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Effects of sublethal levels of tributyltin chloride on a new toxicity test organism, *Liza saliens* (Osteichthyes, Mugilidae): a histological study

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The histopathological effects of 10^{-7} and 10^{-9} M tributyltin(IV)chloride,TBTCl, solutions on different *Liza saliens* organs have been studied by light microscope. The fish were sacrificed after 3–4 h incubation in 10^{-7} M TBTCl solution or after 15 days incubation in 10^{-9} M solution. The observed histopathological changes were dose- and time-dependent. The 10^{-7} M TBTCl concentration resulted in major damage to the gill epithelium, indicating that TBTCl primarily interfered with the respiration, osmoregulation, acid balance and nitrogenous waste excretion processes. After incubation in 10^{-9} M TBTCl solution the fish lived 20 or more days, but many of the organs were altered. Thymus atrophy, reduced spleen and altered head kidney were observed. These histological results indicated that TBTCl interfered with organ immunodefense and altered main metabolic pathways in *Liza saliens*. The presence of melano-macrophage centers, only in TBT-treated liver and spleen, can be considered a tool to facilitate, with other biomarkers, the detection of alterations by toxicants. Regarding the pancreas activity in 10^{-7} M solutions, it has been noted that, in the exocrine cells, very few zymogen granules were still present and the Langerhans islets were more altered. In 10^{-9}M solution the exocrine pancreatic cells had no granules and the islet cells presented degenerative alterations. In addition, TBTCl, which altered the pancreas and gonad morphology, could again be considered an endocrine disrupter even if biochemical data are still necessary. Finally, the *Liza saliens* juveniles could be considered an interesting biological model for experiments with contaminants, due to their ease of adaptation to experimental conditions and food chain position. Copyright © 2006 John Wiley & Sons, Ltd.

KEYWORDS: tributyltin(IV)chloride; *Liza saliens*; histopathology

INTRODUCTION

Organotin compounds are known to be toxic to several organisms, including humans. Their use has been extensive in the recent past, contributing to pollution, especially in aquatic environments, where many organisms live. The paths through which the organotins enter the environment depend on the use of the compound. Their well-known applications are in antifouling paints and agricultural uses, processes through which organotins are released into the environment and, in particular, into the sea. Even if their use has been restricted, they are still used on larger vessels and, as a consequence, tributyltin (TBT) derivatives remain stably bound to marine sediments for long periods. Toxic contamination persists in many locations and TBT levels are found in a variety of organisms.[1–4]

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The effects of organotins on aquatic invertebrate organisms have been studied in many animals, including crabs,[5] molluscs[6] and tunicates.[7,8] Fertilization, embryonic development and larval metamorphosis are seriously affected in tunicates.[9,10] In this way TBT, as with many other pollutants, can produce a reduction not only in their populations, but also in other species of marine biota. Immunotoxic effects of TBT have been reported in molluscs[11] and in tunicates.[12] TBT toxicity has been studied extensively in marine invertebrates, where TBT acts as an endocrine disrupter: in molluscs it produces the so-called 'imposex';[13] in tunicate larvae, it inhibits thyroid hormone synthesis.[10]

Several effects of TBT have been reported on fish. Fish species have been shown to bioaccumulate organotins by two to three orders of magnitude.[14,15] TBT was found to bioaccumulate in salmon held in net pens, and thus to enter the human food chain.[16] Fish from treated pens contained 0.3–0.9 μg/g TBT in muscles.[17] More recently TBT has been found not only in fish,[18] but also in seabirds[19] and marine mammals.[20,21] The uptake of organotin compounds from fish stimulates concern about human health effects. Therefore collection of knowledge about the mechanisms of pollutant bioaccumulation by fish and their target organs is of interest.

As histological responses relate to fitness of individuals, they in turn allow the same extrapolation to population community effects; many authors have studied the histopathological effects of organotins on different organs of fish.[22–25] These studies demonstrate that the possible ecotoxicological impact of TBT or of other organotins is still of concern.

The aim of the work reported on here was to apply histopathology in juvenile fish toxicity studies for rapid analysis of the effects of TBT on different target organs. A coastal species, *Liza saliens*, belonging to the mullets, has been employed as a new toxicity test organism.

MATERIALS AND METHODS

Chemicals

The tributyltin(IV) chloride (TBTCl) was an Alfa Aesar compound (J. Matthey, Kalsruhe, Germany). A 10^{-4} M TBTCl solution was prepared by dissolving the appropriate TBTCl amount in Millipore-filtered sea water (MFSW) containing 7% dimethylsulfoxide (DMSO). Working solutions (pH 7.25–8.50) were obtained by further dilution of the stock solutions in MFSW. Freshly prepared 10^{-7} and 10^{-9} M TBTCl, 0.07% in DMSO, solutions were used 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.[26] The solvent DMSO, used because of the low solubility of TBTCl in MFSW, was a Merck (Darmstadt, Germany) reagent.

Biological material

Liza saliens, Risso, 1810 (Mugilidae, Osteichthyes) juveniles ranging from 150 to 250 mm in body length, were caught from the coasts of Sferracavallo (Palermo). These mugelides live in the coastal waters in contact with sediments; in this way they could be heavily exposed to environmental pollution. They were raised in eight cylindrical fibreglass aquaria, 30 cm in diameter and 30 cm in height, containing 10 l MFSW or MFSW spiked with chemicals. The MFSW was continuously aerated and changed every day. The oxygen levels, between 5.8 and 9.8 mg/l, and the pH, 7.23–8.25, were continuously monitored. The temperature of MFSW was of 19 ± 2 °C and 16 h light and 8 h darkness were maintained during the experiments. The animals were fed every day with *Artemia salina* (SELC, Artemia systems, BAAS-Rode, Belgium). A total of 80 fish were divided equally into four groups as follows:

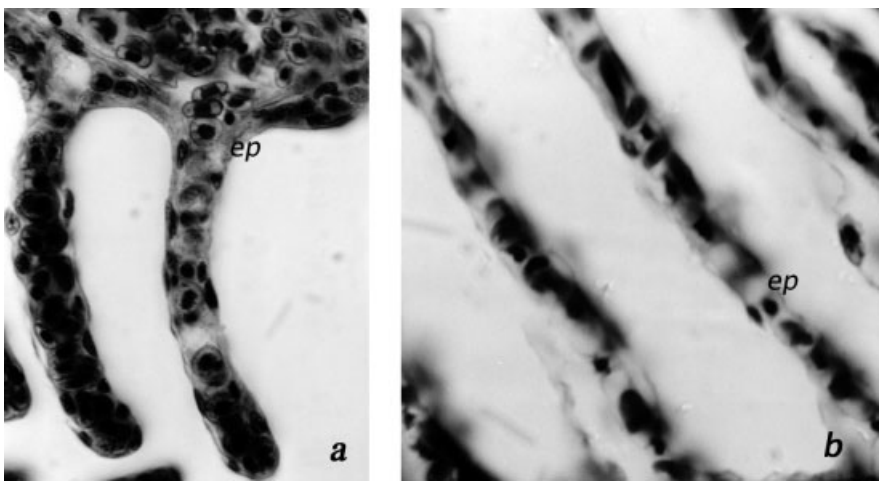

Figure 1. *Liza saliens* gills. (a) Controls: the primary lamellae attached to cartilaginous gill arch and secondary lamellae. These have a single layer of epithelial cells surrounding the capillary. (b) 10^{-9} M TBT-treated individuals: in secondary lamellae, oedema, agglomerates of erythrocytes with altered shape, pycnotic nuclei and reduced plasma. Magnification: a, b = ×720. *ep* = epithelium gill.

