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Toxicity of Endosulfan to *Paratya australiensis* Kemp (Decapoda: Atyidae) and *Jappa kutera* Harker (Ephemeroptera: Leptophlebiidae) in Field-Based Tests

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Changes observed in the macroinvertebrate communities in the rivers of cotton growing regions in Australia are due to the combined effects of a suite of pesticides and herbicides, although impacts observed on common taxa in the Namoi River have been attributed particularly to the organochlorine pesticide endosulfan (Leonard et al. 1999;2000). The greatest concentrations of endosulfan in rivers are likely to result from aerial overspray or storm runoff from adjacent farms, which create acute exposure scenarios. Although rare, overspray can result in endosulfan concentrations in water bodies (1 m deep) exceeding 70 μg/L (Edge et al. 1999), but which may be rapidly diluted in rivers. Runoff from cotton fields during storm events contributes significant loads of endosulfan into rivers, and elevated concentrations of endosulfan resulting from runoff may persist for several days (Muschal 1997). Low-level concentrations of endosulfan persist throughout the pesticide spray season as a result of spray drift and vapour movement of pesticides (Raupach et al. 2001).

Acute effects of endosulfan on macroinvertebrate communities have been shown in mesocosm experiments (e.g. Hose et al. 2002a;b), however, specific responses of key macroinvertebrate taxa are poorly known, or limited to laboratory studies that provide unrealistic test conditions and ignore critical factors that may influence endosulfan toxicity. In this study we aim to address this knowledge gap by assessing the acute toxicity of endosulfan to two common riverine macroinvertebrates, *Paratya australiensis* and *Jappa kutera*, from the Namoi River. Acute toxicity tests are conducted under field conditions with short durations to simulate storm runoff and overspray events. We relate acute toxicity to concentrations of endosulfan and abundances of macroinvertebrates recorded in field studies, and consider the contribution of acute exposure events to changes observed in field populations.

MATERIALS AND METHODS

The decapod crustacean *Paratya australiensis* is a small, transparent and quick-moving shrimp, which lives in a wide range of ecological conditions, but prefers large, still, fresh waters (Williams 1980). *Jappa kutera* is a slow swimming

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mayfly nymph found burrowing in gravel and sediment of stony streams in eastern and northern Australia (Peters and Campbell 1991). Both species are common across southeast Australia and both were found in benthic samples collected along the length of the Namoi River (Leonard et al. 2000).

Toxicity tests were conducted in a system of outdoor stream mesocosms that was constructed on the banks of the Namoi River near Gunnedah, NSW (-30° 58' 30" E, 156° 20' 41" N). The stream mesocosms (hereafter referred to as 'mesocosms') are described in detail by Hose et al. (2002a).

Paratya australiensis were collected from the Namoi River upstream of the cotton growing areas in the catchment. Animals 1.5 – 2.0 cm long were used for the toxicity tests. The animals were acclimated for at least 48 h prior to testing and during this period were fed commercial fish flakes, but were deprived of food for 10 h prior to test initiation. Toxicity tests were conducted in conjunction with two separate mesocosm experiments in January 1998 (Experiments 1 and 2). Detailed descriptions of these experiments, including dosing methods, chemical analysis, dose calculations and benthic macroinvertebrate sampling are given in Hose et al. (2002a;b). The 48 h exposure tests were conducted using river water in 1 L beakers that contained 800 mL of test solution. Beakers were partly submerged in the mesocosm, and thus subject to natural changes in environmental conditions. Each beaker contained five shrimp. Physico-chemical conditions during the tests are given in Table 1.

Table 1. Ranges of water quality variables measured during toxicity tests.

	Turbidity (NTU[1])	Dissolved Oxygen (% Sat.)	Conductivity (µS/cm)	Temperature (°C)	pH
Paratya australiensis					
Experiment 1	-	81-86	252 - 257	25.4 - 26.9	7.6 - 7.8
Experiment 2	6 - 17	72 - 84	262 - 268	25.3 - 26.7	7.4 - 7.9
Jappa kutera					
Experiment 3	19 - 38	30 -118	239 - 376	20.0 - 26.4	8.0 - 9.7

[1] Nephelometric Turbidity Units

The 12 h exposure tests were conducted using small cages that were submerged in the riffle section of each mesocosm. Cages were 10 cm long glass tubes with 2 cm diameter and 500 µm mesh covering both ends. Each cage contained a single animal and there were five cages per stream. During the 12 h exposure period, flow of water into and out of the mesocosms was stopped. Endosulfan was added to the mesocosms and conditions in the mesocosms were kept static for the entire 12 h exposure period. After the 12 h exposure period, the normal flow of water in the mesocosms was resumed. Tests were terminated at 48 h, i.e., 36 h after the 12 h exposure period.

