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Determination and validation of an aquatic Maximum Acceptable Concentration-Environmental Quality Standard (MAC-EQS) value for the agricultural fungicide azoxystrobin☆

Elsa Teresa Rodrigues [a, *], Miguel Ângelo Pardal [a], Cristiano Gante [a], João Loureiro [a], Isabel Lopes [b]

[a] Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal
[b] Department of Biology & CESAM, University of Aveiro, 3810-193 Aveiro, Portugal

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ABSTRACT

The main goal of the present study was to determine and validate an aquatic Maximum Acceptable Concentration-Environmental Quality Standard (MAC-EQS) value for the agricultural fungicide azoxystrobin (AZX). Assessment factors were applied to short-term toxicity data using the lowest EC_{50} and after the Species Sensitivity Distribution (SSD) method. Both ways of EQS generation were applied to a freshwater toxicity dataset for AZX based on available data, and to marine toxicity datasets for AZX and Ortiva® (a commercial formulation of AZX) obtained by the present study. A high interspecific variability in AZX sensitivity was observed in all datasets, being the copepoda *Eudiaptomus graciloides* ($LC_{50,48h}$ = 38 μg L^{-1}) and the gastropod *Gibbula umbilicalis* ($LC_{50,96h}$ = 13 μg L^{-1}) the most sensitive freshwater and marine species, respectively. MAC-EQS values derived using the lowest EC_{50} (≤0.38 μg L^{-1}) were more protective than those derived using the SSD method (≤3.2 μg L^{-1}). After comparing the MAC-EQS values estimated in the present study to the smallest AA-EQS available, which protect against the occurrence of prolonged exposure of AZX, the MAC-EQS values derived using the lowest EC_{50} were considered overprotective and a MAC-EQS of 1.8 μg L^{-1} was validated and recommended for AZX for the water column. This value was derived from marine toxicity data, which highlights the importance of testing marine organisms. Moreover, Ortiva affects the most sensitive marine species to a greater extent than AZX, and marine species are more sensitive than freshwater species to AZX. A risk characterization ratio higher than one allowed to conclude that AZX might pose a high risk to the aquatic environment. Also, in a wider conclusion, before new pesticides are approved, we suggest to improve the Tier 1 prospective Ecological Risk Assessment by increasing the number of short-term data, and apply the SSD approach, in order to ensure the safety of aquatic organisms.

1. Introduction

For retrospective aquatic risk assessment, two types of information are required: exposure levels and toxic effects on non-target organisms, and the risk is expressed as the ratio between exposure concentrations and critical effect concentrations. The latter could be set by an Environmental Quality Standard (EQS) value, which may be generated by applying assessment factors to ecotoxicity data (European Commission, 2011). If a large dataset for different taxonomic groups is available, a probabilistic methodology based on statistical extrapolation techniques such as the Species Sensitivity Distribution (SSD) method might be applied, and therefore lower assessment factors can be used. The SSD approach assembles single-species toxicity data in order to predict hazardous concentrations (HC_x) affecting a certain percentage (x) of species in a community. The most conservative form of this approach uses the lower 95% tolerance limit of the estimated percentage to ensure that the specified level of protection is achieved. Hose and Van den Brink (2004) confirmed this concept of species protection by comparing laboratory-based SSD curves with both local mesocosm

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* Corresponding author.
E-mail addresses: etrodrig@zoo.uc.pt (E.T. Rodrigues), mpardal@zoo.uc.pt (M.Â. Pardal), cgante@student.uc.pt (C. Gante), jloureiro@bot.uc.pt (J. Loureiro), ilopes@ua.pt (I. Lopes).

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experiments and field monitoring data. SSD curves are constructed by fitting a cumulative distribution function to a plot of species toxicity data against rank-assigned percentiles (Wheeler et al., 2002). The greater the number of species tested, the lower the uncertainty of the risk assessment attributable to interspecies differences in sensitivity. In addition, this approach may reduce the uncertainty resulting from differences in the sensitivity of standard test species and those expected to be exposed in nature by also using non-standard test species data. According to Newman et al. (2000), sample size producing HC_5 (hazardous concentration for 5% of species) estimates with minimal variance should range from 15 to 55.

According to international authorities, azoxystrobin (AZX, CAS No. 131860-33-8), the world's No. 1 agricultural fungicide (PAN UK, 2015; Royal Society of Chemistry, 2016; Van Alfen, 2014), is considered to be of low acute and chronic toxicity to mammals, birds and bees (EFSA, 2010; US-EPA, 1997). However, despite the absence of critical areas of concern related to non-target species, an exception was made for aquatic organisms, since a toxicity data gap was identified after the peer-review of the AZX risk assessment of EFSA (2010). In addition, studies on AZX toxic effects on marine organisms are considered scarce by Rodrigues et al. (2013). Therefore, a comprehensive study was designed in order to contribute and timely respond to this critical area of concern, and the median effective concentration for growth rates (EC_{50}) and mortality (LC_{50}) were determined for species representative of several functional and trophic levels of marine ecosystems.

