Chemosphere 182 (2017) 348–355

Contents lists available at ScienceDirect

Chemosphere

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

Toxicity of environmentally realistic concentrations of chlorpyrifos and terbuthylazine in indoor microcosms

Ana Santos Pereira [a, *], Maria José Cerejeira [a], Michiel A. Daam [b]

[a] LEAF, Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017, Lisbon, Portugal
[b] Department of Environmental Sciences and Engineering, Faculty of Sciences and Technology, New University of Lisbon, Quinta da Torre, 2829-516 Caparica, Portugal

HIGHLIGHTS

- Environmentally realistic concentrations were assessed with higher-tier approaches.
- Food-web interactions (indirect effects, recovery of populations) were recorded.
- Terbuthylazine potentiated the effect of chlorpyrifos on feeding rates.
- Zooplankton food-web interactions with multiple chemical stressors need to be evaluated.

ARTICLE INFO

Article history:
Received 8 February 2017
Received in revised form
3 May 2017
Accepted 4 May 2017
Available online 6 May 2017

Handling Editor: David Volz

Keywords:
Model ecosystems
Mixture toxicity
Pesticides
Ecological risk assessment
Zooplankton
Food web interactions

ABSTRACT

Few studies have been conducted into the evaluation of environmentally realistic pesticide mixtures using model ecosystems. In the present study, the effects of single and combined environmentally realistic concentrations of the herbicide terbuthylazine and the insecticide chlorpyrifos were evaluated using laboratory microcosms. Direct toxic effects of chlorpyrifos were noted on copepod nauplii and cladocerans and the recovery of the latter was likely related with the decrease observed in rotifer abundances. Terbuthylazine potentiated the effect of chlorpyrifos on feeding rates of *Daphnia magna*, presumably by triggering the transformation of chlorpyrifos to more toxic oxon-analogs. Possible food-web interactions resulting from multiple chemical (and other) stressors likely to be present in edge-of-field water bodies need to be further evaluated.

1. Introduction

With the modernization and intensification of agricultural practices in the past century, the use of pesticides was initiated to increase yields. As a consequence of pesticide use, water bodies near agricultural areas may become contaminated with pesticide residues through spray drift, drainage, run-off and/or accidental spills (Capri and Trevisan, 1998). Given the variety of pests, diseases and weeds that may need to be combated, it is common practice for several different pesticides to be applied during the growing season to protect crops. Subsequently, freshwater life in edge-of-field water bodies is likely to be exposed to a mixture of compounds (e.g. Stehle and Schulz, 2015). Environmental risk assessment (ERA) of chemicals like pesticides, however, mainly focuses on exposure to individual chemicals, although a number of guidance documents published in the last years have started to indicate how to deal with chemical mixtures (e.g. EFSA, 2013; Bunke et al., 2013; ECHA, 2014; Kienzler et al., 2016). Nevertheless, their use is currently still limited because of a lack of guidance, data, and expertise (Kienzler et al., 2016).

Most scientific studies into mixture toxicity have been conducted using single species tests evaluating concentration series chosen to determine the underlying toxicological model (independent action, concentration addition, and deviations of these). Such concentrations, however, may be considerably above concentrations most often monitored in the environment (Cedergreen,

* Corresponding author.
E-mail address: anapereira@isa.ulisboa.pt (A.S. Pereira).

http://dx.doi.org/10.1016/j.chemosphere.2017.05.032
0045-6535/

2014). Only few studies have evaluated the mixture toxicity of compounds at concentrations likely to occur under real-world conditions (e.g. Banks et al., 2005; Junghans et al., 2006; Laetz et al., 2009; Silva et al., 2015). In addition, the laboratory bioassays that have most often been used in such studies may underestimate the effects of pesticide mixtures in aquatic environments since they do not consider potentially effects in top-down and bottom-up regulation of trophic interactions (Relyea and Hoverman, 2006; Bjergager et al., 2011; Choung et al., 2013).

Model ecosystems (microcosms and mesocosms) are experimental ecosystems that are constructed by collecting parts of natural ecosystems and bringing them together into an artificial housing or by enclosing parts of existing ecosystems in the field (Van den Brink and Daam, 2014). They provide a greater ecological realism than single species tests and since they consider species interactions, top down and bottom up trophic effects may be studied. After reviewing available model ecosystem studies evaluating pesticide mixtures, Verbruggen and Van den Brink (2010) concluded that when pesticides affect the same biological groups (herbicide mixtures, $n = 4$; insecticide mixtures, $n = 5$), synergetic mixture effects are not to be expected. When mixtures of pesticides that affect different biological endpoints (insecticide and herbicide mixtures, $n = 5$) are evaluated, increased indirect effects are often noted due to food web interactions (Verbruggen and Van den Brink, 2010).

