Ecotoxicology and Environmental Safety 149 (2018) 72–79

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

The ecotoxicity of binary mixtures of Imazamox and ionized ammonia on freshwater copepods: Implications for environmental risk assessment in groundwater bodies

Walter D. Di Marzio[a,*], Marco Cifoni[b,c], María E. Sáenz[a], Diana M.P. Galassi[b], Tiziana Di Lorenzo[c]

[a] Programa de Investigación en Ecotoxicología, Departamento de Ciencias Básicas, Universidad Nacional de Luján - Comisión Nacional de Investigaciones Científicas y Técnicas CONICET, Argentina

[b] Department of Life, Health and Environmental Sciences, University of L'Aquila, Via Vetoio 1, Coppito 67100, L'Aquila, Italy

[c] Institute of Ecosystem Study - CNR - National Research Council of Italy, Via Madonna del Piano 10, 50019 Sesto Fiorentino, Florence, Italy

ARTICLE INFO

Keywords:
Imazamox
Ammonia
Mixture
Copepods
Groundwater
Stygofauna

ABSTRACT

Groundwater bodies are impacted by substances such as pesticides and N-fertilizers, which usually occur in the environment as complex mixtures rather than isolated pollutants. The threat that these mixtures pose to groundwater-dwelling organisms is still poorly understood. The aims of the present study were to test the acute effect of a binary mixture of a herbicide (Imazamox) and NH_4^+ on epigean (*Eucyclops serrulatus*) and hypogean (*Diacyclops belgicus*) freshwater copepod species. In addition, to evaluate if the effect of the mixture can be explained by referencing non-interaction models or by more complex interaction models; and the implications for groundwater risk assessment. Compared with the action of the compounds evaluated separately, the effects of Imazamox and NH_4^+ in the binary mixture were more than additive or synergistic for both species. MixTox models evidenced a dose ratio and dose level deviations from concentration addition and independent action traditional models. The hypogean species was three times more sensitive to NH_4^+ that the epigean species when assayed as a single chemical. However, *D. belgicus* was only 1.13 times more sensitive than *E. serrulatus* when NH_4^+ was assayed in the mixture. The use of an integrated approach for substances that are known to interact in groundwater, should include copepods species as test organisms.

1. Introduction

Chemical pollution represents a threat to aquatic ecosystems, which poses a risk to aquatic organisms and human health (Malaj et al., 2014). According to WATERBASE, the European Environmental Agency database of EU fresh- and marine water bodies (www-eea.europa.eu/data-and-maps/data/waterbase-transitional-coastal-and-marine-waters-8), 34% of EU groundwater monitoring sites demonstrated ionized ammonia (NH_4^+) concentrations exceeding quality standards in the period 2000–2011 (EPA, 2010). Although the NH_4^+ concentration has been decreasing in EU surface water bodies in the last decades, it still remains higher than the natural level in several groundwater bodies as a result of agricultural treatments, such as crop fertilization by ammonia-N (NH_4^+ + NH_3) compounds (EEA, 2010, 2015). Pesticides, in association with N-fertilizers, have also been widely detected in surface and groundwater bodies at concentrations exceeding the current EU quality standard (0.1 µg/L; EEA, 2015). The Water Framework Directive (WFD, 2000/60/EC) requires setting environmental quality standards (EQSs) for substances of EU-wide concern (such as pesticides) and threshold values (TVs) for substances of national or local concern (such as NH_4^+). TVs are set by each Member State (EC, 2014a). According to Annex V, point 1.4.3 of the WFD, a waterbody is in good chemical status when the concentrations of pollutants, both as individual compounds and as compounds in mixtures, comply with the relative EQSs and TVs (EC, 2014b).