Jappa kutera nymphs were collected from Lowry Creek, near its confluence with

the Namoi River, upstream of the town of Manilla, NSW. This site is relatively unimpacted by pesticides. Mayfly nymphs used for the tests were <10 mm in length (excluding caudal filaments). Toxicity tests were conducted during a 48 h endosulfan exposure experiment in January 1999 (Experiment 3) using the same cages as described for the *P. australiensis* tests.

Endosulfan was applied to the mesocosms for 48 h, with the toxicant renewed every six hours over this period. The flow of water into and out of the mesocosms was stopped and conditions in the mesocosms were kept static for each 6 h period. Separate cages containing 10 animals were exposed for either 12 or 48 h. Two cages were placed in the riffle section of each mesocosm, one cage for each exposure period. After 12 h exposure, one cage was removed from treatment mesocosms and placed in the clean water of a randomly selected control mesocosm for the remainder of the test. Cages in the control mesocosms were also removed and replaced to account for the effects of these manipulations. The cages receiving the 48 h exposure remained in the original mesocosms for the duration of the experiment. After 48 h, all cages were removed from the mesocosms and live mayflies within them were counted.

Toxicity data were analysed for LC1 and LC50 values (and 95% confidence intervals) using the maximum likelihood regression procedures in the Probit v1.5 program (U. S. Environmental Protection Agency, Cincinnati, OH). T-tests were used to compare mortality in solvent and water only controls. Data were first arcsine transformed and tested for normality and heteroscedascity. If not significantly different, data were pooled for LC1 and LC50 analyses. We include LC1 values as an approximate no-effect concentration.

Data on *J. kutera* and *P. australiensis* abundance in the Namoi River during the 1997/98 pesticide spray seasons are taken from Leonard et al. (2000). Detailed methodologies for its collection and analysis are given therein. These data were analysed for differences in the abundance of *J. kutera* and *P. australiensis* between reference and impacted sites at each time, and among reference, and test sites over time using two-sample t-tests ($\alpha = 0.05$) for unequal variances (Sokal and Rohlf 1981).

Endosulfan concentrations were measured using passive samplers deployed in the Namoi River over a period of approximately 30 days (Leonard et al. 2000). Leonard et al. (2002) reported concentration factors for endosulfan isomers (α and β) and endosulfan sulfate (the principal toxic metabolite) entering such passive samplers. Concentration factors were averaged for the three components and used to estimate average daily exposure concentrations in the field over the preceding month that the sampler was deployed.

RESULTS AND DISCUSSION

Both *P. australiensis* and *J. kutera* were acutely sensitive to endosulfan (Table 2). The sensitivity of both species increased significantly with increasing exposure

period. On the basis of the 48 h LC1 and LC50 values, *P. australiensis* was more sensitive to endosulfan than *J. kutera*.

In our tests under field conditions, *P. australiensis* was considerably more sensitive to endosulfan than in a previous laboratory test (Table 2), but the large difference in sensitivity is easily attributable to the previous study using nominal concentrations and being conducted in laboratory water. A more appropriate comparison lies with the related atyid shrimp *Caridina* sp. for which a measured 48 h LC50 value of 4.0 µg/L was determined in tests using animals and water from the Mehi River, also in the cotton-growing region of NSW (Sunderam 1990). The 48 h LC50 of 0.51 µg/L places *P. australiensis* among the most acutely sensitive of the locally occurring macroinvertebrate taxa that have been tested, and considerably more sensitive to endosulfan than a number of other crustacean species (see Hose et al. 2003 for review). Of the local taxa that have been tested, only the caddis fly larvae *Cheumatopsyche* sp. (48 h LC50 0.4 µg/L, Leonard et al. 1999) is more sensitive.

Table 2. Summary of lethal concentration (LC1 and LC50) values for *Paratya australiensis* and *Jappa kutera* exposed to technical endosulfan in field-based single species tests with 12 and 48 h exposure periods. Concentrations in µg/L.

