(IV) compounds exposure such as inhibition of cleavage of fertilized eggs, interference with the formation of the mitotic spindle, damages affecting chromosome structure, and electron dense precipitate formation in organelles.

To correlate embryonic arrests with the metabolic pathways, and especially to understand why cellular organelles first undergo chemical damages, biological investigations include evaluation of DNA, RNA, protein, glucose, lipids and ATP contents.

DNA, RNA, proteins, glucose, lipids, and ATP fractions are extracted and isolated by modified Schneider methods [33].

Metabolites determinations are carried out following standard methods: (a) DNA, RNA and lipid contents are determined according to standard methods described by Dische and Schwarz [10], Brown [11], Marsh and Weinsterin [12], respectively. (b) Protein content determinations are carried out according to the Lory [13] modified method, after precipitation with 5% TCA and collection of the insoluble material as pellet with a 10,000*g* centrifugation. (c) Finally, Gluc and ATP contents are, usually, determined according to the GOD–POD–PAP method.

Organotin(IV) compounds are strong neurotoxins and induce thymus atrophy and bile duct damage. The metabolism involves subsequent dealkylation reactions; accordingly, $[R_3Sn(IV)]^+$, or $[R_4Sn(IV)]$ exposure results in systematic exposure to the $[RSn(IV)]^{3+}$-, and $[R_2Sn(IV)]^{2+}$ compounds. Although dimercaptosuccinic acid did not reduce the $[Bu_2Sn(IV)]^{2+}$-induced mortality in mice, it reduced thymus and bile duct damage more efficiently than did DMP (2,3-dimercaptopropanol) and it was also an antidote in rats [14–17].

Two women were poisoned by drinking red wine containing $[Me_3Sn(IV)]^+$ added for homicidal purposes. One woman died after one week, with multiorgan failure despite intravenous dimercaptosuccinic acid chelation. The other gradually recovered from severe neuropsychiatric symptoms over several months. She had been chelated

for several weeks with oral dimercaptosuccinic acid, which apparently improved her clinical condition [16,17].

Organotin(IV) compounds are a widely studied class of metal-based antitumour drugs [18–20]. Their intensive investigation has led to the discovery of compounds with excellent in vitro antitumour activity, but, in many cases, disappointingly low in vivo potency or high in vivo toxicity [18–21]. The design of improved organotin(IV) antitumour agents is unfortunately hampered by the paucity of information concerning the cellular targets of these compounds and their mechanism of action, although inhibition of mitochondrial oxidative phosphorylation appears to be an important mode of toxicity [19,20].

Three primary factors are involved: in the structure–activity relationships for organotin(IV) derivatives $(L)_xR_nSnX_{4-n}$: the natures of the organic group {R}, of halide or pseudohalide $\{X_n\}$, and of donor ligand. Examination of the structures of {Sn} compounds containing a N-donor atom and tested for antitumour activity revealed that in the active {Sn} complexes the average Sn–N bond lengths were >239 pm, whereas the inactive complexes had Sn–N bonds <239 pm, which implies that predissociation of the ligand may be an important step in the mode of action of these complexes, while the coordinated ligand may favour transport of the active species to the site of action in the cells, where they are released by hydrolysis.

With regard to the data published on all the {Sn} derivatives, it can be concluded that $[R_2Sn(IV)]^{2+}$ compounds generally exhibit higher antitumour activity than those of the corresponding mono-, tri- and tetraorganotin(IV) or the inorganic {Sn} derivatives, and within the diorganotin(IV) class, the highest activity is exerted by the $[Et_2Sn(IV)]^{2+}$ and $[Ph_2Sn(IV)]^{2+}$ complexes.

Recent work demonstrated, that in the R_2SnL (R = Bu, L is the dianion of glycyltyrosine, glycyltryptophane, leucytirosine, leucylleucine, valylvaline and alanylvaline) complexes all the ligands act as dianionic tridentate ligands coordinating through the $-COO^-$, NH_2 and N-peptide groups, whereas in Ph_3SnHL the ligand acts as a bidentate coordinating through $-COO^-$ and NH_2 groups. The R_2SnL complexes are monomeric, and the polyhedron around the {Sn} is a TBP with the Bu groups and $N_{peptide}$ in the *eq* positions, while the *ax* positions are occupied by a carboxylic {O} and the amide {N} atom. In $Ph_3Sn(HL)$ the structure is intermediate between pseudotetrahedral and *cis*-TBP, with the N-amino and two phenyl groups in *ax* positions. All the complexes were tested against seven cancer cell lines of human origin, viz., MCF-7, EVSA-T, WiDr, IGROV, M19, MEL A498 and H226. $Ph_3Sn(HL)$ displays the lowest ID_{50} values of the compounds tested and reported in this publication. Its activity is comparable to those of methotrexate and 5-fluorouracil. All the $[Bu_2Sn(IV)]^{2+}$ compounds exhibit lower in vitro activities than $[Ph_3Sn(IV)]^{+}$ derivatives; however, they do provide significantly higher activities than that of etoposide and *cis*-platin [21] (see Table 1). Some of the complexes showed above were tested for a wide spectrum of bacteria (*Escherichia coli*, *Rhizobium meliloti*, *Pseudoma putita*, and *Aeromonas formicans*) and fungi (*Aspergillus niger*, *Pencillium chrysogenum*, *Aureobasidium pullulans* and *Verticillium dahliae*) and were found to be active. The LD_{50} values are (>500 mg/kg) for albino rats. Some of the complexes also exhibit very high anti-inflammatory activity [22]. The same authors published the paper on the structure–activity relationship of the di- and triorganotin(IV) derivatives of amino acids and peptides [23]. Some triorganotin(IV)] complexes exhibit good anti-inflammatory activities comparable to that of phenylbutazone.

3. Some results of biological studies

Organotin(IV) compounds are ecotoxicants whose action depends on their structure. The mechanism of toxic effect of organotin(IV) compounds is fairly complicated, and it cannot be regarded as thoroughly studied. It is assumed that these compounds are capable of reacting with cell membranes, finally leading to their decay, accelerating ion exchange processes, and inhibiting oxidative and photochemical phosphorylation. Among $R_nSnX_{(4-n)}$ compounds, the most toxic are the R_3SnX [24–26].

