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Lipid and fatty acid variations in *Ciona intestinalis* ovary after tri-*n*-butyltin(IV)chloride exposure

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Reduction of total lipids (TL) content and significant variations of triglyceride (TG) and phospholipid (PL) fractions were observed as a consequence of exposure of *Ciona intestinalis* ovaries to tributyltin chloride (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 prostaglandine 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 TBTCI. Copyright © 2004 John Wiley & Sons, Ltd.

KEYWORDS: triglycerides; phospholipids; fatty acids; TBTCI; ovary; *Ciona intestinalis*

INTRODUCTION

The negative effects of organotin (IV) compounds on animal cells and, in particular, on the reproductive system of marine organisms living in heavily polluted seawater, have been reported by several authors.[1,2]

Investigations carried out on ascidian gametes showed reduction of fertilization power and of sperm motility, together with abnormal embryonic development, following exposure to organotin (IV) compounds.[3,4]

Among the potential toxic effects induced by exposure to organometallic complexes are blocking of enzyme functional sites, macromolecule conformation modification and, in particular, membrane permeability. According to previous research, the susceptibility of unfertilized ascidian eggs to toxicants could be related to their interaction with their egg membrane,[4] which depends on several metabolic events following fertilization. Artificial activation and normal fertilization are accompanied by a transient increase in the concentration of free Ca^{2+} ions in the ascidian egg cytoplasm.[5–10]

The release of intracellular Ca^{2+} ions triggers a cortical contraction wave, meiosis, a general egg reorganization and DNA duplication, so that each membrane perturbation causes different cell modification processes. Transmission electron microscopy (TEM) observations indicate alterations in mitochondrial and nuclear membranes.[11–13]

Moreover, reduction in lipid content was observed, with respect to the control value, in ovaries exposed to 10^{-5} and 10^{-7} M TBTCL or other organometallic complex solutions.[14,15]

Although organotin (IV) derivatives exert a number of important cellular, biochemical and molecular effects, relatively few data are available on the interaction of these toxicants with processes that regulate membrane metabolism.

High toxicity of organotin (IV) derivatives is at least partly attributable to their solubility in lipids, compared with

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inorganic tin, so that these compounds represent a possible way to explore their sensitivity correlated to the membrane lipid composition.[16]

In fact, membrane permeability has been demonstrated to be influenced, during metal-provoked stress, by lipid membrane composition, which modifies the stress response.[17]

Tributyltin (TBT) toxicity is markedly influenced by membrane fatty acid composition. In *Saccharomyces cerevisiae*, the enrichment of the culture medium with linoleic acid (PUFA, 18:2 n-6) has been associated with elevated TBT resistance, despite increased TBT uptake by these cells, which also show reduced loss of vitality when exposed to TBT compared with cells grown in the absence of a fatty acid supplement.[17]

Recent results indicate that, in C*iona intestinalis*, the female reproductive system is heavily affected at a biochemical level by the chemical pollutant TBTCl.[15] In particular, a reduction of lipid content with respect to the control value in ovaries exposed to 10^{-5} and 10^{-7} M TBTCl solutions has been observed.

Because of the importance of the TBT effect at the reproductive level, as shown by a significant reduction in total lipid content, the variation of the lipid fraction induced by TBTCl has been investigated in the present paper, analyzing the fatty acid profile of lipids extracted from *C. intestinalis* ovaries, following exposure to TBTCl solutions.

MATERIAL AND METHODS

Chemicals

Sterile sea water (SSW) was obtained by filtering and pasteurizing at 80 °C normal sea water containing chloromycetin at 100 μg/cm^3. Tributyltin (IV) chloride (TBTCl) was an Alfa Aesar compound (MA, USA). A 0.1 mM TBTCl solution was prepared by dissolving TBTCl in SSW containing 0.07% v/v dimethylsulfoxide (DMSO). Then, 10^{-5} and 10^{-7} M solutions were obtained by dilution and their total tin content was checked by a Perkin Elmer model 3100 atomic absorption spectrometer, equipped with a Perkin Elmer model 100 flow injection analysis system for atomic spectroscopy, according to standard procedure. The solvent, DMSO, was used because of the low solubility of TBTCl in SSW (Merck, Darmstadt, Germany).

