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In vitro effects of triclosan and methyl-triclosan on the marine gastropod *Haliotis tuberculata*

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ABSTRACT

Triclosan (2,4,4′-trichloro-2′-hydroxy-diphenyl ether; TCS) is an antibacterial agent incorporated in a wide variety of household and personal care products. Because of its partial elimination in sewage treatment plants, TCS is commonly detected in natural waters and sediments. Moreover, due to its high hydrophobicity, TCS accumulates in fatty tissues in various aquatic organisms. TCS can be converted into methyl-triclosan (2,4,4′-trichloro-2′-methoxydiphenyl ether; MTCS) after biological methylation. In this study, the acute cytotoxicity of TCS and MTCS in short-term *in vitro* experiments was assessed on cell cultures from the European abalone *Haliotis tuberculata*. The results showed that morphology and density of hemocyte are affected from a concentration of 8 μM TCS. Using the XTT reduction assay, TCS has been demonstrated to decrease hemocyte metabolism activity in a dose- and time-dependent exposure. The IC_{50} was evaluated at 6 μM for both hemocyte and gill cells after a 24 h-incubation with TCS. A significant cytotoxicity of MTCS was also observed from 4 μM in 24 h-old hemocyte culture. Our results reveal a toxic effect of TCS and MTCS on immune (hemocytes) and/or respiratory cells (gill cells) of the abalone, species living in coastal waters areas and exposed to anthropogenic pollution.

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1. Introduction

Triclosan (TCS; CAS registration number 3380-34-5), is a diphenyl ether referred to as 5-chloro-2-(2,4-dichlorophenoxy)phenol or 2,4,4′-trichloro-2′-hydroxy-diphenyl ether. TCS has been used in various consumer products since 1968 as an antiseptic, disinfectant, preservative in cosmetics and household cleaning products, and in textiles and kitchen utensils (see review, Bedoux et al., 2012). The antibacterial activity of TCS is due to the specific inhibition of fatty acid synthesis, required for building cell membranes (Schweizer, 2001). Among the different benefits of TCS, Chen et al. (2010) have demonstrated that TCS can be introduced in tooth-binding micelle formulations to inhibit biofilm formation and to treat preformed biofilm to the tooth surface. TCS is among the most commonly detected organic wastewater compounds for frequency and concentration (Fuchsman et al., 2010; Lyndall et al., 2010; Brausch and Rand, 2011). Although a part of TCS degrades during wastewater treatment (Onesios et al., 2009), TCS has been found in many environmental samples (Bedoux et al., 2012). TCS was detected in effluents (Sabaliunas et al., 2003; Gomez et al., 2007; Vieno et al., 2007; Kantiani et al., 2008; Peng et al., 2008; Fair et al., 2009; Kumar et al., 2010), and in marine sediments (Agüera et al., 2003; Fernandes et al., 2011). TCS presents moderate water solubility of 12 mg/L (Reiss et al., 2002) and high hydrophobicity. So, TCS is highly adsorbed to particulate materials (Orvos et al., 2002; Chu and Metcalfe, 2007) and bioaccumulates in fish tissue (Adolfsson-Erici et al., 2002; Orvos et al., 2002), and in the filamentous algae *Cladophora* spp. at 100–150 μg/kg fresh weight (Coogan et al., 2007). Recently, Gatidou et al. (2010) have detected TCS in 50% of the eighteen samples of the Mediterranean mussel *Mytilus galloprovincialis* which were collected from different sites along the Thermaikos Gulf and Lesvos Island (Greece) with levels ranging from <LOD to 2578 μg/kg (d.w.). Under these conditions, methyl-triclosan (MTCS or 2,4,4′-trichloro-2′-methoxydiphenyl ether; CAS registration number 4640-01-1) has been found as the main transformation product of TCS (Bedoux et al., 2012). MTCS is reported to be more persistent in the environment than TCS (Lindström et al., 2002; Balmer et al., 2004). Although MTCS is generally less prevalent in the environment than TCS, its mechanism of action is similar and can occur at measurable levels even when TCS is below the limit of detection (Lindström et al., 2002; DeLorenzo et al., 2008; Hinther et al., 2011). At common pH of surface waters (7 to 9),

