Synergy in microcosms with environmentally realistic concentrations of prochloraz and esfenvalerate

Maj-Britt A. Bjergager [a,*], Mark L. Hanson [b], Linda Lissemore [c], Nikki Henriquez [d], Keith R. Solomon [d], Nina Cedergreen [a,**]

[a] Department of Basic Sciences and Environment, Faculty of Life Sciences, University of Copenhagen, Thorvaldsensvej 40, DK-1871 Frederiksberg, Denmark
[b] Department of Environment and Geography, University of Manitoba, Winnipeg, MB, Canada
[c] Pesticides and Trace Contaminants Section, Laboratory Services Division, University of Guelph, ON, Canada
[d] Centre for Toxicology, School of Environmental Sciences, University of Guelph, ON, Canada

ARTICLE INFO

ABSTRACT

Laboratory experiments have shown that azole fungicides enhance the toxic effect of pyrethroid insecticides towards the aquatic crustacean *Daphnia magna*. Due to their sorptive properties the pesticides may, however, be less bioavailable in natural environments, possibly rendering them less toxic to aquatic organisms. In the present study, the synergistic potential of azoles on pyrethroids in natural environments was assessed by treating 18 outdoor aquatic microcosms with concentrations of the pyrethroid esfenvalerate at 0.167, 0.333, or 0.833 μg/L either alone or in combination with 90 μg/L of the azole prochloraz. Pesticide concentrations and the zooplankton and phytoplankton communities were assessed prior to pesticide application and at days 0, 1, 2, 4, 7, 14, 21, and 28 after pesticide application. DT_{50}-values for disappearance of the pesticides from the water of 4.7 days and 30 h were observed for prochloraz and esfenvalerate, respectively. The monitored communities showed larger decreases in abundance of cladoceran, copepods, and chironomids in treatments with esfenvalerate in combination with prochloraz compared to treatments with esfenvalerate alone. No systematic effects were observed in populations of Ostracoda. Adverse effects on populations of cladocerans and copepods occurred between day 2 and day 7 and, though copepods in general were less sensitive than cladocerans to both esfenvalerate alone and in combination with prochloraz, the potentiation factors for the two taxa were similar. Thus, comparison of EC_{20}-values estimated on the basis of concentration–response curves for days 2, 4, and 7 showed that prochloraz enhanced the toxicity of esfenvalerate four to sixfold for copepods and three to sevenfold for cladocerans. Rotifers were not significantly affected by any of the treatments, though there was a tendency of a population increase when cladoceran and copepod populations decreased. In all invertebrate populations that showed response to the pesticide treatments, indications of stabilisation or the beginning of recovery occurred between day 7 and day 14 and full recovery was observed in some of the less affected populations of cladocerans, copepods, and chironomids after 28 days. The occurrence of the synergistic interactions between prochloraz and esfenvalerate in the microcosms and at environmentally realistic concentrations implies that the synergistic interactions may also take place in invertebrate communities in natural ponds and ditches being exposed to azoles and pyrethroids via for example runoff or drift. The question of how to deal with synergy between chemicals in the environment from a regulatory perspective is briefly discussed.

1. Introduction

The inclusion of mixture effects in the risk assessment of contaminants in the environment has been discussed for decades.

Models such as concentration addition (CA) and independent action (IA) have been developed that allow an estimation of the toxicity of mixtures on the basis of the toxicity of the single compounds. In most cases, these models give quite accurate estimations of the toxicity of mixtures (Belden et al., 2007). Some compounds can, however, enhance the toxicity of other compounds. Deneer (2000), Belden et al. (2007), and Cedergreen et al. (2008) all reported that, in approximately 5% of the studies reviewed, the observed effect was more than twofold greater than estimated from concentration addition. Azole fungicides

* Corresponding author. Tel.: +45 353 32426.
** Corresponding author. Tel.: +45 353 33397.
E-mail addresses: mban@life.ku.dk (M.-B.A. Bjergager),
ncf@life.ku.dk (N. Cedergreen).

are among the compounds that can enhance the effect of other xenobiotics. Previous studies have shown enhancement of the toxicity of pyrethroid insecticides towards both aquatic and terrestrial species such as the honeybee *Apis mellifera* (Colin and Belzunces, 1992; Pilling and Jepson, 1993; Pilling et al., 1995; Meled et al., 1998; Vandame and Belzunces, 1998; Papaefthimiou and Theophilidis, 2001). Laboratory experiments with the standard aquatic test organism *Daphnia magna* showed that the imidazole prochloraz can enhance the toxicity of the pyrethroids esfenvalerate by a factor of 6 (Cedergreen et al., 2006) and alpha-cypermethrin by a factor of 12 (Nørgaard and Cedergreen, 2010). Nørgaard and Cedergreen (2010) furthermore showed that the triazoles epoxiconazole and propiconazole enhance the toxicity of alpha-cypermethrin six- and sevenfold, respectively.