Organotin(IV) compounds could be involved in other biological processes occurring in cells, specifically in peroxide oxidation of lipids. The latter process is very important

Table 1
In vitro anti-tumourial activity (ID_{50} ng/ml) values of compounds 1–7, in comparison with some reference compounds used clinically

Cell line	1	2	3	4	5	6	7	DOX	TAX	MTX	DDPt	5FU	ETO
MEL A498	138	196	336	155	134	332	30	90	<3	37	2253	143	1314
EVSA-T	21	64	74	56	32	84	7	8	<3	5	422	475	317
H226	57	133	177	108	78	105	11	199	<3	2287	3269	340	3934
IGROV	25	72	118	90	46	199	6	60	<3	7	169	7	580
M19	73	182	205	150	89	139	16	16	<3	23	558	23	505
MCF-7	40	93	150	96	51	478	10	10	<3	18	699	18	2594
WiDr	284	424	420	295	265	332	8	11	<3	<3	967	<3	150

MEL A498, renal cancer; EVSA-7, mammary cancer; H226, lung cancer; IGROV, ovarian cancer; M19 melanoma; MCF-7, mammary cancer; WiDr, colon cancer.

1, Bu_2Sn-Gly-Tyr; 2, Bu_2Sn-Gly-Trp; 3, Bu_2Sn-Leu-Tyr; 4, Bu_2Sn-Leu-Leu; 5, Bu_2Sn-Val-Val; 6, Bu_2Sn-Ala-Val; 7, Ph_3Sn-Gly-Leu.

DOX doxorubicine, TAX taxol, MTX methotrexate, DDPt cisplatin, 5FU flurouracil, ETO etoposide.

Abbreviations are in footnotes [21].

from the viewpoint of physiology, and it follows a radical chain mechanism [27].

Petrosyan et al. studied peroxide oxidation of oleic[(*Z*)-octadecenoic] acid in the presence of complexes derived from R_nSnCl_{4-n} and phosphatidylcholine [OP(O)(OH)OCH_2CH_2–$N(Me)_3$, PChol] which is a short-chain analog of phospholipids, as well as to compare the effects of the complexes $(R_3SnCl)_2$–PChol (R = Me, Ph), R_2SnCl_2–PChol (R = Me, Bu), and $RSnCl_3$–PChol (R = Me, Ph) with that produced by the organotin parents [28].

Acceleration of peroxide oxidation of lipids in cells leads to accumulation of hydroperoxides, decay of cell membranes, and various pathologies in living bodies. Insofar as organotin(IV) compounds exhibit electron acceptor properties, it was presumed that their toxicity originates from interaction with electron-donor groups in biomolecules. Reactions of organotin(IV) compounds with phosphorus containing biomolecules, such as phospholipids, ATP, nucleic acids, etc., were shown to inhibit the synthesis of phospholipids and their intracellular transport, which may be responsible for the antiproliferative activity of organotin(IV) derivatives [29,30].

It must also be taken into account that organotin(IV) compounds can react with phosphorus-containing fragments of biomolecules in cell membranes to form complexes with dative Sn–O–P bonds. In this case, the mechanism of peroxide oxidation of lipids may change [31,32].

The health consequences of chronic exposure to low levels of organotin(IV) compounds are unknown, although human and animal studies demonstrate that acute exposures can result in either neurotoxicity or immunotoxicity depending on the specific compound involved. For example, acute human exposure to high doses of trimethyltin(IV) (TMT), due to accidental poisoning, has resulted in memory deficits, seizures, altered emotional affect, hearing loss, disorientation and death [33–36].

4. Biological effects of the parents organotin(IV) compounds

TBT is an agent showing a high toxic effect to aquatic life: even at low (nanomolar) aqueous concentrations it causes chronic and acute poisoning of the most sensitive aquatic organisms, such as algae, zooplankton, molluscs and the larval stage of some fish [37].

TBT, used as antifouling agents, is accumulated in aquatic molluscs and it exerts an endocrine disrupting action on them [38].

Molluscs, as a group, are widely known to exhibit the highest tissue burdens and the highest bioaccumulation factors of TBT among marine organisms. The overall impact of imposex varies among species. In some, such as *Ilyanassa obsolete*, the imposed male tract does not appear to interfere with the breeding activity nor alter the population ecology of the species. However, in others, such as the muricids *Nucella lapillus* and *Urosalpinx cinerea*, the oviduct structure may become so modified that reproduction is inhibited, resulting in population decline and eventual localized extinctions [39–41].

Although there has been little evidence of imposex reducing the reproductive capacity in buccinid whelks, imposex has been suggested as a contributing cause to the recent extinction of the common whelk, *Buccinum undatum*, from the Dutch Wadden Sea [42]. Also the tropical muricid *Thais distinguenda* develops imposex in a time-dependent manner after transplantation to a TBT contaminated site [43]. The anatomical aberration is assumed to result from the inhibition by TBT of aromatase cytochrome P450, which catalyzes the aromatization of androgens to estrogens [44].

Histone acetylation is important for the regulation of gene expression and is catalyzed by histone acetyltransferase (HAT). Some organotin(IV) compounds – TBT and triphenyltin(IV) (TPT) – enhanced HAT activity of core histones in a dose-dependent way and other endocrine disrupting chemicals (EDCs) did not affect HAT activity. Organotin(IV) compounds have various influences on the physical function including the hormone and immune systems, embryogenesis, and development. Dibutyl- and diphenyltin(IV), metabolites of TBT and TPT, respectively, also promoted HAT activity, but monobutyltin, monophenyltin, and inorganic tin had no effect. Further, TBT and TPT enhanced HAT activity when nucleosomal histones were used as substrates. These data indicate that the organotin(IV) compounds have unique effects on HATs independent of their EDC activities and suggest that the varied toxicities may be caused by aberrant gene expression following altered histone acetylation [45].

Patricolo et al. reported endocrine disruption effects of TBT on metamorphosis of ascidian larvae of *Ciona intestinalis* [46].

Triorganotin(IV) compounds appear to inhibit the mitochondrial function in at least three ways by: (1) causing large-scale swelling at high concentrations; (2) mediating Cl^-/OH^- exchange across membranes; (3) inhibiting oxidative phosphorylation or ATP hydrolysis, like oligomycin [47]. The last process is usually assumed to be the most significant one, although binding of $[Ph_3Sn(IV)]^+$ to the cell wall was concluded to be responsible for the toxicity of *Ceratocystis ulmi* (*C. ulmi*) [48]. The triorganotin(IV)-mediated anion exchange across the mitochondrial membrane, which is electro-silent, i.e. it involves neutral R_3SnX species, may also interfere with ATP synthesis or hydrolysis.