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MTCS was not or very slowly degraded under natural sunlight when TCS degradation rate ranged from 0.3 to 18 day^{-1}. MTCS has also a higher potential to bioaccumulate, since it is more lipophilic (Lindström et al., 2002; Poiger et al., 2003; Balmer et al., 2004; Bester, 2005; DeLorenzo et al., 2008). *In vitro* and *in vivo* acute toxicity tests showed that both TCS and MTCS could also affect aquatic species such as algae (Wilson, 2003; Kuster et al., 2007; DeLorenzo and Fleming, 2008; DeLorenzo et al., 2008; Franz et al., 2008; Harada et al., 2008; An et al., 2009; Liu et al., 2009; Stevens et al., 2009), crustaceans and fishes (Orvos et al., 2002; Ishibashi et al., 2004; Tatarazako et al., 2004). Very few studies are available about TCS toxicity on mollusks, and especially gastropods, which are widespread along coastal areas and are particularly affected by anthropogenic pollution (Ward and Kach, 2009; Gatidou et al., 2010). *In vitro* and *in vivo* genotoxicity of TCS has been shown in the freshwater mussel *Dreissena polymorpha* for a concentration of 0.1 μM and 0.3 μM respectively (Binelli et al., 2009a,b). Cytotoxicity of TCS was also tested on marine bivalves. A concentration of 1 μM induced destabilization of hemocytes lysosomal membrane in the mussel *M. galloprovincialis* (Canesi et al., 2007). In the clam *Ruditapes philippinarum*, 0.001 μM TCS decreased total hemocyte count (THC) (Matozzo et al., 2011). Based on these studies, the ecotoxicity of TCS seems to depend on the exposure time and the species. To our knowledge, no study has been reported on MTCS ecotoxicity using marine organisms.

The European abalone *Haliotis tuberculata* is a marine benthic gastropod living in coastal areas along the eastern Atlantic to the west coast of Africa. As abalone feed on macro-algae, that can bioaccumulate TCS and MTCS (Coogan et al., 2007), it is a relevant model organism to study the toxicity of these chemicals. Furthermore, abalones have been previously shown to serve as sensitive indicator species for coastal pollutions (Gorski and Nugegoda, 2006; Zhu et al., 2011). Primary cultures derived from mollusk tissues have been successfully used for cytotoxicity tests (Domart-Coulon et al., 2000; Canesi et al., 2007; Binelli et al., 2009a; Matozzo et al., 2011). As shown in marine bivalves, short-term primary cultures from abalone target tissues would help in the assessment of marine pollutants. Since hemocytes play a key role in digestion, metabolite transport, shell repair (Mount et al., 2004) and immune system (Adema et al., 1991; Gopalakrishnan et al., 2009), they provide a suitable model to study *in vitro* toxicity of pollutants. Because gills are the first organ exposed to exogenous molecules, *in vitro* toxicity tests on gill cells could give information on the effect of toxicants on respiratory function.

The aim of this study was to assess the acute cytotoxicity of TCS and MTCS in short-term *in vitro* experiments in hemocyte and gill cell cultures of the abalone *H. tuberculata*. We used previously developed primary cultures of hemocytes to determine the effect of TCS and by-product MTCS on immunity cells (Auzoux-Bordenave et al., 2007). Explant primary cultures of gill cells were also established for *in vitro* toxicity assays of TCS on respiratory cells.

2. Materials and methods

2.1. Source and maintenance of animals

European adult abalones (*H. tuberculata*), 90 mm in shell length, were collected from the north coast of Brittany (Roscoff, France). Animals were maintained at the laboratory in an 80 L-tank supplied with seawater from the Atlantic Ocean (exchange rate 2% per hour). Natural conditions of water temperature, salinity and photoperiod were used. Abalones were fed once a week with the red algae *Palmaria palmata*. Two days before experiments, abalones were starved and seawater was UV treated to avoid contamination.

2.2. Primary cultures of hemocytes

Hemolymph was sampled with a syringe from the branchial cavity of the abalone. For each assay, 5 mL of hemolymph from a single animal were used. Hemolymph was filtered on a 40 μm-sieve and then diluted in 2 volumes of a sterile anticoagulant solution (383 mM NaCl, 115 mM glucose, 37 mM sodium dihydrogen citrate, 11 mM EDTA, 100 U/mL penicillin, and 100 μg/mL streptomycin, diluted in ultra-pure water, pH 7.5) and 1 volume of an antiseptic solution (200 U/mL penicillin, 200 μg/mL streptomycin, 250 μg/mL gentamicin, and 2 μg/mL amphotericin B, diluted in filtered sea water, pH 7.4). Cell density was evaluated using a Malassez grid and cells were plated in 96-well plates (100,000 cells/well). Two hours after seeding, as cells adhered to the bottom of the wells, solutions were replaced by 90 μL/well of modified Leibovitz L-15 medium (346 mM NaCl, 7 mM KCl, 5 mM $CaCl_2$, 4 mM $MgSO_4$, 19 mM $MgCl_2$, 4 mM L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, 200 μg/mL gentamicin, and 1 μg/mL amphotericin B) adjusted to 1100 mmol/L and pH 7.4. Plated cells were incubated at 19 ± 0.2 °C in a humidified incubator, in the dark, for 24 h before any *in vitro* experiments.

2.3. Gill explant cultures

Cells from abalone gills were obtained from 3-day-old explant primary culture. For each assay, gills from a single animal were dissected and washed in the antiseptic solution (see Section 2.2.) for two days. Gills were then minced into 2–3 mm^3 explants that were placed to adhere onto the bottom of plastic 6-well plates. After 5 min, explants were covered with modified Leibovitz L-15 medium and incubated for 3 days at 19 ± 0.2 °C in a humidified incubator, in the dark. Explants and gill cells were collected and filtered on a 40 μm mesh filter. The cell suspension was then resuspended in modified Leibovitz L-15 medium. After estimating cell density, 90 μL of gill cells were plated in 96-well plates (200,000 cells/well) and incubated at 19 ± 0.2 °C in a humidified incubator, in the dark for 24 h before any *in vitro* experiments.