Experiments *in vivo*

Adult *C. intestinalis* individuals were collected from Mazara del Vallo harbour, Sicily. Two hundred ovaries were removed from the animals by dissection, washed in SSW, divided into five batches and reduced to small pieces with steel needles to deliver oocytes at various stages: the first ovary group was cultured in SSW and used as a control, and the second one in SSW containing 0.07% DMSO to test its isolated effect. Two more ovary groups were cultured in 10^{-5} and 10^{-7} M TBTCl solutions, containing 0.07% DMSO. The last one was cultured in a 10^{-5} M TBTCl solution in SSW. After 5 h incubation at room temperature, ovaries from the five batches were washed several times in SSW and frozen at −80 °C until analysis. All experiments were repeated in triplicate ($n = 3$).

Biochemical analyses

Total lipids were extracted from the ovaries by homogenization in dichloromethane: methanol (1:1) solution, added with 2,6-di-tert-butyl-4-methylphenol (BHT), 0.01%, as an antioxidant, according to the Folch method.[18] Total lipids were separated by absorption chromatography on a silica column, using chloroform to eluate triglycerides and methanol to eluate phospholipids. Lipids were dried using a rotary evaporator, then suspended in *n*-hexane and stored under a nitrogen atmosphere at −20 °C until analysis. The fractions were quantitatively determined by a gravimetric method.[18]

Fatty acid methyl esters (FAMEs) from total lipids and from lipid fractions were prepared by acid-catalyzed transmethylation for 1 h at 100 °C, using tricosanoic acid (23:0) as internal standard.[19] Methyl esters were extracted by *n*-hexane, then dried by centrivap, weighted and suspended in c-hexane (1% v/v).

The FAMEs analysis was performed using a Perkin Elmer Autosystem XL gas chromatograph, equipped with a flame ionization detector and fitted with a fused silica capillary column (Omegawax 320; Supelco Inc., Bellefonte, USA); helium was used as carrier gas. The injector, detector and column temperatures were 250, 300 and 200 °C, respectively. Individual FAMEs were identified in comparison with known standards (mixed fish oil PUFA, Supelco) and quantified by Turbochrome navigator software.

Results were expressed as the average of several percentage (%) in weight determinations [$\pm\sigma$ (σ = standard deviation)]. Total lipid, TL, percentage was expressed as g total lipids/100 g of ovary dry weight, while triglycerides or phospholipids percentages were expressed as g triglyceride (TG) or phospholipid (PL)/100 g of TL. Differences between treatments were analyzed by one-way statistical method of analysis, ANOVA (analysis of variance between groups), and reported as significant when probability $p \leq 0.05$.

RESULTS

Total lipids and lipid classes

The TL, TG and PL percentage values observed in *C. intestinalis* ovaries are reported in Table 1. The difference between the two control samples (SSW and SSW containing 0.07% DMSO) and the two treatments at 10^{-5} M TBTCl solutions was not significant.

The maximum value of percentage TL was observed in the control SSW samples (8.4 ± 0.8%) and in samples cultured in SSW containing 0.07% DMSO (8.0% ± 0.5%).