Dutch elm disease continues to devastate the diminishing population of American elm trees. The pathogenic fungus responsible for the disease, *C. ulmi* causes a blockage in the vascular tissue, which can lead to the eventual death of the elm. In explorations of the expectation that the incorporation of biologically active entities into a triorganotin(IV) system would lead to the formation of potent biocides [49], a number of $[Ph_3Sn(IV)]^+$ compounds with simple biologically active anionic groups were synthesized and first investigated spectroscopically [50]. The ambiguous spectroscopic data led to further crystallographic investigations on

two of the Sn–S bound compounds; these complexes have been shown to be especially active against *C. ulmi* [51]. The results of the studies on the complexes of several $[Ph_3Sn(IV)]^+$ carboxylates and of some 1:1 addition compounds of Ph_3SnCl and 2,3-disubstituted thiazolidin-4-ones indicate that the carboxylates in the solid state are monomeric with a T_h {Sn} atom = 2.14–2.54 mm s^{-1} the only exception being the furan-2-carboxylic acid derivative, which is polymeric. The Ph_3SnCl adducts are TBP ($|\Delta_{exp}|$ = 2.97–3.08 mm s^{-1}) with the three {Ph} groups in a non-coplanar *eq* plate. These complexes are effective inhibitors of *C. ulmi* [52].

The 2,3-disubstituted thiazolidin-4-ones [53] are compounds with a wide range of biological activity. Several $[Ph_3Sn(IV)]^+$ complexes of these type of ligands have a TBP structure with the three {Ph} groups in the *eq* plane in non-coplanar positions. These complexes are effective inhibitors of *C. ulmi* [54].

Thyroid hormones (THs) are present in ascidian larvae (Urochordatae), and their function is related to the control of metamorphosis. Invertebrates do not have thyroid tissues; nevertheless, some of them possess thyroid hormones and their precursors [54]. Among the invertebrates able to synthesize THs, adult ascidians have phylogenetic importance, as the body plan of their larvae is a basic model of vertebrate morphogenesis.

Ascidians and amphioxus, which are protochordates, together with the ammocoete of the lamprey, a primitive chordate, concentrate iodide and synthesize THs in a subpharyngeal afollicular endostyle. This structure is considered a thyroid homologue. In the larva of the lamprey, the endostyle reorganizes into a follicular thyroid at metamorphosis to the adult, but in protochordates it never transforms into a follicle. A close histological resemblance of the ammocoete and the protochordates shows the homology of these organs. The endostyle is able to carry out thyroid biosynthesis, and the conclusion is that the characteristic molecules of the thyroid gland are already present in protochordates, the ancestors of vertebrates. Using immunohistochemistry, the presence of tyrosine (T4) in normal larvae of *C. intestinalis* has been localized to mesenchymal cells, many of which will be the future blood cells. It was demonstrated that THs of ascidian larvae are strongly affected by TBT, which not only blocks metamorphosis, but also reduces by 70% the amount of the hormone. In vertebrates, EDCs act on thyroid biosynthesis, impairing the production of THs, or blocking hormone-receptor binding. TBT is a compound that can also react directly or indirectly with a hormone in invertebrates, altering its structure or interfering with its biosynthesis; indeed, data indicate that TBT is an ED in ascidians, invertebrates lacking thyroid follicles, which possess, however, THs in larval tissue. This xenobiotic probably alters and destroys almost all T4 molecules present in mesenchymal cells and blocks its neosynthesis. Even in larvae exposed to the lowest TBT concentration used in this study, despite the integrity of all tissues, T4 is found only in a few cells and the content of the hormone is substantially decreased, as independently confirmed by radioimmunological assay RIA even after 3 h of exposure [55].

The negative effects of TBT have been observed in the bivalve larval development of *Crassostrea gigas* [56], *Mytilus edulis* [57], *Mercenaria mercenaria* [58], *in Sparus aurata* [59], in *Nassarius reticulates* [60] and in the hermaphroditic snails *Phisa fontinalis* [61] and *Adelomelon brasiliana* [62].

Since some molluscs are edible and TBT exerts a variety of toxic actions on mammals, an adverse effect of TBT on human health is one concern [63,64].

The water pollutant TBT is known to stimulate apoptosis. Induction of apoptosis has been reported by TBT in several species of aquatic organisms such as in the blu mussel *Mytilus galloprovincialis* [65,66] and in tissues of the marine sponge *Geodia cydonium* [67].

It has also been reported that TBP triggers apoptosis in cultured hepatocytes of *Salmo salor* [68] and in trout hepatocytes, through a step involving Ca(II) efflux from the endoplasmic reticulum or other intracellular pools and by mechanisms involving cysteine proteases, such as calpains, as well as the phosporylation status of apoptotic proteins such as Bcl-2 homologues [69–71].

Orrenius et al. hypothesized that in trout hepatocytes the rise in cytosolic Ca(II) level stimulates endogenous endonuclease activity and initiates thymocyte apoptosis [72]. In addition, Chow et al. [73] invoke a capacitative Ca(II) entry for TBT-induced cell death. Their report shows that the increase in the cytosolic free Ca(II) concentration ($[Ca(II)]_i$) occurs through a three-step mechanism: (i) release of Ca(II) from the intracellular store(s); (ii) inhibition of the Ca(II) extrusion system; (iii) activation of Ca(II) influx.

Induction of apoptosis has been reported in various mammalian cell lines [74,75].

In previous studies, it has been reported that TBT induces apoptosis in isolated thymocytes at concentrations which are relevant to those causing thymus atrophy in vivo [76–79]. TBT can also induce apoptosis in PC12 cells and in human T-lymphoblastoid CEM cells. While the mechanism of TBT-induced apoptosis is still unknown, it has been reported that TBT stimulates thymocyte apoptosis by a mechanism independent of protein synthesis and under conditions where intracellular ATP levels are severely depleted [80].

Marinovich et al. described the events linked to the process in HL-60 promyelocytic cells after triphenyltin chloride treatment: in particular increase of intracellular calcium, alteration of actin polymerization and induction of DNA degradation [81].

Cima and Ballarin revealed apoptosis induced by TBT in haemocytes of the ascidian *Botryllus schlosseri* [82]. The cellular death for apoptosis is an integrant part of embryonic development, with organized and regulated biochemical events, as intracellular signal transduction, ordered enzyme cascades and targeted cell deletion, in response to a variety of stimuli, when various structures

no longer needed, must be removed [83]. Spontaneous apoptosis in sea urchin larvae of *Paracentrotus lividus* has been found by Roccheri et al. as a physiological event for the development of the adult [84].

