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Toxicity of Organotin Compounds on Embryos of a Marine Invertebrate (*Styela plicata;* Tunicata)

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In order to clarify the interaction mechanism between organotin compounds and organisms, the effects of these compounds on the development of a benthonic filter-feeding invertebrate were studied. Embryos of the ascidian *Styela plicata* were obtained in laboratory by cross-fertilization and their development was followed *in vivo* after incubation with 0.1, 1, and 10 μ*M* organotin compounds for various exposure times. Moreover, embryos selected at opportune stages after incubation with 10 μ*M* tributyltin (TBT) or triphenyltin (TPT) for 1 hr were observed at the electron microscope to recognize cell alterations. Results indicate that organotins significantly affect all stages of ascidian development in a dose- and time-dependent manner and the most sensitive stages are gastrula and neurula. These compounds are able to block development, giving rise to anomalous embryos with irreversible effects. The order of inhibition appears to be strongly dependent on the organotin liposolubility: TBT > dibutyltin (DBT) > monobutyltin (MBT) and TPT > tricyclohexyltin (TCHT). The mitosis block of blastomeres in the early stages may be related to an inhibition of the microtubule polymerization. Observations with light and electron microscopes reveal globe-shaped blastomeres with large intercellular spaces in the morula and gastrula stages, suggesting a toxic damage with alteration of the cytoskeleton. Moreover, the occurrence of electron-dense precipitates of organotins in the inner membrane of mitochondria and morphological changes of their cristae suggest an inhibitory effect on oxidative phosphorylation which is conspicuous in the gastrula stage. In this stage, the size of the electron-dense aggregates grow from 50–70 to 110–170 nm, while at the same time the alteration of the cristae increases.

INTRODUCTION

Organotin compounds are a widely used class of tin chemicals, which have found commercial applications and are industrially synthesized in large amounts. Their uncontrolled use may cause profound effects and a long-term environmental impact on natural aquatic environments. Butyltin compounds (TBT and is degradation products, i.e., DBT and MBT) are leached out from antifouling paints; triphenyltin (TPT) and tricyclohexyltin (TCHT) compounds have a source in the run-off waters of treated plants on which they are used as pesticides. The risk of bioaccumulating organotin compounds is high for aquatic organisms, because these compounds are easily accumulated by dietary and branchial uptake into the lipophilic compartments and their elimination from the body is slow (Yamada *et al.,* 1994).

There is much concern about their hurtful effects on vertebrates in which these compounds accumulate in specific target organs, i.e., brain, liver, and lymphatic tissues. Carcinogenity is not demonstrated (WHO, 1980), but both TBT and TPT are reported to be hepato- and immunotoxic (Verschuuren *et al.,* 1970; Funahashi *et al.,* 1980; Vos *et al.,* 1984; Snoeji *et al.,* 1985; Elferink *et al.,* 1986; Guta-Socaciu *et al.,* 1986; Devries *et al.,* 1991; Dacasto *et al.,* 1994; Ueno *et al.,* 1994; Bressa *et al.,* 1996). Evidence of teratogenic effects from TBT and TPT, but not TCHT, has been reported (FAO/WHO, 1971). In fish they cause embryonic and larval mortality or malformations (Tas *et al.,* 1990; Fent, 1991, 1992; Fent and Meier, 1992, 1994), and the same effects have been observed on mammalian embryos (Nemec, 1987).

Their toxicity on marine invertebrates is to date demonstrated mostly in molluscs in which TBT and TPT exposure includes shell thinning (Waldock and Thain, 1983) and imposex (Smith, 1981; Horiguchi *et al.,* 1994). Among filter-feeding marine invertebrates, bivalves undergo an embryonic block in the early developmental stages (May *et al.,* 1993) and benthonic tunicates suffer from a blocking or delay in embryonic development (Mansueto *et al.,* 1993a,b).

Since the lagoon of Venice is a particular coastal area with shallow waters that incompletely turn over, in which the resident benthonic population suffers the negative impact of organotin compounds for bioaccumulation, filter-feeder organisms such as tunicates were used as indicators of water pollution. In fact, in the latter a significant immunodepression was recently demonstrated after exposure to organotin compounds (Cima *et al.,* 1995). There is still scanty information concerning the mechanisms of action of the organotin compounds, but their lipophilicity might play a key role in bioaccumulation and toxicity (Leo, 1975).