Exposure/Test	LC1	LC50	Laboratory LC50
Paratya australiensis			
12 h Experiment 1	0.31 (0.01 -0.77)	6.35 (3.25-52.59)	
12 h Experiment 2	0.18 (0.03-0.42)	3.01 (1.84-5.09)	
48 h Experiment 1	0.03 (0.00-0.10)	0.96 (0.47 -2.30)	11.1 (8.8-13.4)[1]
48 h Experiment 2	0.06 (0.00-0.15)	0.51 (0.22-1.05)	
Jappa kutera			
12 h Experiment 3	0.05 (0.00-0.37)	6.68 (1.79-15.60)	
48 h Experiment 3	0.50 (0.00-1.62)	3.10 (0.26-5.39)	1.3 (0.8-2.2)[2] 2.0 (1.6-2.6)[2]

[1] Nominal concentration in clean water (Sunderam 1990).
[2] Measured concentration in Namoi River water (Leonard et al. 1999)

In contrast to *P. australiensis*, the sensitivity of *J. kutera* was similar to that in previous laboratory tests, which determined 48 h LC50 values of 1.3 and 2.0 µg/L (Leonard et al. 1999, Table 2). Our 48 h LC50 value for *J. kutera* was also similar to the measured 48 h LC50 value (2.1 µg/L) derived for benthic mesocosm populations (Hose and van den Brink 2004).

Peak concentrations of endosulfan in rivers resulting from aerial overspray or storm runoff are likely to be acutely toxic to both species. Overspray can cause concentrations of endosulfan up to 70 µg/L in waterbodies 1 m deep (Edge et al. 1999). Such concentrations are an order of magnitude greater than the 12 h LC50 values for both species, suggesting that acute effects from overspray are likely even for shorter exposure periods.

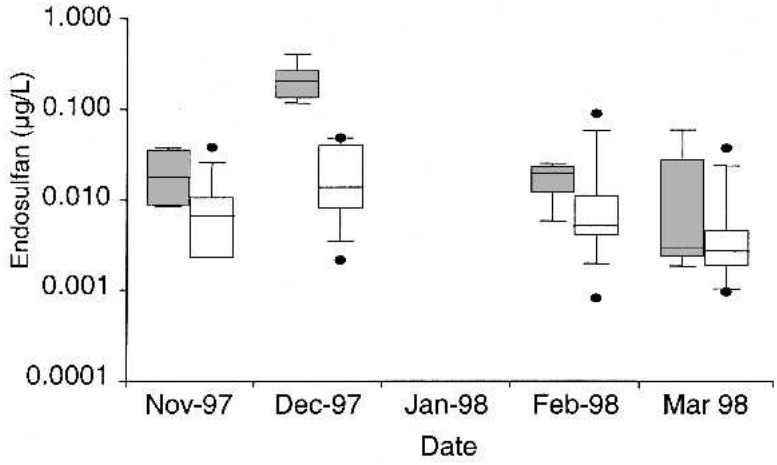

Figure 1. Box plots showing the average daily endosulfan concentrations extrapolated from passive samplers collected during 1997–98 pesticide spray season from pesticide impacted (shaded, n=5) and reference (unshaded, n=12) sites on the Namoi River (data from Leonard et al. 2000). Boxes represent the 25th and 75th percentiles centred about the mean, with the median (50th percentile) shown as a horizontal line within the box. The error bars represent the 10th and 90th percentiles and symbols (●) denote extreme values.

Total endosulfan concentrations exceeding 1.75 µg/L were measured in rivers during a storm event in the cotton-growing region of NSW, with elevated endosulfan concentrations persisting for 48 h (Muschal 1997). Interpolation of our 48 h toxicity data suggest that the peak concentration (1.75 µg/L) would result in 91% and 23% mortality in *P. australiensis* and *J. kutera* populations, respectively. On the basis of these data, an isolated storm event is likely to contribute substantially to the impacts on *P. australiensis* and *J. kutera* populations in the field. However, riverine endosulfan concentrations of this magnitude occur less than 1% of the time in this region (Muschal and Warne 2003).

Persistent low-level concentrations of endosulfan occur in rivers throughout the pesticide spray season as a result of spray drift and vapour movement (Raupach et al. 2001). Although influenced partly by concentrations during storm events, the passive samplers provide a time-integrated measurement of endosulfan in the river that reflects closely the day-to-day concentrations caused by drift and vapour movement. The average daily concentrations of endosulfan at impacted sites over the pesticide spray season ranged from 0.005 to 0.40 µg/L, with the highest concentrations occurring in December (0.11-0.40 µg/L) and presumably causing the greatest impact (Fig 1). At reference sites, the overall range of endosulfan was 0.01 to 0.09 µg/L (Fig 1). If concentrations at the reference sites reflect no effect values, these findings validate the current Australian water quality guideline trigger value for endosulfan (0.03 µg/L, ANZECC and ARMCANZ 2000) for protecting populations of our test species.