The TL content underwent a progressive reduction compared with the control both in SSW (8.4% ± 0.3) and

Table 1. Percentage of TL, TG and PL observed in ovaries of *Ciona intestinalis*[a]

	Control in SSW	Control in SSW containing 0.07% DMSO	10^{-7} M TBTCl solution in SSW containing 0.07% DMSO	10^{-5} M TBTCl solution in SSW containing 0.07% DMSO	10^{-5} M TBTCl solution in SSW
% TL	8.4	8.0	7.1	6.3	5.3
σ	0.8	0.5	0.3	1.2	0.8
ANOVA	a	a	b	c	c
% TG	80.3	77.3	69.3	55.0	50.0
σ	4.1	2.8	3.2	2.6	3.0
ANOVA	a	a	b	c	c
% PL	18.0	20.0	29.2	42.0	47.0
σ	2.3	1.8	3.1	3.6	2.9
ANOVA	a	a	b	c	c

[a] σ = standard deviation; ANOVA = statistical analysis method (see Materials and methods); the values with different letters (a–c) are statistically significantly different.

in SSW containing 0.07% DMSO (8.0% ± 0.5) with increase in TBTCl content from 10^{-7} M (7.1 ± 0.3%) to 10^{-5} M solutions (6.3 ± 1.2%; $p < 0.05$).

The TG percentage showed a significant reduction from 80.3 ± 4.1% in the control, to 69.3 ± 3.2 and 55.0 ± 2.6%, respectively in the samples cultured in 10^{-7} and 10^{-5} M TBTCl solutions in SSW containing 0.07% DMSO ($p < 0.05$). This progressive reduction was positively related to TL content ($r = 0.985$).

The PL fraction underwent a progressive significant increase from 18.0 ± 2.3% in the control to 29.2 ± 3.1 and 42.0 ± 3.6% in samples maintained, respectively, in 10^{-7} and 10^{-5} M TBTCl solution containing 0.07% DMSO.

Qualitative analyses of the lipid fractions implied observation of the fatty acid (FA) profile.

Fatty acid profiles

The FA profiles of the two principal lipid fractions are shown in Tables 2 and 3.

Triglycerides

In the TG fraction, the largest class was represented by monounsaturated fatty acids (MFA), which were subject to a progressive increase from 30.21 ± 1.23 (control SSW), to 34.02 ± 1.27% in samples cultured in the presence of the higher concentration of TBTCl in SSW, showing an inverse relationship to the total lipid content (Table 2).

This trend was followed by palmitoleic acid (16 : 1 n-7), which increased from 4.22 ± 0.11 to 6.55 ± 1.69%, vaccenic acid (18 : 1 n-7), which showed a progressive increment from 6.67 ± 0.33 to 8.63 ± 1.70%, and gadoleic acid (20 : 1 n-9; from 1.58 ± 0.03 to 8.23 ± 1.43%) in samples cultured with TBTCL solutions in SSW. Oleic acid (18 : 1 n-9) represented the principal fatty acid, which showed an increment from 17.74 ± 0.76 to 18.58 ± 0.61% in samples cultured with 10^{-5} M TBTCI solution containing 0.07% DMSO, and a reduction to 10.61 ± 2.12% in samples cultured in 10^{-5} M TBTCI solution in SSW (Table 2).

The decrease of saturated fatty acid (SFA) was positively correlated to the TL decrease.

At PUFA n-3 level, the principal variations were observed in the stearidonic (18 : 4 n-3) and docosahexaenoic (DHA) (22 : 6 n-3) acid profiles (Table 2). Concentration of Stearidonic acid was 5.88 ± 0.52% in samples cultured in SSW and significantly increased to 8.03 ± 1.34% in samples cultured at higher TBTCI concentrations in SSW solutions ($p < 0.05$).

Concentration of DHA, in contrast, was significantly lower in samples cultured at higher TBTCI concentrations (3.29% ± 0.99), compared with the samples cultured in SSW (7.03% ± 2.58) ($p < 0.05$). The PUFA n-6 fraction remained stable during the treatment.