Hence, in the present study a procedure was used to reveal the effects of organotin compounds on the embryonic development using the solitary ascidian *Styela plicata* as a selected

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biosensor system of the Venice lagoon environment and to elucidate their intracellular mechanisms of action. This species is a widespread fouling ascidian which lives in shallow waters within a wide range of temperatures and endures both considerable levels of various contaminants and variations of salinity (Kott, 1972). It was possible to determine and compare the embryotoxicity of various butyl-, phenyl-, and tricyclohexyl tin compounds at different concentrations on selected development stages. Furthermore, ultrastructural alterations during the early embryonic stages were investigated to detect the intracellular target and to compare it with those observed in embryos of *Ciona intestinalis* exposed to TBT (Mansueto *et al.*, 1993a).

MATERIALS AND METHODS

Animals

S. plicata specimens were collected from the lagoon of Venice and reared in the Stazione Idrobiologica of Chioggia and C.I.V.V. (Centro Ittico Valli Venete) of Pellestrina isle (Venice). They were placed in filtering basins and drains with water current at various temperatures. During the reproductive periods, i.e., spring and autumn, when the water temperature was about 15–20°C, some adults were taken away and transferred to the laboratory of the Department of Biology of Padova University where they were maintained in large aquaria and fed with unicellular algae and Liquifry Marine (Liquifry Co., Dorking, Surrey, UK) for 15–20 days.

Organotin Compounds

Monobutyltin chloride (MBTC), dibutyltin chloride (DBTC), tributyltin chloride (TBTC), and tricyclohexyltin chloride (TCHTC) were first dissolved at a 10 m*M* concentration in 95% ethanol. Triphenyltin chloride (TPTC), triphenyltin acetate (TPTA), and triphenyltin hydroxide (TPTH) were first dissolved at a 10 m*M* concentration in dimethylsulfoxide (DMSO). These solutions were then diluted, at the final concentrations of 0.1, 1, and 10 μM, in filtered sea water. All chemicals were purchased from Sigma. The exposure concentrations were selected on the basis of the available data of the environmental contamination in Italy: for TBT they are 3.93 μg/liter (Bacci and Gaggi, 1989) and 3.18 μg/liter (Chiavarini *et al.*, 1991) in the Tyrrhenian Sea.

Experimental Procedure

In every experiment eggs and spermatozoa of at least three specimens were removed from gonoducts and transferred in cupping glasses containing filtered sea water at 25°C and pH 8.2 for cross-fertilization. Ten minutes after fertilization sperm excess was removed and the sea water was renewed. In controls the fertilized eggs became swimming larvae in about 10–12 hr. This is comparable to the development of *C. intestinalis* embryos at a similar range of temperatures (Satoh, 1994). The phases of development were classified following those used for another solitary ascidian by Satoh (1994) and the following stages were distinguished: fertilized egg, two to four cells, morula, gastrula, neurula, middle tailbud embryo, swimming larva, and initial metamorphosis. The *in vivo* observed samples were the following: (1) controls, i.e., fertilized eggs in filtered sea water containing 0.1% 95% ethanol (control for TBTC) or 0.1% DMSO (control for TPTC), and developed up to the swimming larva stage; (2) embryos exposed to the selected organotin concentrations soon after fertilization and beginning from two- to four-cell, gastrula, and neurula stages until controls reached the swimming larva stage.

Data were expressed as *embryonic development index*, i.e., the percentage of embryos reaching a certain development stage, and were analyzed using a log-linear analysis (STAT SOFT 1991 PACKAGE CSS).

Light and Electron Microscopy

A Zeiss Tessovar stereomicroscope and a Leitz Dialux 22 light microscope were used for the *in vivo* observations and micrographs, respectively.

At the end of the experiments selected embryos at the stages of two to four cells, morula, and gastrula were exposed for 1 hr to 10 μM TBTC or TPTC for transmission electron microscope (TEM) investigations. Embryos at the same stage but not exposed to the organotin compounds were used as controls. Samples were fixed for 1 hr in 2.5% glutaraldehyde in 0.2 *M* Na-cacodylate buffer and 1.7% NaCl, pH 7.4, at 4°C and post-fixed for 40 min in 1% OsO_4 in the same buffer at 4°C. Then they were dehydrated in ethanol and embedded in Epon 812. The sections were cut on a LKB ultrotome. Thick sections (1.0 μm) were stained with a filtered solution of 1% toluidine blue and 1% Na-tetraborate in distilled water and then observed with a Leitz Dialux 22 light microscope. The thin sections were briefly counterstained with uranyl acetate and examined in a TEM Hitachi H600.