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experimental controls—(1) reared in tanks with MFSW and (2) reared in tanks with 0.07% DMSO solution in MFSW; experiments with TBTCl—(3) reared in tanks with 10^{-7} M TBTCl, 0.07% DMSO in MFSW and (4) reared in tanks with 10^{-9} M TBTCl, 0.07% DMSO in MFSW.

The experiments were carried out from 5 h to 20 days. Five fish were sampled from each tank. Several fish were raised for 15 days in MFSW in order to obtain evidence of eventual stress causes.

Histology

The *Liza saliens* juveniles were anesthetized with 0.01% MS-222 (tricaine methanesulfonate; Sigma) and fixed in Bouin's liquid, pH 7.20, for 5 days at room temperature. The fish were dehydrated through a graded series of ethanol, infiltrated and embedded in paraplast. The histological specimens were cut into 5 µm transverse sections on a rotary microtome, stained with Gomori's trichrome; nuclei were stained black, cytoplasm/muscle red and collagen/connective tissue green. Light microscope observations were carried out using a Leitz Diaplan microscope and photographs were obtained using Kodak Tmax films.

RESULTS

The individuals fixed soon after capture, and those after 15 days incubation in MFSW, did not have alterations in their organs: their histomorphology was as described in treatises.[27] The *Liza saliens* juveniles, incubated in 10^{-7} M TBTCl solution, died within 30 min to 5 h, with obvious branchial anaemia and asphyxia signs.

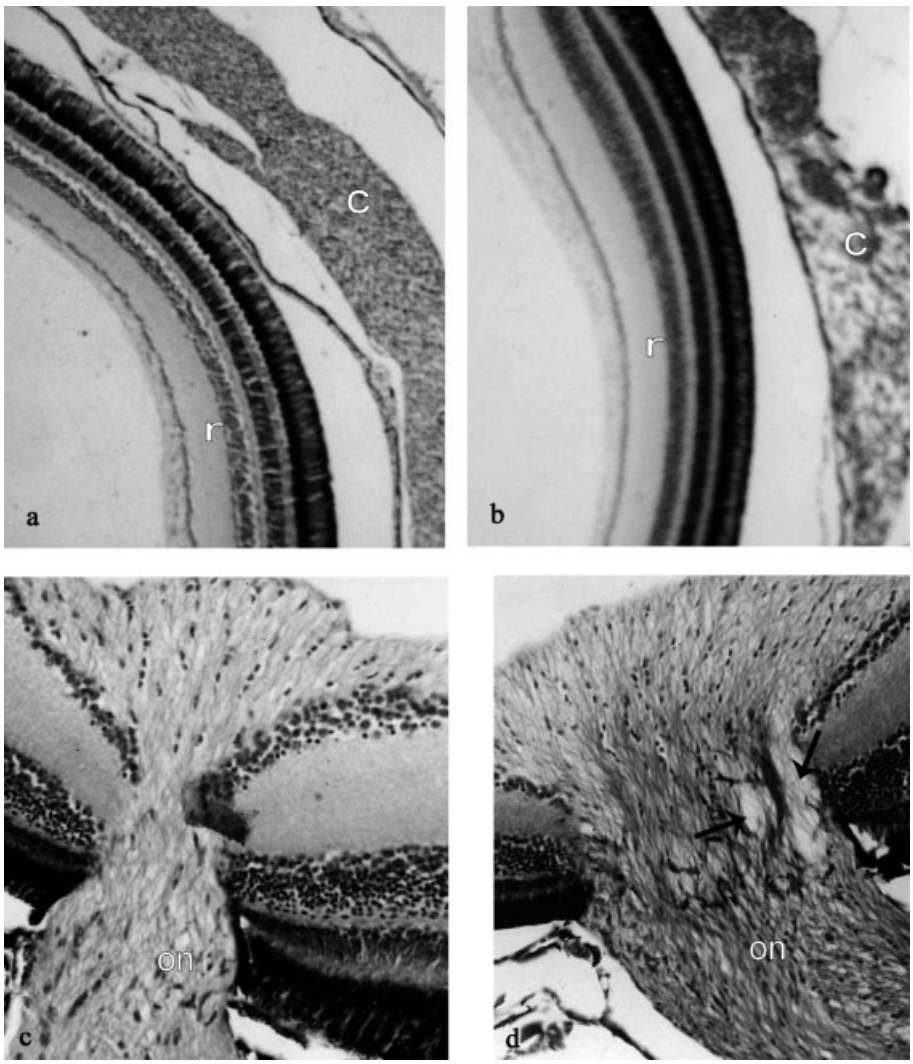

Figure 2. *Liza saliens* eye. (a) Controls: the choroid is a highly vascular layer between sclera and retina, the latter being composed of several layers. (b) 10^{-9} M TBT-treated individuals: the choroid body is almost destroyed and retina layers show irregular arrangement. (c) Controls: optic nerve. (d) 10^{-9} M TBT-treated individuals: the optic nerve is vacuolated in some tracts (arrows); *c* = choroid; *r* = retina; *on* = optic nerve. Magnification: a, b = ×115; c, d = ×290.

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In 10^{-9} M solution, after 15 days of treatment, the following anatomopathology signs were observed: branchial necrotic lesions, cutaneous depigmentation, desquamation, increase of volume of the abdomen, movement in jerks, convulsions. Five individuals incubated in 10^{-7} M TBTCl solution for 3 h and five incubated in 10^{-9} M for 20 days, were sectioned. The exemplars treated in 10^{-7} M TBTCl solution showed heavily compromised organs, above all at gill level. In those treated with 10^{-9} M solution, some alterations were observed in various organs. The fish raised in 0.07% DMSO solution were, *in vivo,* identical to the controls. Also, the organ morphology did not present any alteration.

Gills

The gills of control groups were constituted of primary lamellae attached to a cartilaginous gill arch, and of secondary lamellae. These had a single layer of epithelial cells surrounding a capillary [Fig. 1(a)]. In individuals exposed in 10^{-9} M TBTCl, lamellae showed severe changes: the primary lamellae were often thickened; in the secondary lamellae, often fused, separation of the epithelium from the capillary, epithelial lifting (oedema), dilation and blood vessels congestion were observed [Fig. 1(b)].

Eye

In the controls, an adipose eyelid was present in mullets as a protective structure. The cornea was constituted by a squamous epithelium, a corneal stroma and an endothelium. The sclera, the lens and the retina were the other components. This latter was divided into 10 distinct layers where cellular bodies, axons and dendrites were present. The choroid or rete mirabile was a highly vascular layer between the sclera and the retina [Fig. 2(a)].