Phospholipids

The FA profile of the PL fraction is reported in Table 3. The principal FA classes are represented by MFA and PUFA n-3, then PUFA n-6 and SFA. From a quantitative point of view MFA and PUFA n-6 were positively correlated to TL content, showing the same characteristic decrease. The significant decrease of MFA (32.96 ± 1.88%), in samples treated with the higher TBT concentration, compared with the control (37.84 ± 1.87%), was inversely related to the increase in PUFA n-3, from a mean value of 29.45 ± 5.72% in samples cultured in SSW to a mean value of 44.7 ± 1.08% in 10^{-5} M TBTCI solution, due to the significant increase of 18 : 4 n-3 and DHA (Table 3).

SFAs were quite stable in the various samples. At PUFA n-6 level, significant decrease in samples treated with the higher concentration of TBTCI was observed due to the significant decrease of 18 : 2 n-6 from a value of 9.79 ± 0.21% in SSW samples, to 3.92 ± 0.57% in samples treated with 10^{-5} M TBTCI SSW solution.

DISCUSSION

Organotin (IV) derivatives are a group of highly toxic organometallic compounds which have been widely used

Table 2. Fatty acids classes composition (percentage of total fatty acids) of the triglycerides observed in ovaries of *Ciona intestinalis*, exposed to different experimental conditions[a]

Numerical symbol of fatty acids[b]	Control in SSW		Control in SSW containing 0.07% DMSO		10^{-7} M TBTCl solution in SSW containing 0.07% DMSO		10^{-5} M TBTCl solution in SSW containing 0.07% DMSO		10^{-5} M TBTCl solution in SSW	
	Average	σ	Average	σ	Average	σ	Average	σ	Average	σ
14 : 0	3.51	0.33	3.43	0.12	3.22	0.12	3.21	0.10	2.61	0.66
16 : 0	18.87	0.68	18.51	0.26	17.29	0.58	17.55	0.65	19.53	1.79
16 : 1 n-7	4.22	0.11	4.22	0.07	4.11	0.14	4.13	0.17	6.55	1.69
16 : 2 n-4	0.12	0.02	0.46	0.08	0.16	0.08	0.23	0.03	0.00	0.00
16 : 3 n-4	0.68	0.17	0.92	0.01	0.64	0.01	0.62	0.15	0.00	0.00
18 : 0	4.72	0.02	4.18	0.08	4.08	0.10	4.38	0.15	3.88	0.23
18 : 1 n-9	17.74	0.76	18.43	0.39	18.71	0.52	18.58	0.61	10.61	2.12
18 : 1 n-7	6.67	0.33	7.40	0.16	7.68	0.25	8.02	0.28	8.63	1.70
18 : 2 n-6	11.96	0.46	12.56	0.26	12.57	0.35	12.49	0.41	10.69	1.61
18 : 3 n-3	5.08	0.38	5.46	0.11	5.43	0.17	5.26	0.14	5.89	1.58
18 : 4 n-3	5.88	0.52	5.81	0.14	6.33	0.20	6.00	0.15	8.03	1.34
20 : 1 n-9	1.58	0.03	2.25	0.18	2.19	0.13	2.31	0.02	8.23	1.43
20 : 4 n-6	0.78	0.13	0.59	0.08	0.35	0.01	0.70	0.05	0.49	0.08
20 : 4 n-3	0.78	0.34	0.62	0.06	0.35	0.03	0.65	0.05	0.65	0.11
20 : 5 n-3	10.38	1.42	10.64	0.29	12.84	0.40	12.77	0.13	11.02	3.77
22 : 6 n-3	7.03	2.58	4.50	1.58	4.05	2.40	4.31	1.90	3.29	0.99
SFA	27.11	1.04	26.11	0.46	24.59	0.80	25.15	0.90	26.03	1.28
MFA	30.21	1.23	32.30	0.81	32.68	1.04	33.05	1.08	34.02	1.27
PUFA n-3	29.14	5.24	27.04	2.19	29.00	3.19	29.00	2.37	28.88	1.88
PUFA n-6	12.74	0.59	13.15	0.34	12.92	0.36	12.56	0.91	11.18	1.08

[a] σ = standard deviation;
[b] see Table 4 for names and symbols of higher fatty acids.