RESULTS

Embryonic Exposure

As different embryonic stages of tunicates are differently affected by organotin compounds and some stages are more sensitive than others (Mansueto *et al.*, 1993a,b), four initial exposure stages were chosen, i.e., soon after fertilization, two to four cells, gastrula, and neurula, to study the effect of these compounds on *S. plicata* embryonic development. The observed effects are reported in Table 1. In controls, all embryos reached the larval stage. Therefore, ethanol and DMSO in the concentrations used in the experiments did not affect development and survival.

The statistical comparison of the observed frequencies of the six embryonic stages, i.e., two to four cells, morula, gastrula, neurula, middle tailbud embryo, and larva, demonstrates the following significant results. Among the butyltin compounds, TBTC is the most embryotoxic, blocking development to the larval stage with effects from 1 μM; DBTC causes similar

TABLE 1

Percentage of Survival of *Styela plicata* Embryos Incubated at 25°C in Different Solutions of Organotin Compounds

Compound	μM/liter	Observed stage	Initial exposure stage			
			Postfertilization	2–4 cells	Gastrula	Neurula
TBTC	0.1	2–4 cells	—	—	—	—
		Morula	30.7	26.7	—	—
		Gastrula	—	20	—	—
		Neurula	11.6	6.6	48.3	34.8
		Middle tailbud	—	—	—	—
		Larva	57.7	46.7	51.7	65.2
	1	2–4 cells	—	—	—	—
		Morula	81.4	79.4	—	—
		Gastrula	11.6	10.3	3.3	—
		Neurula	7	10.3	80	35.3
		Middle tailbud	—	—	16.7	64.7
		Larva	—	—	—	—
	10	2–4 cells	—	100	—	—
		Morula	—	—	—	—
		Gastrula	—	—	82.4	—
		Neurula	—	—	17.6	100
		Middle tailbud	—	—	—	—
		Larva	—	—	—	—
DBTC	0.1	2–4 cells	—	—	—	—
		Morula	4	—	—	—
		Gastrula	20	20	—	—
		Neurula	4	16	52.8	40
		Middle tailbud	4	4	5.1	6.7
		Larva	68	60	42.1	53.3
	1	2–4 cells	—	—	—	—
		Morula	—	5.5	—	—
		Gastrula	15	11.2	—	—
		Neurula	25	55.5	60	62.5
		Middle tailbud	5	5.5	5	6.2
		Larva	55	22.3	35	31.3
	10	2–4 cells	—	—	—	—
		Morula	40	52.9	—	—
		Gastrula	40	32.3	—	—
		Neurula	20	14.8	65	33.3
		Middle tailbud	—	—	35	66.7
		Larva	—	—	—	—
MBTC	0.1	2–4 cells	—	—	—	—
		Morula	—	—	—	—
		Gastrula	—	—	—	—
		Neurula	17.2	42.1	42.9	14.3
		Middle tailbud	5.7	5.3	—	—
		Larva	77.1	52.6	57.1	85.7
	1	2–4 cells	—	—	—	—
		Morula	—	—	—	—
		Gastrula	—	—	—	—
		Neurula	13	48.4	58.6	25
		Middle tailbud	3.2	3.1	—	—
		Larva	83.8	48.5	41.4	75
	10	2–4 cells	—	—	—	—
		Morula	—	—	—	—
		Gastrula	—	—	—	—
		Neurula	26	48.5	53.8	40
		Middle tailbud	3.7	3	—	—
		Larva	70.3	48.5	46.2	60

