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Rapid Communication

Toxicity and bioavailability of atrazine and molinate to the freshwater shrimp (*Paratya australiensis*) under laboratory and simulated field conditions

Yin Latt Phyu[a,b,*], M.St.J. Warne[b,c,1], R.P. Lim[a,b]

[a]*Department of Environmental Sciences, Institute for Water and Environmental Resource Management, University of Technology, Sydney (UTS), Westbourne Street, Gore-Hill, NSW 2065, Australia*
[b]*EPA/UTS Centre for Ecotoxicology, P.O. Box 29, Lidcombe, NSW 1825, Australia*
[c]*Ecotoxicology and Water Science Section, New South Wales Environment Protection Authority (EPA), Australia*

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Abstract

Acute (96-h) semistatic toxicity tests were conducted by exposing the freshwater shrimp, *Paratya australiensis*, to atrazine and molinate in laboratory water and in river water both with and without sediment. The median lethal concentrations (LC50) and 95% fiducial limits of atrazine for *P. australiensis* in laboratory water in the absence and presence of sediment were 9.9 (8.6–11.5) and 6.8 (5.4–8.5) mg/L, respectively, while the corresponding values in river water were 9.8 (8.5–11.2) and 6.5 (5.4–7.8) mg/L, respectively. For molinate, the LC50 values in laboratory water in the absence and presence of sediment were 9.2 (7.0–12.1) and 9.0 (6.8–12.0) mg/L, respectively and the corresponding values in river water were 8.7 (6.4–11.8) and 8.2 (6.6–10.2) mg/L, respectively. Neither the river water nor the presence of sediment significantly ($P<0.05$) reduced the bioavailability of either chemical to *P. australiensis*. This was unexpected, as studies with other aquatic organisms have shown that sediment significantly reduced the bioavailability of these chemicals.

Keywords: Atrazine; Molinate; Shrimps; LC50; Herbicide; Toxicity

1. Introduction

The use of herbicides in agriculture has undoubtedly contributed to increased crop yields; however, off-site migration may cause contamination of nearby natural waterways (Ohyama et al., 1986, 1987; Yamagishi and Akiyama, 1981). Atrazine and molinate are both very extensively used herbicides. For example, over 24 million kg of atrazine was applied to corn and related silage crops and over 12 million kg of molinate were used annually between 1995 and 1997 in the USA (National Agricultural Statistics Service, 1997). In the Murrumbidgee Irrigation Area (MIA), which is just one agricultural region of NSW, Australia, 81 384 kg of the active ingredient of molinate and 2552 kg of the active ingredient of atrazine are used annually (Simpson and Haydon, 1999). Atrazine and molinate have aqueous solubilities of 30 and 880 mg/L, respectively, and logarithms of their octanol–water partition coefficients of 2.5 and 2.9, respectively (Tomlin, 2000).

Atrazine, due to its high aqueous solubility and only moderate ability to adsorb onto soils (Francioso et al., 1992), is readily leached from sites of application to

*Corresponding author. Department of Environmental Sciences, Institute for Water and Environmental Resource Management, University of Technology, Sydney (UTS), Westbourne Street, Gore-Hill NSW 2065, Australia. Fax: +61-2-95144079.
E-mail address: ylphyu@uts.edu.au (Y.L. Phyu).
[1]Current address: Centre for Environmental Contaminants Research, CSIRO Land and Water, PMB 2, Glen Osmond, South Australia 5064, Australia.

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surface drains or water courses (Williams et al., 1991). In soil column leaching studies, molinate leached more readily than other thiocarbamate herbicides, with very little of the molinate applied to the surface remaining in the upper 3 inches of the soil column (Gray and Weierich, 1968). Molinate has a half-life of 8–25 days but can persist for up to 160 days in flooded soils (Tomlin, 2000). The half-life of atrazine ranges from 15 to 20 days in an estuarine water/sediment microcosm (Jones et al., 1982). In a similar flask microcosm study (Mersie et al., 1998), atrazine primarily degraded to hydroxyatrazine (HA), and a large fraction of the residue remained bound to the sediment even after 252 days of incubation. The potential impact of atrazine on biota associated with sediment would diminish with time because of dilution in the aqueous phase, transformation to HA, and bound residue formation (Mersie et al., 2000).