2.4. Toxicants

TCS (Irgasan) and MTCS (target molecules) were purchased from Sigma-Aldrich. Considering their low solubility in water, TCS and MTCS were dissolved in DMSO. DMSO was diluted in modified Leibovitz L-15 medium at the concentrations used in the study and the mixture was incubated with hemocyte and gill cell cultures to test its potential proliferating or cytotoxic effect. For both cultures, DMSO showed no significant effect up to 2%. The solutions used in the study ranged between 0 and 0.3% of DMSO, thus, DMSO was considered as a suitable solvent for assaying TCS and MTCS toxicities in abalone cell cultures. Fresh 4 mM stock solutions in DMSO were prepared for each experiment and immediately used. Each solution was diluted in modified Leibovitz L-15 medium and 10 μL was added to experimental wells. Negative controls were performed with 10 μL of the modified Leibovitz L-15 medium. Cell cultures were incubated for 24 h at 19 ± 0.2 °C, in the dark.

2.5. Light microscopy

Plated cells were observed twice a day to control their behavior and morphology with and without target molecules. Cells were observed with an inverted phase contrast microscope (Telaval 3, Zeiss, Germany) and pictures were taken with an Olympus digital camera.

2.6. Cytotoxicity assay

Cell viability was evaluated using the XTT assay (Roche Laboratory, France), based on the reduction of a tetrazolium salt into yellow formazan salt by active mitochondria (Mosmann, 1983). As for the MTT assay, previously adapted to marine mollusk cells, the XTT assay

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provides a global evaluation of the number of viable cells (Domart-Coulon et al., 2000). For each experiment, 50 μL of XTT/PMS mixture (0.02:5) was added to each well containing 100 μL of cells with TCS or MTCS. Plates were incubated for 6 h for hemocytes and 18 h for gill cells, at 19 °C in the dark. The intensity of the coloration was measured by spectrophotometry at 490 nm with a 655 nm reference wavelength (Bio-Rad plate reader).

2.7. Data analyses

Data obtained *in vitro* were the means ± SD of 6 replicates for three abalones. Cell viability percentages were calculated as following: the difference between the mean of six optical density (OD) values measured for one molecule dilution with cells and without cells was first calculated to normalize data. Values were transformed into percentage considering that cells incubated without target molecules represent 100% of viability. Statistical analyses were performed using the Fisher's Least Significant Difference (LSD) test, with a significant level at $P \leq 0.05$.

3. Results

3.1. Abalone primary cell cultures

Circulating cells in abalone were used to be sampled by an incision into the foot (Auzoux-Bordenave et al., 2007). The hemolymph was directly sampled into the branchial cavity in order to reduce contaminants. For gill cells, the explant method was used instead of a dissociation protocol that could damage the cells. The natural migration of gill cells outside the explant is much less aggressive for cells and their functionality can be preserved. Both cell systems were heterogeneous, containing various cell types that were present in the tissue of origin. In two day-old hemocyte culture, cells

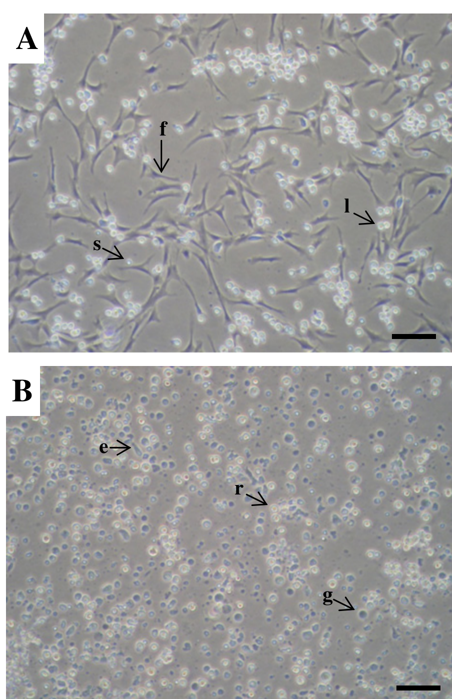

Fig. 1. Primary culture of abalone hemocytes and gill cells. (A) Two day-old culture of hemocytes. Cells were seeded at 4×10^6 cells/well in a 6-well plate and grown at 19 °C in a modified Leibovitz L-15 medium. Hemocytes are represented by small (s) and large (l) hyalinocytes. Adherent hemocytes display pseudopodia (f). (B) One day-old culture of gill cells seeded at 2×10^5 cells/well in a 6-well plate and cultured at 19 °C in modified Leibovitz L-15 medium. The culture is characterized by glandular (g) epithelial columnar (c) and round (r) cells. Scale-bar = 50 μm.

