# CATCHMENT-SCALE ECOLOGICAL RISK ASSESSMENT OF PESTICIDES

By

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## STATEMENT OF ORIGINALITY

The material in this thesis is the original work of the author unless otherwise stated. No part of this thesis has been previously accepted for the award of any other degree or diploma in any university.

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## PUBLICATIONS

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## ABSTRACT

Ecological risk assessment (ERA) used to support pesticide management decisions at the catchment-scale can deliver environmental protection while retaining farm production benefits. Presently, ERA's in Australia are typically performed to evaluate farm-scale ecological impacts from pesticides. However, as pesticide exposure in rivers is usually a result of the activities of more than one farmer affected by spatially and temporally explicit factors such as climate, hydrology, geomorphology and land uses, a catchment-scale pesticide management approach seems a logical progression. The aim of this thesis was to investigate the potential effectiveness of applying ERA adapted to the catchment-scale as a pesticide management tool for agricultural catchments.

Initially, a catchment-based ERA of diuron, prometryn and endosulfan use in the Gwydir River catchment, NSW, Australia, was used to identify possible aquatic exposure sites. The classic phases of problem formulation, analysis and risk characterisation were established. The problem formulation phase identified hazard concerns and established assessment endpoints specific for areas that were considered to have high (95% of species protected 95% of the time) or lower (90% of species protected 95% of the time) ecological value. The analysis phase identified that the likely exposure sources of diuron, prometryn and endosulfan in the reaches of the Gwydir River catchment were agriculture, characterised using available ecotoxicology and regulatory exposure monitoring information using continuous probability distributions.

The characterisation of risk involved comparing distributions of exposure and ecotoxicity as species sensitivity distributions (SSDs) to estimate the probabilities that the endpoints were being exceeded (Solomon *et al*., 2000). With the exception of prometryn, significant risk from diuron (maximum risk = 8.95%) and endosulfan (maximum risk = 7.86%) exposure was found to occur in some reaches of the Gwydir River catchment. These areas, considered as "hot spots", predominated where intensive agricultural production was most prevalent.

An uncertainty evaluation identified a number of information shortfalls in this ERA. These gaps included permanency of ecological effect resulting from pulse exposures, conservative risk estimation that was based on exposure data from a sampling regime targeted when

chemical use and rainfall were more prevalent, and, for the purpose of supporting risk management, identification of specific areas in the catchment contributing chemical loads in areas of concern. These uncertainties led to the need for further research.

The consideration of organism recovery under a pulse exposure scenario likely to be observed in the Gwydir River catchment was investigated in a laboratory toxicity study. This study tested the potential for two duckweed species (*Lemna minor* and *L. gibba*) to recover from a seven day diuron pulse. The duckweed species were exposed to a range of diuron concentrations (0.3-200 μg $L^{-1}$) for seven days, and placed in to clean growth media for a further seven days to simulate a recovery phase.

Exposure toxicity and recovery were evaluated through plant and frond counts, and wet and dry weights. The inhibition of growth was used as the toxicity metric by comparing growth response with control (0 μg $L^{-1}$) populations. Significant growth inhibition of *L. minor* ($EC_{50}$ = 34.9 and 52.8 μg $L^{-1}$ for dry weight and frond count, respectively) and *L. gibba* ($EC_{50}$ = 50.3 and 47.6 μg $L^{-1}$ dry weight and frond count, respectively) was observed at the end of the seven day exposure. By the end of the seven day recovery phase, growth inhibition compared to the controls were shown to decline for a range of treatment exposure concentrations to the point that inhibition was not significantly different from the control for both *L. minor* (50 and 100 μg $L^{-1}$, for dry weight and frond count, respectively) and *L. gibba* (200 and 50 μg $L^{-1}$, for dry weight and frond count, respectively). With reference to the literature, growth inhibition was determined to be in response to photosynthesis inhibition (Haynes *et al*., 2000; Fai *et al*., 2007). Population recovery for treatment concentrations greater that observed in the Gwydir River catchment suggested was a clear reversal of this effect. It was concluded that both *L. minor* and *L. gibba* could sufficiently recover from a prolonged exposure event, suggesting the possibility of keystone aquatic plants and algae resilience to diuron exposure occurring in the Gwydir River catchment.

To clarify the risk characterisation uncertainty of diuron in the Gwydir River catchment, a spatial exposure modelling framework was required. A modelling procedure with the capacity to provide a daily time series exposure concentration pointing to catchment areas contributing to chemical load was selected. This framework involved combining two models, a chemical fate model (Pesticide Root Zone Model, PRZM) and a chemical routing model

(Riverine Water Quality model, RIVWQ). The inputs to these models were obtained and processed from readily accessible databases and/or literature. Required inputs included soil, land use and weather station information; characteristic agronomic practices for different land uses and their respective label application rates. In accordance with the chemical labels all maximum application rates were used for the respective land uses of cotton, wheat, chickpea, canola and pasture in the simulations. To account for the full range of in season applications two scenarios were required to be run. Specifically, pre- (i.e. chemical incorporated in the top 4 cm of soil) and post-emergence (chemical applied directly to the surface) were simulated for cotton.

The simulation results showed that under the post-emergence application regime the highest chemical loading for streams was predicted. This was the result of chemical being more readily available at the surface to be entrained in runoff. It was found that the post-emergence application scenario reflected more closely the peak concentration magnitude and timing observed in the monitoring data. However, when compared with monitoring data, the model framework was unable to predict peak exposure concentrations on precisely the same dates. This indicated a degree of error in the model predictions, an outcome likely to be the result of uncertainties in the model inputs, especially with respect to pesticide applications, timing and crop rotation scenarios. However, the modelling framework did perform in a way that was consistent with chemical fate and fugacity principles. Subsequently, from these different scenarios, the sub-catchments contributing chemical loads were able to be identified, potential pulse durations were characterised as were their probabilities of re-occurrence, with the longest pulse exceeding the toxicity threshold lasting 6-9 days. It was concluded that this approach to estimating exposure at the sub-catchment level could be a useful tool for catchment management, devising and directing risk management strategies associated with monitoring. However, this will require further calibration and validation for effective use as a risk management tool.

The outputs of this thesis are suggested to provide justification for further development of catchment-based ERA strategies in Australia. These would include site specific chemical loading,employ probabilistic risk characterisation and account for ecosystem biodiversity value and resilience. This strategy would take ERA in Australia from the top-down approach now taken by national regulators to a bottom-up alternative, inclusive of catchment managers

operating at the local level interacting directly with stakeholders. Pesticide exposure concerns identified through an ERA should then be addressed through the implementation of a management strategy. This strategy would utilise outputs from spatial modelling supported by monitoring, as a basis for directing management to areas of a catchment where it is most needed. The thesis concludes that managing pesticide exposure in agricultural catchments with more informed ERA can provide a sounder basis for pesticide use in crop production coexisting with ecosystem protection.

## ACKNOWLEDGEMENTS

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I love ya's all!

# ABBREVIATIONS

| | |
|---|---|
| ABARE | Australian Bureau of Agricultural and Resource Economics |
| AGGA | Australian Government: Geoscience Australia |
| ANZECC | Australia and New Zealand Environmental and Conservation Council |
| APVMA | Australian Pesticides and Veterinary Medicines Authority |
| BCPC | British Crop Protection Council |
| BOM | Australian Government Bureau of Meteorology |
| BRGCMA | Borders River-Gwydir Catchment Management Authority |
| CMA | Catchment management Authority |
| NRM | Natural Resource Management |
| NSW | New South Wales |
| NSWWI | New South Wales Government: Water Information |
| SPCC | New South Wales State Pollution Control Commission |
| USEPA | United States Environmental Protection Agency |
| IPM | Integrated Pest Management |
| IRAC | Insecticide Resistance Action Committee |
| IUPAC | International Union of Pure and Applied Chemistry |

| | |
|---|---|
| %OC | Organic carbon fraction |
| a.i. | Active ingredient |
| ANOVA | Analysis of Variance |
| BMP | Best Management Practices |
| *Bt* | *Bacillus thuringensis* |
| $b_{exp}$ | y-intercept parameter of the exposure distribution |
| $b_{tox}$ | y-intercept parameter of the SSD |
| $C_1$ | Concentration in phase 1 |
| $C_2$ | Concentration in phase 2 |
| Caq | Concentration of chemical in solution phase |
| Cs | Concentration of chemical on solid phase |
| DCA | 3,4-dichlorophenylaniline |
| DEM | Digital Elevation Model |

| | |
|---|---|
| $EC_{50}$ | Concentration that effects 50% of a population |
| EMS | Environmental Management System |
| ERA | Ecological Risk Assessment |
| FC | Field Capacity |
| GIS | Geographical Information System |
| GM | Genetically Modified |
| HORC | Highest Observable Recovery Concentration |
| HRAC | Herbicide Resistance Action Committee |
| $HC_5$ | Hazard concentration affecting 5% of species in an SSD |
| $HC_{10}$ | Hazard concentration affecting 10% of species in an SSD |
| $IC_{50}$ | Median Inhibition Concentration |
| $K_{12}$ | Partition coefficient between phase 1 and phase 2 |
| $K_d$ | Soil-water partition coefficient |
| $K_{OC}$ | Organic carbon partition coefficient |
| $K_{OW}$ | Octanol-water partition coefficient |
| $K_{sat}$ | Saturated soil hydraulic conductivity |
| $LC_{50}$ | Lethal concentration that is lethal to 50% of a population |
| LOEC | Lowest Observable Effect Concentration |
| LOQ | Limit of Quantitation |
| MAE | Mean Absolute Error |
| $m_{exp}$ | Gradient parameter of the exposure regression |
| $m_{tox}$ | Gradient parameter of the SSD regression |
| NA | Not Applicable |
| NASA | North American Space Agency |
| NOEC | No Observable Effect Concentration |
| HSPF/NPSM | Hydrological Simulation Program Fortran-Non Point Source Model |
| NR | Not Reported |
| PRZM-RIVWQ | Pesticide Root Zone Model-RIWerine Water Quality |
| PSII | Photosystem II |
| $Q_A$ | Primary quinone electron acceptor |
| $Q_B$ | Second quinone electron acceptor |
| RCII | Reaction centre II |
| RMSE | Root Mean Square Error |

| | |
|---|---|
| SSD | Species sensitivity distribution |
| WP | Wilting Point |

| | |
|---|---|
| $^{o}C$ | Degrees centigrade |
| g | Grams |
| L | Litres |
| m | Meters |
| s | Seconds |
| Pa | Pascal |

Unit prefixes

| | |
|---|---|
| m | milli ($10^{-3}$) |
| µ | micro ($10^{-6}$) |
| k | kilo ($10^{3}$) |

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**CHAPTER 1**

# REVIEW OF CATCHMENT-SCALE ECOLOGICAL RISK ASSESSMENT OF PESTICIDES IN AUSTRALIA

## 1.1 INTRODUCTION

Ecological risk assessment (ERA) is a tiered framework (Figure 1.1) designed to evaluate the likelihood that adverse effects toward ecological groups (including humans) may result from exposure to stressors related to human activity, while accounting for complexity and uncertainty (Norton *et al.*, 1992; USEPA, 1998). The approach has been recommended to be used to manage ecological risk from pesticides in agricultural environments around the world. The principal goal of ERA is to provide risk managers with a means to implement strategies that can minimise concerns regarding exposure in defined environments.

Pesticides are used extensively in agricultural production. They deliver strong production benefits by protecting crops from attacking pests (Pimentel *et al.*, 1978; Stephenson, 2003), an action that is achieved by the chemicals ability to target and alter the function of deliberately selected biochemical pathways or functions. However, as pesticides are applied in open systems, contamination of ecosystems is possible through various processes affecting their fate (Wauchope, 1978; Schnoor, 1992; Scheunert, 1993; Kookana *et al.*, 1998; Mackay, 2001). The extent to which contamination can occur is influenced by the physicochemical and fugacity properties of the compound (Schnoor, 1992; Scheunert, 1993; Kookana *et al.*, 1998; Mackay, 2001), the significance of which is measured by the toxicity of the compound to organisms contained in the defined ecosystem (Wauchope, 1978; USEPA, 1998; Suter II, 2007). The physical and chemical processes involved are complex; hence, ERAs are commonly used to comprehensively evaluate the potential for ecologically significant contamination to occur in a defined environment.

The ERA framework evolved from human health risk assessment that was developed by the United States Government to characterise carcinogenic impacts on humans (Landis, 2003), modified to include other groups of ecological species (Landis, 2003; Suter II, 2007). Specifically, the ERA framework consists of three tiers, problem formulation, analysis and characterisation (Figure 1.1), with pre- and post-evaluation tiers to respectively plan and present the outcomes of an ERA to concerned stakeholders, as well as a feedback mechanism (data acquisition, verification and monitoring) so that modifications of an ERA can be made when new information becomes available (USEPA, 1998; Suter II, 2007). The outcome of an ERA should support management decisions aimed at addressing exposure risk concerns (Dorr *et al*., 2007).

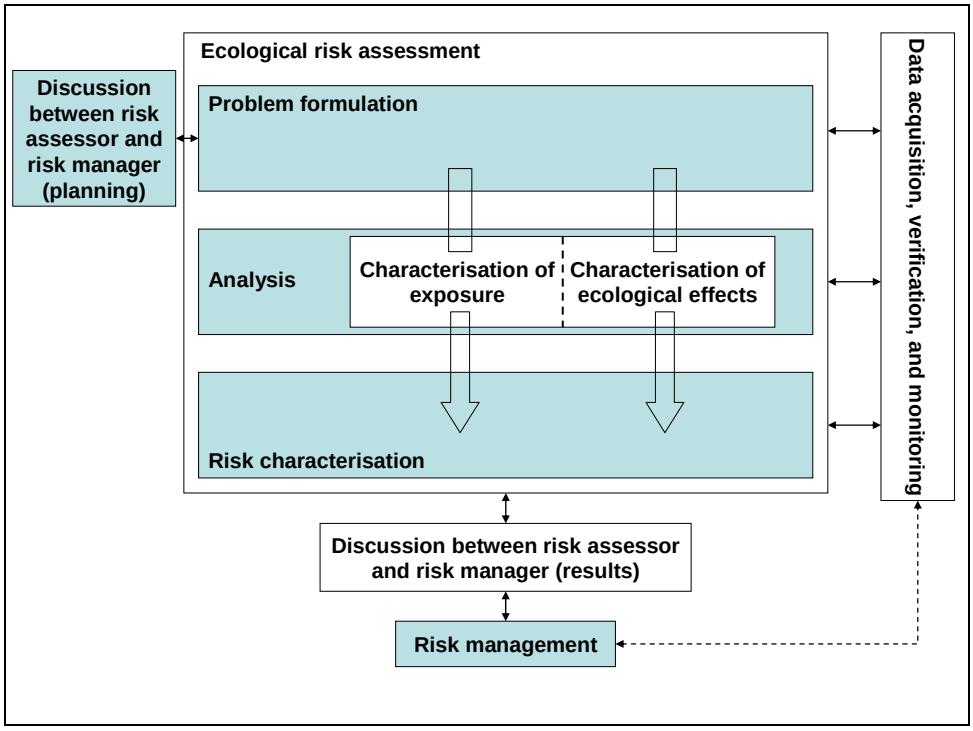



**Figure 1.1** USEPA ecological risk assessment framework (reproduced from USEPA 1998).

As the management of natural resources around the world becomes increasingly implemented at the catchment-scale, it is only logical that the ERA framework is optimised to account for this variation in scale (Hart *et al*, 2006). This approach to ecosystem management has been justified in response to water quality being dependent on catchment processes (Wauchope, 1978; Serveiss, 2002; Hart *et al.*, 2006; Serveiss and Ohlson, 2007). It is expected that the management of agrochemicals should also reflect this management scale (Hart *et al*., 2006). Subsequently, the ERA approach has been modified to operate at catchment-scales (Serveiss, 2002). However, accounting for the complexity within catchment boundaries is challenging. This has resulted in the development of more advanced spatial approaches by applying certain aspects of ERA to geographical information systems (GIS).

This chapter introduces the physicochemical characteristics of three pesticides that are the focus of this thesis, diuron, prometryn and endosulfan, and their fate in the environment. These three pesticides are relevant to the catchment data available which will then be used to exemplify approaches to analysis within this thesis. The review further evaluates the application of ERA as a framework for managing pesticides at different scales, with special focus on application of spatial tools. The review will end with a description of a study catchment that is the focus of this thesis.

## 1.2 PESTICIDES

A pesticide is any substance or mixture of substances that can prevent, destroy, repel, or mitigate any pest (USEPA, 2010). A pest is defined as an unwanted organism that causes damage to crops, humans or other animals, a classification that includes insects, rodents, and other animals, weeds, fungi or microorganisms (USEPA, 2010). There are a number of different pesticides available for use in agricultural production differing in both physicochemical properties and in the biochemical pathways or processes that they target. Such design characteristics cause pesticides to behave differently in the environment, but they can be exploited to improve their effectiveness in controlling the organisms they target. However, the ability of these chemicals to target certain biochemical pathways is not limited to pest organisms and can extend to impact non-target organisms. Consequently, the factors that affect the fate of pesticides from the site of application become important, especially in ecologically sensitive areas of catchments.

This section reviews the types, activities and classes of pesticides, the extent of pesticide use worldwide, the factors that determine the fate of pesticides in agricultural catchments and characteristic exposure scenarios that typically occur in surface water of agricultural catchments. Special focus has been placed on three contrasting pesticides − diuron, prometryn and endosulfan − to provide a practical example of the understanding and benefits of the applying catchment-scale ERA for management purposes, and that they have been characterised to potentially pose ecological problems in different environments, such as the Great Barrier reef (Bainbridge, 2009; Lewis *et al*., 2009), on sugarcane (Stork *et al*., 2008) and cotton farms (Rose, 2006), and in the rivers of northern New South Wales, Australia (Muschal and Warne, 2003).

### *1.2.1 Types, activity and classes of pesticides available for use*

Pesticides are grouped according to the organisms that they target, and by the chemical functional groups associated with their chemical structure. The latter characteristic has often been described to control a chemical group's ability to target certain biochemical pathways. Specifically, the Herbicide Resistance Action Committee (HRAC) (2005) and Insecticide Resistance Action Committee (IRAC) (IRAC, 2005) have respectively classified herbicides and insecticides according to their site of action and chemical functional groups. Further, the British Crop Protection Council (BCPC) (2006) summarised the large number of different

available active pesticides for their physicochemical properties, mode of action, environmental fate and ecotoxicity.

*1.2.1.1 Properties of three pesticides*

The chemical structures of diuron, prometryn and endosulfan, along with the highlighting of their characteristic chemical functional groups, are shown in Figures 1.2a-c and selected chemical and physical properties are summarised in Table 1.1.

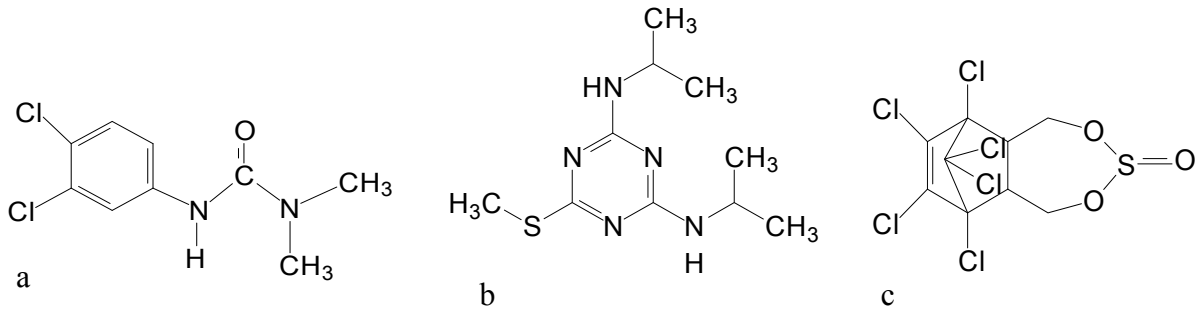



**Figure 1.2.** Chemical structures of (a) diuron, (b) prometryn and (c) endosulfan

**Table 1.1.** Selected physicochemical properties of diuron, prometryn and endosulfan (Source: British Crop Protection Council, 2006).

| Property | Diuron | Prometryn | Endosulfan |
|---|---|---|---|
| IUPAC Name | 3-(3,4-dichlorophenyl)-1,1-dimethylurea | $N^2$,$N^4$-diisopropyl-6-methylthio-1,3,5-triazine-2,4-diamine | (1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-en-2,3-ylenebismethylene) sulfite |
| CAS No. | 330-54-1 | 7287-19-6 | 115-29-7 |
| Chemical family | Urea | Triazine | Cyclodiene organochlorine |
| Composition | Single isomer | Single isomer | Mixture of two stereoisomers, i.e. α-endosulfan and β-endosulfan |
| Vapour pressure (mPa) | $1.1 \times 10^{-3}$ (at 25°C) | 0.165 (at 25°C) | 0.83 (2:1 mixture of α- and β- isomers at 20 °C) |
| $\log K_{OW}$ | 2.85 | 3.1 | α- = 4.74<br>β- = 4.79 |
| Henry constant (Pa $m^3$ $mol^{-1}$) | $7.0 \times 10^{-6}$ | $1.2 \times 10^{-3}$ | α- = 1.48 (at 20°C)<br>β- = 0.07 (at 20°C) |
| Solubility in water (mg $L^{-1}$) | 37.4 | 33 (at 22°C) | α- = 0.32 (at 22 °C)<br>β- = 0.33 (at 22 °C) |

Diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea) (Figure 1.2a) is classified as a urea herbicide (HRAC, 2005; British Crop Protection Council, 2006). It is slightly polar (Figure 1.2a), moderately soluble in water (37.4 mg $L^{-1}$; Table 1.1), non-volatile (vapour pressure = $1.1 \times 10^{-3}$ mPa; Table 1.1), and partitions more strongly into organic phases (Log $K_{OW}$ = 2.85; Table 1.2). It is formulated as a suspension concentrate, water dispersible granule and wettable powder (British Crop Protection Council, 2006).

Prometryn ($N^2$,$N^4$-diisopropyl-6-methylthio-1,3,5-triazine-2,4-diamine) (Figure 1.2b) is classified as a triazine herbicide (HRAC, 2005; British Crop Protection Council, 2006). It is slightly polar (Figure 1.2b), moderately soluble in water (33 mg $L^{-1}$; Table 1.1), volatile (vapour pressure = 0.165 mPa; Table 1.1), and partitions more strongly into organic phases (Log $K_{OW}$ = 3.1; Table 1.1). It is commonly sold as either smoke pellet (or smoke generator), suspension concentrate or wettable powder (British Crop Protection Council, 2006).

Endosulfan (1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-en-2,3-ylenebismethylene) sulphite) is classified as a cyclodiene organochlorine insecticide (IRAC, 2005; British Crop Protection Council, 2006). It is chemically composed of two isomers α- and β-endosulfan; is non-polar

(Figure 1.2 c), hydrophobic (solubility in water = 0.32 and 0.33 mg $L^{-1}$, for α- and β-endosulfan respectively; Table 1.1), highly volatile (vapour pressure = 0.83 mPa; Table 1.1); and partitions more readily into organic phases (log $K_{OW}$ = 4.74 and 4.79, for α- and β-endosulfan respectively; Table 1.1). It is available as formulations of capsule suspension, dustable powder, emulsifiable concentration, granule, suspension concentrate, ultra-low volume suspension, wettable powder and powder concentrate (British Crop Protection Council, 2006).

Apart from their differing chemical structures, a number of physicochemical properties distinguish these compounds affecting their environmental behaviour. The polarity of these compounds affects their solubility in water and ability to partition between organic and water phases. For example, diuron and prometryn exhibit higher solubilities in water over that of non-polar endosulfan. These compounds are all hydrophobic as indicated by the octanol:water partition coefficients (log $K_{OW}$) being greater than one. However, endosulfan is the most hydrophobic followed by prometryn then diuron, significantly affecting their fate. Even more important is their relative volatility, with endosulfan largely being dispersed as vapour (Kennedy *et al*., 2001), prometryn to an intermediate extent and diuron barely at all.

*1.2.1.2 Mode of action of three pesticides*

The toxicity of a pesticide (or any chemical) is defined by a chemical's mode of action and the required dose. Specifically, each pesticide uniquely targets and disrupts the function of certain biochemical processes, if the pesticide is available at the affected site in sufficient concentration. The modes of action of diuron, prometryn and endosulfan are reviewed to provide insight into the unique abilities of these compounds at targeting biochemical sites, significant in their ecological impacts.

The herbicides diuron and prometryn are both photosynthesis inhibitors. Diuron is more commonly applied to the soil surface where it is sufficiently soluble in water to be absorbed through the roots by mass transport; prometryn is commonly applied to the soil or foliage where it is absorbed both through the roots and leaves of the target weed (British Crop Protection Council, 2006). Once inside the plant tissues, the chemicals are translocated acropetally in the xylem to the leaves (British Crop Protection Council, 2006). There they target the photosystem II (PSII) apparatus in photosynthesis in chloroplasts by binding to the

second quinone electron acceptor ($Q_B$) of the reaction centre II (RCII) D1 protein (Renger, 1986; Zer and Ohad, 1995; Khan and Molin, 1996; Gooddy *et al.*, 2002; British Crop Protection Council, 2006). This action restricts the electron flow through the PSII causing alterations in the free-energy state of the primary plastoquinone redox couples $Q_A/Q_A^-$ by preventing the reoxidation of the $Q_A^-$ (Renger, 1986; Khan and Molin, 1996; Krieger-Liszkay and Rutherford, 1998; British Crop Protection Council, 2006; Eullaffroy *et al.*, 2009). Inhibition of tryptic reactions results, restricting the production of glucose required in chemical energy generation in plants (Zer and Ohad, 1995; Krieger-Liszkay and Rutherford, 1998).

Endosulfan targets neurons in insects. It is commonly applied as spray over the top of a crop and enters the target insect through contact and ingestion (British Crop Protection Council, 2006). Once inside the organism, endosulfan acts on gamma-aminobutyric acid (GABA)-gated chloride channel receptors that regulate the flow of chloride ions in nerve cells (Möhler, 1989; Casida, 1993; Bloomquist, 1996). Endosulfan blocks these channels creating a large potential differential at the channel gate resulting in neuroexcitation (Möhler, 1989; Casida, 1993; Bloomquist, 1996). At sufficient concentrations convulsions and death may occur (Bloomquist, 1996).

It is clear that diuron and prometryn, compared to endosulfan, target separate biochemical pathways to elicit their toxic action. Non-target organisms with similar biological functions are only vulnerable to such activity when they become exposed in their habitats. However, exposure of habitats to pesticides is possible through environmental persistence and various fate processes and even non-target effects may be of significance for risk.

### *1.2.3 Persistence and environmental fate of pesticides: factors that lead to pesticide contamination of catchment water bodies*

It is well documented that pesticides can move from the site of application. The extent of translocation is the outcome of the chemicals persistence, and the physicochemical interactions that occur between the pesticides and the environment it is applied (Schnoor, 1992; Kookana *et al.*, 1998; Louchart *et al.*, 2000; Mackay, 2001). This section describes the mechanisms of persistence in the environment, and the processes that can result in the physical translocation of chemicals away from the site of application.

*1.2.3.1 Factors that influence the persistence of a chemical at the site of application*

A pesticide applied to a crop sorbs to the soil and/or foliage. The pesticide partitions between all the available phases, soil, foliage, water and gas components of the applied surface and the immediate area; dissipation and degradation begins immediately (Schnoor, 1992; Mackay, 2001; Ding *et al.*, 2002; Wauchope *et al.*, 2002). When these distribution processes combine, depending on physicochemical interactions, they can either reduce or enhance the degradation of a chemical making it respectively more or less persistent. Ultimately, the rate of degradation of a pesticide and its environmental half-life is a combination of physical and that denatures the active compounds. The ability of these chemicals to persist and move to different environmental phases can provide an insight to its potential toxicity to organisms of different trophic levels.

*Pesticide partitioning*

A pesticide's ability to partition between phases has been defined as dependent on the fugacity of the pesticide and physicochemical properties of the interacting phases (Mackay, 2001). Partitioning is the outcome of thermodynamic equilibrium at equal chemical potential, establishing chemical concentration gradients between phases, for example, soil organic matter and water solution, soil and air, or solution and air (Mackay, 2001; Ding *et al.*, 2002). The extent of partitioning is the outcome of physicochemical properties of the pesticide and the partitioning media, with desorption occurring concomitantly to maintain equilibrium in situations where concentrations in either phase change (Schnoor, 1992; Mackay, 2001; Ding *et al.*, 2002; Wauchope *et al.*, 2002).

The distribution of pesticides between different phases is described by sorption-desorption potentials (Schnoor, 1992; Mackay, 2001; Ding *et al.*, 2002; Wauchope *et al.*, 2002). The preference for a chemical to partition to one phase over another is described by a partition coefficient $K_{12}$ (Equation 1.1). This coefficient describes the differences in pesticide concentration between two phases, $C_1$ and $C_2$. Often, soil/water partition coefficients ($K_d$) are determined to support environmental fate studies (Equation 1.2). A coefficient of greater than one indicates preference for the $C_1$ phase over that of the $C_2$, and in the case of $K_d$ suggests a preference for the soil phase ($Cs$) over the solution phase ($C_{aq}$). Finally, the organic carbon partition coefficient ($K_{OC}$) characterises the influence that organic carbon fraction ($\%OC$) in soil has on $K_d$, and is described by Equation 1.3.

$$K_{12} = \frac{C_1}{C_2} \qquad (1.1)$$

$$K_d = \frac{C_S}{C_{aq}} \qquad (1.2)$$

$$K_{OC} = \frac{K_d}{\%OC} \qquad (1.3)$$

Other non-linear approaches to defining partitioning, including Freundlich ($K_f$) and Langmuir have been described by van Loon and Duffy (2005).

Appendix 1 summarises *$K_d$'s* and *$K_{OC}$'s* for diuron, prometryn and endosulfan in a range of different soil types. As these compounds are more or less hydrophobic all of these pesticides reside strongly in the solid phase, with $K_d$'s being greater than one. Specifically, the soils with higher organic carbon content have a stronger sorbing capacity than soils with lower organic carbon content. This has been shown for diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea) (Gaillardon, 1997; Baskaran and Kennedy, 1999; Gouy *et al.*, 1999; Roy *et al.*, 2000; Gooddy *et al.*, 2002; Yang and Sheng, 2003a; Yang and Sheng, 2003b; Sheng *et al.*, 2005; Landry *et al.*, 2006; Liyanage *et al.*, 2006; Burns *et al.*, 2008), prometryn (Baskaran and Kennedy, 1999; Seol and Lee, 2000; Fingler *et al.*, 2004; Simpson, 2007) and endosulfan (Ghadiri and Rose, 2001; Marshall and Rutherford, 2002; Kumar and Philip, 2006) (See Appendix 1 for details).

Specifically, the diuron $K_d$ reported in the literature range for cropped soils 1.94 L kg$^{-1}$ for a sandy loam in Roujin, France to 39.3 L kg$^{-1}$ for a redoxic hydrosol in Emerald, Australia (Simpson, 2007); 1.78 L kg$^{-1}$ for a cracking clay in Narrabri, Australia (Baskaran and Kennedy, 1999) to 164.73 L kg$^{-1}$ for a clay in Sicily, Italy (Fingler *et al.*, 2004); and 21-295 L kg$^{-1}$ for endosulfan in sediment (Peterson and Batley, 1993) and 231.5 L kg$^{-1}$ for a medium clay in Brazil (Laabs and Amelung, 2005). Importantly, these ranges demonstrate variable partitioning rates between soil types and regions (See Appendix 1 for details). However, it is important to note the greater ability of diuron to re-distribute to the aqueous phase, given its greater water solubility.

*Degradation*

Degradation, collectively, is a process that results in the transformation of the parent compound into physicochemically different daughter compounds (Baer and Calvet, 1999; Jacobson *et al.*, 2005). Different degradation processes operate in the field and are characterised to be either biotic or abiotic in nature.

Biotic degradation mechanisms include plant and microbial metabolism of the parent compound (Sheets, 1964; Kookana *et al.*, 1998). In soil, biotic transformations are those that occur in or are enzyme catalysed within or outside the cells soil organisms (Scheunert, 1993). The activity of the enzymes is often mediated by environmental conditions such as pH, water content, temperature and substrate concentration (Baer and Calvet, 1999; Knox *et al.*, 2001). In soil, microbial degradation of pesticides is influenced by competition with other organic substrates in soil (Baer and Calvet, 1999; Gooddy *et al.*, 2002). Biotic degradation has been reported to occur for diuron (Gooddy *et al.*, 2002), prometryn (British Crop Protection Council, 2006) and endosulfan (Peterson and Batley, 1993; Awasthi *et al.*, 2000; Kennedy *et al.*, 2001; Leonard *et al.*, 2001). Degradation mechanisms and degradation products (degradates) reported in the literature are summarised in Table 1.3 and explained in more detail below.

Abiotic degradation mechanisms include chemical and photochemical transformation (Sheets, 1964; Kookana *et al.*, 1998). Chemical degradation reactions are mediated by reactive chemical species or molecular functions within soil, or by catalysis with chemical species, such as metal oxides and organic or mineral surfaces (Scheunert, 1993). The extent by which a pesticide undergoes chemical degradation is the outcomes of its chemical properties, with processes of most interest in the field include oxidation, reduction and hydrolysis. Photochemical degradation is a mechanism mediated by a parent compound that has been exposed to light. The interaction results in a breakdown of chemical bonds, depending on the nature of the media which the chemical is contained.

Diuron, prometryn and endosulfan have been reported to undergo photochemical degradation. However, only endosulfan has been reported to undergo chemical transformation, where as diuron and prometryn have not owing to their chemical stability. The mechanisms and

degradation products resulting from abiotic degradation reported in the literature are summarised in Table 1.2.

**Table 1.2** Summary of chemical, biological and photochemical degradation mechanisms and daughter products of diuron, prometryn and endosulfan reported in the literature

| Pesticide | Mechanism | Daughter compound IUPAC name | Daughter compound common name | Reference |
|---|---|---|---|---|
| Chemical degradation | | | | |
| Endosulfan | Oxidation of sulphate functional group in water | 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9ahexahydro-6,9-methano-2,4,3-benzodioxathiepine-3,3-dioxide | Endosulfan sulphate | Kennedy et al. (2001); (NRA, 1998) |
| Endosulfan | Alkaline hydrolysis of the sulphite functional group (pH 7-9) in water | 1,4,5,6,7,7-hexachlorobicyclo[2.2.1]hept-5-ene-2,3-dimethanol | Endosulfan diol | Kathpal et al. (1997); Kaur et al. (1998); Shivaramaiah et al. (2005); (NRA, 1998) |
| Biological degradation | | | | |
| Diuron | Aerobic microbial oxidation leading to demethylation of the urea group and hydrolysis | 3,4-dichlorophenylaniline | DCA | Gooddy et al. (2002) |
| Prometryn | Metabolism in plants and microbes oxidising methylthio group | 2,4-diamino-1,3,5-triazine | Deisopropyl prometryn | British Crop Protection Council (2006) |
| Endosulfan | Aerobic microbial oxidation of sulphite functional group | 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9ahexahydro-6,9-methano-2,4,3-benzodioxathiepine-3,3-dioxide | Endosulfan sulphate | Peterson and Batley (1993); Awasthi et al. (2000); Kennedy et al. (2001); Leonard et al. (2001) |
| Endosulfan | Metabolism by microbes and plants cause alkaline hydrolysis of the sulphite functional group | 1,4,5,6,7,7-hexachlorobicyclo[2.2.1]hept-5-ene-2,3-dimethanol | Endosulfan diol | Peterson and Batley (1993); Awasthi et al. (2000); Kennedy et al. (2001); Leonard et al. (2001); Sethunathan et al. (2004) |
| Endosulfan | Aerobic microbial oxidation of sulphite functional group | 4,5,6,7,8,8-hexachloro-1,3,3a,4,7,7ahexahydro-4,7-methano-isobenzofuran | Endosulfan ether | Sethunathan et al. (2004) |
| Photochemical degradation | | | | |
| Diuron | Photolysis of diuron in water resulted in heterolytic substitution of chlorine by hydroxyl groups | 3-(4-chloro-3-hydroxyphenyl)-1,1-dimethylurea | Not Reported | Jirkovsky et al. (1997) |
| Diuron | Irradiation of diuron on sand results in elimination or oxidation of methyl groups | (3,4-dichlorophenyl)-urea; and 3-(3,4-dichlorophenyl)-1-formylurea | Not Reported | Jirkovsky et al. (1997) |
| Prometryn | Photochemical degradation in water by cleavage of the thiomethyl and isopropyl groups | 2,4-diamino-1,3,5-triazine | Deisopropyl prometryn | Kiss et al. (2007) |
| Endosulfan | Ultraviolet irradiated of endosulfan resulted in transformation of sulphite functional group | 1,4,5,6,7,7-hexachlorobicyclo[2.2.1]hept-5-ene-2,3-dimethanol; and 4,5,6,7,8,8-hexachloro-1,3,3a,4,7,7ahexahydro-4,7-methano-isobenzofuran | Endosulfan diol; and Endosulfan ether | Archer et al. (1972); Barcelo-Quintal et al. (2008) |

The time taken for a pesticide to degrade in the different environmental phases is characterised by a half-life. A half-life is defined as the time taken for half of the current chemical mass to degrade. Pesticide half-lives have most commonly been characterised for chemical dissipation in soil, water and plant material, assumed to be first order processes. Appendix 1 presents a summary of diuron, prometryn and endosulfan half-lives reported in field and laboratory studies for soil, water and plant material over the world. Collectively, the different degradation processes described earlier contribute to the persistence of the pesticide in the specific phase. For example, prometryn dissipation in water is influenced by temperature and light with shorter half-lives reported for prometryn in water exposed to light (55-137 days; Navarro *et al.*, 2004; Vela *et al.*, 2004) and prometryn in water exposed to light and a high temperature of 40$^{o}$C (half-life = 75-106 days; Vela *et al*., 2004); relative to experiments performed in the dark, half-lives of prometryn ranged 66-263 days (Navarro *et al*., 2004) and 128-168 days (Vela *et al*., 2004). Dissipation of endosulfan in water is strongly influenced by increasing pH, being very rapid in alkaline conditions above pH 8 (Peterson and Batley, 1993; Kennedy *et al*., 2001).

The reported half-lives of diuron, prometryn and total endosulfan residues in soil range 15-80.7 days, 6.2-22.0 days, and 3.1-77 days, respectively. Further to these, Pablo and Hyne (2009) reported the half-life of endosulfan in sediment and gravel fractions of a mesocosm pond ranged 31-207 days. Navarro *et al*. (2000) reported α- and β-endosulfan dissipation in sterile marine sediment (half-life α- and β- endosulfan = 17.4 days and 16.1 days) to be longer than that of unsterile marine sediment (half-life α- and β- endosulfan = 8.5 days and 8.0 days), suggesting that microbial activity played a significant role in dissipation in marine sediments. Rouchaud *et al*. (2000) reported that when compared to a single diuron application; continuous application over 12 years was reported to reduce the half-life from 80.7 days to 37.3 days, suggesting enhanced microbial activity resulted through adaptation from continuous exposure.

The half-lives for diuron, prometryn and endosulfan in water range 3.2-38.3 days, 2.1-168 days and 0.9-77 days, respectively. The half-life of total endosulfan residues in plant material ranges 0.43-1.6 days, no readily available studies have been identified reporting dissipation of diuron and prometryn in plant material.

*Transport*

At the same time as undergoing partitioning and degradation processes, pesticides can be transported from the site of application in runoff, leaching water, and air. As previously mentioned partitioning determines which phase a pesticide will be in and indicates subsequent transport. The chemical load available for translocation is also dependent on dissipation processes that may have occurred since the application of the pesticide (Simpson, 2007). The remainder of this section describes the movement of pesticides and the typical concentration scenarios likely observed in catchments.

Pesticides can be transported in runoff as dissolved, sediment- and colloidal-bound load (Crossan *et al.*, 2002; Simpson, 2007); and leached down a soil profile in a colloidal or dissolved form (Crossan *et al.*, 2002). During rainfall events, pesticides can enter solution phase as water disrupts the equilibrium state of soil-water or foliage-water interaction causing a pesticide to desorb (Scheunert, 1993; Williams *et al.*, 1995; Kookana *et al.*, 1998). The extent of desorption is dependent on the chemical load in solution and the partitioning potential defined by the $K_d$ of the pesticide and sorbing matrix. Further, the pesticides with greater solubility, such as diuron and prometryn, have a significant potential to partition in to solution over that of less soluble compounds such as endosulfan. The chemical that enters the solution phase can leach passed the soil vadose zone with infiltrating water or be transported in overland flow or runoff, the rates of which are dependent on soil properties that are spatially variable.

The velocity of runoff in sloping terrain may be sufficient to entrain and suspend soil or dispersed colloidal material containing sorbed (either adsorbed or absorbed) pesticides. As diuron, prometryn and endosulfan all have strong tendencies to reside in the solid phase is an indication that the largest pesticide loads are commonly bound to sediment (Wauchope, 1978). These are particularly prominent for runoff events that have high velocity occurring in high sloping landscapes (Wauchope, 1978).

Pesticides can be transported in wind as a gas or bound to soil particles as dust. Like water and soil, pesticides partition into the atmosphere through air-water and air-soil partitioning potentials that is stimulated by concentration gradients and temperature (Mackay, 2001). Such a mechanism is controlled by the vapour pressure of a compound and the temperature of the matrix. Pesticides that partition into the gas phase are transported in wind, and under

sufficiently windy conditions be transported bound to soil. For example, endosulfan being a volatile compound (vapour pressure = 0.83 mPa for a 2:1 mixture of α- and β- isomers at 20 $^{o}$C; British Crop Protection Council, 2006) is well characterised to be transported as vapour as well as by aerial drift as spray droplets (Kennedy *et al.*, 2001), whereas limited literature have indicated vapour as a significant transport route for diuron and prometryn. However, prometryn with a similar volatility to endosulfan indicates transport in the gas phase is possible as well as spray drift. Comparatively, diuron having low volatility two to three orders of magnitude lower suggests that transport in the gas phase would be a very minor route. The material entrained in air may then deposit downwind from the site of application. Such theoretical insights into possible pesticide behaviour offer a risk assessor *a priori* knowledge to integrate and test in the assessment process.

Pesticides transported in wind and runoff may contaminate non-target water bodies in agricultural catchments (Williams *et al.*, 1995). The concentrations of pesticides in surface waters of catchments have been characterised to be temporally highly variable (Wauchope, 1978; Skark *et al.*, 2004). The duration and magnitude of the runoff pulses is dependent on the hydrological responsiveness of the catchment, climate, soil characteristics, land use and pesticide loads. Such attributes vary spatially, and is subsequently reflected in spatial variability in exposure. The concentrations in these streams can sometimes reach levels that may affect ecological groups (Wauchope, 1978). The occurrence of such events is the outcome of natural resource management strategies and can be described by application of ecological risk principals.

## 1.3 NATURAL RESOURCE MANAGEMENT AND PESTICIDE USE IN AUSTRALIA

Pesticides are considered non-point source pollutants, as there is no single source of contamination. Unless comprehensive monitoring is undertaken, elucidating the sources of pesticide contamination in agricultural catchments is difficult. The common means which non-point source pollutants are managed in catchments is through integrated catchment-wide approaches (Seymour and Ridley, 2005). Such approaches have commonly been adopted to manage water quality in catchments of Australia (Mitchell and Hollick, 1993; Johnson *et al.*, 1996; Hu, 1999; Bryan *et al.*, 2009) and around the world (Hu, 1999; Hess *et al.*, 2010). However, the management of pesticides in Australia has most commonly been limited to the farm-scale, typically driven within an industry.

Integrated water resource management, catchment management or Environmental Management System (EMS), is an approach used in Australia and in other countries to manage water quality for human and ecological needs (Seymour and Ridley, 2005). The approach involves managing the natural resources using incentive-based strategies unique to a catchment enacted through co-operation and co-ordination of government organisations, management groups (e.g. Landcare), experts and stakeholders (Mitchell and Hollick, 1993; Johnson *et al.*, 1996; Seymour and Ridley, 2005). Most state governments of Australia have established natural resource management organisations, commonly known as Catchment Management Authorities (CMA) or Natural Resource Management (NRM).

The issues that are dealt with by CMA's include pests, vegetation, and soil and water quality monitoring (Mitchell and Hollick, 1993; Johnson *et al.*, 1996; Seymour and Ridley, 2005). In the case of water quality salinity, acidity, eutrophication and sediment are monitored rigorously. However, characterising potential pesticide contamination and the ecological groups they may affect is beyond their scope due mainly to limitations in skills, resources and technology that enables them to manage at the local level (Hart *et al*., 2006). Typically, pesticide concerns are directed to state government departments, such as environment protection authorities (EPA) that respond when contamination incidents that detrimentally effect local organisms, e.g. fish kills. Such evaluations are only limited to the site of contamination (NEPC, 1999), and rarely consider catchment-scale evaluations in Australia (Hart *et al*., 2006). However, the spatial and temporal variability in pesticide applications on farms and the catchment processes of runoff and drift that influence their fate confirms them being diffuse source pollutants. It would therefore be expected that pesticides be monitored and managed routinely like other diffuse source pollutants, e.g. salinity and sediment, through management strategies on a catchment-scale basis. Determining if contamination is ecologically detrimental can be investigated through ERA adapted to the catchment-scale (Serveiss *et al.*, 2000; Serveiss, 2002; Hart *et al.*, 2006; Serveiss and Ohlson, 2007), an approach that has been considered for use in Australia previously (e.g. Hart *et al.*, 2006; Kookana *et al.*, 2006) but not implemented.

## 1.4 ECOLOGICAL RISK ASSESSMENT

Conceptually, ERA is a decision support framework designed to aid decision making where information may be deficient (USEPA, 1998; Suter II, 2007). ERA is used predominantly by regulators in a range of different applications, e.g. contaminated site evaluation (NEPC,

1999), with varying rules, depending on what purpose it will serve in supporting management decisions. It essentially serves as a model that environmental regulators around the world use for a specific application, but it is not applied dynamically. If applied to the catchment-scale ERA could serve as a dynamic decision support tool for managing pesticides in a range of different environments that reflect the local catchment management goals with the intention of protecting ecosystem health (Hart *et al*., 2006), while maintaining the production benefits that pesticides provide. The classic phases of the ERA framework (USEPA, 1998) of problem formulation, risk analysis and risk characterisation are briefly described.

### *1.4.1 Problem formulation phase*

Problem formulation is the phase that develops the organizing framework for the ERA. The purpose of the phase is to summarise the complex environmental concerns, impacts and relationships observed in the catchment, and formulate preliminary hypotheses about why ecological effects have or may occur as a result of human activities (USEPA, 1998; Cormier *et al.*, 2000; Suter II, 2007). It makes use of available information that characterise ecological resources potentially at risk, stressors, and observed or anticipated ecological effects, to describe the nature of the problem and identify measurable attributes that can be used as measures of exposure and effect, and assessment endpoints. Eventually, a conceptual model is developed to describe the interrelationships among resources, stressors and effects, and provide a focus for the assessment and a plan for the analysis phase of the ERA (USEPA, 1998; Cormier *et al.*, 2000; Serveiss *et al.*, 2000; Suter II, 2007).

### *1.4.2 Analysis phase*

The analysis phase utilises the relationships presented in the conceptual model and develops them to focusing on the most important stressors, their exposure pathways and ecological effects (USEPA, 1998; Serveiss *et al.*, 2000). Specifically, exposure is commonly characterised through targeted sampling or exposure modelling of defined areas. The toxicity posed by these compounds to different ecological groups; and toxicity through continuous exposure laboratory studies

This is achieved by taking targeted measurements, modelling or extrapolation from field or laboratory data to describe existing conditions and subsequently characterise the exposure and effects. An insight to the relationships that exist between the stressors behaviour in the

environment and the effects on organisms that result from exposure is developed (USEPA, 1998; Serveiss *et al.*, 2000; USEPA, 2002a; USEPA, 2002b).

In taking measurements of existing conditions, a quantitative approach is often not possible to characterise each exposure and effect scenario. The quantification of risks may involve targeting single species or chemicals that represent simplified scenarios as opposed to complex and variable situations observed in the environment. It is therefore important to acknowledge that ecosystems are exposed to multiple stressors and are also likely to exhibit incomplete information. Subsequently, scientific judgement and a weight of scientific evidence approach is recommended in order to address information gaps in estimating exposure or effects (Solomon *et al.*, 1996; USEPA, 1998; Serveiss *et al.*, 2000; USEPA, 2002a; USEPA, 2002b). Further, the risk analysis may focus on the ecological effects posed by a stressor of concern, or seek associations with multiple stressors. As a final step, the uncertainty of all the information utilised must be conveyed.

### *1.4.3 Risk characterisation*

Risk characterisation is the final phase in ERA. In risk characterisation, the likelihood and significance of adverse effects resulting from exposure of stressors determined. Two major steps are taken in this phase, risk estimation and risk description. A number of different approaches are commonly used to estimate risk. These conceptually involve comparing the exposure and stressor-response profiles developed in the analysis phase. Estimation methods include hazard quotient (e.g. Nabholz 1991); risk indexes (e.g. Xuan and Zang, 2011); comparing statistical distributions of exposure and effect (e.g. Solomon *et al*. 1996); or using models (e.g. Burmaster and Anderson, 1994; Campbell *et al.*, 2000; Pollino *et al.*, 2007; Park *et al.*, 2008). Risk description interprets the estimated risk from the perspective of likely ecosystem responses resulting from exposure. This includes defining the organisms possibly being affected with respect to the toxicity thresholds set when defining the assessment endpoints, and how these responses translate in to a wider ecosystem effects (e.g. Solomon *et al.*, 1996). Finally, uncertainties that arise from the estimation of risk are characterised.

### *1.4.4 Risk communication and risk management*

The information conceived throughout the ERA is collated and reported. When new information becomes available, the ERA framework expects that such information be used to assess if it changes the outcome of any interpretations of risk that may have previously been

determined. It may well be that as a result of the implementation of management practices has resulted in sufficient ecological protection to warrant relaxing of imposed regulations, or vice versa.

## 1.5 METHODS FOR CHARACTERISING RISK AND COMMON UNCERTAINTIES

Pesticide exposure can be estimated using several different methods. Typically, risk estimation involves comparing exposure profiles with ecotoxicity profiles developed in the analysis phase. Depending on the level of rigour preferred for the risk assessment, or its tier, a range of approaches have been developed to suit. For example, in lower tier assessments, preliminary or screening "hazard quotients" are typically used (Nabholz, 1991; Campbell *et al.*, 2000). Higher tier risk assessments involve comparing statistical distributions of exposure and ecotoxicity effect (Solomon *et al.*, 1996; Campbell *et al.*, 2000; Park *et al.*, 2008); and/or using Monte Carlo (Burmaster and Anderson, 1994), and Bayesian networks (Pollino *et al.*, 2007) models. Specifically, Bayesian approaches are often parameterised using knowledge elicitation only making such approaches highly qualitative (Pollino *et al.*, 2007; Suter II, 2007). Further, the data requirements such approaches are often limiting in ERA, and the time and effort required to parameterise Bayesian approaches has been characterised to be overwhelming (Pollino *et al.*, 2007), and was therefore not considered in this thesis. Of the remaining approaches to risk characterisation, this section describes the different approaches used to characterise ecological risk, and the limitations and uncertainties that are often encountered.

### *1.5.1 Hazard quotient*

Lower tier ERAs establish if a compound poses a hazard to an ecosystem. This is characterised by using a hazard quotient (HQ) that compares the highest measured or predicted exposure concentration ($C_e$) with a toxicity threshold concentration ($C_{tox}$) (Equation 1.4). When the quotient exceeds one this result indicates some level of hazard (Volosin and Cardwell, 2002). However, this concept is sometimes misinterpreted to indicate "risk" (Chen and Liu, 2006; Ward *et al.*, 2007), and has been revealed by Solomon and Sibley (2002); and Tannenbaum *et al*. (2003) to be a common problem in ERA. Put simply, this relationship is not a measure of risk as it lacks a probabilistic paradigm indicating likelihood of exposure (Volosin and Cardwell, 2002; Tannenbaum *et al.*, 2003). As a result, for accuracy, the HQ should only be limited to gauge the necessity for further exposure assessment using

probabilistic techniques where the quotient exceeds one (Solomon *et al.*, 1996; USEPA, 1998; Volosin and Cardwell, 2002; Tannenbaum *et al.*, 2003).

$$HQ = \frac{C_e}{C_{tox}} \qquad (1.4)$$

*1.5.2 Probabilistic risk characterisation*

The USEPA has now established a preference for using probabilistic approaches to characterise risk (USEPA, 1998). This involves comparing distributions of exposure with a distribution of species sensitivity (SSD). Conceptually, the degree of overlap of these logarithmic distributions (Figure 1.3 a) defines whether exposure occurring in a defined ecosystem is likely to affect proportions of a model ecosystem, that is described in a joint probability curve (Figure 1.3 b). Based on these relationships, the level of ecosystem disturbance is gauged by how many species toxicity percentiles are exceeded, further translated to different ecological groups based on trophic interactions, where possible.

A detailed method for applying probabilistic risk assessment (PRA) has been described by Solomon *et al*. (2000). The following sections detail the important steps of this probabilistic risk characterisation including collection of exposure information and development of SSDs, as well as risk interpretation.

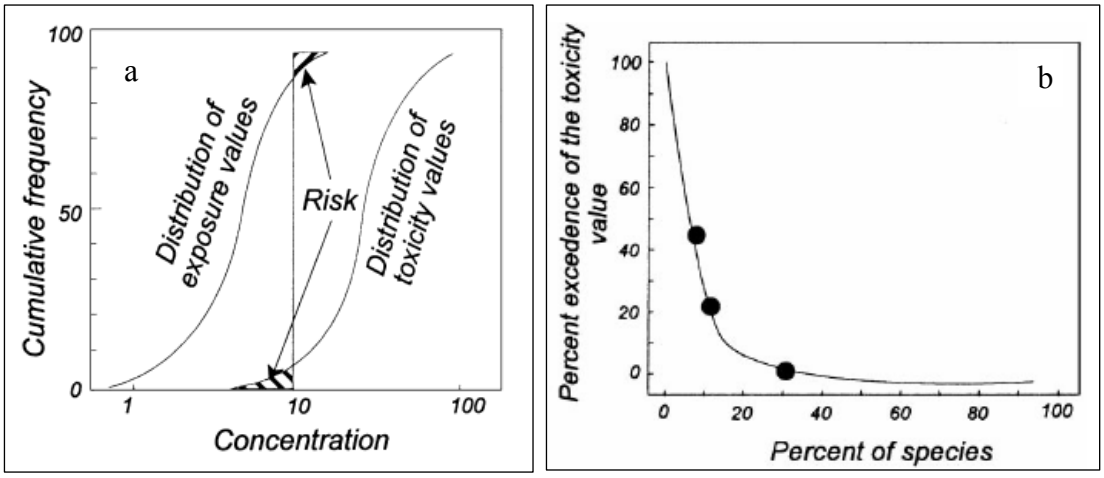



**Figure 1.3** (a) Relating exposure and toxicity (also known as an SSD) distributions to characterise risk; and (b) a joint probability curve that highlights the level of risk showing the decline in likelihood of exceedance when more species are considered (taken from Solomon *et al*. 2000).

*1.5.3 Data requirements*

*1.5.3.1 Data requirements to characterise pesticide exposure*

In performing ERAs pesticide exposures in streams are conventionally characterised by taking field measurements or through modelling of the target compound(s) (USEPA, 1998; Serveiss *et al.*, 2000; Holvoet *et al.*, 2007). Methods of monitoring include grab sampling for water analysis and passive sampling using concentration by inert solvents or exchange materials. Monitoring campaigns schemes are characteristically intensive in order to develop a comprehensive pesticide exposure profile that accounts for the full range and probability of events occurring. It may be conducted randomly or calibrated to pesticide application schedules and this fact recorded. This section reviews the different approaches of grab and passive sampling, and exposure modelling techniques used to evaluate chemical exposure in catchments.

Grab sampling involves inserting a vessel under or down-current of a discharge with the container opening of the container facing upstream at least 0.5 m below the water surface. A number of samples are taken in sequence to characterise concentrations temporally, and where possible at a number of different points to account for spatial variation in exposure. For example, El-Kabbany *et al*. (2000) characterised exposure for a range of organic pesticides in the El-Haram Giza region of Egypt, by carrying out targeted sampling in various reaches of the Giza region. Comoretto *et al*. (2007) investigated pesticide exposure in the Rhône River delta, France and was able to report on seasonal and spatial variation in exposure. A large dataset consisting of pesticide exposure data for the reaches of Gwydir River catchment in northern New South Wales, Australia, as part of the Gwydir Water Quality Project (GWQP), used the grab sampling approach and has been identified as potential value for supporting the development of catchment-scale ERA. Depending on the nature of the target compounds, pesticides are either measured directly in the water or after extraction and concentration before being measured using High Performance Liquid Chromatography (HPLC), enzyme-linked immunosorbent assay (ELISA), or gas chromatography (GC) analytical techniques. Although the grab sampling may adequately characterise the exposure being exhibited in the environment temporally and spatially, the time and resources required to execute an adequate investigation is considered exorbitant.

Another sampling approach developed in the previous 10-15 years involves the use of passive samplers. Passive samplers are increasingly being used in the determination of long-term

pesticide exposure, particularly at low levels. They range in design and characteristically use material that is able to sorb and concentrate specific analytes (Alvarez *et al.*, 2004; Hyne *et al.*, 2004; Petty *et al.*, 2004). The chemical nature of the sorbent varies according to the chemical nature of the target contaminant, which the target analyte generally has a high affinity. The devices are immersed in the water over an extended period of time, after which the sampler is removed and the analytes are eluted from the sorbing material and analysed (El-Kabbany *et al.*, 2000; Alvarez *et al.*, 2004; Hyne *et al.*, 2004; Petty *et al.*, 2004) in a suitable solvent. The total residues eluted from a passive sampler is determined as a load and is considered to be an integral of the concentration during the time the sampler is spent immersed in the water body. The time interval average concentration is estimated from the load sorbed to the sorbent and the average discharge of the reach (Alvarez *et al.*, 2004; Hyne *et al.*, 2004; Petty *et al.*, 2004).

Passive samplers have been used in a number of different studies to characterise pesticide exposure. For example, Hyne *et al*. (2004) used a passive sampler to estimate the concentration of α-endosulfan, β-endosulfan, endosulfan sulfate and chlorpyrifos-ethyl for periods of 7-22 days in the Gwydir River and Namoi River catchments, Australia. Petty *et al*. (2004) investigated time integrated concentrations of organochlorine pesticides, polycyclic aromatic hydrocarbons, organophosphate pesticides, and pharmaceutical chemicals in waste water discharging into a constructed wetland located in the Columbia, Missouri, USA. Hyne *et al*. (2004) and Petty *et al*. (2004) found that the passive samplers used were able to predict within reasonable limits average pesticide concentrations despite the wide variation in river-water concentrations through time. The major benefits of using passive samplers are that they minimise cost, and maximise the precision and breadth of application in characterising exposure (Alvarez *et al.*, 2004; Hyne *et al.*, 2004; Petty *et al.*, 2004). However, Hyne *et al*. (2004) concluded that passive samplers cannot be used for direct measurement of event concentrations and their calibration is imprecise.

Where data from exposure monitoring is insufficient, exposure models have been used by various authors to supplement exposure data. Solomon *et al*. (1996) predicted and compared exposure of atrazine in runoff water from treated fields using the USEPA's PRZM (Pesticide Root Zone Model) and the USDA's GLEAMS model. Parker *et al*. (2007) simulated atrazine, metolachlor, and trifluralin transport in the Sugar Creek sub-catchment of the White River catchment, Indiana, United States using three catchment-scale models, the Soil Water

Assessment Tool (SWAT), the Nonpoint Source Model (NPSM), a modified version of the Hydrologic Simulation Program-Fortran (HSPF), and the Pesticide Root Zone Model-Riverine Water Quality (PRZM-RIVWQ). Multimedia models have also been developed that predict the fate of chemicals based on fugacity principles (see Mackay, 2001).

*1.5.3.2 Species sensitivity distributions*

Determining ecological effects resulting from exposure involves comparing environmental monitoring data with that of ecotoxicity. Often, ecotoxicity data is unavailable for catchment-specific organisms, and ecosystems for that matter (Posthuma *et al.*, 2002a). This problem can be addressed by using a species sensitivity distribution (SSD).

An SSD is a statistical distribution that combines, following a screening process, all single species continuous exposure laboratory ecotoxicity studies. The effect endpoint concentrations gained from these studies are collated and developed in to a cumulative frequency distribution described by a function (Posthuma *et al.*, 2002a). The assumption being that the exposure concentrations observed in the environment will affect a proportion of organisms in an ecosystem, with the fraction of organisms affected corresponding to measured exposure concentrations (Solomon *et al.*, 2000; Solomon and Takacs, 2002).

The SSD approach is commonly used to define toxicity thresholds in setting environmental quality criteria, guidelines for judging the quality of an environmental resource. For example, in Australia the Australian and New Zealand Environment Conservation Council (ANZECC) set environmental protection standards using the SSD approach, by defining a hazard concentration reflective of a certain percentage of the species of organisms ($HC_x$), typically 5% ($HC_5$) (ANZECC, 2000). That is, 95% of the different organisms should be protected. Variations on thresholds are recommended to be applied to distinguish the value and importance of an ecosystem (Solomon *et al.*, 1996; ANZECC, 2000), in the case of ANZECC (2000) these range from 99% for the most ecologically valuable sites, to 90% for the least, with 90-95% protection used in most ERAs (e.g. Solomon *et al.*, 1996; Solomon *et al.*, 2000; Muschal and Warne, 2003; Carriger and Rand, 2008; Schuler and Rand, 2008; Wang *et al.*, 2009).

The toxicity data used to develop SSDs originate from standard laboratory continuous exposure toxicity studies (Posthuma *et al.*, 2002a; Suter II, 2007). These studies involve

exposing an organism to a range of concentrations and track changes in the populations for a set period of time (e.g. OECD, 1992; USEPA, 1996; OECD, 2004; Brain and Solomon, 2007). The interaction between exposure dose and organism response (such as mortality of a given fish species if exposed to different concentrations of the pesticide) is characterised in a stressor-response curve (Figure 1.4; Serveiss *et al.*, 2000). The responses recorded include No Observable Effect Concentration (NOEC), Lowest Observed Effect Concentration (LOEC), both determined by differences of statistical significance; and as single reference value such as an $LC_{50}$ (lethal concentration effecting 50% of the test population) or $EC_{10}$ (concentration causing an effect to 10% of the population) depending on the scenario being described and the best approach for its presentation. Standard testing procedures have been developed to support the chronic and acute response of aquatic plants (OECD, 1998b; OECD, 2006; Brain and Solomon, 2007), fish (OECD, 1984; OECD, 1992), and vertebrates and invertebrates (OECD, 1998a; OECD, 2004) resulting from exposure. The information from these studies are readily accessible from the literature or toxicity databases, such as the USEPA ECOTOX(icology) database (USEPA, 2009).

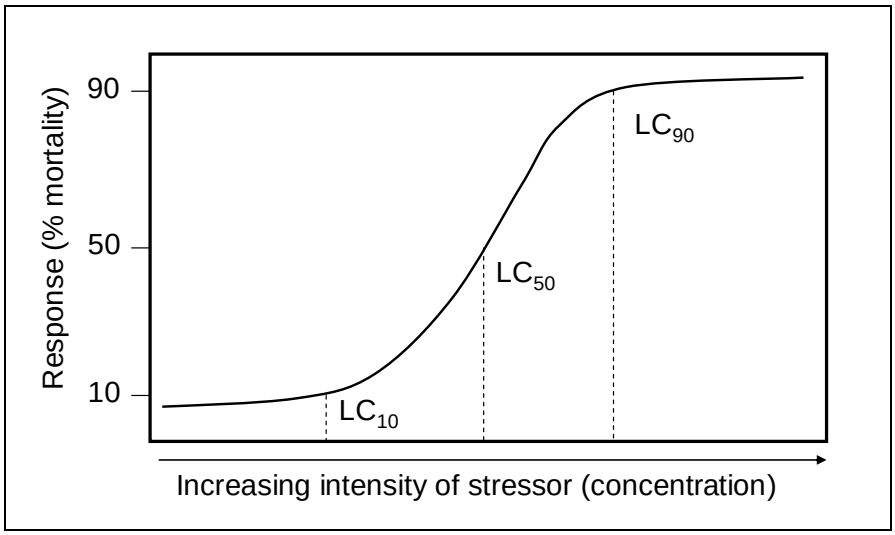



**Figure 1.4** Simplified stressor response relationship of response (% mortality) and increasing intensity of stressor [concentration] (optimised after Serveiss *et al*. 2000).

*Data selection in species sensitivity distributions*

Since the duration of exposure contributes to that results in the observed response, accounting for uncertainty in pulse durations often involves producing two separate SSDs. These account for data on acute *versus* chronic exposure durations in the SSD data selection. Criteria used to select single-species toxicity data are related to test endpoint, duration, effect type, effect measurement and exposure type. Most often toxicity endpoints of median lethal concentration

($LC_{50}$), median effect concentrations ($EC_{50}$) and median inhibition concentration ($IC_{50}$) for aquatic animals and plants (including algae and macrophytes) are used (USEPA, 2009). Lowest observed (LOEC) and no observed (NOEC) effects concentration are commonly excluded because of their lack of precision and statistical rigour, variability at representing effects and low dataset abundance (Laskowski, 1995; Kooijman, 1996; Posthuma *et al.*, 2002b; Suter II, 2007). Similarly, toxicity studies reporting effect concentrations exceeding the water solubility of the toxicant are normally excluded but included in the rank calculation (Solomon and Takacs, 2002; Carriger and Rand, 2008), and De Zwart (2002) suggests salt and freshwater toxicity data be combined.

