January, August & Final Reports

REPORTS

Part 1 - Summary Details

Please use your TAB key to complete part 1 & 2.

CRDC Project Number: **UTS2C**

January Report:	☐	Due 29-Jan-01
August Report:	☐	Due 03-Aug-01
Final Report:	☒	Due within 3 months of project completion

Project Title: Relationship between Pesticides in Passive Samplers to River Water Concentrations and Macroinvertebrates.

Project Commencement Date: 1/7/99 **Project Completion Date:** 1/7/02

Research Program: Best Management Practice and Environment

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Part 3 – Final Report Format

The points below are to be used as a guideline when completing your final report.

1. Outline the background to the project.

In previous studies we have indicated a relationship between a decline in macroinvertebrate population densities and riverine endosulfan concentrations measured using passive samplers (Hyne et al., 1999; Leonard et al., 2000). These passive samplers, constructed of low density polyethylene membrane bags containing the solvent 2,2,4-trimethylpentane (TRIMPS), were then used in the Department of Land and Water Conservation (DLWC) NW region water quality program for comparison to traditional grab sampling procedures (Muschal, 1999). The TRIMPS detected three pesticides in river water that were not detected by routine manual sampling. The TRIMPS were also able to show that endosulfan and profenofos concentrations were higher downstream of irrigated agriculture than upstream of this area (Muschal, 1999). Environment Australia has also drawn attention to the utilisation of passive samplers in the Existing Chemical Review Program (ECRP) for endosulfan and for the registration of certain organophosphorus pesticides.

There was a need to develop a field validated model of the operation of passive samplers. The kinetics of pesticide uptake and release from the passive samplers needed to be understood. The influences of changes in river flow, turbidity and biofouling or ageing on the absorption of pesticides into the passive samplers also needed to be assessed. In addition, the influence of solvent type and frequency of sampling needed to be assessed in laboratory studies.

2. List the project objectives and the extent to which these have been achieved.

The objectives of the project were:

1. Determine in the laboratory, in the first instance, the effectiveness of TRIMPS in estimating river water concentrations for alpha and beta endosulfan, endosulfan sulfate and organophosphorus pesticides and then to field validate the laboratory model being developed in the rivers of northern NSW.

2. In relation to the implementation of BMP, to determine the concentrations of pesticides entering the Namoi River using *in situ* TRIMPS and correlate these pesticide concentrations to population densities of dominant riverine macroinvertebrates.

3. Review and examine the use of acetylcholinesterase activity of the head segment of riverine invertebrates at various developmental stages as a potential indicator of exposure to organophosphorus pesticides.

We investigated using a laboratory continuous flow system the kinetics of the pesticide uptake into, and release from, the passive samplers containing trimethylpentane. We found that the non-polar pesticides, with a log Kow > 3.5 obeyed first-order kinetics during their uptake into, and release from, the passive samplers. A manuscript describing the results was recently published in the international scientific journal, *Environmental Toxicology and Chemistry*.

We have also completed the analyses of the January 2001 and January 2002 field data and have compared the predicted concentrations for total endosulfan and chlorpyrifos to daily measured river water concentrations, when the cumulative measured values were >0.005 µg/L. In the 2001 study, the daily riverine total endosulfan concentrations (<0.001 to 0.066 µg/L) were low, but chlorpyrifos-ethyl was below the detection limits (<0.01 µg/L). The water concentrations of total endosulfan predicted from the passive samplers concentrations using the laboratory-derived concentration factors, were within 40-170% of the accumulated mean daily water concentrations. In the 2002 study, the daily river water total endosulfan concentrations were consistently low (0.001 to 0.005 µg/L), but chlorpyrifos-ethyl was detected in the river water with daily concentrations in the range of <0.01 to 0.24 µg/L. The water concentrations of chlorpyrifos-ethyl predicted from the passive samplers concentrations were within 63-113% of the accumulated mean daily water concentrations and gave a linear regression with an r^2 of 64%.

We reviewed the literature on the general use of biochemical measurements that can be used as individual biomarkers of impaired biological function in invertebrates. The primary focus of the review was on the measurement of biochemical biomarkers in riverine macroinvertebrates under field conditions, where effects of exposure to environmental chemicals at different levels of biological organisation could be examined. A manuscript describing the results has been submitted to the international scientific journal, *Ecotoxicology and Environmental and Safety* and is *in press*. We have also completed a study on the acetylcholinesterase activity of the head segment of nymphs of the mayfly, *Jappa kutera*, at various developmental stages. The effect of pesticides on the acetylcholinesterase activity of *J. kutera* nymphs was also evaluated.

4. How has your research addressed the Corporations three outputs: Sustainability, profitability and international competitiveness, and/or people and community?

Sustainability of Natural Resources

This project evaluated the use of passive samplers containing trimethylpentane as means of estimating river contamination with pesticides in the laboratory and field. The results confirm that passive samplers can detect very low concentrations of certain pesticides in river water that would remain undetected using standard sampling procedures. Passive samplers can also be used to provide estimates of riverine contamination with certain pesticides at relatively low cost.

People and Communities

There is public concern for the health of our waterways and an expectation that freshwater quality will be improved. Passive samplers provide a relatively inexpensive method of estimating riverine contamination and the impact of improved practices through BMP and the reduction of insecticide use through the introduction of Bt cotton.

