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Toxicity testing of simvastatin, sertraline, 4-MBC, propylparaben and triclocarban using zebrafish and sea urchin embryos bioassays

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INTRODUCTION:

In the past decade, many emergent compounds, including some active substances and ingredients of Pharmaceuticals and Personal Care Products (PPCPs) have been detected in water at levels that can negatively impact aquatic ecosystems (Lapworth et al., 2012; Jiang et al., 2013). The recent knowledge of their occurrence has raised concerns about human health effects and ecosystem risks. Although these compounds are frequently detected at concentrations that are not likely to induce adverse effects in humans and may be too low to cause acute effects in other organisms, there is still a serious lack of information about the effects in non-target species, particularly considering chronic exposure or effects resulting from interactions between them (Lapworth et al., 2012).

Pharmaceuticals and Personal Care Products enter the aquatic environment from different point and non-point sources and wastewater treatments plants cannot ensure complete removal of many compounds, and therefore they may be present at significant concentrations in effluents (Jiang et al., 2013). Hence, it is essential to understand the effects of these substances on aquatic organisms. Owing to the large number of new chemicals that must go through toxicity testing, short-term early-life-stages have been frequently used as an alternative to long-term exposures due to its high sensitivity and logistic advantages.

OBJECTIVES:

The main aim of the present work was to assess the toxicity of five emerging pollutants: simvastatin, sertraline, triclocarban, propylparaben and 4-methylbenzylidene camphor (4-MBC) during the embryonic development of zebrafish (*Daniorerio*) and the sea urchin (*Paracentrotuslividus*).

MATERIALS AND METHODS:

After fertilization, zebrafish and sea urchin eggs were randomly allocated to 24 wells plate. The experimental solutions were obtained by diluting the stock solutions of the five selected compounds in artificial seawater (sea urchin assays) or in freshwater (zebrafish assays). All solutions were prepared in order to have a final DMSO concentration of 0.01%. The 24-wells plates of zebrafish assay were incubated at 26.5ºC during 80h and under the same photoperiod conditions as the zebrafish stock. The medium was renewed daily in order to maintain oxygen and toxic nominal concentrations constant during the assay and to remove fungi or other organisms that could develop in the well. Sea urchin embryos were incubated at 20ºC in dark for 48h and were fixed by adding three drops of 37% formaldehyde at the end of exposure. Zebrafish embryos observations were performed at 8, 32 and 80 hours post fertilization and different parameters were recorded (Table 1), while sea urchin observations were only performed at the end of the assay and larvae length of fixed organisms was measured.

Table 1-Endpoints recorded at 8, 32 and 80hours post fertilization (hpf) in zebrafish assay

Endpoint	8hpf	32hpf	80hpf
Mortality rate	*	*	*
75% of epiboly stage	*		
Abnormal cell growth	*		
Head abnormalities		*	*
Tail abnormalities		*	*
Eyes abnormalities		*	*
Yolk-sac abnormalities		*	*
Pericardial edema		*	*
Heart rate		*	*
Hatching rate			*
Muscular involuntary contractions[(1)]			*

[(1)]Only for 4-MBC exposure

RESULTS AND DISCUSSION:

All selected compounds induced significant effects on the embryonic development of both test species after individual exposure (Table 2). These effects were compound and concentration dependent. However our results show that sea urchin embryos were more sensitive than zebrafish embryos. Regarding the relative toxicity, simvastatin showed the highest toxicity in zebrafish bioassay, while triclocarban was the most toxic compound in sea urchin bioassay. Furthermore, triclocarban and 4-MBC induced significant effects in sea urchin embryos at concentrations close to reported concentrations in surface water.

Table 2 - Overview of NOEC and LOEC values of selected PPCPs reported in this study. Maximal concentrations of selected PPCPs reported in the literature in surface water and in WWTPs influents and effluents. (µg/L)

	Zebrafish		Sea urchin		Maximal concentrations		
PPCP	NOEC	LOEC	NOEC	LOEC	WWTPs Influents	WWTPs Efluents	Surface water
SIMV	5.0	50.0	2.0	5.0	0.09	0.001	0.0001
SER	1000.0	10000.0	4.0	10.0	0.02	0.015	0.57
4-MBC	500.0	5000.0	0.8	2.0	6.5	2.7	*0.799
PP	1000.0	10000.0	160.0	400.0	2.8	0.021	0.207
TCC	100.0	1000.0	0.256	0.64	50.0	> 10.0	6.75

CONCLUSION:

This study highlights the risk of these compounds to aquatic ecosystems. Hence, it is important to conduct more comprehensive studies on possible chemical interactions in the environment and the mechanisms involved in order to perform more reliable risk assessment and to implement guidelines for the protection of the aquatic environment.

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