Ecotoxicology and Environmental Safety 72 (2009) 441–449

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Mixture toxicity assessment of wood preservative pesticides in the freshwater amphipod *Gammarus pulex* (L.)

Olivier Adam, Pierre-Marie Badot *, François Degiorgi, Grégorio Crini

Chrono-environment Department, UMR 6249 CNRS - Université de Franche-Comté, Place Leclerc, F-25030 Besançon Cedex, France

ARTICLE INFO

Article history:
Received 6 February 2008
Received in revised form
23 July 2008
Accepted 27 July 2008
Available online 2 September 2008

Keywords:
Wood preservative
Mixture toxicity
Additive
Gammarus pulex
Cypermethrin
Propiconazole
Tebuconazole
IPBC

ABSTRACT

All over the world, insecticides and fungicides are used to protect wood against pathogens. To document the environmental toxicity of wood preservative mixtures, freshwater amphipods *Gammarus pulex* (L.) were submitted to organic pesticides given independently or in mixtures. When given independently at environmentally realistic concentrations, propiconazole and tebuconazole (triazoles fungicides) were not toxic for *G. pulex*, 3-iodo-2-propinyl butyl carbamate (IPBC, fungicide) was moderately toxic, and cypermethrin (pyrethroid insecticide) was extremely toxic. 96-h LC_{50} were, respectively, 4703, 1643, 604, and 0.09 $\mu g\,L^{-1}$. When amphipods were submitted to a mixture mimicking the composition of a commercial solution (18.2% of cypermethrin, 45.8% propiconazole, 17.2% tebuconazole, 18.8% IPBC), the overall toxicity was equal to that of the most toxic component, namely cypermethrin. But, when organisms were submitted to the real commercial mixture containing pesticides, solvents and additives, the toxic effects were markedly higher. Moreover, a third mixture with only 0.002% cypermethrin showed lethality 2.5–18-fold higher than those predicted by the commonly used models. The present results show that toxicity of wood preservative mixtures cannot be assessed starting only from the toxicities of each single component. Furthermore, the present data strongly suggest that the environmental impacts of wood preservative mixtures might be frequently underestimated.

1. Introduction

All over the world, wood, especially from coniferous trees, may be attacked by pathogenic agents such as xylophagous insects or lignivorous fungi. To avoid alterations of the wood mechanical qualities, and consequently economic loss or lifespan reduction, treatments with insecticides and fungicides at different stages of production were given in tree nurseries, during wood storage, or at the sawmill stage (Rayzal, 1998; Juntunen and Kitunen, 2003) .

In the past, European sawmills were frequently water-powered and thus they have been established very close to the forests in basin heads which are particularly vulnerable aquatic ecosystems. The risk of wood preservative transfers and impacts into the aquatic environment has been recognized as very high in such areas (Gifford et al., 1996; Lyytikäinen et al., 2001; Hingston et al., 2002, 2006). Following chronic or accidental pollution, wood preservatives are known to exert marked effects in macrobenthic and fish communities (Kingsbury and Kreutzweiser, 1987; Kreutzweiser and Kingsbury, 1987; Mian and Mulla, 1992; Lebkowska et al., 2003).

Wood preservative commercial solutions very often contain insecticide and fungicide mixtures. Propiconazole, tebuconazole, 3-iodo-2-propinyl butyl carbamate (IPBC), and cypermethrin are among the most frequently used chemicals to protect wood. Two of these pesticides, propiconazole and tebuconazole are triazole fungicides, displaying similar physiological effects: they are 14α-demethylase inhibitors and also referred to as ergosterol biosynthesis inhibitors via cytochrome P450 inhibition (Egaas et al., 1999; Iwasa et al., 2004). Tebuconazole is frequently used in agricultural areas (Berenzen et al., 2005) and propiconazole is one of the most widely distributed pesticides in the world (Castillo et al., 1997; Kreuger, 1998; Kronvang et al., 2003). IPBC is a halogenated unsaturated carbamate fungicide mainly used as wood preservative (Bailey et al., 1999). Juergensen et al. (2000) hypothesized that its fungicidal property was related to the terminal iodine, whereas Jarrad et al. (2004) proposed that carbamate pesticides could act on different physiological targets by disturbing the acetylcholine esterase activity. Another commonly used pesticide in commercial mixture is cypermethrin, a pyrethroid insecticide which exerts very severe toxic effects on aquatic invertebrates. Synthetic pyrethroids are among the most widely used insecticides around the world (Hill et al., 1994; Amweg et al., 2005). Pyrethroids act by slowing the gating of the voltage-dependent sodium channels, thus leading to a sustained

* Corresponding author. Fax: +33 381 665 797.
E-mail address: pierre-marie.badot@univ-fcomte.fr (P.-M. Badot).

0147-6513/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.ecoenv.2008.07.017

membrane depolarization of motor neurons (Bradbury and Coats, 1989).