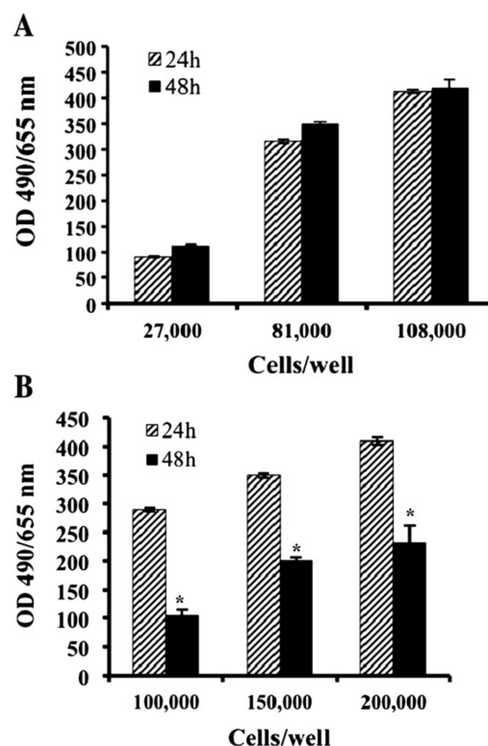

Fig. 2. XTT response according to cell density and time. (A) Hemocytes were plated at three densities: 27,000, 81,000 and 108,000 cells/well for 24 and 48 h. (B) Gill cells were plated at three densities: 100,000, 150,000 and 200,000 cells for 24 and 48 h. Data are the means ± SD of six values expressed as OD 490/655 nm × 1000. Asterisks indicate significant differences between 24 h and 48 h, $P < 0.05$.

presented two main morphologies as previously described (Travers et al., 2008). Using inverted phase contrast microscopy, we distinguished small hyalinocytes, 5–7 μm in diameter with a round shape, and large hyalinocytes, 8–12 μm in length (Fig. 1A). Adhering hyalinocytes displayed thin pseudopodia and formed cellular networks. In gill cell culture, the main cell type was amoeboid-like (3–11 μm), together with glandular cells and round cells (Fig. 1B). Only a few epithelial columnar cells (17 μm) were observed, that firmly adhered to the well bottom (Fig. 1B). For both hemocytes and gill cell primary cultures, cell density as well as incubation time have been previously adjusted using the XTT assay (Fig. 2). A density of 100,000 hemocytes cultured for 48 h (Fig. 2A) and a density of 200,000 gill cells cultured for 24 h (Fig. 2B) provided significant OD range for further cytotoxicity assays.

3.2. Effect of triclosan on hemocyte cultures

The effect of TCS on cell morphology and behavior was observed by phase contrast microscopy after 24 h of incubation with increasing TCS concentrations. In control hemocyte cultures, the two categories of cells previously described can be distinguished into small and large hyalinocytes with a majority of adhering hyalinocytes displaying pseudopodia (Fig. 3A). Hemocytes incubated with 6 μM of TCS were less numerous but no morphological difference was observed and numerous hemocytes were still bonded (Fig. 3B). However, 8 μM of TCS significantly affected cell morphology and density (Fig. 3C). All the cells were resuspended in the medium and no cell was adherent to the well bottom.

The short-term toxicity of TCS was tested in abalone hemocyte using the XTT reduction assay. Results showed that TCS decreased hemocyte XTT response in a dose- and time-dependent manner (Fig. 4). In 24 h and 48 h-old cultures, 80% to 90% of hemocytes were lost at a concentration of 8 μM of TCS. IC_{50} values were

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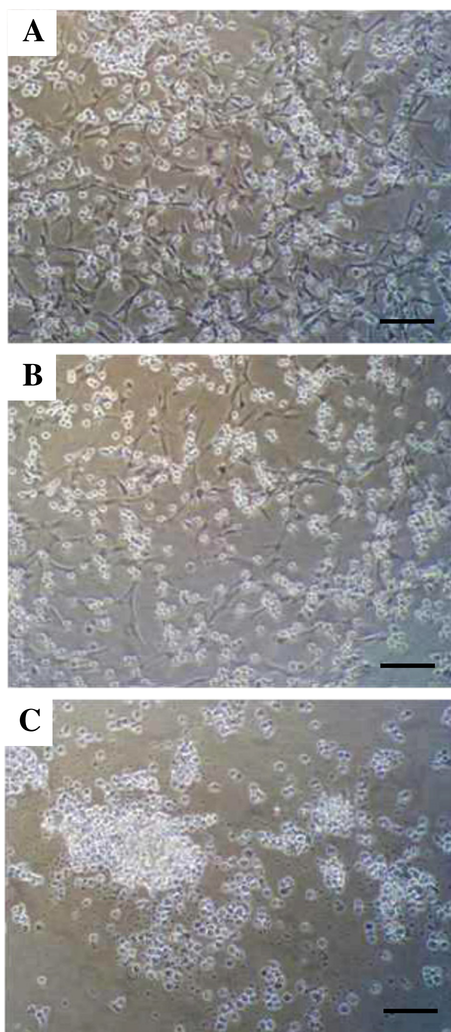

Fig. 3. Effect of TCS on abalone hemocytes.Hemocytes were cultured in 96-well plates and incubated for 24 h at 19 °C in modified Leibovitz L-15 medium without TCS (A), with 6 μM TCS (B) or with 8 μM TCS (C). Cells were observed with an inverted phase contrast microscope. Scale-bar = 50 μm.