5. Detail the methodology and justify the methodology used.

Design of laboratory passive sampler study

The uptake rates of hydrophobic compounds into passive samplers are dependent on both intrinsic and extrinsic factors. Intrinsic factors include the hydrophobicity of the contaminant as measured by the octanol-water partition coefficient (Kow); diffusivity of the molecules that have to pass through the aqueous boundary layer and the polyethylene membrane; and exposure time (Huckins et al., 1993; Booij et al., 1998; Gale 1998). The Kow of a compound is a measure of the affinity (solubility) of the compound for the trimethylpentane in the TRIMPS compared to the ambient water. Therefore, high uptake rates and low release rates are expected for compounds with high Kow values. However, as Kow and molecular mass increase, the diffusivity of the molecule generally decreases (Gale, 1998). The uptake of organic contaminants into passive samplers is also influenced by the deployment time, design of the sampler and concentrations in the surrounding water. The first two factors can be manipulated to give linearly proportional uptake kinetics, for a range of concentrations in the ambient water, thus avoiding equilibrium kinetics (Huckins et al., 1993; Booij et al., 1998).

The first objective of the laboratory study was to determine if the TRIMPS performed as a kinetic sampler in the uptake of a variety of pesticides in a laboratory continuous flow-through system. The pesticide uptake is considered kinetic sampling if the rate of mass transfer to the reference phase (solvent of the passive sampler) is linearly proportional to the ratio in chemical activity of the contaminant between the water and the reference phases (Peterson et al., 1995; Booij et al., 1998). The next objective was to determine the concentration factor (based on a time-dependent proportionality constant) for each pesticide. These estimates of concentration factor will be useful in the determination of the pesticides' average river water concentration knowing the concentration in the TRIMPS.

Pesticide release experiments were also undertaken. The TRIMPS were spiked with known amounts of pesticides. The half-lives for pesticide release was determined from regression equations based on natural logarithm of the initial percentage concentration versus time.

Passive sampler (TRIMPS) preparation

The TRIMPS consisted of prefabricated low density polyethylene (LDPE) membrane bags (Scubs Brand, Schur Consumer ProductsA/S, Vejle, Denmark), approximately 3 cm x 10 cm and with a mean wall thickness of 40 µm (95% CI=39-42 µm, n = 40). The LDPE bags were pre-soaked overnight in 2,2,4-trimethylpentane (Nanograde, Mallinckrodt Laboratory Chemicals, Phillipsburg, NJ, USA) to leach out compounds adsorbed on the bags. The 2,2,4-trimethylpentane solvent (10 ml) was then added to each bag and sealed as described previously (Leonard et al., 1999; 2000).

Field macroinvertebrate study

The field macroinvertebrate study was designed to examine the relationships between the population densities of the selected study taxa to pesticide concentrations in the Namoi River as measured by the *in situ* passive samplers. Densities of the macroinvertebrates at six reference sites were compared to those at six exposed sites in November, December, January, February and March of the 1998/99 cotton-growing seasons. The sites were the same reference sites and the most six downstream sites that were monitored previously in the 1997/98 season (Hyne et al., 1999; Leonard et al., 2000).

Design of field permeability reference compounds study

Since some of the permeability reference compounds (PRCs) that were selected to investigate are also target compounds, the study was undertaken in the Namoi River either prior to or on completion of the pesticide spray season for cotton. During these periods the river discharge was either regulated or unregulated during repeated experiments. At the selected site [Site G, (Leonard et al, 2000)], 24 TRIMPS that were spiked with tributylphosphate (10 mg/L) plus endosulfan sulfate (20 µg/L) and 12 TRIMPS with no added PRCs were placed inside each of 36 large rock filled nylon mesh bags (0.8 mm mesh). The mesh bags were secured by polyester cord in the water column to four rows of 1.8-m high metal fencing posts hammered into the substrate. Four spiked and two blank TRIMPS were removed after days 7, 14, 21, 28, 42 and 56. The half-lives for the PRCs release was determined from regression equations based on natural logarithm of the initial percentage concentration versus time, after correction for any absorbed pesticide determined in the blank TRIMPS.

Design of field passive sampler study

The experimental design consisted of three spatial replicates measured over a four-week period. The three sites chosen in the 2000/2001 cotton season were sites 5, J, and 7 in the Namoi River, described in previous field studies between the towns of Narrabri and Wee Waa, New South Wales (NSW) [Leonard et al, 2000]. In the 2001/2002 cotton season, the three sites were in the Gwydir River catchment and included two sites (site numbers 418053 and 41810111) used as sampling sites by the NSW Department of Land and Water Conservation (Muschal, 2000). All the study sites had pebbly substrate and in past seasons have been exposed to significant concentrations of total endosulfan as well as profenofos and chlorpyrifos, particularly in December through to the end of February [Leonard et al, 2000; Muschal, 2001]. The studies took place over a four-week duration in January each season.

At each of the three sites the environmental parameters of pH, conductivity, temperature, dissolved oxygen and flow velocity were continuously measured using a water quality multiprobe (Hydrolab Corporation, Austin, TX, USA) and a water flow velocity sensor (Mace Instruments, Sydney, Australia). The multiprobe was orientated immediately above the pebbly substrate attached to a triangular-shaped steel bracket that was secured by three metal fencing posts hammered into the substrate. The water flow sensor was also attached to the steel bracket so that the sensor was

orientated upstream with no structural impediment. A data logger (Campbell Scientific Australia P/L, Thuringowa Central, Australia) operated by a solar powered trailer was programmed to record the water quality and river flow hourly. The measurements were grouped to give mean values every eight hours for 22 days. Eight hours was selected as the time period because this was the most practical period for collecting the river water samples accumulated by the auto-samplers (ISCO 3700, USA) secured in the trailers. Turbidity was also measured daily at each site with a Hach portable turbidimeter (Model 2100P, Hach Company, Loveland CO, USA). Rainfall was measured continuously at each site with a rainfall gauge and was recorded by the data logger in the trailers.