Recent studies have shown that cysteine and histidine residues are the primary biological ligands for organotin(IV) compounds [85] and that vicinal dithiols rather than monothiols constitute a general target for organotin(IV) [86].

Billingsley et al. [87] have identified a small membrane proteine, stannin (SNN), containing vicinal dithiols at the membrane interface, which mediates the selective neurotoxic activity of TMT in mammalians by triggering neuronal apoptosis in the hippocampus.

In fact organotins posses a high specificity of action as neurotoxins [88]. TMT and TET are known to cause damage in the central nervous system (CNS). While TMT causes lesions in specific regions of the hippocampus and neocortex, TET damage is localized within the spinal cord [88]. Interestingly, it has been found that in mammalian organs such as brain, liver and kidneys, organotin(IV)s are progressively dealkylated to inorganic Sn(IV) [89].

Aschner suggested that astrocytes may represent an important link in the CNS damage produced by TMT because of evidence of prominently swollen astrocytes without neuronal involvement after in vivo exposure [90]. In vivo exposure to TMT also produces a transient increase in the specific cell marker glial fibrillary acidic protein (GFAP) [91]. In vitro exposure to TMT also disrupts the glutamate transporter associated with astrocytes and stimulate cytokine release from these cells [92].

It has been also shown the neurotoxicity of DBT in aggregatin brain cell cultures. DBT causes general cytotoxicity and affects the myelin content of cholinergic neurons [93].

The higher molecular weight organotin(IV)s, such as TBT and TPT, are known to be immunotoxic and to cause renal and hepatic damage. TBT at environmentally-relevant concentrations increases intracellular concentration of Ca(II) ($[Ca(II)]_i$) in murine thymocytes by increasing membrane Ca(II) permeability and releasing Ca(II) from intracellular stores [73,94] and it induces apoptotic change in plasma membranes [95].

Okada et al. examined the effects of TBT on cellular content of glutathione (GSH) in rat thymocites using a flow cytometer and 5-chloromethylfluorescein diacetate, a fluorescent probe for monitoring the change in the cellular content of GSH. TBT at nanomolar concentrations reduced the cellular content of GSH. There is an important implication on the TBT-induced depletion of cellular GSH since GSH has an important role in protecting the cells against oxidative stress and chemical and metal intoxications. TBT-induced decrease in cellular content of GSH in thymocytes may increase the vulnerability of the immune system [96].

TBT and TPT are membrane-active molecules, and their mechanism of action appears to be strongly dependent on organotin(IV) lipophilicity [97,98]. They function as ionophores and produce haemolysis [99], release Ca(II) from sarcoplasmic reticulum [100], alter phosphatodylserine-induced histamine release [101], alter mitochondrial membrane permeability [102], and perturb membrane enzymes [103,104].

Organotin(IV) compounds have been shown to affect cell signalling: they activate protein kinase C [105] and increase free arachidonic acid through the activation of phospholipase A_2 [106].

Hydrophobicity of organotin(IV) compounds suggests that their interaction with membranes may play an important role in their toxic mechanism. In this respect, the understanding of the interaction of organotin(IV) compounds with the lipid component of membranes is of considerable interest. Fluorescence polarization measurements suggested that the effect of TBT on liposomal membranes is dependent on the anion moiety [107].

Studies on the release of liposome bound praseodymium19 indicated that the lipophilicity and polarity of organotin(IV) compounds and the surface potential and environment of the lipid molecules are important factors in their interaction with membranes [108].

From the study of the interaction of several organotin(IV) compounds (differing in their polar and hydrophobic moieties) with erythrocytes it was concluded that different effects can result from this effect, being more evident in the case of TBTCl [109].

In summary, organotin(IV) compounds are incorporated into very important phospholipids of eukaryotic membranes, i.e. phosphatidylserine, where they perturb its thermotropic and structural properties.

Organotin(IV) compounds interact in a different quantitative way with phosphatidylserine than with phosphatidylcholine and phosphatidylethanolamine. The evidence supports the hypothesis that organotin(IV) compounds are located in the upper part of the phosphatidylserine palisade. The butyl and phenyl groups intercalate between the initial methylene segments, perturbing their packing and affecting the hydration of the interfacial region [110,111].

According to the different effects of TBTCl and TPTCl on the fluidity of the acyl chains and the hydration of the interfacial region of phosphatidylserine, it seems than TBTCl is located more deeply in the phospholipid palisade that TPTCl, which is closer to the lipid–water interface. The observed interaction between organotin(IV) compounds and phosphatidylserine promotes physical perturbations, which could affect membrane function and may mediate some of their toxic effects [112].

The genotoxic, cytotoxic and ontogenetic (embryo–larval) or developmental effects of TBT, were investigated in various cell lines.

Di- and trimethyl, -dibutyl and -diphenyltin(IV), all as chloride were tested for toxicity towards spindle structure and microtubules in V79 Chinese hamster cells [113]. All compounds showed a concentration dependent inhibition of microtubules. An effect on the rate of polymerization

was suggested for tributyl- and triphenyltin(IV). The results further indicate that the inhibition of microtubule assembly is through direct interaction with tubulin but does not involve the sulphydryls of the protein. Thus, the organotin seems to act through two different cooperative mechanisms, inhibition of microtubule assembly and interaction with hydrophobic sites. The latter mechanism might involve Cl^-/OH^- exchange across cellular membranes.

It seems that this anionic exchange would be relevant also to explain interactions of trialkyltin (TAT) compounds (TET, tripropyltin chloride, TBP chloride) with the mitochondria. The current view of this phenomenon is that, these compounds, by exploiting the Cl^- and OH^- gradient in energized mitochondria, behave as electroneutral OH^-/Cl^- exchangers. The crucial point of this new mechanism is that TATs enter the mitochondria as lipophilic cations $[(alkyl)_3Sn(IV)]^+$ and not as electroneutral compounds. The influx is followed by extrusion of the TAT compounds as electroneutral hydroxy compounds $(alkyl)_3SnOH$ [114].

The embryotoxic effects of both TBT and its degradation products, resulting in altered or blocked embryonic development, have also been observed in the sea urchin *Paracentrotus lividus*, probably owing to the interference of organotin(IV)s with intracellular calcium homeostasis during skeleton deposition [115].

The cytotoxic effects of TBTCl, on the neurulation process of the ascidian *Ciona intestinalis* have been evaluated [116]. Exposure of the embryos at early neurula stage in 10^{-5} and 10^{-7} M TBTCl solutions for 1–2 h provoked the irreversible arrest of their development.