in agriculture and marine industries, mainly for their biocide properties.[16]

The toxicity of lipophilic organometallic derivatives, e.g. tributyltin (IV) and other derivatives, towards embryonic development in marine organisms, such as *C. intestinalis*, has been previously demonstrated. Reduction of vitality and death of embryos, contemporary to decrease of all principal biochemical molecules, such as proteins, lipids and D-glucose, have also been observed in the ovaries of this species when exposed to TBTCI solutions.[14,15]

The enhanced toxicity of organotin derivatives is at least partly attributable to their solubility in lipids and because of the fact that the lipid composition of organisms is influenced by environmental variations; this response is very interesting in the evaluation of the toxicity of metal species in the natural environment.[20] Further interesting work has demonstrated that TBT toxicity towards the model eukaryote *S. cerevisiae* is markedly influenced by membrane fatty acid composition.

Enrichment with the PUFA linoleic acid (18 : 2 n-6) was associated with high TBT resistance, despite increased TBT uptake by these cells.[21] The total lipid content, determined both in control samples and in samples exposed to TBTCI solutions, confirms its significant decrease at higher TBTCI concentrations. Such a decrease may cause, in addition to the observed damage to embryos exposed and damage to mithocondria,[11] evident ATP and vitality loss in embryos exposed to organotins.[14]

From a quantitative point of view, significant variations of TG and PL contents occurred. In particular, an evident TG decrease and PL increase, compared with the controls, were observed in the sample exposed to higher TBTCI concentration solutions, contemporary with a significant TG decrease in the same samples. This situation probably caused an increment in membrane fluidity, because of the structural properties of the PL fraction, having high long—chain fatty acid concentrations. The toxicity of organotin compounds is closely related to their interaction with biological membranes;[20,22,23] plasma membrane permeability and dissipation of mitochondrial electrochemical gradient are commonly observed during TBT exposure in marine yeast.[24,25] It is possible to suppose that the TG and PL gradients observed, compared with the controls, in samples exposed to 10^{-5} M TBTCI comprise an adaptive mechanism of cells, for resistance to the pollutant.

In the TG fraction, an increase in monounsaturated fatty acids occurs, while PUFA n-3 and PUFA n-6 are quite stable in all samples. It is noticeable, however that, at the PUFA n-3 level, a significant DHA decrease in samples exposed to

Table 3. Fatty acids classes composition (percentage of total fatty acids) of the phospholipids observed in ovaries of *Ciona intestinalis*, exposed to different experimental conditions[a,b]