TABLE 1—*Continued*

Compound	μM/liter	Observed stage	Initial exposure stage			
			Postfertilization	2–4 cells	Gastrula	Neurula
TPTC	0.1	2–4 cells	—	—	—	—
		Morula	—	—	—	—
		Gastrula	—	—	—	—
		Neurula	13	—	30.7	18.4
		Middle tailbud	9.6	—	—	—
		Larva	77.4	100	69.3	81.6
	1	2–4 cells	73.7	—	—	—
		Morula	21	92.6	—	—
		Gastrula	—	—	84.6	—
		Neurula	5.3	7.4	15.4	100
		Middle tailbud	—	—	—	—
		Larva	—	—	—	—
	10	2–4 cells	—	100	—	—
		Morula	—	—	—	—
		Gastrula	—	—	70.4	—
		Neurula	—	—	29.6	100
		Middle tailbud	—	—	—	—
		Larva	—	—	—	—
TPTA	0.1	2–4 cells	—	—	—	—
		Morula	—	—	—	—
		Gastrula	54.5	25	30	—
		Neurula	36.4	50	10	14.3
		Middle tailbud	9.1	12.5	—	14.3
		Larva	—	12.5	60	71.4
	1	2–4 cells	—	—	—	—
		Morula	—	47.4	—	—
		Gastrula	82.4	36.9	21.4	—
		Neurula	17.6	15.7	71.4	84.6
		Middle tailbud	—	—	7.2	15.4
		Larva	—	—	—	—
	10	2–4 cells	—	100	—	—
		Morula	—	—	—	—
		Gastrula	—	—	71.4	—
		Neurula	—	—	28.6	100
		Middle tailbud	—	—	—	—
		Larva	—	—	—	—
TPTH	0.1	2–4 cells	—	—	—	—
		Morula	—	—	—	—
		Gastrula	35	—	—	—
		Neurula	45	61.1	45.8	21
		Middle tailbud	5	22.2	4.2	15.8
		Larva	15	16.7	50	63.2
	1	2–4 cells	—	—	—	—
		Morula	—	50	—	—
		Gastrula	58.4	40	43.7	—
		Neurula	33.3	10	56.3	73.4
		Middle tailbud	8.3	—	—	26.6
		Larva	—	—	—	—
	10	2–4 cells	—	100	—	—
		Morula	—	—	—	—
		Gastrula	—	—	42.8	—
		Neurula	—	—	50	88.8
		Middle tailbud	—	—	7.2	11.2
		Larva	—	—	—	—

TABLE 1—*Continued*

Compound	μM/liter	Observed stage	Initial exposure stage			
			Postfertilization	2–4 cells	Gastrula	Neurula
TCHTC	0.1	2–4 cells	—	—	—	—
		Morula	—	—	—	—
		Gastrula	—	—	—	—
		Neurula	50	28.6	28.6	21.4
		Middle tailbud	9.1	—	14.3	14.3
		Larva	40.9	71.4	57.1	64.3
	1	2–4 cells	—	—	—	—
		Morula	—	—	—	—
		Gastrula	—	—	—	—
		Neurula	62.5	75	12.5	8.4
		Middle tailbud	37.5	25	87.5	91.6
		Larva	—	—	—	—
	10	2–4 cells	—	—	—	—
		Morula	36.3	45.5	—	—
		Gastrula	36.3	31.8	35	—
		Neurula	27.4	22.7	60	26.6
		Middle tailbud	—	—	5	73.4
		Larva	—	—	—	—

Note. Initial exposure stages are indicated. Data were recorded 12 hr after fertilization. Controls developed 100% of swimming larvae.

results at 10 μM. MBTC is the least active compound; in fact, at the maximum employed concentration of 10 μM, 50% of the embryos is still capable of normal metamorphosis. At all the experimented concentrations, these three compounds significantly affect the embryonic development at all the stages. In particular, the neurulae which were blocked after direct exposure and those derived from the gastrulae exposed to these compounds appear extremely anomalous compared to controls, revealing spherical blastomeres which are separated from each other by large spaces. The latter morphological alteration also occurs in anomalous gastrulae (Figs. 1a and 1b). All the larvae exposed to 0.1 μM TBTC, and several of those exposed to 1 μM DBTC, did not metamorphose, appearing motionless and having twisted tails. Among the triphenyltin compounds, exposure to 0.1 μM TPTA of the postfertilization stage inhibits larval development. The latter is not affected by the other two phenyltin compounds, which, however, block metamorphosis. Exposure to 10 μM TPTC, TPTA and TPTH soon after fertilization hinders embryonic cleavage and at the two- to four-cell stage blocks further development, as observed for TBTC. TCHTC has an effect comparable to that of TPTH.

Therefore, all these organotin compounds significantly affect the exposed stages, with the gastrulae and neurulae appearing to be the most sensitive stages.

