DOI: http://doi.org/10.21698/simi.2022.ab17

VIBRIO FISCHERI BIOLUMINESCENCE INHIBITION ASSAY FOR ACUTE TOXICITY PREDICTION OF PHARMACEUTICAL FROM WASTEWATERS AND SURFACE WATER: A REVIEW

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Keywords*: bioluminescence, metabolites, pharmaceuticals, Vibrio fischeri*

Introduction

The pollution caused by some pharmaceutically active compounds (PhACs) has been an emerging issue linked to a very well-documented risks such as bacterial resistance to PhACs or endocrine disruption in humans, terrestrial and aquatic organisms. Bacterial resistance could be acquired by increasing their genes resistance expression, genes mutation or by PhACs genes resistance lateral transfer. Pharmaceutical residues could enter into the environment as a waste products generated by their manufacture, usage and disposal. More and more scientific data pointed out the need of studying the PhACs effects on the aquatic environment. Newly ecotoxicological data provided essential information for native forms of pharmaceuticals and their transformation products (metabolites, degradation products and selected enantiomers) resulted from hydrolysis, photolysis and biodegradation processes. In spite of the fact that degradation products could be more toxic and more stable over time, they were not being sufficiently studied to identify and monitor their effects on the environment, especially on the aquatic systems.

Materials and methods

The luminescent bacteria test using *Vibrio fischeri* is a widely accepted as an ecotoxicological test on bacterial biological model and it has been standardized by ISO (International Organization for Standardization). It is predominantly used to detect and quantify the toxicity of wastewaters, aqueous eluates and leachates from landfills. The SR EN ISO 11348-3: 2009 method mainly used lyophilized bacteria and this has been extensively used in the Biotests - Biological Analysis Laboratory of INCD ECOIND. Briefly, the ecotoxicological effect of various pollutants has been quantified based on the natural luminescence inhibition, emitted by luminescent bacteria *Vibrio fischeri* cultures. The bacterial strain in a growth medium (6-8.5 pH, NaCl 20% to adjust the osmolality) was incubated at 15±1°C in presence of various pollutant concentrations. The more the pollutant had a negative effect on bacteria, the more the bioluminescence was inhibited. The bioluminescence was quantified by a BioFix Lumi''system and Microtox 500, dedicated for acute tests and the pollutant concentration-effect relationship was calculated using standard linear regression analysis. The test endpoints, IC50 and NOEC values were calculated, too.

Results and conclusions

In the Biotests - Biological Analysis Laboratory of INCD ECOIND, ecotoxicity studies have been routinely performed on pharmaceutical substances from the analgesic and anti-inflammatory group, such as diclofenac, ibuprofen, naproxen, ketoprofen, acetamonofen, indomethacin, carbamazepine, caffeine. Ecotoxicity tests were performed by using a wide range of biological models such as *Vibrio fischeri*, *Cyprinus carpio* and *Daphnia magna*. These studies performed on more biological models offered the advantage of more reliable and specific toxicity degree data for each pharmaceutical products present in surface waters (Mures, Arges, Danube) or wastewater treatment plant's influents and effluents. The luminiscent bacteria showed the highest sensitivity to the toxic effects of all PhACs, mentioned above, with the exception of caffeine. Diclofenac, ketoprofen, naproxen, ibuprofen and carbamazepine showed harmful effects for a concentration range between 16.21 and 54.21 mg/L. The acetaminophen and indomethacin induced a toxic bacterial effect at concentrations smaller than 10 mg/L.

Table 1. Acute toxicity data on aquatic biota: fish, crustaceans and bacteria

PhACs	Effect concentrations (mg/L) ± STDEV					
	Cyprinus carpio		*Daphnia magna*		*Vibrio fischeri* and other bacteria	
	LC_{50}	NOEC	EC_{50}	NOEC	IC_{50} / MTC	NOEC
Diclofenac	109.64 ± 2.12	10.84 ± 0.4	53.70 ± 1.5	0.45 ± 0.02	17.37 ± 1.01	1 ± 0.1
Acetaminophen	245.47 ± 3.7	25 ± 0.9	11.02 ± 0.8	1 ± 0.01	7.02 ± 0.5	0.5 ± 0.01
Ketoprofen	64.56 ± 1.6	0.92 ± 0.03	43.65 ± 1.02	0.56 ± 0.01	16.21 ± 1	1 ± 0.1
Indomethacin	79.43 ± 1.5	0.85 ± 0.02	22.38 ± 1	0.43 ± 0.01	7.94 ± 0.5	0.5 ± 0.01
Naproxen	269.15 ± 3.2	25 ± 0.1	46.72 ± 1.3	1 ± 0.02	19.95 ± 1.2	2 ± 0.1
Ibuprofen	158.48 ± 2.3	10 ± 0.5	104.71 ± 2.3	5 ± 0.05	39.89 ± 1.2	1.5 ± 0.6
Carbamazepine	42.60 ± 1.3	0.8 ± 0.04	21.87 ± 1.02	1.2 ± 0.1	54.21 ± 1.5	0.4 ± 0.02
Caffeine	229.08 ± 2.9	20 ± 0.5	162.18 ± 1.6	10 ± 0.09	88.6 ± 1.7	10 ± 1

L(E)C50- median lethal/effect concentration; IC5-median inhibitory concentration; NOEC- no observed effect concentrations; MTC –microbial toxicity concentration

A more accurate and realistic estimation of pharmaceutical products fate, effects and risk once into surface waters in Romania is based on a seasonal and long-term toxicological testing to collect enough data which could be analyzed together with other synergistic risk studies. In the last years, ecotoxicity studies have shown that monitoring of metabolites and various degradation products from pharmaceuticals, which are present into the environment, should not be neglected. Therefore, further studies are needed to fully understand pharmaceuticals fate in the environment, their chronic effects as well as the overall effects of pharmaceuticals combinations, as they are normally present into the environment.