Given their extensive use and their physicochemical properties, it is not surprising that atrazine and molinate are routinely found in waterways in agricultural regions. For example, average atrazine concentrations in Lakes Erie and Ontario in the USA ranged from approximately 20 to 35 ng/L (Schottler et al., 1998). In Australia, atrazine was detected in 50% of observation wells in the irrigation areas near Shepparton, Victoria (Bauld et al., 1992), while in the irrigation areas and pine plantations of southeast South Australia it was detected in groundwater at four of the eight sites tested, with concentrations ranging from <0.02 to 2.0 μg/L (Stadter et al., 1992). Similarly, molinate was regularly detected in most agricultural drains in the MIA and often exceeded the Australian water quality guidelines (ANZECC (Australian and New Zealand Environment and Conservation Council), 1992) both for drinking water and for the protection of the aquatic environment (Bowmer et al., 1998).

The presence of these herbicides in waterways has led to concerns about potential deleterious impacts on nontarget aquatic organisms (including plants) and/or aquatic ecosystems. The vast majority of toxicity tests are conducted using highly purified laboratory water under highly controlled conditions. However, natural water may contain dissolved organic matter, suspended particulate matter and sediment. Such phenomena can affect the toxicity and bioavailability of herbicides (e.g., Akkanen et al., 2001; Nikkila et al., 2001; Phyu et al., 2004).

The objective of this study was therefore to determine the effects that river water and sediment have on the toxicity and bioavailability of atrazine and molinate to the freshwater shrimp, *Paratya australiensis*. This study is the second in a series investigating these issues for a suite of organisms that cover different trophic levels and taxonomic groups (Phyu et al., 2004).

2. Materials and methods

2.1. Test solutions

Both the atrazine (6-chloro-N^2-ethyl-N^4-isopropyl-1,3,5-triazine-2,4-diamine; CAS No. 1912-24-9) and molinate (s-ethyl *N*,*N*-hexamethylenethio carbamate; CAS No. 2212-67-1) used in this study were reagent-grade technical material ($\geqslant$97% purity). The atrazine was kindly provided by Novartis Crop Protection Australasia Pvt., Ltd. Stock and working solutions of atrazine were prepared in analytical-grade acetone (99% purity) as a carrier solvent. Molinate solutions were prepared without a carrier solvent in reverse osmosis water. The herbicide stock solutions were stored in a freezer at −4 °C. All working stock solutions were made immediately prior to use.

2.2. Collection of water, sediment, and shrimp

River water and shrimps (*P. australiensis*) were collected from the Upper Colo River (33°26′ south, 150°51′ east) located approximately 97 km to the northwest of Sydney, Australia. The site of collection is immediately downstream of the Wollemi National Park. This river is a "protected river," indicating that it is one of the cleanest and least polluted rivers in NSW, Australia (NSW and Department of Environment and Planning, 1983; Birch et al., 1998). The river water was transported back to the laboratory and then stored in a cold room at 4 °C. The physicochemical parameters, i.e., dissolved oxygen content, conductivity and pH, when the river water was collected were in the ranges 93.6–94.7%, 246–252 μS/cm, and 6.89–6.93, respectively. The organic carbon (TOC) content in the river water is 6 mg/L. The shrimps were transported back to the laboratory in an insulated container that was aerated.

The sediment used in the toxicity tests was collected from the riverbank (33°27′ South, 150°52′ East) approximately 5 km downstream from that used for the collection of water. Only the top 2–3 cm of sediment were collected. This was placed in plastic bags and transported to the laboratory. The sediment was sieved using a 1-mm mesh sieve to remove large particles and then stored in a dark cold room at 4 °C for no longer than 2 weeks prior to use. The percentage of sand, silt, and clay in the sediment was 78%, 12%, and 10%, respectively. The total organic carbon content, effective cation exchange capacity (ECEC), pH, and specific moisture content of the sediment were 8.6%, 9.35 mgq/100 g-dry weight basis, 5.4, and 37.5%, respectively. The river water and sediment were analyzed by GC with a nitrogen phosphorus detector (NPD) detector for herbicides using the methods described below, prior to use in the tests. The analytical results showed that the