The Guidance Document n. 27 (TGD) is the official EU technical document for deriving both EQSs and TVs (EC, 2011). TGD highlights the importance of ecotoxicological data in this process (EC, 2011). The base set of taxa that TGD suggests using consists of algae and/or macrophytes, the cladoceran *Daphnia* and fish (EC, 2011). However, none of these taxa dwell in groundwater habitats (Gibert et al., 1994). For many years, several researchers have warned about using surface water species to infer the sensitivity of groundwater taxa due to relevant differences in their metabolism (Hose, 2007; Avramov et al., 2013; Di Lorenzo et al., 2014, 2015c). TGD also highlights the importance of deriving EQSs and TVs for mixtures of substances (EC, 2011). In

* Corresponding author.
E-mail addresses: ecotoxicologia@aae.org.ar, wdimarzio@conicet.gov.ar (W.D. Di Marzio).

https://doi.org/10.1016/j.ecoenv.2017.11.031
Received 7 May 2017; Received in revised form 2 November 2017; Accepted 10 November 2017
0147-6513/ © 2017 Elsevier Inc. All rights reserved.

general, contaminants do not occur as isolated substances in groundwater but rather as complex mixtures. As scientific evidence indicates that the toxicity of a mixture of chemicals can be different from that of the individual compounds as a result of antagonistic or synergistic effects, EQSs and TVs for mixtures are necessary.

The objectives of the present study were: (i) to test the acute effect of a binary mixture (MIX) of a herbicide (Imazamox) and NH_4^+ on epigean and hypogean freshwater copepod species belonging to the same family (i.e., with a relatively close phylogenetic relationship); (ii) to determine if the effect detected in the MIX bioassay can be explained by reference non-interaction models, such as a concentration addition (CA) or an independent action (IA) model, or by more complex interaction models, such as those in which the two chemicals have synergistic or antagonistic effects; (iii) to discuss the implications for groundwater risk assessment.

We selected the copepod taxon because, with more than 1100 species living in groundwater, it is by far the most abundant and species-rich group in groundwater and associated ecosystems (Galassi, 2001). For the purposes of this study, we selected two Cyclopoida, Cyclopidae species, the hypogean *Diacyclops belgicus* and the epigean *Eucyclops serrulatus*.

Finally, we investigated the effect of the binary MIX of NH_4^+, dosed by ammonium nitrate, and the herbicide Imazamox, for the following reasons: 1) EU intensive cereal agriculture is known to be associated with a high use of N-fertilizers in the forms of ammonium nitrate and urea (Erisman et al., 2007), as well as herbicides such as Imazamox (ARPAP, 2014); 2) albeit NH_4^+ is in equilibrium with NH_3 in water depending on pH and temperature, NH_4^+ only is listed in the Annex II Part B of the Groundwater Daughter Directive – GDD − 2006/118/EC as a pollutant for which the Member States have to consider establishing TVs in accordance with the Article 3 of the GDD; 3) Imazamox application to wheat requires the addition of N-fertilizers, such as urea and ammonium nitrate (Geier and Stahlman, 2009); also the increased use of Clearfield® technology, which is based on the use of both the herbicide imazamox and resistant (IMI-R) sunflower, wheat, oilseed rape and rice hybrids, resulting in an increased IMA application (http://agronotizie.imagelinenetwork.com/materiali/Varie/File/syngenta2013/syngenta-girasole2-013.pdf) 4) information from environmental fate studies indicates that Imazamox does not persist in shallow surface waters, but the groundwater ubiquity score (GUS) index for imazamox suggests it poses a leaching risk (Milan et al., 2017) and could persist in environment with low oxygen concentrations (EFSA, 2016); and 5) gaps in the ecotoxicological data concerning the risk posed to aquatic organisms by Imazamox have been recently identified (EFSA, 2016). For these reasons, the environmental fate of imazamox deserves investigation, especially given that its use is likely to keep growing into the future (Milan et al., 2017).

2. Materials and methods

2.1. Test chemicals, test organisms and culture conditions

The experiments were carried out with a binary MIX of NH_4^+, dosed by ammonium nitrate (pure crystalline solid, CAS No. 6484-52-2) and the herbicide Imazamox (commercial formulation 3.7%, Beyond, BASF, Italy, CAS No. 114311-32-9). NH_4^+ concentrations were measured at the beginning and the end of the tests by Hach method #8038, adapted from Standard Methods for the Examination of Water and Wastewater 4500-NH3 B-C, using a DR3900 Hach spectrophotometer. The limit of detection was 20 μg/L. Imazamox is highly stable in water, at least within the assayed time period (USEPA, 2008). However, the nominal concentrations were confirmed at the end of each test by HPLC-UV according to Mastan et al. (2016). The system was equipped with a reversed phase C18 analytical column of 250 mm × 4.6 mm and a particle size of 5 μm (Phenomenex Luna-C18). The injected sample volume was 10 μL. The mobile phases were acetonitrile and 0.1% ortho phosphoric acid (30:70 v/v). The flow rate used was 1.2 mL/min. The detector wavelength was 254 nm. The limit of detection was 0.5 mg/L. pH was measured daily in the test chambers by Oakton 1100 pHmeter.