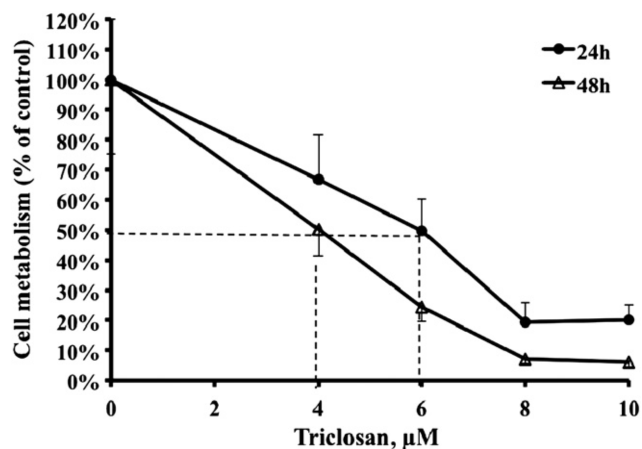

Fig. 4. Cytotoxic effect of TCS on abalone hemocytes.Hemocytes were cultured in 96-well plates and incubated at 19 °C in a modified Leibovitz L-15 medium. Hemocytes were incubated for 24 h or 48 h with increasing concentrations of TCS. Each point represents the mean ± SEM of three values and is expressed as a percent of control with no drug. Dashed line allowed the determination of IC_{50}.

estimated at 6 ± 1 μM and 4 ± 0.6 μM for 24 h and 48 h of incubation, respectively (Fig. 4).

3.3. Effect of triclosan on gill cell cultures

The light microscopy observations of gill cells showed a morphological effect of TCS in 24 h-old culture (Fig. 5). Many highly refringent cells were observed by phase contrast microscopy in cultures without TCS (Fig. 5A). This refringency indicated a good state of the cells. In culture incubated with 3 μM of TCS, very few cells were refringent revealing a negative effect of TCS at these concentrations (Fig. 5B). At a concentration of 8 μM TCS, only few cells were refringent (Fig. 5C). The 24 h toxicity of TCS on gill cells was evaluated with the XTT assay. XTT response dropped to 50% of cell activity from 4 μM of TCS (Fig. 6). IC_{50} was determined at 6 ± 2 μM of TCS after 24 h.

3.4. Effect of methyl-triclosan on abalone cell cultures

MTCS is one of TCS by-products (Bedoux et al., 2012). The cytotoxicity of this molecule was tested on hemocyte and gill cell models using XTT reduction assay (Fig. 7). Cytotoxicity of MTCS was observed on hemocytes from 4 μM in 24 h-old culture (Fig. 7A). In gill cell culture, no significant effect of MTCS was observed in the range of 1 to 4 μM as a preliminary result (Fig. 7B).

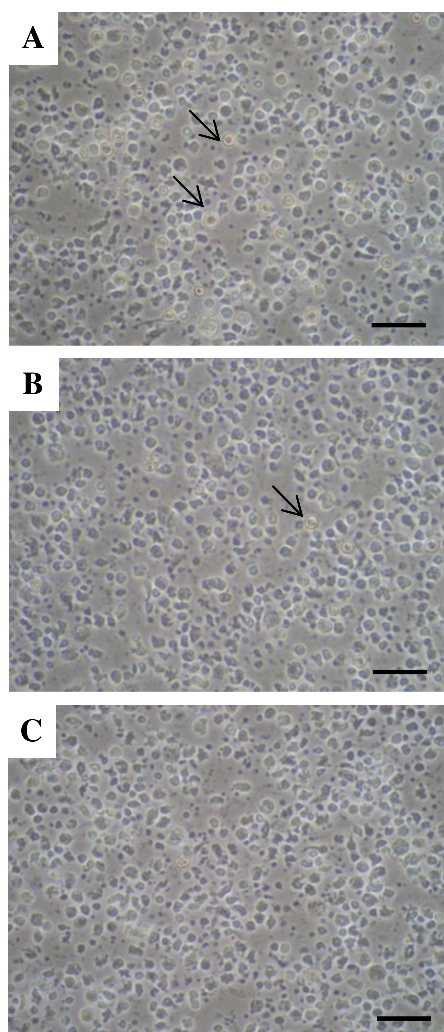

Fig. 5. Effect of TCS on abalone gill cells.Gill cells were cultured in 96-well plates and incubated for 24 h at 19 °C in modified Leibovitz L-15 medium without TCS (A), with 3 μM TCS (B) or with 8 μM TCS (C). Cells were observed with an inverted phase contrast microscope. Arrows indicate highly refringent viable cells. Scale-bar = 25 μm.

