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Probabilistic risk assessment of diuron and prometryn in the Gwydir River catchment, Australia, with the input of a novel bioassay based on algal growth

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ABSTRACT

A probabilistic risk assessment of the selected herbicides (diuron and prometryn) in the Gwydir River catchment was conducted, with the input of the EC_{50} values derived from both literature and a novel bioassay. Laboratory test based on growth of algae exposed to herbicides assayed with a microplate reader was used to examine the toxicity of diuron and prometryn on the growth of *Chlorella vulgaris*. Both herbicides showed concentration dependent toxicity in inhibiting the growth of *Chlorella* during the exposure period of 18–72 h. Diuron caused more toxicity as judged by growth rates than prometryn. Thalaba Creek at Merrywinebone was identified as the 'hotspot' for diuron and prometryn risk in the Gwydir catchment. The use of microplate assays coupled with probabilistic risk assessment is recommended for rapid assessment of ecotoxicity of indigenous species, allowing identification of locations in river catchments requiring environmental management.

1. Introduction

Herbicides are the most widely used of chemical pesticides for agricultural production and landscape management. For example in the U.S., herbicides account for about 70 percent of all pesticides used (Kellogg et al., 2002). The environmental risk of herbicides should be evaluated near sites of use, even though basic ecotoxicological tests have been conducted before they can be registered for marketing. For example, diuron and prometryn, which are both used globally are photosynthetic PSII herbicides that are considered only slightly or moderately toxic to mammals and humans; however concerns have arisen because they are members of a class claimed to be carcinogenic, or may affect the development as reproductive toxins (Orton et al., 2009; Kegley et al., 2010). For this reason, more reliable evidence is needed to test these claims and investigate their ecological effects.

The Gwydir River catchment is an agriculturally and ecologically important catchment located in northern New South Wales, Australia. The land use in the catchment is predominantly agricultural production, with grazing being the dominant practice and a variety of cropping practices with cereal and irrigated cotton being the dominant types. The Gwydir wetlands, an area of ecological significance in Gwydir catchment, supports many migratory and native bird species, as well as many other fresh water organisms, including crustaceans, native and exotic fish species. The aquatic ecosystem may be in danger from runoff contaminated with herbicides such as diuron and prometryn, which are used in the watershed. A suitable way was needed to assess the risk of the herbicides in this catchment.

Probabilistic risk assessment (PRA) allows more quantitative evaluation of the overall risk of polluting chemicals to ecosystems rather than relying on data for single species (Solomon et al., 2000), preferably given the availability of toxicity data on local biota. Fast and accurate methods for evaluating the toxicity and estimating risk are urgently needed to obtain the necessary data for effective PRA.

A number of bioassays have been reported for the assessment of chemical toxins on aquatic ecosystems; inter alia, these include bacterial tests, fish, coral, algae and water flea tests et al. (Jones et al. 2003; Huang et al. 2005; Relyea 2009). Microalgae are considered to be sensitive indicators of various toxicants (Bengtson-Nash et al., 2005; Ma et al., 2006) because they are frequently found in aquatic environments, and are a vital component for primary production, responding rapidly to environmental changes due to their unicellular structure and short generation times.

The standard bioassays of algae, i.e. ISO 8962 (1989) and the OECD 201 (1984), are proven methods for evaluating the toxicity of chemicals. However, the experimental formats are labor intensive, specifying large sample volumes, requiring large areas of benchspace,

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and generating large volumes of waste. Since the 1990s, many researchers have attempted to optimize the procedure, performing tests at microscale using cuvettes, scintillation tubes, or microplates (Arensberg et al., 1995; Nguyen-Ngoc et al., 2009; Paixao et al., 2008; Rojiekova et al., 1998). The 24- or 96-well algal growth inhibition assay performed in a microplate, gave comparable results to standard flask bioassay for the tested algae (Thellen et al., 1990; Rojiekova et al., 1998; Eisentraeger et al., 2003; Horvatic et al., 2007; Riedl and Altenburger, 2007). This simpler method has been used in routine toxicity testing, pollutant phytotoxicity screening, and screening of sensitivity of algae. In terms of pollutants, many heavy metals, and organic pollutants, have been tested with these micro-scale methods, however literature reports of assessments for the herbicides diuron and prometryn are rare (Gomez De Barreda Ferraz et al., 2004; Gregor and Maralek, 2005; Paixao et al., 2008).

In this paper, a bioassay based on growth of algae exposed to herbicides assayed with a 96-well microplate reader was used to examine the effect of diuron and prometryn on the growth of *Chlorella*. These experimental data were further supplemented by undertaking a risk assessment of diuron and prometryn in the Gwydir River catchment using the EC_{50} values generated from the laboratory test and the US EPA ECOTOX database, in order to develop and validate a novel bioassay on evaluating the toxicity of herbicides on algae and to investigate the potential effectiveness of applying PRA of diuron and prometryn in the Gwydir River catchment of northern New South Wales. This information will have potential value for risk management of pesticides used in this catchment.

2. Materials and methods

2.1. Algal cultures

Chlorella vulgaris was obtained from School of Biological Sciences, University of Sydney and grown in MBL medium (Nicholls, 1973). Cultures were grown in a semicontinuous manner in 500 mL Erlenmeyer flasks, where 100 mL of the total 200 mL culture was discarded daily and 100 mL of fresh medium was added to the culture. Cultures were kept on an orbital shaker at 120 rpm and 25 °C and under continuous white lights at about 50 μmol of quanta (400–700 nm) m^{-2} s^{-1}.