Morphological and ultrastructural observations suggested that most probably there are two principal causes determining the neurulation process block. The first is due to the TBT effects of inhibiting the polymerization and/or degradation of microfilaments and microtubules, proteins that constitute the cytoskeleton. The lack of orientation and extension of both microtubules and microfilaments of actin prevent the shape changes and mobility of neural plate blastomeres indispensable to the neurulation process. The second cause is certainly determined by the ultrastructural modification which mitochondria undergo. The ultrastructural anomalies shown by these organules are so serious as to impede their proper functionality with consequent inhibition of oxidative phosphorylation and ATP synthesis, remarkable metabolic processes that occur during ascidian neurulation.

Mansueto et al. [117] suggested that the susceptibility of embryos to toxicants could be firstly related to their interaction with egg membrane where they could provoke changes of permeability, of transmembrane potential, and of receptors distribution which could in turn drastically interfere with normal cell physiology.

Cima et al. observed that TBT alters, immediately after the entry of spermatozoon into the cortex of egg, the normal fertilization cytoplasmic movements which are strictly connected to the plasma membrane [118]. TBT derivatives alter, also, the intracellular Ca(II) homeostasis through inhibition of the membrane Ca(II) ATPase which activated several processes causing, at first, microfilaments and microtubules disassembly or chromosomal disorders, alteration of cytoplasmic organelles and cell metabolism [119].

A molecular variation of plasma membrane has been reported by Puccia et al. Reduction of total lipids (TL) content and significant variations of triglyceride (TG) and phospholipids (PL) fractions were observed as a consequence of the exposure of *Ciona intestinalis* ovaries to TBTCl solutions. In particular, an evident TG decrease and a PL increase were observed, which probably provoked an increment in membrane fluidity, because of the high concentration of long chain fatty acids and, as a consequence, PL. This could be a cell adaptive standing mechanism towards the pollutants, as observed in *Saccharomyces cerevisiae*. Also the increase in the content of the polyunsaturated fatty acids (PUFA), important in the synthesis of compounds such as prostaglandin which are present in the ovary in a stress situation, was probably a consequence of a defense mechanism to the stress provoked by the presence of TBTCl [120].

The morphological aspects of *Styela plicata* fertilization after treatment with TBTCl are described by means of scanning and transmission electron microscopy investigations [121]. Alterations have been shown both on female and male gametes; spermatozoa, all the egg envelopes and the mitochondria of the egg cortical cytoplasm are modified in relation to incubation time. As a consequence, the damage to gametes blocks sperm–egg interaction and fertilization does not occur.

The ultrastructural aspects of fertilization in *S. plicata* are examined in order to compare the resistance of this species against the pollutant and to extend the study of the fertilization process using scanning electron microscopy (SEM). The most relevant alterations, in relation to incubation time, are observed in the follicle cells and the vitelline coat: a few follicle cells are present with a very elongated shape and the vitelline coat is highly damaged until breakage. The vitelline coat shows a different rearrangement: craters, blebs and holes appear as a new surface organization; also, test cells show signs of degeneration. The mitochondria of the egg cortical cytoplasm start to become damaged after 2 h of incubation in 10^{-5} M TBTCl solution. It is well known that triorganotins can disturb mitochondrial activity, binding to a component of the ATP synthase complex and inhibiting mitochondrial ATP synthesis, and thus disturbing the proton gradient [122].

It has been suggested that mitochondria serve as mediators of TBT effects and gene-regulatory signalling pathways [123].

No spermatozoa are seen on the egg surface or on the vitelline coat. After 5 h of treatment with 10^{-5} M TBTCl, a few spermatozoa, with very anomalous heads, have been detected. The absence of spermatozoa on the egg surface or on the vitelline coat could be explained by the absence of

the follicle cells, which, in *S. plicata*, primarily play an attracting function [124].

It was previously shown that TBTCl solution, either 10^{-5} or 10^{-7} M, induces anomalies in spermatozoa, unfertilized and fertilized eggs of *A. malaca*. In particular, the follicle cells detach from eggs and the test cells show anomalies in their nucleus and granules. Moreover, damaged spermatozoa are observed in the vitelline coat, but never in the egg cortical cytoplasm after incubation for 3 h in 10^{-5} and 10^{-7} M TBTCl. This signifies that fertilization does not occur [125].

The reproductive perturbation caused by TBTCl in ascidians result in altered functionality or even gametes death, leading to the species being unable to reproduce. As far as the ability of different species to contrast the effects of TBTCl, we can deduce that gametes of *S. plicata* seem to be more resistant than those of *A. malaca*.

In fact, in *S. plicata*, a prolonged time of incubation and/or higher concentrations of TBT are necessary to detect the anomalies that prevent the fertilization process. However, in any case, fertilization does not occur for at least three reasons: the absence of follicle cells necessary for sperm–egg interaction and the strong anomalies of the vitelline coat, which is considered to be the site of the species-specific binding, and the lack of mobility and alterations of the spermatozoa [126,127].

When the antitumour activity of cisplatin, *cis*-$Cl_2Pt(NH_3)_2$, was discovered, several research groups started to investigate the possible therapeutic applications of other metal-based, often organometallic, compounds. The organotin(IV) compounds that were first tested were those that were available or easily synthesized, like tri- or diorganotin(IV) halides.

The in vivo testing of tetraorganotin(IV) compounds showed that they are inactive, whereas organotin(IV) halides and their complexes with amines and other ligands exhibit borderline activities against P388 or L1210 leukemias [127–133].

The in vivo pre-screenings against these two leukemias used initially by the National Cancer Institute (NCI) were later replaced by in vitro pre-screenings against a panel of human tumour cell lines [134–147]. This is also the procedure that was used when organotin(IV) compounds were tested by the Rotterdam Cancer Institute.

Seven human tumour cell lines were chosen for the panel that was used: MCF-7 and EVSA-T (two mammary cancers), WiDr (a colon cancer), IGROV (an ovarian cancer), M19 (a melanoma), MEL A498 (a renal cancer) and H226 (a lung cancer).

The main disadvantage of organotin(IV) halides for antitumour testings is that, when they are dissolved in water, the pH of the solution dramatically decreases because the Cl–Sn bonds are converted into water–tin bonds; the formed compounds then lose protons, yielding first organotin(IV) hydroxides that are afterwards possibly converted into insoluble bis(triorganotin) oxides or diorganotin(IV) oxides. Because di- or triorganotin(IV) carboxylates do not suffer from the same disadvantage, many series of these compounds were synthesized in order to determine their cytotoxic or antitumour properties. It has been shown that such derivatives, when dissolved in water, indeed remain intact for long periods.