Different approaches to selecting toxicity data for exposure duration and exposure type have been reported. For example, Maltby *et al*. (2005) sorted for data sets test durations in the range 2 to 21 days for fish, 1 to 7 days for invertebrates, 2 to 28 days for macrophytes, and 1 to 7 days for algae, when comparing insecticide SSDs. Solomon *et al*. (2000) used a 48 h exposure duration species $EC_{50}$ and $LC_{50}$ for crustaceans, insects and fish. De Zwart (2002) unified reported exposure durations to 96 h (4 days). Further sorting of single species the exposure type is sometimes used with experiments exhibiting continuous flow-though systems given preference over experiments that were replacing exposure solution at intervals and static systems that were not replacing exposure solutions (Solomon and Takacs, 2002). Where there maybe more than one data point remaining for a single species, the geometric mean is normally calculated to favour the more sensitive studies (Solomon *et al.*, 2000; Solomon and Takacs, 2002; Maltby *et al.*, 2005; Rand *et al.*, 2010).

Finally, the collated ecotoxicity data are commonly grouped according to taxonomic groups. Potential differences in toxicity between taxonomic groups are evaluated. Often as a result of the stressor mode of action specific taxonomic groups may be more sensitive, e.g. herbicide toxicity is more relevant for plants than say invertebrates. Solomon and Takacs (2002) recommends grouping organisms found to be significantly different in sensitivity to a target compound.

*Examples of applying probabilistic risk characterisation, and how risk is interpreted*

Probabilistic risk characterisation has been used in a number of regional- and catchment-scale studies. For example, Solomon *et* al. (1996) characterised exposure risk posed by atrazine in surface waters of North America. Mushcal and Warne (2003) established the risk posed by

eight pesticides, including diuron, prometryn and endosulfan, to aquatic ecosystems the Northern Rivers region of New South Wales, Australia. Wang *et al*. (2009) evaluated the exposure risk posed by a range of organochlorine pesticides, including endosulfan, in a segment of Huaihe River, China. Carriger and Rand (2008) evaluated the chronic risks posed by five pesticides, including endosulfan, in the surface waters adjacent to the Everglades and Biscayne National parks; and Schuler and Rand (2008). In all cases, risk is interpreted by relating fractions of the SSD with those of exposure. The probability that each toxicity threshold is exceeded is estimated and summarised as joint probability curves. From this, the specific organisms being affected are further translated to characterise wider ecosystem responses, with special reference to impacts occurring at different trophic levels.

## 1.6 UNCERTAINTIES AND THEIR IMPORTANCE IN MANAGEMENT

When completing an ERA, authors usually describe where uncertainties are likely to influence the interpretation of risk. These have implications for exposure and toxicology data used to characterise risk. This section describes common forms of uncertainty characterised in large-scale ERAs and the implications these may have for risk managers.

It is often the case that uncertainties in ERA arise from the interpretation of ecological effects with respect to transient exposures observed in agricultural catchments (i.e. pulses). For example, Cardwell *et al*. (1999) concluded in a study evaluating the ecological risk posed by the antifoulant tributyltin (TBT) in surface water of the USA, that the underlying assumption that exposure will simply result in a permanent ecological effect was uncertain. Similar uncertainty was identified by, Schuler and Rand (2008) in an ERA of herbicides, including diuron and prometryn, in freshwater ecosystems of South Florida. The nature of exposure pulses in catchments was likely to influence impacts whether chronic or acute toxicity exposure endpoints are used in SSDs (Reinert *et al.*, 2002; Suter II, 2007). As exposure pulses are often poorly characterised, this also contributes to the reason both acute and chronic SSDs are developed to minimise such uncertainty. However, the ability for organisms to recover has been identified to be a significant uncertainty in ERAs (Handy, 1994; Solomon *et al.*, 1996; Reinert *et al.*, 2002; Cedergreen *et al.*, 2005; Carriger and Rand, 2008; Schuler and Rand, 2008), and exposure pulses characteristic of study catchments should be considered when assessing the ability to protect species diversity.

The large-scale ERAs described in this review have involved using end-of-catchment data to characterise exposure risk resulting from operations occurring upstream. However, for the purposes of risk management these approaches are limited in their ability to identify the likely sources of exposure such that an informed risk manager would require to implement appropriate mitigation strategies. New approaches, combining catchment-based ERA with geographical information systems (GIS) are being developed to operate at the site-specific level. These could allow a risk manager to be able to direct resources for management where they are needed in a more cost-effective way.

## 1.7 CONCLUSIONS AND PROJECT AIMS

It is acknowledged that pesticides play an important role in protecting crops from attacking and competing pests. However, there is a compelling case to employ the best management possible to minimise ecological risk. This thesis seeks to examine this proposition with reference to three pesticides that have been historically important to the cotton industry and the Cotton Catchment Communities CRC, that funded this study. These three chemicals, two herbicides diuron and prometryn, and the insecticide endosulfan, differ in their ability to elicit an effect on target organism, defined by each pesticide's mode of action and the dose required. However, it is clear that this intended action is not limited to effects on target organisms. This is accentuated by the fact that, because of their properties of persistence and translocation in catchments, these pesticides have the potential to be translocated from their site of application in runoff and groundwater and to be deposited in catchment streams. Determining if such transport events result in ecological damage in ecosystems is the primary justification for ERA.

Ecological risk assessment in Australia is currently used by regulators to register pesticides (APVMA, 2007) and by industries to evaluate farm-specific issues (NPEC, 1999). In principle, the use of ERA in Australia could be extended to evaluate farm-specific issues within agricultural industries and used for management of ecological risk (Hart *et al*., 2006). However, as pesticides in surface waters are likely the result of loadings from more than one farm, there is little field evidence that the capacity to deal with this complexity at the catchment-scale needed for pesticide management is occurring. Although higher tier risk analysis has been suggested to be a logical progression to better understanding of exposure risk dynamics (Hart *et al.*, 2006; Kookana *et al.*, 2006), ERA optimised for management at the catchment-scale is concluded to be limited in application in Australia. Amongst the

defined roles of catchment management authorities (CMAs), it is clear that pesticide management in waterways based on ERA can be included.

This literature review provides a necessary background to examine application of ERA at the catchment-scale. The common means of characterising risk is to relate distributions of exposure and species sensitivity (Solomon *et al.*, 1996; Carriger and Rand, 2008; Schuler and Rand, 2008; Rand *et al.*, 2010). Achieving such analysis effectively requires comprehensive data sets and fortunately such data is available for the Gwydir River catchment of Northern New South Wales, Australia. The review has shown that ecosystem level effects can be translated as joint probability curves. However, these studies identified uncertainties in the exposure and toxicity information used to characterise risk (Handy, 1994; Solomon *et al.*, 1996; Reinert *et al.*, 2002; Cedergreen *et al.*, 2005; Carriger and Rand, 2008; Schuler and Rand, 2008). One such uncertainty relates to accounting for pulse exposure and whether this would translate to assessments of permanent ecological effects (Handy, 1994; Reinert *et al.*, 2002; Cedergreen *et al.*, 2005).

This thesis aims to:

1. Develop a catchment-scale ERA to evaluate specific sites in an agricultural catchment that may be experiencing some level of ecological concern,
2. Review and integrate pulse exposure characteristics into a catchment risk assessment model,
3. Overcome the deficiencies with sampling/exposure data across different regions by evaluating a spatial exposure modelling framework for the purpose of providing greater insight to catchment-scale exposure risk concerns, and
4. Provide a frame work to apply catchment-scale ERA within the management goals of a local catchment-management organisation.

It is hypothesised that such a multifaceted approach will allow testing of the hypothesis that ERA can be used more effectively to manage risk from pesticides in Australia.

**CHAPTER 2**

# A PROBABILISTIC ECOLOGICAL RISK ASSESSMENT OF DIURON, PROMETRYN, AND ENDOSULFAN IN THE GWYDIR RIVER CATCHMENT

## 2.1 INTRODUCTION

In 1985 the New South Wales State Pollution Control Commission (SPCC) concluded that pesticides reaching rivers of agricultural catchments of inland northern NSW may be of ecological concern (Muschal and Warne, 2003). The Gwydir River catchment is one of many agricultural catchments located in this region. Managing pesticides to prevent contamination in this catchment is essential as its river network plays a vital role to supplying water for irrigation, livestock, general domestic use and areas of ecological significance (Muschal and Warne, 2003; BRGCMA, 2010). Given that the quality of surface water resources is the outcome of the land use practices and hydrological and physicochemical characteristics of the interacting transport medium (Wauchope, 1978), evaluating whether pest and catchment management strategies developed since 1985 sufficiently minimise contamination to a level that protects ecosystems requires a catchment-based ERA approach (Serveiss *et al.*, 2000; Serveiss, 2002; Hart *et al.*, 2006).

Catchment-based ERA may provide an advanced means by which pesticide exposure can be investigated for its ecological significance (Serveiss *et al.*, 2000; Serveiss, 2002; Hart *et al.*, 2006; Serveiss and Ohlson, 2007). The framework consists of three different phases: problem formulation, analysis and risk characterisation (USEPA, 1998; Serveiss *et al.*, 2000; Serveiss, 2002; Serveiss and Ohlson, 2007). As introduced in Chapter 1, the phases draw on the best information available to firstly describe the problem (problem formulation), then to identify and characterise information sources (analysis phase), and using this information, determine whether such pesticides are impacting on ecological groups (risk characterisation). The risk characterisation phase has the power to determine if the level of risk is significant.

Often the hazard quotient approach is used to characterise the level of risk. However, this approach lacks the probabilistic paradigm inherent in true risk (Tannenbaum *et al.*, 2003), and such probabilistic approaches are the preferred method of characterisation (Solomon *et al.*, 1996; USEPA, 1998; Serveiss *et al.*, 2000; Serveiss, 2002; Solomon and Takacs, 2002; Tannenbaum *et al.*, 2003; Serveiss and Ohlson, 2007) because of their more quantitative nature. Conceptually, the probabilistic approach interprets the significance of exposure levels by comparing frequency distributions of exposure with toxicity thresholds devised from species sensitivity distributions (Solomon *et al.*, 1996; USEPA, 1998; Solomon *et al.*, 2000). The probability of exceeding a toxicity threshold (defined in the assessment endpoints) provides a benchmark for risk and the probability of reoccurrence defines its significance

(ANZECC, 2000; Serveiss *et al.*, 2000; Solomon *et al.*, 2000; Serveiss, 2002; Serveiss and Ohlson, 2007).

The three pesticides of interest − diuron, prometryn and endosulfan − were described in Chapter 1 and have been used in cropping production practices of the Gwydir River catchment region (Kennedy *et al.*, 2001; Raupach *et al.*, 2001; Silburn and Glanville, 2002). As these compounds have previously been characterised to be mobilised in runoff on farms in various environmental fate studies (Chapter 1) and indeed identified to be of possible ecological concern within or emerging from many other agricultural catchments (Raupach *et al.*, 2001; Lewis *et al.*, 2009; Packett *et al.*, 2009), and regions (Muschal and Warne, 2003) of Australia. They were selected for this catchment-based ERA for Gwydir River catchment.

To investigate whether any exposure from diuron, prometryn and endosulfan was likely to be of ecological concern in the reaches of the Gwydir River catchment, monitoring data was obtained from the New South Wales Department of Water and Energy for the years 1991-2007. To evaluate the risk posed by diuron, prometryn and endosulfan in the reaches of the Gwydir River catchment, an ERA following the protocol of USEPA (1998) and using probabilistic risk characterisation techniques was conducted to identify exposure "hotspots" at the sub-catchment level.

## 2.2 DESCRIPTION OF THE GWYDIR RIVER CATCHMENT

The Gwydir River catchment is an agriculturally and ecologically important catchment located in northern New South Wales, Australia (Figure 2.1). Its boundary extends from west of Armidale and Guyra on the New England Tablelands, to Collarenebri occupying an area of 26,500 $km^2$. At the eastern end, toward the headwaters, extensive undulation is observed. Heading west towards Moree and beyond, the terrain becomes less undulating and the stream morphology becomes increasingly braided, draining at its mouth in to the Darling Basin (Figure 2.2).

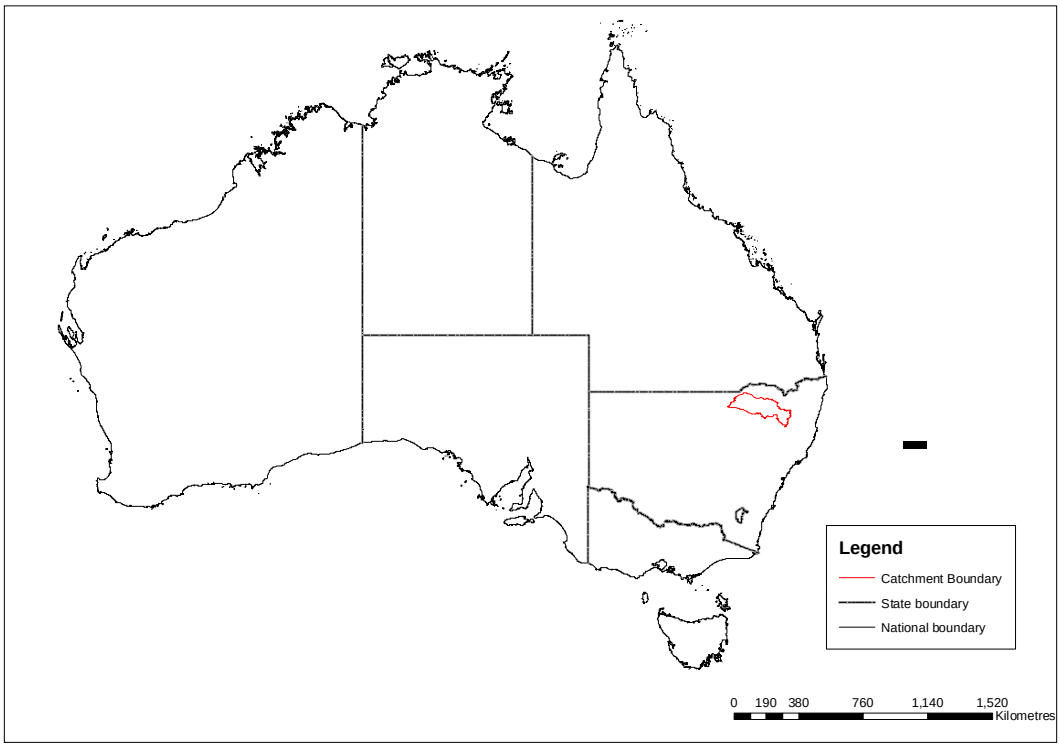



**Figure 2.1** Location of the Gwydir River catchment, Australia (Source: AGGA[1], 2007).

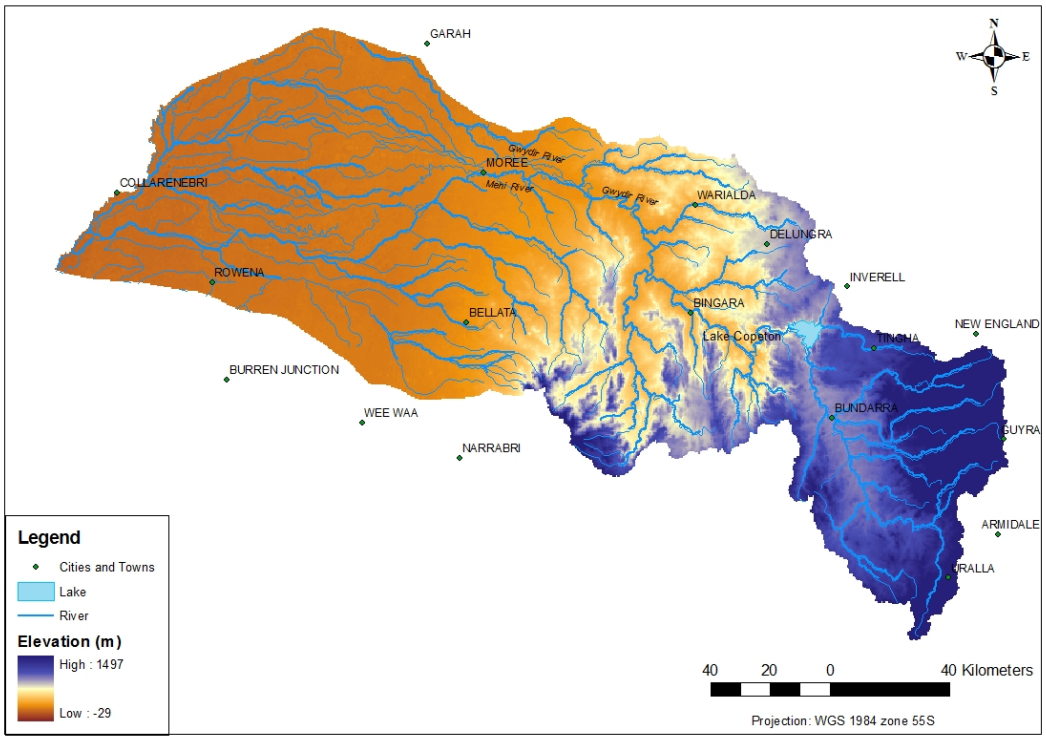



**Figure 2.2** Digital elevation (m), rivers, lakes and town locations profile of the Gwydir River catchment (Source: NASA[2], 2010).

[1] AGGA refers to Australian Government: Geoscience Australia

[2] NASA refers to the North American Space Agency

The main channels in the catchment are the Gwydir and Mehi Rivers. To support irrigated agriculture, the Gwydir River catchment is heavily regulated with Copeton Dam being the largest dam that forms Lake Copeton located approximately 90 km downstream from the headwaters (BRGCMA, 2010). A weir network regulates the flow of the streams in the catchment to control the movement of irrigation water in the Gwydir River catchment (Kingsford, 2000). Specifically, Tareelaroi weir causes a major divergence where the Mehi River forms from the waters of the Gwydir River.

The climate in the Gwydir River catchment is classified as temperate to subtropical. The rainfall in the catchment, determined from Moree Aerodrome weather station is summer dominant, with the highest average rainfall and maximum temperatures occurring in January (Figure 2.3; BOM[3] 2008). The mean annual rainfall at Moree Aerodrome is 595.3 mm, and the mean maximum and minimum temperatures are 26.6°C and 12.3°C, respectively (BOM 2008).

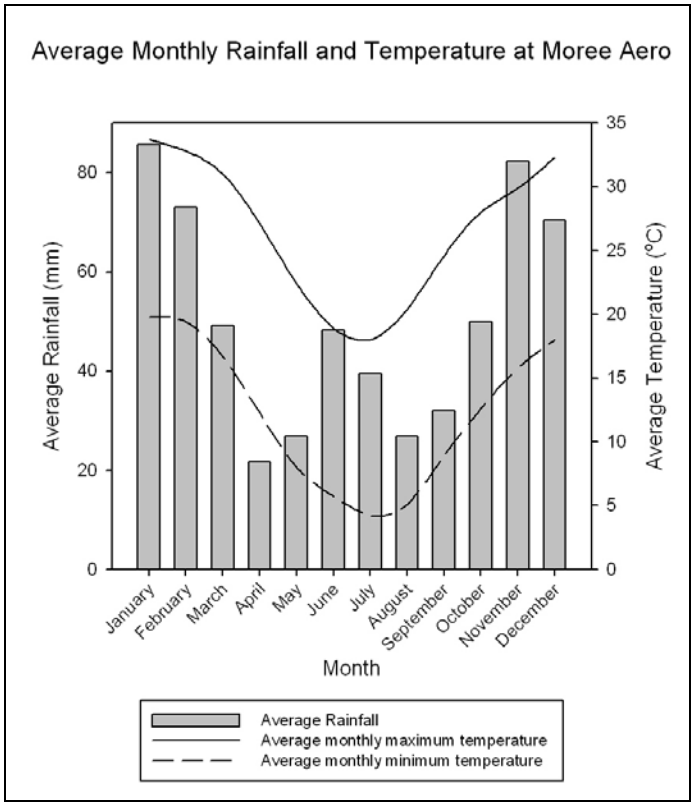



**Figure 2.3** Climate profile of monthly average rainfall and temperature at Moree Aerodrome weather station located in the Gwydir River catchment (Source: BOM, 2008).

[3] BOM refers to the Australian Government: Bureau of Meteorology

The catchment supports a diversity of land uses. 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 (Figure 2.4). The majority of the cropping in the catchment is concentrated in the western end of the catchment, with the largest proportion being of dry land type; however a large proportion is also irrigated (ABARE[4], 2006). There are also some urban areas in the catchment, with the largest town being Moree Shire located in the centre of the catchment (Figure 2.2).

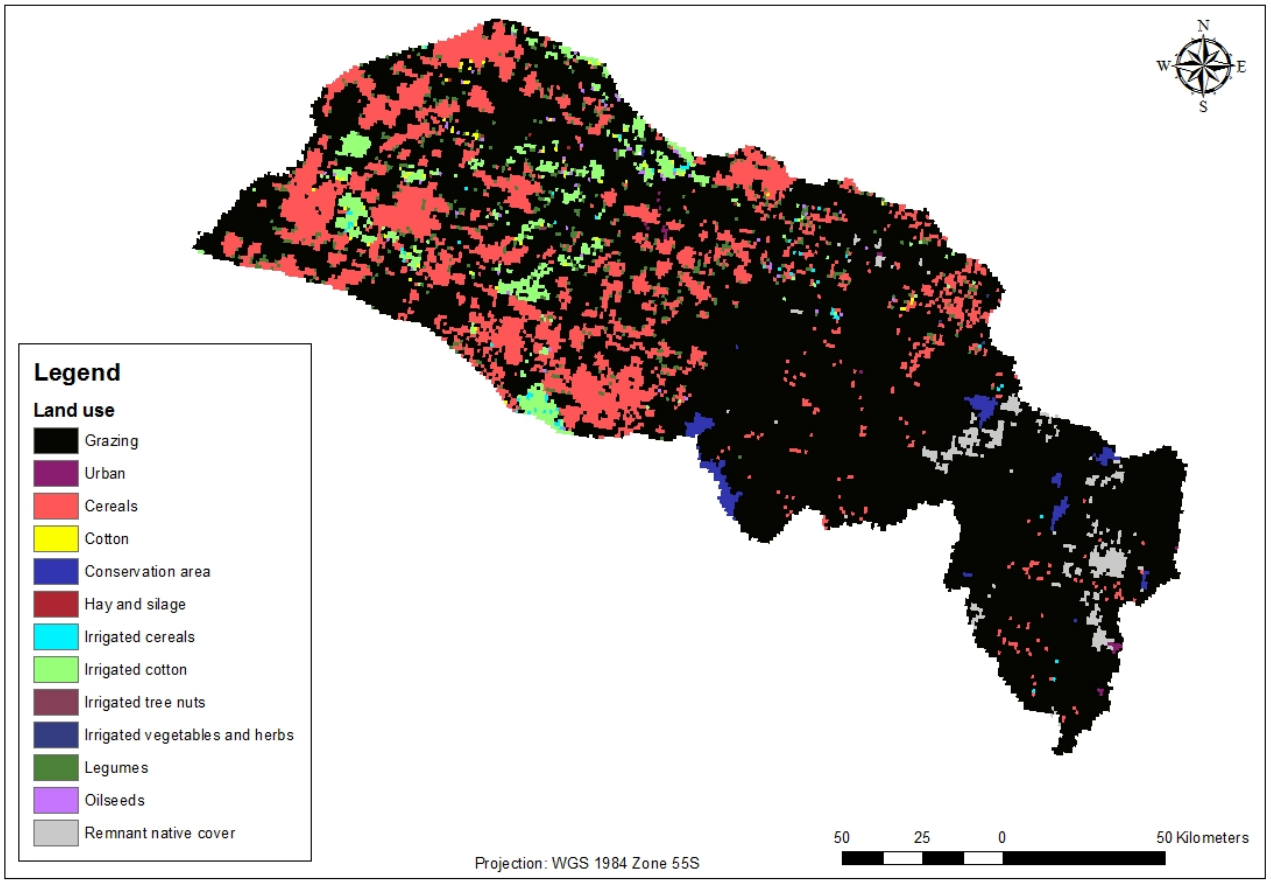



**Figure 2.4** Map of 2001-02 land use in the Gwydir River catchment (Source: ABARE[5], 2006).

One area of ecological significance in the areas of largest agricultural production is the Gwydir Wetlands. The Gwydir wetlands is a terminal-delta wetland located 60 km West of Moree (Kelleway *et al*. 2010; BRGCMA[6], 2010). It is considered of high ecological significance as the area supports many migratory and native bird species, as well as many other fresh water organisms, including crustaceans, native and exotic fish species (Kingsford,

[4] ABARE refers to the Australian Government: Australian Bureau of Agricultural and Resource Economics
[5] ABARE refers to Australian Government: Australian Bureau of Agricultural and Resource Economics
[6] BRGCMA refers to Border Rivers-Gwydir Catchment Management Authority

2000; Kelleway *et al.*, 2010). As a result it has been protected under the Ramsar convention on wetlands since 1999 (BRGCMA, 2010).

## 2.3 PROBLEM FORMULATION

To understand how diuron, prometryn and endosulfan exposure may be occurring in the waterways of the Gwydir River catchment requires a comprehensive characterisation of the stressors behaviour. This involves characterising their physicochemical properties, intended uses in relation to their modes of action, recommended application regimes and their fate in the environment (USEPA, 1998; Cormier *et al.*, 2000; Suter II, 2007). Understanding how these chemicals behave can provide explanation for exposure events of possible ecological significance that can later be used to support the development of management strategies. Endpoints, also known as ecosystem protection goals, and a conceptual model that relates human activities, stressor sources, the form that the stressor is likely to be transported and occur in the waterways, possible ecological effects, are presented to provide further focus for this ERA (USEPA, 1998; Cormier *et al.*, 2000; Serveiss *et al.*, 2000; Suter II, 2007).

### *2.3.1 Characteristics of diuron, prometryn and endosulfan*

This section of the problem formulation phase summarises the physicochemical characteristics of diuron, prometryn and endosulfan; the extent and purpose of their use; and their likely fate in the Gwydir River catchment.

#### *2.3.1.1 Physicochemical characteristics, mode of action and intended use of diuron, prometryn and endosulfan*

The physicochemical characteristics (solubility in water, vapour pressure, Henry's constant, soil-water partition coefficient ($K_d$), half-lives, and breakdown products) of diuron, prometryn and endosulfan have been described in Chapter 1. Table 2.1 summarises the relevant physicochemical characteristics that influence their fate and define their persistence in the environment, together with their chemical family, mode of action and intended use. Specifically, the intended uses of these compounds are directly related to their modes of action, which a brief description is also provided.

**Table 2.1** Relevant physicochemical properties of diuron, prometryn and endosulfan (Source: BCPC, 2006, unless otherwise stated)

| Property | Diuron | Prometryn | Endosulfan |
|---|---|---|---|
| Chemical family | Urea | Triazine | Cyclodiene organochlorine |
| Mode of action | PSII inhibition | PSII inhibition | GABA inhibitor |
| Intended use | Herbicide | Herbicide | Insecticide |
| Vapour pressure (mPa) | 1.1 x $10^{-3}$ (at 25°C) | 0.165 (at 25°C) | 0.83 (2:1 mixture of α- and β- isomers at 20 °C) |
| log$K_{OW}$ | 2.85 [a] | 3.1 | α- = 4.74<br>β- = 4.79 |
| Henry's constant (Pa $m^3$ $mol^{-1}$) | 7.0 x $10^{-6}$ | 1.2 x $10^{-3}$ | α- = 1.48 (at 20°C)<br>β- = 0.07 (at 20°C) |
| Solubility in water (mg $L^{-1}$) | 37.4 | 33 (at 22°C) | α- = 0.32 (at 22 °C)<br>β- = 0.33 (at 22 °C) |
| $K_d$ (L $kg^{-1}$) | 3.24-5.71[a] | 1.78-6.04 [a] | 165.79-231.50[e] |
| Half-life (days) | 49[b] | 6.2-7.0[c] | 7.1[d] |

[a] Source: Baskaran and Kennedy (1999)
[b] Source: Stork *et al*. (2008)
[c] Source: Suzuki and Otani (2004)
[d] Source: Kennedy *et al*. (2001)
[e] Source: Laabs and Amelung

Diuron, prometryn and endosulfan are used in a range of crop production practices to target certain pests. The organisms that they target are an outcome of their respective modes of action (Table 2.1; and detailed in Chapter 1). As both diuron and prometryn target and inhibit the PSII apparatus of plants (Chapter 1), in crop production they are registered for control of broadleaf weeds and annual grasses in a range of crop production practices (Farrell and Johnson, 2005; British Crop Protection Council, 2006; Farrell, 2008; Nufarm, 2009a; Nufarm, 2009c; OzCrop, 2009), and in urban and industrial settings (British Crop Protection Council, 2006; Nufarm, 2009a). As endosulfan targets and disrupts the GABA-gated chloride channel in the nerves of animals (Table 2.1; and detailed in Chapter 1), in crop production it is used to control a range of insect pests. These insects include redlegged earth mite, blue oat mite, rough bollworm, aphids, thrips, cotton looper, jassids, cotton tip worm, cutworms, green vegetable bug, native budworm, cotton bollworm, and web-spinner caterpillar (Farrell, 2008; Nufarm, 2009b).

*2.3.1.2 Extent of diuron, prometryn and endosulfan use in the Gwydir River catchment*

Characterising the extent by which diuron, prometryn and endosulfan are used in the Gwydir River catchment indicates exposure sources. The extent of diuron, prometryn and endosulfan use in the Gwydir River catchment is unknown. However, the areas in the catchment that may use diuron, prometryn and endosulfan in pest management can be elucidated by relating spatial distribution of land use (Figure 2.4) with the authorised uses of these pesticides. Such

uses are detailed on pesticide safety labels, industry best practice guidelines, as well as through consultation with pest managers.

According to pesticide labels diuron, prometryn and endosulfan are registered for use on all or some of these types of land uses in the Gwydir River catchment (Nufarm, 2009b; Nufarm, 2009a; Nufarm, 2009c; OzCrop, 2009). Specifically, diuron is registered for use in cotton, grass seed crops, cereal (wheat, barley, oats, triticale and cereal rye), pulse crops (including chickpeas, faba beans, lentils, narbon beans, field peas, and vetch), pasture (perennial grass seed crops and lucerne) and oilseed crops (canola, safflower, linseed and sunflower), as well as in urban and industrial weed management (Farrell, 2008; Nufarm, 2009a). Prometryn is registered for use in cotton, chickpea and perennial grass seed crops (including Sirocco, Phlaris, Demeter Fescue, Currie, Cocksfoot, and Medea ryegrass) and sunflower crop production practices, but is not registered for use in urban and industrial weed management practices (Farrell, 2008; Nufarm, 2009c; OzCrop, 2009). The use of endosulfan is restricted to insect control in canola, cereal, pulse (including chickpeas, cowpeas, pigeon peas, adzuki beans, faba beans, field peas, navy beans, mung beans, lupins and soy beans) and cotton crops (Farrell, 2008; Nufarm, 2009b). The registered uses of diuron, prometryn and endosulfan are mostly restricted to broad acre cropping. This was confirmed following discussions with local pest managers including Moree shire council (*Pers. com.* 2010) and agronomists (Landmark, Moree agronomist: *Pers. com.* 2010) that these pesticides are only used in broad-acre cropping.

*2.3.1.3 Recommended application regimes for diuron, prometryn and endosulfan*

Pesticide use on farm is usually managed independently by the growers and agronomists. The application regimes of diuron, prometryn and endosulfan used by farmers are detailed on the product label that has been approved by the APVMA as well as through industry endorsed integrated pest management (IPM) strategies. All of these approaches support an application regime that reflects crop type, pest pressure, and measures to limit off-site movement (Mensah, 2002; Farrell and Johnson, 2005; Llewellyn *et al.*, 2007; Farrell, 2008; Holloway *et al.*, 2008; Nufarm, 2009b; Nufarm, 2009a; Nufarm, 2009c; OzCrop, 2009; Cotton CRC extension team, 2010; Whitehouse, 2011; Cotton CRC extension team, 2009). This section details the recommended diuron, prometryn and endosulfan application regimes for the different land uses in the Gwydir River catchment with reference to pesticide labels and recommended pest management strategies by industry, where applicable.

Modern agriculture pesticide use strategies are commonly designed to enhance the efficacy of the chemical application, and limit off- and on-site ecological impacts, which has an obvious economic benefit (Simpson, 2007). The application regimes recommended on labels and in industry pest management strategies for diuron, prometryn and endosulfan application for land uses in the Gwydir River catchment are outlined in Appendix 2. In general, diuron applications should be made on moist soil before weeds emerge (Farrell and Johnson, 2005; Farrell, 2008; Nufarm, 2009a; Cotton CRC extension team, 2010; Cotton CRC extension team, 2009). Restrictions include no applications to be made on light sandy or gravelly soils, rotation crops should not be replanted after two years of diuron use, one year for cotton and lucerne, and cotton, corn and grain sorghum should not be planted the following spring (Nufarm, 2009a). Similarly, prometryn applications (Appendix 2) should be made on moist soil when weeds are young and actively growing (Nufarm, 2009c; OzCrop, 2009). One restriction on prometryn is that treated cotton crop fields should not be recultivated six months after cotton harvest (Nufarm, 2009c; OzCrop, 2009; Cotton CRC extension team, 2009; 2010). In both cases measures are recommended to be taken to minimise the off-site and subsequent contamination potential of non-target ecosystems (Nufarm, 2009a; Nufarm, 2009c; OzCrop, 2009).

Endosulfan applications are strictly limited to pre-emergent (before the crop emerges from the top soil) use in canola, cereals and pulses and pest pressure in cotton (Appendix 2). Except for orchard crops, other crops are limited to a maximum of two full coverage endosulfan applications per crop per season unless irrigation tail water and up to 25 mm of rainfall can be captured on farm (Nufarm, 2009b). Applications of endosulfan are recommended to not be made if heavy rain, likely to generate runoff, is forecasted at greater than 50% probability within two days of application, when irrigating, to waterlogged soil or while water remains in crop furrows, unless tail water can be captured (Nufarm, 2009b). Further, irrigation water should not be applied two days after endosulfan application unless tail water can be captured on farm (Nufarm, 2009b). Endosulfan use on cotton is limited to 2.205 kg a.i. $ha^{-1}$ $season^{-1}$, where irrigation tail water and up to 25 mm of rainfall can be captured on farm, and 1.470 kg a.i. $ha^{-1}$ $season^{-1}$ where irrigation tail water and up to 25 mm of rainfall cannot be captured on farm (Nufarm, 2009b; Cotton CRC extension team, 2010; Cotton CRC extension team, 2009). Furthermore, aerial spray is restricted cotton areas with a downwind boundary greater than 750 m (Nufarm, 2009b; Cotton CRC extension team, 2010; Cotton CRC extension team, 2009). In all applications, it is also recommended that measures

be taken to minimise the off-site and subsequent contamination potential of endosulfan to non-target ecosystems.

Deviations from these application regimes are dependent on pest management strategies adopted by individual farmers in the Gwydir River catchment (Simpson, 2007). For example, IPM involves managing pests by using a variety of methods to maintain pest numbers below a threshold to ensure that crop damage and harm to beneficial pests are minimised using the least possible amount of pesticide (Mensah, 2002; Llewellyn *et al.*, 2007; Holloway *et al.*, 2008; Whitehouse, 2011). IPM also requires that chemicals be rotated with other actives to minimise the onset of resistance, a common problem resulting from the intensive use of a single active (Llewellyn *et al.*, 2007; Holloway *et al.*, 2008; Whitehouse, 2011). The IPM approach to pest management is endorsed by the Australian cotton industry with methods outlined in their pest management guides (Farrell and Johnson, 2005; Farrell, 2008; Cotton CRC extension team, 2010; Cotton CRC extension team, 2009), and are also being adopted by other industries as a way of sustainably managing pests, e.g. in Australian grain production (Llewellyn *et al.*, 2007; Holloway *et al.*, 2008). Subsequently, the spatial variation in land use in the Gwydir River catchment depending on the extent which different strategies are adopted indicates that the likely use of diuron, prometryn and endosulfan varies temporally and spatially. Such characteristics are factors that contribute to the exposure hazard in Gwydir River catchment.

*2.3.1.4 Environmental fate of diuron, prometryn and endosulfan*

The environmental fate, including details regarding degradation and transformation, partitioning and transportation, of diuron, prometryn and endosulfan have been characterised in Chapter 1. It was revealed that their fate is strongly attributed to their physicochemical properties. Being of varying hydrophobicity, these compounds were shown to partition to the solid phase when treated soil comes in to contact with water (Simpson, 2007; Appendix 1). The readily available literature review indicated that these compounds were likely to be transported in sediment loads of runoff, with variable fractions being transported in solution (Stork *et al*., 2008), the magnitudes being defined by soil-water partition coefficients ($K_d$) and the total water flow. It was also determined that even though endosulfan and prometryn are more volatile, transportation in the atmosphere is considered to be a minor route of aquatic exposure (Raupach *et al.*, 2001).

### *2.3.2 Ecological resources of importance in the Gwydir River catchment*

To determine which ecosystems in the Gwydir River catchment were at risk from diuron, prometryn and endosulfan contamination, the following was considered: (1) where, when and in what quantities the pesticides are used, (2) the mechanisms that move the pesticides from the site of application, and (3) available monitoring data to support exposure characterisation (Solomon *et al.*, 1996). This approach identifies distinct environments in the Gwydir River catchment to provide focus for this ERA.

Diuron, prometryn and endosulfan have been described to be used extensively and have the potential to be transported from the site of application in runoff (either dissolved or bound to sediments). Therefore the aquatic ecosystems of the Gwydir River catchment annually receiving runoff from fields treated with diuron, prometryn and endosulfan may be exposed to these active compounds. The extent to which these pesticides were present in aquatic environments has been evaluated via a surface water pesticide monitoring scheme for some reaches of the Gwydir River catchment for the period 1991-2007. This data was made available by the NSW Department of Water and Energy for this ERA. No available data characterising the terrestrial contamination of these compounds was identified and is therefore not considered in this ERA, but would be considered if such information were available.

Two distinct aquatic ecoregions of the Gwydir River catchment were considered in this ERA. These are the Gwydir wetlands and, broadly, all other aquatic ecosystems. Of particular concern is the Gwydir Wetlands, a terminal-delta wetland located in the central western end of the Gwydir River (BRGCMA[7], 2010). It is considered of high ecological significance as the area supports many migratory and native bird species, as well as many other fresh water organisms, and is protected under the Ramsar convention on wetlands in 1999 (BRGCMA, 2010). The land use surrounding the wetland is predominantly agriculture; hence the exposure from diuron, prometryn and endosulfan is possible. As this is a protected wetland the area is deemed to have significant ecological value to the community, therefore lower pesticide concentration tolerance was given for this area of the catchment.

[7] BRGCMA refers to Border Rivers-Gwydir Catchment Management Authority

The remaining aquatic ecosystems broadly accounts for all other stream reaches. As many of these reaches have been, to some extent, disturbed during agricultural development, a limit for distinguishing what is an acceptable or unacceptable level of risk is necessary to protect the ecosystem, as it would otherwise be considered socially unacceptable. Subsequently, a different risk tolerance level, one that reflects its disturbed nature, has been incorporated in this risk assessment (See Assessment endpoints in Section 2.3.3).

As the load of these three pesticides are likely contained in the solution or sediment phases of runoff and according to their modes of action, a number of aquatic organism classes are susceptible to exposure in the aquatic ecoregions of Gwydir River catchment. These organisms ventilate and feed in the water column. They comprise of pelagic[8] and demersal[9] fish, holo-[10] and meroplankton[11], epibenthos[12], bethic[13] and epibenthic[14] filter feeders, algae and macrophytes. According to the modes of action of diuron, prometryn and endosulfan, certain groups of organisms are more sensitive to exposure than others (Solomon and Takacs, 2002). For example, diuron and prometryn with modes of action that target photosynthesis are more effective on aquatic plant and algal populations than vertebrates and invertebrates, whereas endosulfan is more toxic towards vertebrates and invertebrates. Subsequently, distinguishing the ecological groups for their sensitivity toward these compounds is necessary, as each group plays a unique role in ecosystem assemblage resilience (van Straalen, 2002).

### *2.3.3 Assessment endpoints*

The endpoints for this ERA were formulated based on the differing sensitivities of test organisms to the pesticides diuron, prometryn and endosulfan; and ecoregion class. The Gwydir wetlands, perceived to have the greatest ecological significance, required the greatest protection. According to an SSD, the concentration thresholds used to characterise adverse effects in this study were the regulatory endpoints for protecting ecological groups in both protected areas and those areas that are disturbed. In this case, the protected Gwydir River wetlands were taken to require 95% of the most sensitive organisms to be protected 95% of

[8] Living in the water column
[9] Living above the benthic region, but below the pelagic zone of the water column
[10] Planktonic for their entire life-cycle
[11] Planktonic for only a part of their life cycles
[12] Fish and invertebrates living on the sediment's surface
[13] Live on, in or near the river bed
[14] Live on the surface of bottom sediments in waterbody

the time (ANZECC, 2000). All other aquatic ecosystems were required to have 90% of the organisms protected 95% of the time. The setting of the relevant concentration thresholds will be described in the analysis (Section 2.4) and applied in the risk characterisation phases (Section 2.5).

### *2.3.4 Conceptual model*

The information presented allowed for the development of a conceptual model, presented in Figure 2.5. The key areas that are considered important for defining the level of diuron, prometryn and endosulfan exposure to the ecosystems of the Gwydir River catchment include: human activities, possible stressor sources, stressor effects and endpoints. The relationships that exist between these key areas provide insight to the interactions that exist between sources and endpoints to provide focus for this ERA.

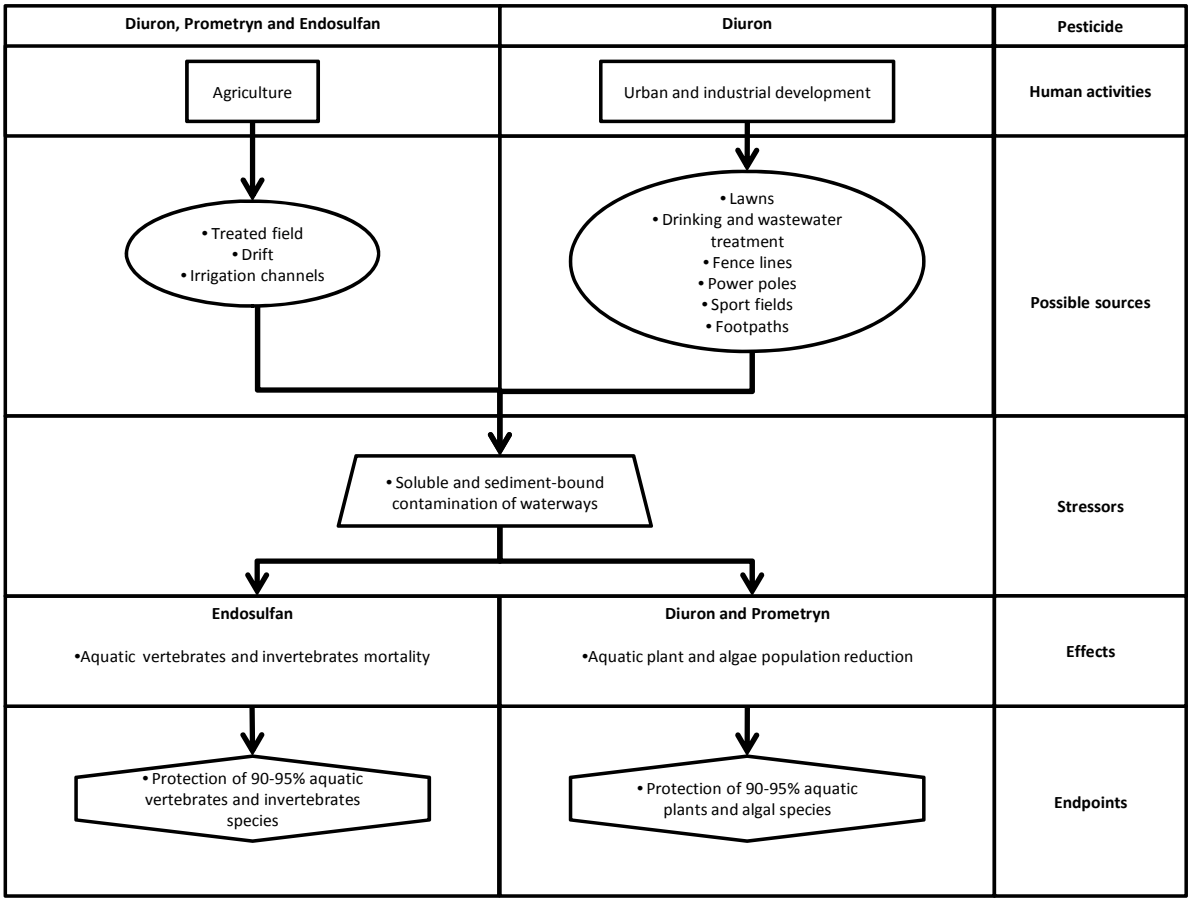



**Figure 2.5** Conceptual model relating diuron, prometryn and endosulfan acting as aquatic ecosystem exposure sources and their effects for the ERA assessment endpoints in the Gwydir River catchment.

The human activities in the Gwydir River catchment identified to use diuron, prometryn and endosulfan were grouped according to their registered uses. These activity groups included agriculture, and urban and industrial development. As the common purpose of using diuron and prometryn is to control weeds, the sources of diuron and prometryn exposure resulting from use in agriculture include treated fields and irrigation channels (Silburn and Glanville, 2002; Silburn *et al.*, 2002; Nufarm, 2009a; Nufarm, 2009c; OzCrop, 2009) and drift that may result during application. However, drift of these herbicides is considered a management issue reasonably well controlled and therefore not considered a significant exposure route.

In urban and industrial development, potential diuron and prometryn exposure sources include treated lawns, fence lines, power poles, sporting fields and foot paths (Nufarm, 2009a; Nufarm, 2009c; OzCrop, 2009). Similarly, the sources of possible endosulfan exposure from use in agriculture were likely to be treated farms (Kennedy *et al.*, 2001; Raupach *et al.*, 2001; Silburn and Glanville, 2002; Silburn *et al.*, 2002; Nufarm, 2009b). Endosulfan is not registered for uses outside of agriculture.

According to the land use profile of this catchment, there is possible widespread use of diuron, prometryn and endosulfan in the Gwydir River catchment. The processes that will lead to indirect exposure in these waterways from these compounds is defined by the prevailing pest pressure that influences the rate of use of these chemicals; also important is the climatic conditions under which they are applied, the treated areas prior experience, the management practices employed by the users to limit their movement from the site of application and their physicochemical properties (Wauchope, 1978). In all cases, the most common means by which indirect aquatic exposure may result in the reaches of the Gwydir river catchment is through surface runoff in rainfall or irrigation events (Wauchope, 1978; Schnoor, 1992; Scheunert, 1993; Lennartz *et al.*, 1997; Kookana *et al.*, 1998; Silburn and Glanville, 2002; Silburn *et al.*, 2002; Rose *et al.*, 2005a; Shivaramaiah *et al.*, 2005; Rose, 2006; Silburn and Hunter, 2009). The pesticides may be transported and deposited from the surface water as soluble and sediment sorbed loads (Lennartz *et al.*, 1997; Kookana *et al.*, 1998; Leonard *et al.*, 2001; Silburn and Glanville, 2002; Silburn *et al.*, 2002; Rose *et al.*, 2005a; Shivaramaiah *et al.*, 2005; Rose, 2006; Silburn and Hunter, 2009). The non-target aquatic organisms likely to be most directly affected by diuron and prometryn exposure are plant and algal populations; for endosulfan, vertebrate and invertebrate animal populations. The effects determining endpoints considered significant for plants and algae are reductions

in population growth, whereas death is the most significant endpoint considered for vertebrates and invertebrates.

The endpoints for this ERA were devised with reference to the Gwydir River catchment management goals defined by the Border Rivers-Gwydir Catchment management authority (BRGCMA, 2010) and those recommended by the ANZECC (2000) water quality guidelines. The devised endpoint goal for this risk assessment was *the protection of the abundance and diversity of the ecological groups within the ecoregions* of the Gwydir River catchment (BRGCMA, 2010). The endpoints for the two previously defined Gwydir wetlands and all other ecoregions were distinguished according to the type of ecosystem being protected.

### *2.3.5 Outcome of problem formulation*

The problem formulation phase provided a clear focus for this risk assessment. Using readily available information diuron, prometryn and endosulfan were characterised for their physicochemical properties, the extent and purpose of their use with respect to their modes of action and land use information and possible application regimes employed under the respective land uses. By profiling the environmental fate of diuron, prometryn and endosulfan a level of indirect exposure was considered to occur in the reaches of the Gwydir River catchment that drain these agricultural areas. However, it is unknown whether this exposure will result in ecological effects.

## 2.4 ANALYSIS PHASE

The analysis phase, utilising the relationships presented in the conceptual model, characterises the exposure pathways and ecological effects resulting from the direct exposure of diuron, prometryn and endosulfan to ecological groups (USEPA, 1998; Serveiss *et al.*, 2000). The exposure analysis defines the likely sources of the stressors, and their spatial and temporal distribution in the catchment. The ecological impacts possibly resulting from such contamination are characterised in the effects analysis, taking into account the range of available toxicity data. Importantly, this phase presents a comprehensive summary of information that is later used to support the risk characterisation phase of this ERA.

*2.4.1 Exposure analysis of diuron, prometryn and endosulfan in the reaches of the Gwydir River catchment*

*2.4.1.1 Sources of diuron, prometryn and endosulfan exposure in the Gwydir River catchment*

It was concluded in the problem formulation phase that runoff originating from or passing through treated areas was the dominant indirect exposure source for diuron, prometryn and endosulfan in the reaches of the Gwydir River catchment. The runoff water could contain both soluble and sediment-bound applied chemicals, their concentrations a direct result of the amount applied, the subsequent proportion available for transport − a manifestation of crop type and pest pressure − and the frequency and application timing to the rainfall events (Wauchope, 1978; Schnoor, 1992; Scheunert, 1993; Ahmad and Kookana, 2007; Silburn and Kennedy, 2007; Simpson, 2007).

*2.4.1.2 Spatial and temporal distribution of diuron, prometryn and endosulfan use in the Gwydir River catchment*

The spatial and temporal use of diuron, prometryn and endosulfan in the Gwydir River catchment is perceived to be highly variable. This inherent variability must be considered with an assessment to ensure exposure is addressed adequately. This variability can be explained by:

1. The spatial variation in land use;
2. Crop rotations adopted by various farmers;
3. Likely pesticide application periods; and
4. New pest management technologies.

*Spatial variation in land use, possible crop rotations and their associated conventional pesticide application periods*

Under the different cropping land uses of the Gwydir River catchment − including cotton, cereal crops, legumes, grass seed crops, and oilseed − it is likely that each farm will adopt different pest management strategies. These pest management strategies are expected to follow a crop rotation schema adopted by each farmer. In the Gwydir River catchment it is expected that irrigated cropping systems will adopt a fixed crop rotation scheme, as availability of water is more certain than their dryland counterparts (Hulugalle and Scott, 2008). Dryland cropping systems are perceived to adopt a more opportunistic regime in the Gwydir River catchment (Hulugalle and Scott, 2008). Typical crop rotations under the

different land uses in the Gwydir River catchment have been investigated through literature searches and are summarised in Table 2.2.

For the different land uses, a number of unique crop rotation characteristics have been identified for irrigated cotton, and cereal cropping farms. For example, for irrigated cotton farms, winter wheat is considered the favoured rotation crop, with 74% of growers in the Gwydir River catchment either sowing a 1:1 or 2:1 cotton-wheat rotation (Table 2.2; Hickman *et al.*, 1998; Hulugalle and Scott, 2008). Rotation schemes for cereal crops, with wheat considered as the primary production crop, are likely to rotate with other grain (e.g. sorghum), leguminous (e.g. chickpea, mung bean, etc), or oil seed crops (e.g. sunflower, and canola), all selected according to soil water conditions (Table 2.2).

**Table 2.2** Example crop rotations by possibly adopted by different land uses of the Gwydir River catchment as described in the literature.

| Land use | Description | Example rotation schema[a] | References |
|---|---|---|---|
| Irrigated cotton | 2:1 cotton-winter rotation | SC, WF, SC, WF, SC, WR, SF, WF | Hickman *et al*. (1998); Cooper (1999); and Hulugalle and Scott (2008) |
| | 1:1 cotton-winter rotation | SC, WR, SF, WF, SC | Hickman *et al*. (1998); Cooper (1999); and Hulugalle and Scott (2008) |
| | Summer rotation | SC, WF, SR, WF, SC | Hulugalle *et al*. (2001) |
| Dryland cotton | Opportunistic | Under ideal conditions rotations are assumed to be similar to irrigated cotton | Hulugalle and Scott (2008) |
| Cereal | Continuous wheat | WW, SF, WW, SF, WW, SF, WW, SF, WF, SF, WW | Felton *et al*. (1995); and Thomas *et al*. (2011) |
| | Wheat, chick pea rotation | WW, SF, WCp, SF, WW | Thomas *et al*. (2011) |
| | Faba bean, Canola, Wheat | WFb, SF, WCa, SF, WW | Thomas *et al*. (2011) |
| | Soghum, Chickpea, Mung bean, wheat | WW, SGs, WCp, SGs, WF, SMb, WW, SF, WF, SF, WCp | Thomas *et al*. (2011) |
| Legume | Rotation with cotton (see above) | | |
| | Rotation with cereal (see above) | | |

[a] SC: Summer cotton; WF: Winter fallow; WR: Winter rotation; SF: Summer fallow; SR: Summer rotation; WW: Winter wheat; WCp: Winter chickpea; WFb: Winter faba bean; WCa: Winter canola; SGs: Summer grain sorghum; SMb: Summer mung bean

In conjunction with the range of possible crop rotation schemes adopted by farmers in the Gwydir River catchment, pest management strategies are likely to change in accordance with the type of crop(s) grown each year. It is expected that such practices will lead to some spatial and temporal variability in diuron, prometryn and endosulfan use, translating to the exposure profiles being observed in the reaches of the Gwydir River catchment.

Through review of available literature, the likely periods in any year where diuron, prometryn and endosulfan are likely to be applied under the different crops is summarised in Table 2.3. It can be concluded from this information that the majority of the cropping practices where diuron, prometryn and endosulfan applications are likely to be made will occur in an 11 month period between June and April. Diuron and prometryn are typically applied for the purpose of controlling weeds prior to and for some period after crop emergence and to defoliate cotton just prior to harvest (Table 2.3). Endosulfan is typically applied in response to pest pressure in cotton and for crop pre-emergence periods under cereal and legume crop systems. Diuron and endosulfan applications are currently restricted to once per season (Farrell, 2008; Nufarm, 2009b; Nufarm, 2009a). Previously, endosulfan use has been described to range between 3-4 applications in an environmental fate study conducted during 1993-1996 in Central and Northern NSW (Kennedy *et al.*, 2001); and up to 10 times in the tropical irrigated cotton production region of Emerald, Queensland (Simpson, 2007). However, more recently the use of diuron, prometryn and endosulfan applications have been restricted because of drought and the introduction of transgenic cotton varieties between the years 2000-2007. It is unclear what application strategies (i.e. pre- or post-emergent) had previously been employed for diuron and prometryn. The extent and timing of their applications in the field contributes to the load of pesticide available for transport in the field (Wauchope, 1978; Simpson, 2007).

**Table 2.3** Application periods of diuron, prometryn and endosulfan in the Gwydir River catchment by land use as reported in the literature.

| Land use | Pesticide | Purpose | Period of application | References |
|---|---|---|---|---|
| Cotton | Diuron[a] | Planting and pre-emergence. | October. | Hulugalle *et al*. (2001); Farrell (2008); and Nufarm (2009a) |
| | | Crop post-emergence. | November-December. | Hulugalle *et al*. (2001); Farrell (2008); and Nufarm (2009a) |
| | | Defoliation. | April. | Hulugalle *et al*. (2001); Farrell (2008); and Nufarm (2009a) |
| | Prometryn | Fallow maintenance prior to planting. | August. | Hulugalle *et al*. (2001); Farrell (2008); OzCrop (2009); and Nufarm (2009c) |
| | | Crop planting and pre-emergence. | October. | Hulugalle *et al*. (2001); Farrell (2008); OzCrop (2009); and Nufarm (2009c) |
| | | Crop post-emergence. | Variable (when weeds are less than 8 cm high). | Hulugalle *et al*. (2001); Farrell (2008); OzCrop (2009); and Nufarm (2009c) |
| | Endosulfan[a] | Applying for pest pressure. | Ground application allowed 1 October-15 January; and Aerial application allowed 15 Novemeber-15 January on cotton higher than 20 cm. | Felton *et al*. (1995); Hulugalle *et al*. (2001); Farrell (2008); and Nufarm (2009b) |
| Cereal crops (Wheat, barley and oats) | Diuron[a] | Crop post-emergence weed control | June | Felton *et al*. (1995); Hulugalle *et al*. (2001); and Nufarm (2009a) |
| | Endosulfan[a] | Insect control at pre-emergence | May | Felton *et al*. (1995); Hulugalle *et al*. (2001); and Nufarm (2009b) |
| Legume | Diuron[a] | Weed control at crop sowing | May-June | Felton *et al*. (1995); Hulugalle *et al*. (2001); and Nufarm (2009a) |
| | Prometryn | Weed control immediately after crop planting | May-June | Felton *et al*. (1995); Hulugalle *et al*. (2001); OzCrop (2009); and Nufarm (2009c) |
| | Endosulfan[a] | Insect control at pre-emergence | May-June | Felton *et al*. (1995); Hulugalle *et al*. (2001); and Nufarm (2009b) |
| Grass seed crops | Diuron[a] | Broad weed control | Variable (at weed emergence) | Nufarm (2009a) |
| | Prometryn | Broad weed control | Variable (when weeds are young) | Nufarm (2009c); and OzCrop (2009) |
| Oilseed (sunflower) | Prometryn | Weed control at crop pre-emergence | August | Nufarm (2009c); and OzCrop (2009) |
| Irrigation channels and drainage ditches | Diuron[a] | Weed control in channels and drainage ditches | When channels are not in use, June-December | Hulugalle *et al*. (2001); and Nufarm (2009a) |

[a] Chemical currently restricted to only one application in a season

*Cultural pest management strategies likely to impact on the temporal exposure profile of diuron, prometryn and endosulfan in the reaches of the Gwydir River catchment*

A number of management strategies have been devised by certain industries to minimise the use and ecological hazard posed by pesticides in the Gwydir River catchment. One successful example is the Australian cotton industry, which, in response to heavy criticism and economic losses experienced in the late 1990s resulting from drift issues and contamination of produce (Crossan *et al.*, 2007), developed best management practices (BMP) (Williams and Willams, 2000). Management strategies introduced by the BMP program included the introduction of genetically modified (GM) cotton varieties expressing insect toxins, the education of growers to reduce the broad-spectrum applications and other risk reduction methods, such as on farm water retention and recycling (Williams and Willams, 2000; Kennedy *et al.*, 2001; Rose *et al.*, 2005b) and remediation using constructed wetlands (Williams and Willams, 2000; Rose *et al.*, 2005a; Rose *et al.*, 2005b; Crossan *et al.*, 2007). The most important pest management strategy incorporated in cotton BMP likely to influence the exposure profile of diuron, prometryn and endosulfan was the integrated pest management (IPM) strategy.

The principal aims of cotton IPM were to minimise pesticide use, enhance the role that beneficial organisms play at predating problem insects and to minimise development of pest resistance (Fitt, 2000). Cotton IPM was made possible by the adoption of transgenic cotton varieties (Crossan *et al.*, 2007). A number of transgenic cotton crop varieties have been adopted for their ability to resist the major pest in cotton production, the cotton boll worm (*Helicoverpa sp.*); and those that favour the use of herbicides that pose lower ecological hazard (e.g. glyphosate). Examples include Roundup Ready® GM cotton which is a variety resistant to the herbicide glyphosate; and Bollgard®II cotton that has adopted two genes (*Cry1Ac* and *Cry2Ab*) from the soil borne bacteria *Bacillus thuringensis* (Bt) that provides the plant with the trait for producing proteins toxic to the cotton boll worm (*Helicoverpa sp.*) (Fitt, 2000). Using classic breeding techniques, these traits have been combined to produce what are called "stacked" varieties, such as Bollgard®II Roundup Ready® (Fitt, 2000; Crossan *et al.*, 2007).

Since their introduction to the cotton industry in the 1999/2000 season, approximately 80% of the commercial cotton growing area for the 2004/05 growing season contains at least one

genetic enhancement (Crossan *et al.*, 2007; Kennedy *et al.*, 2011). The outcome from using these crops is a reduction in residual herbicide use (e.g. diuron) and the number of insecticide applications, especially of endosulfan (Fitt, 2000; Crossan *et al.*, 2007; Kennedy *et al.*, 2011). It is likely that the spatial, temporal and cultural changes in pest management strategies adopted under the different land uses in the Gwydir River catchment are likely to translate in to the exposure profiles of diuron, prometryn and endosulfan in the reaches of the catchment. The spatial variation in land use, and their affiliated crop rotations and pest management strategies are considered important components.

*2.4.1.3 Exposure assessment of diuron, prometryn and endosulfan in the reaches of the Gwydir River catchment*

An exposure assessment of diuron, prometryn and endosulfan concentrations in the reaches of the Gwydir River catchment was conducted using available information. Exposure data for the reaches of the Gwydir River catchment for diuron, prometryn and endosulfan was obtained by request from the NSW Department of Water and Energy's Gwydir Water Quality Project in 2007. The names and locations (including longitude and latitude GPS coordinates) of these sites are summarised in Figure 2.6 (and Appendix 2). This section describes the means by which the exposure data for diuron, prometryn and endosulfan was collected and analysed under the Gwydir Water Quality Projects protocol, and methods used to summarise the exposure information in to a more manageable format.

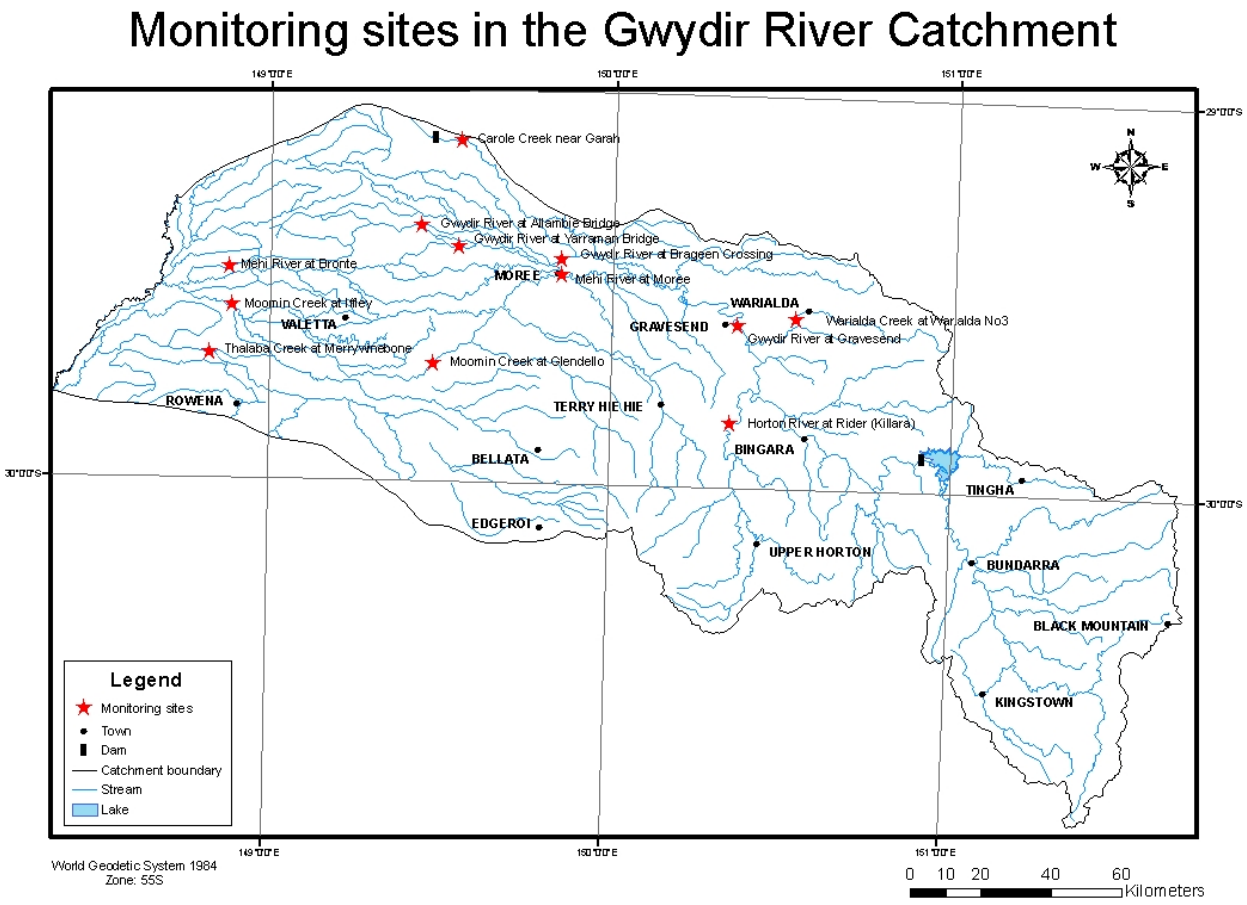



**Figure 2.6** Map of pesticide monitoring locations in the Gwydir River catchment (optimised from the New South Wales department of Water and Energy).