In the natural environment, aquatic organisms are most often exposed to a large range of potentially toxic chemicals at the same time (Kreuger, 1998; Neumann et al., 2002, 2003; Berenzen et al., 2005). Furthermore, pesticides are generally used as commercial mixtures, whereas the vast majority of available toxicity data deals with the effects of single pure chemicals. Two basic concepts have been generally used for predicting multiple mixture toxicity: concentration addition (CA, Loewe and Muischnek, 1926) and independent action (IA, Bliss, 1939). The CA concept is devoted to similarly acting toxicants. Sprague (1970) proposed a derived concept: the toxic unit approach (TU). In this hypothesis any component can be replaced by another if they display the same action mechanism as long as the corresponding relative toxic potency allows to obtain a similar toxic unit. It has been proved that this model provides highly accurate predictions of mixture toxicity when all of the components have a strictly similar mode of action, regardless of their levels and ratios in the mixture (Faust et al., 2001; Zwart and Posthuma, 2005; Junghans et al., 2006). However, the CA model is not adapted to mixtures with components having dissimilar modes of action because it leads to an overestimation of the toxicity of such mixtures (Faust et al., 2003).

The IA model is based on dissimilar actions of mixture components. In this approach, the toxicity of each component is independent and cannot be replaced by another. The basic idea of this approach is that different compounds act on different physiological systems within the exposed organisms and lead to a common toxicological endpoint. This model provides accurate predictions of the mixture toxicity when all of the components have dissimilar modes of action, regardless of their levels and ratios in the mixture (Faust et al., 2003). However, the IA model is not adapted to mixtures with similar acting components because it leads to an underestimation of the overall toxicity (Faust et al., 2001; Junghans et al., 2006). Recently, Zwart and Posthuma (2005) proposed a mixed two-step approach for mixed-model (MM) calculations. The predictability assessment of the available models is still an opened question (Backhaus et al., 2003; Zwart and Posthuma, 2005; Junghans et al., 2006).

In this context, we assess the environmental toxicity of wood preservative mixtures on aquatic biota starting from commercial mixtures and then compare it to model predictions based on single chemical toxicity data. The freshwater amphipod *Gammarus pulex* (L.) (Crustacea, Amphipoda) has been chosen as test-organism because of its ecological and ecotoxicological importance. This crustacean species is one of the most widespread invertebrates in European streams and it is a major component of the biomass of many streams (Welton, 1979). As a detritus feeder, *G. pulex* plays a key role in nutrient cycling in freshwater systems (Welton, 1979) and *Gammarus* species are among the most eaten prey for many fish species (Bollache et al., 2006). *G. pulex* is known to be sensitive to a wide range of pollutants and to be among the most sensitive aquatic invertebrates (Helson and Surgeoner, 1986; Mian and Mulla, 1992; Schulz and Liess, 1999; Wogram and Liess, 2001; Cold and Forbes, 2004; Van Wijngaarden et al., 2004; Bloor et al., 2005). This amphipod species can be easily grown in the laboratory and has been recommended for use in toxicity tests (McCahon and Pascoe, 1988a, b).

Freshwater amphipods *G. pulex* (L.) were exposed to propiconazole, tebuconazole, IPBC, and cypermethrin given separately or in mixtures. Then, mixture toxicities were modelled using CA, IA, and MM. The modelled toxicity was compared with the measured mixture toxicity.

2. Materials and methods

2.1. Chemicals

Solvents were purchased from Fluka (Buchs, Switzerland). Florisil© 100–200 mesh used in chemical analysis was purchased from Supelco©. Propiconazole, tebuconazole, cypermethrin and IPBC (Pestanal®) were purchased from Sigma-Aldrich GmbH (Seelze, Germany).

Chemicals, CAS numbers, purity and solvent used for dilution are listed in Table 1. These chemicals were diluted in mineral water (Volvic® from Danone) that has constant physico-chemical characteristics (Table 2). These dilutions were made immediately before use.

2.2. Test organisms

G. pulex (L.) free from parasites were collected from an unpolluted stream (Ruisseau de la Fontaine des Ermites, France, N47°24′43″ E006°03′32″). Individuals were acclimated in freshwater (Volvic®) to laboratory conditions at least 10 days prior to testing. *G. pulex* (L.) were fed with *Alnus glutinosa* leaves following McCahon and Pascoe (1988a). Temperature was maintained at 15 °C during their stay in the laboratory and we only used large individuals (>6 mm) for acute toxicity tests, following Stephenson (1983).

The present study was conducted in accordance with national and institutional guidelines for the protection of animal welfare.

2.3. Acute toxicity tests

Ten *G. pulex* adults (>6 mm) were randomly chosen and inserted into a test chamber (a 100 mL glass container) that was maintained at 15 °C. For each acute test concentration, six replicates were used. The mortality was observed after 24, 48, 72, and 96 h of exposure.

Table 2
Composition of mineral water (Volvic©) used for dilutions and laboratory acclimatization of *Gammarus pulex*

Chemicals	Concentration ($mg\,L^{-1}$)
Calcium	11.5
Magnesium	8.0
Sodium	11.6
Potassium	6.2
Bicarbonates	71.0
Chlorites	13.5
Nitrates	6.3
Sulfates	8.1
Silica	31.7
Total mineralization (dried residues at 180 °C)	130 mg
pH	7.0

Table 1
Main features of the tested chemicals: cypermethrin, IPBC, propiconazole, tebuconazole, and solvents used for dilutions

Chemicals	CAS no.	Molecular weight ($g\,mol^{-1}$)	Log K_{ow}	Purity (%)	Solvent	Maximum solvent concentration (% v/v)
Cypermethrin	52315-07-8	416.32	6.6	97.0	Acetonitrile	0.01
IPBC	55406-53-6	281.09	2.8	97.0	Acetone	0.10
Propiconazole	60207-90-1	342.23	3.7	98.6	1-Methoxy-2-propanol	0.07
Tebuconazole	107534-96-3	307.80	3.7	99.6	Acetone	0.17