Pesticides are rarely used individually, and additives such as stabilizers, carrying solvents or emulsifiers are added to the final-product (Walker et al., 2001). Accordingly, it has already been shown that commercial formulations of pesticides can be more toxic than their active ingredients (e.g., Mesnage et al., 2014; Puglis and Boone, 2011). The AZX active ingredient is presently registered under different trade names, such as Abound®, Amistar®, Ortiva®, among others. The latter is a mixture of declared hazardous components which are reported in its Safety Data Sheet: 22.9% weight/weight of AZX and 10–20% weight/weight of propane-1,2-diol (Syngenta, 2010). Since sensitivities may be compared by means of the SSD concept (Leung et al., 2001), both Ortiva and AZX SSD curves were plotted to find whether Ortiva is more toxic to marine communities than its active ingredient.

A general strategy to assess the risk of pesticides for marine environments consists of applying safety factors to the risk level calculated based on freshwater toxicity data (ECHA, 2015). Since the available ecotoxicological data on AZX derive mostly from assays with freshwater species (Rodrigues et al., 2013), an SSD curve could also be generated for freshwater species so as to compare sensitivities of both marine and freshwater species by means of the SSD concept (Leung et al., 2001).

The main goal of the present study was to determine and validate a water column Maximum Acceptable Concentration (MAC)-EQS value in line with the European Commission (2011) for AZX. To attain this main goal, three specific objectives were delineated:

1) Determining whether the commercial formulation Ortiva is more toxic than its active ingredient AZX.
2) Comparing the sensitivity of marine species to AZX with that of freshwater species.
3) Determining if MAC-EQS values generated using SSD curves are more protective and conservative than those derived using the lowest EC_{50}.

Since the statistical extrapolation SSD approach for aquatic regulatory purposes is still under debate (Del Signore et al., 2016), this comprehensive study may provide important insights on this subject. In addition, the present study contributes to the establishment of EQSs in the field of water policy under the Water Framework Directive, and allows AZX regulatory risk characterization.

2. Material and methods

2.1. Ethical statement

All animal experiments were conducted in accordance with the ethical guidelines of the European Union Council (Directive 2010/63/EU) and the Portuguese Agricultural Ministry (Decreto-Lei 113/2013) for the protection of animals used for experimental and other scientific purposes. The person in charge of experimental procedures with live animals has accreditation for the use of live animals for scientific purposes (category C) according to the Federation of European Laboratory Animal Science Associations (FELASA) education and training guidelines, granted by the Portuguese General Directorate of Veterinary.

2.2. Marine experimental design

Short-term toxicity assays using both the AZX analytical standard and the commercial formulation Ortiva fully complied with internationally recognized guidelines and protocols (Table 1). The selected species include both standard and non-standard test species, such as non-pathogenic bacteria (*Vibrio fischeri*), microalgae, rotifers (*Brachionus plicatilis*), macrocrustaceans (*Artemia franciscana*), gastropod molluscs (*Rissoa parva* and *Gibbula umbilicalis*) and fish (*Solea senegalensis*). In order to have phytoplankton representativeness, microalgae were chosen from among four phylogenetic groups: Bacillariophyceae (the pennate diatom *Phaeodactylum tricornutum* and the centric diatom *Thalassiosira weissflogii*), Cryptophyceae (*Rhodomonas lens*), Eustigmatophyceae (*Nannochloropsis gaditana*) and Haptophyceae (*Isochrysis galbana*). With a single exception, the *R. parva* assay, all lethal assays were performed using early life stages, larvae or juveniles, as they generally tend to be more sensitive to pollutants than later life stages (Buchwalter et al., 2004; Mohammed, 2013).

2.3. Analytical standard and Ortiva solutions

Azoxystrobin PESTANAL analytical standard (99.9% purity) was purchased from Sigma-Aldrich (31697). Stock standard solutions were prepared in *pro analysis* grade acetone and stored at −18 °C. The fungicide Ortiva was kindly provided by the tree nursery Almeida Rodrigues Viveiros Agrícolas Lda (Coimbra, Portugal). Ortiva intermediate solutions and both AZX and Ortiva exposure media were freshly prepared on the day of use in reconstituted marine water (tropic marin salt, Tropical Marine Centre) using ultra-pure water purified with a Milli-Q Biocel System (Millipore) at salinities presented in Table 1. In the case of the *V. fischeri* assay, the exposure medium was prepared in diluent supplied by Microtox (Modern Water), whereas for the *B. plicatilis* and *A. franciscana* assays, the exposure media were prepared using reagent grade chemicals supplied by MicroBioTest kits: Rotoxkit M and Artoxkit M, respectively. Nominal concentrations were confirmed using a validated chemical method according to section 2.4: the solutions used to start the serial dilutions in the bacteria and microalgae assays, and the exposure solutions collected at the end of the lethal assays. The concentrations used in the statistical analysis were attained by calculating the geometric mean of nominal and measured concentrations, as recommended by Traas (2001).