*Protocol of water sampling and pesticide analysis under the Gwydir Water Quality Project*

The sampling protocol conducted under the NSW Department of Water and Energy's Gwydir Water Quality Project has been described previously by Muschal and Warne (2003). Pesticide concentrations were determined by taking grab samples from 13 different locations in the Gwydir River catchment for the period 1991 and 2007. Grab samples were taken weekly during the cotton "spray seasons" and either fortnightly or monthly during, what was considered to be, the "non-spray" season (Muschal and Warne, 2003). The samples were prepared using the USEPA Method 3510 for liquid/liquid extraction and analysed by gas chromatography and high performance liquid chromatography (USEPA 1986, 1989; as cited by Muschal and Warne, 2003). Fifteen years (1991-2006) of diuron, prometryn, technical endosulfan (α+β endosulfan), endosulfan sulphate and total endosulfan surface water monitoring data was used in this exposure assessment.

*Development of exposure distributions*

To characterise the range of concentrations of diuron, prometryn, technical endosulfan and endosulfan sulphate, continuous probability distributions were developed. Distributions were constructed by ranking the data for increasing concentration. Where concentrations were recorded to be less than the limit of quantitation (LOQ), half of the LOQ was assigned (Solomon *et al.*, 2000). Following this, a Weibull percentile ranking *%Rank* was assigned to the ranked data using the equation:

$$\%Rank = \frac{n}{N+1} \times 100\% \qquad (2.1)$$

where $n$ is the rank for one observation and $N$ is the total number of observations.

To develop the distribution, multiple percentile ranks for a single concentration, such as those recorded as less than the LOQ, were excluded except for the highest *%Rank* for that concentration. The exposure *%Rank* values were converted to probabilities and the $\log_{10}$ of the concentration were calculated. The data was plotted with the logarithm of concentration as the independent variable and normalised rank percentile as the dependent variable (Solomon *et al.*, 2000; Solomon and Takacs, 2002). A linear regression was then fit to the data using the equation:

$$y = mx + b \qquad (2.2)$$

Where $y$ is the *%Rank* converted probability, $m$ is the slope of the regression, $x$ is the $\log_{10}$ of the concentration, and $b$ is the y-intercept of the regression. The regressions were performed using the statistical software SigmaPlot 10.0 (Systat Software), where the coefficients of determination ($r^2$) and regression parameters were recorded to determine the 90$^{th}$ percentile exposure concentration. For reference purposes, the median and maximum exposure concentrations were also determined.

*2.4.1.3.1 Exposure assessment of diuron in the Gwydir River catchment*

The sampling periods, median, 90$^{th}$ percentile, and maximum diuron concentration along with the date recorded, and exposure distributions regression parameters are summarised for

each monitoring site in Table 2.4. In all cases, the median diuron concentration was determined to be less than the LOQ of 0.1 µg $L^{-1}$. The highest 90$^{th}$ percentile diuron concentration was recorded at Thalaba Creek, Merrywinebone (4.6 µg $L^{-1}$), with the remaining sites ranging from the LOQ up to 0.5 µg $L^{-1}$ (Table 2.4). The highest maximum concentration was recorded at Carole Creek, Mungindi Road (82.1 µg $L^{-1}$) on 27 October 1998 with the remaining sites maximum concentrations ranging from the LOQ up to 77.9 µg $L^{-1}$ (Table 2.4). It can be concluded that for all sites the majority of the samples collected recorded diuron concentrations less than the LOQ.

**Table 2.4** Summary of measurement period, total number (*n*) of observations, and median, 90th percentile and maximum concentrations of diuron calculated using the regression parameters for each monitoring site in the Gwydir River catchment.

| | | | Exposure concentration of diuron (µg $L^{-1}$)[a] | | | Distribution regression parameters (y = mx + b) | | |
|---|---|---|---|---|---|---|---|---|
| Site Name | Years measured | n | Median | 90th percentile | Maximum (Date: d/m/yyyy) | m | b | $r^2$ |
| Thalaba Creek, Merrywinebone | 1991-2006 | 273 | <LOQ | 4.6 | 77.9 (10/4/1994) | 0.69 | 0.96 | 0.98 |
| Mehi River, Bronte | 1991-2006 | 296 | <LOQ | 0.5 | 36.5 (19/9/2006) | 0.93 | 1.61 | 0.94 |
| Gwydir River, Brageen Crossing | 1991-2007 | 293 | <LOQ | 0.5 | 16.6 (4/1/1994) | 0.76 | 1.55 | 0.98 |
| Moomin Creek, Iffley | 1991-2006 | 265 | <LOQ | 0.5 | 13.4 (12/2/1996) | 0.78 | 1.58 | 0.94 |
| Gwydir River, Allambie bridge | 2002-2007 | 53 | <LOQ | 0.3 | 5.0 (16/6/2005) | 0.84 | 1.64 | 0.95 |
| Carole Creek, Garah | 2002-2007 | 51 | <LOQ | 0.2 | 12.5 (13/8/2002) | 0.54 | 1.54 | 0.96 |
| Moomin Creek, Glendello | 2002-2007 | 49 | <LOQ | 0.2 | 2.9 (15/11/2005) | 0.80 | 1.85 | 0.82 |
| Carole Creek, Mungindi road | 1991-2001 | 230 | <LOQ | 0.1 | 82.1 (27/10/1998) | 0.46 | 1.76 | 0.98 |
| Horton River, Rider (Killara) | 2002-2007 | 52 | <LOQ | <LOQ | <LOQ | NA | NA | NA |
| Gwydir River, Gravesend road bridge | 1992-2007 | 207 | <LOQ | <LOQ | <LOQ | NA | NA | NA |
| Gwydir River, Yarraman bridge | 2002-2007 | 55 | <LOQ | <LOQ | <LOQ | NA | NA | NA |
| Mehi River, Moree | 2002-2007 | 55 | <LOQ | <LOQ | <LOQ | NA | NA | NA |
| Warialda Creek, Warialda No. 3 | 2002-2007 | 55 | <LOQ | <LOQ | <LOQ | NA | NA | NA |

[a] <LOQ signifies measurements determined to be less than the limit of quantitation

NA signifies not applicability of linear regression to the dataset due to lack of data exceeding the LOQ

*2.4.1.3.2 Exposure assessment of prometryn in the Gwydir River catchment*

The sampling periods, the median and 90$^{th}$ percentile prometryn concentrations, and the maximum prometryn concentration along with the date recorded are summarised for each monitoring site in Table 2.5. The median prometryn concentrations at all sites were determined to be less than the LOQ of 0.1 µg L$^{-1}$ (Table 2.5). The highest 90$^{th}$ percentile prometryn concentration was determined at Thalaba Creek, Merrywinebone (0.5 µg L$^{-1}$), with the remaining sites having 90$^{th}$ percentile concentrations ranging from 0.2 µg L$^{-1}$ to the LOQ (Table 2.5). The highest maximum prometryn concentration was recorded at Carole Creek, Mungindi Road (47.0 µg L$^{-1}$) on 27 October 1998, with the remaining sites having maximum concentrations ranging from the LOQ up to 7.9 µg L$^{-1}$ (Table 2.5). It can be concluded that for all sites the majority of the samples collected recorded prometryn concentrations less than the LOQ.

**Table 2.5** Summary of measurement period, total number (*n*) of observations, and median, 90th percentile and maximum concentrations of prometryn calculated using the regression parameters for each monitoring site in the Gwydir River catchment.

| | | | Exposure concentration of prometryn (µg $L^{-1}$)[a] | | | Distribution regression parameters (y = mx + b) | | |
|---|---|---|---|---|---|---|---|---|
| Site Name | Years measured | n | Median | 90th percentile | Maximum (Date: d/m/yyyy) | m | b | $r^2$ |
| Thalaba Creek, Merrywinebone | 1991-2006 | 274 | <LOQ | 0.5 | 7.9 (7/1/1992) | 1.11 | 1.51 | 0.97 |
| Moomin Creek, Glendello | 2002-2007 | 49 | <LOQ | 0.2 | 0.6 (15/11/2005) | 0.90 | 2.12 | 0.88 |
| Moomin Creek, Iffley | 1991-2006 | 265 | <LOQ | 0.1 | 1.7 (15/2/2005) | 0.95 | 2.18 | 0.94 |
| Carole Creek, Mungindi road | 1991-2001 | 230 | <LOQ | <LOQ | 47.0 (27/10/1998) | 0.41 | 2.06 | 0.93 |
| Carole Creek, Garah | 2002-2007 | 51 | <LOQ | <LOQ | 2.6 (12/1/2004) | 0.18 | 2.00 | 1.00 |
| Mehi River, Bronte | 1991-2006 | 297 | <LOQ | <LOQ | 0.9 (26/10/1999) | 1.08 | 2.51 | 0.92 |
| Gwydir River, Brageen Crossing | 1991-2007 | 294 | <LOQ | <LOQ | 1.2 (22/1/1995) | 0.74 | 2.27 | 0.85 |
| Warialda Creek, Warialda No. 3 | 2002-2007 | 55 | <LOQ | <LOQ | 0.1 (23/5/2007) | 1.17 | 3.32 | 1.00 |
| Gwydir River, Allambie bridge | 2002-2007 | 53 | <LOQ | <LOQ | 0.1 (15/2/2005) | 1.17 | 3.31 | 1.00 |
| Horton River, Rider (Killara) | 2002-2007 | 52 | <LOQ | <LOQ | <LOQ | NA | NA | NA |
| Gwydir River, Gravesend road bridge | 1992-2007 | 207 | <LOQ | <LOQ | <LOQ | NA | NA | NA |
| Gwydir River, Yarraman bridge | 2002-2007 | 55 | <LOQ | <LOQ | <LOQ | NA | NA | NA |
| Mehi River, Moree | 2002-2007 | 55 | <LOQ | <LOQ | <LOQ | NA | NA | NA |

[a] <LOQ signifies measurements determined to be less than the limit of quantitation

NA signifies not applicability of linear regression to the dataset due to lack of data exceeding the LOQ

*2.4.1.3.3 Exposure assessment of endosulfan in the Gwydir River catchment*

The sampling periods, the median and 90$^{th}$ percentile concentrations, and the maximum concentration along with the date recorded for technical endosulfan, endosulfan sulphate, and total endosulfan are summarised for each monitoring site in Table 2.6. All sites returned median concentrations of technical and total endosulfan, and endosulfan sulphate less than the LOQ (0.01 µg L$^{-1}$). Few sites exhibited endosulfan sulphate and total endosulfan median concentrations greater than the LOQ with the highest observed at Thalaba Creek, Merrywinebone (0.03 µg L$^{-1}$) and remaining sites ranging from the LOQ up to 0.01 µg L$^{-1}$ (Table 2.6).

Many of the sites had 90$^{th}$ percentile endosulfan concentrations below the LOQ. The highest α- and only β-endosulfan 90$^{th}$ percentile concentration was determined to occur in Carole Creek, Mungindi Road (0.02 µg L$^{-1}$ and 0.01 µg L$^{-1}$, respectively; Table 2.6). The highest endosulfan sulphate and total endosulfan 90$^{th}$ percentile concentrations occurred in Thalaba Creek, Merrywinebone (0.21 and 0.24, respectively; Table 2.6). Other 90$^{th}$ percentile α-endosulfan, β-endosulfan, endosulfan sulphate and total endosulfan concentrations recorded at the remaining sites ranged from the LOQ up to 0.13 µg L$^{-1}$ (Table 2.6).

**Table 2.6** Summary of measurement period, total number (*n*) of observations, and median, 90th percentile and maximum concentrations of technical endosulfan (α-+β-endosulfan), endosulfan sulphate and total endosulfan exposure samples calculated using the regression parameters for each monitoring site in the Gwydir River catchment.

| | | | | Exposure concentration of endosulfan (µg $L^{-1}$)[a] | | | Distribution regression parameters (y = mx + b) | | |
|---|---|---|---|---|---|---|---|---|---|
| Site name | Years measured | *n* | Isomer or degradate | Median | 90th percentile | Maximum (Date: d/m/yyyy) | m | b | $r^2$ |
| Thalaba Creek, Merrywinebone | 1991-2006 | 274 | Technical endosulfan | <LOQ | <LOQ | 0.80 (9/12/1997) | 0.44 | 2.46 | 0.89 |
| | | | Endosulfan sulfate | 0.03 | 0.21 | 2.21 (10/1/1994) | 1.26 | 2.05 | 0.98 |
| | | | Total endosulfan | 0.03 | 0.24 | 2.21 (10/1/1994) | 1.21 | 1.98 | 0.98 |
| Carole Creek, Mungindi road | 1991-2001 | 230 | Technical endosulfan | <LOQ | 0.03 | 1.62 (6/12/1993) | 0.78 | 2.63 | 0.98 |
| | | | Endosulfan sulfate | <LOQ | 0.1 | 2.24 (6/12/1993) | 1.09 | 2.46 | 0.98 |
| | | | Total endosulfan | <LOQ | 0.12 | 3.86 (6/12/1993) | 1.05 | 2.30 | 0.98 |
| Mehi River, Bronte | 1991-2006 | 296 | Technical endosulfan | <LOQ | <LOQ | 0.17 (30/11/1998) | 0.84 | 3.33 | 0.97 |
| | | | Endosulfan sulfate | 0.01 | 0.08 | 0.69 (30/11/1998) | 1.57 | 3.08 | 0.97 |
| | | | Total endosulfan | 0.01 | 0.08 | 0.86 (30/11/1998) | 1.51 | 2.96 | 0.97 |
| Carole Creek, Garah | 2002-2007 | 51 | Technical endosulfan | <LOQ | <LOQ | <LOQ | NA | NA | NA |
| | | | Endosulfan sulfate | <LOQ | <LOQ | 0.02 (10/1/2005) | 1.07 | 3.89 | 1.00 |
| | | | Total endosulfan | <LOQ | <LOQ | 0.02 (10/1/2005) | 1.07 | 3.89 | 1.00 |
| Gwydir River, Brageen Crossing | 1991-2007 | 294 | Technical endosulfan | <LOQ | 0.02 | 0.22 (7/2/1995) | 0.96 | 3.02 | 0.91 |
| | | | Endosulfan sulfate | <LOQ | 0.1 | 1.86 (4/1/1994) | 1.24 | 2.55 | 0.98 |
| | | | Total endosulfan | <LOQ | 0.13 | 1.91 (4/1/1994) | 1.18 | 2.41 | 0.98 |
| Moomin Creek, Iffley | 1991-2006 | 265 | Technical endosulfan | <LOQ | <LOQ | 0.89 (9/12/1996) | 0.54 | 2.81 | 0.91 |
| | | | Endosulfan sulfate | 0.01 | 0.12 | 1.60 (9/12/1996) | 1.26 | 2.50 | 0.99 |
| | | | Total endosulfan | 0.01 | 0.12 | 2.49 (9/12/1996) | 1.22 | 2.43 | 0.98 |
| Gwydir River, Allambie bridge | 2002-2007 | 53 | Technical endosulfan | <LOQ | <LOQ | <LOQ | NA | NA | NA |
| | | | Endosulfan sulfate | <LOQ | <LOQ | 0.05 (14/12/2004) | 0.59 | 2.78 | 0.93 |
| | | | Total endosulfan | <LOQ | <LOQ | 0.05 (14/12/2004) | 0.59 | 2.78 | 0.93 |
| Moomin Creek, Glendello | 2002-2007 | 49 | Technical endosulfan | <LOQ | <LOQ | <LOQ | NA | NA | NA |
| | | | Endosulfan sulfate | <LOQ | <LOQ | 0.05 (14/12/2004) | 0.48 | 2.64 | 0.94 |
| | | | Total endosulfan | <LOQ | <LOQ | 0.05 (14/12/2004) | 0.48 | 2.64 | 0.94 |
| Horton River, Rider (Killara) | 2002-2007 | 52 | Technical endosulfan | <LOQ | <LOQ | <LOQ | NA | NA | NA |
| | | | Endosulfan sulfate | <LOQ | <LOQ | <LOQ | NA | NA | NA |
| | | | Total endosulfan | <LOQ | <LOQ | <LOQ | NA | NA | NA |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Gwydir River, Gravesend road bridge | 1992-2007 | 207 | Technical endosulfan | <LOQ | <LOQ | 0.02 (4/1/1994) | 0.67 | 3.71 | 0.98 |
| | | | Endosulfan sulfate | <LOQ | <LOQ | 0.01 (3/1/1996) | 0.82 | 4.24 | 1.00 |
| | | | Total endosulfan | <LOQ | <LOQ | 0.02 (4/1/1994) | 0.86 | 4.06 | 1.00 |
| Gwydir River, Yarraman bridge | 2002-2007 | 55 | Technical endosulfan | <LOQ | <LOQ | <LOQ | NA | NA | NA |
| | | | Endosulfan sulfate | <LOQ | <LOQ | <LOQ | NA | NA | NA |
| | | | Total endosulfan | <LOQ | <LOQ | <LOQ | NA | NA | NA |
| Mehi River, Moree | 2002-2007 | 55 | Technical endosulfan | <LOQ | <LOQ | <LOQ | NA | NA | NA |
| | | | Endosulfan sulfate | <LOQ | <LOQ | <LOQ | NA | NA | NA |
| | | | Total endosulfan | <LOQ | <LOQ | <LOQ | NA | NA | NA |
| Warialda Creek, Warialda No. 3 | 2002-2007 | 55 | Technical endosulfan | <LOQ | <LOQ | <LOQ | NA | NA | NA |
| | | | Endosulfan sulfate | <LOQ | <LOQ | <LOQ | NA | NA | NA |
| | | | Total endosulfan | <LOQ | <LOQ | <LOQ | NA | NA | NA |

[a] <LOQ signifies measurements determined to be less than the limit of quantitation

NA signifies not applicability of linear regression to the dataset due to lack of data exceeding the LOQ

Many sites recorded maximum α-endosulfan, β-endosulfan, endosulfan sulphate, and total endosulfan concentrations greater than the LOQ. The highest maximum α-endosulfan, endosulfan sulphate and total endosulfan concentrations were recorded in Carole Creek, Mungindi Road on 6 December 1993 (1.24, 2.24, and 3.86 μg $L^{-1}$, respectively; Table 2.6). The highest maximum β-endosulfan was recorded in Moomin Creek, Iffley on 9 Decemeber 1996 (0.51 μg $L^{-1}$; Table 2.6). Other maximum α-endosulfan, β-endosulfan, endosulfan sulphate and total endosulfan concentrations recorded at the remaining sites ranged from the LOQ up to 2.49 μg $L^{-1}$ (Table 2.6).

*2.4.2 Toxicity assessment of diuron, prometryn and endosulfan*

The endpoints for this ERA were to protect 90-95% of the organisms in the Gwydir River catchment ecosystems, as described in Section 2.3.3. Because of a lack of toxicity information on species unique to the Gwydir River catchment, exercises in collecting available laboratory and field toxicity studies on diuron, prometryn and endosulfan was undertaken. Assuming that the sensitivity of species between regions are not likely to be significantly different (Hose and Van den Brink, 2004; Maltby *et al.*, 2005) the collated information was used to develop species sensitivity distributions. This approach, by accounting for a wide range of species toxicity, assumes that the likely sensitivity of organisms in the Gwydir River catchment fall within the range characterised by the distribution. This section presents the approach used to develop the toxicity databases and SSD development for diuron, prometryn and endosulfan.

*2.4.2.1 Method of ecotoxicity data collection and selection for use in the development species sensitivity distributions (SSD)*

All available single species aquatic ecotoxicology data on diuron, prometryn and endosulfan was obtained from the USEPA ECOTOX (ECOTOXicology) (2009) database and literature searches. As the exposure data could not prescribe some level of certainty on pulse durations, SSDs were developed to account for a range of exposure durations. Data selection criteria were developed from methods described by Solomon *et al*. (2000) and Maltby *et al*. (2005).

*Data selection*

Criteria used to select single-species toxicity data were related to test endpoint, duration, effect type, effect measurement and exposure type. The test endpoints used were the chronic median lethal concentration ($LC_{50}$), median effect concentrations ($EC_{50}$) or median inhibition concentration ($IC_{50}$) for aquatic animals and plants (including algae and macrophytes), respectively, collated from the USEPA's ECOTOX database (USEPA, 2009). Lowest observed (LOEC) and no observed (NOEC) effects concentration endpoints were excluded from the SSD because of their lack of statistical rigour, variability at representing effects and low dataset abundance (Laskowski, 1995; Kooijman, 1996; Posthuma *et al.*, 2002; Suter II, 2007). Studies that were found to report concentrations exceeding the water solubility of these compounds were also excluded from the SSD regression, however were included in the rank calculation (Solomon and Takacs, 2002; Carriger and Rand, 2008). Following the recommendations of de Zwart (2002), salt and freshwater toxicity data were combined.

As it was unknown how long the exposure pulses in the Gwydir River catchment were lasting, exposure duration ranges were used to minimise this uncertainty. The effects measures were examined to account for lethality in animals (including mortality and immobility) and declines in population growth rate for aquatic macrophytes and algae. The species were sorted for test durations in the range 2 to 21 days for fish, 1 to 7 days for invertebrates, 2 to 28 days for macrophytes, and 1 to 7 days for algae (Maltby *et al*. 2005). Where more than one toxicity value was available for a single species the exposure type was evaluated for maintenance of exposure concentration, with experiments exhibiting continuous flow-through systems were given preference over experiments that were replacing exposure solution at intervals and static systems that were not replacing exposure solutions (Solomon and Takacs, 2002). Where more than one toxicity value remained for a single species, the geometric mean was calculated (Solomon *et al.*, 2000; Solomon and Takacs, 2002; Maltby *et al.*, 2005; Rand *et al.*, 2010). An acute to chronic ratio was not determined in this study, as the data selection criteria accounted for this range of exposure durations. As suggested by Solomon and Takacs (2002), groups of organisms found to be significantly more sensitive were separated. This was determined using one-way analysis of variance (ANOVA) testing in SigmaPlot 10.0 (Systat Software).

*Development of species sensitivity distributions (SSD)*

The collated toxicity data was then used to construct SSDs. The toxicity data was ranked and a Weibull percentile ranking *%Rank* calculated using the same approach as Equation 2.1. Species sensitivity distributions consisting of all data and taxonomic groups displaying significantly different levels of exposure sensitivity were constructed. The *%Rank* was converted to probabilities and a linear regression performed (Equation 2.2), with the $EC_{50}$ and/or $LC_{50}$ concentrations used as the independent variable and *%Rank* as the dependent. The coefficients of determination ($r^2$), and regression parameters were recorded ($m_{tox}$ and $b_{tox}$; the slope and regression intercept of the SSD, respectively) to estimate the required $HC_x$'s (Solomon *et al.*, 2000; Solomon and Takacs, 2002).

*Estimation of hazard concentrations ($HC_x$)*

For all SSDs, the 5th and 10th percentile hazard concentrations (HC) toxicity concentrations were calculated to reflect the thresholds set in the problem formulation phase. For all SSDs hazard concentrations of the 5th ($HC_5$) and 10th ($HC_{10}$) percentile of each SSD was calculated using the regression parameters. The hazard concentration affecting X% of species ($HC_x$) was determined by:

$$HC_x = 10^{\frac{\text{Prob}(X)-b_{tox}}{m_{tox}}} \qquad (2.3)$$

Where *Prob*(*X*) is the percentile of the SSD which the hazard concentration is to be calculated, $b_{tox}$ is the y-intercept parameter, and $m_{tox}$ is the gradient parameter of the SSD regression (from Equation 2.2).

*2.4.2.2 Summary of diuron, prometryn and endosulfan toxicity and resultant SSDs*

The outcome of the data selection method used and their associated SSDs for diuron, prometryn and endosulfan are summarised in this section.

*Diuron*

Diuron aquatic species toxicity obtained from the ECOTOX database is summarised in Appendix 2. The most sensitive organism was a species of Blue-green algae *Synechococcus sp.*, ($EC_{50}$ = 0.55 µg $L^{-1}$). The most tolerant was *Ctenopharyngodon idella* (Grass carp) ($LC_{50}$ = 31,000 µg $L^{-1}$). The data was arranged in to taxonomic groups of algae, macrophytes, fish,

amphibians and invertebrates. A non-parametric one-way ANOVA Dunn's test on ranks determined significantly different levels of sensitivity between the taxonomic groups ($P < 0.01$; Appendix 2). Specifically, the algae and macrophyte taxonomic groups were found to be significantly more sensitive to diuron exposure than fish, amphibian and invertebrate taxonomic groups ($P < 0.05$; Appendix 2). Algae and macrophytes taxonomic groups were found to not be significantly different, as were fish, amphibians and invertebrates ($P > 0.05$; Appendix 2).

To distinguish the levels of species sensitivity to diuron, it was necessary to construct three SSDs representing the sensitivities of algae and macrophytes; fish, amphibians and invertebrates; and all taxonomic groups (Figure 2.7). Their regression outputs and $HC_5$ and $HC_{10}$ toxicity thresholds are given in Table 2.7. In all cases, the linear regression explained the variability reasonably well ($r^2 = 0.91$-$0.98$). The SSD consisting of all taxonomic groups confirms a clear separation in sensitivity that is subsequently represented in the two SSDs of fish, amphibians and invertebrates; and algae and macrophytes (Figure 2.7). The estimated $HC_5$s and $HC_{10}$s for each SSD were, respectively, 1.0 and 1.7 µg $L^{-1}$ for algae and macrophytes; 697.0 and 1151.0 µg $L^{-1}$ for fish, amphibians and invertebrates; and 0.8 and 3.4 µg $L^{-1}$ for both taxonomic groups (Table 2.7).

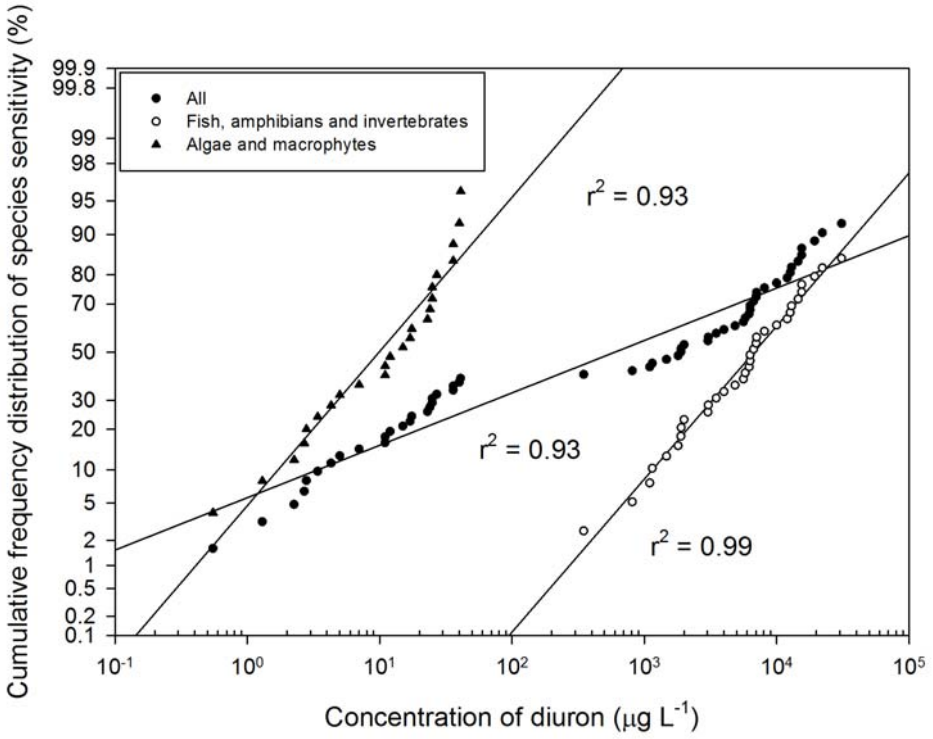



**Figure 2.7** Diuron species sensitivity distributions for all; fish, amphibians and invertebrate; and algae and macrophyte taxonomic groups

**Table 2.7** Diuron species sensitivity linear regression parameters and estimated $HC_5$ and $HC_{10}$ for each taxonomic group.

| Taxonomic group | n[a] | $b_{tox}$ | $m_{tox}$ | $r^2$ | $HC_5$ (µg $L^{-1}$) | $HC_{10}$ (µg $L^{-1}$) |
|---|---|---|---|---|---|---|
| Algae and macrophytes | 24 (24) | -1.67 | 1.68 | 0.93 | 1.0 | 1.7 |
| Fish, amphibians and invertebrates | 37 (33) | -6.39 | 1.67 | 0.99 | 697.0 | 1151.0 |
| All taxonomic groups | 61 (57) | -1.59 | 0.57 | 0.93 | 0.8 | 3.4 |

[a] n represents the total count of studies used to develop the SSD, including geometic means of the same species; and numbers in brackets represent the number of points used in the regression estimate.

*Prometryn*

Prometryn aquatic species toxicity obtained from the ECOTOX database is summarised in Appendix 2. The most sensitive organism was a diatom species *Navicula pelliculosa* ($EC_{50}$ = 1 µg $L^{-1}$). The most tolerant was the Mayfly species *Cloeon dipterum* ($EC_{50}$ = 40,000 µg $L^{-1}$). The data was arranged in to taxonomic groups of algae, macrophytes, fish, amphibians and invertebrates. A non-parametric one-way ANOVA Dunn's test on ranks determined significantly different levels of sensitivity to prometryn between the taxonomic groups ($P < 0.01$). Specifically, the algae and macrophyte taxonomic groups were found to be significantly more sensitive to prometryn exposure than fish, amphibian and invertebrate

taxonomic groups ($P < 0.05$; Appendix 2). Algae and macrophyte taxonomic groups were found to not be significantly different, as were fish, amphibians and invertebrates ($P > 0.05$; Appendix 2).

To distinguish the levels of species sensitivity to prometryn, it was necessary to construct three SSDs representing the algae and macrophyte; fish, amphibians and invertebrates; and all taxonomic groups (Figure 2.8). Their SSD regression outputs, and $HC_5$ and $HC_{10}$ toxicity thresholds are given in Table 2.11. In all cases, the linear regression explained the variability in species sensitivity reasonably well ($r^2 = 0.93$-$0.97$). The SSD consisting of all taxonomic groups confirmed a clear separation in sensitivity that is subsequently represented in the two SSDs of algae and macrophytes, being the most sensitive; and fish, amphibians and invertebrates (Figure 2.8). The estimated $HC_5$s and $HC_{10}$s for the respective SSDs were 1.4 and 2.6 μg $L^{-1}$ for algae and macrophytes; 1380.0 and 2011.1 μg $L^{-1}$ for fish, amphibians and invertebrates, and 0.9 and 3.2 μg $L^{-1}$ for all species (Table 2.8).

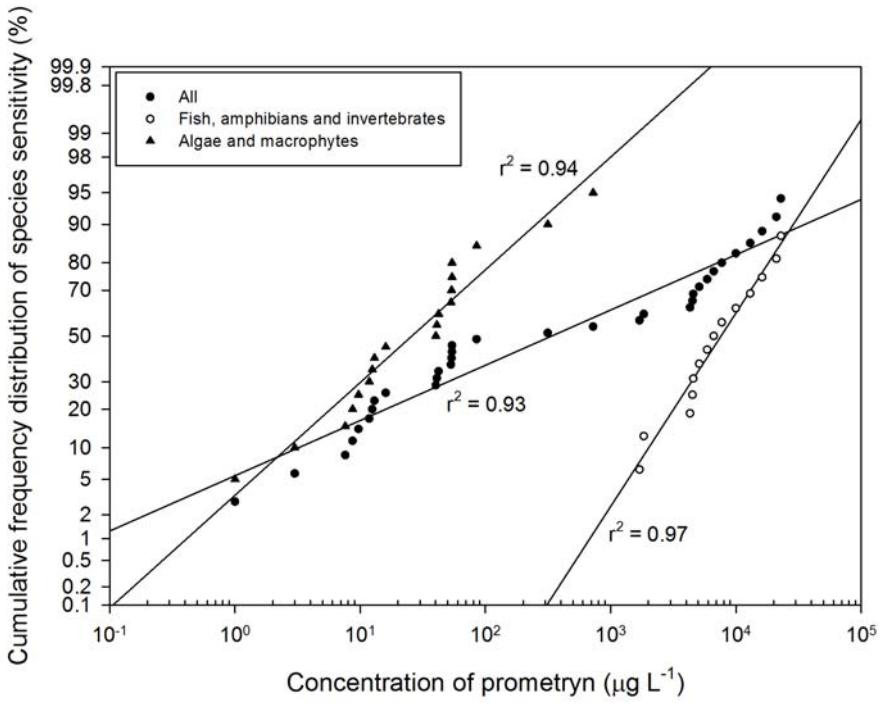



**Figure 2.8** Prometryn species sensitivity distributions for all; fish, amphibians and invertebrate; and algae and macrophyte taxonomic groups

**Table 2.8** Prometryn species sensitivity linear regression parameters and estimated $HC_5$ and $HC_{10}$ for each taxonomic group.

| Taxonomic group | n[a] | $b_{tox}$ | $m_{tox}$ | $r^2$ | $HC_5$ (µg $L^{-1}$) | $HC_{10}$ (µg $L^{-1}$) |
|---|---|---|---|---|---|---|
| Algae and macrophytes | 19 (19) | -1.83 | 1.29 | 0.94 | 1.4 | 2.6 |
| Fish, amphibians and invertebrates | 15 (14) | -8.62 | 2.22 | 0.97 | 1380.0 | 2011.1 |
| All taxonomic groups | 34 (33) | -1.60 | 0.63 | 0.93 | 0.9 | 3.2 |

[a] n represents the total count of studies used in the *%Rank* calculation, including geometic means of the same species; and numbers in brackets represent the number of points used in the regression estimate.

*Endosulfan*

The toxicity data obtained from the ECOTOX database for technical endosulfan (consisting of both α- and β- endosulfan combined) and endosulfan sulphate were treated separately, following the precedent set by Rand *et al*. (2010). The ecotoxicity data for technical endosulfan and endosulfan sulphate, as an outcome of the data selection process, are given in Appendices 2.3.3.

Following the data selection processes, no algal or macrophyte toxicity data were suitable for the SSD development of both technical endosulfan and endosulfan sulphate. A large number of toxicity data was available for the invertebrate, fish and amphibian taxonomic groups for technical endosulfan, and a relatively smaller number of toxicity studies for endosulfan sulphate. The most sensitive organism to technical endosulfan and endosulfan sulphate was the invertebrate *Penaeus duorarum,* Northern Pink Shrimp ($LC_{50}$ 7= 0.04 µg $L^{-1}$) and *Jappa kutera*, Mayfly ($LC_{50}$ = 0.04 µg $L^{-1}$), respectively. Conversely, the most tolerant organisms to technical endosulfan and endosulfan sulphate were the invertebrates *Physa fontinalis*, Bladder Snail ($LC_{50}$ = 316.2 µg $L^{-1}$); and equally *Aedes aegypti*, Yellow Fever Mosquito, *Artemia salina*, Brine Shrimp, *Chironomus riparius*, Midge, *Physa fontinalis*, Bladder Snail, *Daphnia magna*, Water Flea ($LC_{50}$ = 316.2 µg $L^{-1}$; Appendices 2.8.1 and 2.8.2, respectively ).

A non-parametric one-way ANOVA Dunn's test on ranks found no taxonomic groups displaying significantly different levels of sensitivity to technical endosulfan (P >0.05; Appendix 2) and endosulfan sulphate (P = 0.164; Appendix 2). SSDs consisting of technical endosulfan and endosulfan sulphate toxicity data are respectively given in Figures 2.9 and 2.10. To improve these regression responses, similar to that of Rand *et al*. (2010), two separate SSDs composed of fish and amphibians, and invertebrates taxonomic groups were produced for both technical and endosulfan and endosulfan sulphate (Figures 2.9 and 2.10, respectively). The log-linear regressions for technical endosulfan and endosulfan sulphate

taxonomic groups improved from $r^2$ of 0.93 and 0.96, to $r^2$ of 0.96-0.97 and 0.98-0.99, respectively (Table 2.9). The separation of the different taxonomic groups enabled the variability observed down the more sensitive end of the distribution to be explained. Using the SSD regression parameters (Table 2.9), the $HC_5$s and $HC_{10}$s for technical endosulfan were determined to be 0.12 and 0.45 µg $L^{-1}$, 0.19 and 0.34 µg $L^{-1}$, and 0.11 and 0.30 µg $L^{-1}$ for invertebrates, fish and amphibians, and all taxonomic groups, respectively (Table 2.9); which are consistent with Carriger *et al*. (2008). Endosulfan sulphate $HC_5$s and $HC_{10}$s were similarly determined to be 0.25 and 0.59 µg $L^{-1}$, 0.46 and 1.53 µg $L^{-1}$, and 1.42 and 1.29 µg $L^{-1}$ for invertebrates, fish and amphibians, and all taxonomic groups, respectively (Table 2.9).

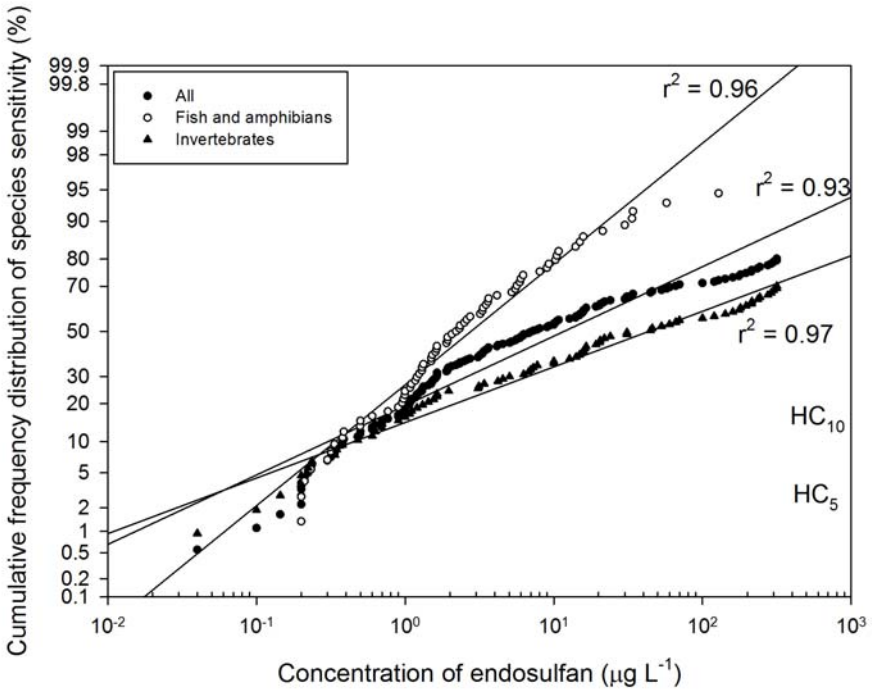



**Figure 2.9** Technical endosulfan species sensitivity distributions for all; fish and amphibians; and invertebrate taxonomic groups.

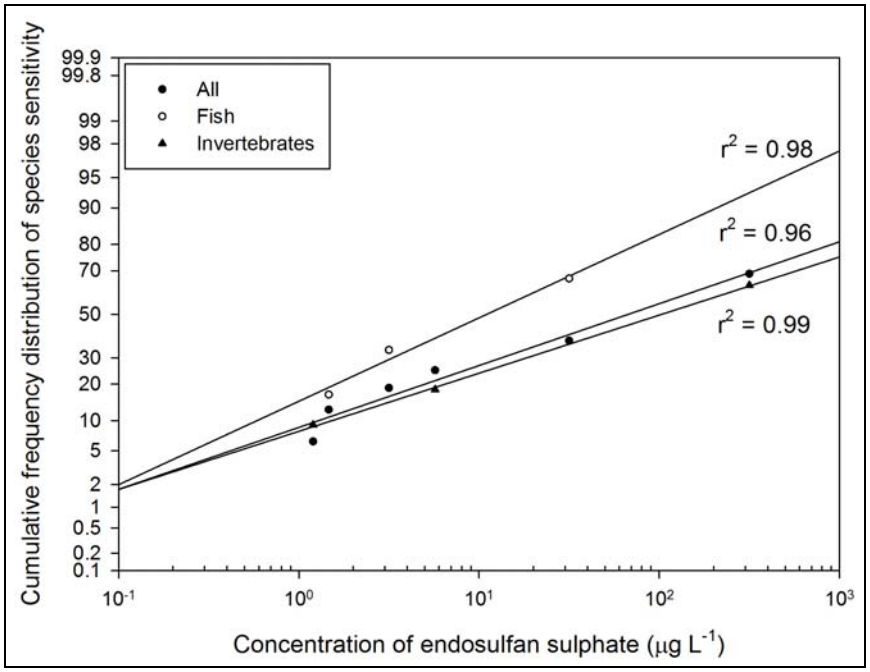



**Figure 2.10** Endosulfan sulphate species sensitivity distributions for all; fish; and invertebrate taxonomic groups.

**Table 2.9** Technical endosulfan and endosulfan sulphate species sensitivity linear regression parameters and estimated $HC_5$ and $HC_{10}$ for each taxonomic group

| Taxonomic group | n[a] | $b_{tox}$ | $m_{tox}$ | $r^2$ | $HC_5$ (µg $L^{-1}$) | $HC_{10}$ (µg $L^{-1}$) |
|---|---|---|---|---|---|---|
| Technical endosulfan | | | | | | |
| Invertebrates | 106 (75) | -1.06 | 0.65 | 0.97 | 0.12 | 0.45 |
| Fish and amphibians | 73 (70) | -0.62 | 1.41 | 0.96 | 0.19 | 0.34 |
| All taxonomic groups | 179 (145) | -0.86 | 0.81 | 0.93 | 0.11 | 0.30 |
| Endosulfan sulphate | | | | | | |
| Invertebrates | 5 (3) | -1.05 | 1.01 | 0.98 | 0.25 | 0.59 |
| Fish | 7 (3) | -1.41 | 0.70 | 1.00 | 0.46 | 1.53 |
| All taxonomic groups | 11 (6) | -1.36 | 0.75 | 0.96 | 0.42 | 1.29 |

[a] n represents the total count of studies used in the *%Rank* calculation, including geometic means of the same species; and numbers in brackets represent the number of points used in the regression estimate.

## 2.5 RISK CHARACTERISATION

### *2.5.1 Introduction*

The final phase in this risk assessment was the risk characterisation, which involves two steps: risk estimation and risk description, according to the format followed (USEPA, 1998). The risk estimation combined the exposure and toxicity profiles to estimate the risk that diuron, prometryn and endosulfan exposure in the Gwydir River catchment posed to the assessment endpoints. A description of the risk was then used to interpret the significance of

these estimations by comparing the output with regulatory guidelines. Uncertainty in all of the phases of this risk assessment were identified and described.

*2.5.2 Risk estimation*

The probability that the concentrations of diuron, prometryn and endosulfan were exceeding the toxicity threshold limits at the monitoring locations were characterised as an overlap of the species sensitivity and exposure distributions (Solomon *et al.*, 2000). The probability of measuring a concentration greater than the $HC_x$ concentration (*Risk*) was calculated as:

$$Risk = 1 - (m_{\exp}.HC_x + b_{\exp}) \times 100 \qquad (2.4)$$

Where $m_{exp}$ is the slope of the log-transformed exposure regression and $b_{exp}$ is the intercept of the log-transformed regression line of the exposure distributions (taken from Tables 2.4-2.6). The probabilities of exceeding either the $HC_5$ or $HC_{10}$ toxicity thresholds, according to the site specific assessment endpoints, were estimated using all available exposure data and on an annual basis. Using the georeferenced locations of the monitoring sites, *Risk* was estimated using all data and was further summarised in a map using ArcGIS 9.3 (ESRI, USA).

*2.5.3 Joint probability curves*

Joint probability curves were constructed, using the method of Solomon *et al*. (2000) to characterise the probability that a wide range of toxicity thresholds, 0.1-99.9% of species, were being exceeded. This involved determining the toxicity thresholds for these percentiles, using Equation 2.3, and calculating the *Risk* that each threshold was being exceeded, using Equation 2.4, at each monitoring site. Joint probability curves were constructed with *Risk* as the dependent variable, and the X-th species sensitivity thresholds as the independent variable.

*2.5.4 Estimated and interpretation of diuron, prometryn and endosulfan exposure risk in the Gwydir River catchment*

The probability that diuron, prometryn and endosulfan concentrations in the reaches of the Gwydir River catchment were exceeding endpoint toxicity thresholds was estimated. For brevity, an example estimating the probability of exceeding the $HC_{10}$ is presented for the site

that posed the highest diuron risk, Thalaba Creek, Merrywinebone. The remaining demonstrations of risk characterisation are given in Appendix 2.

*Risk estimation of diuron in the Gwydir River catchment*

By comparing the distributions of exposure with species sensitivity (Figure 2.11) the probability that the diuron concentrations at each monitoring site in the Gwydir River catchment was exceeding their relevant toxicity threshold for algae and macrophytes; fish, amphibians and invertebrate; and all taxonomic groups are presented in Table 2.10. The sites where the toxicity thresholds of the most sensitive taxonomic group, algae and macrophytes, were exceeded for more than 5% of the time was determined to occur at Thalaba Creek, Merrywinebone (exceeding the $HC_{10}$ 13.2% of the time; Table 2.10), Gwydir River, Brageen crossing (exceeding the $HC_5$ 5.87% of the time; Table 2.10), and Gwydir River, Allambie bridge (exceeding the $HC_5$ 4.94% of the time; Table 2.10). The remaining sites and the risk posed to the fish, amphibian and invertebrate taxonomic group, either displayed some, or no risk (Table 2.10). However, between the sites, Figure 2.12 reveals that much of the diuron exposure risk posed to the macrophyte and algae taxonomic group was concentrated at the western (downstream) end of the catchment where much of the crop production occurs (see land use map Figure 2.4; Section 2.2). Therefore, the level of *Risk* estimated to be occurring at the monitoring sites of the Gwydir River catchment is directly attributed to the adjacent intensive cropping land uses which these streams drain.

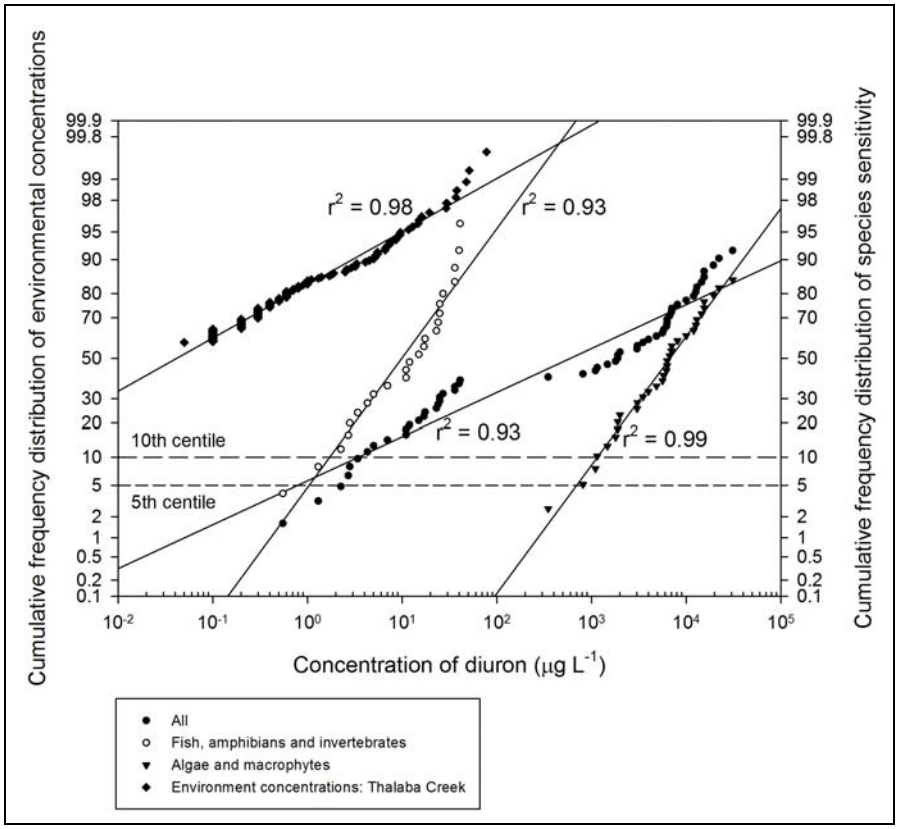



**Figure 2.11** Example of relating distribution of diuron environmental concentrations at Thalaba Creek, Merrywinebone with species sensitivity distributions of All; Fish, amphibians and invertebrates; and algae and macrophytes.

**Table 2.10** Estimates of the risk that diuron concentrations at the monitoring sites of the Gwydir River catchment are exceeding the $HC_5$ and $HC_{10}$ toxicity thresholds for the algae and macrophytes; fish, amphibians and invertebrates; and all taxonomic groups.

| | Probability (%) of diuron exposure exceeding the toxicity threshold by taxonomic group | | |
|---|---|---|---|
| Site | Algae and macrophytes | Fish, amphibians and invertebrates | All taxonomic groups |
| Thalaba Creek, Merrywinebone[a] | 13.20 | 0.11 | 9.66 |
| Gwydir River, Brageen Crossing[b] | 5.87 | 0.01 | 6.77 |
| Gwydir River, Allambie bridge[b] | 4.94 | 0.00 | 5.81 |
| Carole Creek, Garah[a] | 4.81 | 0.07 | 3.53 |
| Moomin Creek, Iffley[a] | 3.87 | 0.00 | 2.42 |
| Mehi River, Bronte[a] | 3.35 | 0.00 | 1.88 |
| Carole Creek, Mungindi road[a] | 3.11 | 0.08 | 2.34 |
| Moomin Creek, Glendello[a] | 2.09 | 0.00 | 1.23 |
| Warialda Creek, Warialda No. 3[a] | 0.00 | 0.00 | 0.00 |
| Horton River, Rider (Killara)[a] | 0.00 | 0.00 | 0.00 |
| Gwydir River, Gravesend road bridge[b] | 0.00 | 0.00 | 0.00 |
| Gwydir River, Yarraman bridge[b] | 0.00 | 0.00 | 0.00 |
| Mehi River, Moree[a] | 0.00 | 0.00 | 0.00 |

[a] $HC_5$ toxicity threshold used in risk estimation.
[b] $HC_{10}$ toxicity threshold used in risk estimation.

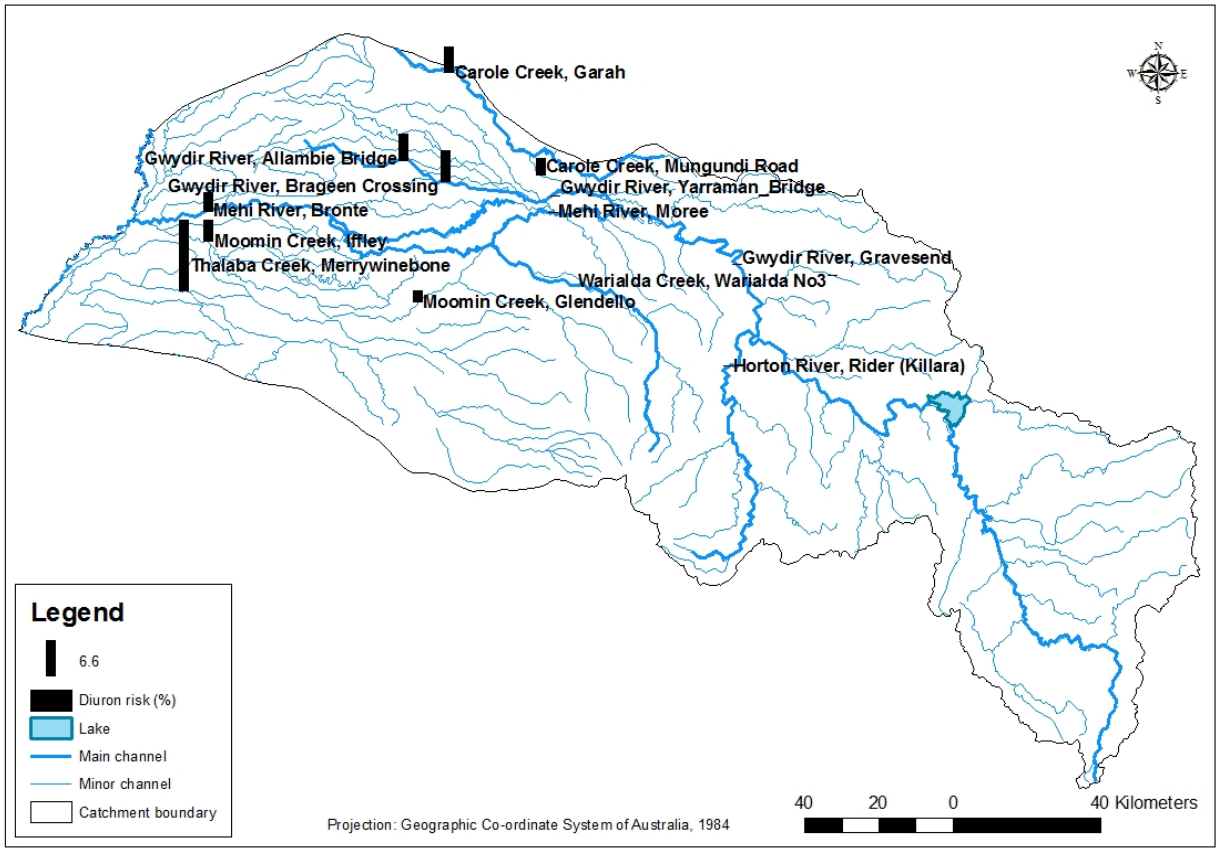



**Figure 2.12** Map of displaying the level of estimated diuron risk, represented by the height of the black bars, at the monitoring sites of the Gwydir River catchment.

With reference to the sites displaying the highest level of risk, Thalaba Creek, Merrywinebone; Gwydir River, Brageen crossing; and Gwydir River, Allambie bridge, the level of risk significance can be modified to incorporate the condition and purpose of the channel. For example, Thalaba creek, Merrywinebone is a highly modified irrigation channel regularly experiencing low discharges, and combined with the intensive cropping practices employed on the adjacent land uses undoubtedly contributes to its characteristically higher risk. This catchment is considered of low ecological significance and it may be that 13.2% exceedence may not be cause for concern to the adjacent and downstream stakeholders, a characterisation that should be confirmed by catchment managers. Comparatively, the monitoring sites along the Gwydir River that drain in to the Gwydir wetlands warrant less tolerance and so the risk observed at the monitoring sites of Gwydir River, Brageen crossing and at Gwydir River, Allambie bridge are a cause for concern.

Between the monitoring years, the risk that diuron posed to taxonomic groups of all species; algae and macrophytes; and fish, amphibians and invertebrate for each site that registered

some level of risk were found to be variable (Figure 2.13). The highest exposure *Risk* for any one year occurred at Thalaba Creek, Merrywinebone in 2003 where it was estimated that 55.25% of the time the algae and macrophyte $HC_{10}$ was exceeded. Other sites displaying exposure *Risk* where their toxicity thresholds could not be protected 95% of the time include Carole Creek, Garah (21.44% in 2002); Gwydir River, Brageen Crossing (15.40% in 1992); Moomin Creek, Iffley (15.5% in 1996); Mehi River, Bronte (20.02% in 2006); Gwydir River, Allambie Bridge (17.73% in 2004); Carole Creek, Mungindi Road (11.85% in 1995); and Moomin Creek, Glendello (8.9% in 2005).

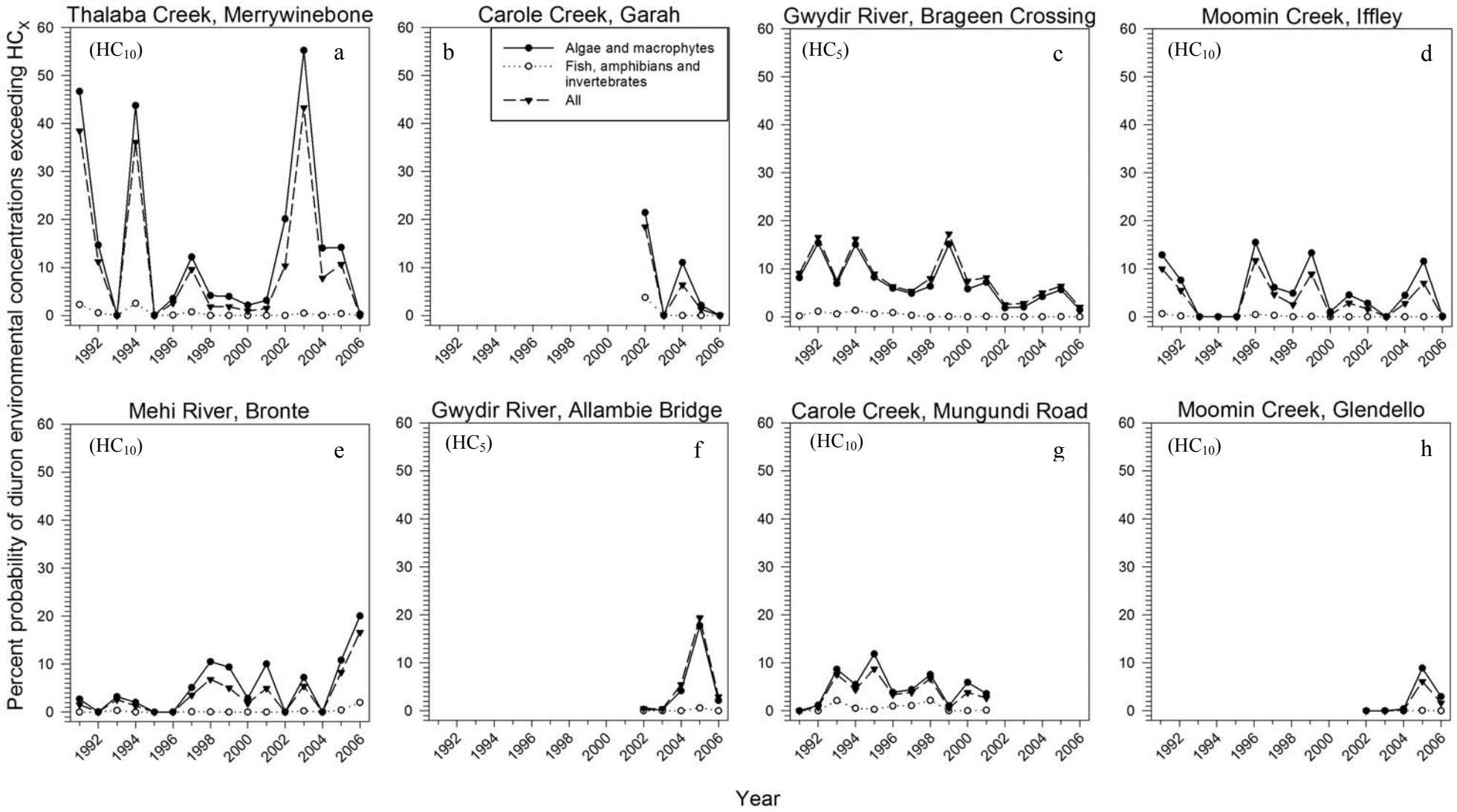



**Figure 2.13** Annual risk of diuron exposure exceeding the $HC_5$ or $HC_{10}$ ($HC_x$) at (a) Thalaba creek, Merrywinebone (1991-2006); (b) Carole Creek, Garah (2002-2006); (c) Gwydir River, Brageen Crossing (1991-2006); (d) Moomin Creek, Iffley (1991-2006); (e) Mehi River, Bronte (1991-2006); (f) Gwydir River, Allambie Bridge (2002-2006); (g) Carole Creek, Mungindi Road (1991-2001); and (h) Moomin Creek, Glendello (2002-2006). Symbols in brackets indicate the toxicity threshold used to calculate risk, with respect to assessment endpoints.

Joint probability curves of all sites having displayed some level of diuron risk toward all; and algae and macrophytes taxonomic groups are shown in Figures 2.14 a-d. All sites including Thalaba creek, Merrywinebone; Carole Creek, Garah; Gwydir River, Brageen Crossing; Moomin Creek, Iffley; Mehi River, Bronte; Gwydir River, Allambie Bridge; Carole Creek, Mungindi Road; and Moomin Creek, Glendello were determined to exhibit higher probabilities of exceeding lower diuron toxicity thresholds, declining exponentially with increasing percent species toxicity thresholds (Figures 2.14 a-d). When compared to other sites, Thalaba Creek, Merrywinebone displayed higher probabilities of exceedance for higher diuron toxicity thresholds confirming its characteristically higher level of risk (Table 2.10). Specifically, some extreme events affecting plant and algae populations in the order of 80-90% of species were occurring. Such rare events could have lead-on effects to higher trophic groups, including invertebrates and fish such as the macroinvertebrate *Cherax destructor* (Common Yabby), and *Nermatalosa erebi* (Bony bream), native to the Gwydir River catchment, that have been shown in a trophic level interaction study to rely on algae and macrophytes for sustenance (Kelleway *et al.*, 2010). However, in all cases the probability of events affecting higher percent species toxicity thresholds was low, as the joint probabilities curved close to the axes (Figures 2.14 a-d).

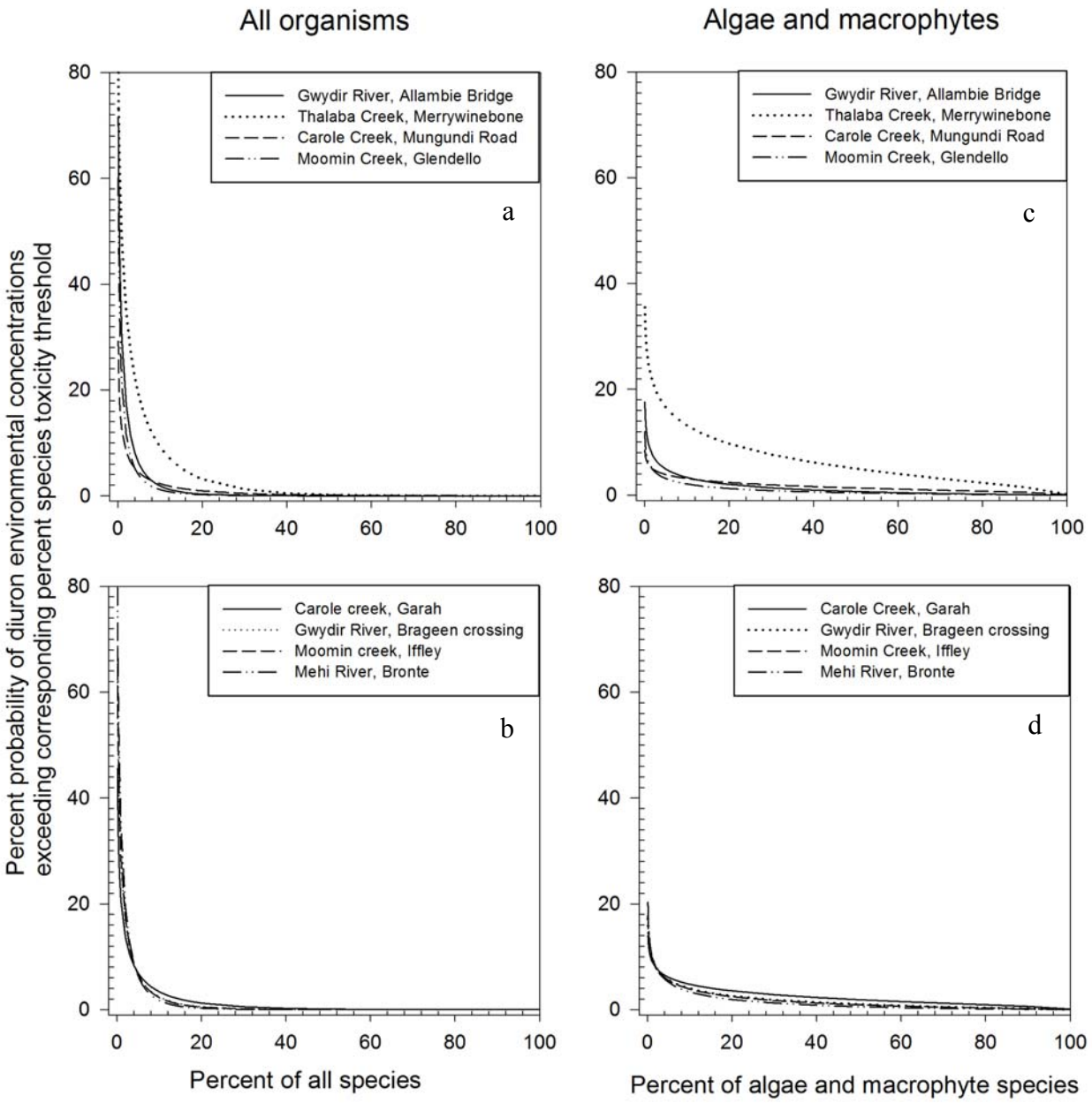



**Figure 2.14** Joint probability curves depicting the probability that diuron concentrations are exceeding toxicity thresholds of percent species of (a, b) all; and (c, d) algae and macrophyte taxonomic groups at the monitoring sites (a, c) Gwydir River, Allambie bridge; Thalaba Creek, Merrywinebone; Carole Creek, Mungindi road; and Moomin Creek, Glendello; and (b, d) Carole Creek, Garah; Gwydir River, Brageen crossing; Moomin Creek, Iffley; and Mehi River, Bronte, in the Gwydir River catchment.

*Risk estimation of prometryn in the Gwydir River catchment*

By comparing the distributions of exposure with species sensitivity (Appendix 2) the probability that the prometryn concentrations at each monitoring site in the Gwydir River catchment was exceeding their relevant endpoint toxicity threshold for algae and macrophytes; fish, amphibians and invertebrate; and all taxonomic groups are presented in Table 2.11. The site found to pose the highest exposure *Risk* to the most sensitive taxonomic group of algae and macrophytes was Thalaba Creek, Merrywinebone, exceeding the $HC_{10}$ toxicity threshold 2.41% of the time (Table 2.11). All other sites showed either no *Risk* or the toxicity threshold was protected 95% of the time. Similar to diuron, the estimated *Risk* between the sites (Figure 2.15) showed that the prometryn exposure posed to the macrophyte and algae taxonomic group was concentrated at the western (downstream) end of the catchment, where much of the crop production occurs (see land use map Figure 2.4; Section 2.2). Therefore, the minor level of *Risk* estimated to be occurring at the monitoring sites of the Gwydir River catchment is directly attributed to the adjacent intensive cropping land uses which these streams drain.

