

# Single and binary mixture toxicity of ibuprofen and paracetamol to *Daphnia magna*

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Pharmaceuticals are a diverse group of compounds biologically active, produced to treat human and animal diseases. They enter the environment mainly in two ways, waste water effluent from human sources and agricultural runoff from veterinary treatments. As removal of a large number of pharmaceuticals from sewage treatment processes is not complete, mixtures of pharmaceuticals are discharged into the receiving waters in detectable amounts. The aim of this study was to assess the acute and chronic effects of paracetamol, ibuprofen and its mixture, at ecologically relevant concentrations in the freshwater crustacean *Daphnia magna*.

The acute toxicity tests followed the OECD standard guideline No. 202. Five concentrations, with four replicates, of paracetamol (PAR), ibuprofen (IBU) and its binary mixture, a control with M4 media and DMSO 0,1%, were tested. Five juveniles, less than 24h of age, were randomly assigned to each 100mL glass bottle with the different test solutions. For PAR 0.75, 1.5, 3, 6, 12 mg/L were used. IBU concentrations were 2.5, 5, 10, 20, 40 mg/L. Mixture tests were conducted using proportions of the respective LC50 of PAR and IBU: 25%PAR:75%IBU, 50%PAR:50%IBU, 75%PAR:25%IBU, 100%PAR, 100%IBU. Survival was determined at 24h and 48h of exposure. LC50 was determined by probit analysis. *The chronic assay* was adapted according to the OECD 211 guideline. After the 48h acute exposure, the surviving daphnids were transferred to fresh M4 medium, fed daily with *Chlorella vulgaris* culture  $3 \times 10^5$  cell.mL<sup>-1</sup>, at 20°C and 14h:10h photoperiod. Media was renewed very other day and daphnids survival and reproduction output was followed until day 22.

Both pharmaceuticals individually tested had a significant effect on *Daphnia* juveniles at 48h of exposure, LC50 values were 20 mg/L for ibuprofen and 6 mg/L for paracetamol which are in accordance with values found on previous research works. In the chronic test with ibuprofen, there was mortality at 5, 10 and 20 mg/L and the percentage of mortality was higher at the highest concentration, as expected. The total number of juveniles per female was higher at control and at the highest concentration of ibuprofen (20 mg/L). At concentrations 2.5, 5 and 10 mg/L, the total number of juveniles decreased and were of similar value. Age at first reproduction was similar at all the concentrations tested and was 12.3 days.

In the chronic test with paracetamol, there was mortality at all the treatments and varied between 13% at 1.5mgL<sup>-1</sup> and 19% at the other treatments. The total number of juveniles, was higher than the control at the lowest paracetamol concentrations and decreased at the highest concentration. In the mixture test, mortality was higher at IBU 100% and PAR75%:IBU25%. The total number of juveniles increased with increasing amount of paracetamol and was higher at the IBU100% treatment. Age at first reproduction increased one day in all the mixture treatments.

This study showed toxic effects of both pharmaceuticals paracetamol and ibuprofen alone and increased toxicity with their mixture on *Daphnia magna* survival and reproduction.