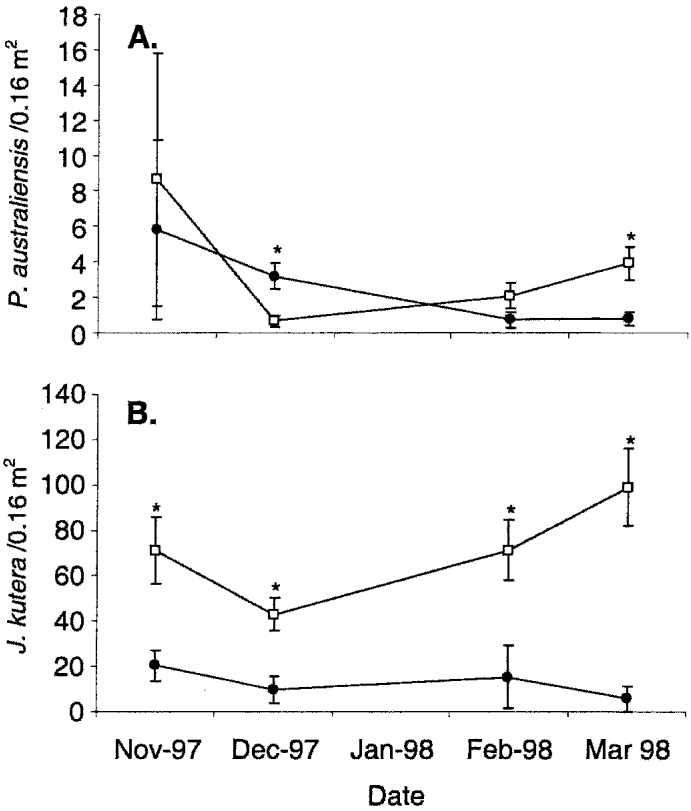

Figure 2. Mean (± standard error) abundance of A. *Paratya australiensis* and B. *Jappa kutera* nymphs in benthic samples collected during 1997–98 from reference (□) and pesticide impacted (●) sites on the Namoi River (data from Leonard et al. 2000). Asterisk (*) indicates significant difference in abundance ($P<0.05$) between reference and impact sites at that time.

Average daily concentrations of endosulfan at impact sites in December 1997 were below the 48 h LC1 value for *J. kutera*, thus suggesting average daily conditions in the river are not acutely toxic. However, impacts to *P. australiensis* populations might be expected since concentrations at all impact sites exceeded the 48 h LC1 values, and furthermore, the highest average daily concentration (0.40 µg/L) extrapolates to 42% mortality. Alarmingly, even the low average daily concentrations of endosulfan detected at reference sites are similar to the LC1 values for *P. australiensis*. Although detectable acute impacts at this level are unlikely, there is a likelihood of chronic effects occurring.

The mean abundances of *P. australiensis* were significantly different between reference and test sites in December and March, and for *J. kutera*, on all occasions. The apparent declines in abundance from November to December for both species (Fig. 2) were not statistically significant, in part due to large

variability in abundances in November, but we suggest these changes are biologically significant. At reference sites, this decline is in response to high river discharges from irrigation releases from the upstream dam (Leonard et al. 1999) rather than pesticide concentrations that were low at the time (Fig 1), but for impacted sites, the decline in abundance is likely to be a combination of these factors. Most importantly, however, the abundances of *P. australiensis* and *J. kutera* at reference sites increased significantly from December to March, but abundances in impacted sites further declined or remained the same over this period (Fig 2). This means that any chronic impacts on *P. australiensis* at reference sites are likely to be minimal. Our toxicity data support Leonard et al. (1999) who attribute this trend to toxic concentrations of endosulfan occurring in December (Fig. 1) that affected recruitment and early instar animals that subsequently failed to develop.

Impacts on field populations of *P. australiensis* are due to acute toxicity of daily endosulfan concentrations. Impacts from daily exposure will be exacerbated by peak concentrations during storm events, which are also acutely toxic. Impacts on this species are likely along the length of the Namoi River because daily concentrations, even in reference sites, are likely to cause chronic effects. For *J. kutera*, daily concentrations are not acutely toxic, but threats to populations exist from storm events and low-level chronic exposures. Future studies will consider the impacts of chronic exposures, particularly on the more sensitive *P. australiensis*.

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