In 10^{-9} M TBTCl solution, the cornea was eroded, the layers retina showed irregular arrangement and the choroid body was almost destroyed [Fig. 2(b)]. The optic nerve, in the control, connected the retina to the diencephalons [Fig. 2(c)]. In treated individuals, the optic nerve vacuolated in some tracts [Fig. 2(d)].

Heart

In control groups, the red cells, which are nucleated, had typical oval shape and biconvex outline. The musculature was constituted by striated fibres [Fig. 3(a)]. In treated individuals (10^{-9} M TBTCl), the muscle fibres of the heart were more collapsed and spaced; the striation was not visible [Fig. 3(b)], and in some cases they were swollen.

Liver

In control groups, the parenchyma of the liver appeared to be composed of polyhedric hepatocytes [Fig. 4(a)]: the hepatocytes appeared with a central nucleus containing one nucleolus; they were arranged in cords. Thin capillaries, the sinusoids, were among the hepatocytes. No melano-macrophage aggregates were seen. The liver of individuals incubated in 10^{-9} M TBTCl seemed more enlarged than that

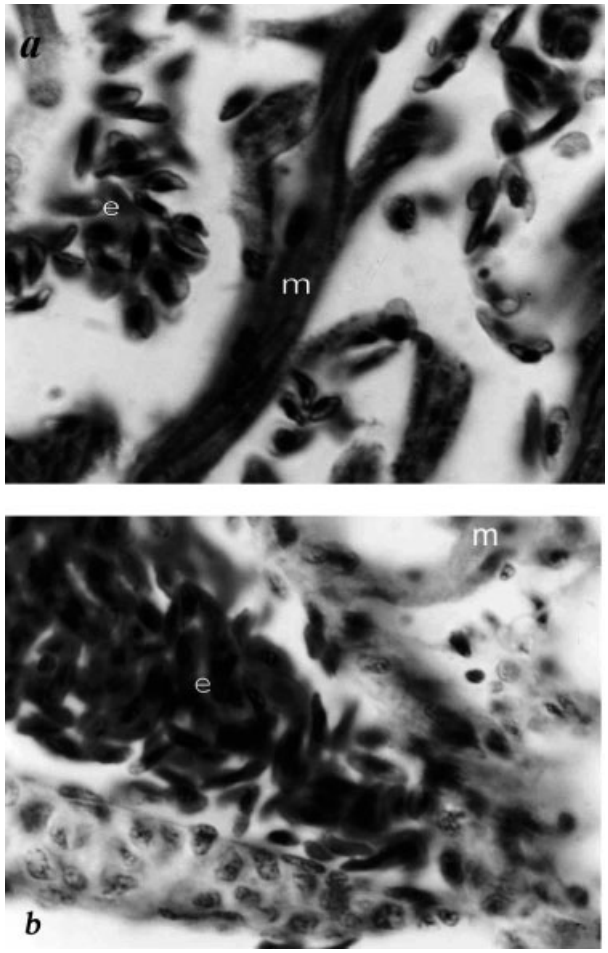

Figure 3. *Liza saliens* heart. (a) Controls: red cells with typical oval shape and biconvex outline; the musculature is constituted by striated fibres. (b) 10^{-9} M TBT-treated individuals: muscle fibres are more collapsed and spaced. Red cells with condensed chromatin and reduced cytoplasm. *e* = erythrocytes; *m* = muscle fibres. Magnification: a, b = ×1150.

of controls, and the blood vessels were more prominent and the big ones destroyed [Fig. 4(b, c)]. In individuals exposed to 10^{-9} M TBTCl, a loss of liver normal architecture with cord disarray was evident [Fig. 4(c, d)]. Sinusoids were dilated and congested. Melano-macrophage aggregates were present, appearing collapsed (data not shown).

Kidney

The histological study of control groups showed that the kidney in the fish was constituted of two parts, the anterior, referred to as the head kidney with hematopoietic, lymphoid and endocrine tissue, and the posterior one [Fig. 5(a)]. In treated individuals (10^{-9} M TBTCl), the head kidney had an altered structure [Fig. 5(b)], with remarkable lymphocyte depletion. In the controls, the glomerulus showed the bunch of fine blood vessels [Fig. 5(c)]. The cells of tubules were,

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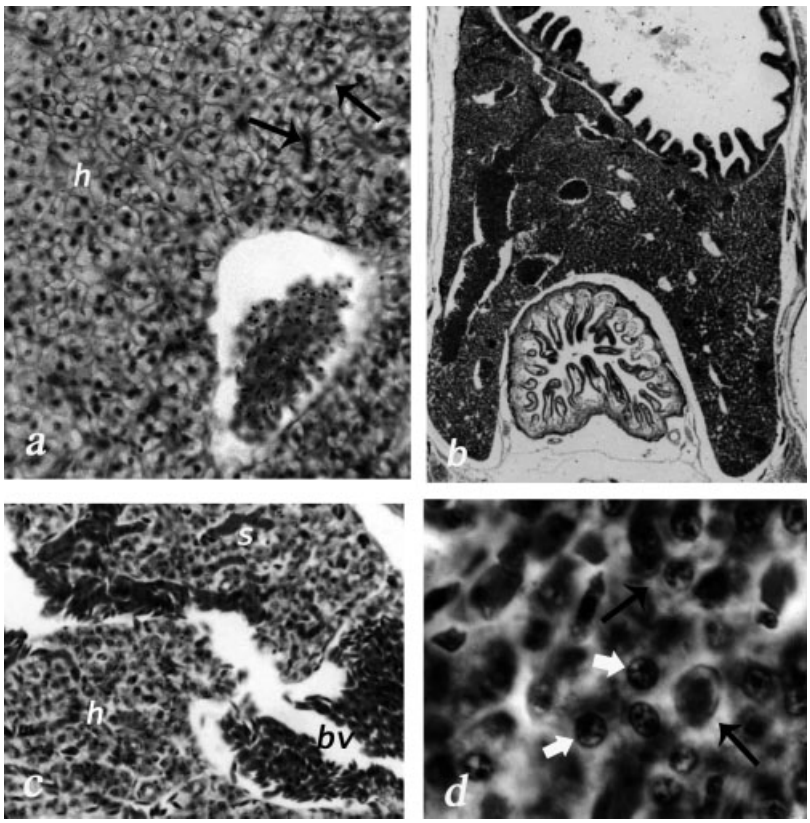

Figure 4. *Liza saliens* liver. (a) Controls: parenchyma composed of polyhedric hepatocytes with central nucleus, and sinusoids (arrows) among hepatocytes. (b) 10^{-9} M TBT-treated individuals: blood vessels; (b, c) the big ones are destroyed; (d) erythrocytes anomalous in shape and with pycnotic nuclei (arrows) and chromatin of hepatocytes fragmented (arrowheads). *e* = erythrocytes, *h* = hepatocytes, *s* = sinusoid; *bv* = blood vessel. Magnification: a = ×288; b = ×46; c = ×320; d, = ×1220.

in some cases, fused with fragmentated chromatin and the glomerulus appeared dilated and distorted [Fig. 5(d)].