Ultrastructural Analysis

In order to elucidate the ultrastructural targets of these compounds, the early embryonic stages (two to four cells, morula, and gastrula) were incubated with 10 μM TBTC and TPTC for 1 hr. These two compounds were chosen because of their worldwide distribution. In spite of the lesser embryotoxicity of TPTC and TPTA, the former was preferred because of the common presence of the chloride anion in TBTC which exhibits similar physicochemical properties.

Spherical-shaped electron-dense precipitates were observed in exposed embryos under TEM investigation and probably represent metal tin aggregates. Their number and size were dependent on the organotin compound used and the stage exposed: TBTC and TPTC precipitates revealed an average diameter of 45 and 55 nm, respectively. Similar electron-dense precipitates (50 to 70 nm) were sometimes also observed in controls.

Two- to Four-Cell Stage

In the embryos exposed to 10 μM TPTC or TBTC for 1 hr, various electron-dense aggregates were present in mitochondria (Fig. 1c), indicating changes of the inner membrane which was reduced to scanty cristae and vesiculation.

Morula Stage

The blastomeres of the embryos likewise exposed were globe-shaped with large intercellular spaces, without changes in intercellular junctions. Electron-dense aggregates were again visible both in mitochondria (Fig. 1d) and scattered in the cytoplasm. Mitochondrial cristae appeared indistinct and mixed with the matrix.

Gastrula Stage

In the embryos likewise exposed, the typical morphology of the cells of embryonic sheets during the differentiation pattern was severely altered, demonstrating globular shapes with large intercellular spaces. Moreover, inside mitochondria the elec-