Test specimens were collected from two different shallow boreholes (B1 and B2) used for gardening on the campus of Consiglio Nazionale delle Ricerche - CNR in Florence (Italy), 300 m apart from each other, in April 2014. Water samples from both bores were tested for 32 chemicals to ascertain the requisite assurance that the test organisms were obtained directly from wild populations in relatively unpolluted areas. Concentrations of NH_4^+, nitrites, nitrates, heavy metals, inorganic pollutants, polycyclic aromatic hydrocarbons, pesticides, and volatile organic compounds were lower than the Italian legislative quality standards. In particular, NH_4^+ concentrations were < 0.01 mg/L in both bores, and the Imazamox concentration was below the limit of detection. The bores were open at the top (70 cm in diameter), allowing the mixing of rainwater with shallow groundwater. Dissolved organic carbon (DOC) was 1.1 and 1.0 mg/L in B1 and B2, respectively. Bacteria were present in approximately 10^6 prokaryotic cells/mL in both bores. Both bores (depth < 10 m) were situated in a shallow quaternary porous aquifer. A phreatobiological net sampler (mesh size 60 μm) was used to collect copepods from the bottom and the water column of the wells. The specimens were transported to the laboratory with the bore water in a cooling box within 10 min after collection. The copepods were sorted using a micropipette under a stereomicroscope at 12× magnification and separated into different groups according to macroscopic differences in morphology. Specimens of each group were then identified at the species level under an optical microscope at 100× magnification using the taxonomic key of Alekseev et al. (2006). Two different species were identified, namely *E. serrulatus* (epigean) and *D. belgicus* (hypogean). Groups of 25 individuals of each species were reared in 25-mL glass beakers in a standard water (Millipore® Milli-Q® deionized water re-mineralized with chemical grade: pH 7.4, hardness 80 mg/L, alkalinity 30 mg/L). They were kept in permanent darkness in a laboratory thermostatic cabinet (Pol-Eko-Aparatura Mod. ST 3) at 15 °C, corresponding to the mean annual temperature of the bore water, which was measured monthly in both bores using a multiparametric probe (ECM MultiTM; Dr. Lange GmbH, Düsseldorf, Germany).

Individuals of *E. serrulatus* can be easily maintained with a standardized algal diet in the laboratory, where they complete their full-life cycle (egg to adult to egg) in approximately 70 days at 15 °C, producing approximately 16 eggs per clutch per female and surviving up to 80 days. In contrast, individuals of *D. belgicus* must be maintained in the laboratory in glass beakers filled with bore water, allowing them to feed on a prokaryotic diet. The development rate of this species is unknown; however, the individuals collected in April 2014 remained alive for approximately one year in the laboratory. Only one female out of 16 produced egg-sacs (6 eggs per sac) within this period and no hatched nauplii were observed. Further information about the ecological features of stygobiotic copepods can be found in Galassi (2001). Due to these differences in the life cycles of the two species, after collecting and sorting, we acclimated 250 adult individuals of each species for the tests, in two different glass beakers (500 mL) with the standard water used for the stock cultures for 3 days prior to each ecotoxicological test. To cope with unexpected events, the number of acclimated organisms was 20% more than that required for the tests (200 organisms for each species). At the end of the tests, the unused organisms were maintained in laboratory stock cultures. Copepod fecal pellets are nutrient-enriched microenvironments that act as hotspots for microbial colonization and consume oxygen in the test vials (Di Lorenzo et al., 2015a). Accordingly, acute tests with copepods are usually performed without food (Di Lorenzo et al., 2014, 2015b, 2015c). However, starvation was required also during the 3-day acclimation in our trials because both *D. belgicus* and *E. serrulatus* produce fecal pellets for three days after stopping eating. For this reason, both species were deprived of food during acclimation to allow the guts to empty completely. The digestive tract was clearly visible at 60 × magnification under a Leica Microsystems M80

Download English Version:

https://daneshyari.com/en/article/8854483

Download Persian Version:

https://daneshyari.com/article/8854483

Daneshyari.com