Thymus

In the control groups, the thymus was present in the dorso-lateral region of the gill chamber, composed by an outer cortex packed with thymocytes and less densely populated inner medulla [Fig. 6(a)]. In the individuals incubated in 10^{-7} M solution, the thymus was present, but with broken capsule, some altered cells with fragmented chromatin. In those treated with 10^{-9} M solution, the histological sections showed completely atrophied thymus; only the stroma deprived of thymocytes was present [Fig. 6(b)].

Pancreas

The pancreas of control groups comprised exocrine and endocrine components. In the latter few Langerhans islets, small organs with endocrine function containing extended capillary networks were present. The exocrine pancreas consisted of acinar basophilic cells with zymogen granules [Fig. 7(a)] responsible for digestion of proteins, fats, carbohydrates and nucleotides. All these components were normal. In individuals incubated in 10^{-7} M TBTCl, the exocrine pancreatic cells showed few zymogen granules while the islets presented degenerative alterations. In 10^{-9} M solution the pancreatic cells were smaller than in controls and their outlines not evident; the zymogen granules were absent in the exocrine cells [Fig. 7(b)]. The Langerhans islets were destroyed.

Spleen

The spleen in control groups appeared to have a typical morphology: the red pulp, specialized in the erythropoiesis, with macrophages and lymphocytes, and the white pulp with leucopoiesis function [Fig. 8(a, b)]. In individuals incubated in 10^{-9} M TBTCl, parts of the spleen were affected: the cells were contracted and absent in some areas; the cells appeared with anomalous shape and structure [Fig. 8(c)]. Large dimension melano-macrophage aggregates were also seen [Fig. 8(d)].

Muscles

In the control groups, the muscles were joined by connective tissue with well evident fibres [Fig. 9(a)]. In treated individuals (10^{-9} M TBTCl), the muscle fibres were spaced, destroyed and with a different coloring from controls [Fig. 9(b)].

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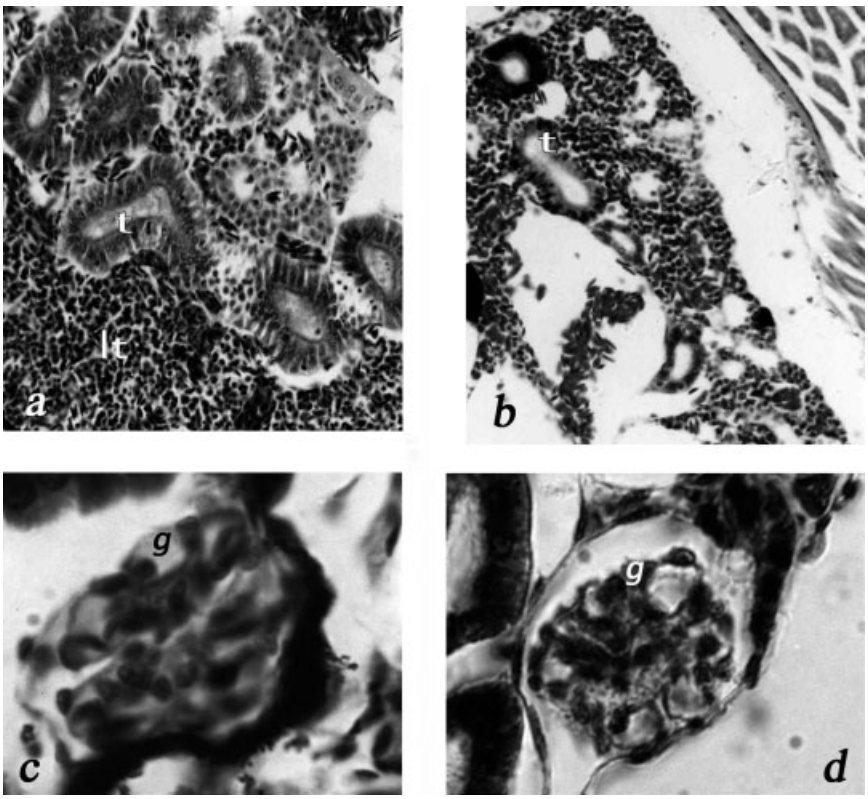

Figure 5. *Liza saliens* kidney. (a) Controls: an anterior one or head kidney, with hematopoietic–lymphoid tissue and a posterior one with tubules. (b) 10^{-9} M TBT-treated individuals: head kidney with altered structure; the hematopoietic-lymphoid tissue is almost destroyed. (c) Control: glomerulus. (d) Glomerulus appears more compact in treated individuals. *t* = tubule; *lt* = lymphoid tissue; *g* = glomerulus. Magnification: a, b = ×288; c, d = ×1150.

Intestine

In control groups, the intestine and the pyloric caeca had numerous villi. In individuals exposed to 10^{-9} M TBTCl, intestinal cells had outlines which were not evident and sometimes fused. The villi were dilated with mucosal epithelium degeneration. Some intestinal villi were presented pluristratified layers. Also those of pyloric caeca were flattened and swollen (data not shown).

Gonad

The individuals were sexually immature. The gonads of controls were restricted in the anterior part of fish and were rich with well-stained germinal cells [Fig. 10(a)]. In the gonad of treated individuals (10^{-9} M TBTCl), many germinal cells were destroyed and the connective stroma was prevalent; other ones were less stained than controls and coerced [Fig. 10(b)].

Cartilage

The cartilage of controls had a matrix where some chondrocytes were present in nests [Fig. 11(a)]. In individuals exposed in 10^{-9} M TBTCl, the chondrocytes had a swollen cytoplasm and fragmented chromatin [Fig. 11(b)]. The matrix appeared dilated and unstained. Some chondrocytes were destroyed.

Erythrocytes

These cells, in the organs of treated fish, appeared to have an altered shape. The nuclei were condensed or had fragmented chromatin, they were less stained than controls and in many cases they gave rise to agglomerates.