Several recent reviews and papers have been devoted to the antitumour properties of organotin(IV) compounds [145–149].

5. Biological effects of organotin(IV) complexes

A judicious choice of the ligand coordinated to the organotin(IV) fragment can modulate the activity of the organotin(IV) complexes and minimize its drawbacks. Generally, their mechanism of action is still unknown: some authors proposed that DNA is the probable target for the cytotoxic activity [150,151]. When deoxyribonucleic acid, DNA, undergoes condensation processes, from aqueous solutions, by charge neutralization of the phosphodiester groups due to addition of cationic species, toroidal structures are detected [152]. DNA condensation is induced also by organotin(IV) derivatives (mono-, di-, triorganotin(IV), salts and complexes) [153].

Casini et al. monitored the interactions in vitro with DNA of two representative organotin(IV) compounds, the dimer of bis[(di-*n*-butyl-3,6-dioxaheptanoato)tin]oxide and tri-*n*-butyltin-3,6,9-trioxodecanoate through various physico-chemical techniques [154]. Both investigated organotin(IV)s exhibit strong in vitro antitumour activities: the cytotoxic effects were previously analyzed on several tumour cell lines of human origin, MCF-7, EVSA-T, WiDr, IGROV, M19Mel, A498 and H226 and found to be larger than those induced by some classical antitumour drugs. The interactions of these organotin(IV) compounds with DNA were investigated through circular dichroism spectroscopy (CD), DNA thermal denaturation analysis and gel electrophoresis methods. The results suggest that the interaction of organotin(IV) compounds with DNA is not sequence- or diagnostic base-specific and therefore most likely occurs at the level of the external phosphate group.

Diorganotin(IV) clinical treatment of certain types of neoplasias placed platinum complexes was reported in [155–158].

Crowe et al. reported the results of a screening 115 diorganotin(IV) halide on the role in anticarcinogenesis [159,160].

The most promising development in the field of antitumour-active organotin(IV) compounds has been achieved by the synthesis and testing of organotin(IV) compounds that contain a polyoxaalkyl moiety linked to tin either by a C–Sn or by a Sn–O bond. Many of these compounds, of which some are very soluble in water, exhibit exceptionally high cytotoxicities against seven human tumour cell lines mentioned above [161–163].

Organotin(IV) complexes of amino acids and their organic derivatives containing the carboxylic O–Sn bond,

display significant antitumour activity and promising potential in many other fields like, wood preservation, polymer chemistry, pesticidal, bactericidal and antifouling agents, etc. [161–173].

New coordination compounds of some organotin(IV)s with *N*-methylglicine (sarcosine) have been tested for in vitro cytotoxic activity against human adenocarcinoma HeLa cells, showing, in some cases, strong activity even at low concentration [174].

Another possibility to constitute a "lead" for a rational development of molecules with antitumour activity, are represented by the organotin(IV)–porphyrin derivatives. In fact the porphyrin ligand possess all the characteristics of an intercalating agent which, by attachment to the ring of opportune linkers, could selectively bring the organotin(IV) ion on the site where the lesion must be operated.

Han and Yang reported the synthesis of organotin(IV)–porphinate based on tris-(4-pyridiniumyl)-porphyrin and tris(*N*-methyl 4-pyridiniumyl)-porphyrin and the activity against P388 and A-549 tumour cell lines and their interactions with DNA [175]. The results show that the antitumour activities of organotin(IV)–porphinate is related to the water solubility of the compounds and the central ion in the porphyrin ring. The interaction between the water-soluble dibutyltin(IV)–porphinate complexes and DNA has been investigated by spectroscopic methods. Electrophoresis test shows that the compound cannot cleave the DNA. According to the electrophoresis test and other results, the cytotoxic activity against P388 and A-549 tumour cells appears not to come from the cleavage of DNA caused by the compounds but from the high affinity of compounds to DNA.

Bis-[diorganotin(IV)-chloro]-protoporphyrin IX complexes have been investigated and evidence of chromosome damages has been seen in early-developing embryos of *Anilocra physodes* L. (Crustacea, Isopoda) following exposure to bis-[dimethyltin(IV)chloro]protoporphyrin IX [176].

The organotin-[*meso*-tetra(4-carboxyphenyl)]porphinate, organotin-[*meso*-tetra(4-sulfonatophenyl)-porphinate and diorganotin(IV) chloro protoporphyrin IX derivatives have been tested for their cytotoxicity also towards immortalizes mouse embryonic fibroblasts (NIH-3T3) and towards early-developing embryos of *Anilocra physodes*, showing that cytotoxicity of the parent organotin(IV) halide may be modulated by using appropriate ligands [177,178].

Embryos at the two-cell stage were incubated in 10^{-5} or 10^{-7} M solutions of various compounds. The most toxic among the tested compounds was tributyltin(IV) [*meso*-tetra(4-carboxyphenyl)porphinate], $(Bu_3Sn)_4$TPPC, since the fertilized eggs were unable to divide into two cells, even at a concentration of 10^{-7} M. This embryonic arrest is correlated with the metabolic pathway. The higher concentration (10^{-5} M) reduced the content of ATP, Glu, lipid, protein and RNA. The cytotoxicity of the $(Bu_3Sn)_4$TPPC derivative included molecular mechanisms: once the compound migrates inside the cell, it may immediately disrupt the cell metabolism of RNA, proteins, lipids, Glu and ATP.

The primary effect seems to be damage to the molecular membrane structure, i.e. to the mitochondrial membrane, or cellular membrane, or both.

It could be possible that, inside the mitochondria, the porphyrin group of $(Bu_3Sn)_4$TPPC might compete with the porphyrin group of cytochrome and therefore it might damage the H-pump for ATP production [179].

In addition, the cytotoxic derivatives diorganotin(IV) and triorganotin(IV) [*meso*-tetra(4-carboxyphenyl)-porphinates, with stoichiometries $[R_2Sn(IV)]_2$TPPC and $[R_3Sn(IV)]_4$TPPC [R = Me, Bu, Ph; $TPPC^{-} = meso$-tetra(4-carboxyphenyl)porphinate^{4-}], namely bis[Me_2-Sn(IV)], bis[Bu_2Sn(IV)], bis[Ph_2Sn(IV)], tetra[Me_3Sn(IV)], tetra[Bu_3Sn(IV)] and tetra[Ph_3Sn(IV)][*meso*-tetra(4-carboxyphenyl)porphinate]s have been used to investigate their effects on the cultured human kidney cell cycle in order to understand further the origin of cell-growth inhibition induced by the above-mentioned chemicals [180].