Numerical symbol of fatty acids[b]	Control in SSW		Control in SSW containing 0.07% DMSO		10^{-7} M TBTCl solution in SSW containing 0.07% DMSO		10^{-5} M TBTCl solution in SSW containing 0.07% DMSO		10^{-5} M TBTCl solution in SSW	
	Average	σ	Average	σ	Average	σ	Average	σ	Average	σ
14 : 0	1.91	0.17	1.98	0.34	1.31	0.37	1.63	0.08	2.24	0.06
16 : 0	3.8	0.18	4.02	0.28	3.40	0.24	3.24	0.06	4.00	0.19
16 : 1 n-7	14.83	0.81	14.81	1.44	11.88	2.54	12.44	0.08	19.00	1.20
16 : 2 n-4	0.73	0.14	0.77	0.02	0.72	0.07	0.65	0.13	0.97	0.01
16 : 3 n-4	0.37	0.13	0.42	0.01	0.39	0.01	0.37	0.01	0.30	0.01
18 : 0	3.80	0.37	3.60	0.16	3.56	0.06	3.06	0.10	3.60	0.22
18 : 1 n-9	13.44	0.34	12.9	0.16	13.07	0.94	11.71	0.38	6.84	1.20
18 : 1 n-7	8.70	0.24	8.27	0.30	7.91	0.59	7.05	0.19	9.35	1.68
18 : 2 n-6	9.79	0.21	9.46	0.02	9.57	0.58	8.90	0.79	3.92	0.57
18 : 3 n-3	3.54	0.01	3.47	0.01	3.47	0.11	3.33	0.07	4.67	1.45
18 : 4 n-3	3.86	0.02	3.65	0.23	3.87	0.16	3.80	0.06	6.18	1.34
20 : 1 n-9	0.87	0.02	1.20	0.09	1.38	0.01	1.27	0.06	2.27	0.43
20 : 4 n-6	0.50	0.05	0.25	0.07	1.22	0.08	0.80	0.01	1.44	0.12
20 : 4 n-3	0.20	0.05	0.80	0.08	0.90	0.13	0.10	0.08	1.71	0.17
20 : 5 n-3	15.85	0.84	15.41	1.14	17.83	0.67	15.22	2.42	15.47	1.99
22 : 6 n-3	6.00	2.11	11.90	1.98	13.90	1.71	14.29	0.24	16.68	1.18
SFA	9.51	0.72	9.60	1.87	8.27	1.47	7.93	0.94	9.90	1.27
MFA	37.84	1.87	37.18	1.98	34.24	1.45	32.47	1.75	32.96	1.88
PUFA n-3	29.45	5.72	35.23	3.61	39.97	3.26	36.74	0.96	44.70	1.08
PUFA n-6	10.29	0.74	9.71	0.77	10.79	1.41	9.70	0.96	5.30	0.89

[a] σ = standard deviation;
[b] See Table 4 for names and symbols of higher fatty acids.

the higher TBT concentrations was observed, independently of the presence of DMSO (Table 2). These data assume a significant value if related to the contemporary increase of DHA in PL profiles (Table 3).

There was a pattern change during the exposure to the pollutant; a PL and a PUFA increase in this fraction, could signify a defense attempt against TBT, as observed in *S. cerevisiae*. In these cells PUFA enrichment was associated with a marked increase in cellular TBT resistance.[21]

It is also important to underline the PUFA n-3 role in the biosynthesis of eicosanoids, a class of compounds including prostaglandin (PG), thromboxane and leucotrienes that are produced by macrophages in stress situations.[26]

Eicosanoids are derived from membrane-associated PUFA via the action of cyclooxygenase or lipoxygenase enzymes. Knight *et al*.[27] studied the eicosanoid-generating capabilities of different tissues and organs from the common sea squirt and demonstrated that the ovary generated significant PG amounts. In this case the presence of TBTCI could stimulate macrophage activity via eicosanoid production. A PUFA increment, in the presence of TBTCI, could be a defense mechanism to the stressor.

Moreover, looking at the fatty acid percentage determination following triglyceride and phospholipid component hydrolysis, a relevant fluctuation, compared with the control percentage, could be observed. The above-mentioned considerations would suggest an increment of lipase activity which must occur, as a consequence of TBTCL treatment, before the analytical separation of triglyceride and phospholipid components.

As previously shown,[14] TBTCL treatment increases Ca^{2+} content, and this ion, as is known, is a lipase activator; on the other hand, other bivalent ions, such as Zn^{2+}, Co^{2+} and Mg^{2+} are also strong lipase activators.[28–30] We do not know if Sn^{2+} can similarly be a lipase activator. However, following TBTCI treatment, spots of metallic tin could be observed in mitochondria cristae[11,31] where, as is known, an electron flux for oxidative phosphorylation occurs. In that region, reduction of tri-*n*-butyltin (IV) to Sn^{2+}, and finally to metallic tin, is not improbable. In such conditions, Sn^{2+}, as well as previously mentioned bivalent ions, could be a lipase activator. The Sn^{2+} ionic radius, according to Pauling, is 1.12 Å; this value approaches the ionic radius of Ca^{2+} (0.99 Å), which is known to be a lipase activator, rather than the one of ions such as Zn^{2+} (0.74 Å), Mg^{2+} (0.75 Å) and Co^{2+} (0.72 Å), which are also well known lipase activators. Therefore, Sn(II) could be a lipase enzyme activator owing to the fact that, in spite of its ionic radius, it could be inserted