DISCUSSION

The experiments above on *Liza saliens* juveniles showed that TBT chloride, even at low concentrations, altered several organs including eye, gills, muscles, liver, intestine, pancreas and heart. Moreover, it originated thymus atrophy, spleen reduction and kidney alteration. Cartilage was also altered. Most of these alterations have been reported in the literature on different fish species.[22–25,28–30]

The significance of the toxicant-induced changes in intestine included malabsorption, secondary parasitic infections and protein-losing enteropathies. Liver toxicity resulted in altered fat metabolism and digestion. Structural lesions of gills could affect respiration, osmoregulation, acid–base balance

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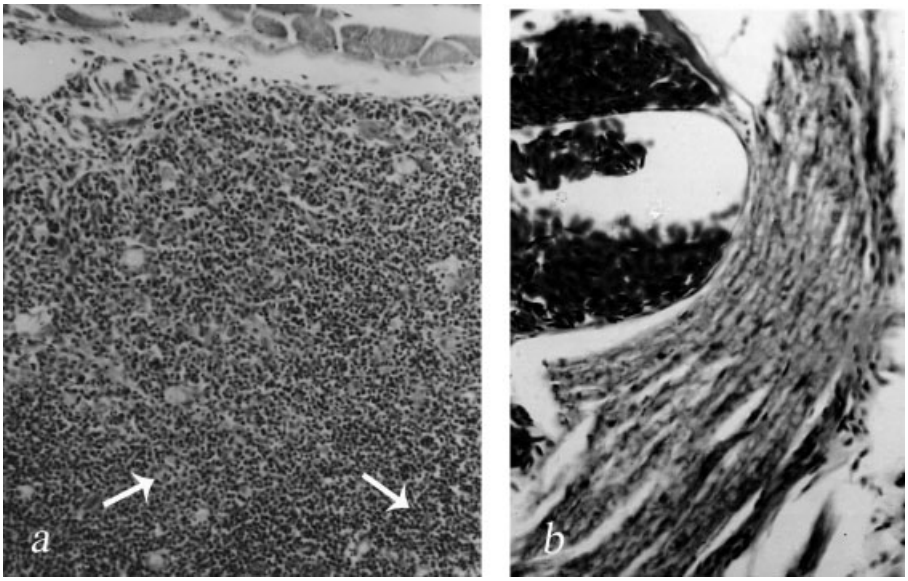

Figure 6. *Liza saliens* thymus. Controls: (a) outer cortex packed with thymocytes (arrows). (b) 10^{-9} M TBT-treated individuals: thymus completely atrophied; the stroma is deprived of cells. Magnification: a, b = ×140.

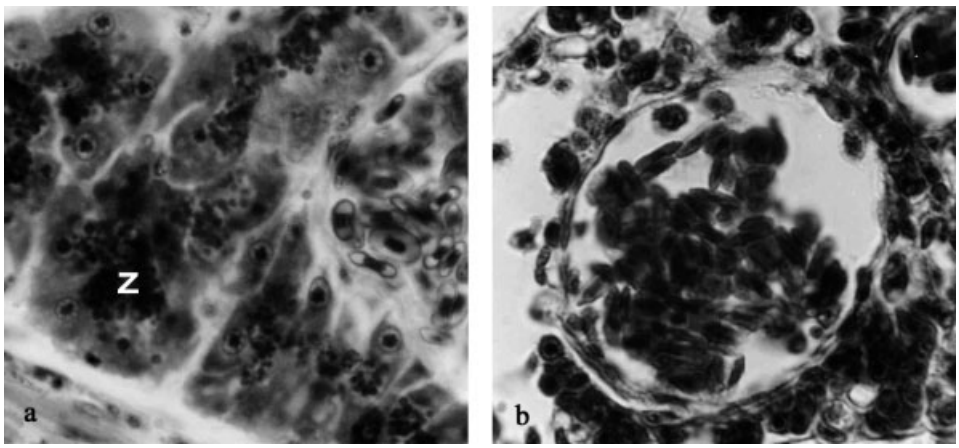

Figure 7. *Liza saliens* pancreas. (a) Controls: exocrine pancreas consists of acinar basophilic cells with zymogen granules. (b) 10^{-9} M TBT-treated individuals: pancreatic cells smaller than in controls. Zymogen granules absent, a blood vessel destroyed with erythrocytes flattened. *z* = zymogen granules. Magnification: a, b = ×1010.

and excretion of nitrogenous waste. The alteration of muscle cells impaired the fish movement. The damage to the eye could also impair behavioral parameters linked to feeding, defense and reproduction mechanism.

Vacuoles were observed in the optic nerve, with the choroid body destroyed and the retina layers altered. Lesions in the optic nerve have been observed in trout after TBT treatment.[31] Histological changes by TBT have been described by Fent and Meier[22] in *Phoxinus phoxinus* eye. ^{113}Sn-TBT has been found in some areas of the nervous system of fish by Rouleau *et al*.[32]

Besides direct mortality, TBT could also cause sublethal adverse effects. In *L. saliens* pancreas, the exocrine activity may be suppressed or reduced because very few granules of enzyme activity are present in the treated individuals, suggesting a reduced exocrine pancreas activity, although biochemical verification of this is required. It is well known that organotins influence several enzymes systems:[33,34] triphenyltin(IV)chloride, TPTCl, was found to reduce the activity of zymogens (trypsinogen and chymotrypsinogen) in the *Pagrus major* liver[35] and TBT and TPT affected the microsomal monooxygenase system in the *Mullus barbatus* liver.[36] Langerhans islets are altered or destroyed by TBT treatment, which probably affects their endocrine function. In addition, in the treated individuals, the spleen seemed of reduced size; the thymus was atrophied and the head kidney appeared with altered structure. The organs possess immune

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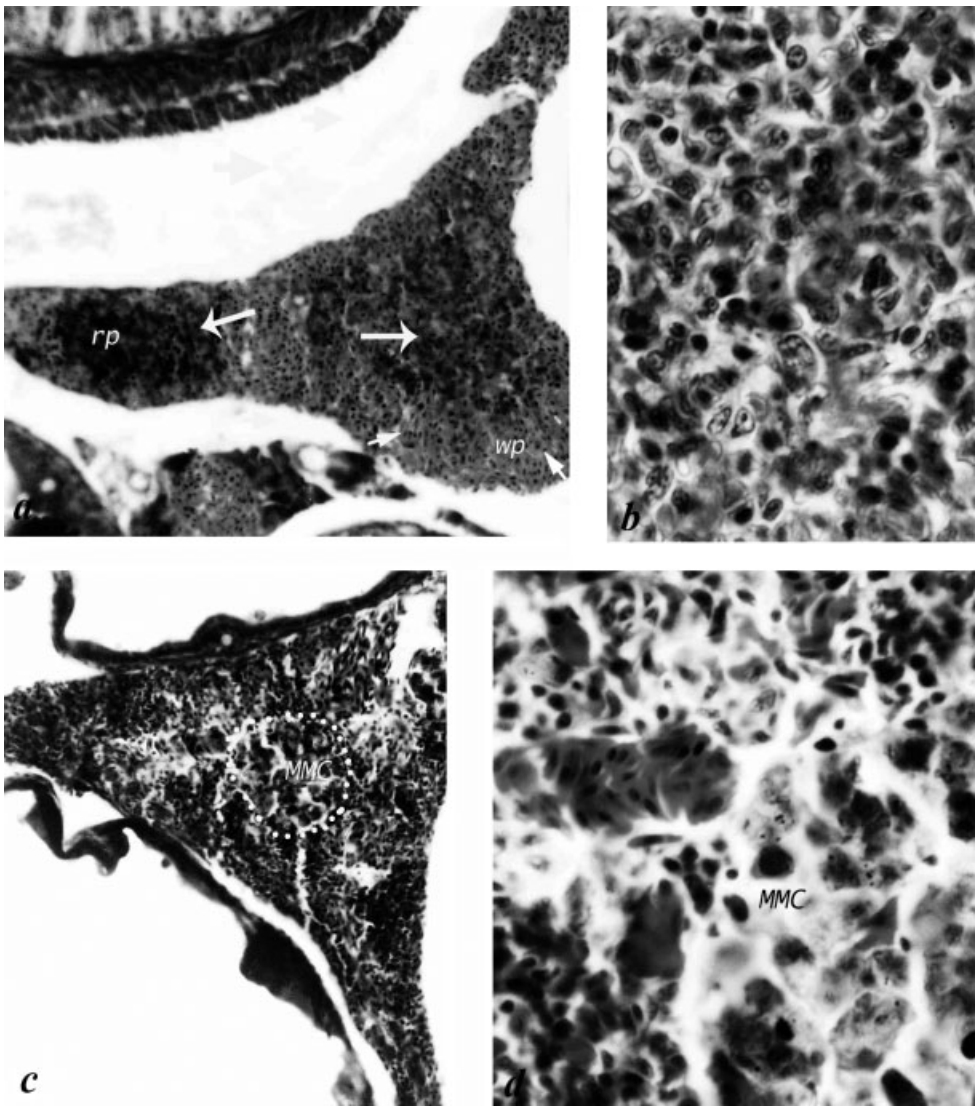