**Table 2.11** Estimates of the risk that prometryn concentrations at the monitoring sites of the Gwydir River catchment are exceeding the $HC_5$ and $HC_{10}$ toxicity thresholds for the algae and macrophytes; fish, amphibians and invertebrates; and all taxonomic groups.

| | Probability (%) of prometryn exposure exceeding the toxicity threshold by taxonomic group | | |
|---|---|---|---|
| Site | Algae and macrophytes | Fish, amphibians and invertebrates | All taxonomic groups |
| Thalaba Creek, Merrywinebone[a] | 2.41 | 0.00 | 1.91 |
| Carole Creek, Garah[a] | 1.92 | 0.50 | 1.85 |
| Carole Creek, Mungindi road[a] | 1.28 | 0.03 | 1.16 |
| Moomin Creek, Glendello[a] | 0.63 | 0.00 | 0.51 |
| Moomin Creek, Iffley[a] | 0.48 | 0.00 | 0.38 |
| Gwydir River, Brageen Crossing[b] | 0.87 | 0.00 | 1.31 |
| Mehi River, Bronte[a] | 0.15 | 0.00 | 0.11 |
| Gwydir River, Allambie bridge[b] | 0.03 | 0.00 | 0.06 |
| Warialda Creek, Warialda No. 3[a] | 0.01 | 0.00 | 0.00 |
| Horton River, Rider (Killara) [a] | 0.00 | 0.00 | 0.00 |
| Gwydir River, Gravesend road bridge[b] | 0.00 | 0.00 | 0.00 |
| Gwydir River, Yarraman bridge[b] | 0.00 | 0.00 | 0.00 |
| Mehi River, Moree[a] | 0.00 | 0.00 | 0.00 |

[a] $HC_{10}$ toxicity threshold using in risk estimation
[b] $HC_5$ toxicity threshold used in risk estimation

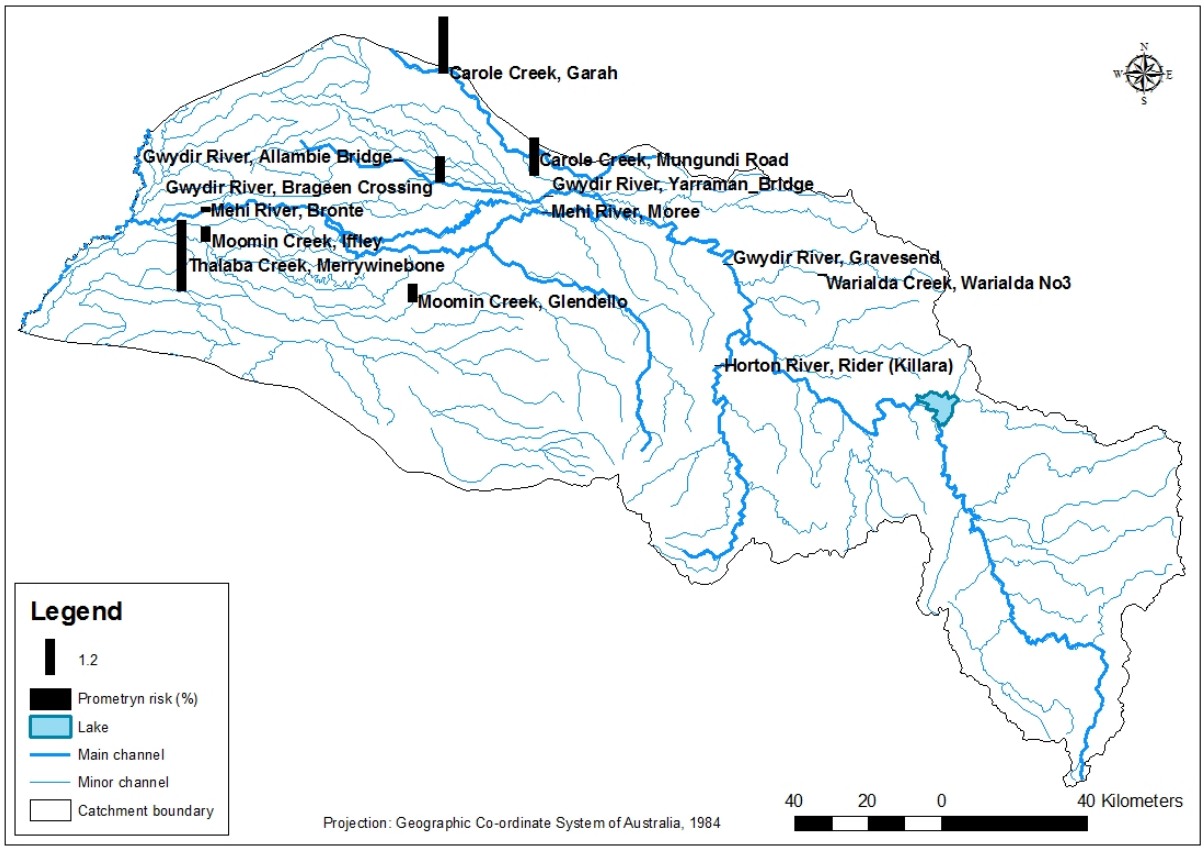



**Figure 2.15** Map of displaying the level of estimated prometryn risk, represented by the height of the black bars, at the monitoring sites of the Gwydir River catchment.

Although exposure risk from prometryn was shown to protect the species toxicity threshold 95% of the time, the *Risk* between monitoring years was determined to be variable and in some cases exceeding this protection threshold for algae and macrophytes (Figure 2.16). The highest prometryn exposure *Risks* by monitoring year was determined to occur at Thalaba Creek, Merrywinebone in 1992 (16.63%); Carole Creek, Garah in 2004 (8.31%); Moomin Creek, Glendello in 2004 (6.90%); Gwydir River, Brageen crossing in 1994 (6.89%); Carole creek, Mungindi road in 1998 (5.42%); Moomin creek, Iffley in 1991 (5.63%).

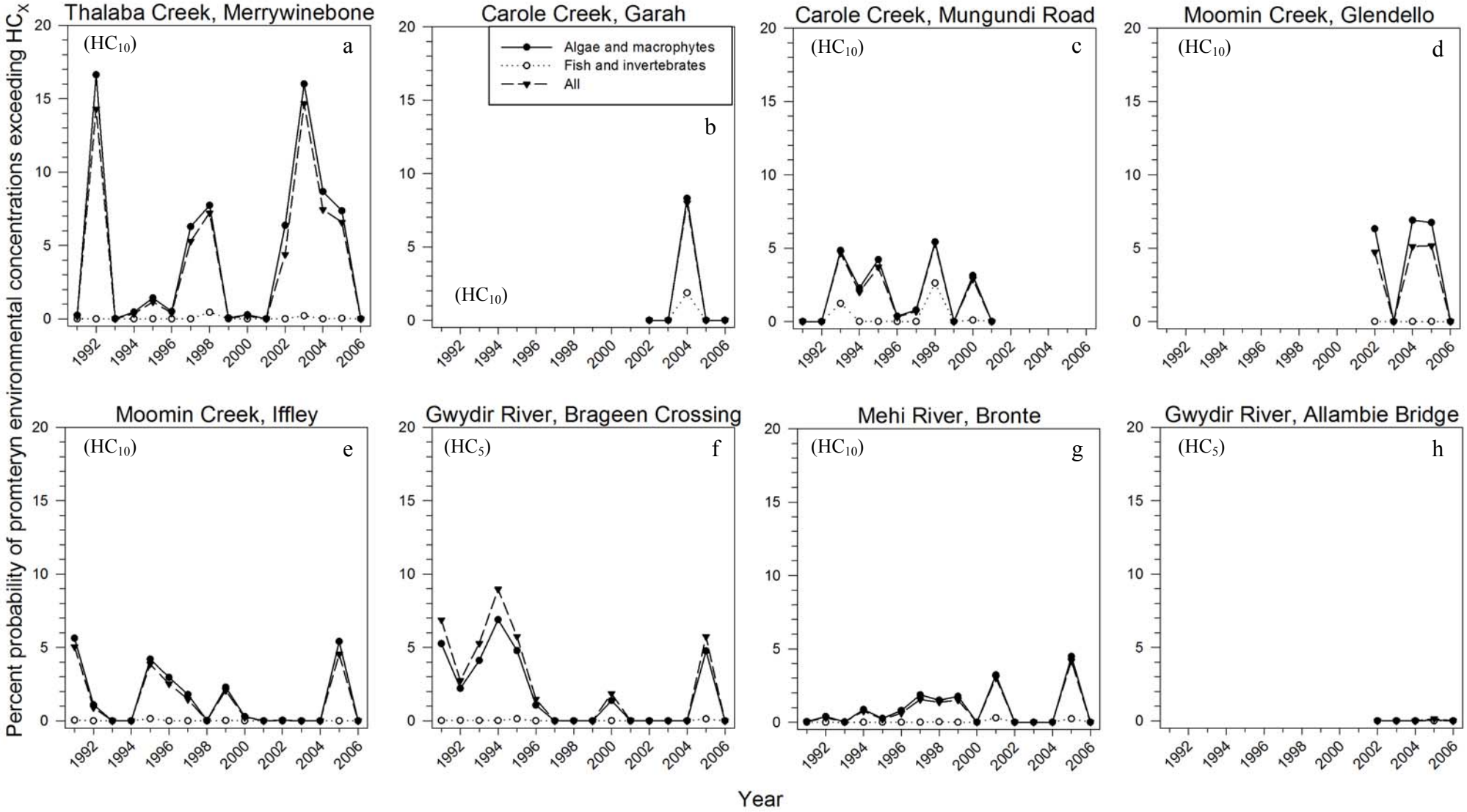



**Figure 2.16** Annual risk of prometryn exposure at (a) Thalaba creek, Merrywinebone (1991-2006); (b) Carole Creek, Garah (2002-2006); (c) Carole Creek, Mungindi Road (1991-2001); (d) Moomin Creek, Glendello (2002-2006); (e) Moomin Creek, Iffley (1991-2006); (f) Gwydir River, Brageen Crossing (1991-2006); (g) Mehi River, Bronte (1991-2006); and (h) Gwydir River, Allambie Bridge (2002-2006). Symbols in brackets indicate the toxicity threshold used to calculate risk, with respect to assessment endpoints.

Joint probability curves showing prometryn *Risk* toward all species combined; and algae and macrophytes taxonomic groups are shown in Figures 2.17 a-d. All sites including Thalaba creek, Merrywinebone; Carole Creek, Garah; Gwydir River, Brageen Crossing; Moomin Creek, Iffley; Mehi River, Bronte; Gwydir River, Allambie Bridge; Carole Creek, Mungindi Road; and Moomin Creek, Glendello were determined to exhibit higher probabilities of exceeding lower prometryn toxicity thresholds, declining almost exponentially with increasing percent species toxicity thresholds (Figures 2.17 a-d). When compared to other sites, Thalaba Creek, Merrywinebone displayed slightly higher probabilities of exceedence for higher prometryn toxicity thresholds confirming its characteristically higher level of risk (Table 2.11). However, in all cases the probability of events affecting higher percent species toxicity thresholds was considered low (Figures 2.17 a-d).

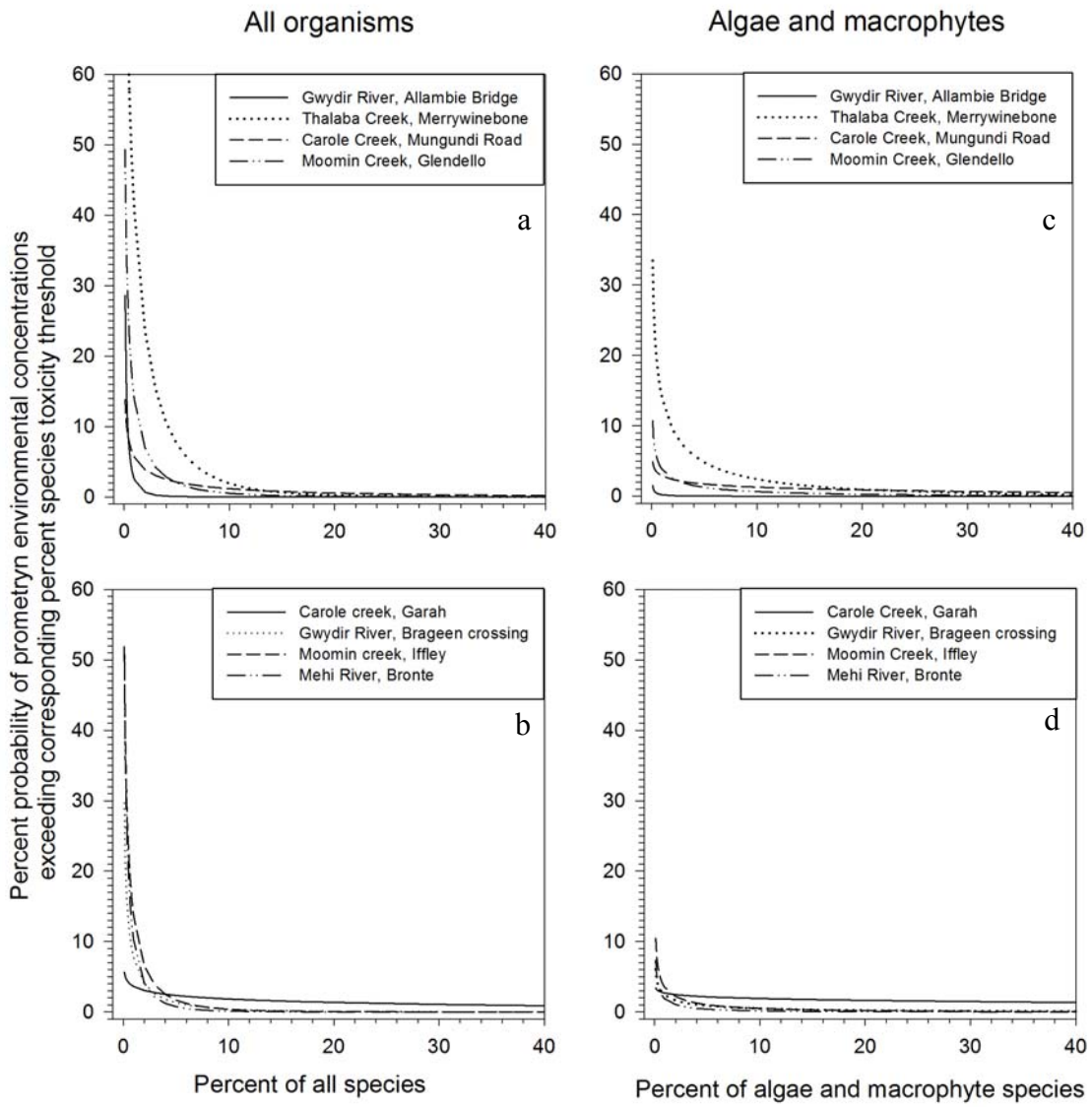



**Figure 2.17** Joint probability curves depicting the probability that prometryn concentrations are exceeding toxicity thresholds of percent species of (a, b) all; and (c, d) algae and macrophyte taxonomic groups at the monitoring sites (a, c) Gwydir River, Allambie bridge; Thalaba Creek, Merrywinebone; Carole Creek, Mungindi road; and Moomin Creek, Glendello; and (b, d) Carole Creek, Garah; Gwydir River, Brageen crossing; Moomin Creek, Iffley; and Mehi River, Bronte, in the Gwydir River catchment.

*Risk estimation of endosulfan in the Gwydir River catchment*

Using the same approach as diuron and prometryn, the distributions of exposure with species sensitivity were compared to evaluate technical endosulfan, endosulfan sulphate and total endosulfan exposure *Risk* in the Gwydir River catchment (Appendix 2). This estimation was made for the relevant toxicity threshold for invertebrates; fish and amphibians; and all taxonomic groups, which the results are given in Table 2.16. The *Risks* posed by technical endosulfan and endosulfan sulphate were not high, as 95% of the time the relevant toxicity thresholds were protected. However, combining the concentrations of technical endosulfan and endosulfan sulphate in formulating risk for total endosulfan yielded the highest exposure risk in the Gwydir River catchment. Two sites had significant total endosulfan exposure risk toward all taxonomic groups, these were Gwydir River, Brageen crossing (10.19%; Table 2.12) and Thalaba Creek, Merrywinebone (8.77%; Table 2.12). Similar to diuron and prometryn, the highest endosulfan exposure risks were found to occur in the western end of the catchment (Figure 2.18), confirming its likely source from agricultural production.

**Table 2.12** Estimates of the risk that technical endosulfan (α- + β-endosulfan), endosulfan sulphate and total endosulfan concentrations at the monitoring sites of the Gwydir River catchment are exceeding the $HC_5$ and $HC_{10}$ toxicity thresholds for the algae and macrophytes; fish, amphibians and invertebrates; and all taxonomic groups.

| | | Probability (%) of endosulfan exposure exceeding the toxicity threshold by taxonomic group | | |
|---|---|---|---|---|
| Site name | Isomer or degradate | Invertebrates | Fish and amphibians | All |
| Gwydir River, Brageen Crossing[a] | Technical endosulfan | 1.59 | 1.02 | 1.84 |
| | Endosulfan sulphate | 3.45 | 1.62 | 1.85 |
| | Total endosulfan | 8.95 | 6.01 | 10.19 |
| Thalaba Creek, Merrywinebone[b] | Technical endosulfan | 1.06 | 1.23 | 1.30 |
| | Endosulfan sulphate | 3.91 | 1.12 | 1.43 |
| | Total endosulfan | 5.87 | 7.86 | 8.77 |
| Carole Creek, Mungindi road[b] | Technical endosulfan | 0.90 | 1.17 | 1.29 |
| | Endosulfan sulphate | 1.36 | 0.39 | 0.50 |
| | Total endosulfan | 2.63 | 3.54 | 3.96 |
| Moomin Creek, Iffley[b] | Technical endosulfan | 0.43 | 0.52 | 0.56 |
| | Endosulfan sulphate | 1.37 | 0.32 | 0.42 |
| | Total endosulfan | 2.22 | 3.16 | 3.61 |
| Gwydir River, Allambie bridge[a] | Technical endosulfan | 0.00 | 0.00 | 0.00 |
| | Endosulfan sulphate | 0.76 | 0.49 | 0.53 |
| | Total endosulfan | 1.24 | 0.94 | 1.37 |
| Mehi River, Bronte[b] | Technical endosulfan | 0.12 | 0.17 | 0.19 |
| | Endosulfan sulphate | 0.33 | 0.04 | 0.06 |
| | Total endosulfan | 0.72 | 1.20 | 1.45 |
| Moomin Creek, Glendello[b] | Technical endosulfan | 0.00 | 0.00 | 0.00 |
| | Endosulfan sulphate | 0.57 | 0.31 | 0.35 |
| | Total endosulfan | 0.66 | 0.78 | 0.84 |
| Carole Creek, Garah[b] | Technical endosulfan | 0.00 | 0.00 | 0.00 |
| | Endosulfan sulphate | 0.01 | 0.00 | 0.00 |
| | Total endosulfan | 0.02 | 0.03 | 0.04 |
| Gwydir River, Gravesend road bridge[a] | Technical endosulfan | 0.09 | 0.06 | 0.11 |
| | Endosulfan sulphate | 0.01 | 0.00 | 0.00 |
| | Total endosulfan | 0.05 | 0.03 | 0.06 |
| Warialda Creek, Warialda No. 3[b] | Technical endosulfan | 0.00 | 0.00 | 0.00 |
| | Endosulfan sulphate | 0.00 | 0.00 | 0.00 |
| | Total endosulfan | 0.00 | 0.00 | 0.00 |
| Horton River, Rider (Killara)[b] | Technical endosulfan | 0.00 | 0.00 | 0.00 |
| | Endosulfan sulphate | 0.00 | 0.00 | 0.00 |
| | Total endosulfan | 0.00 | 0.00 | 0.00 |
| Gwydir River, Yarraman bridge[a] | Technical endosulfan | 0.00 | 0.00 | 0.00 |
| | Endosulfan sulphate | 0.00 | 0.00 | 0.00 |
| | Total endosulfan | 0.00 | 0.00 | 0.00 |
| Mehi River, Moree[b] | Technical endosulfan | 0.00 | 0.00 | 0.00 |
| | Endosulfan sulphate | 0.00 | 0.00 | 0.00 |
| | Total endosulfan | 0.00 | 0.00 | 0.00 |

[a] $HC_5$ toxicity threshold used in risk estimation
[b] $HC_{10}$ toxicity threshold using in risk estimation

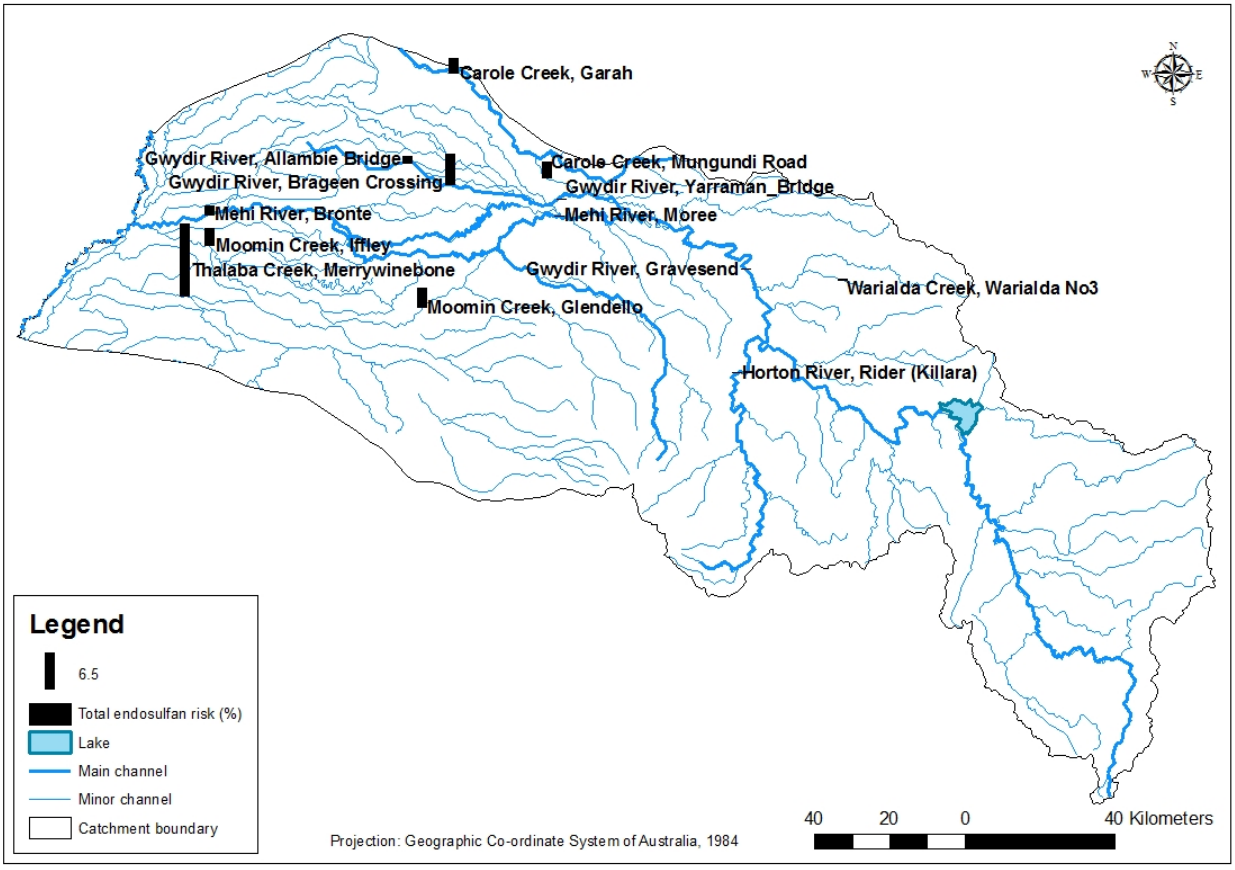



**Figure 2.21** Map of displaying the level of estimated total endosulfan risk, represented by the height of the black bars, at the monitoring sites of the Gwydir River catchment.

Although the majority of sites did not show risk greater than 5% of the time from technical endosulfan, endosulfan sulphate, and total endosulfan in the Gwydir River catchment, the annual risk showed the level of risk to be variable between monitoring years (Figure 2.19-21). At some sites, the exposure risk exceeded their respective toxicity thresholds for more than 5% of the time for invertebrates; fish and amphibians; and all taxonomic groups. The highest significant endosulfan exposure risks by monitoring year was determined to occur at Thalaba Creek, Merrywinebone in 1992 (12.17% in 1991, 26.80% in 1992, and 45.71% in 1992, for technical endosulfan, endosulfan sulphate and all taxonomic groups respectively[15]); followed by Gwydir River, Brageen crossing in 1994 (12.88% in 1995, 16.89% in 1994, and 33.92% in 1992); Moomin creek, Iffley (5.81% in 1996, 12.26% in 1991, and 18.52% in 1991); Carole creek, Mungindi road (7.09% in 1993, 8.30% in 1995, and 17.83% in 1995);

[15] The maximum significant annual risk posed by technical endosulfan, endosulfan sulphate and total endosulfan are given for the remaining sites in the same order.

Mehi River, Bronte (NS[16], NS, and 14.19% in 1991); and Gwydir River, Allambie bridge (NS, NS, and 5.92% in 2004), as shown in Figures 22-24[17]. Variability between the years is likely to be an outcome of variations in endosulfan use in response to insect pressure and the prevalence of environmental fate processes, described in Chapter 1, operating to move the chemical away from the site of application (Wauchope, 1978). However, the prevalence of exceeding the 5% recurrence threshold declined between 1998 and the end of the sampling period, especially at Gwydir River, Brageen crossing; Moomin creek, Iffley; and Gwydir River, Brageen crossing. This is likely due to a transition in the cotton industry to adopting insect resistant transgenic cotton varieties and the implementation of BMP (Fitt, 2000; Crossan et al., 2007).

[16] NS refers to no significant risk level determined between monitoring years for that compound.

[17] Figures referred to in order of Technical endosulfan, endosulfan sulphate and total endosulfan.

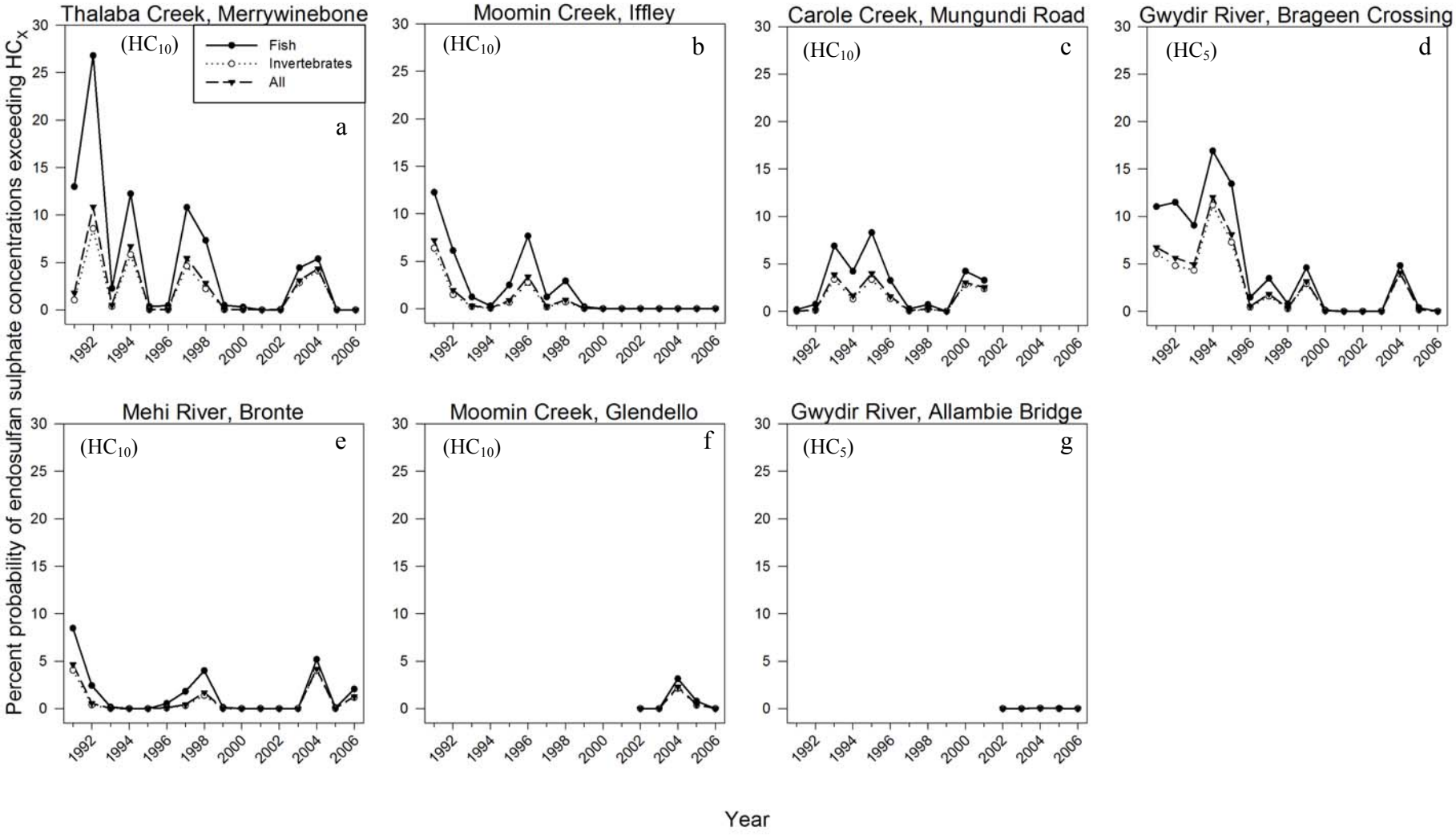



**Figure 2.19** Annual risk of endosulfan sulphate exposure at (a) Thalaba creek, Merrywinebone (1991-2006); (b) Moomin Creek, Iffley (1991-2006); (c) Carole Creek, Mungindi Road (1991-2001); (d) Gwydir River, Brageen Crossing (1991-2006); (e) Mehi River, Bronte (1991-2006); (f) Moomin Creek, Glendello (2002-2006); and (g) Gwydir River, Allambie Bridge (2002-2006). Symbols in brackets indicate the toxicity threshold used to calculate risk, with respect to assessment endpoints.

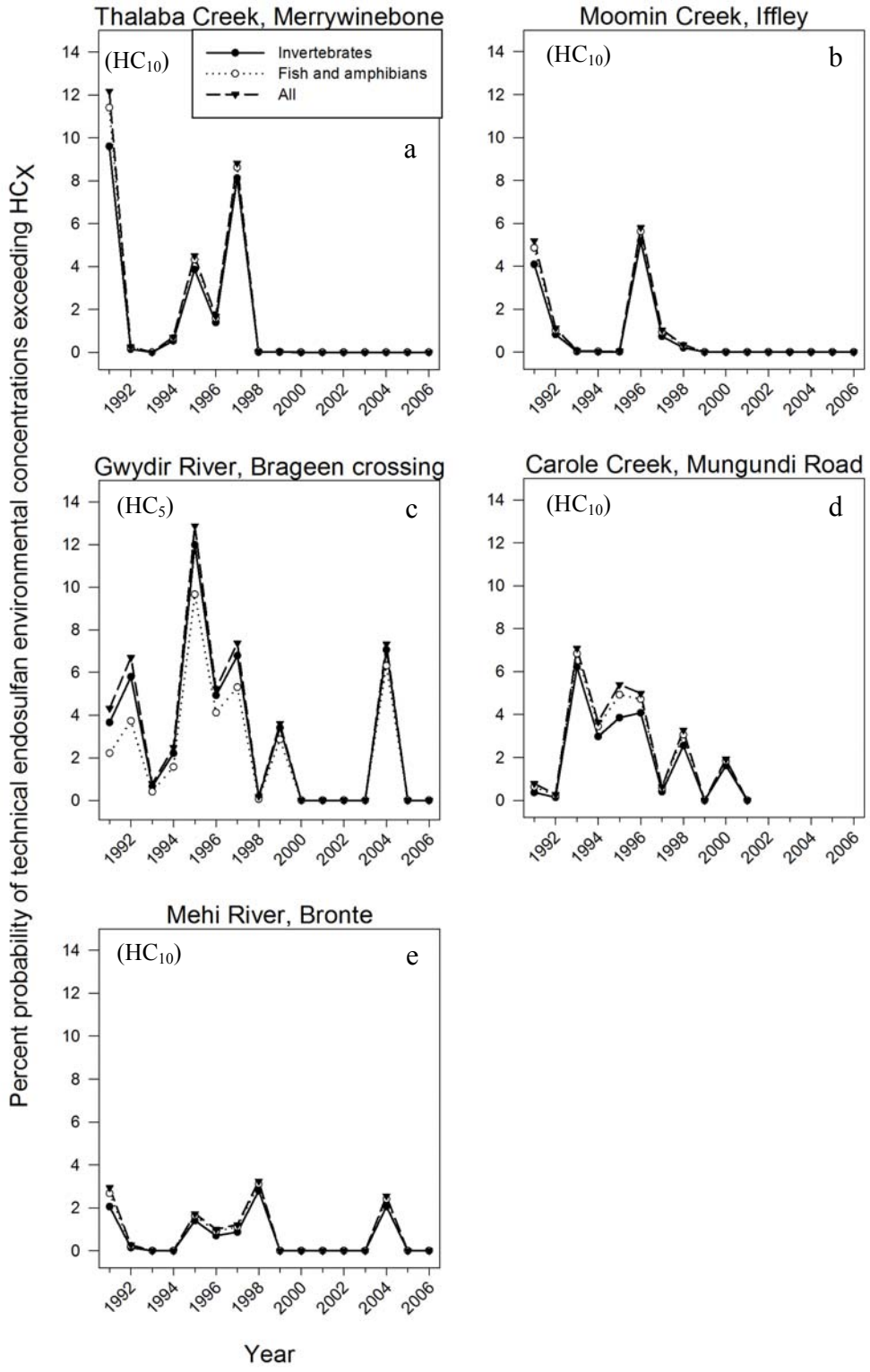
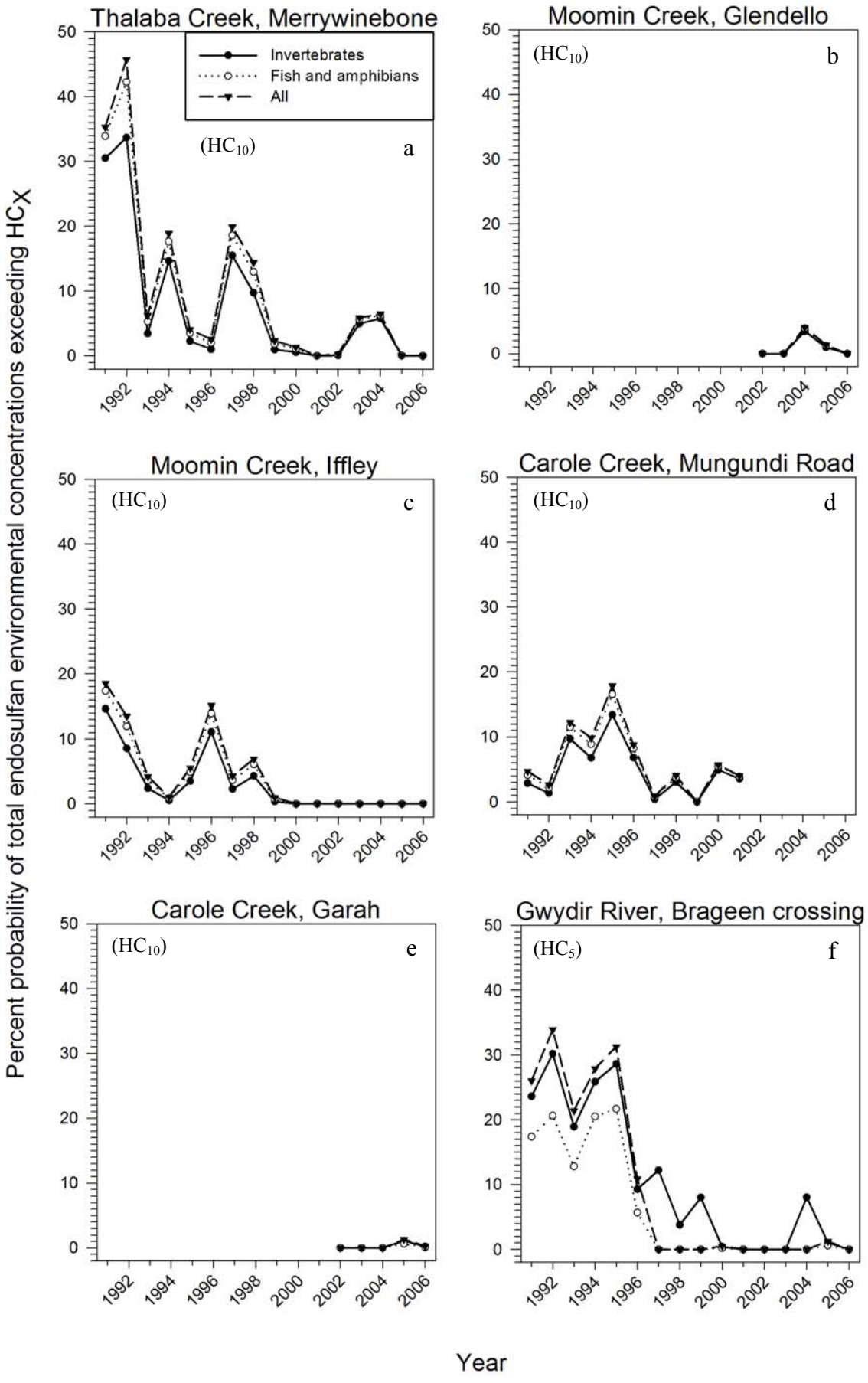



**Figure 2.20** Annual risk of technical endosulfan exposure at (a) Thalaba creek, Merrywinebone (1991-2006); (b) Moomin Creek, Iffley (1991-2006); (c) Gwydir River, Brageen Crossing (1991-2006); (d) Carole Creek, Mungindi Road (1991-2001); and (e) Mehi River, Bronte (1991-2006). Symbols in brackets indicate the toxicity threshold used to calculate risk, with respect to assessment endpoints.


Thalaba Creek, Merrywinebone
Invertebrates
Fish and amphibians
All
(HC10)
a
Moomin Creek, Glendello
(HC10)
b
Moomin Creek, Iffley
(HC10)
c
Carole Creek, Mungundi Road
(HC10)
d
Carole Creek, Garah
(HC10)
e
Gwydir River, Brageen crossing
(HC5)
f
Percent probability of total endosulfan environmental concentrations exceeding HCX
Year

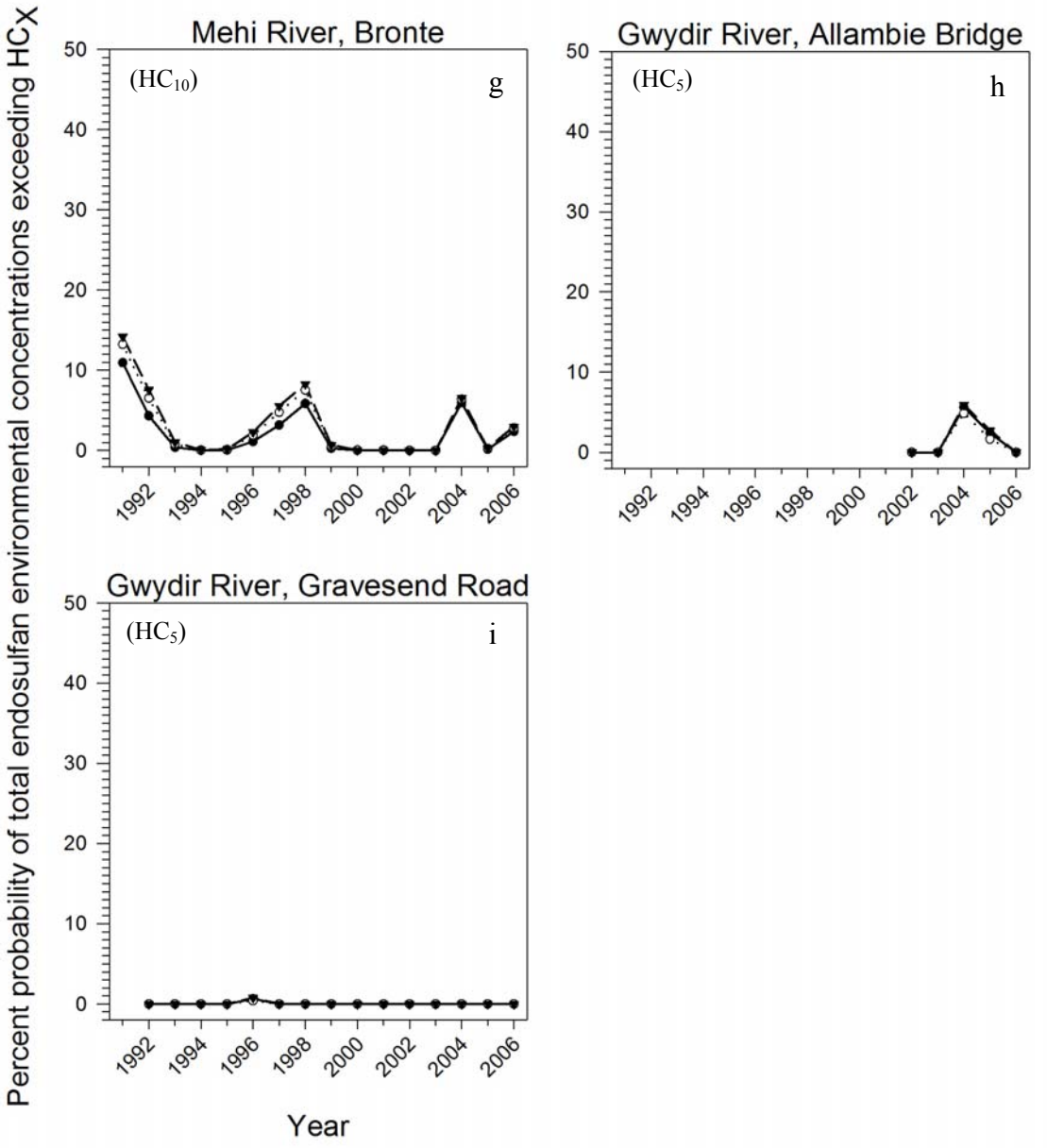



**Figure 2.21** Annual risk of total endosulfan exposure at (a) Thalaba creek, Merrywinebone (1991-2006); (b) Moomin Creek, Glendello (2002-2006); (c) Moomin Creek, Iffley (1991-2006); (d) Carole Creek, Mungindi Road (1991-2001); (e) Carole Creek, Garah (2002-2006); (f) Gwydir River, Brageen Crossing (1991-2006); (g) Mehi River, Bronte (1991-2006); and (h) Gwydir River, Allambie Bridge (2002-2006); and (i) Gwydir River, Gravesend Road (1991-2006). Symbols in brackets indicate the toxicity threshold used to calculate risk, with respect to assessment endpoints.

The joint probability curves for technical endosulfan, endosulfan sulphate and total endosulfan were developed for each site that displayed some level of risk (Figures 2.22-24 a-d). The joint probability curves characterising the level of risk posed to all taxonomic groups and fish demonstrates that with increasing percent species toxicity threshold, the risk of exceedance declined exponentially. That is, the level of risk at each site was highest for lower percent species toxicity thresholds. However, Thalaba creek, Merrywinebone was estimated to have a higher range of species exceedance, confirming its characteristically higher endosulfan sulphate and total endosulfan risk, relative to other monitoring sites of the Gwydir River catchment (Figures 2.22-24 a and c). In the case of fish, the probability of exceeding the majority of percent species toxicity thresholds was low (Figures 2.22-24 c and d).

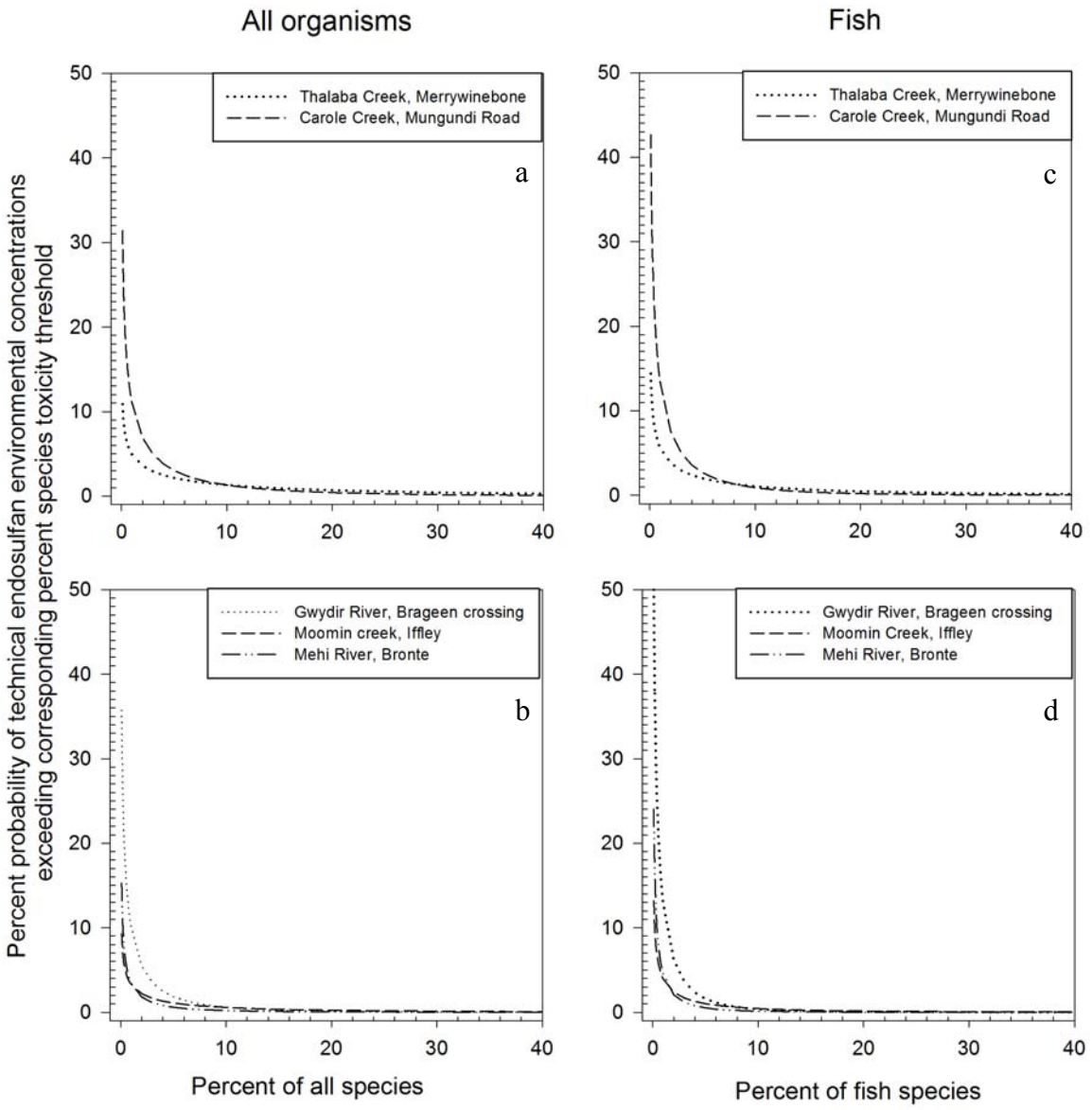



**Figure 2.25** Joint probability curves depicting the probability that technical endosulfan concentrations are exceeding toxicity thresholds of percent species of (a, b) all; and (c, d) fish taxonomic groups at the monitoring sites (a, c) Thalaba Creek, Merrywinebone; and Carole Creek, Mungindi road; and (b, d) Carole Creek, Garah; Gwydir River, Brageen crossing; Moomin Creek, Iffley; and Mehi River, Bronte, in the Gwydir River catchment. Symbols in brackets indicate the toxicity threshold used to calculate risk, with respect to assessment endpoints.

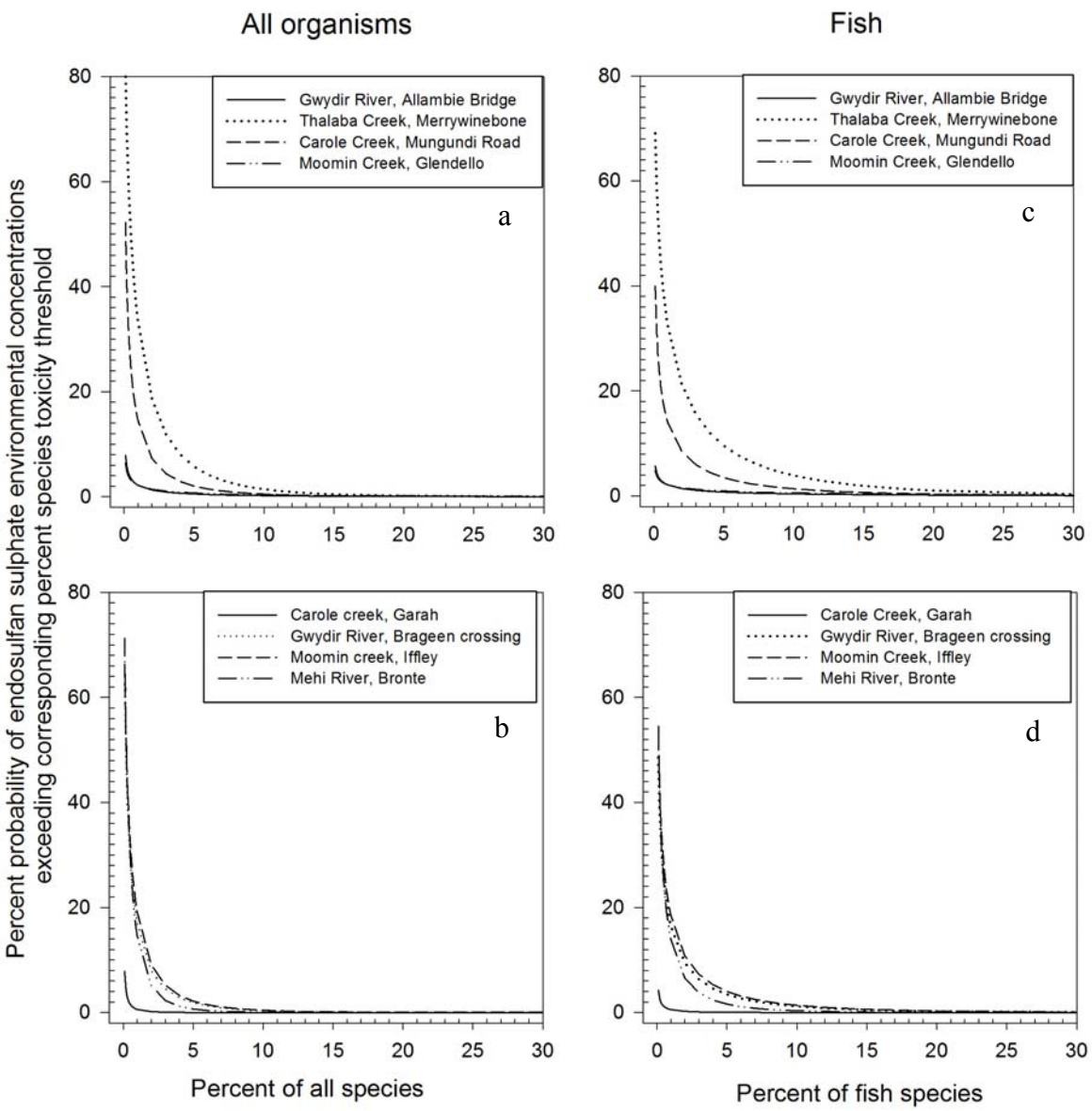



**Figure 2.26** Joint probability curves depicting the probability that endosulfan sulphate concentrations are exceeding toxicity thresholds of percent species of (a, b) all; and (c, d) fish taxonomic groups at the monitoring sites (a, c) Thalaba Creek, Merrywinebone; and Carole Creek, Mungindi road; and (b, d) Carole Creek, Garah; Gwydir River, Brageen crossing; Moomin Creek, Iffley; and Mehi River, Bronte, in the Gwydir River catchment.

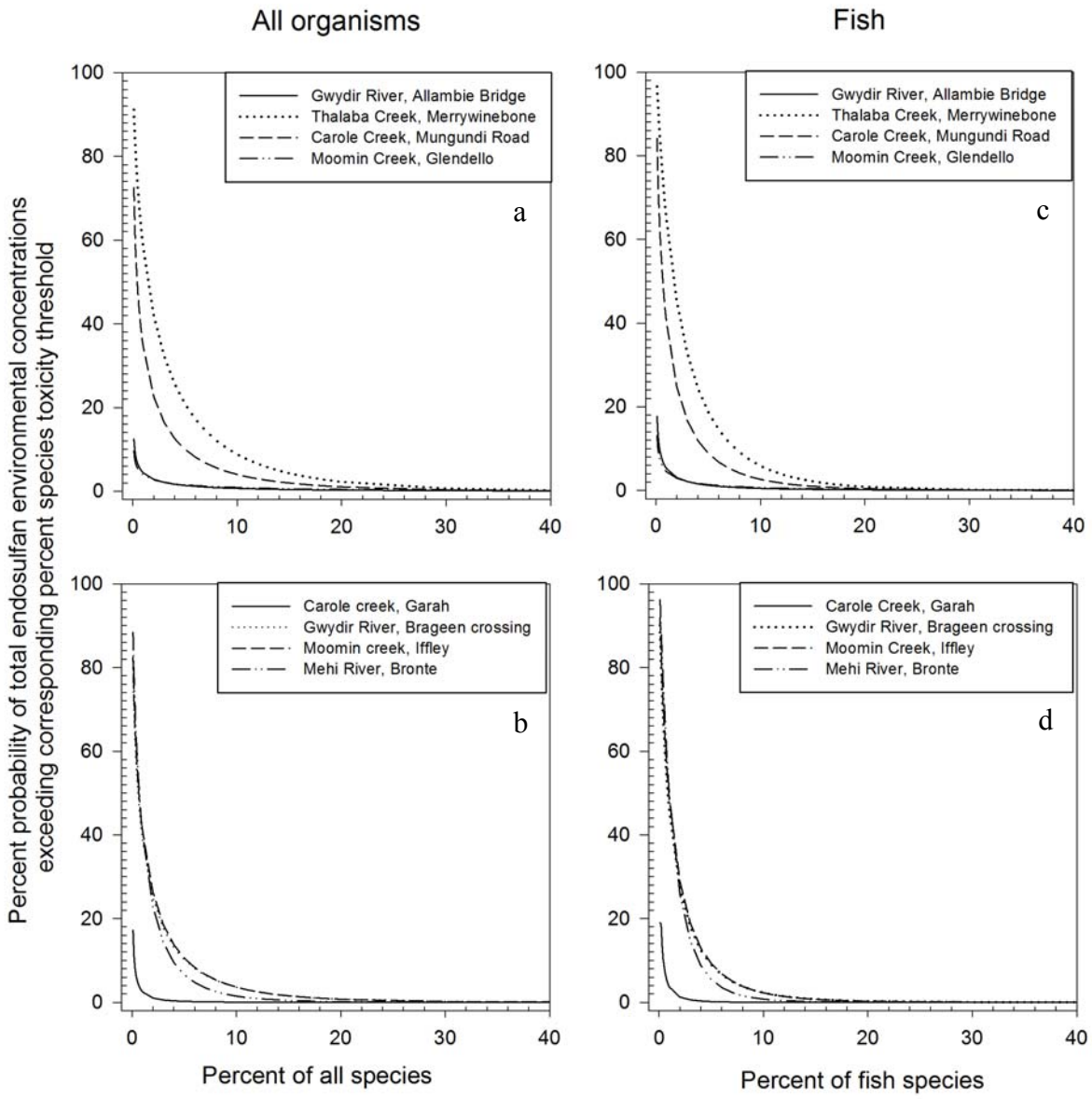



**Figure 2.27** Joint probability curves depicting the probability that total endosulfan concentrations are exceeding toxicity thresholds of percent species of (a, b) all; and (c, d) fish taxonomic groups at the monitoring sites (a, c) Thalaba Creek, Merrywinebone; and Carole Creek, Mungindi road; and (b, d) Carole Creek, Garah; Gwydir River, Brageen crossing; Moomin Creek, Iffley; and Mehi River, Bronte, in the Gwydir River catchment.

## 2.6 UNCERTAINTY

This ERA of diuron, promteryn and endsulfan is the most comprehensive to date for the Gwydir River catchment. However, this has not proceeded without uncertainty. Uncertainty analysis identifies and quantifies, where possible, the uncertainty encountered in the phases of problem formulation, analysis and risk characterisation. It is anticipated that by identifying uncertainties in this assessment, they may be reduced to minimise uncertainties in the future. Solomon *et al*. (1996) described uncertainties in ERA originate from three sources:

1. Incomplete knowledge of components that should be known;
2. Systematic errors that can include computational, analytical, or data transformation errors; and
3. Non-systematic errors that include, random or stochastic errors and variability that comes from the system being assessed.

This section characterises the uncertainties identified for the Gwydir River catchment risk assessment of diuron, prometryn and endosulfan exposure.

### *2.6.1 Uncertainty in exposure assessment*

*Systematic bias associated with sampling frequency*

The sampling regime used by the NSW department of water and energy was to evaluate pesticide concentrations observed in the reaches of the Gwydir River catchment during times of high chemical use (Muschal and Warne, 2003). This sampling was especially targeted at evaluating the chemical exposure occurring during the cotton growing season, the period which the greater sampling prevalence was conducted, and is coincidently the period of higher rainfall. To support a probabilistic approach to risk assessment with the intention of accounting for catchment-scale exposure, this sampling approach gave unequal weighting to the high exposure periods and may have implications for risk characterisation. Furthermore, it was determined that the time accounted for between monitoring dates. A catchment-scale modelling approach would be useful to fill this data gap.

*Error associated with chemical analysis*

Diuron, prometryn and endosulfan were analysed after being extracted from water using a liquid/liquid extraction method (Muschal and Warne, 2003). As the original documentation from the NSW Department of Water and Energy detailing the extraction and analysis protocol is not readily available, it is unclear to what extent chemical loss or concentrating of

the chemical may be occurring prior to analysis. This may increase or decrease the level of exposure occurring at the monitoring sites, enhancing the uncertainty in the risk characterisation. Without access to the methodology, or ability to reanalyse samples if an error was found, then the data would have to be assumed to be free from significant systematic error. This outcome is contrary to the uncertainty analysis standard set by Solomon *et al*. (1996) who were able to demonstrate the level of uncertainty in atrazine exposure based on a detailed sampling protocol. Since this ERA involved comparing exposure with a stringent endpoint toxicity threshold emphasises the importance of being able to characterise the uncertainty during sample analysis.

*Uncertainty in exposure sources*

As this study was able to comprehensively evaluate exposure risk in the various reaches of the Gwydir River catchment, areas in the sub-catchments contributing to the level of risk could not be confirmed. For the purposes of risk management, this uncertainty has significant implications for catchment managers needing implement management strategies in sub-catchments. To overcome this deficiency, a spatial exposure modelling exercise may be able to evaluate chemical loading at the scale useful for catchment-managers to implement management strategies. This is conducted in Chapter 4.

### *2.6.2 Uncertainty in ecological effects data*

Relevant ecotoxicity data was used to characterise the ecological relevance of diuron, prometryn and endosulfan exposures occurring in the Gwydir River catchment. Ecotoxicity datasets consisted solely of laboratory-based studies and were synthesised in to an SSD. Organisms being grouped according to their taxonomic groups allowed for trophic level evaluation, with particular emphasis on the more sensitive groups used to characterise exposure risk. This approach was expected to minimise the uncertainty in ecological effects characterisation and provide greater certainty to the range of possible ecological responses through characterising the impact posed to keystone species.

Specifically, trophic level effects were possibly occurring under extreme diuron exposure events in Thalaba Creek, Merrywinebone; and Carole Creek, Mungindi road. Such events were found to not be consistently occurring, as the joint probability curves suggested. However, such one-off events may be important, especially if the effects were determined to

translate to affecting higher trophic groups, as hypothesised through evaluation of the trophic interaction work of Kelleway *et al*. (2010). Subsequently, determining whether extreme exposure events in the reaches were lasting long enough to elicit the effect predicted by the SSD is important for confirming such a response, as is its potential reversibility (Handy, 1994; Reinert *et al.*, 2002; Cedergreen *et al.*, 2004; Cedergreen *et al.*, 2005). That is, given the toxicity endpoints for plant and algae were reduction in population growth and biomass production by 50%, not death, highlights an uncertainty if such an effect endpoint is permanent when the event passes. For example, photosynthetic organisms exposed to diuron have displayed rapid recovery in photosynthesis for coral (Negri *et al.*, 2005), tropical sea grass species (Haynes *et al.*, 2000), following a short exposure pulse. Such an interaction is likely to translate in to population and biomass recovery, with limited effect on higher trophic organisms. However, the effect from endosulfan on fish is more certain, given its highly acute toxic mode of action (Chapter 1). Further quantitation through simulated pulse exposure toxicity studies unique to the Gwydir River catchment is required for diruon, as is a characterisation of the range of possible pulse exposure durations that could be achieved through spatial exposure modelling.

Finally, this study did not account for ecosystem responses observed in cosm studies (e.g. Hose and Van den Brink, 2004; Tlili *et al.*, 2008). Such studies are deemed important for characterising the uncertainty in the effect that diuron, prometryn and endosulfan impose on changes in ecosystem trophic interactions. Although important, cosm studies require specialist equipment and resources not readily available in this study. Such ecotoxicity studies should be included in the future to gain an understanding to the ecosystem effects if there are sufficient concerns regarding the ecological behaviour of the target, in a manner similar to Solomon *et al*. (1996).

### *2.6.3 Uncertainty in risk characterisation*

Using probabilistic distributions to characterise the full range of pesticide exposure scenarios likely to be observed in catchments is considered the most robust approach to support estimation of risk (Solomon *et al.*, 1996; Solomon *et al.*, 2000; Tannenbaum *et al.*, 2003). Such a distribution requires a randomised sampling regime. However, as the sampling strategy in the Gwydir River catchment was biased for high chemical application periods, such an approach was likely to influence the true exposure scenarios represented by the

distribution. Contrary to the thoughts of Muschal and Warne (2003), such a sampling regime would likely elevate the probability of exposure exceeding the toxicity thresholds limits, in some cases exceeding the 95% regulatory ecosystem protection thresholds. This is only further conflicted by the fact that the sampling data used in this ERA only accounted for between 2.9 and 6.4% of the time across all monitoring sites, from the beginning date to the end date of the sampling exercise (Appendix 2). Although it may be that most of the ecological impact will happen around these times and could therefore remain the focus, in such cases, characterisation of exposure pulses is necessary to provide greater certainty in the required dose to elicit ecological effect. Further, the time between pulses where species population may have periods of reprieve are also important, if such sampling approaches were used in the future.

## 2.7 CONCLUSIONS

The different phases of problem formulation, analysis and characterisation were used effectively to evaluate the ecological concerns that the pesticides diuron, prometryn and endosulfan may be posing toward the most vulnerable non-target organisms in the reaches of the Gwydir river catchment. The problem formulation phase of this ERA was able to characterise relevant properties of diuron, prometryn and endosulfan that are likely to influence their fate in the Gwydir River catchment. Comparing label and pest management guide application information with spatial land use information the uses these compounds in the Gwydir River catchment was considered to pose a hazard to aquatic organisms. Assessment endpoints were devised based on readily available water quality guidelines to set the thresholds to determine exposure risk significance for two distinct ecoregions, the Gwydir wetlands and all other ecosystems, each requiring different levels of protection. This phase set the scene for the ensuing analysis and risk characterisation phases.

The analysis phase presented an exposure and ecotoxicity assessment of diuron, prometryn and endosulfan. A database consisting of extensive pesticide monitoring data at different locations of the catchment was made available. Limited ecotoxicity data was available for organisms native to the Gwydir River catchment; however ecotoxicity data was collated from a readily accessible database. Using these datasets, exposure and species sensitivity distributions were developed to respectively characterise the full range of exposures occurring in the Gwydir River catchment, and ecotoxic responses to diuron, prometryn and

endosulfan. Toxicity thresholds were devised from the SSDs from which to base exposure significance.

By comparing probabilistic distributions of species sensitivity with exposure and evaluating the overlap at the prescribed toxicity thresholds, the significance of the diuron, prometryn and endosulfan exposure occurring in the sub-catchments of the Gwydir River catchment was estimated. Using all available data, no significant prometryn exposure concerns were identified. However, the monitoring sub-catchments of Thalaba Creek, Merrywinebone; and Gwydir River, Brageen Crossing were found to exhibit significant diuron and endosulfan exposure risk; and Gwydir River, Allambie Bridge was found to have limited diuron exposure risk.

Importantly, the sites displaying any level of exposure risk were concentrated at the western end of the Gwydir River catchment where most of the crop production occurred. It was therefore concluded that the level of risk posed by diuron, prometryn and endosulfan was directly attributed to upstream land uses occurring in the sub-catchments of the Gwydir River catchment.

However, an uncertainty analysis identified a number of flaws in this risk assessment. The sampling strategy used to characterise the exposure in the reaches of the Gwydir River catchment was described to be biased for periods of a year where pesticides were applied on cotton and for periods of high runoff, elevating the estimated risk and marginalising the relevance of the periods where concentrations are low. The ecotoxicity data used in this assessment was entirely laboratory based and did not consider the changes in ecosystem assemblages commonly observed in cosm studies, or the potential for any of the organisms to recover following a pulsed exposure. Further research was identified to be needed in the areas of:

1. Evaluating permanency of effect in under pulse exposure relevant to the Gywydir River catchment
2. Spatial exposure modelling to characterise exposure pulses and identify areas in sub-catchments contributing pesticide loads to streams.