Table 4. Systematic and trivial names and symbols for higher fatty acids presented in the text

Numerical symbols	Systematic names	Trivial names
14 : 0	Tetradecanoic acid	Myristic
16 : 0	Hexadecanoic acid	Palmitic
18 : 0	Octadecanoic acid	Stearic
16 : 1 n-7	9-Hexadecenoic acid	Palmitoleic
16 : 2 n-4	9, 12-Hexadecadienoic acid	Hexadecadienoic
16 : 3 n-4	7, 10, 13-Hexadecatrienoic acid	Hexadecatrienoic
18 : 1 n-7	11-Octadecenoic	Vaccenic
18 : 1 n-9	*cis*-9-Octadecenoic acid	Oleic
18 : 2 n-6	*cis*, *cis*-9, 12-Octadecadienoic acid	Linoleic
18 : 3 n-3	9, 12, 15-Octadecatrienoic acid	α-Linolenic
18 : 4 n-3	6, 9, 12, 15-Octadecatetraenoic acid	Stearidonic
20 : 1 n-9	9-Eicosenoic acid	Gadoleic
20 : 4 n-6	5, 8, 11, 14-Eicosatetraenoic acid	Arachidonic
20 : 4 n-3	8, 11, 14, 17-Eicosatetraenoic acid	Eicosatetraenoic
20 : 5 n-3	5, 8, 11, 14, 17-Eicosapentaenoic acid	EPA
22 : 6 n-3	4, 7, 10, 13, 16, 19-Docosahexaenoic	DHA
SFA	Saturated acids	
MFA	Monounsatured acids	
PUFA	Polyunsaturated fatty acids	

into lipase enzymes as Zn^{2+} and the other ions are. Actually it is not clear if and how Sn^{2+} can increase Ca^{2+} levels, but experimental data with TBTCL show that it occurred.

Ca^{2+} is a lipase activator and its activity increases the concentration of many fatty acids, as found in this work. This mechanism involves an increase of glycerophosphate and phosphatase enzyme activity,[30] as previously found.[15] The lipase activity increase damages cytochrome C[32] and this, in turn, causes ATP decrease.[15]

As is known, the higher ATP content originates from oxidative phosphorylation following the citric Krebs cycle. The acetyl–CoA group of this cycle derives first from glycolysis, then from oxidative degradation of fatty acids. In effect, ATP decrease is correlated to D-glucose increase and to the total lipid content decrease.[15] Another significant aspect of the TBTCL action on ascidia ovary culture concerns the percentage increase in arachidonic fatty acid (20 : 4 n-6). It is known that this breaks down to PGE1 prostaglandine and is responsible for two important physiological events: tissue inflammation and smooth muscular contraction.

Because of the relevant roles of PUFA in determining membrane fluidity, biosynthesis of the molecules involved in stress response and cell signal transmission, other investigations are needed to understand the effects of TBTCI in the lipid profile of marine organisms.