Figure 8. *Liza saliens* spleen. (a, b) Controls: red pulp with erythrocytes (arrows) and lymphocytes (arrowheads) and white pulp. No macrophage aggregates present. (c, d) 10^{-9} M TBT-treated individuals: parts of spleen are not present; the cells are contracted. A melano-macrophages aggregate of large dimension is recognizable (dotted line). Red pulp = rp; white pulp = wp; MMC = melano-macrophage. Magnification: a, c = ×290; b, d = ×1150.

and hematopoietic functions, and are essential for fish health. Grinwis *et al*.[37] noted a significant reduction of thymus induced by TBTO in *Platichthys flesus*. Thymus atrophy by TBTO has been demonstrated in *Poecilia reticulata* and *Oritias latipes*.[28,29] A variety of environmental contaminants, such as the organotins, exert immunotoxic damage on mammals species;[38,39] the organotin(IV) effects included thymus and spleen atrophy, suppression of T-cell dependent immunity and suppression of tumoricidal activity.[40,41] As regards molecular mechanisms involved in the thymus atrophy due to organotin compounds exposure, several studies have underlined the pivotal role played by apoptosis.[42] Another aspect to consider is the presence of melano-macrophage centers (MMC) in liver and spleen of treated juvenile *L. saliens* fish. They are not present in these control organs. Alterations in MMC have been seen after exposure of fish to individual toxicants.[43–45] In these aggregates the macrophages contain pigments, such as hemosiderin, lipofuscin and melanin[46] Although the roles are poorly understood, their innate immune function can be stated broadly as the sequestering of exogenous and endogenous substances for storage, destruction or detoxification. The number of MMC in the kidney and spleen was increased in dab exposed to high concentration of sewage sludge[47] or in Atlantic cod exposed to crude oil[48] or in fish from other sites.[49] Although the function of these components is unclear, they were absent in controls and it is possible to suppose that their presence could be a toxicant response in fish juveniles exposed to

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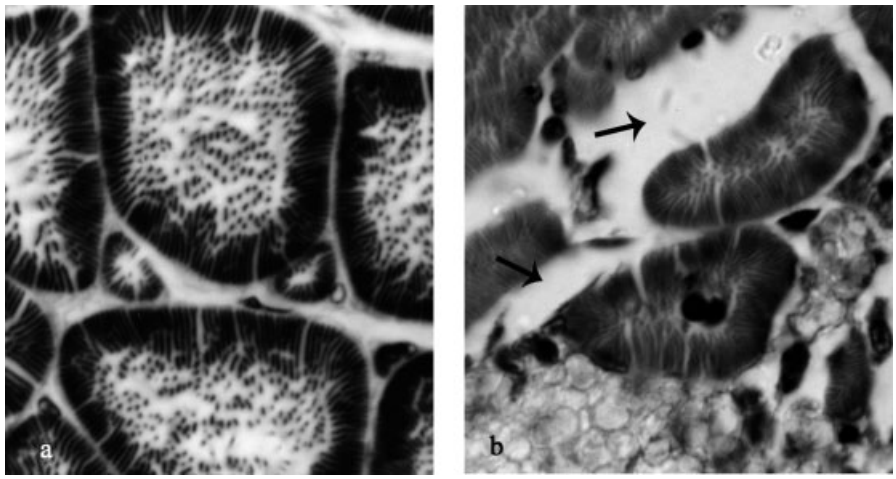

Figure 9. *Liza saliens* muscles. (a) Controls: muscles joined by connective tissue and fibres are well evident. (b) 10^{-9} M TBT-treated individuals: muscles fibres spaced (arrows), partly destroyed and showing different staining from controls. Magnification: a, b = ×1150.

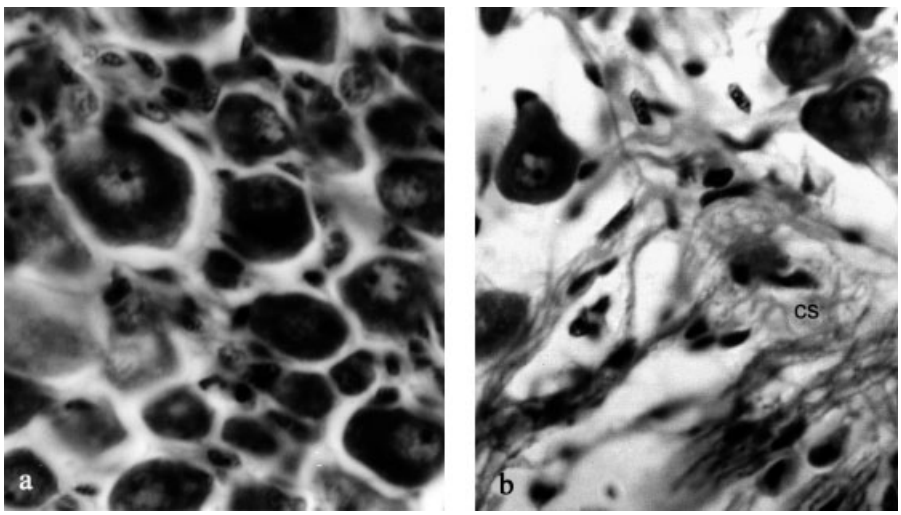

Figure 10. *Liza saliens* gonad. (a) Controls rich of well-stained goni. (b) 10^{-9} M TBT treated individuals: many germinal cells destroyed and less stained than in controls. connective stroma = *cs*. Magnification: a, b = ×1150.

TBTC, indicating, in this case, cellular modification due to the presence of toxicant, preceding a toxic effect at critical targets. In this respect MMC is a potentially useful biomarker in environmental degradation of natural sites.