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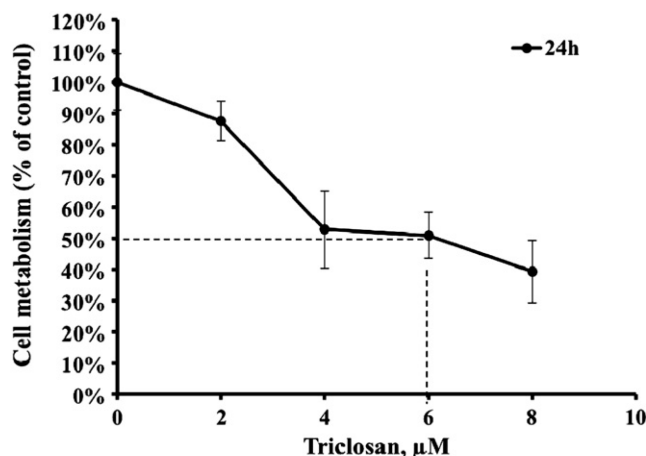

Fig. 6. Cytotoxic effect of TCS on abalone gill cells.Gill cells were subcultured in 96-well plates and incubated at 19 °C in modified Leibovitz L-15 medium. Cells were incubated for 24 h with various concentrations of TCS. Each point represents the mean ± SEM of three values and is expressed as a percent of control with no drug. Dashed line allowed the determination of IC_{50}.

4. Discussion

TCS is considered as a ubiquitous pollutant, widely detected in all types of environmental compartments from nM/L to several μM/L and is accumulated in biota and in humans (Bedoux et al., 2012). Toxicity and ecotoxicity studies show a variety of organisms sensitive to TCS exposure (Dann and Hontela, 2011). Human exposure to TCS has been reviewed by Rodricks et al. (2010). The effects can be evaluated by using representative organisms, bacteria, plants, fishes, birds, protozoa and mammals (Capdevielle et al., 2008; Chalew and Halden, 2009; Fuchsman et al., 2010; Lyndall et al., 2010; Brausch and Rand, 2011). Many studies reported the toxic effect of TCS on freshwater organisms, like amphibians, fishes and crustaceans (Dann and Hontela, 2011), considering that these species are the most exposed organisms to wastewater treatment plant effluents that drain into rivers and estuarine systems (DeLorenzo and Fleming, 2008; DeLorenzo et al., 2008). Concerning marine species, the TCS toxicity was only assessed in very few species. Bivalvia are the most studied organisms for the effect of pollutants because of their sensitivity to bioaccumulate organic compounds by filtering the water (Quinn et al., 2006; Canesi et al., 2007; Binelli et al., 2009a,b; Gatidou et al., 2010; Kookana et al., 2011).

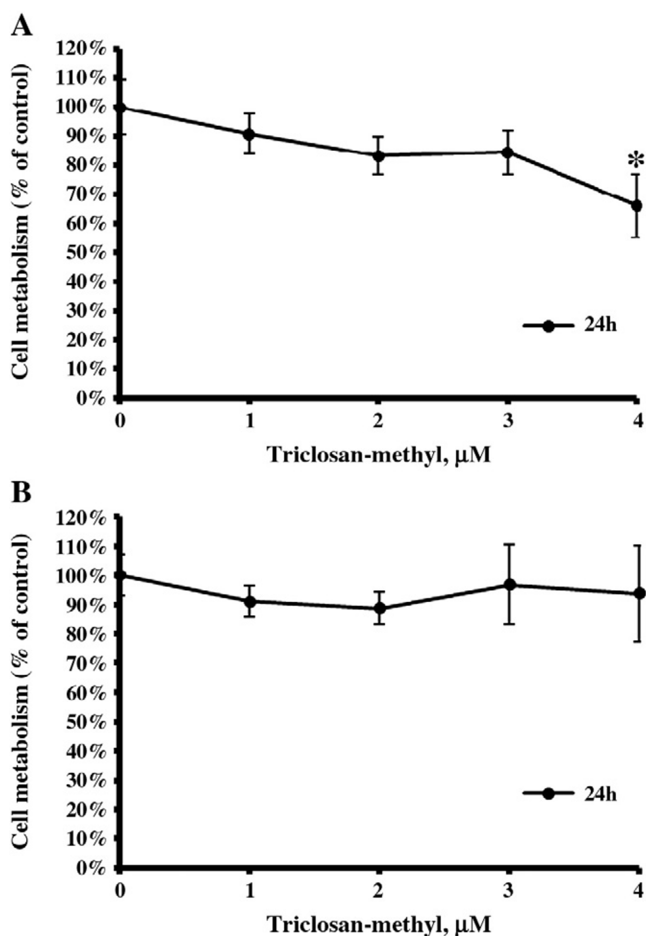

Fig. 7. Cytotoxic effect of MTCS on abalone hemocytes and gill cells.(A) Hemocytes were plated at 100,000 cells/well and incubated at 19 °C in modified Leibovitz L-15 medium with increasing concentrations of MTCS. The asterisk indicates a significant difference between 4 μM and 0 μM ($P<0.05$). (B) Gill cells were plated at 200,000 cells/well and incubated at 19 °C in modified Leibovitz L-15 medium with increasing concentrations of MTCS. Data are the means ± SD of six values expressed in percentage of control.