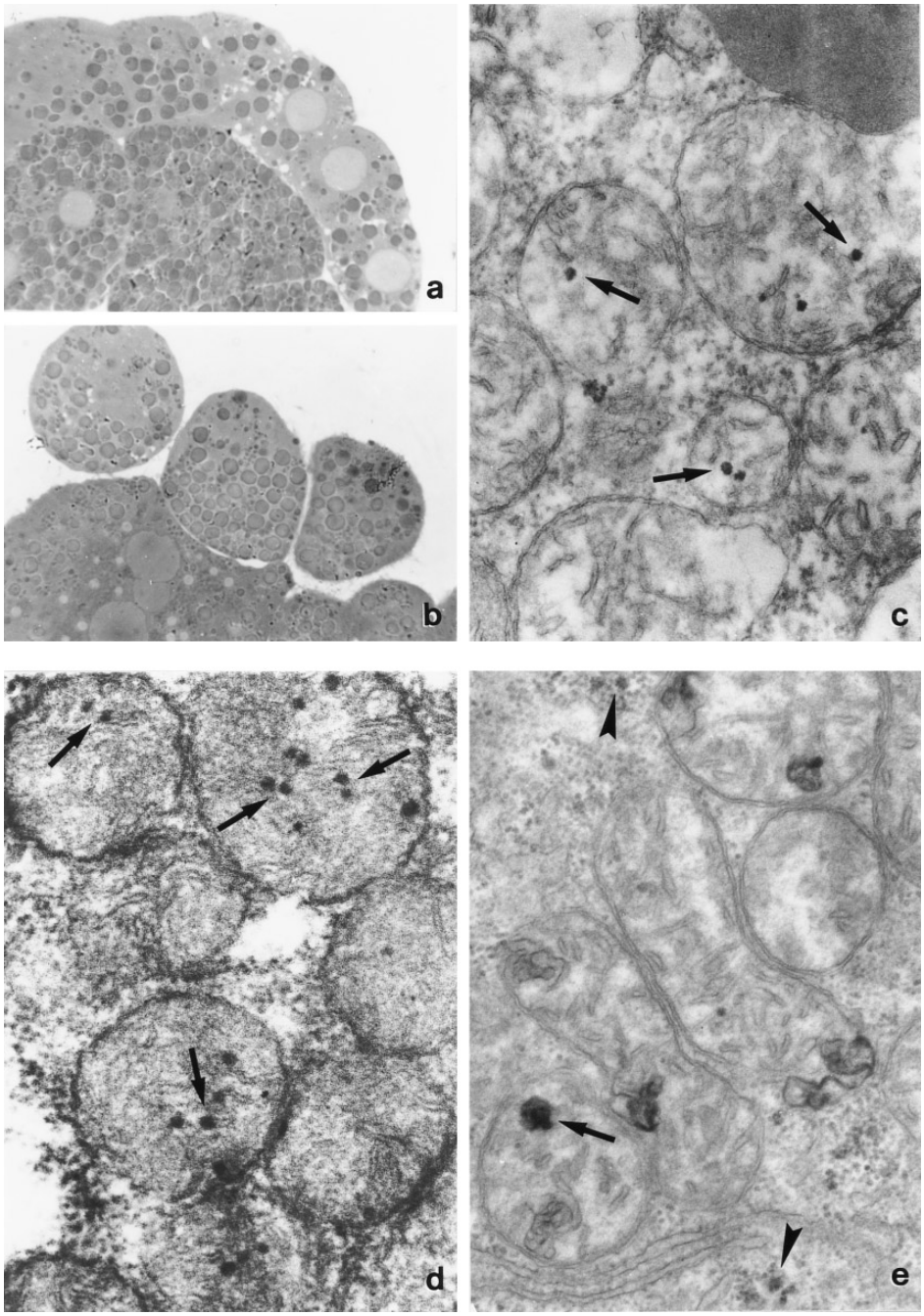

FIG. 1. *Styela plicata* embryos. Control (a) and treated stages with 10 μM TBTC (b,d,e) or TPTC (c) for 1 hr. (a,b) Semithin sections; detail of a normal (a) and anomalous gastrula (b); the latter demonstrates some spherical-shaped cells of the sheet, separated by large intercellular spaces. (c,d,e) Thin sections; detail of cytoplasm with clusters of altered mitochondria. At the 2- to 4-cell stage (c), mitochondria reveal scanty cristae, vesiculations, and minute electron-dense precipitates (arrows); at morula (d), mitochondria present indistinct cristae and numerous electron-dense precipitates (arrows); at gastrula (e), mitochondria contain large electron-dense aggregates (arrow) together with folded fragments of cristae; some smaller precipitates are in the cytoplasm (arrowheads). (a,b) ×450; (c) ×31,400; (d) ×49,000; (e) ×30,000.

tron-dense aggregates were remarkable in size (110–170 nm), i.e., larger than in the previous exposed stages (Fig. 1e). Mitochondrial cristae were strongly modified up to small vesicles and folded fragments. Smaller aggregates (70 nm) appeared numerous in the cytoplasm (Fig. 1e), but clearly distinguishable from ribosomes in size and electron density.

DISCUSSION

The results of this study demonstrate that the early stages of *S. plicata* embryonic development undergo remarkable and irreversible dose- and time-dependent alterations after exposure to the tested organotin compounds. In the more severe re-

sponses, the embryotoxicity is declared *in vivo* as a block of the exposed stage and ultrastructurally as remarkable changes mainly in mitochondria. However, the concentrations used were higher than those having effects in mammals and fish, probably because in tunicate embryos the egg envelopes act as protective barriers against contaminants in sea water (Mansueto *et al.*, 1984) and, on the other hand, at least some of these embryonic envelopes contain metals and seem to be able to take them up from the sea water (Botte *et al.*, 1979).

It must be emphasized that the organotin compounds reveal a high affinity for the cell membranes. Therefore, they can easily penetrate into cells, causing toxic damages up to cell death because of ATP loss and phospholipid oxidation (Gray *et al.*, 1987). It is difficult to compare the classes of organotin derivatives, even if the chemical nature of the alkyl, aryl, or cyclohexyl group has a strong influence on the physical and biological properties. Moreover, a singly charged anion, i.e., chloride in MBTC, DBTC, TBTC, TPTC, and TCHTC, or an anionic organic group, i.e., acetate in TPTA and hydroxide in TPTH, influences their solubility and volatility (WHO, 1980). Hence, in the toxic responses of the exposed embryos of *S. plicata*, MBTC, TPTH, and TCHTC appear less active, probably owing to their less lipophilic property. Moreover, cyclohexyl groups of TCHT compounds undergo metabolism in animals by scission from the Sn atom (Blair, 1975). The order of inhibition of the embryonic development is strongly dependent on the organotin liposolubility: TBTC > DBTC > MBTC and TPTA > TPTC > TPTH $\geqslant$ TCHTC.