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REFERENCES

1. Colborn T, Clemmens C. *Chemically Induced Alterations in Sexual Functional Development: the Wildlife/Human Connection*. Princeton Scientific: Princeton, NJ, 1992.
2. Giesy JP, Bowerman WW, Mora MA, Verbrugge DA, Othoudt RA, Newsted JL, Puccia E, Messina C, Cangialosi MV, D'Agati P, Mansueto C, Pellerito C, Nagy L, Mansueto V, Scopelliti M, Fiore T and Pellerito L. *Arch. Environ. Contam. Toxicol.* 1995; **29**: 309.
3. Mansueto C, Pellerito L, Girasolo MA. *Acta Embryol. Morphol. Exp.* 1989; **10**(3): 237.
4. Mansueto C, Lo Valvo M, Pellerito L, Girasolo MA. *Appl. Organometal. Chem.* 1993; **7**: 95.
5. Steinhardt RA, Marzia D. *Nature* 1973; **241**: 400.
6. Bevan SJ, O'Dell DS, Ortolani G. *Cell Differ.* 1977; **6**: 313.
7. Sawada T, Osanai K. *Wilhelm Roux's Arch.* 1981; **190**: 208.
8. Jeffery WR. *Science* 1982; **216**: 545.
9. Dale B. *Exp. Cell Res.* 1988; **177**: 205.
10. Speksnijder JE, Corson DW, Sardet C, Jaffe LF. *Dev. Biol.* 1989; **135**: 182.
11. Mansueto C, Gianguzza M, Dolcemascolo G, Pellerito L. *Appl. Organometal. Chem.* 1993; **7**: 391.
12. Villa L, D'Agati P, Mansueto C, Pellerito C, Scopelliti M, Fiore T, Nagy L, Pellerito L. *Appl. Organometal. Chem.* 2003; **16**: 106.
13. Mansueto C, Villa L, D'Agati P, Marciano V, Pellerito C, Fiore T, Scopelliti M, Nagy L, Pellerito L. *Appl. Organometal. Chem.* 2003; **17**: 553.
14. Mansueto C, Puccia E, Maggio F, Di Stefano R, Fiore T, Pellerito C, Triolo F, Pellerito L. *Appl. Organometal. Chem.* 2000; **14**: 229.
15. Puccia E, Mansueto C, Cangialosi MV, Fiore T, Di Stefano R, Pellerito C, Triolo F, Pellerito L. *Appl. Organometal. Chem.* 2001; **15**: 213.
16. Fent K. *Crit. Rev. Toxicol.* 1996; **26**: 1.
17. Gadd GM. *New Phytol.* 1993; **124**: 25.
18. Folch J, Lees M, Stanley GHS. *J. Biol. Chem.* 1957; **226**: 497.

19. Lepage G, Roy CC. *J. Lipid Res.* 1984; **25**: 1391.
20. Gadd GM. *Molecular Biology and Biotechnology of Extremophiles*, Herbert RA, Sharp RJ (eds). Blackie; Chapman and Hall: Glasgow, New York, 1992; 225–227.
21. Masia A, Avery SV, Zoroddu MA, Gadd GM. *FEMS Microbiol. Lett.* 1998; **167**: 321.
22. Cooney JJ, Wuertz SJ. *Industr. Microbiol.* 1989; **4**: 375.
23. Gadd GM. *FEMS Microbiol. Rev.* 1993; **11**: 297.
24. Laurence OS, Cooney JJ, Gadd GM. *Microb. Ecol.* 1989; **7**: 185.
25. Ambrosini A, Bertoli E, Zolese G. *Appl. Organometal. Chem.* 1996; **10**: 53.
26. Secombes CJ. Macrophage activation in fish. In: *Modulators of Fish Immune Response*, Stolen J and Fletcher TC (eds), SOS Publications: Fair Haven, NY, 1994; Vol. I.
27. Knight J, Taylor GW, Wright P, Clare AS, Rowley AF. *Biochim. Biophys. Acta* 1999; **1436**: 467.
28. Gulati R, Saxena RX, Gupta R, Yadav RP, Davidson SW. *Process Biochem.* 1999; **35**: 459.
29. van Heerden E, Litthauer D, Verger R. *Enzyme Microb. Tech.* 2002; **30**: 902.
30. Waite M. *Handbook of Lipid Research. The Phospholipase*. Hanahan DJ (ed.). Plenum Press: New York, 1987.
31. Gianguzza M, Dolcemascolo G, Mansueto C, Pellerito L. *Appl. Organometal. Chem.* 1996; **10**: 405.
32. Fruton JS, Simmonds S. *General Biochemistry*, 2nd edn. Wiley: New York, 1963; 580.