Given the above, there is a clear need for model ecosystem studies that evaluate environmentally realistic mixtures of pesticides, especially for mixtures containing pesticides with different modes of action. s-Triazine herbicides (e.g. terbuthylazine) and organophosphorus insecticides (e.g. chlorpyrifos) are commonly used in agricultural areas and are among the most commonly detected pesticides in surface waters worldwide, including Portugal (Canccapa et al., 2016; Schreiner et al., 2016; Silva et al., 2015; Van Wijngaarden et al., 2005; Wacksman et al., 2006). Three studies previously evaluated the mixture toxicity of terbuthylazine and chlorpyrifos in laboratory bioassays with the cladoceran *Daphnia magna* and the green algae *Raphidocelis subcapitata* (Pérez et al., 2013a, b; Munkegaard et al., 2008). To the best of our knowledge, however, this mixture has hence never been evaluated at the community level neither at environmentally realistic concentrations. The aim of the present study was therefore to evaluate the effects of the herbicide terbuthylazine and the insectide chlorpyrifos using indoor model ecosystems. The two pesticides were evaluated individually and in two mixtures using concentrations measured or likely to occur in a Portuguese agricultural area. The ecological effects of the two compounds and implications for their risk to aquatic life are discussed.

2. Material and methods

2.1. Experimental design

Fourteen microcosms were situated in a laboratory devoid of daylight and maintained at 24–28 °C with a photoperiod (fluorescent lamp; light intensity 295 $\mu E/m^2$ s) of 12 h to simulate Mediterranean conditions (Van Wijngaarden et al., 2005). Each microcosm consisted of a glass cylinder (diameter 20 cm; height 50 cm), filled with 13 L water obtained from an uncontaminated pond at *Instituto Superior de Agronomia* (Lisbon, Portugal). Additional zooplankton was collected from the same pond by passing pond water through a zooplankton net (mesh size, 55 μm; Hydrobios, Kiel) and equally distributed (500 mL) over the microcosms. The microcosms were also inoculated with less than 24-h old *D. magna* obtained from ephippia (Microbiotests, Ghent, Belgium). Microcosms were allowed to stabilise for 1 week, after which treatments were assigned randomly to the microcosms. Subsequently, the systems were monitored for several endpoints (see below) during an experimental period of four weeks. Water losses due to evaporation were replenished once a week with demineralized water throughout the experiment.

2.2. Pesticide treatments and analyses

Terbuthylazine (TBZ; Chemical Abstracts Service [CAS] number 5915-41-3; purity 98.6%) and chlorpyrifos (CPF; CAS number 2921-88-2; purity 98%) were purchased from Sigma–Aldrich. Treatment levels of terbuthylazine (8.5 μg/L) and chlorpyrifos (0.17 μg/L), individually and as a binary mixture, were selected from concentrations measured simultaneously in the "Lezíria Grande de Vila Franca de Xira" agricultural area, situated in the vicinity of the River Tagus Estuary Natural Reserve (Portugal). Terbutrylazine (mean ± SD = 0.33 ± 1.2 μg/L; max. = 8.5 μg/L) and chlorpyrifos (mean ± SD = 0.56 ± 2.6 μg/L; max. = 12 μg/L) co-occurred in approximately half (48%) of the 54 samples taken in May to July 2014. In line with the maximum concentration of 8.5 μg/L terbuthylazine measured at this field site, similar (maximum) concentrations have been reported in several other studies (5.6–9.6 μg/L; Baillie, 2016; Knauer, 2016; Tsaboula et al., 2016). However, based on the predicted environmental concentrations reported in the draft assessment report of terbuthylazine, concentrations up to 31 μg/L may be expected for application scenarios in South Europe (EC, 2007). In line with this, Otto et al. (1999) reported a maximum terbuthylazine concentration of 47 μg/L in surface waters following its application in an Italian field trial. Wenneker et al. (2010) showed that concentrations of terbuthylazine in local surface water due to point sources linked to use of sprayers in arable farming were even 100 μg/L or higher. A concentration level of 85 μg terbuthylazine/L was therefore also included to represent a realistic worst-case exposure scenario. Regarding chlorpyrifos, the maximum concentration of 12 μg/L was only measured once in the field and may be expected to lead to a complete elimination of zooplankton (e.g. Daam and Van den Brink, 2007; Rubach et al., 2010; Van Wijngaarden et al., 2005). Subsequently, only the more frequently measured concentration of 0.17 μg chlorpyrifos/L was included as a treatment level, the more as this concentration is close to the EC50 (48 h, immobility) value determined for *D. magna* in our laboratory (unpublished data). Subsequently, the following six treatments were made:

(1) Control (CTR): no pesticide treatment
(2) 0.17 μg chlorpyrifos/L (CPF 0.17)
(3) 8.5 μg terbuthylazine/L (TBZ 8.5)
(4) 85 μg terbuthylazine/L (TBZ 85)
(5) 0.17 μg chlorpyrifos/L + 8.5 μg terbuthylazine/L (MIX 8.5)
(6) 0.17 μg chlorpyrifos/L + 85 μg terbuthylazine/L (MIX 85)

Single applications of the different pesticide treatments were made to two microcosms for each treatment. Before application, sub-samples were taken from the stock solutions for determination of nominal concentrations. Acetonitrile was used as a solvent for both stock solutions and kept below 0.1 mL/L as recommended in OECD (2002). Applications were made by evenly distributing appropriate aliquots of these stock solutions over the water surface of the microcosms, followed by gentle stirring of the water layer with a glass rod. Four other systems were only treated with water containing acetonitrile in a concentration corresponding to that in the pesticide-treated microcosms to serve as controls.

Concentrations of the pesticides in the water were determined 2 days before and 0.25 (6 h), 1, 4, 7, 14 and 28 days after application of the test substances. Depth-integrated water samples of approximately 50 mL were taken from the microcosms by means of a glass