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## A. FISCHERI BIOREACTIVITY TOWARD DIFFERENT ANALGESICS

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### **Introduction**

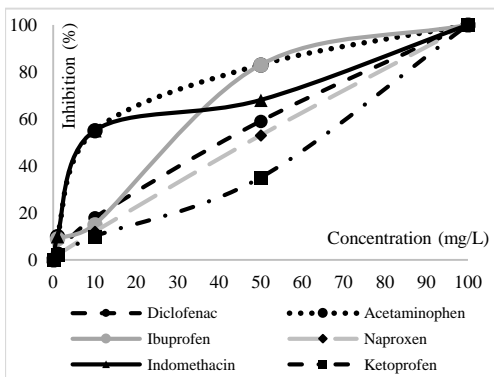
The manufacture and use of pharmaceuticals compounds (PhACs) is one of the great benefits of today's society. However, the increased use of these substances has resulted in continuous and uncontrolled releases to the environment for many years, as complex mixtures, mainly through the effluents of waste water treatment plants, as well as the use of biological sludge in agriculture. Two serious consequences of environmental pollution with these compounds are the adaptation of microorganisms and viruses to the active substance and the disruption of the endocrine system in humans and in terrestrial and aquatic organisms. The ecotoxic effects of PhACs in aquatic ecosystems become an indisputable necessity. The European research and relementation communities required evaluation of the toxic effects of PhACs on aquatic organisms at different trophic levels (microorganisms, invertebrate or vertebrate).

### **Materials and methods**

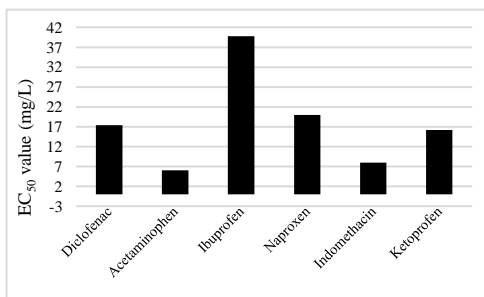
The tested analgesic compounds were: diclofenac, acetaminophen, ibuprofen, naproxen, indomethacin and ketoprofen. All tested substances had analytical purity >98%. The bacteria *Aliivibrio fischeri* (marine bioluminescent bacteria, NRRL B-11177) was chosen as biological model in order to estimate the adverse effect of PhACs on microbiological communities from aquatic systems. The gram-negative marine bacteria emit luminescence as a metabolic product, a phenomenon that could be affected by the toxicity of some chemicals. The light intensity of the luminescent bacteria is measured using the Microtox® M500 testing system (according to SR EN ISO 11348-3:2008). The luminescence was quantified before and after a 30 minutes incubation period in the presence of toxic substance, and the results are compared with a control. The intensity difference between the sample and the control is associated with the effect of the sample on the organisms: inhibition or stimulation. The experimental tests were leaded for five serial concentration in the range of 0.01 mg/l to 100 mg/l for each PhAC. Two control tests were also carried out: (1) dilution medium and (2) dilution medium with methanol in case of ibuprofen and indomethacin. Before of test beginning, normal survival conditions of the bacterium have been assured, respectively the pH and salinity adjustments and has been eliminated the interferences determinate by the samples turbidity.

### Results and conclusions

The effect of PhACs on bacteria light intensity is measured for each tested concentration. Start to 1 mg/l inhibition effects were registered ( $\leq 10\%$ ). At 100 mg/l the luminescence inhibition was totally (100%). Also, similar inhibition effects for diclofenac, naproxen and ketoprofen was observed, probably due to the chemical structural similarities (Figure 1).



**Figure 1.** Dose-response of *Aliivibrio fischeri* expressed as inhibition (%) to PhACs.



**Figure 2.**  $EC_{50}$  (mg/L) for *Aliivibrio fischeri* in presence of PhACs.

Also, similar  $EC_{50}$  values for diclofenac, naproxen and ketoprofen was observed, probably due to the chemical structural similarities.

In conclusion the experimental studies highlighted that all six analgesics have toxic effects, inhibiting the bacterial metabolism, measured by luminescence inhibition. The bacteria model used was a proper and fast indicator of analgesics toxicity.

### Acknowledgements

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Base on luminescent inhibitions (%), the toxic concentration for 50% of tested bacteria  $EC_{50}$  after 30 minutes of contact were estimated. Figure 2 show the results of  $EC_{50}$  for each tested compound. All samples had  $EC_{50}$  values (30min)  $< 50$  mg/L and therefore may exhibit harmful effects on luminescent bacteria.

Taking into account the toxicity classification criteria specified in the European legislation, acetaminophen and indomethacin have  $EC_{50}$  values  $< 10$  mg/L, which classify them as toxic for bacteria. Diclofenac ibuprofen, naproxen and ketoprofen induce a moderate toxic effects.