**CHAPTER 3**

# EXPOSURE AND RECOVERY OF *LEMNA GIBBA* AND *LEMNA MINOR* TO A SEVEN DAY DIURON PULSE

## 3.1 INTRODUCTION

Ecosystems sometimes endure periodic exposures of varying concentrations of contaminants (Reinert *et al.*, 2002; Skark *et al.*, 2004; Suter II, 2007). Probabilistic risk assessments performed in Chapter 2 showed temporal variability in diuron, prometryn and endosulfan exposure that translated into different levels of risk in the sub-catchments of Gwydir River catchment. The frequency distributions of exposure and effect, as detailed earlier are useful for evaluating a set of single events. The toxicity end-points used in the SSDs were developed from standardised laboratory ecotoxicity data that is assumed to translate to a fractional effect on diverse ecosystem species when exposure occurs.

The limitations of these toxicity end-points such as hazard quotients used for single events, or even the series of events as used in the probabilistic characterisation, is that they focus on the proportion of the population that is affected at that point in time (Posthuma *et al.*, 2002a; Posthuma *et al.*, 2002b; Reinert *et al.*, 2002; Cedergreen *et al.*, 2005). It follows that the species populations within an ecosystem that are not killed or irreversibly affected by an exposure event may recover (Handy, 1994; Suter II, 2007). This chapter addresses such possible recovery scenarios by analysing the response of *Lemna gibba* and *Lemna minor* to a seven day pulse exposure of diuron, a scenario observed through observations made for flow data of the Gwydir River catchment.

These two macrophytes, swollen duckweed (*Lemna gibba*) and common duckweed (*Lemna minor*), are sometimes used as reference species to assess the toxicity of compounds (Hanson *et al.*, 2003; Brain *et al.*, 2004a; Brain *et al.*, 2004b). They are small freshwater aquatic monocotyledonous plants, 2-6 mm in diameter, floating or submerged and commonly found in rather lentic eutrophic streams, ditches, ponds and wetlands (Vaughan and Baker, 1994; Lemon *et al.*, 2001). They reproduce almost exclusively by recruitment of asexual propagules that originate from reproductive pockets that radiate from a single node and develop through branching and fragmentation into separate decumbent units called fronds (Lemon and Posluszny, 2000). The daughter fronds detach becoming independent plants with the same form and structure as the mother frond. When compared to *L. minor*, *L. gibba* exhibits characteristically gibbous fronds resulting from its ability to swell from enlargement of air sacs on the underside of the fronds (Pieterse, 1975).

Both these species of duckweed have been tested for diuron toxicity previously (e.g. Teisseire *et al.*, 1999; Teisseire and Vernet, 2000; Okamura *et al.*, 2003b). However, their ability to recover following exposure to diuron has not. To support the ERA presented in Chapter 2, this experimental study attempts to simulate an exposure pulse by investigating the ability of *L. gibba* and *L. minor* to recover following a continuous seven-day exposure to different concentrations of diuron.

## 3.2 MATERIALS

### *3.1.1 Lemna gibba and Lemna minor culture*

Cultures of axenic *Lemna minor L.* and *Lemna gibba L.* were originally obtained from a laboratory colony culture at the University of Waterloo (Waterloo, ON, Canada). In the laboratory they were maintained separately under axenic conditions in 250 mL Pyrex Erlenmeyer flasks containing 100 mL sterile Hoagland's growth media (see later) stoppered with cotton wool wrapped in cheesecloth. The cultures were kept under constant uniform cool fluorescent lights at room temperature (23±2 $^{o}$C) and a selection of five seemingly healthy (exhibiting no chlorosis) plants were transferred aseptically to fresh sterile Hoagland's growth media (100 mL) contained in 250 mL Erlenmeyer flasks every 7-10 days.

### *3.1.2 Chemicals*

The analytical grade chemicals used in this experiment and diuron are listed in Table 3.1.

**Table 3.1** Chemicals used in the pulse exposure experiment.

| Chemical | Company and location |
|---|---|
| $MgSO_4.7H_2O$ | Fisher scientific, USA |
| $KNO_3$ | Mallinckrodt Baker, St Louis, Missouri, USA |
| $Ca(NO_3)_2.4H_2O$ | Mallinckrodt Baker, St Louis, Missouri, USA |
| $KH_2PO_4$ | EMD Merck KGaA, Darmstadt, Germany |
| $H_3BO_3$ | Fisher scientific, Hampshire, New Hampshire, USA |
| $MnCl_2.4H_2O$ | Mallinckrodt Baker, St Louis, Missouri, USA |
| $Na_2MoO_4.2H_2O$ | Mallinckrodt Baker, St Louis, Missouri, USA |
| $ZnSO_4.7H_2O$ | Mallinckrodt Baker, St Louis, Missouri, USA |
| $CuSO_4.5H_2O$ | Fisher scientific, USA |
| EDTA | Fisher scientific, USA |
| $FeCl_3.6H_2O$ | Fisher scientific, USA |
| NaOH | Fisher scientific, USA |
| HCl | Mallinckrodt Baker, St Louis, Missouri, USA |
| Diuron (80% a.i. w/w)[a] | DuPont, Mississauga, ON, Canada |

[a] The acronym a.i. refers to the amount of active ingredient content of the compound.

## 3.3 METHODS

### *3.3.1 Stock solutions*

Stock solutions were prepared to support both the growth media and diuron exposure media used this experiment. A modified version of Hoagland's E+ growth media was used in all experiments. Four stock solutions (Table 3.2) used in its formulation were prepared in distilled water and stored in the refrigerator (4$^{o}$C) for the life of the experiment.

**Table 3.2** Stock solutions used for the preparation of Hoagland's growth media solution.

| Stock solution number | Chemical | Amount dissolved in 1 L of distilled water (g) |
|---|---|---|
| 1 | $MgSO_4.7H_2O$ | 50.0 |
| 2 | Each dissolved in sequence | |
| | (i) $KNO_3$ | 75.8 (add 6mL of 6 M HCl to facilitate dissolution) |
| | (ii) $Ca(NO_3)_2.4H_2O$ | 59.0 |
| | (iii) $KH_2PO_4$ | 34.0 |
| 3 | Each dissolved in sequence | |
| | (i) $H_3BO_3$ | 2.86 |
| | (ii) $MnCl_2.4H_2O$ | 3.62 |
| | (iii) $Na_2MoO_4.2H_2O$ | 0.12 |
| | (iv) $ZnSO_4.7H_2O$ | 0.22 |
| | (v) $CuSO_4.5H_2O$ | 0.08 |
| 4 | Each dissolved in sequence | |
| | EDTA[a] | 9.0 (add 8 mL of 6 M KOH to facilitate dissolution) |
| | $FeCl_3.6H_2O$ | 5.3 |

[a] The acronym EDTA refers to the compound Ethylenediaminetetraacetic acid

### *3.3.2 Preparation of Hoagland's E+ growth media*

To prepare 1 L of Hoagland's E+ growth media, volumes of the four stock solutions (Table 3.3) were added to approximately 400 mL of distilled water, and the pH adjusted to 4.4-4.8 using 1 mol $L^{-1}$ HCl and/or 1 mol $L^{-1}$ NaOH. The volume was made up to 1 L in a volumetric flask using distilled water. The media was transferred to Erlenmeyer flasks and stoppered with cotton wool wrapped in cheese cloth, or Schott autoclave bottles, where necessary. The media was autoclaved using the liquid setting on an AMSCO Eagle Series 3000 3021-S Stage 3 (Gravity) (STERIS Corporation Mentor, OH, USA) autoclave.

**Table 3.3** Composition of chemical concentrations in Hoagland's growth media.

| Stock number | Volume added for 1 L of Hoagland's solution (mL) | Chemical in stock | Final concentration in Hoagland's solution (mg $L^{-1}$) |
|---|---|---|---|
| 1 | 10 | $MgSO_4.7H_2O$ | 500.00 |
| 2 | 20 | $KNO_3$ | 1515.20 |
| | | $Ca(NO_3)_2.4H_2O$ | 1180.00 |
| | | $KH_2PO_4$ | 680.00 |
| 3 | 1 | $H_3BO_3$ | 2.86 |
| | | $MnCl_2.4H_2O$ | 3.62 |
| | | $Na_2MoO_4.2H_2O$ | 0.12 |
| | | $ZnSO_4.7H_2O$ | 0.22 |
| | | $CuSO_4.5H_2O$ | 0.08 |
| 4 | 1 | EDTA | 9.00 |
| | | $FeCl_3.6H_2O$ | 5.30 |

*3.3.3 Preparation of the diuron stock solution*

Diuron exposure media (0, 0.3, 3, 25, 50, 100 and 200 µg $L^{-1}$) were prepared from a 1 mg $L^{-1}$ diuron stock solution containing 1.25 g (±0.1 mg) of 80% a.i. w/w (DuPont, Mississauga, ON, Canada) dissolved in 1 L of sterilized Hoagland's growth media (see later). From this, 1 L stock solutions of 0, 0.3, 3, 25, 50, 100 and 200 µg $L^{-1}$ of diuron were prepared in Hoagland's growth media by serial dilution. The concentrations of the stocks were confirmed using Diuron ELISA test kit (Abraxis, USA) and a Bio-Rad Model 680 microplate reader at 450 nm (Hercules, CA, USA) (See Appendix 3).

*3.3.4 Method for initial seven day exposure of Lemna gibba and Lemna minor to different concentrations of diuron in Hoagland's growth media.*

Two three-frond *L. gibba* and *L. minor* plants (healthy in appearance, i.e. not exhibiting chlorosis) were selected and transferred to six replicate polystyrene petri-dishes (Fisher Scientific, USA) containing 40 mL of Hoagland's solution containing diuron (0, 0.3, 3, 25, 50, 100 and 200 µg $L^{-1}$). The petri-dishes were set up in a randomized block design on trays lined with black cardboard and placed in an E-15 Conviron (Winnipeg, Canada) growth chamber managed by a CMP 3244 digital circuit control box, with temperature maintained at 25 ± 2 $^oC$ and light intensity 111 ± 12 µE $m^{-2}$ $s^{-1}$ measured at various points in the growth chamber. After four days, the exposure media was removed using a vacuum hose to evacuate the solution into a collection flask and replaced with fresh diuron media (40 mL). The petri-dishes were replaced in the growth chamber and diuron exposure continued for a further three days.

After a total of seven days of exposure to diuron, the petri-dishes were removed from the growth chamber. The total numbers of plants and fronds in each petri-dish were visually counted and photographed. All of the plants from three of the six petri-dishes were removed from their dishes (including their roots), blotted dry on paper towels, placed in tared envelopes, weighed for fresh weight on an analytical balance (±0.01 mg), and then oven dried (60$^{o}$C) for 24 hours (Environment Canada, 2006; OECD, 2006b). The envelopes were removed from the oven and placed in a dessicator and allowed to cool to room temperature. The dried plants were removed from the envelopes and weighed in weigh boats to determine their dry weight (Environment Canada, 2006). The plants contained in the remaining three petri-dishes were kept for recovery.

*3.3.5 Method for seven day recovery of Lemna gibba and Lemna minor to different concentrations of diuron in Hoagland's growth media.*

From the remaining three petri-dishes two apparently healthy three-frond plants were selected from each dish, rinsed in distilled water three times and placed into their respective (triplicate) new petri-dishes containing fresh Hoagland's (40 mL). The petri-dishes were then placed in the growth chamber, under the same conditions as for the exposure to diuron, to simulate a recovery phase. The plants were left in the growth chamber for a total of seven days, with the growth media being replaced after four days. After seven days the number of plants and fronds in each test vessel were counted and photographed, and the frond area and fresh and dry weights were determined as above.

*3.3.6 Calculations*

Using the plant and frond counts, and fresh and dry weights for both exposure and recovery the reduction in yield, differences from the control and modeling to determined effects concentrations ($EC_x$) were carried out according to the methods reported in Environment Canada (2006); OECD (2006a); and Brain and Solomon (2007).

*3.3.6.1 Reduction in yield*

Per cent reduction in yield ($\%I_y$) was calculated for each treatment using equation 3.1, where $b_C$ is the difference of the final biomass (g) and the starting biomass for the control group (g), and $b_T$ is the difference of the final and starting biomasses in the treatment group (g).

$$\%I_y = \frac{(b_C - b_T)}{b_C} \times 100 \qquad (3.1)$$

*3.3.6.2 Statistical analysis*

Comparing treatments and assembling growth-response curves were done using various statistical methods and relevant effects parameters were calculated following the methods described in Environment Canada (2006); OECD (2006a); and Brain and Solomon (2007).

To characterize the differences between treatments in both exposure and recovery scenarios parametric and non-parametric statistical approaches were used (OECD, 2006a). Where normality ($P>0.05$) and equal variance ($P>0.05$) was assumed, analysis of variance (ANOVA) Dunnett's test was used in SigmaPlot 11.0 (Systat Software inc., USA). Alternatively, where normality and equal variance could not be assumed, a non-parametric ANOVA Dunnett's test based on ranks was used in SigmaPlot 11.0. From this, where significant differences from the control were observed ($P<0.05$) a lowest observed effect concentration (LOEC) ($P<0.05$) and no observable effect concentration (NOEC) ($P>0.05$) were determined for plant and frond counts, and wet and dry weights in exposure. Similarly, for the recovery scenario, the highest concentration that exhibited recovery that was not significantly lower than the control, referred to as the highest observable recovery concentration (HORC) ($P<0.05$), were determined.

Effects concentrations ($EC_x$) inhibiting plant and frond production, wet and dry weight and fresh weight production by 10% ($EC_{10}$), 20% ($EC_{20}$) and 50% ($EC_{50}$) were estimated by fitting a four parameter logistic regression (Equation 3.2) to the per cent inhibition data using SigmaPlot 11.0. Where $y$ refers to the per cent inhibition, $y_{min}$ refers to the minimum inhibition response (%), $y_{max}$ maximum inhibition response (%), $x$ refers to the exposure concentration (µg $L^{-1}$), $d$ is the median number (µg $L^{-1}$) and $b$ refers to the slope of the curve at its midpoint.

$$y = y_{\min}\left(\frac{y_{\max} - y_{\min}}{1 + \left(\frac{x}{d}\right)^b}\right) \qquad (3.2)$$

## 3.4 RESULTS

The results for the *Lemna minor* and *Lemna gibba* pulse exposure to diuron in Hoagland's growth media are presented separately, and the results from all statistical analysis are given in Appendix 3.

### *3.4.1 Lemna minor*

#### *3.4.1.1 Results for initial seven day growth period in Hoagland's growth media containing diuron.*

The average plant and frond counts, and fresh and dry weights for the initial seven day growth period in Hoagland's growth media containing different concentrations of diuron for *L. minor* are summarised in Table 3.4. The average *L. minor* plant and frond counts, and wet and dry weights generally decreased with increasing concentration after seven days with slight, but not significant, increases observed at lower treatment concentrations of 0.3 and 3 µg $L^{-1}$ (Table 3.4). Using ANOVA, the Dunnett's test detected significant differences from the control for concentrations greater than 50 µg $L^{-1}$ ($P = 0.01$) and greater than 25 µg $L^{-1}$ ($P=0.00$) for plant and frond counts, respectively. Similarly, significant differences from the control for the average wet and dry weights were determined for concentrations greater than 50 µg $L^{-1}$ ($P = 0.01$) and 25 µg $L^{-1}$ ($P = 0.02$) respectively (Table 3.4).

**Table 3.4** Summary of *Lemna minor* average plant and frond counts, and fresh and dry weights (mg), with their respective standard deviations at the end of seven days exposure to different concentrations of diuron (μg $L^{-1}$) in Hoagland's growth media.

| Concentration of diuron (μg $L^{-1}$) | Average plant count (n =6) | Average frond count (n=6) | Average wet weight (mg) (n=3) | Average dry weight (mg) (n=3) |
|---|---|---|---|---|
| 0 | 15 ± 3 | 79 ± 6 | 110.1 ± 15.3 | 6.9 ± 1.6 |
| 0.3 | 15 ± 3 | 79 ± 11 | 111.3 ± 24.7 | 6.6 ± 0.9 |
| 3 | 16 ± 3 | 82 ± 11 | 107.9 ± 21.1 | 6.9 ± 0.5 |
| 25 | 14 ± 3 | 60 ± 6[a] | 81.4 ± 19.9 | 4.2 ± 0.2[a] |
| 50 | 9 ± 1[a] | 44 ± 7[a] | 57.5 ± 9.8[a] | 2.8 ± 0.8[a] |
| 100 | 7 ± 2[a] | 25 ± 4[a] | 61.9 ± 15.4[a] | 1.7 ± 0.5[a] |
| 200 | 4 ± 1[a] | 11 ± 2[a] | 8.6 ± 4.7[a] | 0.2 ± 0.2[a] |

[a]Significant differences ($P<0.05$) from the control determined based on Dunnett's ANOVA test, assuming normality ($P>0.05$) and equal variance ($P>0.05$).

A summary of the no observable effect (NOEC) and lowest observed effects concentration (LOEC) for *Lemna minor* determined for plant and frond counts, and wet and dry weights, along with their significance level (P-value) is shown in Table 3.5. The same NOECs and LOECs levels were the same for dry weight and frond count, and wet weight and plant count effects endpoints (Table 3.5). For dry weight and frond counts, the NOECs and LOECs were respectively determined to be 3 and 25 μg $L^{-1}$ ($P_{dry\ weight} = 0.02$; $P_{frond\ count} = 0.00$). Wet weight and plant count NOECs and LOECs were respectively determined to be 25 μg $L^{-1}$ and 50 μg $L^{-1}$ ($P_{wet\ weight} = 0.01$; $P_{plant\ count} = 0.01$).

**Table 3.5** Summary of NOECs and LOECs for *Lemna minor* determined by one-way ANOVA and comparison with Dunnett's test ($P \leq 0.05$) for exposure to diuron at the end of seven days.

| Endpoint | Control | NOEC (μg $L^{-1}$)[a] | LOEC (μg $L^{-1}$)[a] | Dunnett's P-value |
|---|---|---|---|---|
| Plant count | 15 ± 3 | 25 (14 ± 3) | 50 (9 ± 1) | 0.01 |
| Frond count | 79 ± 6 | 3 (82 ± 11) | 25 (60 ± 6) | 0.00 |
| Wet weight (mg) | 110.1 ± 15.3 | 25 (81.4 ± 19.9) | 50 (57.5 ± 9.8) | 0.01 |
| Dry weight (mg) | 6.9 ± 1.6 | 3 (6.9 ± 0.5) | 25 (4.2 ± 0.2) | 0.02 |

[a] numbers in brackets correspond to the average endpoint measurement together with their standard deviation.

Dose-response curves for *L. minor* per cent reductions in wet and dry weights, and frond and plant counts relative to the control, are shown in Figures 3.1a-d. The minimum and maximum inhibition yield for plant and frond counts, wet and dry weights ranged -4.2 to 83.4% (for the concentrations 0.3 and 200 μg $L^{-1}$, respectively), -4.1 to 92.7% (for the concentrations 3 and 200 μg $L^{-1}$, respectively), -2.0 to 99.8% (for the concentrations 0.3 and 200 μg $L^{-1}$, respectively) and -2.9 to 104.1% (for the concentrations 0.3 and 200 μg $L^{-1}$, respectively), respectively. In all cases the four parameter logistic model explained dose-responses

reasonably well with adj. $r^2$ ranging 0.7-0.95 (Table 3.6). From these fits, the $EC_{10}$, $EC_{25}$ and $EC_{50}$ were estimated by applying the output parameters in Table 3.6 to the dose response model. The calculated $EC_x$s were different over the measured effects endpoints with $EC_{10}$s lowest for the wet and dry weights; the dry weight and frond counts were the lowest of the $EC_{25}$s; and the $EC_{50}$s were more sensitive for dry weight and frond counts (Table 3.6).

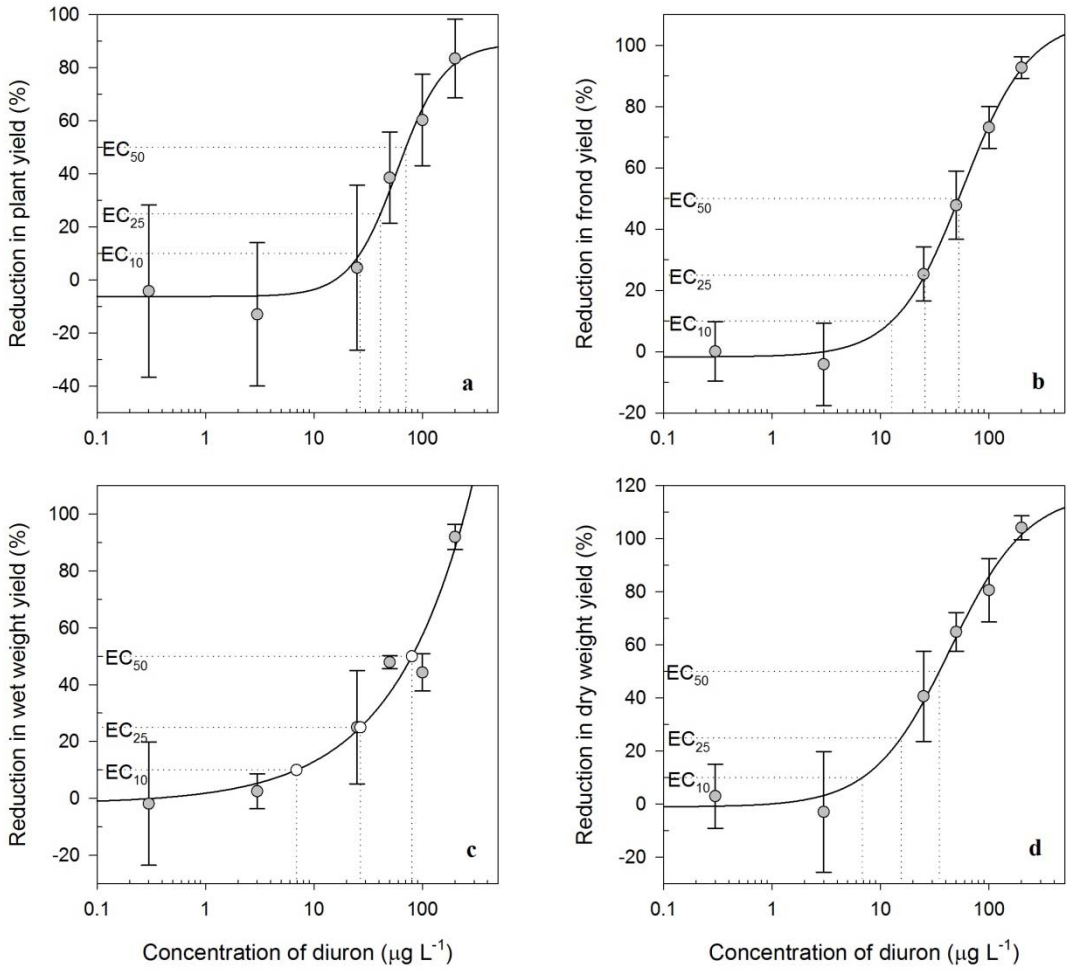



**Figure 3.1** Four-parameter logistic dose-response curves of *L. minor* per cent reduction in (a) plant and (b) frond counts, and (c) wet and (d) dry weights yields, relative to the control, at the end of seven days exposure to different concentrations of diuron in Hoagland's growth media (µg $L^{-1}$).

**Table 3.6** Summary of *L. minor* $EC_{10}$, $EC_{25}$ and $EC_{50}$ for effect endpoints of wet and dry weight, and plant and frond count estimated from their respective best model fit along with the output model parameters and significance of the fit (adj. $r^2$).

| Endpoint | $EC_{10}$ (µg $L^{-1}$) | $EC_{25}$ (µg $L^{-1}$) | $EC_{50}$ (µg $L^{-1}$) | Model | Model parameters | Adjusted $r^2$ |
|---|---|---|---|---|---|---|
| Plant count | 26.7 | 41.1 | 70.6 | Logistic | $y_0$ = -6.22; $y_{max}$ = 89.04; $d$ = 58.83; $m$ = 2.00 | 0.70 |
| Frond count | 12.8 | 25.7 | 52.8 | Logistic | $y_0$ = -1.72; $y_{max}$ = 108.46; $d$ = 57.52; $m$ = 1.41 | 0.95 |
| Wet weight | 6.9 | 26.9 | 80.1 | Logistic | $y_0$ = -1.86; $y_{max}$ = 2901041.42; $d$= 6229660579.86; $m$ = 0.60 | 0.85 |
| Dry weight | 6.8 | 15.5 | 34.9 | Logistic | $y_0$ = -1.06; $y_{max}$ = 117.50; $d$ = 43.83; $m$ = 1.22 | 0.91 |

*3.4.1.2 Results for seven day recovery period in Hoagland's growth media following diuron exposure.*

The results for the seven day recovery of *L. minor* in Hoagland's growth media, following exposure to diuron, are summarised in Table 3.7. The results show variable recovery responses, with increases not significantly different from the control in the frond count and wet weight with increasing treatment concentration from 0-3 µg $L^{-1}$. However, significant differences from the control ($P<0.01$) were observed for higher treatment concentrations of 100 and 200 µg $L^{-1}$. Based on these differences the HORC for *L. minor* were 50 µg $L^{-1}$ for the average frond count, and 100 µg $L^{-1}$ for plant count, and wet and dry weights.

**Table 3.7** Summary of *Lemna minor* average plant and frond counts, and fresh and dry weights, with their respective standard deviations, after seven days recovery from different exposure to diuron (µg $L^{-1}$) in Hoagland's growth media.

| Concentration of diuron (µg $L^{-1}$) | Average plant count (n =3) | Average frond count (n=3) | Average wet weight (mg) (n=3) | Average dry weight (mg) (n=3) |
|---|---|---|---|---|
| 0 | 23 ± 6 | 132 ± 18 | 135.5 ± 20.1 | 15.1 ± 3.8 |
| 0.3 | 23 ± 7 | 148 ± 9 | 163.4 ± 12.3 | 14.7 ± 1.0 |
| 3 | 21 ± 3 | 137 ± 13 | 154.4 ± 14.6 | 13.1 ± 2.0 |
| 25 | 16 ± 1 | 112 ± 11 | 127.3 ± 17.5 | 10.5 ± 1.5 |
| 50 | 18 ± 6 | 118 ± 11 | 127.3 ± 8.8 | 12.0 ± 1.1 |
| 100 | 15 ± 2 | 93 ± 12 [a] | 109.9 ± 21.8 | 8.7 ± 2.0 |
| 200 | 8 ± 1 [a] | 55 ± 9 [a] | 75.4 ± 19.2 [a] | 4.9 ± 1.2 [b] |

[a] Significant differences ($P<0.05$) from the control determined based on Dunnett's ANOVA test, assuming normality ($P>0.05$) and equal variance ($P>0.05$).
[b] Significant differences determined from Dunnett's ANOVA rank test, not assuming normality ($P<0.05$) and equal variance ($P<0.05$).

The extent of inhibition in yield recorded by the end of the seven day recovery period for the plant and frond counts, and wet and dry weights, are summarised in Figures 3.2a-d. The

minimum and maximum inhibition yield for plant and frond counts, wet and dry weights ranged -2.7 to 67.7%, -13.1 to 60.9%, -22.5 to 45.9% and -1.2 to 70.5%, for the concentration range 0.3 to 200 μg $L^{-1}$, respectively. Interestingly, some of the treatment concentrations showed negative inhibition suggesting a greater growth response relative to the control during recovery. Importantly, these calculated inhibitions in yield were comparatively less than the inhibitions observed for the exposure scenario (Figures 3.1a-d).

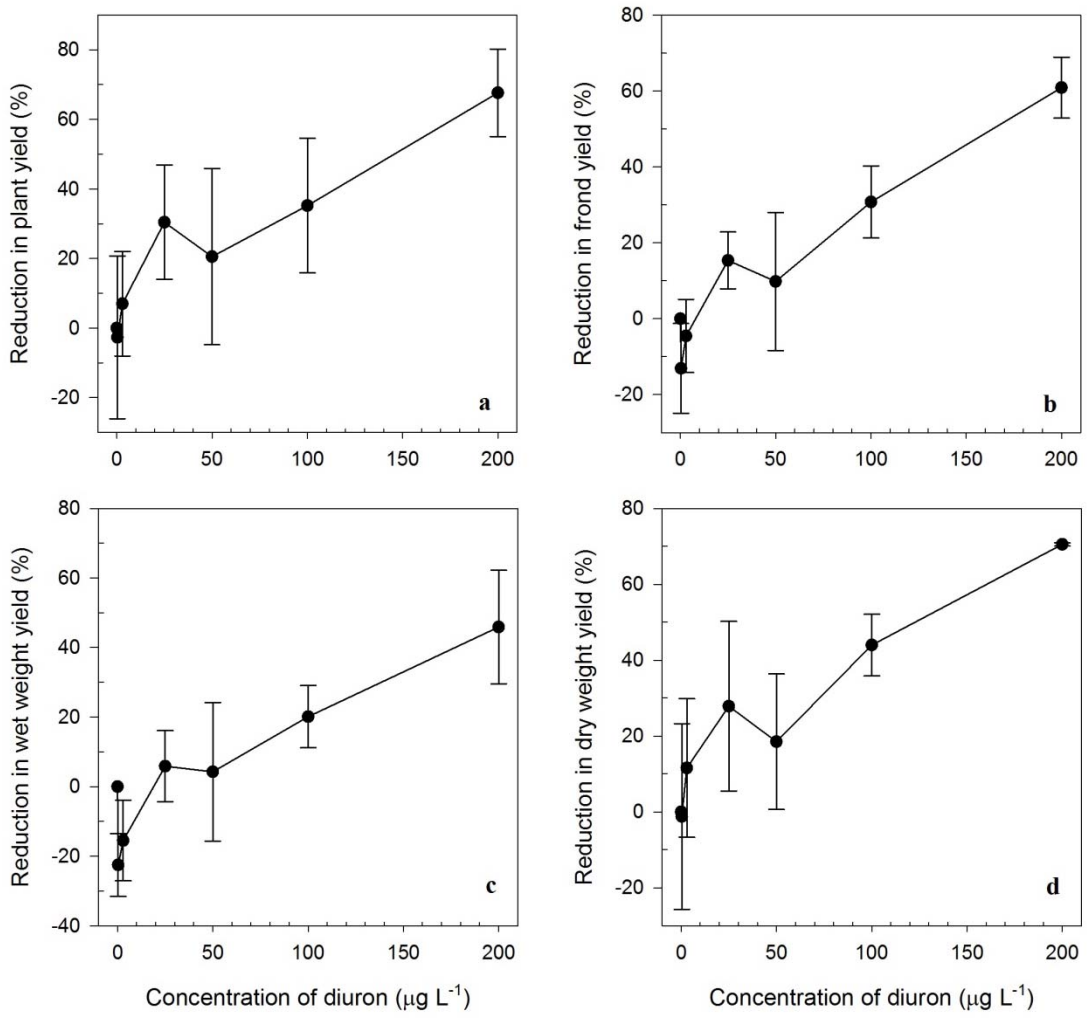



**Figure 3.2** Graphs of different levels of *L. minor* inhibition from diuron exposure (μg $L^{-1}$) at the end of the seven day recovery period for the parameters per cent reduction in (a) plant and (b) frond counts, and (c) wet and (d) dry weights yields relative to the control. Error bars show the standard deviation.

*3.4.2 Lemna gibba*

*3.4.2.1 Results for initial seven day growth period in Hoagland's growth media containing diuron.*

The average plant and frond counts, and fresh and dry weights for the initial seven day growth period in Hoagland's growth media containing different concentrations of diuron for *L. gibba* are summarised in Table 3.8. The average *L. gibba* plant and frond counts, and wet and dry weights generally decreased with increasing concentration after seven days with slight, but not significant, increase in the average plant count for 3 μg $L^{-1}$ treatment (Table 3.8). Using one-way ANOVA, the Dunnett's test detected significant differences from the control for concentrations greater than 50 μg $L^{-1}$ (P = 0.01) and greater than 25 μg $L^{-1}$ (P=0.00) for plant and frond counts, respectively. Similarly, significant differences from the control for the average wet and dry weights were determined for concentrations greater than 50 μg $L^{-1}$ (P = 0.01) and 25 μg $L^{-1}$ (P = 0.02) respectively (Table 3.8).

**Table 3.8** Summary of *Lemna gibba* average plant and frond counts, and fresh and dry weights (mg), with their respective standard deviations at the end of seven days exposure to different concentrations of diuron (μg $L^{-1}$) in Hoagland's growth media.

| Concentration of diuron (μg $L^{-1}$) | Average plant count (n =6) | Average frond count (n=6) | Average wet weight (mg) (n=3) | Average dry weight (mg) (n=3) |
|---|---|---|---|---|
| 0 | 15 ± 3 | 74 ± 12 | 178.8 ± 14.0 | 9.2 ± 0.9 |
| 0.3 | 13 ± 4 | 67 ± 19 | 172.1 ± 7.6 | 9.1 ± 0.5 |
| 3 | 14 ± 3 | 67 ± 13 | 169.9 ± 37.5 | 8.6 ± 0.7 |
| 25 | 10 ± 4 | 53 ± 9 | 130.1 ± 9.7 [a] | 5.6 ± 0.2 [a] |
| 50 | 9 ± 2 [a] | 39 ± 9 [b] | 86.1 ± 22.1 [a] | 3.6 ± 0.7 [a] |
| 100 | 7 ± 2 [a] | 24 ± 3 [b] | 67.9 ± 18.3 [a] | 1.8 ± 0.1 [a] |
| 200 | 5 ± 1 [a] | 14 ± 1 [b] | 15.8 ± 2.7 [a] | 0.9 ± 0.1 [a] |

[a] Significant differences (P<0.05) from the control determined based on Dunnett's ANOVA test, assuming normality (P>0.05) and equal variance (P>0.05).
[b] Significant differences determined from Dunnett's ANOVA rank test, not assuming normality (P<0.05) and equal variance (P<0.05).

A summary of the no observable effect (NOEC) and lowest observed effects concentration (LOEC) for *Lemna minor* determined for plant and frond counts, and wet and dry weights, along with their significance level (P-value) is shown in Table 3.9. The NOEC and LOEC levels were the same for dry weight and frond count, and wet weight and plant count effects endpoints (Table 3.9). For plant and frond counts, the NOECs and LOECs were respectively determined to be 25 and 50 μg $L^{-1}$ ($P_{plant\ count}$ = 0.01; $P_{frond\ count}$ = 0.01). Wet weight and plant count NOECs and LOECs were respectively determined to be 3 μg $L^{-1}$ and 25 μg $L^{-1}$ ($P_{wet\ weight}$ = 0.02; $P_{dry\ count}$ = 0.00).

**Table 3.9** Summary of NOECs and LOECs for *Lemna gibba* determined by one-way ANOVA and comparison with Dunnett's test (P≤0.05) for exposure to diuron at the end of seven days.

| Endpoint | Control | NOEC (µg $L^{-1}$)[a] | LOEC (µg $L^{-1}$)[a] | Dunnett's P-value (LOEC vs Control) |
|---|---|---|---|---|
| Plant count | 74 ± 12 | 25 (10 ± 4) | 50 (9 ± 2) | 0.01 |
| Frond count | 15 ± 3 | 25 (53 ± 9) | 50 (39 ± 9) | 0.01 |
| Wet weight (mg) | 178.8 ± 14.0 | 3 (169.9 ± 37.5) | 25 (130.1 ± 9.7) | 0.02 |
| Dry weight (mg) | 9.2 ± 0.9 | 3 (8.6 ± 0.7) | 25 (5.6 ± 0.2) | 0.00 |

[a] numbers in brackets correspond to the average endpoint measurement together with their standard deviation.

Dose-response curves for *L. gibba* per cent reductions in wet and dry weights, and frond and plant counts relative to the control, are shown in Figures 3.3a-d. The responses show increases in inhibition with increasing diuron concentration. A slight, but not significant, hormesis response was observed for plant yield at 3 µg $L^{-1}$ diuron (Figure 3.3a). Specifically, the per cent inhibition of yield for plant and frond counts, wet and dry weights ranged -0.3 to 79.4% (for the concentrations 3 and 200 µg $L^{-1}$), 11.0 to 88.7% (for the concentrations 3 and 200 µg $L^{-1}$), 3.9 to 99.5% (for the concentrations 0.3 and 200 µg $L^{-1}$) and 0.5 to 99.0% (for the concentrations 0.3 and 200 µg $L^{-1}$), respectively. In all cases the four parameter logistic model was the best fit to describe the responses (adj. $r^2$ = 0.71-0.97; Table 3.10). From this, the $EC_{10}$, $EC_{25}$ and $EC_{50}$ were estimated, of which the output parameters and adjusted coefficient of determination (adj. $r^2$) are summarised in Table 3.10. The calculated $EC_x$s were different over the measured effects endpoints. $EC_{10}$s were lowest for the dry weight and frond count; the plant and frond counts had the lowest of the $EC_{25}$s; and the $EC_{50}$s were more sensitive for plant count and dry weight (Table 3.10).

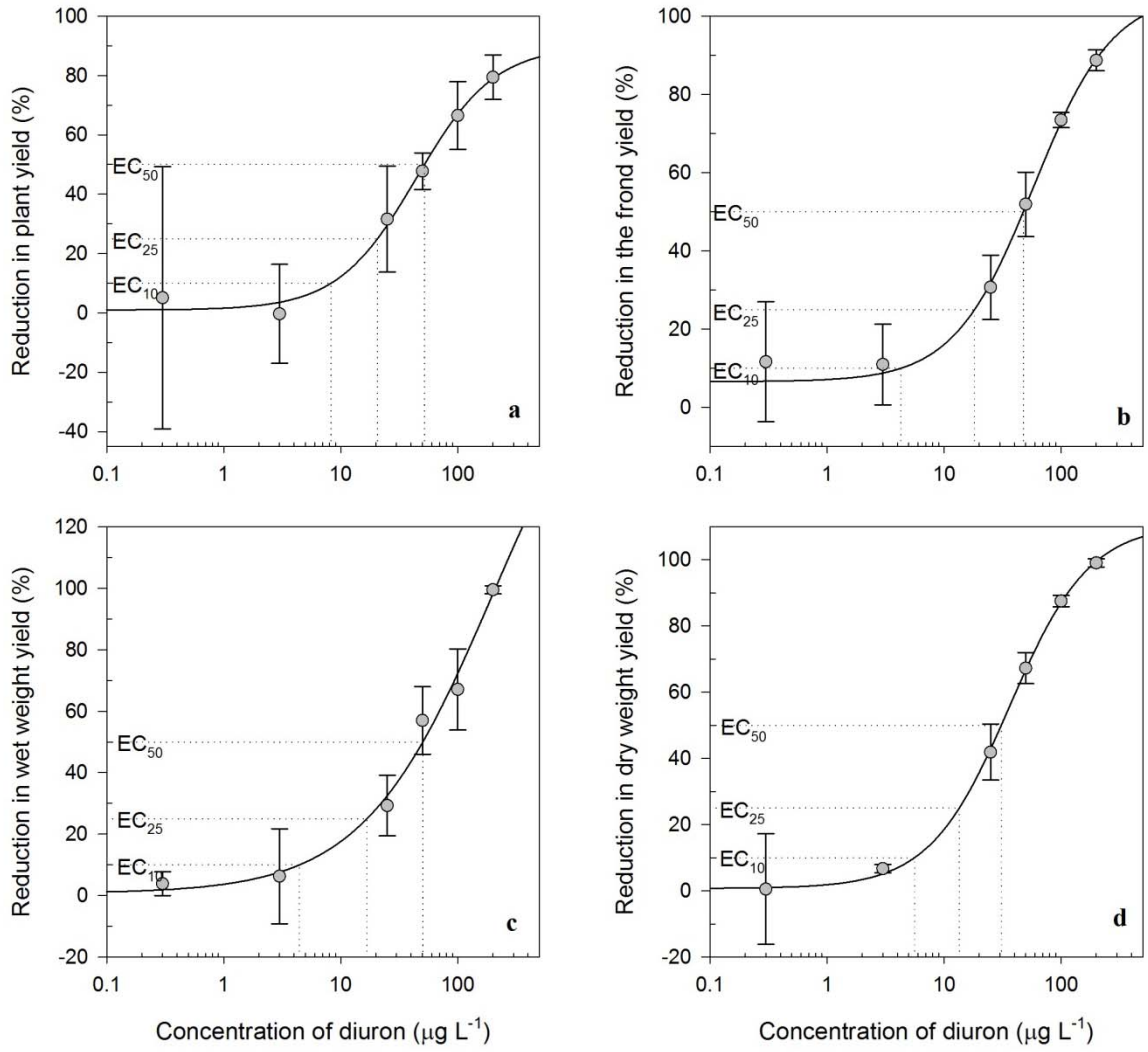



**Figure 3.3** Four-parameter logistic dose-response curves of *L. gibba* per cent reduction in (a) plant and (b) frond counts, and (c) wet and (d) dry weights yields, relative to the control, at the end of seven days exposure to different concentrations of diuron in Hoagland's growth media (µg $L^{-1}$).

**Table 3.10** Summary of $EC_{10}$, $EC_{25}$ and $EC_{50}$ for effect endpoints of wet and dry weight, and plant and frond count estimated from their respective best model fit along with the output model parameters and significance of the fit (adj. $r^2$).

| Endpoint | $EC_{10}$ (µg $L^{-1}$) | $EC_{25}$ (µg $L^{-1}$) | $EC_{50}$ (µg $L^{-1}$) | Model | Model parameters | Adjusted $r^2$ |
|---|---|---|---|---|---|---|
| Plant count | 5.6 | 13.5 | 30.9 | Logistic | $y_0 = 0.71$; $y_{max} = 110.75$; $d = 36.36$; $m = 1.27$ | 0.97 |
| Frond count | 4.4 | 16.7 | 50.3 | Logistic | $y_0 = 0.7455$; $y_{max} = 194.6251$; $d = 197.47$; $m = 0.79$ | 0.95 |
| Wet weight | 8.3 | 20.6 | 51.9 | Logistic | $y_0 = 0.97$; $y_{max} = 89.91$; $d = 44.30$; $m = 1.30$ | 0.71 |
| Dry weight | 4.3 | 18.2 | 47.6 | Logistic | $y_0 = 6.57$; $y_{max} = 106.04$; $d = 58.12$; $m = 1.28$ | 0.93 |

*3.4.2.2 Results for seven day recovery period in Hoagland's growth media following diuron exposure.*

The results for the seven day recovery of *L. gibba* in Hoagland's growth media, following exposure to diuron, are summarised in Table 3.11. The results show variable recovery responses. No significant differences (P = 0.46) were observed between the control and the different treatments for the average frond count. However, significant differences from the control (P<0.01) were found for higher treatment concentrations in the plant counts (200 µg $L^{-1}$), and wet and dry weights (≥100 µg $L^{-1}$) (Table 3.11). Specifically, the HORC was 100 µg $L^{-1}$ for the plant and frond count endpoints and 50 µg $L^{-1}$ for the wet and dry weight end points (Appendix 3).

**Table 3.11** Summary of *Lemna gibba* average plant and frond counts, and fresh and dry weights, with their respective standard deviations, after 7-days recovery from different exposure to diuron (µg $L^{-1}$) in Hoagland's growth media.

| Concentration of diuron (µg $L^{-1}$) | Average plant count (n =3) | Average frond count (n=3) | Average wet weight (mg) (n=3) | Average dry weight (mg) (n=3) |
|---|---|---|---|---|
| 0 | 16 ± 3 | 91 ± 14 | 278.7 ± 39.5 | 16.1 ± 3.2 |
| 0.3 | 15 ± 2 | 95 ± 21 | 267.6 ± 38.6 | 16.2 ± 1.7 |
| 3 | 15 ± 1 | 95 ± 10 | 283.0 ± 51.0 | 15.9 ± 1.6 |
| 25 | 15 ± 2 | 92 ± 5 | 288.3 ± 21.6 | 18.0 ± 0.8 |
| 50 | 14 ± 2 | 87 ± 5 | 227.9 ± 128 | 14.2 ± 0.5 |
| 100 | 17 ± 3 | 93 ± 2 | 181.1 ± 23.5 [a] | 12.2 ± 0.9 [a] |
| 200 | 10 ± 1 [a] | 77 ± 5 | 162.6 ± 26.5 [a] | 10.1 ± 1.3 [a] |

[a] Significant differences (P<0.05) from the control determined based on Dunnett's ANOVA test, assuming normality (P>0.05) and equal variance (P>0.05).

The extent of inhibition in *L. gibba* yield recorded by the end of the seven day recovery period for the plant and frond counts, and wet and dry weights, are summarised in Figures 3.4a-d. The minimum and maximum inhibition yield for plant and frond counts, wet and dry weights ranged -10.2 to 38.7% (for 100 and 200 µg $L^{-1}$, respectively), -5.5 to 14.4% (for 3 and 200 µg L, respectively), -4.6 to 43.1% (for 25 and 200 µg $L^{-1}$, respectively), and -17.4 to 38.1% (for 0.3 to 200 µg $L^{-1}$, respectively), respectively. Interestingly, some of the treatment concentrations showed negative inhibition suggesting a greater growth response relative to the control occurred during recovery. Importantly, these calculated inhibitions in yield are comparatively less than the inhibitions observed for the exposure scenario (Figures 3.3a-d).

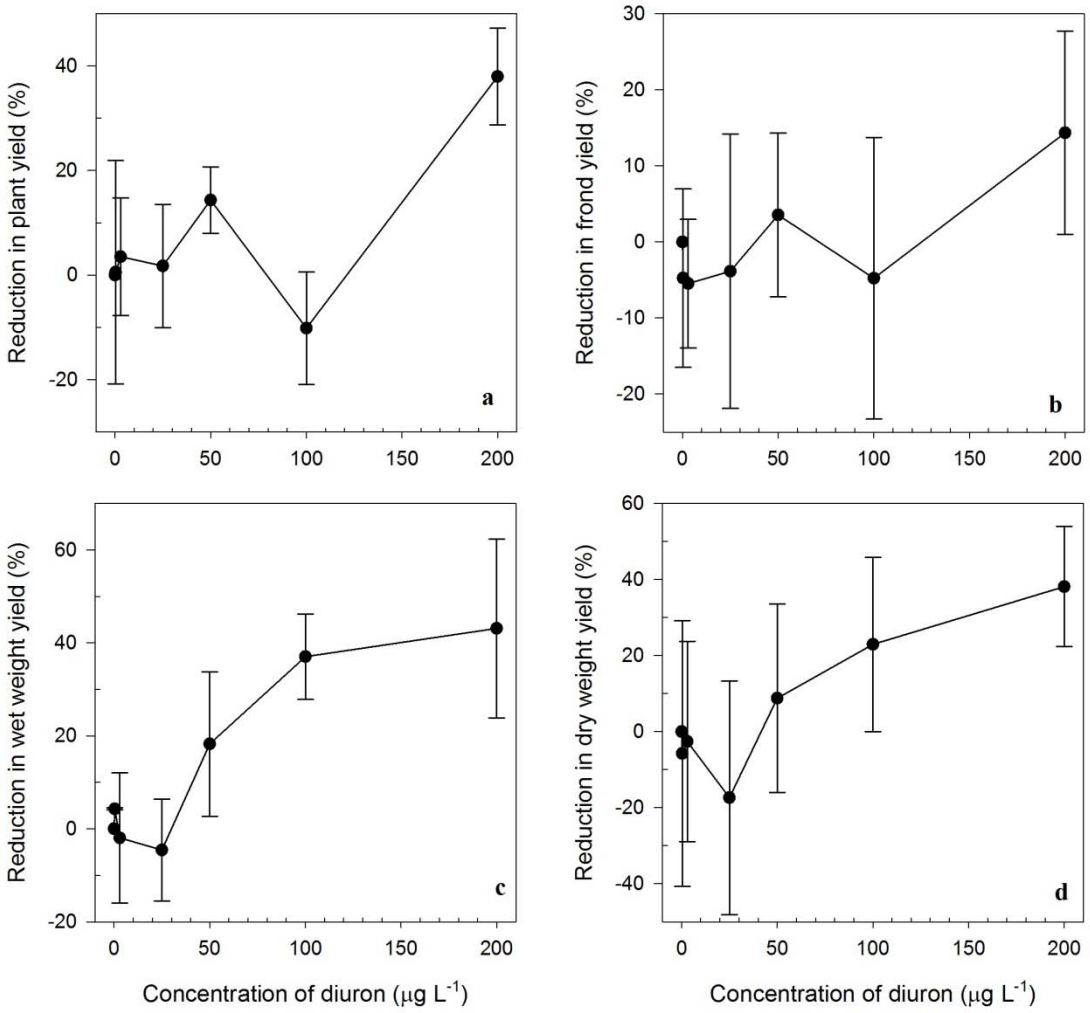



**Figure 3.4** Graphs of different levels of *L. gibba* inhibition from diuron exposure (μg $L^{-1}$) at the end of the seven day recovery period for the parameters per cent reduction in (a) plant and (b) frond counts, and (c) wet and (d) dry weights yields relative to the control. Error bars show the standard deviation.

## 3.5 DISCUSSION

In this study, a seven day diuron pulse deemed sufficient to represent a maximum exposure scenario in the Gwydir River catchment was conducted on the macrophyte species *L. minor* and *L. gibba*. Exposure and recovery responses were quantified, with recovery being defined by the populations growth that is the same or similar to that of the control group following exposure. The results from this experiment demonstrate significant population recovery of *L. minor* and *L. gibba* following a seven-day exposure pulse to different concentrations of diuron. The effects of diuron on the growth of *L. minor* and *L. gibba* are considered with reference to the biochemical pathways diuron targets, their ability to recover from prolonged exposure to the toxicant, experimental errors and the significance of these results for ecological risk assessment on a catchment-scale basis.

*3.5.1 Responses of Lemna minor and Lemna gibba to diuron exposure*

*3.5.1.1 Growth responses of Lemna minor and Lemna gibba to diuron exposure*

The toxic action of diuron resulted in a reduction in plant and frond yields in *L. minor* and *L. gibba* that also translated to the observed reductions in wet and dry weights with increasing treatment concentration (Tables 3.4 and 3.8; and Figures 3.1a-d and 3.3a-d). Similar toxic endpoint responses of NOEC, LOEC, and $EC_{50}$ (see Tables 3.5 and 3.6, and Tables 3.9 and 3.10, respectively, for details in *L. minor* and *L. gibba* toxicity endpoints summaries) have been reported in other studies involving *L. minor* and *L. gibba.* For example, in seven day diuron exposure studies, Teisseire *et al*. (1999) reported a reduction in *L. minor* dry weight yield (LOEC = 5µg $L^{-1}$ and estimated $EC_{50}$ = 25 µg $L^{-1}$); and Okamura *et al*. (2003a) reported reduction in frond counts for *L. minor* and *L. gibba* ($EC_{50}$s = 29 µg $L^{-1}$ and 30 µg $L^{-1}$, respectively). Similar responses to diuron exposure have been reported is also common for other aquatic plants and algae. For instance, Stauber *et al*. (2008) reported reductions in population growth for three diatom species (*Pyrocystis lunula* 24 h exposure NOEC = 10 mg $L^{-1}$, LOEC = 16 mg $L^{-1}$, $EC_{50}$ = 19 mg $L^{-1}$; *Nitzschia closterium* 72 h inhibition of growth rate NOEC = 2 µg $L^{-1}$, LOEC = 6 µg $L^{-1}$, $EC_{50}$ = 17 µg $L^{-1}$; and *Entomoneis c.f. punctulata* 72 h inhibition of growth rate NOEC = 2 µg $L^{-1}$, LOEC = 6 µg $L^{-1}$, $EC_{50}$ = 16 µg $L^{-1}$); and Ma *et al*. (2002), Ma *et al*. (2003) and Ma *et al*. (2006) respectively reported population reductions for 96 h laboratory diuron toxicity experiments for the green algae *Chlorella vulgaris* ($EC_{50}$ = 4.3 µg $L^{-1}$), *Scenedesmus quadricauda* ($EC_{50}$ = 2.7 µg $L^{-1}$) and *Raphidocelis subcapitata* ($EC_{50}$ = 0.7 µg $L^{-1}$). These studies demonstrate consistency in response when exposed to diuron.

*3.5.1.2 Mechanism driving the growth responses of Lemna minor and Lemna gibba when exposed to diuron*

Reductions in population growth of *L.minor* and *L. gibba* observed in this study are the outcome of photosynthesis inhibition. Specifically, diuron's mode of action has been characterised to disrupt the normal function of the PSII apparatus in photosynthesis by binding to the second quinone electron acceptor ($Q_B$) of reaction centre II (RCII) D1 protein (Zer and Ohad, 1995; Gooddy *et al.*, 2002; British Crop Protection Council, 2006) and altering the free-energy state of the primary plastoquinone redox couples $Q_A/Q_A^-$ (Krieger-Liszkay and Rutherford, 1998; Eullaffroy *et al.*, 2009). This action leads to a reduction in

glucose production required in chemical energy generation in plants limiting their growth (Zer and Ohad, 1995; Krieger-Liszkay and Rutherford, 1998).

Reductions in photosynthesis activity have been quantified in diuron toxicity studies involving *L. minor* (Eullaffroy *et al.*, 2009; Knauert *et al.*, 2010). No readily available studies have been identified for diuron photosynthesis inhibition in *L. gibba*. The onset of photosynthesis inhibition is often rapid. Greatest inhibition was reported to occur after 30 min to 1 h, when for algae *Selenastrum capricornutum* following exposure to diuron (Fai *et al.*, 2007); after 2 h for three tropical seagrass species *Cymodocea serrulata*, *Halophila ovalis* and *Zostera capricorni* (Haynes *et al.*, 2000); and 5 h, when *L. minor* was exposed to three different PSII inhibiting herbicides atrazine, isoproturon and prometryn, in separate experiments (Kuster and Altenburger, 2007). To complement this rapid activity, the response to this stress was almost immediate, with reductions in population growth being observed for algae exposed to diuron (Fai *et al.*, 2007) and other PSII inhibiting herbicides (Kuster and Altenburger, 2007; Vallotton *et al.*, 2008). Similar reductions in wet and dry weights were observed in this study (Tables 3.4 and 3.8).

### *3.5.2 The effect of a seven day recovery phase on the growth of Lemna minor and Lemna gibba*

Relative to the exposure scenario, improvements in plant and frond, and wet and dry weight yields *L. minor* (Table 3.7 and Figure 3.2) and *L. gibba* (Table 3.11 and Figure 3.4) at the end of the seven day recovery period were observed for endpoint concentrations NOEC, LOEC, $EC_{10}$, $EC_{25}$ and $EC_{50}$ that would be deemed ecologically detrimental in ecological risk assessments. It was found that fewer treatment groups differed significantly from the controls, except for the higher concentrations of 100 and 200 $\mu g\ L^{-1}$ (Tables 3.7 and 3.11). These responses suggest that the toxic effect of diuron inhibiting the growth of *L. minor* and *L. gibba* through inhibition of photosynthesis activity is not permanent and the onset of recovery was the result of a reversibility in the exposure effects, facilitated by the introduction of the affected macrophytes to the clean growth media.

Pulse studies involving other PSII herbicides suggest that recovery of *L. minor* and *L. gibba* from diuron exposure in this study is the outcome of recovering photosynthesis activity. This was confirmed by Cedergreen *et al*. (2005) who suggested that oxidative damage to the PSII

in *L. minor* caused by the PSII herbicide terbuthylazine could be repaired, an action resulting in restoration of photosynthesis activity within 24 h. Similarly, Valloton *et al.* (2008) reported rapid improvements in PSII effective quantum yield during a recovery phase of the microalgae *Scenedesmus vacuolatus* that had been exposed, in separate experiments, to two PSII inhibiting herbicides, atrazine and isoproturon. Importantly, the restoration of PSII activity in these studies rapidly translated in to organism growth, which is consistent with the results presented in this study. These rapid changes in growth suggest that the effect of diuron on photosynthesis is not permanent. This is likely to be a response of a rapid elimination of the compound from plant cells, and indeed the PSII site, that has translated into reversibility in the mode of action (van Rensen, 1982; Vallotton *et al.*, 2008).

### *3.5.3 Experimental errors and suggested modifications*

Large variations in the results and extensive differences observed between the controls of both the exposure and recovery scenarios (Figures 3.1-3.4) were unexpected occurrences. It has been identified that the cultures of *L. minor* and *L. gibba* being grown under cool fluorescent light and transferred directly from the Erlenmeyer flasks to the test vessel, were not pre-conditioned in the growth chamber prior to commencing the experiment. Changes in the light intensity would have resulted in variation in the growth responses observed in the results. Although this is unlikely to change the outcome of exposure and recovery responses, it is recommended that the macrophytes be cultured under the experimental conditions (including light and temperature) to achieve better uniformity in response (Cedergreen *et al.*, 2005; Brain and Solomon, 2007), together with further replication of the experimental design.

## 3.6 CONCLUSIONS

This study investigated the response of *L. minor* and *L. gibba* growth for a seven day diuron exposure pulse followed by a seven day recovery period evaluating exposure and recovery effects, a situation likely to be observed in the Gwydir River catchment and indeed other catchments. At the end of the exposure period significant inhibition of growth was recorded for both *L. minor* and *L gibba*, as hypothesised. This observation can be confidently explained by the mode of action of diuron, which inhibits photosynthesis. Such inhibition was found to be rapidly reversible in the seven day recovery period when treated plants were replaced in clean growth media, as might be experience in a flowing river system. The significance of the recovery corresponding to inhibition treatment concentrations of NOEC,

LOEC, EC10, EC25 and EC50 levels determined for the exposure period. As these effects endpoints are commonly used in ecological risk characterisation phase to set an ecosystem effect threshold, the ability of effected *L. minor* and *L. gibba* populations to recover from exposure highlighted significant uncertainty in the assumptions contained in ecological risk characterisation phase when applied on a catchment-basis.

**CHAPTER 4**

# SPATIAL EXPOSURE MODELLING OF DIURON IN THE GWYDIR WETLANDS CATCHMENT

## 4.1 INTRODUCTION

Spatial exposure modelling can be used as a management tool to identify areas in a catchment contributing chemical loads to a river. In Chapter 2 it was revealed that there was some ecological risk from diuron occurring in parts of the Gwydir river catchment. Notably, slight risk was identified in the ecologically valuable area of the Gwydir wetlands catchment. This level of risk was determined from data that displayed highly variable levels of exposure. Such variability was thought to be a function of the amount of diuron applied and spatial and temporal differences in environmental attributes, such as soil type, crop cover and the rainfall-runoff events (Wauchope, 1978; Schnoor, 1992; Scheunert, 1993). Sampling is assumed to provide an accurate understanding of the exposure events, based upon a random sampling regime. We can therefore use modelling to assist with characterising the exposure events, ensuring that the maximum likely exposures are accurate, and more importantly, assess the duration of exposure (Reinert *et al*., 2002; Solomon and Takacs, 2002). The validation of the model can also be an important feedback process to identify where more intensive sampling should be undertaken. However, because it is difficult for sampling regimes to capture the level of information required to conduct a comprehensive risk evaluation, exposure modelling can be used to augment sampling.

### *4.1.1 Selection of a spatial modelling framework*

The fate of a pesticide in the environment is governed by a number of complex interactions that include:

- Physicochemical characteristics of the pesticide (Wauchope, 1978; Mackay, 2001; Schnoor, 1996),
- Crop production and pesticide management practices (Wauchope, 1978; Silburn *et al.*, 2002; Simpson, 2007),
- Soil physical and hydrological properties (Wauchope, 1978; Silburn *et al.*, 2002),
- Physicochemical interactions that exist between the pesticide and the target environment (Schnoor, 1996; Mackay, 2001; Silburn *et al.*, 2002), and
- Climatic conditions during and following chemical use (Wauchope, 1978; Silburn *et al.*, 2002; Simpson, 2007).

To evaluate the fate of diuron, models were required that could adequately simulate these processes.

*4.1.1.1 Catchment-scale fate conceptual model*

A two-phase transport conceptual model was developed (Figure 4.1) to identify the relevant pesticide translocation processes in a catchment that would need to be modelled. These translocation processes broadly included chemical loading through field runoff (See conceptual model 1 in Figure 4.1), and translocation through the catchment stream network (See conceptual model 2 in Figure 4.1). These models were required to account for the fluxes in chemical loads from the point of application and while in transit through the catchment.

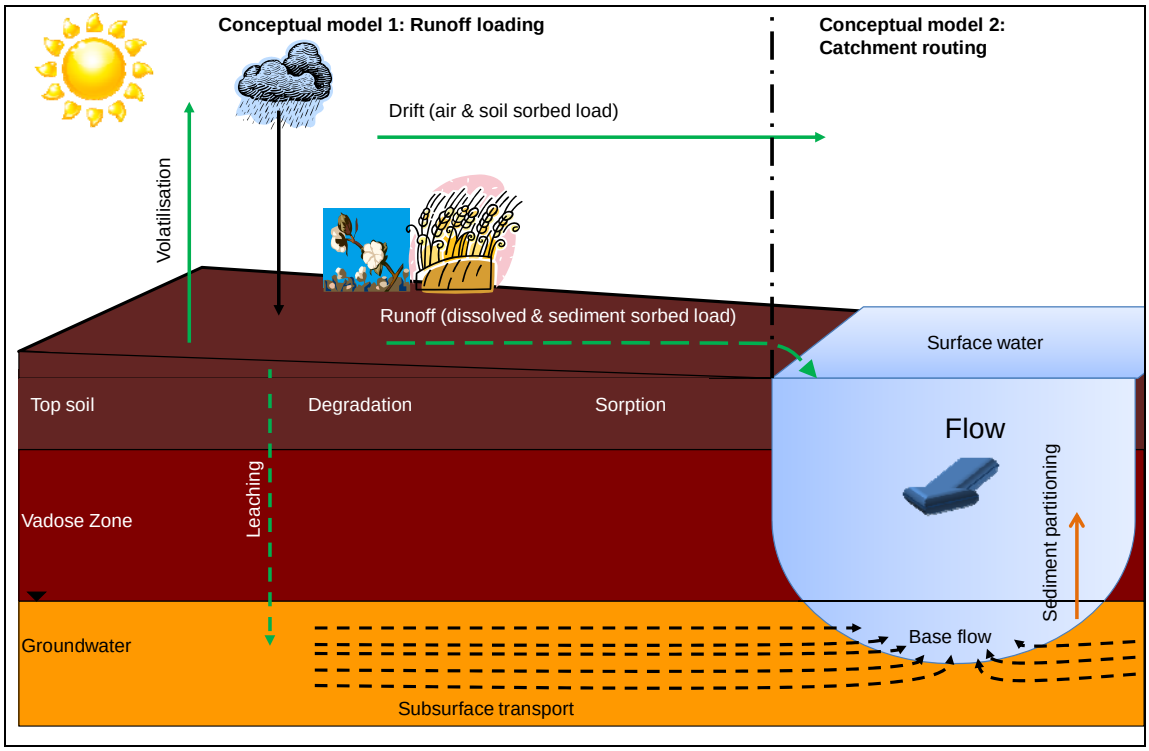



**Figure 4.1** Conceptual model of pesticide fate processes to be modelled in the Gwydir wetlands catchment (adapted from: Kookana *et al*., 1998; and Mackay, 2001).

*4.1.1.2 Selection of a suitable model framework*

A brief review of the literature identified a number of modelling frameworks. Specifically, a review for the USEPA by Parker *et al*. (2008), explored available modelling frameworks that were suitable for simulating pesticide fate in catchments spatially. These models included:

- the Soil Water Assessment Tool (SWAT) (Neitsch *et al.*, 2005),
- Nonpoint Source Model (NPSM) ,
- Hydrologic Simulation Program Fortran-Nonpoint Source Model (HSPF/NPSM) (Bicknell *et al.*, 2005), and
- The Pesticide Root-Zone Model-Riverine Water Quality (PRZM-RIVWQ) model (Williams *et al.*, 2004; Carousel *et al.*, 2005).

The models were evaluated by Parker *et al*. (2008) for potential use in regulatory fate modelling, but were unable to conclude which one was the most suitable. However calibration efforts were thought to rectify this outcome.

These catchment-scale pesticide fate models were briefly reviewed for their suitability in modelling diuron fate in the Gwydir Wetlands catchment based on data availability, model complexity and practicality; whether they had been applied to simulate pesticide fate in Australian conditions previously, and required computer power. It was found that all of these modelling frameworks were able to predict the same output necessary to fulfil the conceptual model requirements. However, the model framework of PRZM-RIVWQ was selected on the basis that (1) it had been applied previously in Australia (Hoogeweg *et al*., 2008), (2) it does not require large amounts of computer power, (3) inputs can be easily manipulated to suit different environments, and 4) PRZM is a regulatory fate model.

### *4.1.2 Aims*

To test the relevance of the estimated diuron exposure risk in the Gwydir wetlands catchment (Chapter 2), spatial exposure modelling was explored in this Chapter as a way to:

1. Overcome deficiencies in exposure data,
2. Characterise exposure pulses, and
3. Identify areas in the catchment contributing to chemical loading.

## 4.2 MATERIALS AND METHODS

This model framework was executed by the author with training and computer programming support provided by Waterborne Environmental Inc. (Leesburg, VA, USA).

### *4.2.1 Description of the chosen framework: PRZM-RIVWQ*

The objective of this modelling study was to estimate diuron exposure concentrations in the reaches of the Gwydir Wetlands catchment. The chosen framework required the initial collection and processing of terrain information to develop a manageable drainage network, consisting of sub-catchments and a link-node stream system. The link-node system defines the model geometry in which the simulated system is divided into a series of discrete volumes (nodes) linked by flow channels.