Adjacent to the triangular-shaped steel bracket at each site, 24 TRIMPS were placed inside each of 24 large rock filled nylon mesh bags (0.8 mm mesh, unless otherwise stated). The mesh bags were secured by polyester cord in the water column to four rows of 1.8 m high metal fencing posts hammered into the substrate. At each site, four TRIMPS were removed every third day after day 7 (2001/2002 season) or day 10 (2000/2001 season) of the experiment until day 22. The mean recovery of trimethylpentane was over 90%.

River water collection and extraction

The collection containers for the river water samples consisted of 350-ml glass bottles in a gas-refrigerated chamber (Barron Refrigeration, Bundaberg, Australia). Twelve of these bottles were filled with 330 ml of river water by the programmed auto-sampler (ISCO 3700, USA) in each 8 hour sampling period (one filled every 40 minutes) at each of the three sites. The completion of each collection over an 8-hour period was set at approximately 12 pm, 8 am and 4 pm. Day 1 was considered to have commenced at midnight after the TRIMPS at each site had been set up during the previous day. At each time period, the twelve individual samples at each site were pooled to give a 4-L water sample. These pooled water samples were taken to a laboratory at either the Australian Cotton Research Institute at Myall Vale (2000/2001 season), or the Moree Technical High School (2001/2002 season), approximately 10 to 45 km from each site. The internal standard 2,2-bis(p-bromophenyl)-1,1-dichloroethane (di-bromo-DDE) was added (0.08µg) to each 4-L sample for measurement of extraction efficiency. The pesticide extraction was undertaken using a glass 5L-separating funnel using 2x 125 ml of dichloromethane (nanograde). The combined extracts were dried with anhydrous sodium sulphate and stored at 4 °C in 250-ml amber glass bottles (Cozpak Pty Ltd, Sydney, Australia) until analysis.

Chemical analysis of pesticides and quality control

Pesticide concentrations in samples from the laboratory experiments were measured using a gas chromatograph with either an electron capture or a nitrogen/phosphorus detector on a DB1 or DB5 column (4°C/min from 100°C to 270°C, Hewlett Packard 5890, Series 11 plus, Wilmington, DE, USA).

Chemical analysis of the river water and passive samplers for total endosulfan concentrations from the field validation experiments were conducted by Sydney Water Ensight Laboratory. The DLWC Arncliffe laboratory analysed the river water and passive samplers for chlorpyrifos-ethyl in samples from the 2001/2002 season. The combined dichloromethane extracts were reduced in volume to 1.0 ml by heating in a 35°C water bath under N_2 atmosphere using a TurboVap® 11 concentration workstation (Linbrook International Pty Ltd, Sydney, Australia). For quality assurance purposes, river water samples were spiked with low concentrations (blank, 0.001, 0.01 and 0.100 ppb) of α-endosulfan, β-endosulfan, endosulfan sulfate, chlorpyrifos-ethyl and profenofos and submitted in triplicate as unknown samples to the external laboratories.

The 2001/2002 season samples were analysed for organophosphorus compounds using Agilent Technologies 5890 Series II Gas Chromatograph fitted with a nitrogen phosphorus detector and a 30m x 0.25-mm DB1 column. Confirmation was done using Agilent Technologies 6890 Gas Chromatograph with a mass selective detector and 30m x 0.22-mm BPX5 column. A second independent laboratory confirmed the concentration of a sub-group of 20 river water extract samples containing chlorpyrifos-ethyl.

Acetylcholinesterase activity of mayfly nymphs

Mayfly nymphs (*Atalophlebia* sp.) (length 4 to 12 mm) were collected from the Namoi River and transported to the laboratory as described previously (Leonard et al., 1999). The nymphs were fed conditioned leaves and aerated continuously. Acetylcholinesterase activity was measured as described by Fisher et al. (2000).

6. Detail results including the statistical analysis of results.

Laboratory passive sampler study

The laboratory continuous flow system delivering known aqueous concentrations of pesticides to passive samplers was used to examine the kinetics of pesticide uptake and release. Experiments were run in triplicate for both uptake and release experiments. In each experiment there were four treatments including a control and three five-fold dilutions of a pesticide mixture that included the usual range of these pesticides measured in the riverine environment of cotton-growing regions of Australia. The results indicated that for non-polar pesticides with a log K_{ow} >3.5, uptake was linear over the 42-d exposure time, and was independent of the treatment concentration. The half-lives for release of these pesticides from the TRIMPS varied from 26 to 130 d in clean water and obeyed first-order kinetics.

The relatively polar pesticides with a log K_{ow} <3.5, had lower uptake rates into the TRIMPS due to their greater affinity for water. In contrast to non-polar pesticides, polar pesticides with a log K_{ow} < 3.5 had reached equilibrium in the solvent in the TRIMPS within 42 days. The release rates of the polar pesticides with a log K_{ow} < 3.5 from the TRIMPS also obeyed first-order kinetics, with the plots of the natural logarithm of pesticide concentration retained in the solvent of the TRIMPS linearly related to time (R^2 values exceeded 0.94). These pesticides, including methyl-parathion (t1/2=3.6 days), tributylphosphate (t1/2=10.4 days), prometryn (t1/2=3.2 days) and molinate (t1/2=3.3 days) were rapidly eliminated from the solvent of the TRIMPS.

