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# Toxicological assessment of ibuprofen, paracetamol and metformin in terrestrial ecosystems

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## INTRODUCTION:

Pharmaceuticals (PhACs) are a class of emerging contaminants that have, in recent years, been associated with potential risks for the environment (Fent, Weston & Caminada, 2006). Pharmaceutical substances are often engineered for persistence, so they remain unaltered as they pass through the organism. This leads to the persistence of these substances when they are excreted, leading to potential environmental problems (European Environment Agency, 2010). Residues of pharmaceuticals have been detected at trace quantities in aquatic systems, with studies finding over 80 PhACs from various prescription classes in sewage, surface and groundwater, in several countries all over the world (Heberer, 2002). These facts make the evaluations of potential effects of pharmaceuticals on ecosystems relevant and necessary. Acute aquatic toxicity has been more extensively studied since aquatic organisms are more easily identifiable as important targets, as they might be exposed to wastewater over their whole life. However, only little is known about the ecotoxicological effects of pharmaceuticals on terrestrial organisms (Fent et al., 2006). Residues of PhACs may reach the terrestrial environment through several sources/routes (Heberer, 2002) but, since removal of PhACs in Wastewater Treatment Plants (WWTPs) is many times not efficient, contamination comes mostly from applying contaminated sludge in agriculture.

While single substances are mostly found in the environment in modest quantities, it's important to note that in the environment, we find complex mixtures of pharmaceuticals and metabolites that may interact (Escher et al., 2011). Assessments of individual substances may therefore be misleading (European Environment Agency, 2010).

Ibuprofen, Paracetamol and Metformin are among the PhACs most consumed in Portugal and their metabolization by the organism is low, therefore it is important to assess their ecotoxicity.

## OBJECTIVES:

Assess if Ibuprofen, Paracetamol and Metformin and their combination effect (Metformin + Ibuprofen, Ibuprofen + Paracetamol and Paracetamol + Metformin) represent a potential risk to terrestrial ecosystems, by studying their effects on earthworms (*Eiseniafetida*) and seeds of lettuce (*Lactucasativa*), garden cress (*Lepidium sativum*) and dwarf bean (*Phaseolus vulgaris*).

## MATERIALS AND METHODS:

A reference toxicity test using boric acid was conducted to assess, under standardized conditions, the sensitivity of a portion of the population of adult earthworms (Environment Canada, 2004).

Acute avoidance tests were conducted using test units that consist of a circular container with an outer diameter of 23 cm, partitioned into a central cylinder with an inner diameter of ~5cm, and six pie-shaped interconnecting compartments each with a capacity for ~350 mL of soil. A series of ~1cm holes enable the free movement of earthworms from the central cylinder (devoid of substrate) to the test compartments and between the compartments. A total of five test units were used, one for each concentration [0.10; 0.32; 1.00; 3.20; 10.00 mg/kg]. An identical weight of negative control soil was transferred to every second test compartment (three/test unit) within each test unit. The remaining compartments were filled with the same

weight of contaminated test soil. Test units were placed in a darkened facility for 48 hours at room temperature ( $22^{\circ}\text{C}\pm 1^{\circ}\text{C}$ ). At that time a side partition was quickly inserted adjacent to the side of each test compartment and the number of worms in each compartment was counted and recorded.

Toxicity tests of PhACs to terrestrial seed plants were conducted by placing a filter paper circle in Petri dishes, adding 2 mL of the contaminant solutions at different concentrations [0.00; 0.10; 0.18; 0.32; 0.56; 1.00; 1.80; 3.20; 5.60; 10.00mg/L] and placing 5 seeds (dwarf bean) or 10 seeds (garden cress and lettuce) in each Petri dish, equally distanced, so they wouldn't interfere with each other's growth (three replicates for each concentration). Petri dishes were covered with lids, sealed and incubated in the dark at room temperature ( $22^{\circ}\text{C}\pm 1^{\circ}\text{C}$ ) for 7 days. After incubation, root lengths were measured (from transition point between hypocotyl and root to root tip) and the seed germination was evaluated using root length of seedlings as endpoint (primary root > 2 mm).

## RESULTS AND DISCUSSION:

Seven-day LC50 was determined to be 4858.7 mg of boric acid/kg soil (dry wt). Expected seven-day LC50 has endpoint values ranging from 3295–4915 mg of boric acid/kg soil (dry wt), meaning results fit within the expected range.

In Acute Avoidance Tests, all worms survived all five concentrations of the three different test contaminants and their mixtures. Results show that the avoidance response of worms to soil contaminated with the PhACs doesn't change with increasing concentrations between 0.10 and 10.00 mg/kg. Earthworms don't avoid soils contaminated with these PhACs, having no preference for clean soils. At the chosen concentrations, acute exposure to these contaminants poses no risk to *Eiseniafetida*. Question stands if higher concentrations could have an effect on earthworms. Tests with higher concentrations would, however, cease to have environmental relevance.

Results indicate that while lettuce seeds exposed to Ibuprofen and to the mixture solution of Ibuprofen + Paracetamol showed a slight raise in root lengths as concentrations increased. In lettuce seeds exposed to Paracetamol, a relation between concentration and root length is not visible. Lettuce seeds exposed to Metformin and to the mixture solution of Metformin + Ibuprofen showed a small decrease in root lengths. Root lengths for garden cress and dwarf bean seedlings exposed to the three contaminants oscillated several times as concentrations increases, and no relation between the concentration of contaminant and the average root growth is visible. Variability between seeds of garden cress and dwarf bean roots length inside each group of seeds may be responsible for the differences between tests with different concentrations of the same contaminant. In lettuce, the variance between groups was not as high. Still, Ibuprofen and Paracetamol didn't appear to have any effect on the growth of seeds, with root lengths being equally arbitrary among concentrations. Even though, there isn't an apparent effect of the three pharmaceuticals in garden cress and dwarf bean, disparity between the length of roots inside and among concentrations groups and contaminants make it impossible to know, for sure, if the contaminants influence the development of the root or not. The only contaminant for which it seemed there was an effect on lettuce root growth was Metformin. However, statistical significant differences between the control group and seeds exposed to Metformin were not detected. Furthermore, variances in root length among groups are similar to those found in lettuce exposed to Paracetamol, where no apparent effect or pattern can be detected, and Ibuprofen, where root lengths increased slightly with increasing test concentrations. Therefore, we can't be sure that the apparent decrease in root lengths of lettuce seedlings is caused by Metformin, as there is a chance that it might be simply a result of the variability existent among the lettuce seeds used in the test.

Chronic toxicity studies with environmentally relevant concentrations might show results in both worms and seeds, seeing as both can take up and accumulate pollutants present in the soil (Wu, Wu, Qiu, Zhong, & Chen, 2011).

## CONCLUSION:

Avoidance results for acute exposure to single PhACs, namely Ibuprofen, Paracetamol and Metformin and their mixtures don't indicate a toxicity of these compounds to *E. fetida*, at environmentally relevant concentrations, with no added toxicity being found for pharmaceutical mixtures. The same conclusion applies to tests using seeds, with no acute toxicity noted for these pharmaceuticals, either for single or combined pharmaceuticals. Acute exposure to Ibuprofen, Paracetamol and Metformin at environmentally relevant concentrations did not prove to have an impact on terrestrial ecosystems. The future realization of chronic studies for the same concentrations of these pharmaceuticals is, however, crucial for the determination of the environmental impact of these contaminants in terrestrial environments. Since several PhACs have been detected in the environment, and to fill the gap existing in studies of toxicity of pharmaceuticals in terrestrial ecosystems, future testing is needed on other PhACs and their interactions in the environment.

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