The cell-cycle-dependent DNA content distribution of cultured cells exposed to these compounds has been analyzed through flow cytometry, a potent technique capable of probing several aspects of drug-induced cytotoxicity. Cultured human kidney cells have been used as a model system, on the premise of greater physiological similarity to the human situation in vivo.

The DNA flow histograms presented, represent *snapshots* of the distribution of nuclei in the various phases of the cell-cycle at the time of fixation. Complexes might synergistically interact with DNA, the porphyrin ligand and the organometallic moieties acting in a concerted fashion and the complex behaving as a pseudo-bifunctional adduct. TBTPPC is the most toxic of the tested compounds, causing cell death, as indicated by the appearance of a sub-G1 peak, that revealed apoptosis.

Pellerito et al. [181] showed apoptosis in the sea urchin *Paracentrotus lividus*, after incubation with four new organotin(IV) chlorin derivatives, [chlorin = chlorin-e6 = 21H,23H-porphine-2-propanoic acid, 18-carboxy-20-(carboxymethyl)-8-ethenyl-13-ethyl-2,3-di-hydro-3,7,12,17-tetramethyl-(2S-*trans*)–]. The results demonstrated that the novel compound $(Ph_3Sn)_3$chlorin $\cdot$ $2H_2O$ was the most toxic derivative, by exerting antimitotic effect very early and by triggering apoptosis in the two-cell stage of sea urchin embryonic development, as shown by light microscope observations through morphological assays. The apoptotic events in two-cell stage embryos revealed: (i) DNA fragmentation, with the TUNEL reaction (terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling); (ii) phosphatidylserine translocation in the membrane, with Annexin-V assay and (iii) cytoplasm blebbing, with the TUNEL reaction.

Several new organotin(IV) complexes with biologically active ligands have been synthesized and evaluated for cytotoxicity. A number of studies of the triorganotin(IV)

compounds, R_3SnX, and diorganotin(IV) compounds, R_2SnXY, indicated that the marked biological activity of the organotins may be due to the transport of either more active species ($[R_nSn_{(4-n)}]^+$, where $n = 2$ or 3) or the molecule as a whole across the cellular membrane, and X or XY group influences only the readiness of delivery of the active part $[R_3Sn(IV)]^+/[R_2Sn(IV)]^{2+}$ into the cell [182,183].

Further, the studies on structure–activity correlation of organotin(IV) compounds reveal that the biologically active compounds should have available coordination positions at tin and relatively stable ligand–Sn bonds [184]. These bonds should have low hydrolytic decomposition.

The interactions of dibutyltin(IV)-thiaminepyrophosphate(DBTPP) and tributyltin(IV)-thiaminepyrophosphate (TBTPP) complexes with Bluescript KS plasmid and immortalized 3T3 fibroblasts were studied [185]. Both compounds have a clear inhibitory effect on the growth of immortalized mouse embryonal fibroblasts (NIH-3T3), TBTPP being much more active. No evidence was found, however, for DNA cleavage by the compounds at molar ratios as high as 1:10 (DBTPP, TBTPP/DNAbase pairs). The cytotoxicity of TBTPP does not seem to be based on direct interaction with DNA, but in the presence of TBTPP (1:10, TBTPP/DNA bp), plasmid DNA seems to be more susceptible to cleavage by UV.

New organotin(IV) ascorbates of the general formulae $R_3Sn(HAsc)$ (where R = Me, Pr, Bu and Ph) and $R_2Sn(Asc)$ (where R = Bu and Ph) have been synthesized and have been assayed for their anti-inflammatory and cardiovascular activity [186]. The anti-inflammatory activity of the organotin(IV)-ascorbates is influenced by the nature of the ligand environment and organic groups attached to tin. Diorganotin(IV) derivatives have been found to show better activity than the triorganotin(IV) derivatives.

The compounds exhibited mild hypotensive activity without affecting the carotid occlusion and noradrenaline response. Such a profile of pharmacological effect is indicative of direct vasodilator action of these compounds.

Biological activity tests on organotin(IV) complexes with a potent anti-hypertensive agent, captopril, were carried towards the embryos of *Ciona intestinalis*.

The main results obtained were as follows:

(1) The ligand does not affect the embryonic development of *C. intestinalis* significantly.
(2) Me_2Sn(cap) and Et_2Sn(cap) do not affect the embryonic development; Bu_2Sn(cap) and $^tBu_2Sn$(cap) exert toxic activity on *C. intestinalis* embryos in the early stages of development. This toxicity is concentration-dependent and is related to the lipophilic properties of the complexes [187].

Cytotoxic study on *C. intestinalis* is reported also for organotin complexes with 6-uracilcarboxylic acid (orotic acid = Hor) that plays an important role in pyrimidine biosynthesis in mammalian systems. The compound which exerts the highest cytotoxic effect is Bu_3SnH_2 or at 10^{-5} M concentration because it blocks embryo development immediately. Me_3SnH_2 or at 10^{-5} M concentration inhibits cell cleavage in the embryos at the 32-blastomere stage, while Bu_2SnHor at the same concentration gives rise to abnormal embryos. Me_2SnHor, is less toxic than the trimethyl, dibutyl and tributyl analogues, since 40% of the total number of treated embryos resulted in normal larvae. The ligand does not affect embryonic development significantly. The results seem to indicate that the chemical species under investigation, especially Bu_3Sn-H_2or, interfere with polymerization of tubulin during the process of cell division in early embryo development [188].

Due to the extensive use as pesticides and fungicidal, large amounts of organotin(IV) compounds have been introduced to various ecosystems. The current emphasis for chemists is on the production of novel types of pesticide that prevent insect, bacteria or fungi resistance and that are environmentally friendly. It has been well established that dithiocarbamates are a group of compounds that are active against fungi and insects [189,190].

A series of triorganotin(IV) dithiocarbamates $R_3SnS_2CNR'_2$ (R = Cy, Ph; $NR'_2 = NEt_2$, $N(Bu)_2$, $N(i\text{-}Bu)_2$, $N(Pr)_2$, $N(CH_2)_5$, NH(Pr), NH(Bu), $NH(^iBu)$) has been synthesized and their insecticidal activities were screened against the second larval instar of the *Anopheles stephensi* Liston and *Aedes aegypti* (L.) mosquitoes that are vectors of human malaria and yellow fever. Results from the screening studies indicated that triorganotin(IV) ditiocarbamates are effective larvicides against both species of larvae [191].