Combining the sub-catchment drainage information with readily available soil, climate and land use information, hydrological response units (HRUs) were developed as unique modelling scenarios (Beven, 2001). These unique scenarios were modelled to provide estimates for their edge of field chemical loadings using the one-dimensional dynamic compartmental model, Pesticide Root Zone Model (PRZM) (Carousel *et al.*, 2005). Edges of field loads were translated to exposure concentrations for each node segment using the Riverine Water Quality Model (RIVWQ) (Williams *et al.*, 2004). Combining these models allowed evaluation of how the variation in management practices may affect exposure to a pollutant at the sub-catchment level, effectively managing ecological risk.

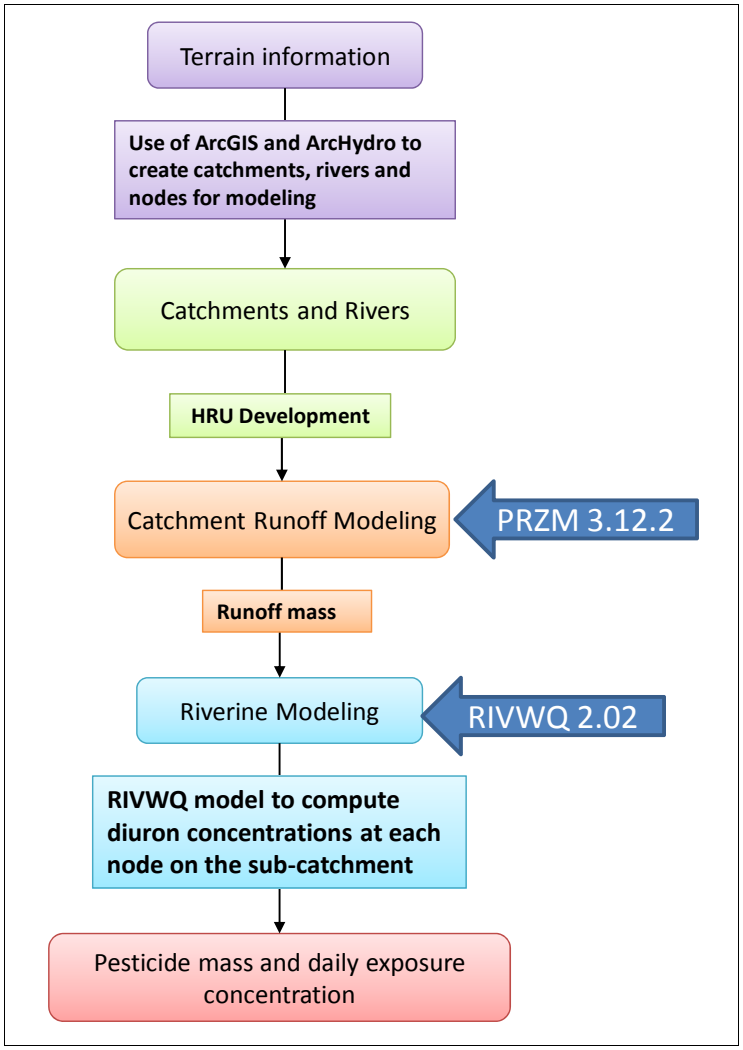



**Figure 4.2** Conceptual diagram of the spatial exposure modelling framework using PRZM-RIVWQ to evaluate diuron loading and concentrations in sub-catchments of the Gwydir wetlands catchment (HRU refers to Hydrological Response Unit; See later for formulation).

#### *4.2.1.1 Description of models used*

*Runoff model*

Water and chemical mass balances were simulated with PRZM, Version 3.12.2 (Carousel *et al.*, 2005). PRZM is a one-dimensional, dynamic, compartmental model that simulates chemical movement through unsaturated soil systems below the crop root and vadose zone. The model also simulates time-varying hydrologic behaviour on a daily time step, including runoff, infiltration, erosion and evapotranspiration (ET). PRZM estimates chemical transport by calculating pesticide uptake by plants, surface runoff, erosion, decay, vertical movement, foliar loss, dispersion, and retardation.

*Receiving water model*

Flow and chemical transport in the Gwydir wetlands catchment was simulated with RIVWQ Version 2.02 (Williams *et al.*, 2004). RIVWQ is capable of accommodating tributary systems, non-uniform flow, and mass loading along the length of the model system. Stream geometries are modelled as link-nodes that divide the system into discrete volumes (nodes) that are connected by links. The model assumes steady-state hydraulics that can change between time periods, where any changes are assumed to be instantaneous throughout the system, and flows are calculated using the continuity equation alone.

RIVWQ in this study was set up to simulate flow generated from PRZM or from readily available discharge gauge information, obtained from the PINNEENA 9.3 (NSWWI, 2008). As there were two points in the Gwydir River wetlands catchment diverting flow from the Gwydir River for irrigation, the available discharge data from all gauge discharge stations were used to simulate flow. However, where there was missing discharge data RIVWQ was set up to use PRZM discharge in its place.

*4.2.2 Requirements of the PRZM-RIVWQ diuron fate modelling framework*

The objective of this study was to evaluate the spatial variation of possible diuron load contributed by the different agricultural land uses to the reaches of the Gwydir wetlands catchment. The computer models PRZM and RIVWQ were combined to respectively evaluate the loads and chemical load transport in streams. To execute this framework, several stages of data collection, and processing was required including:

- Data collection,
- Sub-catchment and link-node stream network development,
- Hydrological response unit (HRU) development,
- Diuron use scenario development,
- Model input development,
- Model execution, and
- Post-processing of outputs.

*4.2.3 Data collection*

The environmental attributes required for the PRZM and RIVWQ models were obtained from readily accessible databases (see Table 4.1). The raw data were further processed to develop hydrological response units. This section details the collection and processing of environmental attributes used to support this modelling approach.

Spatial and temporal data were collected from a number of databases. The required spatial inputs include:

- digital elevation model (DEM) to determine slope and gravitational potential for water flow, and defining sub-catchment boundaries,
- catchment boundary to limit the drainage area,
- river networks for defining the link-node flow network,
- weather station locations for later determination of spatial influence of each weather station and the available climate information for that area,
- stream monitoring locations to aid in defining the links,
- soil properties (soil class, % organic carbon, layer depth, %sand, %silt and bulk density) that affect chemical transport and runoff, and

- land use, determining diuron applications and agronomic practices.

The sources and formats of these attributes are summarised in Table 4.1. The digital mapping software ArcGIS 9.3 was used to process these attributes for the Gwydir River wetlands catchment.

**Table 4.1** A summary of raw data collected, their format and sources which they were obtained to support modelling in the Gwydir River catchment

| Information | Format | Number of stations | Source |
|---|---|---|---|
| Spatial | | | |
| 30 m DEM | Raster | - | NASA (2010) |
| Catchment boundary | Polygon | - | AGGA[a] (2007) |
| Stream network | Polyline | - | AGGA[a] (2007) |
| Land use | Raster | - | ABARE (2006) |
| Weather station locations | Longitude and latitude recorded from database | - | BOM (2008) |
| Water quality monitoring station locations | Longitude and latitude recorded from database | - | NSWWI (2008) |
| Soil properties (soil class and physical properties) | Polygon and raster (respectively) | - | ABARE (2000a; 2000b, respectively) |
| Temporal | | | |
| Daily rainfall | Daily time step | 4 | BOM (2008) |
| Daily wind speed | Monthly daily averages | 3 | BOM (2011) |
| Average daily temperature | Daily maximum and minimum temperature | 2 | BOM (2011) |
| Daily solar | Monthly daily averages | 3 | BOM (2011) |
| Daily evapotranspiration | Monthly daily average | 2 | BOM (2011) |
| Daily discharge | Daily time step | 7 | NSWWI[b] (2008) |
| Chemical information | Labels and literature | - | Farrell (2008); and Nufarm (2009) |

[a]AGGA refers to Australian Government: Geoscience Australia
[b]NSWWI refers to New South Wales Government: NSW water information

*4.2.3.1 Link-node and sub-catchment development*

Links and nodes, and sub-catchments required for this modelling approach were developed using the spatial processing model ArcHYDRO 14. The GIS data inputs required were:

- 30 m DEM, and
- the stream network of the Gwydir River catchment

Firstly, the catchment, having characteristically low undulation, required the DEM to be reconditioned to enhance the ability for ArcHYDRO to estimate the "real" drainage lines. This involved burning the stream network in to the DEM lowering the elevation along the network by 100 m. All sinks in the DEM were then filled; the flow direction and accumulation grids, and stream definition and segmentation grids, and catchment grids and

polygons, and drainage lines were delineated. The Gwydir wetlands sub-catchment (Figure 4.3) polygon and its associated drainage line were then "clipped" as separate layers. The stream network and sub-catchment delineation was limited to flow downstream of the Gwydir River divergence to form the Mehi River, where both rivers are regulated by the Tareelaroi Weir. The selected drainage line and the Gwydir Wetlands catchment were then used for further processing to delineate a link-node and sub-catchment series.

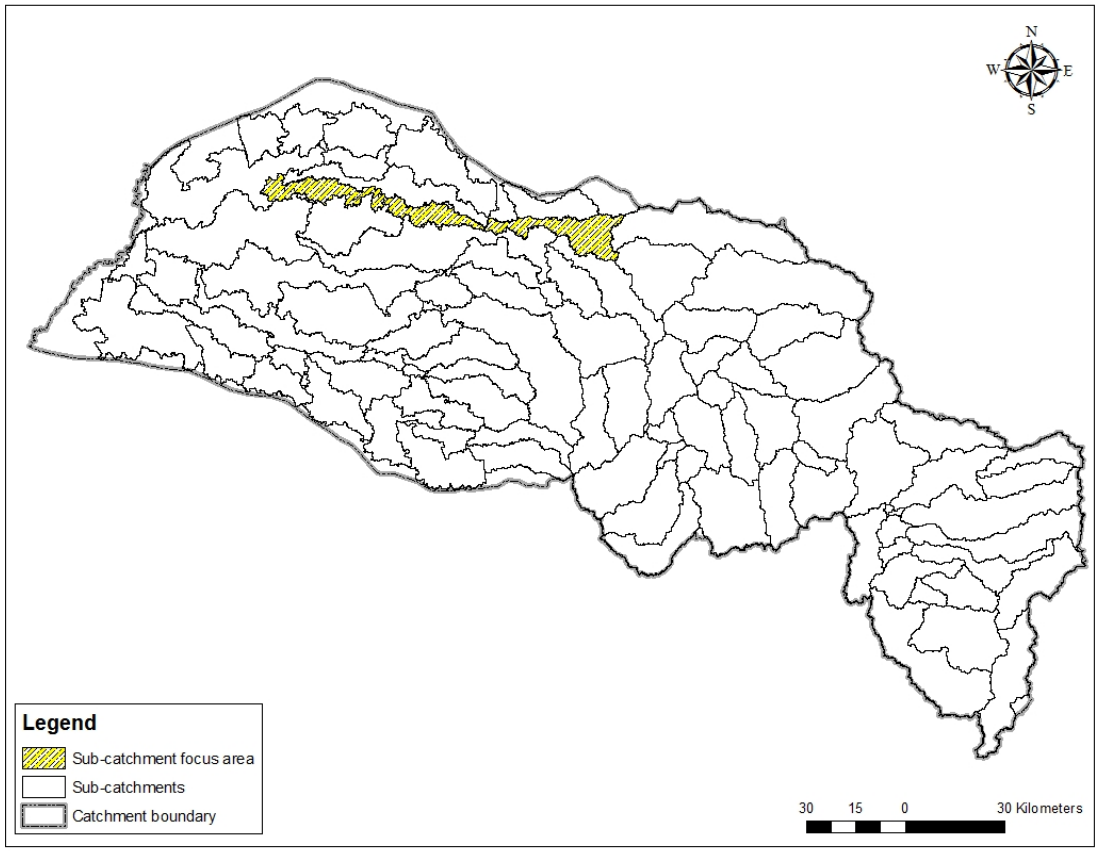



**Figure 4.3** Map of delineated sub-catchments of the Gwydir River catchment, with the Gwydir Wetlands sub-catchment being highlighted.

"Batch points" were "snapped" along the drainage line at 1500-2000 m intervals, giving priority to known discharge gauges and pesticide monitoring locations. These gauge and pesticide monitoring locations were taken from the stream flow database PINNEENA 9.3 (New South Wales Government: NSW water information, 2008) and pesticide monitoring sites of Chapter 2, which their unique identifiers are summarised in Table 4.2. Two batch points, each given a unique identifier, were placed at all intervals with each assigned as either an inlet or an outlet to respectively signify both the ending of a sub-catchment and the beginning of a downstream one. "Batch sub-catchments" were then delineated and manually

edited where anomalous sub-catchment delineations were identified, typically along the regions where the stream network was used to recondition the DEM. A total of 52 sub-catchments were delineated, of which 51 were used in the modelling. A map of the sub-catchments and their associated link-nodes is shown in Figure 4.4.

**Table 4.2** Summary of discharge and pesticide monitoring locations, their names and unique identifiers used in this modelling framework for the Gwydir wetlands sub-catchment.

| Unique identifier[a] | Name | Monitoring type | Location[b] | |
|---|---|---|---|---|
| | | | Latitude | Longitude |
| 418042 | Gwydir River, Downstream Tareelaroi weir | Discharge | -29.4333 | 150.0333 |
| 418036 | Gwydir River, Downstream Boolooroo weir | Discharge | -29.4164 | 149.8818 |
| 418004 | Gwydir River, Yarraman bridge | Discharge and pesticide | -29.4283 | 149.8461 |
| 418063 | Gwydir River, South arm downstream Tyreel offtake regulator | Discharge | -29.4395 | 149.7772 |
| 418053 | Gwydir River, Brageen crossing | Discharge and pesticide | -29.3983 | 149.5475 |
| 418078 | Gwydir River, Allambie bridge | Discharge and pesticide | -29.3478 | 149.4303 |
| 418066 | Gwydir River, Millewa | Discharge | -29.3635 | 149.3694 |

[a] Unique Identifier source: NSWWI (2008)
[b] Locations projected for Geodetic Datum of Australia 1994

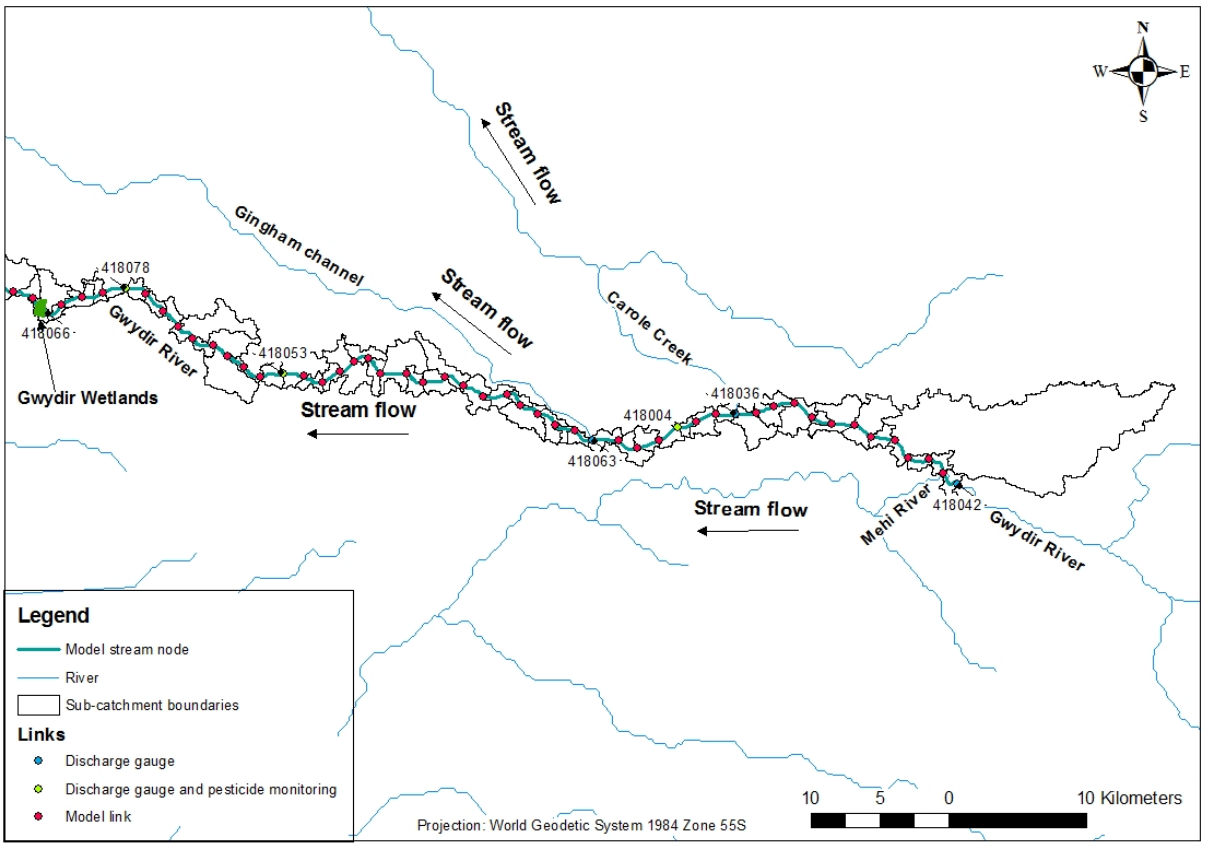



**Figure 4.4** Map of the delineated sub-catchment and link-node network for the Gwydir wetlands catchment.

### *4.2.2.2 Meteorological data collection and processing*

The climate variables required for this modelling approach included the following daily measurements:

- rainfall that drives pesticide foliar extraction and surface runoff,
- temperature influences evaporations, pesticide volatilisation, potential evapotrans-piration, and soil heat fluxes,
- wind speed that facilitates evaporation and air density,
- solar that influences soil albedo, and
- evapotranspiration that defines the crop water use.

These attributes were obtained from the BOM (Table 4.3).

A total of four weather stations were identified to have records of daily rainfall, two stations kept daily maximum and minimum temperature records (determined as averages), three with daily wind speed and solar monthly averages, and two with monthly average of daily evapotranspiration (Table 4.3). Specifically, the daily time step datasets of rainfall and temperature for each weather station had missing data for periods where gauges malfunctioned. This data substituted using data from the closest weather stations. Monthly averages of wind speed, solar, and evapotranspiration were assumed to occur on daily time steps for the modelling period.

**Table 4.3** Locations and measured climatic variables of weather stations in the Gwydir wetlands catchment

| Weather station ID[a] | Name | Location | | Available variables[b] | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Longitude | Latitude | Rainfall | Temperature | Wind | ET | Solar |
| 53033 | Pallamallawa Post Office | -29.4748 | 150.1359 | x | | | | |
| 53070 | Moree (Mallowa) | -29.6202 | 149.3770 | x | | | | |
| 52010 | Weemelah (Crinolyn) | -29.2530 | 149.1207 | x | | | | |
| 53115 | Moree Aero | -29.4914 | 149.8458 | | x | x | x | x |
| 52020 | Mungundi Post Office | -28.9786 | 148.9899 | | x | x | | x |
| 53048 | Moree Comparison | -29.4819 | 149.8383 | | | x | x | x |

[a] Weather station ID's from BOM (2008)
[b] x refers to the availability of the climatic variable for the weather station

Not all the weather station in the catchment kept records of these required climate variables. This deficiency was overcome by developing "generalised weather stations". The generalised weather stations were required to contain daily data for each climate variable for the modelling period. Firstly, the weather stations were characterised for their spatial influences in the catchment by developing Thiessen polygons from a weather station point layer (Figure 4.5). The Thiessen polygons were then "dissolved" or merged to form a single layer, with the weather station identifiers used to indicate the stations required to generate the "generalised weather stations" datasets. Subsequently, five "generalised weather stations" were developed, each with unique in daily rainfall, temperature, wind speed, solar and evapotranspiration (Table 4.5).

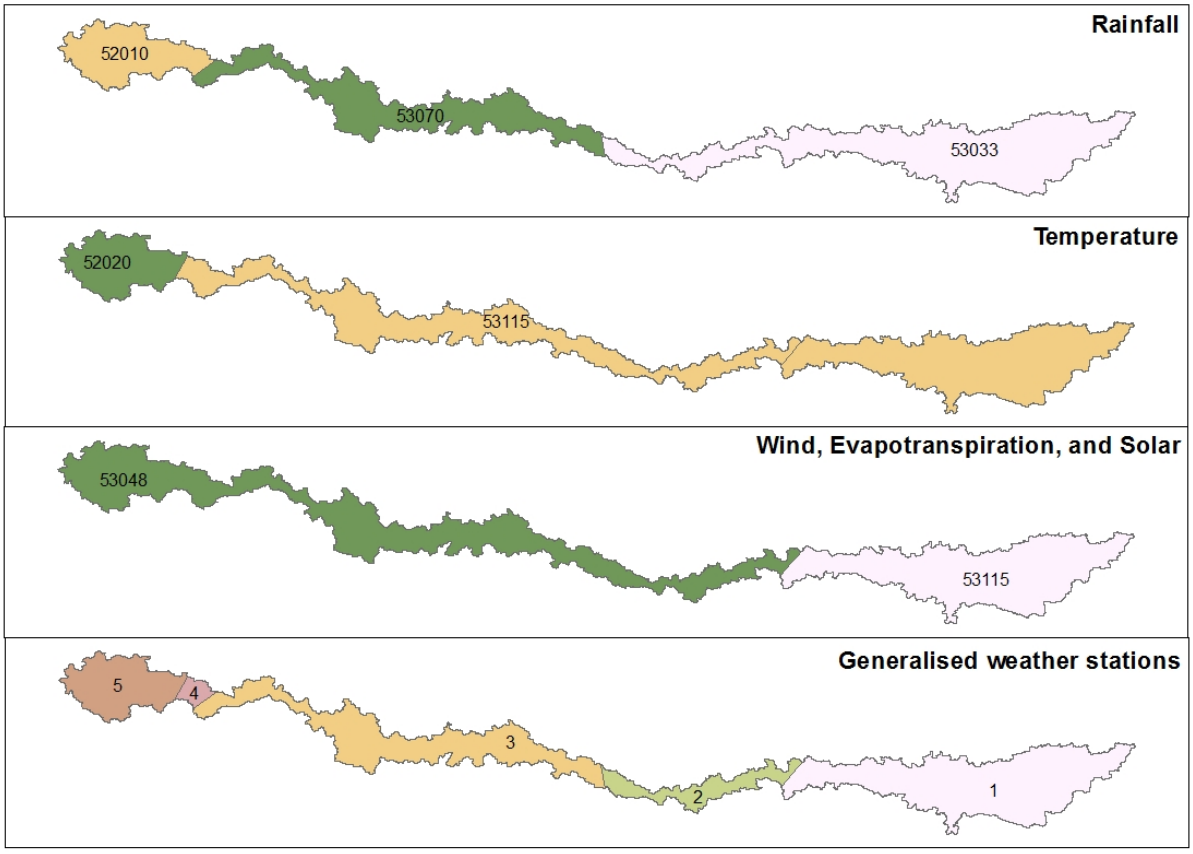



**Figure 4.5** Spatial influences of weather stations in the Gwydir Wetlands catchment defined by Thiessen polygons.

**Table 4.4** Description of generalised weather stations in the Gwydir Wetlands catchment

| Generalised weather station ID | Weather station ID source of climate variable[a] | | | | |
|---|---|---|---|---|---|
| | Rainfall | Temperature | Wind | Solar | Evapotranspiration |
| 1 | 53033 | 53115 | 53115 | 53115 | 53115 |
| 2 | 53033 | 53115 | 53048 | 53048 | 53048 |
| 3 | 53070 | 53115 | 53048 | 53048 | 53048 |
| 4 | 52010 | 53115 | 53048 | 53048 | 53048 |
| 5 | 52010 | 52020 | 53048 | 53048 | 53048 |

[a] Weather station ID source from BOM (2008).

*4.2.3.3 Soils database*

A database of soil physical properties was developed using available spatial soil databases. The spatial data used were unique soil class polygons of the digital Atlas of Australian soils (ABARE, 2000a); and the soil physical properties of %clay, %sand, and %organic carbon, bulk density and horizon depth (ABARE, 2000b).

Using the Gwydir wetlands catchment boundary, the unique soil classes in the Digital Map of Australian soil were "clipped". A total of five unique soil class polygons were obtained for

the modelling area (Figure 4.6). These polygons were then used to clip the soil physical properties layers. From this, statistical analysis using ArcGIS 9.3 on the clipped raster datasets determined the 90$^{th}$ percentile soil physical properties (Table 4.6). Soil moisture characteristics of saturated hydraulic conductivity ($K_{sat}$); available water-holding capacity (AWC); Field capacity (FC); and wilting point (WP) were estimated by entering the %clay, %sand, and %OM in to the Soil Water Characteristics calculator v6.02 (Saxton and Rawls, 2011). These simplified soil groups were collated for use as model inputs in PRZM.

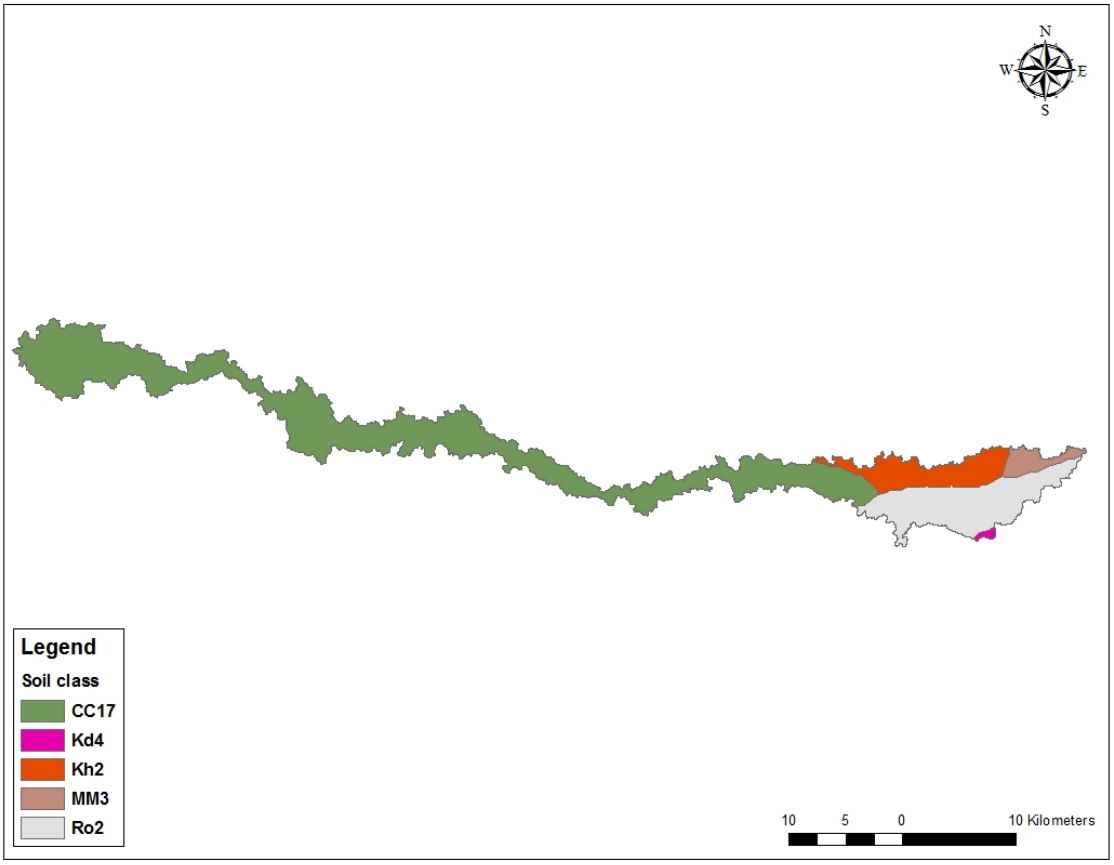



**Figure 4.6** Clipped unique soil classes (map unit) of the Gwydir River sub-catchments obtained from the Digital map of Australian soils (ABARE, 2000a).

**Table 4.5** Summary of 90th percentile soil physical properties by unique soil class (map unit) for the sub-catchments of the Gwydir River catchment.

| Map Unit | CSIRO Code | Subdivision | Layer | Thickness (cm) | %Sand | %Clay | %OC | BD (g $cm^{-3}$) | $K_{sat}$ (mm $h^{-1}$) | AWC (cm $cm^{-1}$) | FC (%) | WP (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CC17 | 16455 | Cracking clay | 1 | 19.7 | 33.0 | 62.0 | 0.31 | 1.25 | 0.04 | 0.12 | 47.6 | 35.8 |
| | | | 2 | 132.6 | 22.0 | 62.0 | 0.34 | 1.45 | 0.33 | 0.12 | 47.1 | 35.2 |
| Kh2 | 16974 | Cracking clay | 1 | 18.5 | 40.0 | 58.0 | 0.32 | 1.37 | 0.01 | 0.12 | 46.6 | 34.5 |
| | | | 2 | 137.4 | 29.0 | 60.5 | 0.35 | 1.53 | 0.16 | 0.12 | 46.3 | 34.2 |
| MM3 | 17376 | Cracking clay | 1 | 13.7 | 34.8 | 62.0 | 0.23 | 1.22 | 0.02 | 0.12 | 47.7 | 35.5 |
| | | | 2 | 132.2 | 22.0 | 62.0 | 0.26 | 1.45 | 0.32 | 0.12 | 47.2 | 35.2 |
| Ro2 | 17412 | Brown duplex | 1 | 20.0 | 50.0 | 47.3 | 0.30 | 1.40 | 0.22 | 0.11 | 39.5 | 28.2 |
| | | | 2 | 137.7 | 24.3 | 62.0 | 0.34 | 1.63 | 0.25 | 0.12 | 47.2 | 35.3 |
| Kd4 | 17432 | Cracking clay | 1 | 17.5 | 33.8 | 62.0 | 0.36 | 1.33 | 0.03 | 0.12 | 47.5 | 35.5 |
| | | | 2 | 150 | 22.0 | 62.0 | 0.37 | 1.43 | 0.33 | 0.12 | 47.1 | 35.2 |
| CC17 | 16455 | Cracking clay | 1 | 19.7 | 33.0 | 62.0 | 0.31 | 1.25 | 0.04 | 0.12 | 47.6 | 35.8 |
| | | | 2 | 132.6 | 22.0 | 62.0 | 0.34 | 1.45 | 0.33 | 0.12 | 47.1 | 35.2 |

*4.2.3.4 Land use*

The land use in the Gwydir River catchment was obtained as a digitised raster (1 $km^2$ grid) format from the Australian Natural Resources database. The raster was transformed to a polygon series and the land uses simplified into classes of conservation, pasture, agroforestry, dry land cereal, oilseed, cotton and legumes; and irrigated cotton (Figure 4.7). The dominant land uses in the Gwydir River wetlands catchment are pasture; and cereal, cotton, and legume cropping production (Figure 4.7). The cropping practices that may potentially use diuron are cotton, grain and legume types; based on the recommended application regimes described on the product labels. The various land uses have ramifications in the modelling operations for hydrology and pesticide application regimes in PRZM. This data will be used to support crop input development (see later).

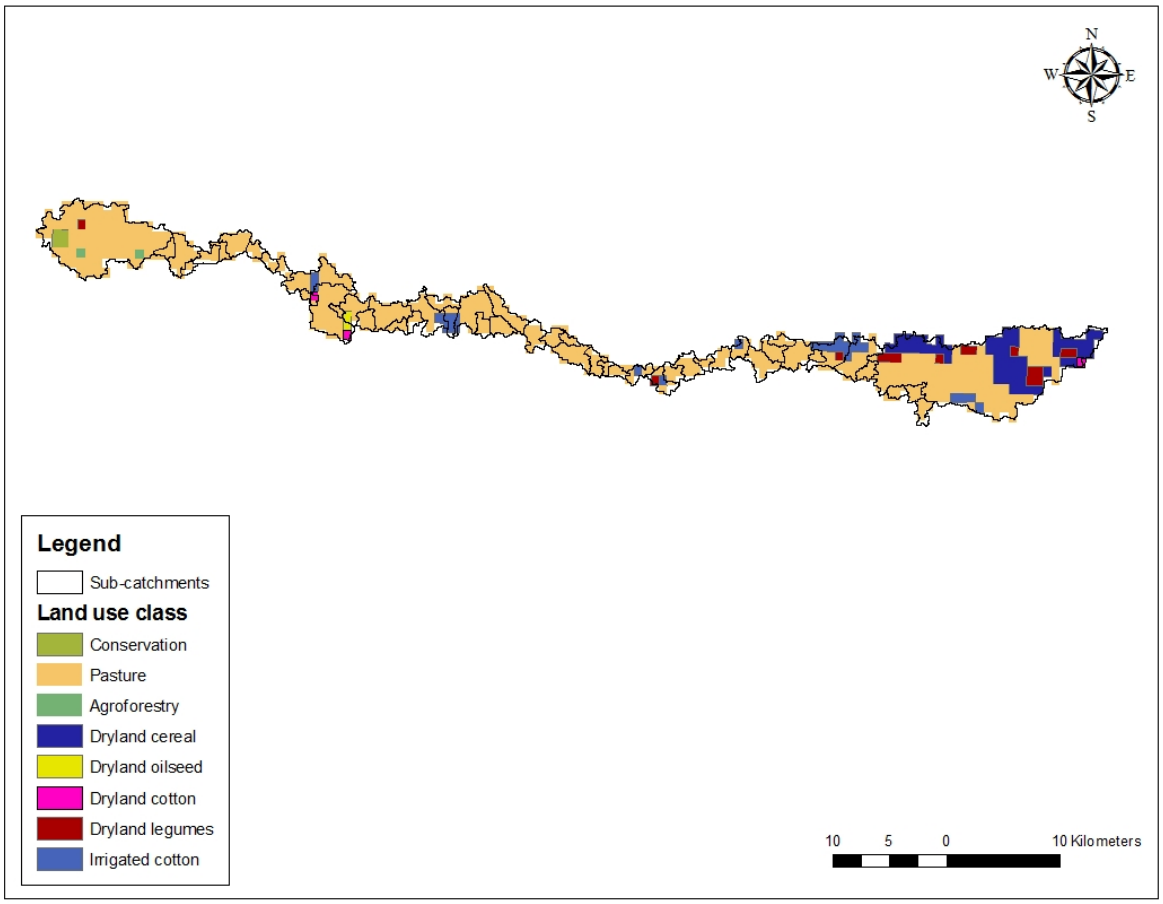



**Figure 4.7** Map of land use polygon classes in the Gwydir wetlands catchment

*4.2.3.5 Hydrological response units*

Hydrological response units (HRU) in the sub-catchments were developed to simplify the model inputs for each PRZM land use scenario unique to the Gwydir wetlands catchment. These HRU's were homogeneous in soil properties, weather stations, and land use classifications. This was developed by dissolving the land use, soil, and generalised weather station Thiessen polygons (Figure 4.8). The HRU output highlighted that a total of 92 unique modelling scenarios were required to be represented in the PRZM modelling for the Gwydir wetlands sub-catchments, of which 25 unique land use, soil and weather station simulations were required in fate modelling.

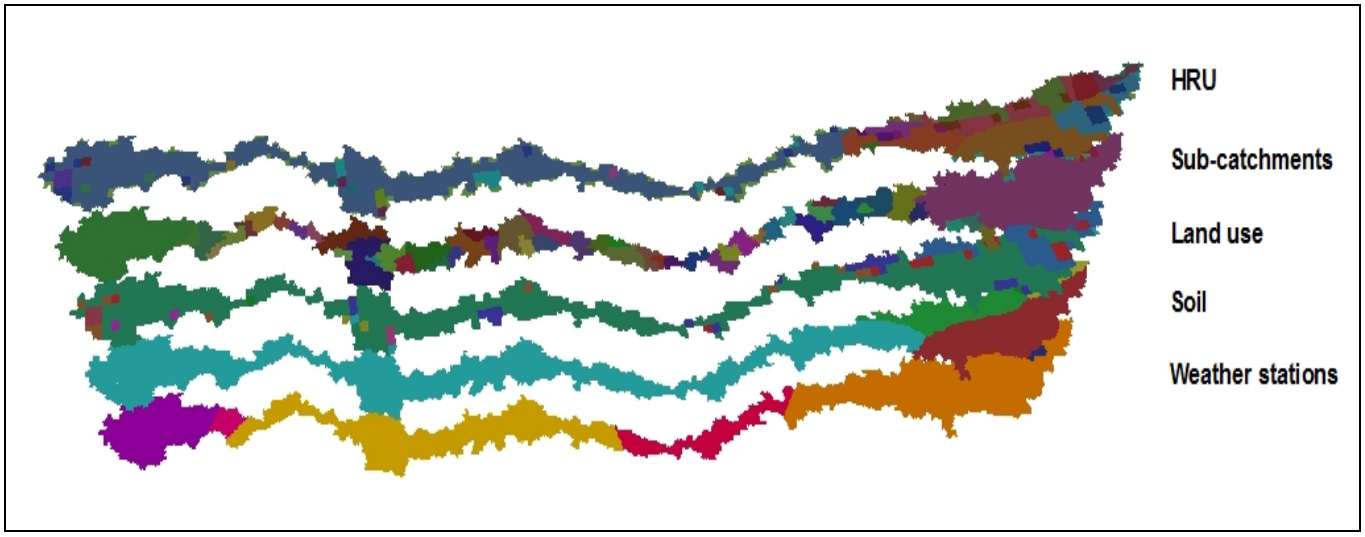



**Figure 4.8** Layer series map depicting the formation of hydrological response units for model simulation, from sub-catchment, land use, soil and weather station polygon layers with each colour representing a different attribute class in the layer.

*4.2.4 PRZM inputs and modelling scenarios*

To support the different HRUs as inputs to PRZM, simplified crop scenarios were devised to test the model framework capability. Physicochemical and environmental fate properties of diuron were collated (as described in Chapter 1) and a crop database was developed to support the input development for the different modelling scenarios.

*4.2.4.1 Crop database and pesticide application scenarios*

A crop input database was developed to characterise the unique PRZM scenario inputs for the different HRUs. The cropping scenarios considered were irrigated and dryland cotton, cereals

(wheat), pasture, legumes (chickpea), and oil seeds (canola). Table 4.7 summarises the different modelling inputs parameters used for each cropping scenario, with input details given in Appendix 4. Critical agronomic and diuron use practices simulated for pasture, dryland cereal (wheat), oilseed (canola), dryland legumes (chickpea), and dryland and irrigated cotton are described.

**Table 4.7** Summary of PRZM 3.12.3 input parameters and their respective lookup variables of Carousel et al. (2005).

| Parameter | PRZM 3.12.3 Code |
|---|---|
| Simulation period | |
| Simulation start date | ISDAY, ISMON, ISTYR |
| Simulation end date | IEDAY, IEMONTH, IEYR |
| Hydrologic Data | |
| Precipitation (cm) | PRECIP |
| Air Temperature ($^oC$) | TEMP |
| Pan Factor | IPEIND |
| Snow Factor (cm/$^oC$) | SFAC |
| Pan Evaporation Flag | IPEIND |
| Evaporation Extraction, Minimum Depth (cm) | ANETD |
| Flag for initial crop | INICRP |
| Crop condition (initial) | ISCOND |
| Erosion | ERFLAG |
| Area (ha) | AFIELD |
| NRCS 24-hour hyetograph | IREG |
| Crop data | |
| Number of crops simulated | NDC |
| Crop number for the different crop | ICNCN |
| Maximum interception storage (cm) | CINTCP |
| Maximum active root depth (cm) | AMXDR |
| Maximum areal coverage (%) | COVMAX |
| Soil surface condition after harvest | ICNAH |
| Runoff curve number for AM-II (fallow, crop, residue) | CN |
| Max. Dry weight of crop (kg $m^{-2}$) | WFMAX |
| Max. Canopy height | HTMAX |
| Crop number | CROPNO |
| Number of cropping periods | NCPDS |
| Day for crop emergence | EMD |
| Month for crop emergence | EMM |
| Year for crop emergence | IYREM |
| Day for crop maturation | MAD |
| Month for crop maturation | MAM |
| Year for crop maturation | IYRMAT |
| Day for harvest | HAD |
| Month for harvest | HAM |
| Year for harvest | IYRHAR |
| Crop number associated with NDC | INCROP |
| Irrigation data | |
| Irrigation flag | IRFLAG |
| Irrigation type | IRTYP |
| Salinity leach factor requirement | FLEACH |
| Percent depletion | PCDEPL |
| Application efficiency | RATEAP |
| Pesticide application data | |
| Number of applications | NAPS |
| Application month, day and year | APM,APD, APY |
| Chemical application method | CAM |
| Incorporation depth (cm) | DEPI |
| Target application rate (kg $ha^{-1}$) | TAPP |
| Application efficiency (fraction) | APPEFF |
| Spray drift | DRFT |
| Filtration parameter for exponential foliar application model | FILTRA |
| Disposition of foliar pesticide after harvest | IPSCND |

| | |
|---|---|
| Plant uptake factor | UPTKF |
| | |
| Pesticide fate data | |
| Soil type | STITLE |
| Soil-water adsorption coefficient, $K_d$ (mL $g^{-1}$) | KD |
| Decay rate, dissolved ($day^{-1}$) | DWRATE |
| Decay rate, adsorbed ($day^{-1}$) | DSRATE |
| | |
| Soil data | |
| Number of horizons | NHORIZ |
| Depth of soil core (cm) | CORED |
| Horizon thickness (cm) | THKNS |
| Layer thickness (cm) | DPN |
| Bulk density (g $cm^3$) | BD |
| Field capacity ($cm^3$ $cm^{-3}$) | THEFC |
| Wilting point ($cm^3$ $cm^{-3}$) | THEWP |
| Initial soil moisture ($cm^3$ $cm^{-3}$) | THETO |
| Organic Carbon (%) | OC |
| Hydrodynamic dispersion ($cm^2$ $day^{-1}$) | DISP |
| Soil drainage parameter (L $day^{-1}$) | AD |

*Pasture*

Pasture was simulated to emerge on 1 August 1991, mature on 2 August 1991 and be harvested on 31 December 2007 (at the end of the model period). Diuron was not simulated to be applied in either modelling scenario, and the pasture was assumed to grow throughout the course of the modelling period

*Dryland cereal (wheat)*

Dry-land wheat was simulated to emerge on 25 May, mature on 25 November and be harvested on 25 December each simulation year (Felton *et al.*, 1995; Carousel *et al.*, 2005). A single diuron application was simulated as a post-emergence at the maximum rate (0.45 kg a.i. $ha^{-1}$; NuFarm 2009a). This involved applying diuron directly to the soil surface, and was assumed to not infiltrate past the top 5 mm of soil. At harvest, crop residues were simulated to remain and were incorporated prior to planting of the next wheat crop in the following season.

*Dryland oilseed (canola)*

Dry land canola was simulated to emerge on 20 May, mature on 14 December and be harvested on 24 December each simulation year (Carousel *et al.*, 2005). Diuron was not simulated to be applied, as it is not registered for use in canola. At harvest, canola crop residues were simulated to remain and were incorporated prior to planting of the next canola crop in the following season.

*Dryland legume (chickpea)*

Dry land chickpea was simulated to emerge on 20 June (Felton *et al.*, 1995; Hulugalle *et al.*, 2001), mature on 5 November (Felton *et al.*, 1995; Hulugalle *et al.*, 2001) and be harvested on 10 November each simulation year (Carousel *et al.*, 2005). A single diuron application was simulated as a pre-emergence on 15 June using the mean rate (0.9 kg a.i. $ha^{-1}$; NuFarm 2009a). This involved applying diuron at sowing directly to the soil surface and incorporating the chemical to a depth of 4 cm (Nufarm, 2009). At harvest, crop residues were simulated to remain and were incorporated prior to planting of the next chickpea crop in the following season.

*Irrigated and dryland cotton*

Irrigated and dryland cotton crops were simulated to emerge on 1 October, mature on 1 April and be harvested on 1 May each simulation year (Hulugalle *et al.*, 2001). A single diuron application was simulated to be applied each year, in separate modelling scenarios, as either as a pre-emergence on 15 June, or post-emergence on 15 November at the maximum rate (1.8 kg a.i. $ha^{-1}$; Farrell, 2008; NuFarm, 2009a). The pre-emergence scenario involved applying diuron at sowing directly to the soil surface and incorporating the chemical to a depth of 4 cm (Nufarm, 2009); and the post-emergence scenario involved applying diuron directly to the soil and was assumed to not infiltrate past the top 5 mm of soil. At harvest, crop residues were simulated to remain and were incorporated prior to planting of the next chickpea crop in the following season.

Specifically, under the irrigated cotton scenario, cotton was simulated to be grown under furrow irrigation system. This irrigation system assumed irrigation water was applied when 55% of the soil moisture was depleted, and that 20% of the applied water runoff the farm as tail water (Carousel *et al.*, 2005).

*4.2.5 RIVWQ inputs*

The inputs to RIVWQ involved firstly defining the stream (link-node) network morphology. These attributes included defining the bank full[18] width (m), depth (m) and discharge ($m^3$ $s^{-1}$), and the length (m) of each node for each link. The bank full widths and depths had previously

[18] Bankfull refers to the maximum discharge for a stream before it breaks its banks

been characterised for some monitoring locations in the database PINNENA 9.3 (New South Wales Government: NSW water information, 2008). For the links that have no morphology information, these attributes were extrapolated for the upstream and downstream links based on the calibration curve of distance downstream and bank full depth (Appendix 4). Bank full widths were further estimated using Google Earth (version 6.03) measurement tool with a polygon of links overlaid to aid in the measurement process. In all cases, the bank full discharges were estimated from discharge and measured height data and the sub-catchment drainage area were collated from the sub-catchment delineation for use in RIVWQ. The model was set-up to use readily available discharge data from PINNENA 9.3 (NSWWI, 2008). However, where discharge data was missing, PRZM runoff estimates were used in its place.

The predicted edge of field masses produced under each HRU was up-scaled to the sub-catchment level from 1 $km^2$ polygons that PRZM simulated. Factors were assigned to the simulation according to how much surface area the homogeneous HRUs occupied in each sub-catchment. These sub-catchment diuron masses became the chemical loading inputs for RIVWQ.

### *4.2.6 Statistical analysis*

*Time series and risk calculation*

Time series concentration outputs of both post- and pre-emergence modelling scenarios for each sub-catchment were plotted against simulation dates using SigmaPlot 11.0, together with monitoring data and the 1992 simulation year for Gwydir River, Allambie bridge. The mean, median and $90^{th}$ percentile exposure concentrations at the three pesticide monitoring sites of Gwydir River, Yarraman bridge; Gwydir River, Allambie bridge; and Gwydir River, Brageen crossing calculated using "describe data" function in SigmPlot 11.0. Characterising whether the predicted exposures were resulting in some form of ecosystem disturbance, diuron exposure *Risk* of exceeding the $HC_5$ (1.0 µg $L^{-1}$; Chapter 2) was estimated for each monitoring sites using the risk estimation method described in Chapter 2.

*Pulse duration characterisation and ecological significance*

Pulse exposure durations were characterised using the diuron exposure concentration time series. These were tested for the number of days the predicted diuron concentration were

either above the monitoring LOQ (0.1 µg $L^{-1}$) and exceeded the diuron $HC_5$. Where concentrations exceeded the monitoring LOQ and the $HC_5$, maximum, median, 90$^{th}$ percentile were determined by using cumulative probability distributions. This estimations was the same as that used to characterise the range of exposure and toxicity data in Chapter 2, except pulse durations were assigned a *%Rank* and plotted where $\log_{10}$ duration (days) was the independent variable, and the *%Rank* on the probability scale was the dependent. The probability of any pulse greater than the monitoring LOQ, and the $HC_5$ lasting a certain duration was similarly defined by a linear regression ($y = mx + b$). Specifically, the probability that a pulse was exceeding a standard 96 h algae exposure toxicity test was estimated (USEPA, 1996), which is in the chronic exposure range greater than 12 h (de Zwart, 2002).

*Error calculation*

To compare the modelling results with monitoring observations, statistical methods used by Parker *et al*. (2007) and Mamy *et al*. (2008). The mean absolute error (MAE), root mean square error (RMSE), and Nash-Sutcliffe coefficient of modelling efficiency (NS) were calculated. Specifically, MAE and RMSE were calculated to quantify the deviation in the model predictions from what has been observed in monitoring data. The MAE was calculated by:

$$\mathrm{MAE} = \frac{\left(\sum |y_i - \hat{y}_i|\right)}{n} \qquad (4.1)$$

And the RMSE was calculated using:

$$\mathrm{RMSE} = \left\{ \frac{\left[\sum (y_i - \hat{y}_i)^2\right]}{n} \right\} \qquad (4.2)$$

Where $y_i$ is the $i$th monitoring observation, $\hat{y}_i$ is the $i$th simulation value, and $n$ is the number of observations.

The NS modelling efficiency parameter was calculated to estimate model performance. NS has a maximum of 1.0 and indicates perfect relationship between model and monitoring data,

and where very negative values indicate poor relationship. An NS of less than 0 suggests that the average of monitoring data is a better predictor for exposure than the simulated output. NS was calculated using:

$$NS = 1 - \frac{\sum (y_i - \hat{y}_i)^2}{\sum (y_i - \bar{y})^2} \qquad (4.3)$$

Where □ is the average monitoring concentration (μg $L^{-1}$).

## 4.3 RESULTS

Time series as well as cumulative frequency distributions were prepared to compare monitoring data from Gwydir River, Yarraman Bridge; Gwydir River, Allambie bridge; and Gwydir River, Brageen crossing with modelled exposure data from both the cotton pre- and post-emergence application simulations.

### *4.3.1 Time series diuron exposure concentration*

The model framework predicted spatially and temporally variable diuron exposure occurring in the reaches of the Gwydir wetlands catchment under the pre- and post-emergence diuron application in cotton. Figure 4.9 shows a typical simulation time series for the year 1992 at Gwydir River, Brageen crossing monitoring site. Specifically, diuron exposure peaks were predicted to occur between the months July-August under post- and pre-emergence scenarios, and November-December for post-emergence scenario only. These peak concentrations were predicted to occur for rainfall events following the time when chemical was simulated to be applied, in wheat, cotton, and chickpea crops. The model output shows, that after a number of rainfall events passed, the frequency and magnitude in diuron exposure declined, which corresponded well with monitoring data, especially under the post-emergence simulation for cotton. However, the model did not always predict peak exposure concentrations on precisely the same date as monitoring.

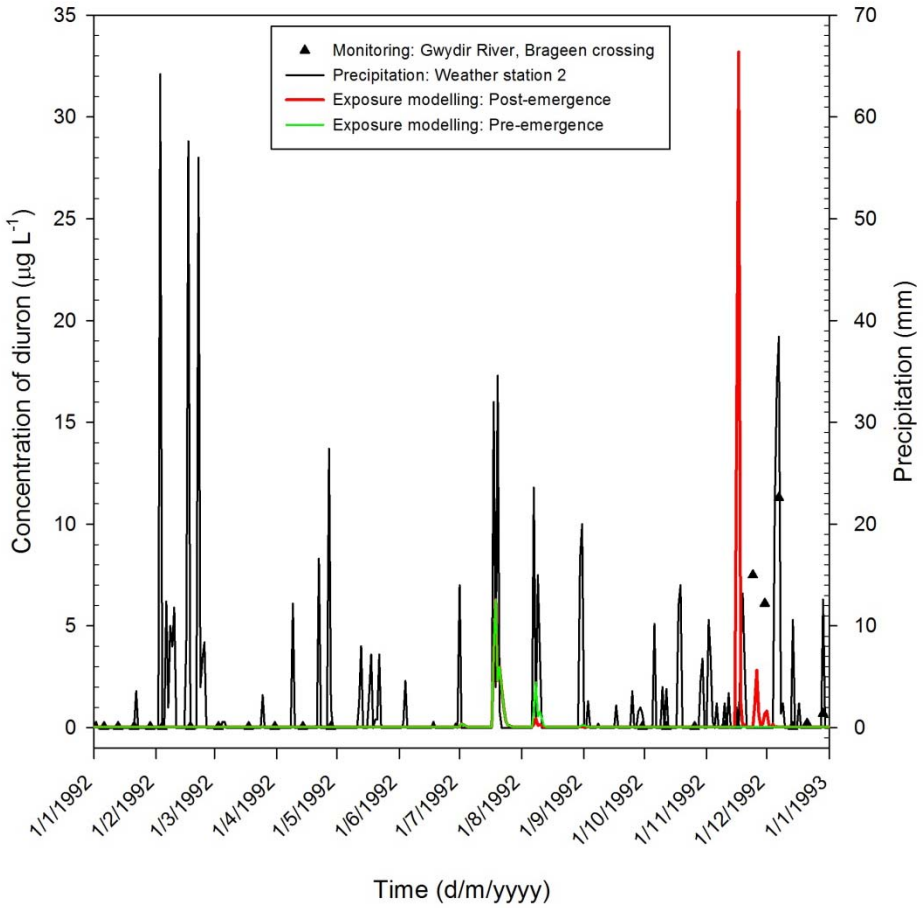



**Figure 4.9** Time series of monitoring and estimated diuron exposure concentrations (µg $L^{-1}$) at Gwydir River, Brageen crossing, for the year 1992 under pre- and post-emergence diuron applications in cotton production.

Peak diuron exposure was consistently predicted by both the post- and pre-emergence model scenarios to be most prevalent between the months July-December at Gwydir River, Yarraman bridge (Figure 4.10a); Gwydir River, Brageen crossing (Figure 4.9b); and Gwydir River, Allambie bridge (Figure 4.10c). However, the model was unable to predict peak exposures on precisely the same date as the monitoring data. This characteristic was observed when monitoring data was compared with all model time series data at Gwydir River, Brageen crossing (Figure 4.10a); and other monitoring sites of Gwydir River, Yarraman bridge (Figure 4.9 b); and Gwydir River, Allambie bridge (Figure 4.10 c). The post-emergence model scenario did predict within the range of monitoring exposure concentrations between the years 1991-2000 at Gwydir River, Brageen crossing and consistently over predicted exposure between the monitoring years 2001-2007. It is difficult to confirm if the model was predicting a similar outcome for the other monitoring sites of Gwydir River, Yarraman Bridge; and Gwydir river, Allambie Bridge due to the lack of monitoring data available for the years 1991-2001.

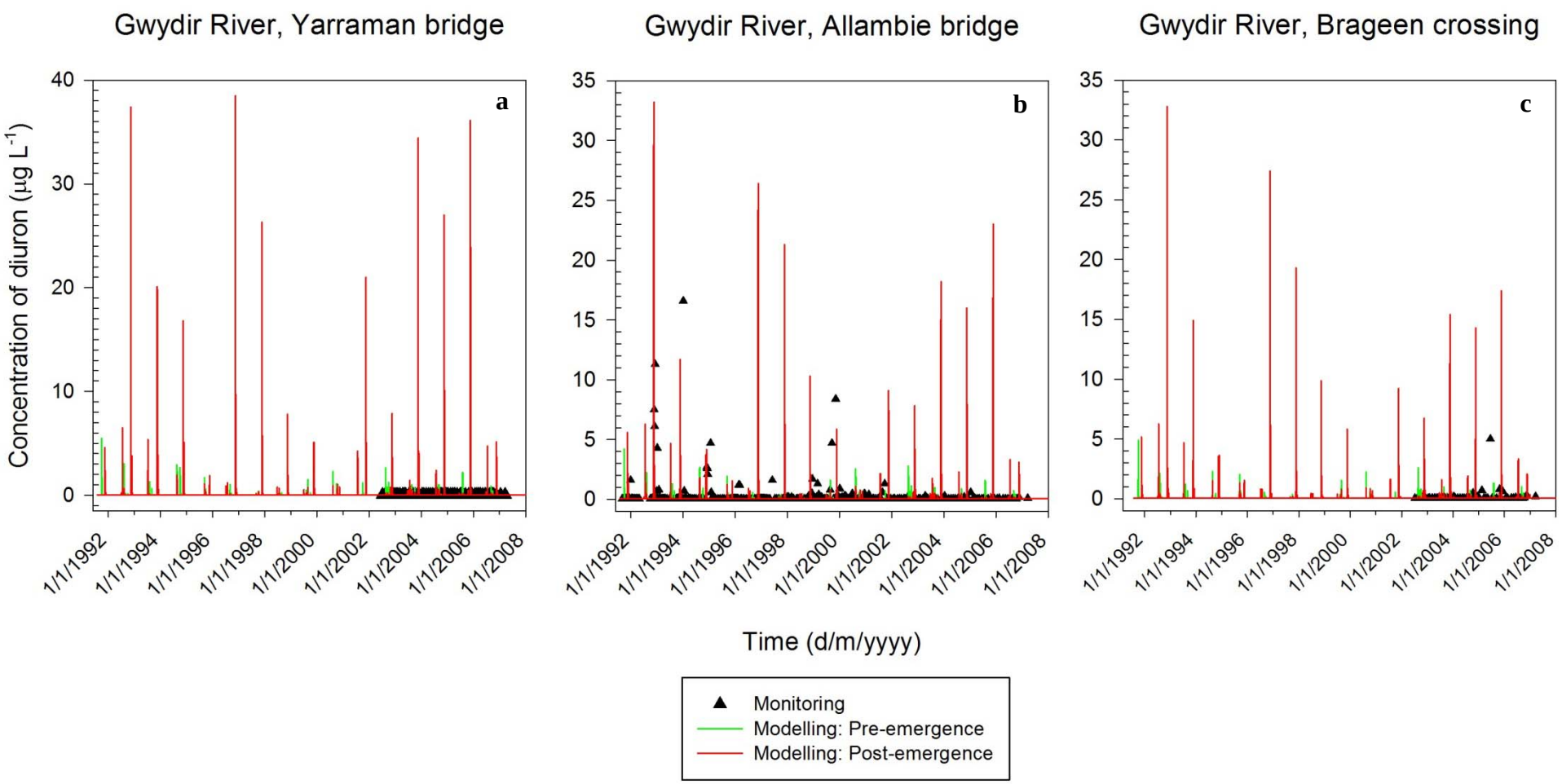



**Figure 4.10** Time series of monitoring and estimated diuron exposure concentrations (µg $L^{-1}$) under pre- and post-emergence diuron applications in cotton production at the monitoring sites Gwydir River, Yarraman bridge; Gwydir River, Allambie bridge; and Gwydir River, Brageen crossing.

The median exposure concentrations in the monitoring and modelling data were less than the monitoring LOQ (Table 4.8), and the 90$^{th}$ percentile concentrations could only be determined for the monitoring data to range from 0.5 μg L$^{-1}$ at Brageen crossing; to 0.3 μg L$^{-1}$ at Allambie bridge. The maximum exposure concentrations, compared with monitoring dates, ranged from 0.5-15.4 μg L$^{-1}$ for the post-emergence scenario, and from less than the monitoring LOQ to 0.6 μg L$^{-1}$ under the pre-emergence scenario.

Using all time series data, the maximum exposure concentrations under the pre-and post-emergence scenarios for all three monitoring sites ranged 32.8-38.5 μg L$^{-1}$ and 6.3-6.5 μg L$^{-1}$, respectively. When compared to the monitoring data, the post- and pre-emergence model scenarios under predicted the maximum exposure by 7% and 96%, respectively, at Gwydir River, Brageen crossing; 90% and 98% at Gwydir River, Allambie bridge; and over predicted by 1900% under the post-emergence scenario only at Gwydir River, Yarraman bridge.

The error and prediction efficiency, respectively quantified by MAE and RMSE, and NS were estimated to vary between monitoring sites and modelling scenarios (Table 4.8). Specifically, the MAE ranged 0.03-0.16 μg L$^{-1}$ between monitoring sites for the post-emergence scenario, and 0.00-0.32 μg L$^{-1}$ for the pre-emergence scenario, with the highest determined for the pre-emergence scenario at Gwydir River, Brageen crossing (MAE = 0.32 μg L$^{-1}$; Table 4.8). In all cases the calculated MAE's were less than their respective observed means for the monitoring sites (Table 4.8). The RMSE ranged 0.17-1.87 μg L$^{-1}$ between monitoring sites for the post-emergence scenario, and 0-0.69 μg L$^{-1}$ for the, with the highest RMSE determined for the post-emergence scenario at Gwydir River, Brageen crossing. All NS modelling efficiencies (Table 4.8), except for the Gwydir River, Yarraman bridge (NS = 1.00) pre-emergence scenario, were found to be negative (-0.02 to -2.4x10$^{31}$), suggesting that the average of the observed data was a better predictor for the monitoring dates than the model.

**Table 4.8** Summary of mean, median and 90th percentile diuron exposure concentrations comparing exposure under monitoring and all modelling dates for post- and pre-emergence scenarios at the monitoring sites of Gwydir River, Yarraman bridge; Gwydir River, Brageen crossin; and Gwydir River, Allambie bridge. Calculated errors of MAE, RMSE, and NS are given together with estimates of *Risk* (%), and the respective cumulative probability distribution regression outputs (*m*, *b*, and $r^2$).

| | | | Exposure concentration of diuron (µg $L^{-1}$) | | | | Error | | | | Distribution regression parameters ($y = mx + b$) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Comparison | n | Mean | Median | 90th centile | Maximum (Date: d/m/yyyy) | MAE[a] (µg $L^{-1}$) | RMSE[b] (µg $L^{-1}$) | NS[c] | *Risk* (%) | *m* | *b* | $r^2$ |
| Gwydir River, Yarraman bridge | Monitoring dates | | | | | | | | | | | | |
| | Monitoring | 55 | 0.05 | <LOQ | <LOQ | <LOQ | | | | 0.00 | NA | NA | NA |
| | Post-emergence | 55 | 0.08 | <LOQ | <LOQ | 1.0 (15/11/2005) | 0.03 | 0.17 | -2.4E31 | 2.45 | 0.28 | 1.96 | 0.68 |
| | Pre-emergence | 55 | 0.05 | <LOQ | <LOQ | <LOQ | 0.00 | 0.00 | 1.00 | 0.00 | NA | NA | NA |
| | All modelling data | | | | | | | | | | | | |
| | Post-emergence | 5997 | | <LOQ | <LOQ | 38.5 (16/11/1996) | | | | 1.66 | 0.49 | 2.12 | 0.95 |
| | Pre-emergence | 5997 | | <LOQ | <LOQ | 6.5 (19/7/1992) | | | | 0.68 | 0.64 | 2.46 | 0.93 |
| Gwydir River, Brageen crossing | Monitoring dates | | | | | | | | | | | | |
| | Monitoring | 293 | 0.37 | <LOQ | 0.5 | 16.6 (4/1/1994) | | | | 5.90 | 0.76 | 1.55 | 0.98 |
| | Post-emergence | 293 | 0.17 | <LOQ | <LOQ | 15.4 (16/11/1992) | 0.04 | 1.87 | -0.63 | 1.84 | 0.39 | 2.08 | 0.92 |
| | Pre-emergence | 293 | 0.05 | <LOQ | <LOQ | 0.6 (5/10/1994) | 0.32 | 1.50 | -0.05 | 0.21 | 0.66 | 2.85 | 0.99 |
| | All modelling data | | | | | | | | | | | | |
| | Post-emergence | 5997 | | <LOQ | <LOQ | 33.2 (17/11/1992) | | | | 0.58 | 0.69 | 2.51 | 0.94 |
| | Pre-emergence | 5997 | | <LOQ | <LOQ | 6.3 (19/7/1992) | | | | 1.42 | 0.55 | 2.18 | 0.96 |
| Gwydir River, Allambie bridge | Monitoring dates | | | | | | | | | | | | |
| | Monitoring | 53 | 0.21 | <LOQ | 0.3 | 5 (16/6/2005) | | | | 4.84 | 0.77 | 1.65 | 0.96 |
| | Post-emergence | 53 | 0.06 | <LOQ | <LOQ | 0.5 (2/12/2003) | 0.16 | 0.69 | -0.03 | 1.21 | 0.50 | 2.24 | 1.00 |
| | Pre-emergence | 53 | 0.05 | <LOQ | <LOQ | 0.1 (16/6/2005) | 0.16 | 0.69 | -0.02 | 0.27 | 0.75 | 2.76 | 1.00 |
| | All modelling data | | | | | | | | | | | | |
| | Post-emergence | 5997 | | <LOQ | <LOQ | 32.8 (19/11/1992) | | | | 1.39 | 0.59 | 2.19 | 0.97 |
| | Pre-emergence | 5997 | | <LOQ | <LOQ | 6.3 (19/7/1992) | | | | 0.58 | 0.70 | 2.51 | 0.94 |

[a] MAE refers to Mean Absolute Error; [b] RMSE refers; [c]NS refers to Nash-Sutcliffe model efficiency.

To demonstrate to what extent the model output would impact risk characterisation, exposure frequency distributions comparing modelled and monitoring data were constructed for all three monitoring sites (Figure 4.11 a-c). With the exception of Gwydir River, Allambie bridge (Figure 4.11 a), these distributions confirm that the model consistently under predicted exposure. However, the post-emergence scenario predicted more closely to the monitoring data at Gwydir River, Allambie Bridge.

Using continuous exposure distributions, the risk that exposure was exceeding the $HC_5$ toxicity threshold for diuron (1.04 μg $L^{-1}$; See Chapter 2) was characterised using all modelling data and modelled data corresponding with monitoring dates. Compared to monitoring data, the model predicted lower exposure risk, with higher risk predicted to occur under post-emergence scenario (1.21-2.45%; Table 4.8) relative to pre-emergence (0-1.42%; Table 4.8). Importantly, the highest exposure risk was predicted to be occurring at Gwydir River, Yarraman Bridge.