The *in vivo* and ultrastructural results indicate that organotins strongly affect all stages of ascidian development, but the most sensitive and critical stage is gastrula. The electron-dense aggregates, observed under TEM in mitochondria and cytoplasm of embryos exposed to 10 μM TBTC and TPTC, are stable structures induced by xenobiotics crossing the cell membranes, while their size may be modified by interaction with other cellular lipophilic compounds (Gray *et al.*, 1987). The present results may provide an explanation for the observations of Mansueto *et al.* (1993a) who described large electron-dense precipitates linked to alterations of the inner membrane of mitochondria in gastrulae of *C. intestinalis* exposed to TBT. The sizes of the aggregates observed in the mitochondria of *S. plicata* are comparable to those reported by Gray *et al.* (1987) in human erythrocytes and increases from 45 to 170 nm as embryonic development proceeds to gastrula, suggesting a contamination of an organotin nature. In addition, for the first time, 55-nm precipitates of TPT in mitochondria were found.

All the organotin compounds are potent inhibitors of oxidative phosphorylation in the mitochondria for which they have a high binding affinity (Aldridge and Cremer, 1955; Aldridge *et al.*, 1977). In particular, triorganotin compounds derange mitochondrial function in three different ways: (i) an oligomycin-like inhibition of coupled phosphorylation, more by TBT than TPT (Stockdale *et al.*, 1970); (ii) an alteration of hydroxide exchange across lipid membranes, producing a reduction of intramitochondrial substrate and phosphate concentrations followed by structural damage (WHO, 1980); (iii) an inhibition of ATP synthesis owing to a reaction of the phenyl groups of TPT with the thiols of the lipoic acid, followed by enzymatic inhibition of lipoic acid–acetyltransferase and lipoamide dehydrogenase (Ascher and Nissim, 1964).

The electron-dense precipitates, sometimes also observed in controls, might be associated with the presence of inorganic tin or other metals in sea water independent of pollution. Tin levels have been demonstrated in sea water (0.003 mg/liter) by Vinogradov (1953), in marine animals (0.2–20 mg/kg) by Bowen (1966), and recently in phlebobranch ascidians by Monniot *et al.* (1993). Nevertheless, the specific localization of electron-dense precipitates in mitochondria led to the exclusion of an exogenous origin by inorganic tin in sea water and to the assumption of an organotin compound source because of their typical lipophilicity. In agreement with these results, a basal contamination by organotin compounds of the lagoon of Venice and a bioaccumulation in mussels have been documented for TBT and TPT (about 0.28 mg/kg as Sn) (Caricchia *et al.*, 1991).

Further mechanisms may underlie the different effects of organotin compounds on embryos of *S. plicata.* It is known that the tin ion of these compounds can interact with proteins, causing conformational changes as other heavy metals (Yallapragada *et al.*, 1990). Activities of many enzymes are thus deranged, such as cytoskeletal microfilaments and microtubules. For the latter it has been demonstrated *in vitro* that the triorganotin compounds TBT and TPT both hinder the polymerization of G-actin in F-actin, followed by disaggregation (Galli *et al.*, 1993), and inhibit polymerization of tubulin with a mechanism of action similar to that of heavy metals (Tan *et al.*, 1978).

From the above data, it is suggested that the inhibition of the microtubule polymerization and then of the mitotic fuse formation causes a cleavage block in the early embryonic stages. In fact, the embryos exposed to the highest concentration of TBTC, TPTA, or TPTC are soon blocked at the treated stage or, if their development continues, proceed toward an anomalous cleavage producing various sized blastomeres and finally a clearly asymmetrical morula.

In the second place, as the inhibition of the microfilament polymerization causes deep changes in the internal organization of the cell and in its morphology, this suggests that such an inhibition is the main cause of the embryonic anomalies observed during the stages of differentiation, like gastrula, neurula, and larva. In fact, observations with light and electron microscopes revealed globe-shaped blastomeres with large intercellular spaces in the gastrula stage, a block of tail motility, and metamorphosis of the larva stage. Besides, the tail was twisted in an unnatural manner, as proof of a probable internal disorganization of the tail tissues.

CONCLUSION

In conclusion, from this study three aspects of the embryotoxicity of organotin compounds were clarified: (i) the identi-

fication of the more sensitive embryonic stages to organotin compounds, (ii) their characteristic accumulation in the inner membrane of mitochondria associated with the inhibition of oxidative phosphorylation as a fundamental mechanism of toxic action on embryonic development, and (iii) their interaction with cytoskeletal proteins explaining both the block and the malformations of the embryonic stages. These results may contribute to a strengthening of the basis of a proper risk evaluation for organotin contaminants in sea water.

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