Preliminary experiments have been undertaken to develop and calibrate a new passive sampler device for more polar pesticides, such as the herbicides molinate and atrazine.

Field macroinvertebrate study

During the 1998/99 cotton-growing season in the Namoi River the population densities of the dominant benthic macroinvertebrates were not significantly different at six downstream sites exposed to the pesticides endosulfan and profenofos, compared to upstream reference sites. Throughout the cotton-growing season, the monthly mean total endosulfan and profenofos concentrations in the passive samplers at the exposure sites were 1- 6 times those at the reference sites. However, the predicted water concentrations, from the measured passive samplers concentrations using the laboratory calibrated concentration factors, were less than the Water Quality Guideline values for these pesticides, except for one occasion. In December 1999, the average total endosulfan concentration across the exposed sites in the river was 0.05 µg/L.

Release of permeability reference compounds- field vs laboratory comparison

We initially investigated the use of permeability reference compounds as a direct measure of potential effects of environmental variables on pesticide release. This is based on the desorption rate of the permeability reference compounds spiked into the TRIMPS at the beginning of the deployment period. We used tributylphosphate and endosulfan sulfate, and found half-lives for release from the TRIMPS deployed in the Namoi River of 7.7 and 16.5 days, respectively. This compares with half-lives for release of 10.4 and 26.5 days for these compounds, in the laboratory continuous flow system. Release of trimethylpentane from the TRIMPS placed in 0.8-mm mesh nylon bags in river water was also used as an indicator for chemical diffusion across the aqueous boundary to examine the effect of environmental factors. Most variability in solvent-release in the field deployed TRIMPS across all sites was explained by time of deployment, and was not significantly influenced by river flow or water temperature.

Field passive sampler study

The study was limited because of the very low concentrations of total endosulfan in the river water during the period of the field validation experiments. As a result, analyses of river water at very low limits of detection were undertaken at Sydney Water by Dr Ahmad, allowing an assessment of the effectiveness of the passive samplers.

Over 22 days in January in the 2000/2001 season and 2001/2002 cotton-growing seasons, river water was collected from three study sites in the Namoi River and the Gwydir River catchment, respectively. Within each 24-h period, three composite river water samples were collected from each of the three sites, adjacent to cotton fields. These composite river water samples from the three sites were taken to the laboratory for extraction with dichloromethane. The completion of each collection period was set at approximately at 12 pm, 8 am and 4 pm. Both the 12 pm and the 8 am composite samples were collected in the morning and delivered to the laboratory. This resulted in an elapsed time period for these two composite samples of approximately 18 hr and 10 hr, respectively, from when the first aliquot had been collected from the river to when the extraction was completed. From the daily measured values obtained, the cumulative mean daily endosulfan and chlorpyrifos concentrations in the river water were calculated at each site for the days that the TRIMPS were removed from the river. These values were then compared to the concentrations of endosulfan and chlorpyrifos measured in the TRIMPS. To compare the cumulative mean daily pesticide concentrations to the predicted concentrations from the passive samplers, the laboratory derived pesticide concentration factors for days 7, 10, 13, 16 19, and 22 (Table 1) were applied to the mean pesticide concentrations measured in the four passive samplers removed on these days. For the predicted total endosulfan concentration, the predicted concentrations for α-endosulfan, β-endosulfan and endosulfan sulfate were calculated from their mean concentrations in the passive samplers, and then added to give a predicted total endosulfan concentration. Predicted pesticide concentrations were compared to the cumulative mean daily concentrations (based on measured values), when the cumulative mean water concentrations were >0.005 µg/L.

Table 1. Concentration factors for pesticide uptake by TRIMPS derived from laboratory kinetic model.

Day	Concentration factor			
	α-Endosulfan	β-Endosulfan	Endosulfan sulfate	Chlorpyrifos
7	294	243	238	482
10	631	525	479	676
13	842	702	624	868
16	1058	884	769	1057
19	1278	1070	915	1244
22	1502	1259	1061	1430

Values shown are from Leonard et al. [2002] and reflect the concentrating capacity of the TRIMPS that facilitates the measurement of low water concentrations of the pesticides.

In the 2001 study in the Namoi River, no chlorpyrifos was detected in the river water and the cumulative mean daily endosulfan concentrations measured from 10 to 22 days were very low at only twice the practical detection level of 0.01 µg/L (Figure 1). The endosulfan concentrations measured in the TRIMPS were dominated by α-endosulfan, indicating that the endosulfan being taken up by the TRIMPS had been only recently sprayed. In contrast, the proportions of the parent isomers and

sulfate metabolite in the river water compared to their corresponding proportions in the TRIMPS indicated that α-endosulfan was being converted to endosulfan sulfate. Figure 1 shows a plot of the the predicted water concentrations from the passive samplers concentrations collected from days 10 to 22 versus the measured accumulated mean daily total endosulfan concentrations in the river water. With the exception of one outlier value, individual measured data values were within 40-170% of the predicted water concentrations from the TRIMPS (Figure 1). Three values from the data set are not shown as the cumulative measured values of the total endosulfan concentrations in the river water were < 0.005 µg/L and the corresponding total endosulfan concentrations accumulated by the TRIMPS were not predictive. The data indicated that a total endosulfan concentration > 10 µg/L in the TRIMPS deployed over a 10 to 22 day period is needed in order to obtain a good estimate of average river water concentrations. The regression equation for the data from the three study sites indicated that the predicted water concentrations were with 8% of the measured cumulative mean daily total endosulfan concentrations in the river water (Figure 1).