Organotin(IV) complexes with isatin and *N*-alkylsatin bisthiocarbonohidrazones are active against Gram positive bacteria at concentrations ranging from 0.7 to 50 μg/ml. None of the tested compounds possess inhibitory properties against fungi up to concentration of 100 μg/ml. Butyl complexes are detected as broad-spectrum compounds, in fact they inhibit the growth also of *P. vulgaris* and *S. typhimurium* Gram negative bacteria. It must be noted that in several cases their growth inhibitory effect is better than that of the free ligands. These compounds were tested for mutagenicity in the *Salmonella*-microsome test structural factors, including steric and electronic constraints imposed by the organo-metallic moieties, may be responsible for the absence of activity [192].

Novák et al. prepared new organotin(IV) compounds containing a {C,N} chelating ligand. These substances represent new types of compounds containing a bulky substituent instead of the halogen atom. The in vitro antifungal activity of the compounds studied was comparable to similar organotin(IV) compounds and antifungal drugs in clinical use [193].

Moreover, it was reported that the organotin(IV) steroidcarboxylates are potent and posses in vitro cytotoxicities against bacteria and fungi [194].

TBT derivatives of six different pharmaceutically active carboxylates were synthesized and their antibacterial activities were tested using ten different bacteria (*B. cereus*, *C. diphtheria*, *E.C.ETEC*, *K. pneumonia*, *P. mirabilis*, *P. aeroginosa*, *S. typhi*, *S. boydii*, *S. aureus*, *S. pyogenes*) relative to the reference drugs ampicillin and cephalexin [195]. Apparently, the function of the ligand is to support the transport of the active organotin(IV) moiety to the site of action where it is released by hydrolysis.

The biocidal activity of triorganotin carboxylates is also related to their structure; species generating a T_h structure in solution are more active.

Due to the emergence of bacterial resistance towards many of the commonly prescribed antibiotics, the development of new antibiotics is urgent.

Many peptide antibiotics have novel structural motifs, such as cyclic structures and are often further modified (such as in β-lactam antibiotics) and conjugated with sugars, lipids, and other molecules.

In this perspective, Pellerito et al. designed new complexes of diorganotin(IV) and triorganotin(IV) with peptide antibiotics [196–199]. It was expected that antibiotic properties of chemotherapic agents may give better properties to organotin(IV) complexes if peptide antibiotics are used as ligands.

The eggs fertilized by *Ciona intestinalis* and *Ascidia malaca* in the 10^{-4} mol dm^{-3} solutions of organotin(IV) chloramphenicol and D-cycloserine derivatives, or incubated 30 min after fertilization, did not cleave into the blastomeres. If they were incubated at the two-cell stage, in the 10^{-4} mol dm^{-3} solutions of organotin(IV) chloramphenicol and D-cycloserine derivatives, development stopped and the cytoplasm of two blastomeres was fragmented into several parts that often could refuse. The fertilized eggs incubated in 10^{-5} mol dm^{-3} solutions of organotin(IV) chloramphenicol and D-cycloserine derivatives developed into anomalous larvae. They were inside the membrane with an open neural plate lacking sensorial organs and a very short tail. Eggs and embryos incubated for 1 h in 10^{-4} mol dm^{-3} $Me_2Snchloramph_2$ and then transferred into normal sea water originated larvae as the controls, but slightly delayed; those incubated in $Me_2Sncyclos_2$ blocked at the two-cell stage with an anomalous disposition of blastomeres [196].

Penicillins are a very important class of β-lactamic antibiotics used in therapy because of their specific toxicity towards bacteria. From a coordination chemistry perspective it has been demonstrated that all the β-lactamic antibiotics possess a number of potential donor sites and they are known to interact effectively with several metal ions and organometallic moieties, originating complexes [196,197].

The cytotoxic activity of dialkyl- and trialkyltin(IV) complexes of the deacetoxy-cephalosporin-antibiotic cephalexin [7-(D-2-amino-2-phenylacetamido)-3-methyl-3-cephem-4-carboxylic acid] (Hceph) as well as triorganotin(IV) complexes of 6-[D-(2)-b-amino-*p*-hydroxyphenyl-acetamido]penicillin (5amoxicillin) and 6-[D-(2)-a-aminobenzyl] penicillin (5ampicillin) has been tested using two different chromosome-staining techniques Giemsa and CMA3, towards spermatocyte chromosomes of the mussel *Brachidontes pharaonis* (Mollusca: Bivalvia). Colchicinized-like mitoses (*c*-mitoses) on slides obtained from animals exposed to organotin(IV) cephalexinate, amoxicillinate and ampicillinate compounds, demonstrated the high mitotic spindle-inhibiting potentiality of these chemicals. Moreover, structural damages such as ''chromosome achromatic lesions'', ''chromosome breakages'' and ''chromosome fragments'' have been identified through a comparative analysis of spermatocyte chromosomes from untreated specimens (negative controls) and specimens treated with the organotin(IV) complexes [198–200].

The multidrug resistance (mdr) reversing effect of the new phenothiazine complexes was tested on mouse T cell lymphoma cell lines. TFP was much more effective at the same concentration than verapamil. The efficacy of some metal coordinating complexes [TFP-Cu(II) and TFP-V(IV)] exceeded the action of TFP alone. CPZ or CPZ–Pt(II) complex had the same or less effect than verapamil or promethazine (Pz) used as a control.

We propose that the compounds mentioned above can form a complex with the regulatory protein or DNA resulting in the inhibition of induction (SOS response). These complexes inhibit the mdr efflux pump by inactivating the P-glycoprotein. We conclude that our data can be exploited in the molecular design of drugs against SOS related biological function. The results show some interaction between the phenothiazine metal coordination complexes and DNA.The increased melting temperatures of DNA in the presence of metal coordination complexes indicates an interaction with DNA and stabilization of the helix. CPZ and TFP can be seen to stabilize the DNA helix by intercalation causing a slight increase in the thermal denaturation temperature. A similar effect was seen with the TFP–Me_2Sn(IV), the CPZ–Me_2Sn(IV) and the TFP–Cu(II) coordination complexes indicating an interaction with the DNA helix whilst metal ions alone showed no significant stabilization of the helix. The chlorides of Pt, Pd and V degraded the DNA resulting in a linear thermal stability profile. The coordination complexes of these metals with CPZ and TFP however decreased the thermal stability of DNA indicating a destabilization of the DNA helix by weakening the hydrogen bonding between the base pairs. The phenothiazines appear to be exerting a protective effect, protecting the DNA against total degradation caused by the metal ions alone [201].

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