In our experiments, erythrocytes appeared with picnotic nuclei having altered shape. In the last few years, Falcioni and Zolese[50] investigated the effect of different organotins on trout nucleated erythrocytes, indicating a plasma membrane perturbation when the process was followed in the presence of TBTCl and TPTCl. A marked genotoxic effect was demonstrated after TBTCl treatment on rainbow trout erythrocytes.[51] Gabrielska *et al*.[52] suggested that the lipophilicity and polarity of organotin compounds, and the surface potential and environment of the lipid molecules, are important factors in the interaction between these compounds and model membranes. Thus, they reduce the erythrocyte plasma membrane mechanical strength and increase the extent of hemolysis under osmotic stress conditions.[53]

As far as the reproductive system is concerned, the *Liza saliens* juveniles were sensitive to perturbation of reproductive cells and this could play a significant role in population decline. Another significant problem of TBT is its effect as endocrine disrupter chemical (EDC): it is known that many environmental contaminants are noted as endocrine disruptors. Some of them are concentrated in glandular tissue where they cause cell death; in this case, the tissue responsible for hormone production was destroyed or significantly reduced. The most frequently reported effects of EDCs on reproductive process were on sex determination, secondary sexual characters, oogenesis, spermatogenesis and the onset

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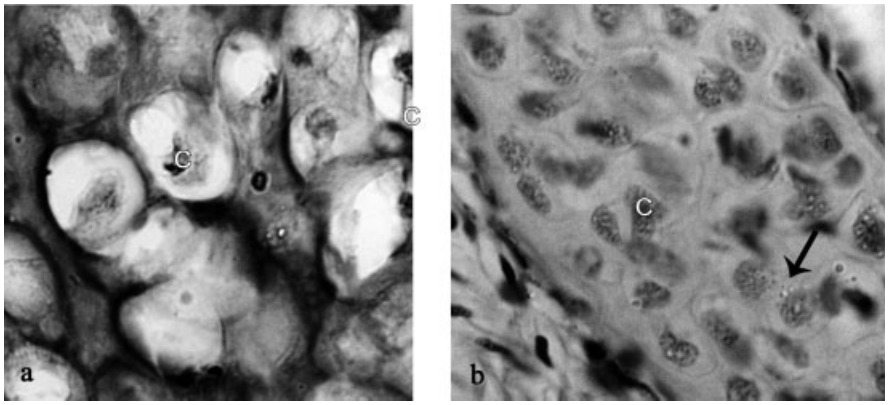

Figure 11. *Liza saliens* cartilage. (a) Controls: in matrix some chondrocytes present in isogenic groups. (b) 10^{-9} M TBT treated individuals: chondrocytes show a swollen cytoplasm, fragmented chromatin, and cytoplasmic blebs (arrow). Matrix appears dilated and less stained; some chondrocytes are destroyed. *c* = chondrocytes. Magnification: a, b = ×1150.

of sexual maturation.[54] The exposure to these compounds during the juvenile life is responsible for genital anomalies, infertility or sexual inversion.[55–57]

The histological observations showed that kidney, pancreas and intestinal cells had irregular cellular outlines. A modification of membrane lipid composition was demonstrated by Puccia *et al*.[58] after treatment of *Ciona intestinalis* ovary. It is possible that the first toxic effect of organotins, in particular of TBT, could be at membrane level from where a series of altered events could be triggered. The cell shrinkage observed in many tissues is an important effect of TBT as it inhibits, probably indirectly, many cellular pumps (ATPases), causing water loss from the cell compartments. For example, in various cell types, TBT-induced inhibition of Ca^{2+}-ATPase has been described and paralleled with an increase in the membrane Ca^{2+} permeability of intracellular organelles, resulting in a loss of Ca^{2+} storage capacity.[59] Thus, this shrinkage may be closely related to an alteration in the Ca^{2+}-transport system associated with the cisternae of smooth endoplasmic reticulum.[60]

The cell chromatin of some of the *Liza* organs appeared fragmentated: many xenobiotics, such as organotins, induce this process which could be the first step in apoptosis in different species.[60–63] Recently, Pellerito *et al*.[64] demonstrated a programmed cell death in *Paracentrotus lividus* 2-cell embryos treated with several organotin(IV)chlorine$_6$ derivatives. It is probable that the phenomenon could also occur in some *L. saliens* cells. Tissue sections could provide mediocre visualization of apoptotic cells, but this could be improved upon by use of specific reactions.

The results of the present study confirm susceptibility of *L. saliens* juveniles to TBTCl and introduce this species as a bioindicator of potential toxicants on fish. The findings may indicate that the pollutant acted as stressor, affecting the overall performance of the fish. In addition, it might be a compound contributing to the reduction of natural populations of *L. saliens*, by altering the reproductive system.

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REFERENCES

1. Chau YK, Maguire RJ, Brown M, Fang F, Batchelor SP. *Water Qual. Res. J. Canada* 1997; **32**: 453.
2. Morcillo Y, Borghi V, Porte C. *Arch. Environ. Contam. Toxicol.* 1997; **32**: 198.
3. Shawky S, Emons H. *Chemosphere* 1998; **36**: 523.
4. Schlenk D, Sapozhnikova Y, Baquirian JP, Mason A. *Environ. Toxicol. Chem.* 2002; **21**: 2138.
5. Weis JS, Weis P, Wang F. *OCEAN'S 87 Conf. Proc. Organotin Symp.* 1987; **4**: 1456.
6. Bryan GW, Gibbs PE, Hummerstone LG, Burt GR. *J. Mar. Biol. Assoc. UK* 1986; **66**: 611.
7. Mansueto C, Lo Valvo M, Pellerito L, Girasolo MA. *Appl. Organomet. Chem.* 1993; **7**: 95.
8. Cima F, Ballarin L, Bressa G, Burighel P. *Ecotoxicol. Environ. Safety* 1998; **40**: 160.
9. Mansueto C, Villa L, D'Agati P, Marcianò V, Pellerito C, Fiore T, Scopelliti M, Nagy L, Pellerito L. *Appl. Organomet. Chem.* 2003; **17**: 553.
10. Patricolo E, Mansueto C, D'Agati P, Pellerito L. *Appl. Organometal. Chem.* 2001; **15**: 916.
11. Fisher WS, Oliver LM, Walker WW, Manning CS, Lytle TF. *Mar. Environ. Res.* 1999; **47**: 185.
12. Cooper EL, Arizza V, Cammarata M, Pellerito L, Parrinello N. *Comp. Biochem. Physiol.* 1995; **112C**: 285.
13. Gibbs PE, Bryan GW. *Tributyltin: Case Study of an Environmental Contaminant*, de Mora SJ (ed.), Cambridge University Press: Cambridge, 1996; 212.
14. Martin RC, Dixon DG, Maguire RJ, Hodson PV, Tkacz RJ. *Aquat. Toxicol.* 1989; **5**: 37.
15. Schwaiger J, Bucher F, Ferling H, Kalbfus W, Negele R. *Aquat. Toxicol.* 1992; **23**: 31.
16. Ellis DV. *Mar. Pollut. Bull.* 1991; **22**: 8.