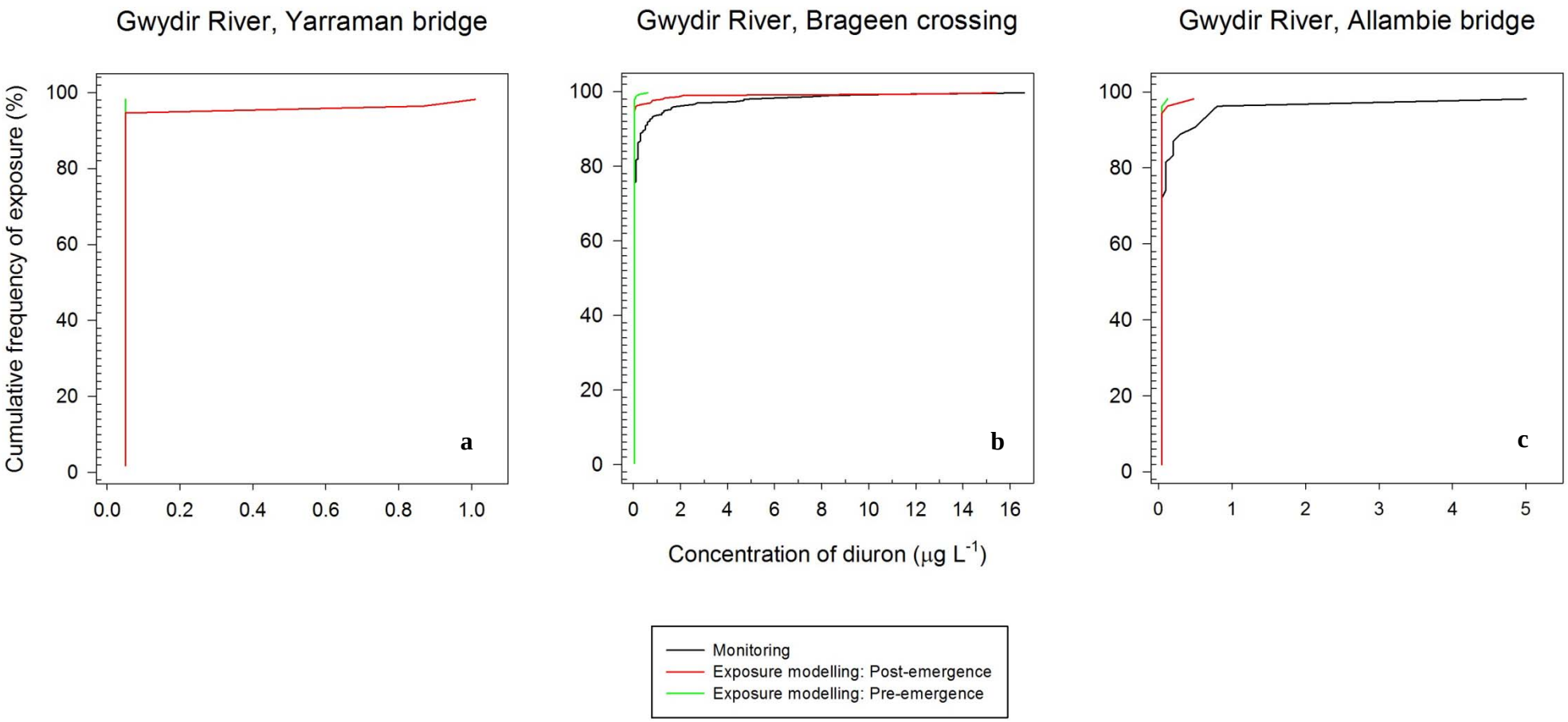



**Figure 4.11** Cumulative exposure frequency (%) distributions for post- (red line) and pre-(green line) emergent modelling scenarios, and monitoring data (black line) for the sites, (a) Gwydir River, Yarraman bridge; (b) Gwydir River, Brageen crossing; and (c) Gwydir River, Allambie bridge.

*4.3.2 Characterisation of diuron exposure pulse duration*

The modelled exposure data was used to estimate the possible duration of diuron exposures occurring in the Gwydir wetlands catchment. Durations exceeding monitoring LOQ and $HC_5$ from pulse duration distributions for the monitoring sites, Gwydir River, Yarraman bridge (Figure 4.12 a-b); Gwydir River, Brageen crossing (Figure 4.12 c-d); and Gwydir River, Allambie bridge (Figures 4.12 e-f). In all cases, the linear regressions satisfactorily explained the variation in the distributions ($r^2$ = 0.96-0.99; Figure 12 a-f).

The maximum, median, 90$^{th}$ percentile, and probability that a chronic algae diuron exposure duration (96 h) estimated to occur, together with the probabilistic pulse exposure duration distribution regression outputs are summarised in Table 4.9. It was found that the maximum pulse duration occurring at the monitoring sites exceeding the LOQ and $HC_5$ were estimated to range 6-13 days and 6-9 days, respectively. The 90$^{th}$ percentile pulse durations for the post-emergence estimated to exceed the LOQ and HC5 ranged 7-8 and 3-7 days, respectively; and ranged 3-6 days and 4-6 days for the for the pre-emergence scenario. The probability that a pulse exposure would last long enough to warrant concerns for chronic toxicity, ranged from 10.03-26.40%. Specifically, lower probabilities were, on most part, estimated to occur under cotton post-emergence diuron application regime.

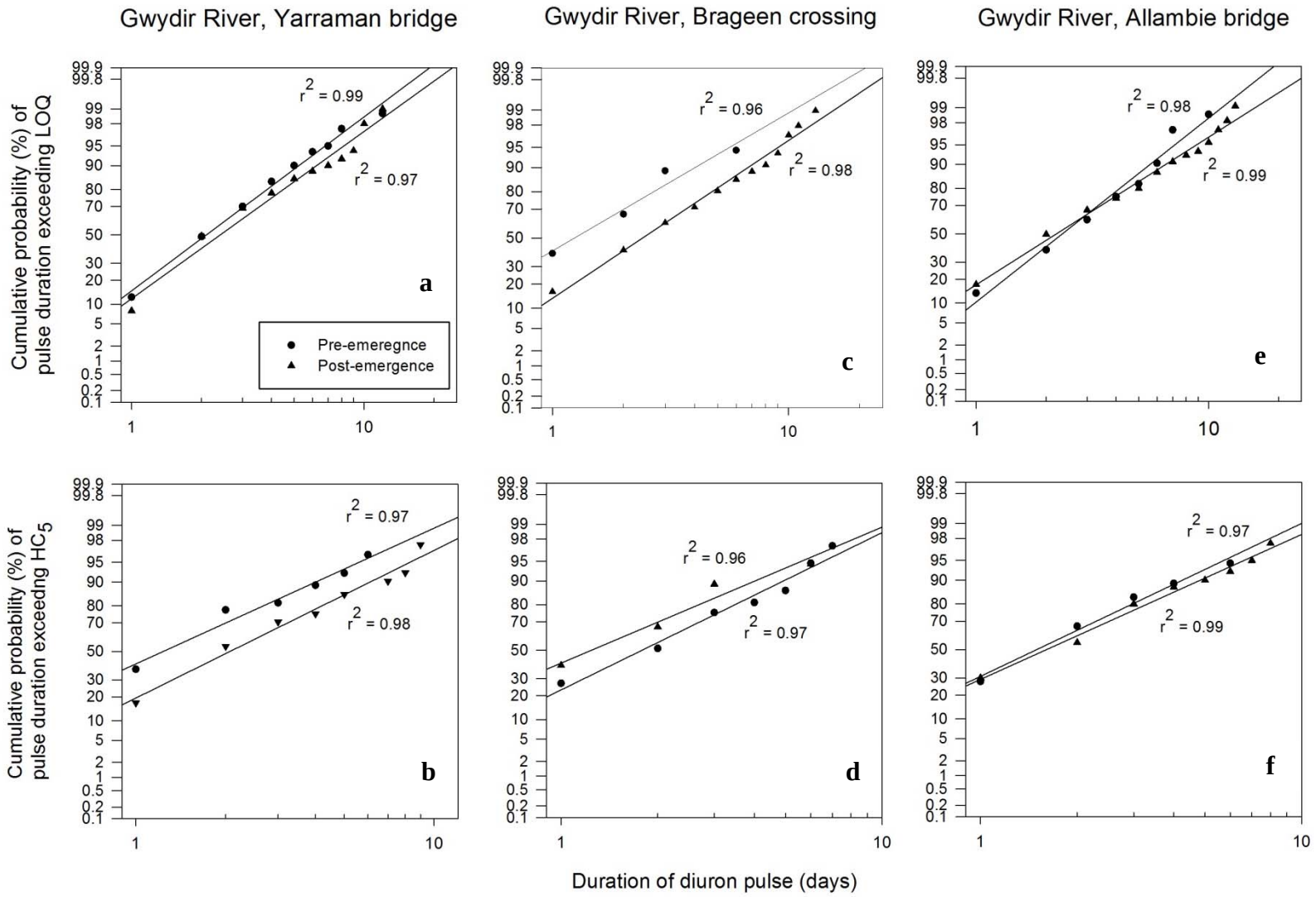



**Figure 4.12** Cumulative probability (%) distributions of exposure pulses exceeding monitoring LOQ (0.1 µg $L^{-1}$) for post- (closed circle) and pre-(closed triangle) emergent modelling scenarios, for the sites, (a, b) Gwydir River, Yarraman bridge; (c, d) Gwydir River, Brageen crossing; and (e, f) Gwydir River, Allambie bridge.

**Table 4.9** Summary of maximum, median and 90$^{th}$ percentile exposure pulses durations (days) exceeding diuron monitoring LOQ (0.1 µg $L^{-1}$), and $HC_5$ for post- and pre-emergence diuron exposure modelling scenarios at Gwydir River, Yarraman bridge; Gwydir River Brageen crossin; and Gwydir River, Allambie bridge monitoring sites. Together with, the probability that any of these pulses will last 96 h, the equivalent of a standard algae toxicity study; and the cumulative probability (%) distribution regression parameters.

| Site | Pulse exposure threshold test for modelling scenario | n | Pulse duration (days) | | | Probability of 96 h algae pulse occurring (%) | Regression parameters (y = mx + b) | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Maximum | Median | 90th Percentile | | m | b | r ² |
| | Exceeding LOQ | | | | | | | | |
| Gwydir River, Yarraman bridge | Post-emergence | 99 | 12 | 3 | 7 | 18.31 | 3.09 | -1.18 | 0.97 |
| | Pre-emergence | 79 | 12 | 3 | 5 | 24.68 | 3.21 | -1.03 | 0.99 |
| Gwydir River, Brageen Crossing | Post-emergence | 96 | 13 | 3 | 8 | 10.11 | 2.87 | -1.10 | 0.98 |
| | Pre-emergence | 17 | 6 | 2 | 3 | 26.40 | 2.51 | -0.24 | 0.96 |
| Gwydir River, Allambie bridge | Post-emergence | 108 | 13 | 3 | 7 | 21.56 | 2.73 | -0.94 | 0.98 |
| | Pre-emergence | 72 | 10 | 3 | 6 | 24.20 | 3.40 | -1.26 | 0.98 |
| | Exceeding $HC_5$ | | | | | | | | |
| Gwydir River, Yarraman bridge | Post-emergence | 40 | 9 | 2 | 7 | 11.39 | 2.73 | -0.86 | 0.98 |
| | Pre-emergence | 26 | 6 | 2 | 4 | 14.45 | 2.51 | -0.23 | 0.97 |
| Gwydir River, Brageen Crossing | Post-emergence | 17 | 6 | 2 | 3 | 15.45 | 2.51 | -0.24 | 0.96 |
| | Pre-emergence | 36 | 7 | 2 | 6 | 10.11 | 2.90 | -0.73 | 0.97 |
| Gwydir River, Allambie bridge | Post-emergence | 17 | 8 | 2 | 5 | 10.03 | 2.68 | -0.55 | 0.99 |
| | Pre-emergence | 39 | 6 | 2 | 4 | 21.75 | 2.83 | -0.50 | 0.99 |

*4.3.3 Estimated diuron sub-catchment loading*

Maps showing the sources of the diuron loads that have resulted in the predicted exposure concentrations in the Gwydir River under pre- and post-emergence scenarios in cotton are shown in Figures 4.13 and 4.14, respectively. In both scenarios, diuron loading occurred at both the eastern and western end of the catchment, with some sub-catchment contributing minor loads. Chemical loading under cotton pre-emergence was predicted to range 0.01-55.6 kg $year^{-1}$. Similarly, pre-emergence application in cotton scenario predicted highest chemical loading (1.0-26.65 kg) to occur in the eastern end of the catchment, with some smaller loads (0.01-1 kg) contributed down-stream.

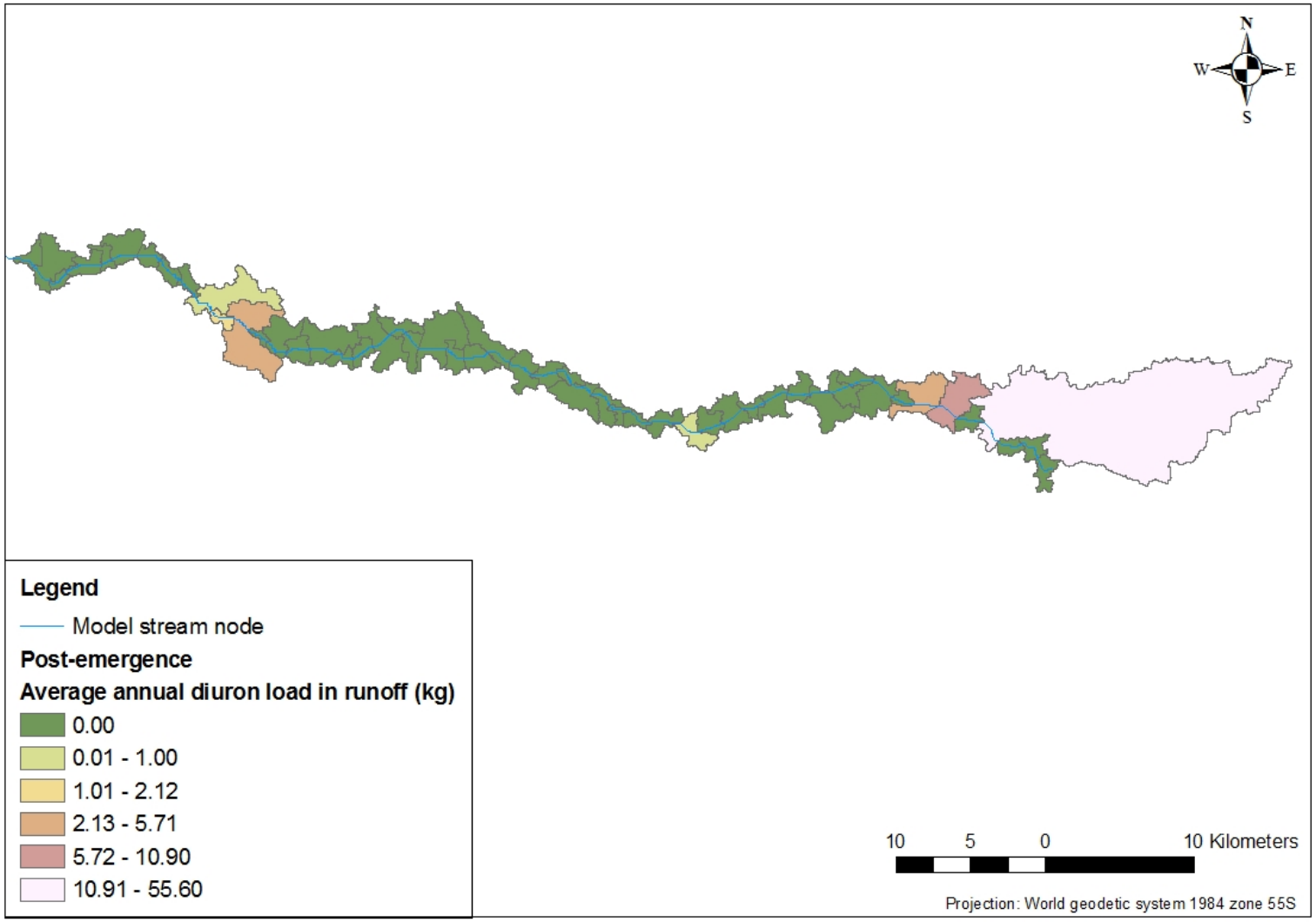



**Figure 4.13** Map of average annual sub-catchment diuron loading (kg) in runoff predicted to occur for the cotton post-emergence scenario.

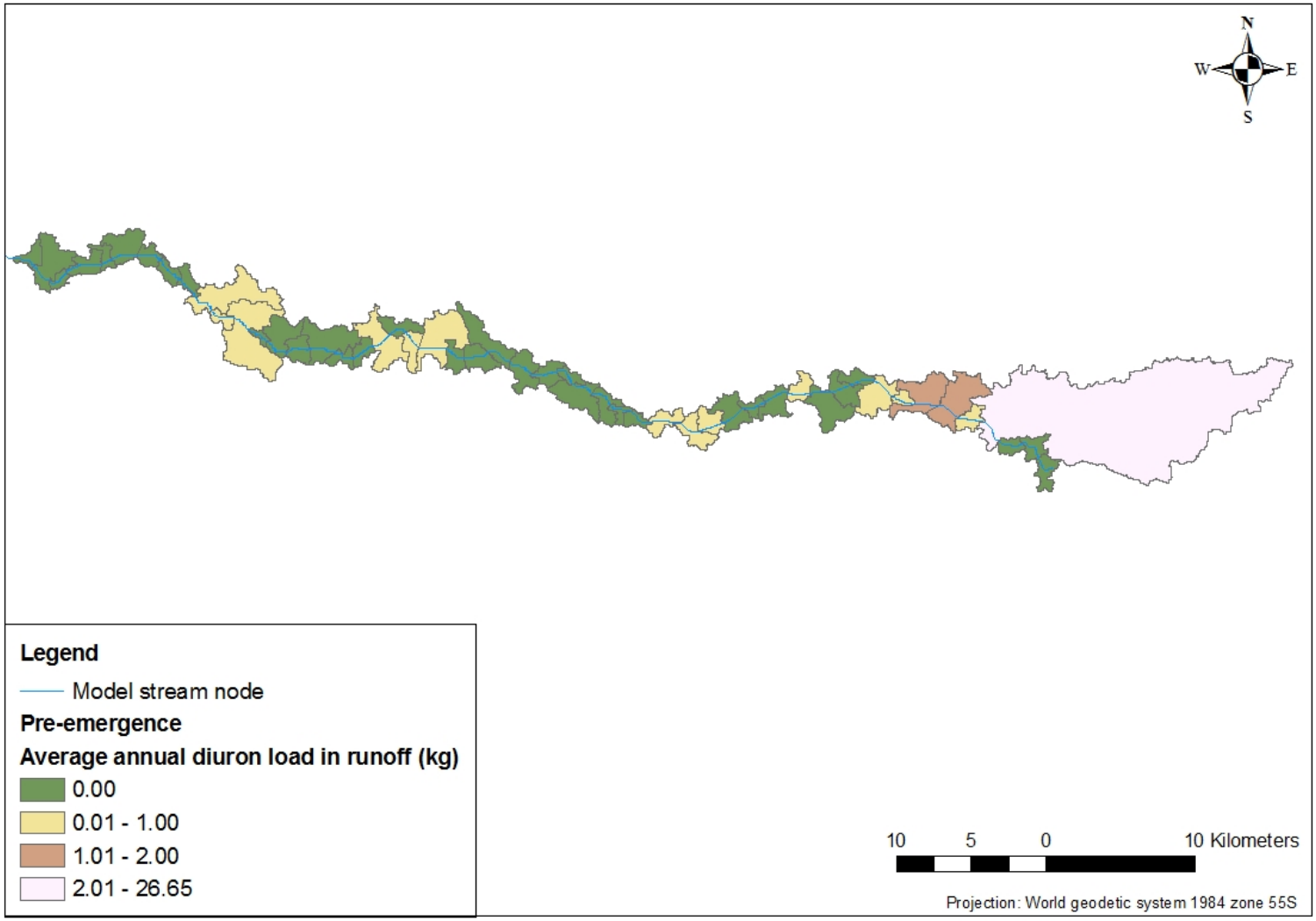



**Figure 4.14** Map of average annual sub-catchment diuron loading (kg) in runoff predicted to occur for the cotton pre-emergence scenario.

## 4.4 DISCUSSION

When a sufficient level of risk posed by a pesticide has been characterised in a catchment, identifying chemical loading sources is critical for developing risk management strategies. Preliminary results applying a spatial exposure modelling framework to identify potential sources of diuron contributing to the level of risk in the Gwydir Wetlands catchment identified in Chapter 2, has been presented. As illustrated in this chapter, this modelling framework is capable of estimating sub-catchment level chemical loading, exposure concentrations, and pulse durations likely to occur in the reaches of the Gwydir wetlands catchment. This model can be discussed for its ability to predict exposures in the reaches of the Gwydir Wetlands catchment, relevance of characterising exposure pulses, uncertainties and limitations influencing the reliability of the results, and considerations for future work.

### *4.4.1 Model framework response*

As shown in the time-series results, the model predicted chemical loading in direct response to rainfall events. This was qualified by exposure peaks corresponding to rainfall events at Gwydir River, Brageen crossing for the modelling year 1992 (Figure 4.9). In all time series results (Figures 4.10 a-c), the consistent July-August peak concentrations corresponded with chemical application occurring in wheat and chickpea production. The peak exposures predicted to occur during November-December for the post-emergence scenario corresponds with chemical application made in the simulated irrigated and dryland cotton cropping systems. However, from the time of chemical application, the prevalence of exposure pulses declined between rainfall events. The ability for the model to predict diuron loading at times when it was available for transport is related to diuron soil-water partitioning ($K_d$) (Mackay, 2001).

In order to maintain thermodynamic equilibrium between the solid and liquid phases of the soil and water interface, diuron would be expected to continuously partition to runoff (Schnoor, 1992; Kookana *et al.*, 1998; Burns *et al.*, 2008; Alister and Kogan, 2010; Kennedy *et al.*, 2011). At the time of application, diuron load available to partition in to solution would be at its highest. With time, chemical loading into runoff is expected to decline as more events pass through the system and as a result of degradation processes (Wauchope, 1978; Louchart *et al.*, 2000; Dores *et al.*, 2009). That is, the first runoff event occurring after application resulted in the highest load predictions, and subsequent events were relatively

smaller. Such events were simulated to occur to the point in each growing season that the diuron sources were marginalised to the point that exposure concentrations were below the monitoring LOQ, an observation consistent with the monitoring data (Figures 4.9; and 4.10 a-c). Such a characteristic is consistent with diuron environmental fate studies (Louchart *et al.*, 2000; Dores *et al.*, 2009; Alister and Kogan, 2010), and agrees with fugacity modelling predictions (Mackay, 2001) and chemical transport principles (Wauchope, 1978; Schnoor, 1992; Kookana *et al.*, 1998; Kennedy *et al.*, 2011).

The magnitudes of diuron exposures predicted to occur under the post-emergence scenario were greater than under the pre-emergence scenario (Figures 4.9; and 4.10 a-c). In the post-emergence scenario diuron was simulated to be applied directly to the soil surface with no incorporation of the chemical. As the maximum amount of chemical was simulated to be is concentrated in the top 5 mm of soil, it is not unreasonable that PRZM-RIVWQ framework predicted the peak chemical concentrations at the magnitude seen in the time series output. That is, a greater chemical load is available at the soil surface for transport and as a rainfall event proceeds, a higher chemical load can transported in the early stages of a runoff event. By contrast, in the pre-emergence application scenario, the chemical was simulated to be distributed within the top 4 cm of the soil, effectively "diluting" the chemical loads available for transport at the soil-water interfaces. Furthermore, since the soil types used in this model were highly deficient in organic carbon (less than 1%), this would suggest there is a preference for diuron to partition to aqueous solution, compared to more carbon rich soils, further contributing to higher chemical loading (Schnoor, 1992; Kookana *et al.*, 1998; Burns *et al.*, 2008). This result suggests that the transportable fraction of diuron in the treated soil was likely to be rapidly depleted with repeated rainfall events.

### *4.4.2 Chemical loading by sub-catchments*

As expected, chemical loading characterised by sub catchments (Figures 4.13 and 4.14) corresponded well with land use. That is, the sub-catchments shown to have higher density of intensive agricultural land uses simulated to apply diuron in crop production were predicted to have higher loadings. As there was little difference between the soil texture grades, organic carbon content (Table 4.5) and relief within the sub-catchment, with only slight spatial climatic difference, these results confirm the influence that land use and affiliated cropping and pest management regimes had on influencing loadings. Specifically, the higher average

loading under the post-emergence crops confirms higher exposure concentrations observed in the monitoring and modelling. This emphasises the strong influence that applying diuron on the soil surface post-emergence of cotton has on its loadings in the Gwydir wetlands catchment. Identifying which sub-catchments were most likely to contribute the greatest loads can provide risk managers with more power to manage risk.

*4.4.3 Pulsed diuron exposure*

The exposure events predicted by the modelling framework took the form of a pulse. The durations of these pulses are the direct outcome of catchment soil hydrological properties and the fluxes in chemical load available for transport, that are both characteristically unsteady (Mackay, 2001). It was determined that the simulated pulses lasted longer under the post-emergence scenario, relative to the pre-emergence (Table 4.9). This is the outcome of the longer interaction of the treated soil surface with runoff water with desorption occurring right up to the point when runoff ceases. Interestingly, for pulses exceeding the $HC_5$ there was a greater chance that a pulse under the pre-emergence scenario would last long enough to result in a chronic effect toward algae. This is the outcome of diuron loads in soil likely to be depleted at slower rate to that of the post-emergence application, due to the protection provided by incorporating the chemical.

The magnitude, duration and frequency of diuron exposure pulses is what will ultimately determine if algal and macrophyte groups identified in Chapter 2 will be affected (Suter II, 2007). Although it appears that there were more days with exposure concentrations below the monitoring LOQ occurring at the monitoring sites, the pulses that last long enough and at a high enough concentration were identified with respect to 96 h standard algal toxicity study (USEPA, 1996) and further judged by the $HC_5$ (Table 4.9). Importantly, these toxicity studies do not account for organism recovery, given the endpoint chosen does not result in the death of the organism.

With reference to the *L. minor* and *L. gibba* seven day pulse toxicity study (Chapter 3), it was shown that the peak exposures in the Gwydir wetlands catchment were unlikely to have effects at the $EC_{50}$ level and pulses were unlikely to be maintained for this seven day duration. However, further assessment of the model performance is needed to confirm if this is likely to be the case. Further evaluation of the interval between exposure pulses would be

necessary to characterise the intervals where an organism has the opportunity to recover, similar to that described by Solomon and Takacs (2002) using atrazine exposure data in Lost Creek, Ohio USA.

Nonetheless, if the model predictions are correct, significant inhibition observed under the pulse durations may require the implementation of management strategies; this need would be reinforced by confirmation of the pulses using targeted monitoring. However, the model has demonstrated the relevance of pulse exposure dynamics in catchment and provides a tool to consider whether these are eliciting effects on non-target ecosystems.

*4.4.4 Model framework performance*

Although the model framework performed in a way that reflected pesticide transport and fugacity principles, the model did not predict peak exposure concentrations on precisely the same dates as the monitoring data. This could be observed in the time series plots at each site and differences in the cumulative frequency plots of the monitoring sites (Figures 4.10 a-c; and Figure 4.11 a-c), and confirmed in the model error estimates of MAE and RMSE, and NS model efficiency estimates, as well as the lower estimation in exposure risk (Table 4.8). With the exception of Gwydir River, Yarraman Bridge monitoring site, the NS model efficiency for the pre-emergence scenario suggested that the average of observed data was a better predictor of exposure than the model.

A previous study by Parker *et al*. (2007) similarly concluded the same outcome for the PRZM-RIVWQ framework while modelling for the herbicides atrazine, metolachlor, and trifluralin in Sugar Creek catchment, USA. However, in another study by Luo and Zhang (2009) PRZM was combined with a spatially distributed flow and transport routing model showed good agreement between predicted and observed data when modelling the organophosphate pesticides diazinon and chlorpyrifos. The level of error from this study suggests that while PRZM diuron fate predictions in response to rainfall events was conceptually accurate in the model output, it is more likely that uncertainty in the model inputs is driving the level of inaccuracy in the model predictions.

*4.4.5Model framework uncertainty*

The ability of the model framework to predict peak chemical concentrations observed in monitoring data was poor. This was likely to be the outcome of input uncertainties. Identified sources of uncertainty include crop inputs, diuron application rates and timing, and land use information. The monitoring process is also subject to uncertainties, possibly being biased temporarily by a focus on rain events or by limits on access to sampling sites during peak flows.

*Crop inputs*

Crop input parameters are what drives the variable hydrologic nature of a cropping system. In this modelling study, simplified intensive monoculture cropping regimes were used as inputs to demonstrate the capabilities of the spatial modelling framework. However, the lack of information regarding crop rotation schedules was identified to be a source of uncertainty. Importantly, different crop rotations have been recommended for use in a range of different cropping practices located in the Gwydir wetlands (See Chapter 2). These are adopted for the purposes of preserving soil health and to promote IPM strategies.

Site-specific crop rotations are likely to influence the suite of chemicals used in the production practices, with respect to their registered uses and industry pest management guides (see Chapter 2). It may well be that between growing seasons diuron is used variably. However, by not accounting for crop rotations has resulted in chemical applications being made without accounting for the uncertain prospect that the chemical may or may not be used, a characteristic likely to vary spatially and temporally. It is likely that the current model scenarios will elevate the diuron loads available for transport over the entire catchment. Such uncertainty can only be minimised through the collection of site specific crop and chemical rotation practices adopted by each farmer.

*Pesticide inputs*

Apart from estimating using available literature, and relating chemical label recommendations with land use maps, it is unknown if, when and how much diuron is being applied under the different cropping practices in the Gwydir wetlands catchment. As it was assumed chemical application rate and timing occurred at the same time every growing season in this study, such timing was likely to influence the occurrence of peak exposure

concentrations. This lack of information may have contributed to the poor correlation of exposure peaks in the model outputs, when compared to the monitoring data. Other factors likely to influence these inputs are the adoption of crop rotations, IPM strategies and the acceptance of herbicide tolerant GM crops since the year 2000 (as discussed in Chapter 2).

*Runoff management*

In some agricultural industries, practices have been devised to manage pesticide contaminated runoff. Management of runoff is increasingly being adopted by different agricultural industries to control pesticide transport. These include collection, storage and recycling of runoff and irrigation tail water (Kennedy *et al.*, 2001; Rose *et al.*, 2005). This is particularly the case in cotton production BMP strategy (Williams and Willams, 2000). The cotton BMP strategy requires the first 25 mm of rainfall be retained on site (Williams and Willams, 2000). Farmers engineer their fields to capture runoff and recycle the water. Such hindrances to water movement will either prevent or delay the chemical load deposition in to the streams. This could allow some of the diuron to degrade *in situ*. Based on the land use map available at the time of modelling this area, it was difficult to elucidate the areas where runoff management was occurring. This simulation did not incorporate such a runoff management strategy and the actual field data indicate that the effect of such possible mitigation by retention was minimal. Clearly, a management system with complete retention was far from operational. However, with a more detailed land use map, these structures should be identified and incorporated into the modelling framework.

*Dealing with uncertainty*

For this modelling framework to be used as a management tool these uncertainties need to be addressed. Much of the uncertainty can simply be reduced through field surveys, or as a real-time strategy through the adoption of a spatially explicit web-based pesticide management survey. It would be expected through a catchment-based ERA management strategy that CMA's have such information on record. Only through improvements in model inputs can the validity of model outputs in supporting ERA be justified.

### *4.4.6 Future research*

It would be expected that a spatial fate modelling framework, such as the one presented in this study, be further developed and calibrated to specific catchments to support risk

management decisions. Furthermore, through sensitivity analysis of model input parameters this model can also have the capacity to development risk management strategies, similar to that of Hoogeweg *et al*. (2008). The effectiveness of such a tool in directing management can only be supported by targeted monitoring to validate the influence of model inputs, especially with regard to characterising pulses.

## 4.6 CONCLUSIONS

This model framework has demonstrated high potential to be adapted as a risk management tool. To reflect all possible diuron label applications, two alternate scenarios, post- and pre-emergence application of diuron in cotton production, were compared. The model outputs reflected expected stream chemical fluxes in response to rainfall events and in-field load depletion, which specific sub-catchments were identified for their chemical loading to the reaches of the Gwydir wetlands catchment. Sub-catchment scale fluxes in chemical loading corresponding with rainfall events agreed with transport and fugacity principles, with higher average loading predicted under the post-emergence scenario. The reality was likely to be intermediate between these extremes, but the predictive data generated suggests that post-emergent application was predominant, possibly reflecting applications to channel banks as well as fields, particularly in earlier years of the study before restrictions were applied by regulators. These fluxes in streams were characterised to take the form of a pulse, with exposure durations and magnitudes characterised to possibly affect algae, with higher pulse magnitudes occurring under the post-emergence scenario.

However, when compared to monitoring data, the model predicted timing of peak concentration poorly. It is expected that a calibration exercise that addresses the crop, pesticide, and site-specific land use input uncertainties, as well as being backed up by pulse exposure monitoring should rectify some of these inaccuracies. This is concluded on the basis that the model framework chemical load predictions in response to rainfall events were conceptually sound. Based on this modelling output, it can be recommended in cotton production that post-emergence application be restricted to manage chemical loss and to mitigate the risk to aquatic organisms. As the sub-catchments contributing the highest diuron loads could be identified, if confirmed by monitoring it can be concluded that this modelling framework has strong potential to support risk management decisions that can only be improved by addressing model input uncertainties.

**CHAPTER 5**

# GENERAL DISCUSSION AND CONCLUSIONS

## 5.1 INTRODUCTION

In Chapter 1 it was concluded that an effective ERA framework had not been established in Australia as a management tool. Although elements of the well-recognised ERA framework were used in some scenarios (e.g. Muschal and Warne, 2003), an integrated approach, making use of GIS technology, was not identified. This thesis aimed to build on the existing framework to identify and characterise exposure risk 'hotspots' in an agricultural catchment of Australia. This chapter seeks to integrate the outputs from the risk analysis at the sub-catchment scale, establish the significance of pulse exposure in ecotoxicity and the utility of spatial modelling to define area-specific chemical loading to support risk management. To complete this dissertation, a series of recommendations will be provided that would support the successful implementation of an ERA framework for the purposes of managing pesticides in catchments.

### *5.1.1 Significance of catchment-scale ecological risk assessment: the Gwydir River catchment experience*

Chapter 2, using readily available monitoring data, evaluated the ecological risk posed by diuron, prometryn and endosulfan at the sub-catchment level in the Gwydir River catchment. Following the standardised ERA protocol (USEPA, 1998), it was found that the risk varied temporally and spatially, most likely reflecting localised chemical and environment interactions, which were characterised in Section 2.3. Unique assessment endpoints distinguished ecologically sensitive areas from those that were experiencing some level of disturbance. By distinguishing these environments resulted in the implementation of sub-catchment specific ecosystem toxicity thresholds that included assigning a $HC_5$ for more sensitive areas, such as the Gwydir wetlands catchment, and $HC_{10}$ for more disturbed areas (ANZECC, 2000). This approach indicated that risk varied greatly within a catchment that allowed for two main insights to be made. Firstly, that scale is important in the application of a probabilistic risk approach. Secondly, the duration of exposure was also considered with respect to acute or chronic exposure and potential recovery.

### *5.1.2 Importance of catchment exposure dynamics: pulse exposure responses*

Sources of pesticide exposure in catchments generally take the form of a pulse (Wauchope, 1978; Handy, 1994; Williams *et al.*, 1995; Reinert *et al.*, 2002). However, the toxicity data commonly used to characterise risk assumes continuous or chronic exposure (Aldenberg *et*

*al.*, 2002; Reinert *et al.*, 2002). The pulse exposure toxicity experiment, presented in Chapter 3, found that two duckweed species, *L. minor* and *L. gibba* growth was able to recover from significant growth inhibition caused during diuron exposure. The concentrations where recovery was possible were in the range of the reported NOEC, LOEC, $EC_{10}$, $EC_{25}$ and even $EC_{50}$ endpoints. Given the highly variable exposure concentrations seen in the Gwydir River catchment (Chapter 2) it would be expected that populations of *L. minor* and *L. gibba* can recover effectively from an exposure pulse lasting up to seven days, with recovery in growth likely to be rapid. Obviously, temporary loss of productivity of plants may have implications for particular species with respect to trophic interactions, but is unlikely to have irreversible implications for biodiversity.

These responses have practical implications for catchment-based ecological risk characterisation, where the traditional approach relies on LOEC, $EC_{10}$, or $EC_{50}$ data from toxicity studies to define the management threshold limits (Solomon and Takacs, 2002; Suter II, 2007). It would seem that the recovery potential of an organism would allow these thresholds for diuron to be considered less stringent given that sensitive photosynthetic organisms are likely to exhibit reversibility of effect (Chapter 3). To overcome this dilemma, two approaches to characterise exposure concerns, with considerations of catchment-based ERA, are suggested.

The first approach intends to account for different pulse durations by considering catchment-specific variations. Likely exposure and recovery windows should be characterised for specific catchments (Reinert *et al.*, 2002). Such characterisations will set pulse duration scenarios to be tested and should be further extended to account for potential irreversible effects. Given that populations are already likely to be established in catchments, the irreversibility of effects should be considered to account for the likelihood of trophic impacts as well as the life-cycle time of the organism (Solomon and Takacs, 2002). As this study suggested (Chapter 3), in agreement with others (e.g. van Rensen, 1982; Vallotton *et al.*, 2008), exposure concentrations that may be deemed detrimental may only temporarily stall algal population regeneration (Solomon and Takacs, 2002). While this outcome may be dependent on the mode of action of the chemical in question, it is clear that such resilient action is probable for diuron.

The second approach involves evaluations of ecosystem resilience using mesocosm approaches. Mesocosm studies commonly investigate the variation in ecosystem responses to exposure by characterising how the suppression of one species or group of organisms might affect the well-being of others (Girling *et al.*, 2000; Boesten *et al.*, 2007; Knauert *et al.*, 2009; Proia *et al.*, 2011; Tlili *et al.*, 2011). For example, growth inhibition of an organism group may provide another less sensitive competing organism with a competitive edge allowing it to populate (Solomon *et al.*, 1996). These studies may also highlight the resilience and adaptive capacities of ecosystems (Solomon *et al.*, 1996). In a more recent study, Tlili *et al*. (2011) compared chronic and acute periphyton community responses to exposure to environmentally relevant concentrations of diuron, and found that diuron adsorbed in to periphyton during each pulse and desorbed 13 h later. It was also shown that subsequent pulses resulted in improved photosynthesis efficiency for chronically exposed periphyton through physiological adaptation. Such studies designed to last for defined pulse exposure durations likely to be observed in a study catchment, such as that characterised through the spatial exposure modelling effort (Chapter 4), are critical. They also provide greater certainty in the interpretation of exposure risk and the implications these have on organism diversity and abundance being observed in catchment ecosystems. However, inferences made from such studies should be further validated in the catchment through ecological surveys.

### *5.1.3 Spatial exposure modelling as a risk management tool*

Chapter 4 developed a spatial modelling approach to estimate diuron exposure occurring outside of the monitoring times in the Gwydir Wetlands catchment. This approach highlighted important uncertainties in spatial exposure modelling. Aside from this, it was also found to have potential use in characterising exposure pulses, development of risk management strategies, and ability to help identify sub-catchment loading points.

Specifically, the model predicted higher load associated with post-emergent applications to cotton that was consistent with the peak concentrations observed in the monitoring data. Although the model response agreed with diuron environmental fate studies (Simpson, 2007; Stork *et al*., 2008), and fugacity and chemical transport principles (Wauchope, 1978; Mackay, 2001), it was unable to predict exposure peaks on precisely the same date as the monitoring data. This was identified to be the outcome of model input uncertainty (Dubus *et al.*, 2003), suggesting that further work is needed to develop site-specific crop management

practices. It is suggested this lack of information could be overcome through the development of a web-based questionnaire where farmers could provide actual farm management information with suitable incentives.

The model framework also estimated the pulse exposure scenarios likely to be observed between sub-catchments. Following calibration and validation, it would be expected that with the ability to estimate exposure pulses provides a sound basis to extend SSD theory to include time-to-effect (Handy, 1994; Reinert *et al.*, 2002; Solomon and Takacs, 2002; Cedergreen *et al.*, 2005) on a predictive basis. That is, toxic action being more accurately estimated from dose, which is a culmination of exposure concentration and duration (Reinert *et al.*, 2002; Solomon and Takacs, 2002). This would provide the platform for which a three dimensional SSD consisting of proportion affected, concentration and time (Suter II, 2007), as a way of predicting organism response to exposure at the back-end of this model framework to estimate risk on an event basis (Travis and Hendley, 2001; Reinert *et al.*, 2002; Solomon and Takacs, 2002). Although this approach appears highly ambitious, it may be limited to simply distinguishing acute and chronic exposure, and characterising risk by comparing with their respective SSDs.

Although this model combination was demonstrated in this thesis on a sub-catchment level, this framework has the potential to evaluate diuron exposure at higher resolution given that PRZM operates on a site-specific basis. In its current state, it is expected that maps generated from this spatial modelling approach can be used to support catchment monitoring efforts, and where unacceptable risk is observed be used to support the development of cost-benefiting and effective management practices through sensitivity analysis. Although this thesis does not seek to endorse the idea that a model result is the true result, it does suggest that, if properly calibrated, it could provide a useful management tool, serving as an indicator while the technology is in its infancy. Such characterisations would need to be further confirmed through characterisation of exposure pulses and ecological surveys.

## 5.2 RECOMMENDATIONS

This thesis has identified some complex interactions that may be occurring at the sub-catchment level. It is proposed that pesticides would be most appropriately managed at this local scale. Management through local organisations, such as CMAs, may provide the

capacity to deal with this level of complexity. Such organisations need to be built into an ERA framework as instigators and investigators that actively involve stakeholders and other relevant risk experts. However, some critical questions need to be considered in executing such a strategy. These include, 1) at what scale and 2) what ecosystem protection thresholds should we be managing for?

Accounting for the complexity in ecosystem response at the sub-catchment level will certainly be a challenge. However, by using the spatial modelling approach presented in this thesis highlights that priority areas can be defined to augment sampling for risk evaluations. This must be further distinguished according to the ecological value of an ecosystem, critical in devising priority areas for greatest level of protection, such as the Gwydir wetlands sub-catchment presented in Chapter 2, and further evaluated in Chapter 4.

It is therefore recommended that a dynamic "bottom-up" ERA strategy targeted at managing pesticides in individual catchments needs to be established in Australia. The current top-down "one size fits all" approach to basing registration and chemical use decisions applied on a national basis, such as that used by the national pesticide regulator the APVMA (Chapter 1), is clearly not compatible with the spatial and temporal variability in exposure risk highlighted in this thesis. Further recommendations include:

1. Establish a national ecotoxicity network that specialises in the evaluation of pesticide toxicity toward local native species, accounts for chronic and acute effects through pulse exposure dynamics to establish ecosystem resilience as well as considering the importance of changes in ecosystem assemblages as a way to justify exposure significance;
2. Develop a regulatory approved spatial exposure modelling framework that can aid risk managers similar to that presented in Chapter 4, as well as a catchment-specific land use management database where farmers can provide pesticide use and land management information to minimise input uncertainties, that can further pre-empt catchment-level risk concerns for an ensuing season; and
3. Develop a risk management network that allows for different management bodies to communicate, and is flanked by a management strategy that risk managers can follow

to implement cost-effective and practical management strategies to suit risk situations (Suter II, 2007).

## 5.3 CONCLUSION

It can be concluded that catchment-scale ERA can serve as a more rational approach to formulating management decisions of pesticides in catchments. However, realising this potential can only be achieved through its implementation in the catchment management framework of Australia. This would require much more cooperation between federal regulators such as the APVMA and departments of agriculture and environment with the catchment and natural resource managers based in the states. However, this could lighten the load of each organisation and raise the effectiveness of environmental protection by enlisting more involvement at the local level. The research from this thesis is suggested to illustrate the potential, but much work remains for it to be developed into a dynamic and effective framework.

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# APPENDICES

**APPENDIX 1 (See next page)**

Diuron, prometryn and endosulfan $K_d$ and $K_{OC}$ reported by publications, with reference to soil texture, location and % organic matter (collated by the author).

| Location | Soil Type | % Organic matter | $K_d$ (L kg$^{-1}$) | $K_{OC}$ (L kg$^{-1}$ $_{OC}$) | Reference |
|---|---|---|---|---|---|
| Diuron | | | | | |
| Roujan, France | Silt loam | 0.60 | 1.94 | 323.00 | Lennartz *et al.* (1997) |
| Mont Pellier, France | Silt loam | 1.00 | 2.40 | 240.00 | Lennartz *et al.* (1997) |
| Phillipines | Variable | 1.05 | 5.5 | 579.00 | Oliver *et al*. (2005) |
| Dijon, France | Dispered Clay loam | 1.36 | 6 ($K_f$) | 441.18 | Gaillardon (1997) |
| Dijon, France | Undispered Clay loam | 1.36 | 4.93 ($K_f$) | 362.50 | Gaillardon (1997) |
| Dijon, France | Undispered Clay loam | 1.36 | 7.17 ($K_f$) | 527.21 | Gaillardon (1997) |
| La Jailliere | Sandy loam | 2.00 | 9.60 | 480.00 | Guoy *et al*. (1999) |
| Arkansas, USA | Sandy Loam | 2.10 | 4.60 | 219.05 | Sheng *et al*. (2005) |
| Arkansas, USA | Sandy Loam | 2.10 | 3.51 | 166.67 | Sheng *et al*. (2005) |
| South Australia | Variable | 2.23 | 14 | 618.00 | Oliver *et al*. (2005) |
| Florida, USA | Carbonatic silt loam | 2.40 | 2.90 | 121.00 | Nkedi-Kizza *et al*. (2006) |
| Florida, USA | Carbonatic sandy loam | 2.50 | 3.18 | 127.00 | Nkedi-Kizza *et al*. (2006) |
| North East, Western Australia | Variable | 2.83 | 14.9 | 536.00 | Oliver *et al*. (2005) |
| Florida, USA | Carbonatic gravel loam | 4.50 | 6.21 | 138.00 | Nkedi-Kizza *et al*. (2006) |
| Florida, USA | Noncarbonatic fine sandy clay loam | 4.60 | 16.84 | 366.00 | Nkedi-Kizza *et al*. (2006) |
| Florida, USA | Noncarbonatic | 44.00 | 186.56 | 424.00 | Nkedi-Kizza *et al*. (2006) |
| Arkansas, USA | 100% Wheat char | 13 | 3594.00 | 27646 | Sheng *et al*. (2005) |
| Arkansas, USA | Sandy Loam (1% Wheat char ammendment) | NR | 32.10 | NR | Sheng *et al*. (2005) |
| Sugarcane region, Queensland, Australia | Yellow chromosol | NR | 12.1 | 1270 | Simpson (2007) |
| Sugarcane region, Queensland, Australia | Grey chromosol | NR | 27.1 | 3390 | Simpson (2007) |
| Sugarcane region, Queensland, Australia | Red chromosol | NR | 27.3 | 2220 | Simpson (2007) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Sugarcane region, Queensland, Australia | Redoxic hydrosol | NR | 39.3 | | 5460 | | Simpson (2007) |
| Narrabri, New South Wales, Australia | Black cracking clay | 0.7 | 3.42 | | 488 | | Baskaran and Kennedy (1999) |
| Wee Waa, New South Wales, Australia | Red sandy soil | 0.65 | 3.24 | | 457 | | Baskaran and Kennedy (1999) |
| Moree, New South Wales, Australia | Brown loam | 1.27 | 5.71 | | 498 | | Baskaran and Kennedy (1999) |
| Prometryn | | | | | | | |
| Narrabri, New South Wales, Australia | Black cracking clay | 0.7 | 1.78 | | 254 | | Baskaran and Kennedy (1999) |
| Wee Waa, New South Wales, Australia | Red sandy soil | 0.65 | 2.75 | | 423 | | Baskaran and Kennedy (1999) |
| Moree, New South Wales, Australia | Brown loam | 1.27 | 6.04 | | 483 | | Baskaran and Kennedy (1999) |
| Sicily, Italy | Clay | 1.3 | 164.73 ($K_f$) | | 12671 | | Fingler *et al*. (2004) |
| Peloponnesos, Greece | Silt loam | 3.7 | 6.75 ($K_f$) | | 182.4 | | Fingler *et al*. (2004) |
| Wales, Great Britain | Loam | 3.45 | 4.22 ($K_f$) | | 122.3 | | Fingler *et al*. (2004) |
| Normandy, France | Silt | 1.55 | 13.16 ($K_f$) | | 849.0 | | Fingler *et al*. (2004) |
| Schleswig-Holstem, Germany | Loam sand | 9.25 | 130.23 ($K_f$) | | 1407.9 | | Fingler *et al*. (2004) |
| Normandy, France | Silt loam | 0.25 | 0.44 ($K_f$) | | 176 | | Fingler *et al*. (2004) |
| Indiana, USA | Sandy loam | 0.36 | 1.37 | | 380 | | Seol and Lee (2000) |
| Indiana, USA | Silt loam | 2.91 | 3.80 | | 130 | | Seol and Lee (2000) |
| Endosulfan | | | | | | | |
| | | | α-endosulfan | β-endosulfan | α-endosulfan | β-endosulfan | |
| Jabiru Lagoon, Northern NSW, Australia | Sediment | 0.50 | 21.00 | 36.00 | 3981.07 | 7943.28 | Peterson & Batley (1993) |
| Psamments, Brazil | Sand | 0.73 | 0.00 | — | 12600.00 | — | Laabs & Amelung (2005) |
| Psamments, Brazil | Sand | 0.73 | 0.00 | — | 7052.00 | — | Laabs & Amelung (2005) |
| Boobora Lagoon, Northern NSW, Australia | Sediment | 0.90 | 76.00 | 99.00 | 7943.28 | 10000.00 | Peterson & Batley (1993) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Boobora Lagoon, Northern NSW, Australia | Sediment | 0.90 | 85.00 | 143 | 10000.00 | 15848.93 | Peterson & Batley (1993) |
| Jabiru Lagoon, Northern NSW, Australia | Sediment | 1.30 | 133.00 | 137.00 | 10000.00 | 10000.00 | Peterson & Batley (1993) |
| Jabiru Lagoon, Northern NSW, Australia | Sediment | 2.20 | 82.00 | 144.00 | 3981.07 | 6309.57 | Peterson & Batley (1993) |
| Ustox, Brazil | Medium clay | 2.64 | 165.79 | — | 6280.00 | — | Laabs & Amelung (2005) |
| Ustox, Brazil | Medium clay | 2.64 | 231.50 | — | 8769.00 | — | Laabs & Amelung (2005) |
| Boobora Lagoon, Northern NSW, Australia | Sediment | 5.30 | 295.00 | 788.00 | 5011.87 | 15848.93 | Peterson & Batley (1993) |

Reported half-lives of diuron, prometryn and endosulfan reported for soil, water and plant media application studies

| Location | Medium | Treatment rate | Half-life (days) | Reference |
|---|---|---|---|---|
| Diuron | | | | |
| Auscott cotton farm water storage, Narrabri, Australia | Water sample (Storage receiving cotton farm runoff) | 50 µg $L^{-1}$ (spiked solution) | 3.3 and 3.2 (light and dark dissipation, respectively) | Rose *et al*. (2007) |
| Apple orchard Gorsem, Belgium | Soil (Loam; 2.9% organic matter) | 3.0 kg $ha^{-1}$ (not applied previously) | 80.7 | Rouchaud *et al*. (2000) |
| Apple orchard Gorsem, Belgium | Soil (Loam; 2.9% organic matter) | 3.0 kg $ha^{-1}$ (used continuously for 12 years) | 37.3 | Rouchaud *et al*. (2000) |
| Sugarcane farm, Burnett catchment, Queensland, Australia | Soil (Sand; 0.9% organic carbon) | 1.6 kg $ha^{-1}$ | 49 | Stork *et al*. (2008) |
| Vinyard, Cassablanca Valley, Chile | Soil (Loam; 0.68% organic carbon) | 2.0 kg $ha^{-1}$ | 24.6-69.2 | Alister and Kogan (2010) |
| Central western Brazil | Soil (Sandy clay loam; 1.55% organic carbon) | 2.0 kg $ha^{-1}$ | 15 | Dores *et al*. (2009) |
| Vinyard, Cassablanca Valley, Chile | Soil (Loam; 1.28% organic carbon) | 2.0 kg $ha^{-1}$ | 15.8 | Kogan *et al*. (2007) |
| Galathera Creek floodway wetland, Narrabri, Australia | Water sample (wetland adjacent cotton farm) | 50 µg $L^{-1}$ (spiked solution) | 20.7 and 23.5 (light and dark dissipation, respectively) | Rose *et al*. (2007) |
| Mollee cotton farm water storage, Narrabri, Australia | Water sample (Farm runoff) | 50 µg $L^{-1}$ (spiked solution) | 4.3 and 3.3 (light and dark dissipation) | Rose *et al*. (2007) |
| Vegetated pond, Cotton farm, Narrabri, Australia | Water sample (pond receiving cotton farm runoff) | 50 µg $L^{-1}$ (spiked solution) | 38.3 and 33.3 (light and dark dissipation, respectively) | Rose *et al*. (2007) |
| Prometryn | | | | |
| Tochigi, Japan | Soil (Loam) | NR | 12.9-22.0 | Suzuki and Otani (2004) |
| Tochigi, Japan | Soil (Clay loam) | NR | 6.2-7.0 | Suzuki and Otani (2004) |
| Calasparra, Murcia, Spain | River water (assessment of light and dark dissipation) | 5 mg $L^{-1}$ spiked solution | 55 and 66 (for light and dark dissipation, respectively) | Navarro *et al*. (2004) |
| Mar Menor Lagoon, Murcia, Spain | Sea water (assessment of light and dark dissipation) | 5 mg $L^{-1}$ spiked solution | 68 and 216 (for light and dark dissipation, respectively) | Navarro *et al*. (2004) |
| El Palmar, Murcia, Spain | Groundwater (assessment of light and dark dissipation) | 5 mg $L^{-1}$ spiked solution | 88 and 263 (for light and dark dissipation, respectively) | Navarro *et al*. (2004) |
| Auscott cotton farm water storage, Narrabri, Australia | Water sample (Storage receiving cotton farm runoff) | 50 µg $L^{-1}$ (spiked solution) | 2.1 and 2.1 (for light and dark dissipation, respectively) | Rose *et al*. (2007) |

| | | | | |
|---|---|---|---|---|
| Galathera Creek floodway wetland, Narrabri, Australia | Water sample (wetland adjacent cotton farm) | 50 µg $L^{-1}$ (spiked solution) | 4.0 and 5.5 (for light and dark dissipation, respectively) | Rose *et al*. (2007) |
| Mollee cotton farm water storage, Narrabri, Australia | Water sample (Farm runoff) | 50 µg $L^{-1}$ (spiked solution) | 2.1 and 2.1 (for light and dark dissipation) | Rose *et al*. (2007) |
| Vegetated pond, Cotton farm, Narrabri, Australia | Water sample (pond receiving cotton farm runoff) | 50 µg $L^{-1}$ (spiked solution) | 4.5 and 4.4 (for light and dark dissipation, respectively) | Rose *et al*. (2007) |
| Milli-Q (Millipore Waters, Canada) water purifier system located at Murcia, Spain | Organic-free purified water | 5 mg $L^{-1}$ (spiked solution) | 165, 106 and 168 (for light + 20°C, Light + 40°C and dark + 20°C, respectively) | Vela *et al*. (2004) |
| Urban drinking water network of Murcia, Spain | Drinking water | 5 mg $L^{-1}$ (spiked solution) | 90, 75 and 128 (for light + 20°C, Light + 40°C and dark + 20°C, respectively) | Vela *et al*. (2004) |
| Wastewater treatment plant, Molina de Segura, Murcia, Spain | Wastewater after physical and biological treatment | 5 mg $L^{-1}$ (spiked solution) | 137, 97 and 156 (for light + 20°C, Light + 40°C and dark + 20°C, respectively) | Vela *et al*. (2004) |
| Endosulfan | | | | |
| Cotton farm of Northern New South Wales, Australia | Cotton plants | 2.25-3.00 kg $ha^{-1}$ | 1.6 | Kennedy *et al*. (2001) |
| Cotton farm of Northern New South Wales, Australia | Water (Farm runoff water samples) | 2.25-3.00 kg $ha^{-1}$ | 1.5 | Kennedy *et al*. (2001) |
| Cotton farm of Northern New South Wales, Australia | Soil (Silty loam; 0.9% organic carbon) | 2.25-3.00 kg $ha^{-1}$ | 7.1 | Kennedy *et al*. (2001) |
| Auscott cotton farm water storage, Narrabri, Australia | Water sample (Storage receiving cotton farm runoff) | 5 µg $L^{-1}$ (spiked solution) | 1.2 and 0.7 (for light and dark dissipation, respectively) | Rose *et al*. (2007) |
| Galathera Creek floodway wetland, Narrabri, Australia | Water sample (wetland adjacent cotton farm) | 5 µg $L^{-1}$ (spiked solution) | 35.9 and 24.7 (for light and dark dissipation, respectively) | Rose *et al*. (2007) |
| Mollee cotton farm water storage, Narrabri, Australia | Water sample (Farm runoff) | 5 µg $L^{-1}$ (spiked solution) | 0.9 and 0.9 (for light and dark dissipation, respectively) | Rose *et al*. (2007) |
| Vegetated pond, Cotton farm, Narrabri, Australia | Water sample (pond receiving cotton farm runoff) | 5 µg $L^{-1}$ (spiked solution) | 4.1 and 1.1 (for light and dark dissipation, respectively) | Rose *et al*. (2007) |
| Mesocosm pond, Namoi River catchment, New South Wales, Australia | Mesocosm water, gravel and sediment | 0.1-10 µg $L^{-1}$ (spiked pond water) | 19, 31 and 38 (for water, gravel and sediment fractions of pond, respectively) | Pablo and Hyne (2009) |

| | | | | |
|---|---|---|---|---|
| Mesocosm pond, Namoi River catchment, New South Wales, Australia | Mesocosm sediment, gravel and sediment | 2-2000 µg $kg^{-1}$ (spiked pond sediment) | 34, 58 and 207 (for water, gravel and sediment fractions of pond, respectively) | Pablo and Hyne (2009) |
| African eggplant (*Solanum macrocarpon*) farm Cotonou, Benin | Soil (sand; 0.65% organic carbon) | 0.7603 kg $ha^{-1}$ (two applications during study) | 8.5 and 17 (for first and second applications, respectively) | Rosendahl *et al*. (2009) |
| African eggplant (*Solanum macrocarpon*) farm Cotonou, Benin | Soil (sand; 0.72% organic carbon) | 0.7603 kg $ha^{-1}$ (two applications during study) | NR and 74 (for first and second application, respectively) | Rosendahl *et al*. (2009) |
| Field tomato (*Lycopersicon esculentum*) crop, Akumadan, Ghana | Soil (sandy loam; 1.3% organic matter) | 0.5 kg $ha^{-1}$ | 6.03 | Ntow *et al*. (2007) |
| Field tomato (*Lycopersicon esculentum*) crop, Akumadan, Ghana | Leaves of field tomato | 0.5 kg $ha^{-1}$ | 0.43 | Ntow *et al*. (2007) |
| Carrasquilla wadi (hypersaline coastal lagoon), Mar Menor catchment, Iberian Peninsula | Seawater | 0.56 mg $L^{-1}$ (spiked solution) | 4.5 and 5.1 (for α-endosulfan and β-endosulfan, respectively) | Navarro *et al*. (2000) |
| Milli-Q (Millipore Waters, Canada) water purifier system located at Murcia, Spain | Organic-free purified water | 0.56 mg $L^{-1}$ (spiked solution) | 28.9 and 77.0 (for α-endosulfan and β-endosulfan, respectively) | Navarro *et al*. (2000) |
| Carrasquilla wadi (hypersaline coastal lagoon), Mar Menor catchment, Iberian Peninsula | Sterile sediment | 1.75 mg $kg^{-1}$ (spiked sediment) | 17.4 and 16.1 (for α-endosulfan and β-endosulfan, respectively) | Navarro *et al*. (2000) |
| Carrasquilla wadi (hypersaline coastal lagoon), Mar Menor catchment, Iberian Peninsula | Unsterile sediment | 1.75 mg $kg^{-1}$ (spiked sediment) | 8.5 and 8.0 (for α-endosulfan and β-endosulfan, respectively) | Navarro *et al*. (2000) |
| Cotton farm, Hisar, India. | Soil (Sandy loam; 0.5% organic matter) | 0.875 kg $ha^{-1}$ | 39.6-42.1 | Kathpal *et al*. (1997) |
| Chick pea (*Cicer arietinum*) farm, Mohanpur, West Bengal, India | Soil (soil properties not reported) | 0.35-0.70 kg $ha^{-1}$ | 4.4-5.0 | Chowdhury *et al*. (2007) |
| Lychee orchard, Northern Thailand | Soil (not reported) | 4.71 kg $ha^{-1}$ | 3.1 and 4.0 (for α-endosulfan and β-endosulfan, respectively) | Ciglasch *et al*. (2006) |
| Rorkee, India | Soil (sandy loam) | 5 µg $g^{-1}$ (spiked soil) | 27.4 and 27.5 (for α-endosulfan | Kaur *et al*. (1998) |

| | | | | |
|---|---|---|---|---|
| | | | and β-endosulfan, respectively) | |
| Rorkee, India | Water (treatment pH 5.5 and 8.0 phosphate buffered saline prepared in distilled water) | 1 mg $L^{-1}$ (spiked solution) | 11.3-11.8; and 5.3-5.0 (for pH 5.5 and 8.0 treatments, respectively) | Kaur *et al*. (1998) |

Recommended diuron, prometryn and endosulfan application regimes as described on labels and in industry pest management guides.

| Chemical | Land use | Application timing | Application rate (kg a.i. $ha^{-1}$) | Conditions | Reference |
|---|---|---|---|---|---|
| Diuron | Cotton | At crop planting and pre-emergence | 0.9-1.8 | Apply at planting or within 7 days of planting and prior to cotton emergence. Seed should be planted 4-5 cm deep and the soil compacted over the seed. Use high rate on soil with high clay or organic matter | Farrell (2008) and Nufarm (2009a) |
| | | Post-emergence | 0.9-1.8 | Applications made when cotton plant is 30 cm high using higher rate on high clay and/or organic matter soil. Not to be applied on light sandy soils low in organic matter. | Farrell (2008) and Nufarm (2009a) |
| | | Defoliation | 0.01-0.2 | Apply when youngest boll is expected to reach harvest is physiologically mature. This occurs when 60-65% cotton bolls are open. | Farrell (2008) |
| | Grass seed crops | At weed emergence | 2.7-3.0 | Apply on crop that is at least one year old using boom spray with higher rates on high clay soils. | Nufarm (2009a) |
| | Phalaris, sirolan and Sirosa | At weed emergence | 1.5 | Apply with boom spray using higher rates on high clay soils. | Nufarm (2009a) |
| | Lucerne | When weeds are small | 0.9-1.7 | Apply to weeds that are very small on lucerne crop that has been grazed and established for 1 year or more | Nufarm (2009a) |
| | Pulses (including chickpeas, faba beans, lentils, narbon beans, field peas, and vetch) | At crop sowing | 0.8-1.0 | Incorporated by sowing using lower rates on sandy soils | Nufarm (2009a) |
| | | Crop pre-emergence | 0.5-0.8 | Application to moist soil before weed or crop emergence using lower rates on sandy soils | Nufarm (2009a) |
| | Wheat, barley and oats | Crop post-emergence | 0.45 | Apply when weeds are 2-4 leaf stage and when crop is past 2-5 leaf stage about 6 weeks after sowing | Nufarm (2009a) |
| | Wheat and barley | Post-emergence | 0.252 | Apply by boom or aircraft when crop is at 3-5 leaf stage, with weeds at 2-5 leaf stage | Nufarm (2009a) |
| | Irrigation channels and drainage ditches | Non-crop season | 36 | Apply in 2000L of water when channel is not in use and prior to expected seasonal rainfall | Farrell (2008) and Nufarm (2009a) |

| | | | | | |
|---|---|---|---|---|---|
| | Rights of way, commercial and industrial areas | Initial treatment | 4.05-16.2<br>16.2-32.4<br>24.3-36 | Apply prior to or during active weed growing season. To be used when rainfall can be expected to take chemical to root zone. Applications rates for regions of annual rain 0-500 mm; 500-1000 mm; and 1000 mm or more, using higher rates on heavy clay and/or high organic matter soils | Nufarm (2009a) |
| Prometryn | Cotton | Maintenance of fallow, prior to planting and weed pre-emergence | 1.1-2.3 | Apply to bare moist soil. If conditions are dry after application, incorporation to a depth of 5cm for treated soil is necessary | Farrell (2008); Nufarm (2009c); OzCrop (2009) |
| | | At crop planting and pre-emergence | 0.8-2.3 | Apply to bare moist soil or irrigate within three days of spraying. | Farrell (2008), Nufarm (2009c), and OzCrop (2009) |
| | | Crop post-emergence | 0.4-2.3 | Apply when weeds are less than 8 cm high | Nufarm (2009c), and OzCrop (2009) |
| | Chickpea | After crop planting | 0.75 | Apply immediately after planting. Application should not be made to rigid or excessively cloddy soils. | Nufarm (2009c), and OzCrop (2009) |
| | Perennial grass seed crop (sirocco, Phlaris, Demeter Fescue, Currie Cocksfoot, Medea ryegrass 3-6 leaf stage) | Less than four true leaves on target weeds | 0.6-1.1 | Apply when weeds are young and actively growing, using higher rates on heavy soils | Nufarm (2009c), and OzCrop (2009) |
| | Sunflower | Crop pre-emergence | 1.4-2.0 | Apply to bare moist soil at within 2-3 days of sowing. Rainfall or irrigation within 10 days is necessary to move the chemical to the weed root zone using higher rates on heavy clay soils and lower rates on light sandy soils. | Nufarm (2009c), and OzCrop (2009) |
| Endosulfan | Canola, Cereals, and pulses (including chickpeas, cowpeas, pigeon peas, adzuki beans, faba beans, field peas, navy beans, mung beans, lupins and soybeans) | Strictly crop pre-emergence | 0.2-0.4 | Apply using a ground rig using 50 L $ha^{-1}$ of water. Use lower rates for broad area spraying of mite infestation prior to seedling emergence. Use higher rates at perimeter where mites are moving to paddock from adjacent areas. | Nufarm (2009b) |

| | | | | |
|---|---|---|---|---|
| Cotton | Apply for pest pressure | 0.8 | Ground applications can be made 1 October-15 January; and aerial application can be made 15 November-15 Janurary on cotton higher than 20 cm. Applications should be made at the first sign of infestation for control of armyworm, cutworm, tipworm, aphids, leaf hopper, silverleaf whitefly, Green vegetable bug, and thrips. To control cotton bollworm, native budworm and rough bollworm, applications should be made at or prior to egg hatching. | Farrell (2008); and Nufarm (2009b) |