In this study, we described for the first time the effects of TCS at a cellular level on the European abalone *H. tuberculata*, a marine benthic gastropod that inhabits coastal waters areas exposed to treated wastewater disposal. Using primary cultures, we demonstrated that TCS has a dose-dependant cytotoxic effect on hemocytes and gill cells at concentrations ranging from 2 to 10 μM. Hemocytes are involved in many functions and are the main immune effector cells (Cheng, 1981). The production of antimicrobial peptides has been reported (Mitta et al., 2000). After 24 h of exposure to TCS, cell metabolic activity was reduced, with an IC_{50} estimated at 6 ± 1 μM for hemocytes and 6 ± 2 μM for gill cells. Furthermore, hemocytes morphology was also affected. Although hemocytes showed adherence to the bottom of the culture plates in control conditions, 8 μM TCS induced a total loss of adherence. Table 1 summarizes TCS toxicity in marine and freshwater species. The lowest concentration of TCS that was cytotoxic for abalone hemocytes (2 μM) is comparable to the concentration that affects lysosomal membrane stability in the marine mussel *M. galloprovincialis* hemocytes (1 μM) (Canesi et al., 2007). In the freshwater mussel *D. polymorpha*, 0.1 μM of TCS for 60 min was shown to induce hemocyte apoptosis (Binelli et al., 2009a). In the clam *R. philippinarum*, 0.001 μM TCS decreased total hemocyte count (THC) (Matozzo et al., 2011). The establishment of abalone gill cell cultures and the use of these cultures for a cytotoxic assay are described in this work for the first time. Gill cells are directly exposed to pollutants since gills are the respiratory organs. IC_{50} estimated for abalone gill cells exposed to TCS for 24 h (6 μM) was identical to that IC_{50} for hemocytes. To date, no data are available on effects of TCS on gill cells. Only one other tissue was investigated in *M. galloprovincialis*, the digestive gland, which represents a significant target of TCS (Canesi et al., 2007). TCS accumulation has been described for freshwater aquatic organisms mainly in lower trophic-level organisms such as algae (Capdevielle et al., 2008), bivalves (Gatidou et al., 2010), invertebrates (Coogan and La Point, 2008; DeLorenzo et al., 2008), fishes (Miyazaki et al., 1984; Adolfsson-Erici et al., 2002; Balmer et al., 2004) and marine mammals (Bennett et al., 2009; Fair et al., 2009). High levels of TCS (0.24–4.4 mg/kg) were reported in the bile of fish, one sample being as high as 120 mg/kg in rainbow trout (*Oncorhynchus mykiss*) exposed to downstream WWTP discharges (Adolfsson-Erici et al., 2002), and in blood plasma of fish in the Detroit River of North America (Valters et al., 2005). Lower amounts were determined in bluegill tissues (Mottaleb et al., 2009), in the bile of wild living fish (Adolfsson-Erici et al., 2002; Ramirez et al., 2009) and in plasma of wild Atlantic bottlenose dolphins (*Tursiops truncatus*) in U.S. estuaries (Fair et al., 2009), in which TCS was found to be between 0.025 and 0.27 μg/kg wet weight. Bioaccumulation factor (BAF) is often evaluated and corresponds to the ratio of the chemical concentration in the organism and the water (CAESAR website). The BAF of TCS has been determined for some animal species. BAF values ranged from 27 in earthworm tissues (Kinney et al., 2008), 44 in stage 66 *Xenopus laevis* larvae and 740 in *Bufo woodhousii*

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Table 1
Acute toxic effect of TCS on aquatic species.