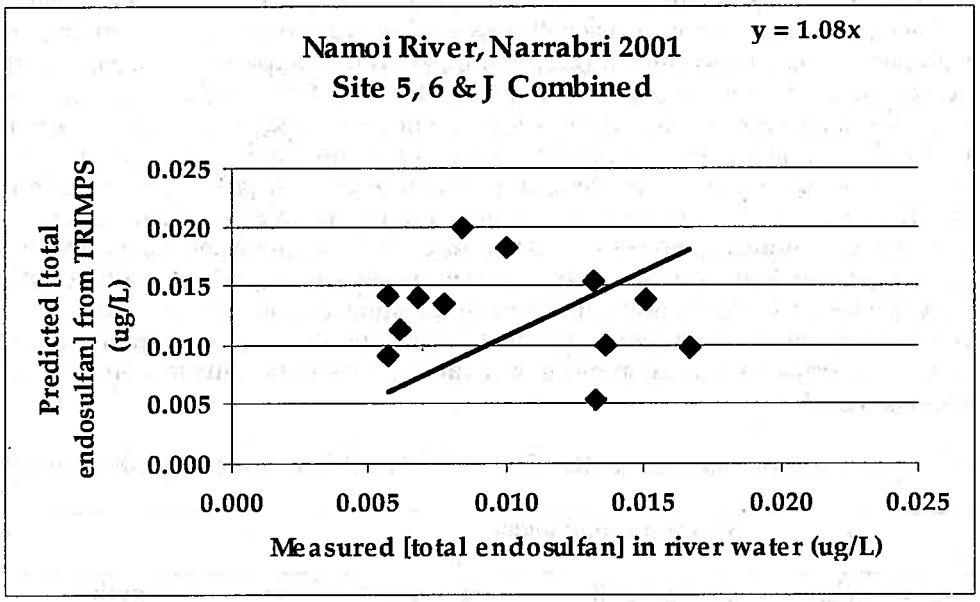

Figure 1. Comparison of the predicted water concentrations from the passive samplers concentrations collected from days 10 to 22 to the measured accumulated mean daily total endosulfan concentrations in the river water. The passive samplers were collected from the river every third day from day 10 to day 22 of the study. Values shown are from three sites in the Namoi River.

In the 2002 study in the Gwydir River catchment, the daily river water total endosulfan concentrations were consistently low (0.001 to 0.005 µg/L). The total endosulfan concentrations were also very low (< 20 µg/L) in the TRIMPS at all three sites. At the site with the highest endosulfan concentrations absorbed by the passive samplers, the predicted total endosulfan water concentrations calculated from these values were compared to the calculated accumulated mean daily total endosulfan concentrations in the river water from the measured river water concentrations. The predicted total endosulfan concentrations were up to an order of magnitude higher than the calculated accumulated mean daily total endosulfan concentrations in the river water which varied from 0.0023 to 0.0028 µg/L. However the predicted values from the passive samplers were equal to or less than 0.003 µg/L, and no value was obtained that incorrectly predicted that the current Australian Water Quality Guideline value for total endosulfan was exceeded. The much higher predicted total endosulfan concentration from the passive samplers compared to the measured water concentrations may be due to additional contact time in the collected water allowing stronger binding to the dissolved organic carbon in the river water at these very low total endosulfan concentrations.

In the 2002 study in the Gwydir River catchment, the measurement of frequent chlorpyrifos-ethyl concentrations in the river water above the practical detection limit was only recorded at one of the sites and the values were in the range of <0.01 to 0.24 µg/L. A plot of the predicted water

concentrations for chlorpyrifos-ethyl from these passive samplers values compared to the calculated accumulated mean daily water concentrations from the measured river concentrations, gave a linear regression with an r^2 of 0.64 (Figure 2). Individual data points were within 63-113% of the predicted water concentrations from the TRIMPS (Figure 2). The regression equation indicated that the predicted water concentrations for chlorpyrifos-ethyl from the TRIMPS were within 75% of the measured cumulative mean daily chlorpyrifos-ethyl concentrations in the river water.