2.2. Chemicals and reagents

Diuron (99.7 percent pure) and prometryn (purity 97 percent) were obtained from Sigma-Aldrich Chemical Company, Castle Hill, Australia. All chemicals used in the media preparation were laboratory reagent standard. The chemical properties of the tested chemicals relevant to their toxicity are shown in appendices Table A.

2.3. Microplate bioassay

The microplate bioassays were performed according to Rojiekova et al. (1998), Eisentraeger et al. (2003) and Paixao et al. (2008) with a modification. The algae were exposed to various dose of diuron (at nominal concentrations 0.125, 0.25, 0.5, 5, 10, 50 μg L^{-1}) and prometryn (at nominal concentrations 0.5, 5, 10, 25, 50, 100 μg L^{-1}) for 72 h. Growth of cultures was measured at 540 nm wavelength using a microplate reader (Multiskan Ascent model 353 from Labsystems, Helsinki) twice a day.

Test cultures containing the desired concentrations of diuron and prometryn and the desired quantity of algal inocula were prepared by dilution with sterile algal medium aliquot of stock solutions of herbicides and algal suspensions. The inocula were obtained from a pre-culture which was incubated semi-continuously under test conditions and used when cells were growing exponentially.

Growth inhibition tests of herbicides were performed in 96-well, sterilized polystyrene microplates, with lids and flat bottoms. The final test volume was 150 μL per well. The microplate bioassays were conducted with twelve replicates of each test concentrations of pesticides, and controls (culture medium and algae).

The microplates were placed on an orbital shaker oscillating at 120 rpm and 25 °C, under continuous white fluorescent lights (~50 μmol m^{-2} s^{-1}). The exact location under the light bank was randomly alternated after each absorbance reading. The microplates were automatically shaken for 10 s at 960 rpm before each reading.

Plots of growth curves were graphed and inspected visually to collect data on variations in growth pattern in addition to calculating apparent exponential doubling times. Growth rates for 72 h were calculated and then the percent inhibition for each treatment replicate was determined. ANOVA and Student Newman–Keuls multiple range test was used to determine if treatments were significantly different from each other. Results were deemed significantly different at the probability level P less than 0.05. EC_{50} (0–72 h) values with standard deviation were estimated by the linear regression of probit of percentage growth on $\log_{10}$ of the concentration of herbicides. All statistical analyses were performed using the software packages SPSS.

2.4. Risk assessment

To provide a context to predict ecological effects in the field, probabilistic risk assessment (PRA) comparing distributions of actual exposure concentrations in surface waters and published species sensitivity data from laboratory toxicity studies including our results was used to define the relationship between measures of effects and assessment end points.

Diuron and prometryn exposure data from 12 monitoring sites were collected from the Gwydir River catchment of northern New South Wales, Australia by the NSW Department of Water and Energy for the period 1991–2007. Sampling was conducted monthly independently of chemical use, but with two or three samples per month collected for months that were perceived to be riskier because of higher rainfall and during the cotton crop growing season.

Species sensitivity distributions (SSDs) for each herbicide were constructed with LC_{50} and EC_{50} values to determine the sensitivity of aquatic animals, plants and algae. Toxicity data employed in the SSDs were developed from toxicity data gathered from the US EPA ECOTOX database (http://www.epa.gov/ecotox), and the results of the laboratory test mentioned above. The measurement endpoints considered all laboratory toxicity data related to growth. 163 records for EC_{50} or LC_{50} of diuron and 51 records of prometryn were selected. Where more than one toxicity value was available for a single species, the geometric mean was calculated to favor the more sensitive studies (Maltby et al., 2005; Rand et al., 2010). Toxicity data for 60 species by diuron and 35 species by prometryn were finally derived to construct the SSD for the two herbicides.

The potential risk of the herbicides in the Gwydir catchment freshwaters were examined by integrating the probability distributions of the exposure concentrations by sites with the SSDs of each herbicide. Distributions of exposure and species sensitivity were constructed by the methods of USEPA (1998), Solomon et al. (2000), and Maltby et al. (2005). The degree of overlap between the exposure distributions and the SSDs was used to estimate the number of species affected given a percentage of the time (Solomon et al., 1996). Where the exposure and species sensitivity distributions overlap at the five percent species sensitivity threshold denotes risk. The probability that the five percent effect threshold (based on Australia standard regulatory approach) can be exceeded at any time can then be determined. When the calculation of risk was completed, the output was applied to Geographical Information Systems software (ArcGIS) allowing for the identification of hotspots for exposure in the catchment.

3. Results

3.1. Microplate bioassays

Plots of growth curves of diuron and prometryn (Fig. 1a and b) showed that the algae displayed exponential growth during the test period. Importantly, both diuron and prometryn showed toxicity in terms of both herbicide concentration and time dependent toxicity in inhibiting the growth of *Chlorella* during the exposure period of 18–72 h (Two-way ANOVA analysis, $P=0.000$ for both the variables of time and herbicides concentrations). Post hoc tests showed that significant inhibition of growth started on the 21st hour, and the inhibition increased significantly at the end of day -2 and day-3 ($P<0.05$).

The average growth rates of algae at 0–72 h for diuron are shown in Fig. 2(a). The concentration of diuron that caused significant effects ($P<0.05$) on the algal growth with respect to control values ranged from 0.125 μg L^{-1} to 50 μg L^{-1}. Reduction in growth rates was observed with an increase of diuron concentrations. The growth inhibition of algae treated with 25 μg L^{-1} and 50 μg L^{-1} of diuron were 36.9 percent and 56.2 percent respectively. The EC_{50} for the growth rates inhibition was calculated as 45 ± 5 μg L^{-1} (Pearsons $R=0.989$).