Both the azole fungicides and the pyrethroid insecticides are widely applied to agricultural fields and may enter the aquatic environment via runoff (Anderson, 1989; de Jonge et al., 1998; Riise et al., 2004; Werner et al., 2004; Weston et al., 2004). Environmental azole concentrations of up to 175 μg/L (Elsaesser and Schulz, 2008) and pyrethroid concentrations of up to 0.72 μg/L (Styczen et al., 2003; Werner et al., 2004) have been reported. Pyrethroids act by binding to the potassium channels in the nerve cells, thereby disrupting normal nerve function (Casida, 1980), whereas the azoles inhibit P450 monooxygenases, of which some are involved in the degradation of xenobiotics in a range of organisms. Thus, the synergistic interaction between azoles and other xenobiotics is hypothesised to be caused by a slower degradation of xenobiotics in the organisms in the presence of azoles (Cedergreen et al., 2006). Synergy through this mechanism will only take place for xenobiotics where the first step in the degradation is oxidation and in organisms that use this mechanism as the main route of degradation of xenobiotics. As both the azoles and pyrethroids have log K_{OW}-values in the range of four and seven, respectively (Tomlin, 2003), they sorb strongly to soil, sediment, and plants. Hence, it was hypothesised that under natural conditions where sorption surfaces are plenty and the pesticide concentrations added within a realistic range, synergy will not be as pronounced as observed in laboratory experiments where there is no organic phase the pesticides can bind to and where pesticides are added to obtain full effects (Cedergreen et al., 2006; Nørgaard and Cedergreen, 2010).

To test this hypothesis and to expand the knowledge of the range of organisms prone to azole induced synergy, the combination effects of the imidazole prochloraz and the pyrethroid esfenvalerate on different zooplankton groups, on chironomids and on the pelagic phytoplankton community were tested. This was accomplished by applying eight different pesticide treatments to 18 outdoor 12,000 L aquatic microcosms located at the University of Guelph (Guelph, ON). The microcosm treatments were selected to include azole and pyrethroid concentrations representative of those measured in the environment near agricultural applications. Nominal initial test concentrations of the pyrethroid esfenvalerate, corresponding to 25%, 10%, and 5% of a full field rate applied to water 30 cm deep were applied to simulate different rates of spray drift or surface runoff. A nominal initial concentration of prochloraz of 90 μg/L was chosen on the basis of the range of measured environmental concentrations (Elsaesser and Schulz, 2008) and laboratory toxicity studies found that this prochloraz concentration corresponded to less than a *D. magna* laboratory EC$_{0.01}$ (Nørgaard and Cedergreen, 2010). Hence, the applied concentration of prochloraz alone was not expected to cause any measurable additional toxicity to the toxicity of esfenvalerate. Effects on the zooplankton and phytoplankton communities were followed from just prior to treatment to the end of the 28 day study.

2. Materials and methods

2.1. Microcosm setup

The experimental design included a series of 18 microcosms of an approximate volume of 12,000 L (water depth: 1 m, diameter: 3.9 m) located at the University of Guelph (Guelph, ON), which have been described in detail in Bestari et al. (1998). Forty-six planting trays (52.1 cm × 25.4 cm × 5.7 cm; ITML Horticultural Products, Brantford, ON, Canada) were filled to a depth of 5 cm with sediment containing 9.96 ± 0.1% organic carbon and placed in a standardised pattern on the bottom of each microcosm to cover approximately 50% of the bottom area. Sediment was added to create natural substrates for aquatic macrophytes and sediment-living organisms. The microcosms were filled with water from an adjacent, well-fed irrigation pond. The water was circulated between the microcosms and the irrigation pond at a rate of 12,000 L/day for one month (starting on May 13th 2008) prior to pesticide treatment to establish identical zooplankton communities in all microcosms. One microcosm was not included in the circulation until May 20th due to replacement of the liner in this microcosm, but this reduction in duration of circulation did not lead to any measurable difference in the populations of invertebrates on day −1 compared to the rest of the microcosms (ANOVA: $p > 0.05$). During the first week of the circulation period, macrophytes (*Elodea canadensis*, *Myriophylum spicatum*, and *Potomageton* spp.) collected from ponds located at Guelph Correctional Park were seeded in the microcosms by introducing equal and sufficient amounts of each species on the water surface and letting them settle and establish in the sediment. Circulation was halted the day before treatment with pesticides (June 16th 2008).

2.2. Pesticide treatment and analysis

Technical grade prochloraz and esfenvalerate were used for treatment of the microcosms. Physicochemical properties, source, and purity are given in Table 1. Treatment solutions were prepared in acetone (99.5%, Caledon), giving an initial acetone concentration of 0.016 mL/L in the microcosms. Solvent controls were included. The following treatments were used: control, solvent controls, 90 μg/L prochloraz, 0.167, 0.333, and 0.833 μg/L esfenvalerate alone and 0.167, 0.333, and 0.833 μg/L esfenvalerate plus 90 μg/L prochloraz. All treatments were duplicated and added in a randomised design to the ponds. The treatment solutions were poured directly into the water column of each microcosm while mixing the water with a paint mixer for 10 min to ensure a homogeneous distribution of the pesticides. Concentrations of prochloraz and esfenvalerate in the water column were measured on days 0 (1 h), 1, 4, 7, 14, and 28 after pesticide application. Samples were extracted into dichloromethane, rotovapped and reconstituted into hexane prior to analysis using GC–MS in SIM mode. Prochloraz and esfenvalerate residues were quantified from a 5 point calibration curve using linear regression ($R^2 > 0.99$). On each sampling day for pesticide residues, three quality control samples were collected. The quality controls consisted of 1 L non-filtered water from the control microcosms spiked with prochloraz and esfenvalerate to give final nominal concentrations of 45,900 μg/L prochloraz and 300 μg/L esfenvalerate, respectively. The quality controls were extracted and analysed according to the same procedure as similar to the rest of the samples.

2.3. Zooplankton sampling and enumeration

Zooplankton communities in the microcosms were established from the established populations supplied by the circulated irrigation pond water. Sampling of zooplankton was conducted the