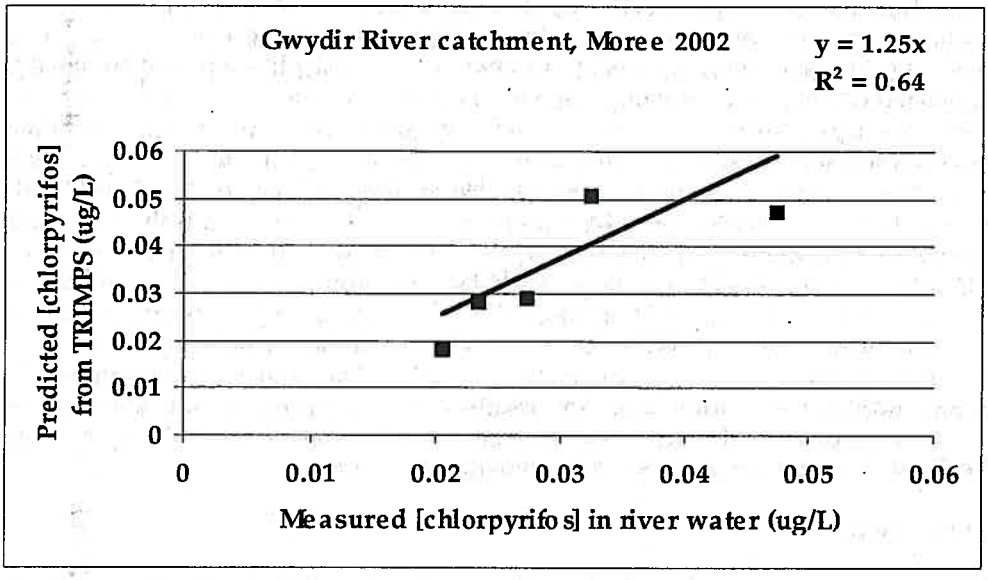

Figure 2. Comparison of the predicted water concentrations from the passive samplers concentrations collected from days 7 to 22 to the measured accumulated mean daily chlorpyrifos-ethyl concentrations in the river water. The passive samplers were collected from the river every third day from day 7 to day 22 of the study. Values shown are from one site in the Gwydir River catchment.

Pesticides uptake by TRIMPS – effect of aperture size of mesh bags

In the 2000/2001 season, the TRIMPS were deployed in the Namoi River at the three study sites inside nylon mesh bags with mesh aperture length of either 800-µm or 1180-µm. Every third day from day 10 until day 22, four TRIMPS, two from a 800-µm mesh bag and two from a 1180-µm bag were removed from the river at each site. Despite the larger sized mesh bags having reduced biofilm on its external surface, comparison of the mean total endosulfan concentrations measured in the TRIMPS contained in the different sized mesh bags, showed no significant difference ($p<0.05$). No biofilm was ever observed on the surface of the polyethylene bags containing the trimethylpentane solvent.

Acetylcholinesterase activity of mayfly nymphs

The acetylcholinesterase activity in homogenates of the head segment from nymphs of the mayfly, *Jappa kutera*, was found to be linear over time and with increases in protein. The acetylcholinesterase activity increased as the body length of the nymphs increased, with increasing length being associated with changes in developmental stage. The mayfly nymphs were assayed for acetylcholinesterase activity in homogenates of the head segment in body length groups of < 4mm, 4-5 mm, 6-7 mm, 8-9 mm and > 10 mm. The acetylcholinesterase activity (11.4 ± 2.1 to 11.9 ± 0.2 µmol/min/g protein) of the first three groups were not significantly different from each other. In contrast, nymphs with body lengths of 8-9 mm and > 10 mm, had greater acetylcholinesterase activity of 20.2 ± 6.1 µmol/min/g protein and 44.9 ± 23.9 µmol/min/g protein, respectively. Chlorpyrifos inhibited the head segment acetylcholinesterase activity in a concentration dependent manner (IC50=0.2 µg/L) , whereas technical endosulfan in the concentration range 1 to 10 µg/L ,had no inhibitory effect.

7. Discuss the results, and include an analysis of research outcomes compared with objectives.

Laboratory passive sampler study

The concentrations of relatively lipophilic pesticides (log K_{ow} > 3.5) taken up into 2,2,4-trimethylpentane-containing passive samplers (TRIMPS) in a laboratory continuous flow system were linearly related to their concentrations in water. This enabled the prediction of river water concentrations of pesticides from measured pesticide concentrations in TRIMPS deployed in the field. Several results established in this investigation support this finding. Firstly, no saturation of the trimethylpentane solvent occurred within the environmental concentration range of these relatively hydrophobic pesticides in the usual deployment time of the TRIMPS (28 days). This was indicated by the pesticides having uptake concentrations into the trimethylpentane solvent of the TRIMPS that were linearly proportional to the concentrations in the water over a 42 d period. Secondly, once in the TRIMPS these pesticides have half-lives of release varying from 26 to 130 d in clean water and obeyed first-order kinetics. For the majority of these pesticides, maximum loss of sorbed compounds during normal deployment times is only a small fraction of the total. Thirdly, solvent-release from the TRIMPS deployed in the Namoi River in NW NSW was used as a indicator for lipophilic chemical diffusion across the aqueous boundary and it was not influenced by river water temperature or flow rate (discharge/channel width) across study sites. The results indicate that the deployment time and the concentrations of pesticides in the river water may explain most variability of pesticide concentrations in the TRIMPS that were deployed in the riverine environment.

Field macroinvertebrate study

No significant differences were found between the mean population densities of the macroinvertebrates at the reference and exposed sites during the 1998/99 cotton-growing season, when the pesticide exposure was on most occasions below water quality guideline values. In previous studies, in the 1995/1996 and 1997/98 seasons, the macroinvertebrate population densities were significantly lower at the exposed sites when pesticide concentrations at the exposed sites were significantly higher. This confirms that the differences in the macroinvertebrate population densities between the reference and exposed sites in the Namoi River, are a sensitive indicator of pesticide exposure. The last three cotton-growing seasons have experienced low insect pest pressure. This has resulted in reduced pesticide applications to the cotton crop and in combination with dry weather conditions, has led to a reduction of riverine pesticide contamination. During the last three cotton seasons, the riverine macroinvertebrates study was discontinued in order to maximise effort on the field passive sampler study during the pesticide-spraying period.

