

Cytotoxic effects of *in vitro* exposure to triclosan on the marine gastropod *Haliotis tuberculata*

Béatrice GAUME^{1,2}, Nathalie BOURGOUGNON¹, Stéphanie AUZOUX-BORDENAVE^{2,3}, Benoit ROIG⁴, Barbara LE BOT⁴, Gilles BEDOUX¹

1 Laboratoire de Biotechnologie et Chimie Marines, EA3884, Université de Bretagne-Sud (Université Européenne de Bretagne), IUEM, Vannes, France

2 UMR BOREA (Biologie des Organismes et Ecosystèmes Aquatiques), MNHN/CNRS 7208/IRD 207/UPMC, Muséum national d'Histoire naturelle, Station de Biologie Marine de Concarneau, Concarneau, France

3 Université Pierre et Marie Curie Paris, 4 place Jussieu, 75 005 Paris, France

4 U1085 IRSET/ LERES EHESP, Advanced School of Public Health, Rennes, France

Corresponding author: Gilles Bedoux, gilles.bedoux@univ-ubs.fr

Keywords: *Triclosan*, *Methyl-triclosan*, *Antibacterial*, *Cytotoxicity*, *Cell culture*, *Marine gastropod*, *Haliotis tuberculata*

Human activity has been increasing exponentially the number and concentrations of molecules rejected in the environment. Some of these chemicals compounds are totally degraded in sewage treatment plants; others are partially eliminated and then found in rivers and ocean. Aquatic flora and fauna are the first organisms impacted by this anthropogenic pollution that has effects on the nervous and endocrine systems. 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, it is one of the most commonly detected organic wastewater compounds for frequency and concentration. Moreover, due to its high hydrophobicity, TCS accumulates in fatty tissues of aquatic organisms. Under these conditions, methyl-triclosan (2,4,4'-trichloro-2'-methoxydiphenyl ether, MTCS) is the main transformation product of TCS. Although MTCS is generally less prevalent in the environment than TCS, its mechanism of action is similar and can occur at measurable levels.

Very few studies are available about TCS toxicity on marine mollusks. The European abalone *Haliotis tuberculata* is a marine gastropod of economic interest which inhabits rocky shores in Brittany. Since abalone is particularly exposed to anthropogenic pollution, it is a relevant model for ecotoxicological studies. The aim of our study was to assess the acute cytotoxicity of TCS in short-term *in vitro* experiments. Primary cultures of hemocytes - circulating cells of abalone - and gill cells were incubated with TCS concentrations ranging from 0 to 10 μM for 24 to 48h. The cytotoxicity of TCS was evaluated through the cell viability using the XTT assay.

The results showed that morphology and density of hemocytes 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 on both immune (hemocytes) and respiratory cells (gill cells) of the abalone, a marine species exposed to anthropogenic pollution.