Class	Organisms	TCS assay		Results		Ref.
		Measurement	Duration	Assay	Value (μM)	
Marine species						
Gastropoda	*Haliotis tuberculata*	Cytotoxicity on hemocytes and gill cells	24 h	IC_{50}	4.00	Present study
Bivalvia	*Ruditapes philippinarum*	Cytotoxicity on hemocytes	7 days	LOEC	0.001	Matozzo et al., 2011
		Cell free hemolymph/decrease of lactate dehydrogenase activity		LOEC	0.001	
		Apoptotic hemocytes/DNA fragmentation		LOEC	0.002	
	Mytilus galloprovincialis	Hemocytes/lysosomal destabilization	30 min	LOEC	1.00	Canesi et al., 2007
Estuarian/Amphidromous species						
Malacostraca	*Palaemonetes pugio*	Acute toxicity (larvae)	96 h	LC_{50}	0.53	DeLorenzo et al., 2008
		Acute toxicity (adults)		LC_{50}	1.05	
Chlorophyceae	*Dunaliella tertiolecta*	Static algal bioassay–biomass	96 h	EC_{50}	0.01	DeLorenzo and Fleming, 2008
Actinopterygii	*Oryzias latipes*	Fertilized eggs, Decrease of hatchability	14 days	LOEC	1.08	Ishibashi et al., 2004
		Acute toxicity (24-h-olf larvae)	96 h	LC_{50}	2.08	
Freshwater species						
Bivalvia	*Dreissena polymorpha*	Apoptosis *(in vivo)*	24 h	LOEC	0.001	Binelli et al., 2009b
		Apoptosis (*in vitro*)	60 min	LOEC	0.10	Binelli et al., 2009a
		Genotoxicity (*in vitro*)	60 min	LOEC	0.10	
Branchiopoda	*Ceriodaphnia dubia*	Growth	7 days	IC_{25}	0.76	Tatarazako et al., 2004
	Daphnia magna	Acute effects	48 h	EC_{50}	1.35	Orvos et al., 2002
Actinopterygii	*Danio rerio*	Fertilized eggs, hatching	9 days	IC_{25}	0.76	Tatarazako et al., 2004
	Pimephales promelas	Acute effects (adults)	96 h	LC_{50}	0.90	Orvos et al., 2002
	Lepomis macrochirus	Acute effects		LC_{50}	1.28	
Amphibia	*Xenopus laevis*	Acute effects (developmental model)	96 h	LC_{50}	1.18	Palenske et al., 2010
	Acris blanchardii			LC_{50}	1.27	
	Bufo woodhousii	Acute effects (larval species)		LC_{50}	0.52	
	Rana sphenocephala			LC_{50}	1.94	

(Palenske et al., 2010), up to around 500 for snails (*Helisoma trivolvis*) (Coogan and La Point, 2008). For algal species (*Cladophora* spp.), BAF values ranged from 900 to 1200 (Coogan et al., 2007). Bioaccumulation potential of TCS in abalone has to be investigated in future works because, overall, if macroalgae like *Cladophora* sp. (Coogan et al., 2007) can bioaccumulate TCS, it means that abalone, which feeds on macro-red-algae, can be exposed to TCS.

In activated sludge, MTCS was formed concomitantly with the removal of triclosan under aerobic conditions (Chen et al., 2011). In the present study, the cytotoxicity of MTCS was evidenced on abalone hemocytes. MTCS was also found in fish at concentrations ranging from 30 to 600 μg/kg (Balmer et al., 2004; Leiker et al., 2009). Concerning aquatic and terrestrial organisms, data show clearly that the risk should be more related to chronic effect (due to bioaccumulation) than acute impact. However the sensitivity of some species (namely microorganisms and aquatic plants) even at environmental concentrations shows that the ecosystems can be disturbed (Fuchsman et al., 2010; Lyndall et al., 2010; Brausch and Rand, 2011).

Cell cultures represent a valuable model to assess the toxicity of PCPs. However, *in vivo* experiments can give additional information on the effects of pollutants. TCS effect was assayed in *M. galloprovincialis* hemocytes both *in vitro* and *in vivo* (Canesi et al., 2007). In this latter work, different parameters were investigated like DNA damage, lysosomal alterations and apoptosis. The results showed that TCS induced significant damages to hemocytes at lower concentrations *in vivo* (1 nM) than *in vitro* (100 nM) (Canesi et al., 2007). Furthermore, it could be very interesting to study the effects of TCS and its derivates on larval development of aquatic species to investigate the real effect of these target molecules on aquatic populations. In a recent study, the effects of TCS and other persistent pollutants were shown on sea urchin embryos and larvae (Anselmo et al., 2011). For a concentration of >0.5 μM of TCS, no eggs were hatched and for the inferior concentrations, 100% of morphological abnormalities was observed in larvae. Nonylphenols and bisphenol A were recently demonstrated as disrupting compounds of the embryonic development of the abalone *Haliotis diversicolor* (Liu et al., 2011) showing that studies on the toxicity on larval development are essential to evaluate the damages caused by pollutants on aquatic populations.

5. Conclusion

This study shows for the first time, an acute *in vitro* toxicity of TCS on abalone. IC_{50} was evaluated at 6 ± 1 μM for hemocytes and 6 ± 2 μM for gill cell cultures. These values indicate a sensibility of this marine organism as reported for freshwater species. The effect of TCS on hemocytes, the immune cells of mollusks, shows a potential risk for abalones. MTCS, a degradation compound of TCS, has also been demonstrated as a cytotoxicant for hemocytes. To date, no studies have been reported on the ecotoxicity of MTCS and this work shows a potential effect of by-products. While these preliminary results were carried out with concentrations higher than those reported in aquatic environment, other experiments will be performed *in vivo*, particularly on embryos and larvae which are reported to be more sensitive to pollutants.

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