Field passive sampler study

Previous modelling studies [Booij et al, 1998; Gale, 1998; Huckins et al, 1999] have shown that the rate limiting step for the uptake and release of hydrophobic chemicals into the sequestering media of passive sampling devices is the passage through the aqueous boundary layer adjacent to the polyethylene membrane. However, changes in flow velocity and turbulence of the surrounding water affects the effective thickness of the external boundary layer of the passive sampling devices [Jeanot and Cantwell, 1997; Porter et al., 2000]. The flow velocity of the study rivers is up to two orders of magnitude larger than the flow velocity of the laboratory continuous flow-through system [Leonard et al, 2000]. However, we have previously shown that in field deployment of the TRIMPS, the external nylon mesh bag acted as a protective barrier that baffled the effect of the river flow velocity and prevented the rapid loss of trimethylpentane from the TRIMPS [Leonard et al, 2002].

Performance reference compounds (PRCs) are a more direct measure of the effect of environmental variables on pesticide release from the TRIMPS. This is based on the desorption rate of PRCs spiked into the TRIMPS at the beginning of the deployment period. The use of permeability standards assumed that isotropic exchange kinetics governs the accumulation and release of pesticides from the TRIMPS [Huckins et al., 2002]. We used tributylphosphate and endosulfan sulfate and which gave

release half-lives from TRIMPS deployed in the Namoi River of 7.7 and 16.5 days, respectively. This compares with half-lives for release of 10.4 and 26.5 days, respectively, for these reference compounds in a laboratory continuous flow system [Leonard et al, 2002]. Since the release rates of the pesticides from the TRIMPS obeyed first-order kinetics [Leonard et al, 2002], the half-lives for release are inversely proportionally to the release rate [Sienko and Plane, 1966]. The half-live for release of endosulfan sulphate from TRIMPS deployed in the field indicated that the release rate was approximately 40% more than that from TRIMPS in the laboratory. This indicates that for endosulfan sulphate and more hydrophobic pesticides, the laboratory kinetic model described previously for the TRIMPS, could be applied to pesticide uptake into and release from TRIMPS in the field.

Despite the low daily riverine total endosulfan (<0.001 to 0.066 µg/L) and chlorpyrifos-ethyl concentrations (<0.01 to 0.24 µg/L) in the two studies, the predicted water concentrations, from the TRIMPS concentrations using the laboratory-derived concentration factors, were within 40-170% of the accumulated mean daily water concentrations. When the cumulative measured river water concentrations were >0.005 µg/L, predicted water concentrations estimated from field deployed passive samplers gave a good estimate of the average water concentation for total endosulfan and chlorpyrifos-ethyl over the period of exposure. The passive samplers provide a means of estimating integrative concentrations in river water of pesticides knowing its concentration in the passive samplers after a known period of deployment. This is particularly useful in river studies where concentrations of pesticides in water are typically changing and are often characterised by long periods of low background concentrations that can be interrupted by pulses of higher than normal concentrations.

Permeability standards.

In order to account for the potential adverse effects of environmental variables on using laboratory-derived calibration factors to estimate average water concentrations for field deployed passive samplers, it is recommended that two permeability standards be used over the log Kow range of the chemicals being investigated. Permeability standards are based on the rate of release of a synthetic organic chemical that is spiked to the sequestering phase of the passive sampler at the beginning of the deployment period, and have been used by several investigators [Booij, et al. 1998; Huckins et al., 2002]. Tributylphosphate, an organophosphate compound, is one chemical suitable for this purpose as it does not occur in the environment and has a laboratory half-life of 10.4 d inside the TRIMPS. Using these standards it will be possible to delete data from field deployed passive samplers that are not within a "normal range" of membrane permeability.

Use of acetylcholinesterase activity as a potential indicator of pesticide exposure

Aquatic macroinvertebrates are commonly used in biological monitoring programs, but their use in biomarker studies has been limited. In order to link a biomarker measurement in individuals to population changes, it is necessary to understand the mechanisms of ecotoxicological damage by the causative agent. Quantitative dose response measurements of the biomarker, will then link the molecular effect of the toxicant to the toxic response of the individual organism. Linkage of whole organism responses to changes in field populations can then be obtained by statistical correlations. The slow induction of monoxygenase activity and P450 in response to contaminants in invertebrates precludes the use of mixed functional oxidases as a biomarker of exposure to environmental contaminants. Two biochemical mechanisms that confer high resistance to organophosphorus and pyrethroid insecticides are detoxification by the over-production of esterases and insensitive acetylcholinesterases. Measurement of the activities of these enzymes in aquatic macroinvertebrates could be used as a biomarker of susceptibility to toxicity (decreased acetylcholinesterase activity) or as a biomarker of resistance (insensitive acetylcholinesterase or increase carboxyesterase activity) that could be linked to changes in population densities. Our preliminary study on the acetylcholinesterase activity of the head segment of nymphs of the mayfly, *Jappa kutera*, indicates that the acetylcholinesterase activity varies with nymph length, which are at various developmental stages. Also, the nymph head acetylcholinesterase activity was inhibited by chlorpyrifos at low environmental concentrations. The ability of these macroinvertebrate biomarkers to predict effects on field populations needs to be validated by studying a known contaminated catchment.

8. Provide an assessment of the likely impact of the results and conclusions of the research project for the cotton industry. Where possible include a statement of the costs and potential benefits to the Australian cotton industry and future research needs.

