Mini Review

Diclofenac, an Emerging Pollutant. Acute Toxicity Study in Fish and Amphibians

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Abstract

Study of emerging pollutants, including pharmaceutical compounds, is nowadays of great interest for the environmental research. Diclofenac is a highly used drug in Argentina. We report different types of acute toxicity studies evaluating diclofenac, using as experimental models fish of the species *Poecillia reticulata* and *Danio rerio* and amphibians of the specie *Rinbella arenarum*. In this study we use the technique recommended by the US Fish and Wild life Service modified by our research group. Our results indicate that diclofenac would not produce acute toxicity effects in the experimental models studied; however from values of 20 mg/L already begin to be seen effects of embryotoxicity.

Keywords: Emerging pollutants; Pharmaceutical compounds; Diclofenac; Acute toxicity; Fish; Embryotoxicity

Introduction

In recent years, the study of emerging pollutants, including pharmaceutical compounds, has been of great interest for environmental research [1]. Drugs are used by a huge proportion of the population in large quantities. An important fraction after ingestion is excreted through urine and feces, continuously entering wastewater [2]. The composition profile and levels found depend on the drug, the country and its consumption.

In Argentina, information about drug concentrations in aquatic environments in the country is scarce or non-existent, especially when compared to other countries such

as Canada, China or the European continent. The lethal or acute toxicity effects of these contaminants on aquatic biota have not yet been studied in detail. In addition we may consider that determined levels also have sublethal and chronic effects.

Diclofenac is a relatively non-selective cyclooxygenase inhibitor drug, it belongs to the family of non-steroidal anti-inflammatory drugs (NSAIDs). It has analgesic, anti-inflammatory and antipyretic properties. Soluble in water and has an action time of 6 to 8 hours; its half-life corresponds to 1 to 3 hours. Undergo liver metabolism and then urinary and biliary excretion of conjugated metabolites [3].

Fish and amphibians have been used in toxicity studies from 1950 [4,5], demonstrating over the time that they are a reliable models for measuring the environmental impact of different substances [6]; our research group has used an adaptation of the proposed technique by Johnson and Finley [7] in several studies [8-10].

We present in this work different types of acute toxicity studies evaluating diclofenac, using as experimental models fish of the species *Poecillia reticulata* and *Danio rerio* and amphibians of the specie *Rinbella arenarum*.

Methodology

Drug

For this experience we use sodium diclofenac (C₁₄H₁₀Cl₂NNaO; PM: 318.13), provided by Laboratorios Puntanos; Lot Number 3011510076, IRDFS 10151926. Hygroscopic white solid. Solubility: 50 mg/mL.

Acute toxicity in fish and amphibians

We use the technique recommended by the US Fish and Wild life Service [7] which was modified to use a smaller amount of test compounds as was reported by Mascotti [8].

Specimens of fish and amphibian were purchased in local businesses and were transferred to our laboratory and placed for 21 days in tanks parked 50 L of water to adapt to new conditions. The water conditions were: pH 7.2, temperature 23 degrees Celsius, soft water and free of chlorinated compounds and initial nitrites.

During that period they were fed 1 time per day with a (Tetramin®) specific food for fish and acelga for anphibians and standardized controlled aeration supply; the value of the ambient temperature was a daily average of 23°C and water replenishment undertaken to maintain the volume of the ponds. For the experiences we selected specimens to 1 cm in length obtained from reproduction of adults.

Ten adults specimens were exposed for 96 hours to each concentration of test drug solutions using five concentrations in each toxicity test (in the range of 5 mg/L to 80 mg/L). Solutions and specimens were placed in a 1 L vessel (ratio of 1 specimen per 100 mL of water) where they were kept until the end of the evaluations. The numbers of dead specimens in each container were removed every 24 hours. The percentage of mortality was assessed at 96 hs. It was determined the lowest concentration of formulated which produced 100% mortality (MC100% M) and the highest concentration that did not cause mortality (MC0% M).

Inhibition of feeding in amphibians

Food consumption was evaluated only in amphibians by adding 2 cm pieces of boiled chard with scrapings and compared the food area consumed by the test lot of 40 mg/L vs. The food area consumed by the control lot.

Embryotoxicity - test in D. Rerio

The embryo hatching studies at Danio Rerio were carried out following methodologies proposed by the OECD [11] or sublethal doses of herbicides (20, 40 and 80 mg/L). Groups of 12 adult of zebra fish (7 male and 5 female) kept at 12/12 hours light/dark conditions were placed in stainless steel mesh spawning cages of 12 L in the morning for spawning.

The embryos were recollected at 24 hrs. The test was carried out in triplicate Each embryo was placed in individual wells of 5 mL of solutions of sodium diclofenac and incubated for 72-96 hrs. The incubation conditions were: static technique (without solution replacement) temperature 23 degrees Celsius, without aeration, seasonal light cycle (November, southern hemisphere). The eggs were observed with magnifying glass each 24 hours.

Results

Indicative evaluation of acute toxicity for diclofenac

We perform simultaneously, the evaluation of diclofenac at 5 (five) different concentrations: 5, 10, 20, 40 and 80

mg/L in adult fish of the species *P. reticulata* and *D. Rerio* as well as in two different Larval stages of amphibians, *R. Arenarum*. In any of the species evaluated and even in the highest concentration (80 mg/L) was observed mortality of individues, in some cases within 96 hs.

In the case of amphibians, the study was carried out during a period of 8 days, during that time they passed to the X larval stage without observing neither mortality nor malformation effects and/or developing it in the larvae.

Inhibición feeding in amphibians

We chose to evaluate a dose of 40 mg/L for 48 hours as a food inhibitor, but we obtained negative results. Proving so that at that dose of drug has no effects on food.

Evaluation in embryos of fish

Following the technique described in the methodology, we evaluated acute toxicity in embryos of *D. Rerio* during a period of 72/96 hours at concentrations of 20, 40 and 80 mg/L obtaining partial mortality effects (30%) at 20 mg/L and totals at 40 and 80 mg/L. Similar results were obtained by other authors using similar doses in this experimental model [12].

Conclusions

Considering the results obtained we can consider that sodium diclofenac would not produce acute toxicity effects in the experimental models studied; however we would must to perform deeper studies because because from values of 20 mg/L already begin to be seen effects of embryotoxicity. Therefore considering that sodium diclofenac is a drug of general use in Argentina this result should be taken into account.

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