17. Short JW, Thrower FP. *Ocean'86 Conference Record*, 4 IEEE Service Center, 1987; 193.
18. Kannan K, Falandysz J. *Mar. Pollut. Bull.* 1997; **34**: 203.
19. Guruge KS, Iwata H, Tanaka H, Tanabe S. *Mar. Environ. Res.* 1996; **44**: 191.
20. Iwata H, Tanabe S, Miyazaki N, Tatsukawa R. *Mar. Pollut. Bull.* 1994; **28**: 607.
21. Focardi S, Corsolini S, Aurigi S, Peccetti G, Sanchez-Hernandez JC. *Appl. Organomet. Chem.* 2000; **14**: 48.
22. Fent K, Meier W. *Arch. Environ. Contam. Toxicol.* 1992; **22**: 428.
23. Visoottiviseth P, Thamamaruitkun T, Sahaphong S, Riengrojpitak S, Kruatrachue M. *Appl. Organomet. Chem.* 1999; **13**: 749.
24. Wang DY, Huang BQ. *Zool. Stud.* 1999; **38**: 189.
25. Pacheco M, Santos MA. *Ecotox. Environ. Safety* 2002; **53**: 332.
26. Pannier F, Astruc A, Astruc M, Morabito R. *Appl. Organomet. Chem.* 1996; **10**: 471.
27. Takashi H. *An Atlas of Fish Histology: Normal and Pathological Features*. Stuttgart Publications: New York, 1982.
28. Wester PW, Canton JH. *Aquat. Toxicol.* 1987; **10**: 143.
29. Wester PW, Canton JH, Van Iersel AAJ, Krajnc EI, Vaessen HAMG. *Aquat. Toxicol.* 1990; **16**: 53.
30. Mauceri A, Tigano C, Ferrito V, Barbaro B, Calderaro M, Ainis L, Fasulo S. *Ital. J. Zool.* 2002; **69**: 195.
31. Triebskorn R, Köhler HR, Körtje KH, Negele RD, Rahmann H, Braunbeck T. *Aquat. Toxicol.* 1994; **30**: 199.
32. Rouleau C, Xiong ZH, Pacepavicius G, Huang GL. *Environ. Sci. Technol.* 2003; **37**: 3298.
33. Laughlin RB, Linden O. *Ambio* 1987; **16**: 252.
34. Tanguy A, Castro NF, Marhic A, Morana D. *Mar. Pollut. Bull.* 1999; **38**: 550.
35. Kuroshima R, Kakuno A, Koyama J. *Nippon Suisan Gakk.* 1997; **63**: 85.
36. Morcillo Y, Porte C. *Aquat. Toxicol.* 1997; **38**: 35.
37. Grinwis GCM, Boonstra A, van den Brandhof EJ, Dormans JAMA, Engelsma M, Kuiper RV, van Loveren H, Wester PW, Vaal MA, Vethaak AD, Vos JG. *Aquat. Toxicol.* 1998; **42**: 15.
38. Malins DC, McCain BB, Landhal JT, Myers MS, Krahn MM, Brown DW, Chan SL, Roubal WT. *Aquat. Toxicol.* 1988; **11**: 43.
39. Vogelbein WK, Fournie JW, Van Veld PA, Huggett RJ. *Cancer Res.* 1990; **50**: 5978.
40. Vos JG, de Klerk A, Krajnc EI, Kruizinga W, Van Ommen W, Rozing J. *Toxicol. Appl. Pharmac.* 1984; **75**: 387.
41. Snoeij NJ, Bol-Schoenmakers M, Penninks AH, Seinen W. *Int. J. Immunopharmacology* 1988; **10**: 29.
42. Dacasto M, Cornaglia E, Nebbia C, Bollo E. *Toxicology* 2001; **169**: 227.
43. van der Weiden MEJ, Bleumick R, Seinen W, van den Berg M. *Aquat. Toxicol.* 1994; **29**: 147.
44. Kranz H, Gercken J. *J. Fish Biol.* 1987; **31A**: 75.
45. Rabitto IS, Alves Costa JRM, Silva de Assis HC, Pelletier E, Akaishi FM, Anjos A, Randi MAF, Oliveira Ribeiro CA. *Ecotox. Environ. Safety* 2005; **60**: 147.
46. Wolke RE. *A. Rev. Fish Dis.* 1992; **2**: 91.
47. Secombes CJ, Fletcher TC, O'Flynn JA, Costello MJ, Stagg R, Houlihan DF. *Comp. Biochem. Physiol.* 1991; **100C**: 133.
48. Khan RA, Kiceniuk J. *Can. J. Zool.* 1984; **62**: 2038.
49. Couillard CM, Hodson PV. *Environ. Toxicol. Chem.* 1996; **15**: 1844.
50. Falcioni G, Zolese G. *Rec. Res. Dev. Comp. Biochem. Physiol.* 2000; **1**: 67.
51. Tiano L, Fedeli D, Moretti M, Falcioni G. *Appl. Organomet. Chem.* 2001; **15**: 575.
52. Gabrielska J, Sarapuk J, Przestalski S. *Z. Naturforsch C* 1997; **52**: 209.
53. Miszta A, Gabrielska J, Przestalski S, Lagner M. *Appl. Organometal. Chem.* 2005; **19**: 736.
54. Crisp TM, Clegg ED, Copper RL, Wood WP, Anderson DG, Baetcke KP, Hoffman JL, Morrow MS, Rodier DJ, Patel YM. *Environ. Health Perspect.* 1998; **106**: 11.
55. Kime DE. *Endocrine Disruption in Fish*. Kluwer Academic:, Dordrecht, 1999.
56. Jobling S, Coey S, Whitmore J, Kime DE, Van Look K, McAllister BG, Beresford N, Henshaw AC, Brighty G, Tyler CR, Sumpter JP. *Biol. Reprod.* 2002; **67**: 515.
57. Nakayama K, Oshima Y, Yamaguchi T, Tsuruda Y, Kang IJ, Kobayashi M, Imada N, Honjo T. *Chemosphere* 2004; **55**: 1331.
58. Puccia E, Messina CM, Cangialosi MV, D'Agati P, Mansueto C, Pellerito C, Nagy L, Mansueto V, Scopelliti M, Fiore T, Pellerito L. *Appl. Organomet. Chem.* 2005; **19**: 23.
59. Girard JP, Ferrua C, Pesando D. *Aquat. Toxicol.* 1997; **38**: 225.
60. Cima F, Ballarin L. *Appl. Organomet. Chem.* 1999; **13**: 697.
61. Grosvik BE, Goksoyr A. *Biomarkers* 1996; **1**: 45.
62. Marinovich M, Viviani B, Corsini E, Ghilardi F, Galli CL. *Exp. Cell Res.* 1996; **226**: 98.
63. Stridh H, Fava E, Single B, Nicotera P, Orrenius S, Leist M. *Chem. Res. Toxicol.* 1999; **12**: 874.
64. Pellerito C, D'Agati P, Fiore T, Mansueto C, Mansueto V, Stocco G, Nagy L, Pellerito L. *J. Inorg. Biochem.* 2005; **99**: 1294.

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