The regression equations established in the laboratory studies relate the concentrating capacity of the passive samplers to the time of deployment of passive samplers for each of the eight lipophilic pesticides. These equations could be used in estimating integrative concentrations in water of a pesticide knowing its concentration in passive samplers after a known period of exposure. This is particularly useful in river studies where concentrations of pesticides in water are typically changing and are often characterised by long periods of low background concentrations that can be interrupted by pulses of higher than normal concentrations.

We have previously shown a link between a decline in macroinvertebrate population densities and riverine endosulfan concentrations measured using passive samplers (Hyne et al., 1999; Leonard et al., 2000). This project has validated the use of passive samplers as a means of estimating river contamination with pesticides, allowing evaluation of the effect of increased transgenic cotton usage, and the implementation of Best Management Practices, on the concentrations of pesticides entering the riverine environment. This validated, easily applied and relatively low-cost method will provide a convenient reference system by which improvements in river water quality can be readily assessed.

The estimated cost to undertake a continuing monitoring program, using passive samplers, to monitor river water quality using a minimum number of sampling points in the Namoi River only or both the Namoi and Gwydir River systems assuming that they were monitored together would be as follows:

	Namoi R (7 sites)	Gwydir River (3 sites)
3 samplers per site = 21 samplers per month for 5 monthly deployments for the Namoi River (NR). 3 samplers per site = 9 samplers per month for 5 monthly deployments for Gwydir River (GR).		
Preparation costs, salary, vehicle, accommodation and food costs	$ 41,000	$ 8,000
TOTAL for both rivers =	**$49,000**	

9. Describe the project technology (eg. commercially significant developments, patents applied for or granted licenses etc).

Not applicable.

10. Provide a technical summary of any other information developed as part of the research project. Include discoveries in methodology, equipment design, etc.

Not applicable.

11. Detail a plan for the activities or other steps that may be taken;

(a) to further develop or to exploit the project technology.

(b) for the future presentation and dissemination of the project outcomes.

A. Continue with the development of the laboratory pesticide uptake model using the continuous flow-through system. The use of various solvents will be investigated to develop a suitable passive sampler to measure polar pesticides.

To undertake a continuing monitoring program, using passive samplers, to monitor river water quality.

B. In addition to the final report, presentations at conferences both national and internationally as well as scientific papers will ensure the findings are published and adopted by the national regulatory community.

We have had several inquiries from other researchers in Victoria, South Australian and Queensland on how to prepare and use the passive samplers for herbicides and insecticides. Groups in South Australian, Victoria and Queensland have commenced studies using the passive samplers (TRIMPS) and others are planning to use them next year.

If sufficient interest, a workshop will be organise on the use of passive samplers.

12. List the publications arising from the research project.

Leonard, A.W., Hyne, R.V., and Pablo, F. (2002). Trimethylpentane-containing passive samplers for predicting time-integrated concentrations of pesticides in water– laboratory and field studies. *Environ. Toxicol. Chem.* 21: 2591-2599.

Hyne, R.V. and Maher, W.A. (2002). Invertebrate Biomarkers: Links to Toxicosis that Predict Population Decline. *Ecotoxicol. Environ. Saf.* 52: in press.

Hose, G.C., Lim, R.P., and Hyne, R.V. (2002). The transport, fate and effects of endosulfan in the Australian freshwater environment. *Australasian J. Ecotoxicol.* in press.

Hyne, R.V., and Pablo, F., Aistrope, M. A. and Leonard, A.W. (2002). Trimethylpentane-containing passive samplers as a tool for predicting time-integrated concentrations of pesticides in water– a model based on laboratory and field studies. Presentation at the Interact 2002 Conference, Sydney, July 2002. Abstract B2 session.

Hyne, R.V., Leonard, A.W., Pablo, F., Ahmad, N. and Kennedy, I. Comparison of pesticide concentrations predicted from field deployed passive samplers to continuous measurements of river water concentrations. *Environ. Toxicol. Chem.* in preparation.

13. Are changes to the Intellectual Property register required?

No.

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Part 4 – Final Report Plain English Summary

Provide a half to one page Plain English Summary of your research that is not commercial in confidence, and that can be published on the World Wide Web.

The major outcome from this research has been to provide information on the effectiveness of passive samplers for assessing river water quality. The data showed that these devices cannot be used to directly measure exact concentrations of pesticides entering rivers and waterways. However, by concentrating such compounds in river water over an extended period of exposure, the study has shown that they can provide information on approximate levels of contamination of river water with particular chemicals, allowing their use to survey river water quality. Once the passive samplers are calibrated for each pesticide they can be deployed in waterways to determine average water concentrations of the pesticides which can then be linked to water quality guideline values. This will provide the basis by which river water quality can be assessed to address local community concerns with pesticide discharges on downstream receiving environments.

The project evaluated with field studies the use of passive samplers containing the solvent, trimethylpentane, for estimating the average river contamination of pesticides that are sparingly soluble in water (log Kow >3.5). This study established that the amount of these pesticides measured in the solvent was related to their average concentration in the river water. Most of the variability of the pesticide concentrations in the solvent could be explained by their concentration in the river water and the length of time of deployment of the passive samplers up to 42 days.

The results confirm that passive samplers can detect very low concentrations of certain pesticides in river water that would remain undetected using standard sampling procedures. Passive samplers can also be used to provide estimates of riverine contamination with certain pesticides at relatively low cost. It is recommended that, based on this study, a small number of passive samplers be deployed each year at selected river locations to provide an ongoing survey of river contamination and to monitor the effectiveness of best management practices in improving river water quality.