**APPENDIX 2**

Summary of the sample collection period for measurement of diuron, prometryn and endosulfan in various sampling locations of the Gwydir River catchment by the NSW department of Water and Energy.

| Site | Years sampled | Location[a] | |
|---|---|---|---|
| | | Latitude | Longitude |
| Carole Creek, Garah | 2002-2007 | 748312.3 | 6775224.0 |
| Gwydir River, Brageen Crossing | 1991-2007 | 747245.1 | 6744963.0 |
| Gwydir River, Allambie Bridge | 2002-2007 | 738145.2 | 6759226.0 |
| Mehi River, Bronte | 1991-2006 | 682585.7 | 6739779.0 |
| Moomin Creek, Glendello | 2002-2007 | 739783.7 | 6712045.0 |
| Carole Creek, Mungindi Rd | 1991-2001 | 773105.0 | 6746980.0 |
| Moomin Creek, Iffley | 1991-2006 | 683130.2 | 6728754.0 |
| Thalaba Creek, Merrywinebone | 1991-2006 | 676593.9 | 6715254.0 |
| Warialda Creek, Warialda No. 3 | 2002-2007 | 842668.6 | 6724136.0 |
| Horton River, Rider (Killara) | 2002-2007 | 823678.1 | 6694581.0 |
| Gwydir River, Gravesend Rd Bridge | 1992-2007 | 826105.0 | 6722504.0 |
| Gwydir River, Yarraman Bridge | 2002-2007 | 776611.6 | 6741329.0 |
| Mehi River, Moree | 2002-2007 | 776434.1 | 6737094.0 |

[a] Location determined using Geodetic System of Australia (GDA) 1984 zone 55 South

Summary of single-species diuron toxicity studies used in SSD development for each taxonomic group.

| Species scientific name | Species common name | End point | Effect measure | Concentration ($\mu g\ L^{-1}$)[a] |
|---|---|---|---|---|
| Algae | | | | |
| *Synechococcus sp.* | Blue-green algae | $EC_{50}$ | Population abundance | 0.55 (1) |
| *Chlorella pyrenoidosa* | Green algae | $EC_{50}$ | Population growth rate | 1.3 (1) |
| *Coccolithus huxleyi* | Coccolithophorid | $EC_{50}$ | Population abundance | 2.26 (1) |
| *Scenedesmus quadricauda* | Green algae | $EC_{50}$ | Population growth rate | 2.7 (1) |
| *Ceramium tenuicorne* | Red Algae | $EC_{50}$ | Population growth rate | 3.4 (1) |
| *Chlorella vulgaris* | Green algae | $EC_{50}$ | Population growth rate | 4.3 (1) |
| *Scenedesmus acutus acutus* | Green Algae | $IC_{50}$ | Population growth rate | 7 (3) |
| *Dunaliella tertiolecta* | Green algae | $EC_{50}$ | Population growth rate | 11 (3) |
| *Pseudokirchneriella subcapitata* | Green algae | $EC_{50}$ | Population growth rate and abundance | 11 (6) |
| *Scenedesmus acutus* | Green algae | $IC_{50}$ | Population abundance | 12 (1) |
| *Nitzschia closterium* | Diatom | $IC_{50}$ | Population growth rate | 17 (1) |
| *Gracilaria tenuistipitata* | Red algae | $EC_{50}$ | Population growth rate | 17.5 (2) |
| *Oscillatoria chalybea* | Blue-green algae | $IC_{50}$ | Population abundance | 23 (1) |
| *Entomoneis punctulata* | Algae | $IC_{50}$ | Population growth rate | 24 (1) |
| *Chlorococcum hypnosporum* | Green algae | $EC_{50}$ | Population growth rate | 25 (1) |
| *Navicula forcipata* | Diatom | $EC_{50}$ | Population growth rate | 27 (2) |
| *Chaetoceros gracilis* | Diatom | $IC_{50}$ | Population abundance | 36 (1) |
| *Scenedesmus subspicatus* | Green algae | $EC_{50}$ | Population abundance | 36 (1) |
| *Phaeodactylum tricornutum* | Diatom | $IC_{50}$ | Population growth rate | 40 (2) |
| *Ulothrix fimbriata* | Green algae | $EC_{50}$ | Population growth rate | 540 (1) |
| *Chlamydomonas moewusii* | Green algae | $IC_{50}$ | Population abundance | 559 (1) |
| *Oscillatoria laetevirens* | Blue-green Algae | $EC_{50}$ | Population biomass | 979 (1) |
| *Skeletonema costatum* | Diatom | $EC_{50}$ | Population abundance | 13000 (1) |
| *Euglena gracilis* | Flagellate euglenoid | $IC_{50}$ | Population abundance | 21911 (1) |
| *Pyrocystis lunula* | Dinoflagellate | $EC_{50}$ | Population abundance | 25787 (2) |
| Macrophytes | | | | |
| *Apium nodiflorum* | European Marshwort | $EC_{50}$ | Growth rate | 2.8 (1) |
| *Myriophyllum spicatum* | Eurasian watermilfoil | $EC_{50}$ | Growth rate | 5 (1) |

| | | | | |
|---|---|---|---|---|
| *Lemna perpusilla* | Minute duckweed | $EC_{50}$ | Growth rate | 15 (1) |
| *Lemna minor* | Common duckweed | $IC_{50}$ | Population growth rate | 25 (1) |
| *Spirodela polyrhiza* | Large duckweed | $EC_{50}$ | Population growth | 41 (1) |
| Fish | | | | |
| *Salmonidae* | Trout family | LC50 | Mortality | 1100 (1) |
| *Salvelinus namaycush* | Lake trout | LC50 | Mortality | 1800 (1) |
| *Morone saxatilis* | Striped bass | LC50 | Mortality | 1893(13) |
| *Oncorhynchus clarki* | Cutthroat trout | LC50 | Mortality | 1900 (1) |
| *Cyprinus carpio* | common carp | LC50 | Mortality | 3046(2) |
| *Oryzias latipes* | Medaka | LC50 | Mortality | 3500 (1) |
| *Carassius auratus* | Goldfish | LC50 | Mortality | 5800 (1) |
| *Oncorhynchus kisutch* | Coho salmon | LC50 | Mortality | 6197 (2) |
| *Mugil cephalus* | Striped mullet | LC50 | Mortality | 6300 (1) |
| *Mugil curema* | White mullet | LC50 | Mortality | 6300 (1) |
| *Cyprinodon variegatus* | Sheepshead minnow | LC50 | Mortality | 6700 (1) |
| *Oncorhynchus mykiss* | Rainbow trout | LC50 | Mortality | 6996 (16) |
| *Lepomis macrochirus* | Bluegill | LC50 | Mortality | 7052(20) |
| *Gambusia affinis* | Western mosquitofish | LC50 | Mortality | 10000 (1) |
| *Pimephales promelas* | Fathead minnow | LC50 | Mortality | 12958 (3) |
| *Tinca tinca* | Tench | LC50 | Mortality | 15500 (1) |
| *Ctenopharyngodon idella* | Grass carp | LC50 | Mortality | 31000 (1) |
| *Carassius sp.* | Carp | LC50 | Mortality | 63000 (1)[b] |
| *Rasbora heteromorpha* | Harlequinfish, red rasbora | LC50 | Mortality | 190000 (1)[b] |
| Amphibians | | | | |
| *Xenopus laevis* | African clawed frog | $LC_{50}$ | Mortality | 8100 (1) |
| *Rana catesbeiana* | Bullfrog | $LC_{50}$ | Mortality | 12700 (1) |
| *Pseudacris regilla* | Pacific chorus frog | $LC_{50}$ | Mortality | 14549 (2) |
| *Rana aurora* | Red-legged frog | $LC_{50}$ | Mortality | 22200 (1) |
| Invertebrates | | | | |
| *Gammarus lacustris* | Freshwater shrimp | LC50 | Mortality | 349 (3) |
| *Gammarus fasciatus* | Freshwater shrimp | LC50 | Mortality | 812 (5) |
| *Americamysis bahia* | Opossum shrimp | LC50 | Mortality | 1149 (2) |
| *Ceriodaphnia dubia* | Water flea | EC50 | Immobilisation | 1472 (4) |
| *Simocephalus serrulatus* | Water flea | EC50 | Immobilisation | 2000 (1) |
| *Palaemon serratus* | Common prawn | LC50 | Mortality | 3027 (2) |
| *Nitocra spinipes* | Harpacticoid copepod | LC50 | Mortality | 4000 (1) |
| *Daphnia magna* | Water flea | LC50 | Mortality | 2757 (5) |
| *Daphnia pulex* | Water flea | LC50 | Mortality | 5624 (3) |
| *Artemia salina* | Brine shrimp | LC50 | Mortality | 12010 (1) |
| *Asellus brevicaudus* | Aquatic sowbug | LC50 | Mortality | 15500 (1) |
| *Hyalella azteca* | Freshwater shrimp | LC50 | Mortality | 19400 (1) |
| *Cloeon dipterum* | Mayfly | LC50 | Mortality | 40000 (2)[b] |
| *Proisotoma minuta* | Collembolan | LD50 | Mortality | 711000 (1)[b] |

[a] Numbers in brackets are *n* studies reporting the same toxic activity for the species
[b] Numbers excluded from final SSD, but included in rank calculation

SigmaPlot output of One-way ANOVA for diuron single species toxicity groups

**One Way Analysis of Variance** Friday, May 20, 2011, 5:29:51 AM

**Data source:** Data 1 in Notebook1

Dependent Variable: Concentration

**Normality Test:** Failed (P < 0.050)

Test execution ended by user request, ANOVA on Ranks begun

**Kruskal-Wallis One Way Analysis of Variance on Ranks** Friday, May 20, 2011, 5:29:51 AM

**Data source:** Data 1 in Notebook1

Dependent Variable: Concentration

| Group | N | Missing | Median | 25% | 75% |
|---|---|---|---|---|---|
| Algae | 25 | 0 | 23.000 | 6.325 | 165.000 |
| Macrophytes | 5 | 0 | 15.000 | 4.450 | 29.000 |
| Invertebrates | 12 | 0 | 3513.500 | 1310.500 | 8817.000 |
| Fish | 16 | 0 | 6248.500 | 2842.000 | 7024.000 |
| Amphibian | 4 | 0 | 13624.500 | 10400.000 | 18374.500 |

H = 32.533 with 4 degrees of freedom. (P = <0.001)

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001)

To isolate the group or groups that differ from the others use a multiple comparison procedure.

All Pairwise Multiple Comparison Procedures (Dunn's Method) :

| Comparison | Diff of Ranks | Q | P<0.05 |
|---|---|---|---|
| Amphibian vs Macrophytes | 40.300 | 3.330 | Yes |
| Amphibian vs Algae | 34.540 | 3.555 | Yes |
| Amphibian vs Invertebrates | 14.375 | 1.380 | No |
| Amphibian vs Fish | 9.844 | 0.976 | Do Not Test |
| Fish vs Macrophytes | 30.456 | 3.295 | Yes |
| Fish vs Algae | 24.696 | 4.276 | Yes |
| Fish vs Invertebrates | 4.531 | 0.658 | Do Not Test |
| Invertebrates vs Macrophytes | 25.925 | 2.700 | No |
| Invertebrates vs Algae | 20.165 | 3.183 | Do Not Test |
| Algae vs Macrophytes | 5.760 | 0.652 | Do Not Test |

Note: The multiple comparisons on ranks do not include an adjustment for ties.

Summary of single-species prometryn toxicity studies used in SSD development for each taxonomic group.

| Species scientific name | Species common name | End point | Effect measure | Concentration ($\mu g\ L^{-1}$)[a] |
|---|---|---|---|---|
| Algae | | | | |
| *Navicula pelliculosa* | Diatom | EC50 | Population abundance | 1 (1) |
| *Anabaena variabilis* | Blue-Green Algae | EC50 | Population abundance | 3 (1) |
| *Skeletonema costatum* | Diatom | EC50 | Population abundance | 7.6 (1) |
| *Scenedesmus acutus* | Green Algae | EC50 | Population growth rate | 8.7 (2) |
| *Scenedesmus quadricauda* | Green Algae | EC50 | Population growth rate | 9.7 (1) |
| *Chlorella fusca ssp. vacuolata* | Green Algae | EC50 | Population growth rate | 12.5 (1) |
| *Pseudokirchneriella subcapitata* | Green Algae | EC50 | Population growth | 16 (2) |
| *Anabaena flosaquae* | Blue-Green Algae | EC50 | Population abundance | 40.1 (1) |
| *Cryptomonas sp.* | Cryptomonad | EC50 | Population growth rate | 42.2 (8) |
| *Dunaliella tertiolecta* | Green Algae | EC50 | Population growth | 53 (1) |
| *Chlorella vulgaris* | Green Algae | EC50 | Population growth rate | 53.6 (1) |
| *Chlorella pyrenoidosa* | Green Algae | EC50 | Population growth | 54 (2) |
| *Lyngbya sp.* | Blue-Green Algae | EC50 | Population growth | 314 (1) |
| *Chlorococcum sp.* | Green Algae | EC50 | Population doubling time | 724.2 (1) |
| Macrophytes | | | | |
| *Lemna gibba* | Inflated Duckweed | EC50 | Population abundance | 11.8 (1) |
| *Lemna perpusilla* | Duckweed | EC50 | Population growth | 13 (1) |
| *Lemna aequinoctiales* | Duckweed | IC50 | Population abundance | 41 (1) |
| *Lemna minor* | Duckweed | EC50 | Population growth rate | 54 (1) |
| *Spirodela polyrhiza* | Large Duckweed | EC50 | Population growth | 85 (1) |
| Fish | | | | |
| *Danio rerio* | Zebra Danio | LC50 | Mortality | 1838 (3) |
| *Oryzias latipes* | Medaka, High-Eyes | LC50 | Mortality | 4300 (1) |
| *Phoxinus phoxinus* | Minnow | LC50 | Mortality | 4500 (1) |
| *Oncorhynchus mykiss* | Rainbow Trout | LC50 | Mortality | 4570 (2) |
| *Cyprinodon variegatus* | Sheepshead Minnow | LC50 | Mortality | 5100 (1) |
| *Carassius auratus* | Goldfish | LC50 | Mortality | 5899 (2) |
| *Cyprinus carpio* | Common Carp | LC50 | Mortality | 6648 (2) |
| *Poecilia reticulata* | Guppy | LC50 | Mortality | 7714 (2) |
| *Lepomis macrochirus* | Bluegill | LC50 | Mortality | 10000 (2) |
| Amphibians | | | | |
| *Rana limnocharis* | Bog Frog | LC50 | Mortality | 22880 (1) |
| Invertebrates | | | | |
| *Americamysis bahia* | Opossum Shrimp | LC50 | Mortality | 1700 (1) |
| *Proisotoma minuta* | Collembolan | LD50 | Mortality | 13000 (1) |
| *Daphnia magna* | Water Flea | EC50 | Immobilisation | 16182 (3) |
| *Mercenaria mercenaria* | Hard Clam | EC50 | Immobilisation | 21000 (1) |
| *Cloeon dipterum* | Mayfly | LD50 | Mortality | 40000 (2)[b] |

[a] Numbers in brackets are *n* studies reporting the same toxic activity for the species

[b] Numbers excluded from final SSD, but included in rank calculation

SigmaPlot output of One-way ANOVA for prometryn single species toxicity groups

**One Way Analysis of Variance** Sunday, May 22, 2011, 12:27:22 AM

**Data source:** Data 1 in Prometryn SSD_20110521

Dependent Variable: Concentration

**Normality Test:** Failed (P < 0.050)

Test execution ended by user request, ANOVA on Ranks begun

**Kruskal-Wallis One Way Analysis of Variance on Ranks** Sunday, May 22, 2011, 12:27:22 AM

**Data source:** Data 1 in Prometryn SSD_20110521

Dependent Variable: Concentration

| Group | N | Missing | Median | 25% | 75% |
|---|---|---|---|---|---|
| Algae | 14 | 0 | 28.050 | 8.700 | 53.600 |
| Macrophyte | 5 | 0 | 41.000 | 12.700 | 61.750 |
| Invertebrate | 7 | 0 | 13000.000 | 503.000 | 19795.500 |
| Fish | 10 | 0 | 5499.500 | 4500.000 | 7714.000 |

H = 22.633 with 3 degrees of freedom. (P = <0.001)

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001)

To isolate the group or groups that differ from the others use a multiple comparison procedure.

All Pairwise Multiple Comparison Procedures (Dunn's Method) :

| Comparison | Diff of Ranks | Q | P<0.05 |
|---|---|---|---|
| Fish vs Algae | 17.693 | 4.056 | Yes |
| Fish vs Macrophyte | 15.700 | 2.721 | Yes |
| Fish vs Invertebrate | 1.229 | 0.237 | No |
| Invertebrate vs Algae | 16.464 | 3.376 | Yes |
| Invertebrate vs Macrophyte | 14.471 | 2.346 | No |
| Macrophyte vs Algae | 1.993 | 0.363 | No |

Note: The multiple comparisons on ranks do not include an adjustment for ties.

Summary of single-species technical endosulfan toxicity studies used in SSD development for each taxonomic group.

| Species scientific name | Species common name | End point | Effect measure | Concentration ($\mu g\ L^{-1}$)[a] |
|---|---|---|---|---|
| Fish | | | | |
| *Nematolosa erebi* | Bony Bream | LC50 | Mortality | 0.2 (1) |
| *Rasbora heteromorpha* | Red Rasbora | LC50 | Mortality | 0.2 (1) |
| *Morone saxatilis* | Striped Bass | LC50 | Mortality | 0.2 (10) |
| *Leiostomus xanthurus* | Spot | EC50 | Mortality | 0.2 (2) |
| *Lagodon rhomboides* | Pinfish | LC50 | Mortality | 0.3 (1) |
| *Carassius auratus* | Goldfish | LC50 | Mortality | 0.3 (5) |
| *Oncorhynchus mykiss* | Rainbow Trout | LC50 | Mortality | 0.3 (14) |
| *Mugil cephalus* | Striped Mullet | LC50 | Mortality | 0.4 (1) |
| *Macquaria ambigua* | Yellowbelly | LC50 | Mortality | 0.4 (2) |
| *Monopterus albus* | Swamp Eel | LC50 | Mortality | 0.5 (3) |
| *Melanotaenia duboulayi* | Eastern Rainbow Fish | LC50 | Mortality | 0.5 (1) |
| *Mugil curema* | White Mullet | LC50 | Mortality | 0.6 (1) |
| *Oreochromis mossambicus* | Mozambique Tilapia | LC50 | Mortality | 0.8 (4) |
| *Salmo trutta* | Brown Trout | LC50 | Mortality | 0.9 (1) |
| *Jenynsia multidentata* | Onesided Livebearer | LC50 | Mortality | 1.0 (2) |
| *Cyprinus carpio* | Common Carp | LC50 | Mortality | 1.0 (2) |
| *Pimephales promelas* | Fathead Minnow | LC50 | Mortality | 1.0 (17) |
| *Fundulus heteroclitus* | Mummichog | LC50 | Mortality | 1.0 (7) |
| *Cymatogaster aggregata* | Shiner Perch | LC50 | Mortality | 1.1 (2) |
| *Heteropneustes fossilis* | Indian Catfish | LC50 | Mortality | 1.1 (1) |
| *Labeo rohita* | Rohu | LC50 | Mortality | 1.2 (2) |
| *Misgurnus anguillicaudatus* | Oriental Weatherfish | LC50 | Mortality | 1.2 (2) |
| *Cyprinodon variegatus* | Sheepshead Minnow | LC50 | Mortality | 1.2 (15) |
| *Barbus sophore* | Two Spot Barb | LC50 | Mortality | 1.3 (2) |
| *Atherinops affinis* | Topsmelt | LC50 | Mortality | 1.3 (1) |
| *Tilapia zillii* | Tilapia | LC50 | Mortality | 1.3 (2) |
| *Ictalurus punctatus* | Channel Catfish | LC50 | Mortality | 1.5 (1) |
| *Menidia beryllina* | Inland Silverside | LC50 | Mortality | 1.5 (1) |
| *Hypophthalmichthys molitrix* | Silver Carp | LC50 | Mortality | 1.5 (9) |
| *Gymnocorymbus ternetzi* | Black Tetra | LC50 | Mortality | 1.6 (1) |
| *Anabas testudineus* | Climbing Perch | LC50 | Mortality | 1.6 (3) |
| *Catla catla* | Catla | LC50 | Mortality | 1.6 (5) |
| *Mystus cavasius* | Catfish | LC50 | Mortality | 1.9 (1) |
| *Bidyanus bidyanus* | Silver Perch | LC50 | Mortality | 1.9 (3) |
| *Barbus stigma* | Barb | LC50 | Mortality | 1.9 (1) |
| *Oncorhynchus kisutch* | Silver Salmon | LC50 | Mortality | 2.1 (2) |
| *Mystus vittatus* | Striped Catfish | LC50 | Mortality | 2.2 (1) |
| *Tilapia nilotica* | Nile Tilapia | LC50 | Mortality | 2.3 (2) |
| *Cirrhinus mrigala* | Hawk Fish | LC50 | Mortality | 2.5 (1) |
| *Salvelinus fontinalis* | Brook Trout | LC50 | Mortality | 2.6 (1) |
| *Tilapia aurea* | Tilapia | LC50 | Mortality | 2.7 (1) |

| | | | | |
|---|---|---|---|---|
| *Leuciscus idus ssp. melanotus* | Carp | LC50 | Mortality | 3.2 (1) |
| *Ctenopharyngodon idella* | Grass Carp | LC50 | Mortality | 3.3 (9) |
| *Scardinius erythrophthalmus* | Rudd | LC50 | Mortality | 3.3 (2) |
| *Macrognathus aculeatus* | Spiny Eel | LC50 | Mortality | 3.5 (1) |
| *Lepomis macrochirus* | Bluegill | LC50 | Mortality | 3.6 (12) |
| *Catostomus commersoni* | White Sucker | LC50 | Mortality | 3.6 (8) |
| *Poecilia reticulata* | Guppy | LC50 | Mortality | 4.1 (3) |
| *Cyprinus carpio ssp. communis* | Carp | LC50 | Mortality | 5.2 (1) |
| *Oryzias latipes* | Medaka | LC50 | Mortality | 5.5 (4) |
| *Gambusia affinis* | Western Mosquito fish | LC50 | Mortality | 5.6 (9) |
| *Tilapia sp.* | Tilapia | LC50 | Mortality | 5.9 (1) |
| *Gasterosteus aculeatus* | Three spine Stickleback | LC50 | Mortality | 6.0 (1) |
| *Channa punctata* | Snake-Head Catfish | LC50 | Mortality | 6.2 (4) |
| *Barbus javanicus* | Barb | LC50 | Mortality | 8.0 (9) |
| *Leuciscus idus* | Golden Orfe | EC50 | Mortality | 9.0 (3) |
| *Channa orientalis* | Smooth-Breasted Snakefish | LC50 | Mortality | 9.3 (13) |
| *Macropodus cupanus* | Paradise Fish | LC50 | Mortality | 10.2 (3) |
| *Clarias batrachus* | Walking Catfish | LC50 | Mortality | 10.5 (11) |
| *Nuria danrica* | Channel fish | LC50 | Mortality | 10.8 (6) |
| *Anguilla japonica* | Japanese Eel | LC50 | Mortality | 14.0 (1) |
| *Barilius bendelisis* | Cyprinid Fish | LC50 | Mortality | 15.8 (2) |
| *Barbus conchonius* | Rosy Barb | LC50 | Mortality | 21.4 (1) |
| *Lepidocephalichthys thermalis* | Loach | LC50 | Mortality | 30.0 (1) |
| *Cyprinus carpio ssp. carpio* | Carp | LC50 | Mortality | 33.6 (1) |
| *Anguilla anguilla* | Common Eel | LC50 | Mortality | 34.0 (18) |
| *Agonus cataphractus* | Hooknose | LC50 | Mortality | 57.4 (1) |
| | | | | |
| Amphibians | | | | |
| *Rana tigrina* | Tiger Frog, Indian Bullfrog | LC50 | Mortality | 2.4 (4) |
| *Rana clamitans* | Green Frog | LC50 | Mortality | 15.0 (2) |
| *Bufo melanostictus* | Common Indian Toad | LC50 | Mortality | 128.5 (3) |
| | | | | |
| Invertebrates | | | | |
| *Penaeus duorarum* | Northern Pink Shrimp | LC50 | Mortality | 0.04 (1) |
| *Eucyclops sp.* | Cyclopoid Copepod | LC50 | Mortality | 0.1 (1) |
| *Acartia tonsa* | Calanoid Copepod | LC50 | Mortality | 0.1 (6) |
| *Alonella sp.* | Water Flea | LC50 | Mortality | 0.2 (1) |
| *Crangon septemspinosa* | Bay Shrimp, Sand Shrimp | LC50 | Mortality | 0.2 (1) |
| *Paratelphusa jacquemontii* | Crab | LC50 | Mortality | 0.2 (3) |
| *Penaeus sp.* | Penaeidean Shrimp | LC50 | Mortality | 0.2 (2) |
| *Palaemonetes paludosus* | Riverine Grass Shrimp | LC50 | Mortality | 0.3 (4) |
| *Daphnia longispina* | Water Flea | LC50 | Mortality | 0.3 (2) |
| *Penaeus indicus* | Indian Prawn | LC50 | Mortality | 0.4 (4) |
| *Macrophthalmus erato* | Mangrove Crab | LC50 | Mortality | 0.5 (1) |
| *Diaptomus sp.* | Calanoid Copepod | LC50 | Mortality | 0.6 (1) |
| *Atalophlebia australis* | Mayfly | LC50 | Mortality | 0.6 (10) |

| | | | | |
|---|---|---|---|---|
| *Cheumatopsyche sp.* | Caddisfly | LC50 | Mortality | 0.7 (6) |
| *Paratya australiensis* | Shrimp | LC50 | Mortality | 0.7 (2) |
| *Cypria sp.* | Ostracod | LC50 | Mortality | 0.9 (1) |
| *Amphipoda* | Scud Order | LC50 | Mortality | 1.0 (1) |
| *Gammarus palustris* | Gammarid Amphipod | LC50 | Mortality | 1.1 (3) |
| *Americamysis bahia* | Opossum Shrimp | LC50 | Mortality | 1.1 (16) |
| *Culex quinquefasciatus* | Southern House Mosquito | LC50 | Mortality | 1.2 (2) |
| *Palaemonetes pugio* | Daggerblade Grass Shrimp | LC50 | Mortality | 1.3 (1) |
| *Jappa kutera* | Mayfly | LC50 | Mortality | 1.4 (16) |
| *Caridina laevis* | Smooth Caridina | LC50 | Mortality | 1.6 (2) |
| *Uca pugilator* | Fiddler Crab | LC50 | Mortality | 1.6 (1) |
| *Crassostrea virginica* | American Oyster | LC50 | Mortality | 1.6 (1) |
| *Paphia laterisulca* | Estuarine Clam | LC50 | Mortality | 2.0 (1) |
| *Crangon sp.* | Caridean Shrimp | LC50 | Mortality | 3.1 (2) |
| *Macrobrachium rosenbergii* | Giant River Prawn | LC50 | Mortality | 3.1 (5) |
| *Palaemon macrodactylus* | Korean Or Oriental Shrimp | LC50 | Mortality | 3.4 (1) |
| *Macrobrachium lamarrei* | Prawn | LC50 | Mortality | 4.2 (4) |
| *Strongylocentrotus droebachiensis* | Green Sea Urchin | LC50 | Mortality | 4.5 (1) |
| *Macrobrachium dayanum* | Freshwater Prawn | LC50 | Mortality | 5.0 (4) |
| *Palaemonetes argentinus* | Caridean Shrimp | LC50 | Mortality | 6.3 (1) |
| *Pteronarcys californica* | Stonefly | LC50 | Mortality | 6.8 (3) |
| *Gammarus lacustris* | Scud | LC50 | Mortality | 7.0 (3) |
| *Hyalella azteca* | Scud | LC50 | Mortality | 7.3 (3) |
| *Gammarus fasciatus* | Scud | LC50 | Mortality | 7.7 (2) |
| *Asellus aquaticus* | Aquatic Sowbug | LC50 | Mortality | 10.0 (1) |
| *Crangon crangon* | Common Shrimp | LC50 | Mortality | 10.0 (1) |
| *Caridina weberi* | Pugnose Caridina | LC50 | Mortality | 12.7 (16) |
| *Crassostrea madrasensis* | Oyster | LC50 | Mortality | 14.1 (1) |
| *Cancer magister* | Dungeness Crab | EC50 | Immobilisation | 14.9 (2) |
| *Atalophlebia sp.* | Mayfly | LC50 | Mortality | 15.6 (7) |
| *Eretes sticticus* | Beetle | LC50 | Mortality | 15.8 (2) |
| *Moina micrura* | Water Flea | LC50 | Mortality | 16.2 (1) |
| *Sigara alternata* | Water Boatman | EC50 | Immobilisation | 16.5 (3) |
| *Paramelita nigroculus* | Amphipod | LC50 | Mortality | 19.2 (1) |
| *Penaeus monodon* | Jumbo Tiger Prawn | LC50 | Mortality | 20.3 (6) |
| *Lamellidens marginalis* | Mussel | LC50 | Mortality | 20.5 (3) |
| *Enallagma sp.* | Damselfly | LC50 | Mortality | 21.9 (3) |
| *Procambarus clarkii* | Red Swamp Crayfish | LC50 | Mortality | 24.0 (1) |
| *Nanosesarma sp.* | Crab | LC50 | Mortality | 31.0 (1) |
| *Lamellidens corrianus* | Bivalve | LC50 | Mortality | 31.0 (3) |
| *Spicodiaptomus chilospinus* | Calanoid Copepod | LC50* | Mortality | 44.7 (2) |
| *Pseudagrion sp.* | Dragonfly | LC50 | Mortality | 46.0 (1) |
| *Chironomus plumosus* | Midge | LC50 | Mortality | 53.0 (1) |
| *Lymnaea luteola* | Pond Snail | LC50 | Mortality | 60.0 (1) |
| *Culex pipiens ssp. quinquefasciata* | Mosquito | LC50 | Mortality | 66.0 (1) |
| *Tigriopus japonicus* | Harpacticoid Copepod | LC50 | Mortality | 70.0 (1) |

| | | | | |
|---|---|---|---|---|
| *Chironomus riparius* | Midge | LC50 | Mortality | 100.0 (1) |
| *Ischnura sp.* | Damselfly | LC50* | Mortality | 119.9 (10) |
| *Culex fatigans* | Mosquito | LC50 | Mortality | 142.3 (2) |
| *Moina macrocopa* | Water Flea | LC50 | Mortality | 160.0 (1) |
| *Eucalanus sp.* | Calanoid Copepod | LC50 | Mortality | 175.0 (1) |
| *Daphnia carinata* | Water Flea | EC50 | Immobilisation | 180.0 (1) |
| *Neanthes arenaceodentata* | Polychaete Worm | LC50 | Mortality | 196.6 (4) |
| *Scylla serrata* | Crab | LC50 | Mortality | 213.9 (2) |
| *Moinodaphnia macleayi* | Water Flea | EC50 | Immobilisation | 215.0 (1) |
| *Litopenaeus stylirostris* | Blue Shrimp | LC50 | Mortality | 235.0 (1) |
| *Acartia sp.* | Calanoid Copepod | LC50 | Mortality | 243.0 (1) |
| *Daphnia magna* | Water Flea | LC50 | Mortality | 275.5 (2) |
| *Lucifer sp.* | Decapod | LC50 | Mortality | 290.0 (1) |
| *Daphnia pulex* | Water Flea | LC50 | Mortality | 300.0 (1) |
| *Aedes aegypti* | Yellow Fever Mosquito | LC50 | Mortality | 316.2 (1) |
| *Physa fontinalis* | Bladder Snail | LC50 | Mortality | 316.2 (1) |

[a] Numbers in brackets are *n* studies reporting the same toxic activity for the species

Summary of single-species technical endosulfan sulphate toxicity studies used in SSD development for each taxonomic group.

| Species scientific name | Species common name | End point | Effect measure | Concentration of endosulfan sulphate ($\mu g\ L^{-1}$)[a] |
|---|---|---|---|---|
| Fish | | | | |
| *Oncorhynchus mykiss* | Rainbow Trout | LC50 | Mortality | 1.5 (3) |
| *Poecilia reticulata* | Guppy | LC50 | Mortality | 3.2 (1) |
| *Carassius auratus* | Goldfish | LC50 | Mortality | 31.6 (1) |
| *Leuciscus idus ssp. melanotus* | Carp | LC50 | Mortality | 31.6 (1) |
| Invertebrates | | | | |
| *Jappa kutera* | Mayfly | LC50 | Mortality | 1.2 (1) |
| *Hyalella azteca* | Scud | LC50 | Mortality | 5.7 (1) |
| *Aedes aegypti* | Yellow Fever Mosquito | LC50 | Mortality | 316.2 (1) |
| *Artemia salina* | Brine Shrimp | LC50 | Mortality | 316.2 (1) |
| *Chironomus riparius* | Midge | LC50 | Mortality | 316.2 (1) |
| *Physa fontinalis* | Bladder Snail | LC50 | Mortality | 316.2 (1) |
| *Daphnia magna* | Water Flea | LC50 | Mortality | 316.2 (1) |

[a] Numbers in brackets are *n* studies reporting the same toxic activity for the species

SigmaPlot output of One-way ANOVA for endosulfan single species toxicity groups

**One Way Analysis of Variance** Wednesday, May 25, 2011, 12:56:16 AM

**Data source:** Data 1 in Notebook1

Dependent Variable: Conc 1 Op (ug/L)

**Normality Test:** Failed (P < 0.050)

Test execution ended by user request, ANOVA on Ranks begun

**Kruskal-Wallis One Way Analysis of Variance on Ranks** Wednesday, May 25, 2011, 12:56:16 AM

**Data source:** Data 1 in Notebook1

Dependent Variable: Conc 1 Op (ug/L)

| Group | N | Missing | Median | 25% | 75% |
|---|---|---|---|---|---|
| Invertebrates | 75 | 0 | 10.000 | 1.130 | 58.250 |
| Fish | 67 | 0 | 1.929 | 0.996 | 5.608 |
| Amphibians | 3 | 0 | 15.000 | 5.509 | 100.112 |

H = 13.264 with 2 degrees of freedom. (P = 0.001)

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.001)

To isolate the group or groups that differ from the others use a multiple comparison procedure.

All Pairwise Multiple Comparison Procedures (Dunn's Method) :

| Comparison | Diff of Ranks | Q | P<0.05 |
|---|---|---|---|
| Amphibians vs Fish | 40.811 | 1.646 | No |
| Amphibians vs Invertebrates | 16.387 | 0.663 | Do Not Test |
| Invertebrates vs Fish | 24.424 | 3.459 | Do Not Test |

Note: The multiple comparisons on ranks do not include an adjustment for ties.

SigmaPlot output of One-way ANOVA for endosulfan sulphate single species toxicity groups

**One Way Analysis of Variance** Thursday, May 26, 2011, 12:02:50 AM

**Data source:** Data 3 in Endosulfan_SULPHATE_ SSD_20110524

Dependent Variable: Concentration

**Normality Test:** Failed (P < 0.050)

Test execution ended by user request, ANOVA on Ranks begun

**Kruskal-Wallis One Way Analysis of Variance on Ranks** Thursday, May 26, 2011, 12:02:50 AM

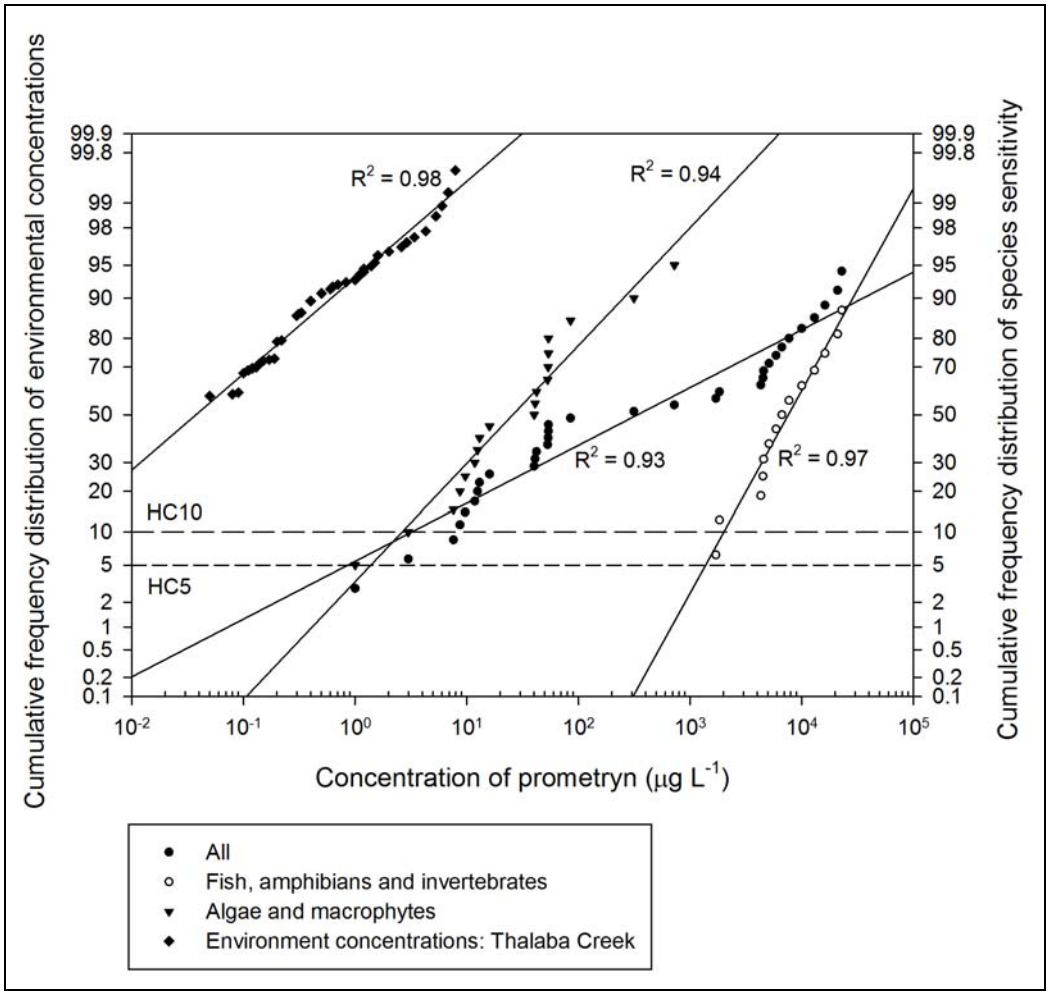
**Data source:** Data 3 in Endosulfan_SULPHATE_ SSD_20110524

Dependent Variable: Concentration

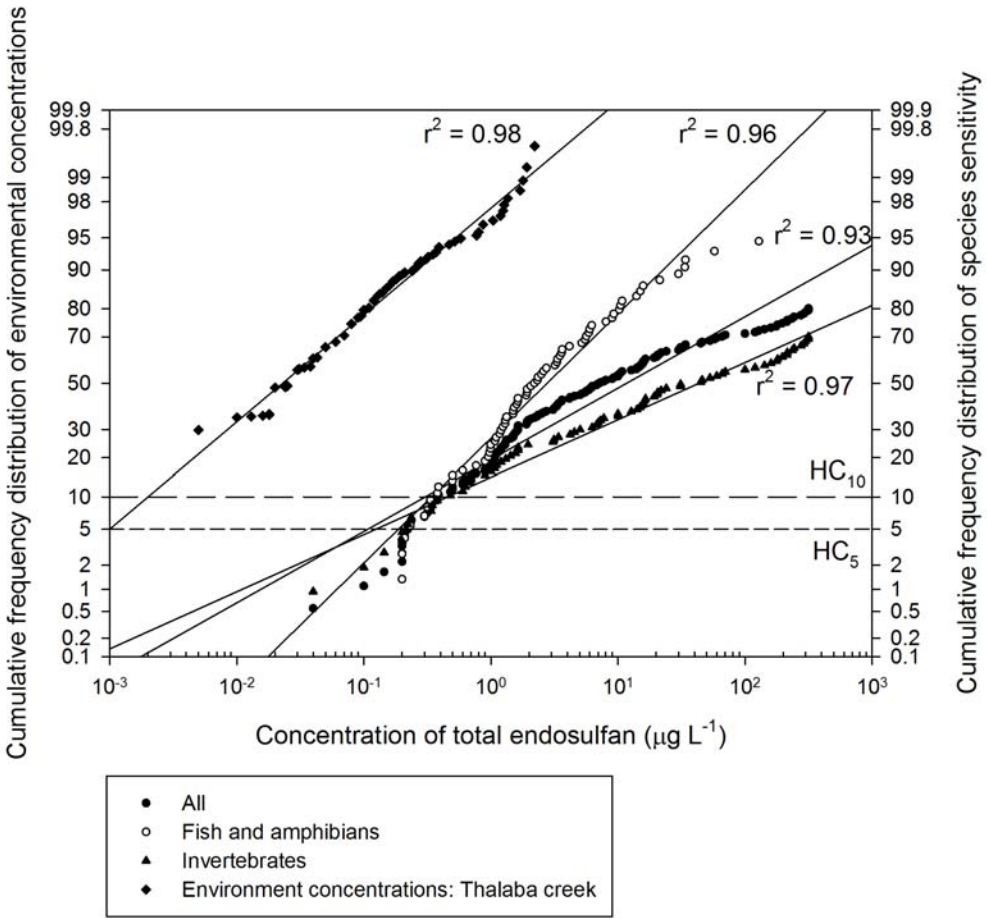
| Group | N | Missing | Median | 25% | 75% |
|---|---|---|---|---|---|
| Invertebrate | 7 | 0 | 316.228 | 83.332 | 316.228 |
| Fish | 4 | 0 | 17.393 | 2.314 | 31.623 |

H = 2.527 with 1 degrees of freedom. P(est.)= 0.112 P(exact)= 0.164

The differences in the median values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.164)




Example of relating distribution of diuron environmental concentrations at Thalaba Creek, Merrywinebone with Species sensitivity distributions of All; Fish, amphibians and invertebrates; and algae and macrophytes.




Example of relating distribution of total endosulfan environmental concentrations at Thalaba Creek, Merrywinebone with Species sensitivity distributions of All; Fish and amphibians; and invertebrates.

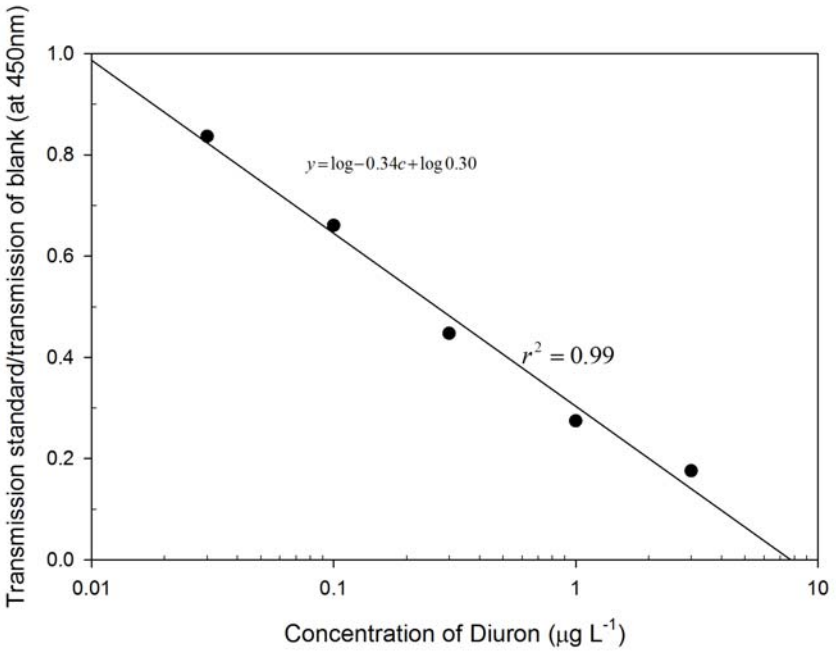
Number of sampling days relative to the time sampling period

| Site | Sample date (d/mm/yyyy) | | Number of days between sampling dates | Number of samples collected | Fraction of time accounted for in sampling (%) |
|---|---|---|---|---|---|
| | Start | End | | | |
| Carole Creek, Garah | 9/07/2002 | 15/01/2007 | 1651 | 51 | 3.1 |
| Gwydir River, Brageen Crossing | 2/09/1991 | 20/03/2007 | 5678 | 294 | 5.2 |
| Gwydir River, Allambie Bridge | 8/07/2002 | 20/03/2007 | 1716 | 53 | 3.1 |
| Mehi River, Bronte | 2/09/1991 | 16/11/2006 | 5554 | 297 | 5.3 |
| Moomin Creek, Glendello | 8/07/2002 | 20/03/2007 | 1716 | 49 | 2.9 |
| Carole Creek, Mungundi Rd | 2/09/1991 | 22/06/2001 | 3581 | 230 | 6.4 |
| Moomin Creek, Iffley | 2/09/1991 | 16/11/2006 | 5554 | 265 | 4.8 |
| Thalaba Creek, Merrywinebone | 2/09/1991 | 16/11/2006 | 5554 | 274 | 4.9 |
| Warialda Creek, Warialda No. 3 | 10/07/2002 | 19/06/2007 | 1805 | 55 | 3.0 |
| Horton River, Rider (Killara) | 10/07/2002 | 19/06/2007 | 1805 | 52 | 2.9 |
| Gwydir River, Gravesend Rd Bridge | 30/09/1992 | 19/06/2007 | 5375 | 207 | 3.9 |
| Gwydir River, Yarraman Bridge | 8/07/2002 | 20/03/2007 | 55 | 1716 | 3.2 |
| Mehi River, Moree | 8/07/2002 | 15/01/2007 | 55 | 1652 | 3.3 |

## APPENDIX 3

### Diuron ABRAXIS ELISA results of exposure standards

Following the analysis of diuron in the Hoagland's growth media using the ABRAXIS test kit, the concentration of diuron in the stock solutions was determined using that calibration curve (0.03-3 μg $L^{-1}$) of the transmission of the standard. The concentrations of the stock exposure solutions are given in the Table immediately below the graph.




ELISA calibration curve of diuron concentration as the independent variable and transmission of standard (0.03-3 μg $L^{-1}$)/transmission of standard blank (0 μg $L^{-1}$) as the dependent variable.

Table of nominal concentrations and the transmission (at 450 nm) results, together with the calculated diuron concentration (μg $L^{-1}$) in stock exposure solutions

| Nominal concentration | **Transmission** replicate 1 | Transmission replicate 2 | Calculated concentration |
|---|---|---|---|
| 0 | 1.238 | 1.558 | 0 |
| 0.3 | 0.508 | 0.596 | 0.43 |
| 3 | 0.38 | 0.217 | 1.69 |
| 25 | 0.305 | 0.261 | 30.80 |
| 50 | 0.305 | 0.274 | 59.59 |
| 100 | 0.304 | 0.307 | 117.83 |
| 200 | 0.353 | 0.289 | 208.33 |

### *LEMNA MINOR:* PULSE EXPOSURE/RECOVERY STATISTICS

## *Lemna minor*: exposure plant count ANOVA

**One Way Analysis of Variance** Monday, December 20, 2010, 10:08:50 PM

**Data source:** Data 1 in LEMNA MINOR

Dependent Variable: # Plants

**Normality Test:** Passed (P = 0.293)

**Equal Variance Test:** Passed (P = 0.076)

| Group Name | N | Missing | Mean | Std Dev | SEM |
|---|---|---|---|---|---|
| 0.000 | 6 | 0 | 14.667 | 3.386 | 1.382 |
| 0.300 | 6 | 0 | 14.833 | 3.488 | 1.424 |
| 3.000 | 6 | 0 | 15.833 | 2.714 | 1.108 |
| 25.000 | 6 | 0 | 13.667 | 3.141 | 1.282 |
| 50.000 | 6 | 0 | 9.333 | 1.033 | 0.422 |
| 100.000 | 6 | 0 | 6.833 | 1.835 | 0.749 |
| 200.000 | 6 | 0 | 3.833 | 1.472 | 0.601 |

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Between Groups | 6 | 777.238 | 129.540 | 19.103 | <0.001 |
| Residual | 35 | 237.333 | 6.781 | | |
| Total | 41 | 1014.571 | | | |

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

Multiple Comparisons versus Control Group (Dunnett's Method) :

Comparisons for factor: **Concentration**

| Comparison | Diff of Means | q' | P | P<0.050 |
|---|---|---|---|---|
| 0.000 vs. 200.000 | 10.833 | 7.206 | -- | Yes |
| 0.000 vs. 100.000 | 7.833 | 5.210 | -- | Yes |
| 0.000 vs. 50.000 | 5.333 | 3.547 | -- | Yes |
| 0.000 vs. 3.000 | 1.167 | 0.776 | -- | No |
| 0.000 vs. 25.000 | 1.000 | 0.665 | -- | Do Not Test |
| 0.000 vs. 0.300 | 0.167 | 0.111 | -- | Do Not Test |

Note: The P values for Dunnett's and Duncan's tests are currently unavailable except for reporting that the P's are greater or less than the critical values of .05 and .01.

## *Lemna minor*: exposure frond count ANOVA

**One Way Analysis of Variance** Monday, December 13, 2010, 6:05:54 PM

**Data source:** Data 1 in LEMNA MINOR

Dependent Variable: # Fronds

**Normality Test:** Passed (P = 0.604)

**Equal Variance Test:** Passed (P = 0.067)

| Group Name | N | Missing | Mean | Std Dev | SEM |
|---|---|---|---|---|---|
| 0.000 | 6 | 0 | 78.833 | 5.981 | 2.442 |
| 0.300 | 6 | 0 | 79.000 | 11.349 | 4.633 |
| 3.000 | 6 | 0 | 81.833 | 11.427 | 4.665 |
| 25.000 | 6 | 0 | 60.167 | 5.845 | 2.386 |
| 50.000 | 6 | 0 | 43.667 | 6.683 | 2.728 |
| 100.000 | 6 | 0 | 25.333 | 4.367 | 1.783 |
| 200.000 | 6 | 0 | 11.167 | 2.137 | 0.872 |

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Between Groups | 6 | 28902.571 | 4817.095 | 84.808 | <0.001 |
| Residual | 35 | 1988.000 | 56.800 | | |
| Total | 41 | 30890.571 | | | |

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

Multiple Comparisons versus Control Group (Dunnett's Method) :

Comparisons for factor: **Concentration**

| Comparison | Diff of Means | q' | P | P<0.050 |
|---|---|---|---|---|
| 0.000 vs. 200.000 | 67.667 | 15.551 | -- | Yes |
| 0.000 vs. 100.000 | 53.500 | 12.295 | -- | Yes |
| 0.000 vs. 50.000 | 35.167 | 8.082 | -- | Yes |
| 0.000 vs. 25.000 | 18.667 | 4.290 | -- | Yes |
| 0.000 vs. 3.000 | 3.000 | 0.689 | -- | No |
| 0.000 vs. 0.300 | 0.167 | 0.0383 | -- | Do Not Test |

Note: The P values for Dunnett's and Duncan's tests are currently unavailable except for reporting that the P's are greater or less than the critical values of .05 and .01.

## *Lemna minor*: exposure wet weight ANOVA

**One Way Analysis of Variance** Monday, December 20, 2010, 9:44:09 PM

**Data source:** Data 1 in LEMNA MINOR

Dependent Variable: FW

**Normality Test:** Passed (P = 0.741)

**Equal Variance Test:** Passed (P = 0.754)

| Group Name | N | Missing | Mean | Std Dev | SEM |
|---|---|---|---|---|---|

| | | | | | |
|---|---|---|---|---|---|
| CONT | 3 | 0 | 110.067 | 15.284 | 8.824 |
| 0.300 | 3 | 0 | 111.333 | 24.698 | 14.260 |
| 3.000 | 3 | 0 | 107.900 | 21.124 | 12.196 |
| 25.000 | 3 | 0 | 81.400 | 19.914 | 11.497 |
| 50.000 | 3 | 0 | 57.467 | 9.817 | 5.668 |
| 100.000 | 3 | 0 | 61.900 | 15.398 | 8.890 |
| 200.000 | 3 | 0 | 8.600 | 4.700 | 2.714 |

| **Source of Variation** | **DF** | **SS** | **MS** | **F** | **P** |
|---|---|---|---|---|---|
| Between Groups | 6 | 25603.372 | 4267.229 | 14.628 | <0.001 |
| Residual | 14 | 4083.940 | 291.710 | | |
| Total | 20 | 29687.312 | | | |

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

Multiple Comparisons versus Control Group (Dunnett's Method) :

Comparisons for factor: **FWDW_CONC**

| **Comparison** | **Diff of Means** | **q'** | **P** | **P<0.050** |
|---|---|---|---|---|
| CONT vs. 200.000 | 101.467 | 7.276 | -- | Yes |
| CONT vs. 50.000 | 52.600 | 3.772 | -- | Yes |
| CONT vs. 100.000 | 48.167 | 3.454 | -- | Yes |
| CONT vs. 25.000 | 28.667 | 2.056 | -- | No |
| CONT vs. 3.000 | 2.167 | 0.155 | -- | Do Not Test |
| CONT vs. 0.300 | 1.267 | 0.0908 | -- | Do Not Test |

Note: The P values for Dunnett's and Duncan's tests are currently unavailable except for reporting that the P's are greater or less than the critical values of .05 and .01.

## *Lemna minor*: exposure dry weight ANOVA

**One Way Analysis of Variance** Monday, December 13, 2010, 6:12:11 PM

**Data source:** Data 1 in LEMNA MINOR

Dependent Variable: Biomass

**Normality Test:** Passed (P = 0.316)

**Equal Variance Test:** Passed (P = 0.255)

| Group Name | N | Missing | Mean | Std Dev | SEM |
|---|---|---|---|---|---|
| 0.000 | 3 | 0 | 6.933 | 1.557 | 0.899 |
| 0.300 | 3 | 0 | 6.633 | 0.896 | 0.517 |
| 3.000 | 3 | 0 | 6.900 | 0.458 | 0.265 |
| 25.000 | 3 | 0 | 4.167 | 0.153 | 0.0882 |
| 50.000 | 3 | 0 | 2.800 | 0.794 | 0.458 |
| 100.000 | 3 | 0 | 1.700 | 0.520 | 0.300 |
| 200.000 | 3 | 0 | 0.233 | 0.231 | 0.133 |

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Between Groups | 6 | 133.883 | 22.314 | 35.392 | <0.001 |
| Residual | 14 | 8.827 | 0.630 | | |
| Total | 20 | 142.710 | | | |

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

Multiple Comparisons versus Control Group (Dunnett's Method) :

Comparisons for factor: **Concentration**

| Comparison | Diff of Means | q' | P | P<0.050 |
|---|---|---|---|---|
| 0.000 vs. 200.000 | 6.700 | 10.334 | -- | Yes |
| 0.000 vs. 100.000 | 5.233 | 8.072 | -- | Yes |
| 0.000 vs. 50.000 | 4.133 | 6.375 | -- | Yes |
| 0.000 vs. 25.000 | 2.767 | 4.267 | -- | Yes |
| 0.000 vs. 0.300 | 0.300 | 0.463 | -- | No |
| 0.000 vs. 3.000 | 0.0333 | 0.0514 | -- | Do Not Test |

Note: The P values for Dunnett's and Duncan's tests are currently unavailable except for reporting that the P's are greater or less than the critical values of .05 and .01.

## *Lemna minor*: Exposure response reduction in plant yield Regression

**Nonlinear Regression - Dynamic Fitting** **Thursday, December 23, 2010, 7:29:15 PM**

**Data Source: Data 4 in LEMNA MINOR**
**Equation: Standard Curves, Four Parameter Logistic Curve**
f1 = min + (max-min)/(1 + (x/EC50)^(-Hillslope))
f = if(x<=0, if(Hillslope>0,min,max), f1)

**Dynamic Fit Options:**

| | |
|---|---|
| Total Number of Fits | 200 |
| Maximum Number of Iterations | 1000 |

**Parameter Ranges for Initial Estimates:**

| | Minimum | Maximum |
|---|---|---|
| min | -200.0000 | 66.6667 |
| max | -100.0000 | 300.0000 |
| EC50 | 0.0000 | 0.9000 |
| Hillslope | -1.0000 | 3.0000 |

**Summary of Fit Results:**

| | |
|---|---|
| Converged | 100.0% |
| Singular Solutions | 43.5% |
| Ill-Conditioned Solutions | 1.0% |

**Results for the Overall Best-Fit Solution:**

| R | Rsqr | Adj Rsqr | Standard Error of Estimate |
|---|---|---|---|
| 0.8485 | 0.7200 | 0.6978 | 22.1902 |

| | Coefficient | Std. Error | t | P |
|---|---|---|---|---|
| min | -6.2206 | 5.2717 | -1.1800 | 0.2453 |
| max | 89.0378 | 19.1201 | 4.6568 | <0.0001 |
| EC50 | 58.8307 | 18.0543 | 3.2585 | 0.0024 |
| Hillslope | 2.0042 | 0.9492 | 2.1115 | 0.0414 |

**Analysis of Variance:**

Analysis of Variance:

| | DF | SS | MS |
|---|---|---|---|
| Regression | 4 | 72781.5004 | 18195.3751 |
| Residual | 38 | 18711.4134 | 492.4056 |
| Total | 42 | 91492.9138 | 2178.4027 |

Corrected for the mean of the observations:

| | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Regression | 3 | 48103.8141 | 16034.6047 | 32.5638 | <0.0001 |
| Residual | 38 | 18711.4134 | 492.4056 | | |
| Total | 41 | 66815.2276 | 1629.6397 | | |

**Statistical Tests:**

| | | |
|---|---|---|
| **Durbin-Watson Statistic** | 1.9817 | Passed |

**Normality Test (Shapiro-Wilk)** Failed (P = <0.0001)

W Statistic= 0.8254 Significance Level = 0.0500

**Constant Variance Test** Passed (P = 0.1451)

**Regression Diagnostics:**

| Row | Predicted | Residual | Stud. Res. |
|---|---|---|---|

**95% Confidence:**

| Row | Predicted | 95% Conf-L | 95% Conf-U | 95% Pred-L | 95% Pred-U |
|---|---|---|---|---|---|

## *Lemna minor*: exposure reduction in frond yield regression

**Nonlinear Regression - Dynamic Fitting** **Thursday, December 23, 2010, 12:19:29 PM**

**Data Source: Inhibition in LEMNA MINOR**
**Equation: Standard Curves, Four Parameter Logistic Curve**
f1 = min + (max-min)/(1 + (x/EC50)^(-Hillslope))
f = if(x<=0, if(Hillslope>0,min,max), f1)

**Dynamic Fit Options:**

| | |
|---|---|
| Total Number of Fits | 200 |
| Maximum Number of Iterations | 1000 |

**Parameter Ranges for Initial Estimates:**

| | Minimum | Maximum |
|---|---|---|
| min | -77.0270 | 25.6757 |
| max | -97.4359 | 292.3077 |
| EC50 | 0.0000 | 75.0000 |
| Hillslope | -1.0000 | 3.0000 |

**Summary of Fit Results:**

| | |
|---|---|
| Converged | 100.0% |
| Singular Solutions | 30.0% |

**Results for the Overall Best-Fit Solution:**

| R | Rsqr | Adj Rsqr | Standard Error of Estimate |
|---|---|---|---|
| 0.9748 | 0.9502 | 0.9462 | 8.6230 |

| | Coefficient | Std. Error | t | P |
|---|---|---|---|---|
| min | -1.7179 | 2.1823 | -0.7872 | 0.4360 |
| max | 108.4590 | 12.8596 | 8.4341 | <0.0001 |
| EC50 | 57.5244 | 12.0023 | 4.7928 | <0.0001 |
| Hillslope | 1.4131 | 0.2984 | 4.7361 | <0.0001 |

**Analysis of Variance:**

Analysis of Variance:

| | DF | SS | MS |
|---|---|---|---|
| Regression | 4 | 101188.0033 | 25297.0008 |
| Residual | 38 | 2825.5629 | 74.3569 |
| Total | 42 | 104013.5662 | 2476.5135 |

Corrected for the mean of the observations:

| | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Regression | 3 | 53892.1963 | 17964.0654 | 241.5924 | <0.0001 |
| Residual | 38 | 2825.5629 | 74.3569 | | |
| Total | 41 | 56717.7592 | 1383.3600 | | |

**Statistical Tests:**

**Durbin-Watson Statistic** 2.1463 Passed

**Normality Test (Shapiro-Wilk)** Failed (P = 0.0370)

W Statistic= 0.9432 Significance Level = 0.0500

**Constant Variance Test** Passed (P = 0.3507)

**Regression Diagnostics:**

| Row | Predicted | Residual | Stud. Res. |
|---|---|---|---|

**95% Confidence:**

| Row | Predicted | 95% Conf-L | 95% Conf-U | 95% Pred-L | 95% Pred-U |
|---|---|---|---|---|---|

## *Lemna minor*: exposure reduction in fresh weight regression

**Nonlinear Regression - Dynamic Fitting** **Thursday, December 23, 2010, 4:54:38 PM**

**Data Source: Data 5 in LEMNA MINOR**
**Equation: Standard Curves, Four Parameter Logistic Curve**
f1 = min + (max-min)/(1 + (x/EC50)^(-Hillslope))
f = if(x<=0, if(Hillslope>0,min,max), f1)

**Dynamic Fit Options:**

| | |
|---|---|
| Total Number of Fits | 200 |
| Maximum Number of Iterations | 1000 |

**Parameter Ranges for Initial Estimates:**

| | Minimum | Maximum |
|---|---|---|
| min | -71.5429 | 23.8476 |
| max | -96.8502 | 290.5507 |
| EC50 | 0.0000 | 60.8209 |
| Hillslope | -1.0000 | 3.0000 |

**Summary of Fit Results:**

| | |
|---|---|
| Converged | 100.0% |
| Singular Solutions | 71.5% |
| Ill-Conditioned Solutions | 28.5% |

**Results for the Overall Best-Fit Solution:**

| R | Rsqr | Adj Rsqr | Standard Error of Estimate |
|---|---|---|---|
| 0.9352 | 0.8746 | 0.8525 | 13.0556 |

| | Coefficient | Std. Error | t | P |
|---|---|---|---|---|
| min | -1.8625 | 6.3675 | -0.2925 | 0.7734 |
| max | 2901041.4217117 | 109482011.9048 | 2.4772E-005 | 1.0000 |
| EC50 | 6229660579.8630 | 4.1806E+014 | 1.4901E-005 | 1.0000 |
| Hillslope | 0.6017 | 0.5196 | 1.1580 | 0.2629 |

**Analysis of Variance:**

Analysis of Variance:

| | DF | SS | MS |
|---|---|---|---|
| Regression | 4 | 39098.4536 | 9774.6134 |
| Residual | 17 | 2897.6419 | 170.4495 |
| Total | 21 | 41996.0956 | 1999.8141 |

Corrected for the mean of the observations:

| | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Regression | 3 | 20215.3476 | 6738.4492 | 39.5334 | <0.0001 |
| Residual | 17 | 2897.6419 | 170.4495 | | |
| Total | 20 | 23112.9895 | 1155.6495 | | |

**Statistical Tests:**

**Durbin-Watson Statistic** 2.4843 Passed

**Normality Test (Shapiro-Wilk)** Passed (P = 0.9687)

W Statistic= 0.9838 Significance Level = 0.0500

**Constant Variance Test** Passed (P = 0.7240)

**Regression Diagnostics:**

| Row | Predicted | Residual | Stud. Res. |
|---|---|---|---|

**95% Confidence:**

| Row | Predicted | 95% Conf-L | 95% Conf-U | 95% Pred-L | 95% Pred-U |
|---|---|---|---|---|---|

## *Lemna minor*: exposure reduction in dry weight yield regression

**Nonlinear Regression - Dynamic Fitting** **Thursday, December 23, 2010, 11:58:22 AM**

**Data Source: Data 3 in LEMNA MINOR**
**Equation: Standard Curves, Four Parameter Logistic Curve**
f1 = min + (max-min)/(1 + (x/EC50)^(-Hillslope))
f = if(x<=0, if(Hillslope>0,min,max), f1)

**Dynamic Fit Options:**

| | |
|---|---|
| Total Number of Fits | 200 |
| Maximum Number of Iterations | 1000 |

**Parameter Ranges for Initial Estimates:**

| | Minimum | Maximum |
|---|---|---|
| min | -74.1945 | 24.7315 |
| max | -107.0423 | 321.1268 |
| EC50 | 0.0000 | 46.9528 |
| Hillslope | -1.0000 | 3.0000 |

**Summary of Fit Results:**

| | |
|---|---|
| Converged | 100.0% |
| Singular Solutions | 32.0% |

**Results for the Overall Best-Fit Solution:**

| R | Rsqr | Adj Rsqr | Standard Error of Estimate |
|---|---|---|---|
| 0.9629 | 0.9272 | 0.9143 | 12.3962 |

| | Coefficient | Std. Error | t | P |
|---|---|---|---|---|
| min | -1.0559 | 4.8124 | -0.2194 | 0.8289 |
| max | 117.5001 | 24.9594 | 4.7076 | 0.0002 |
| EC50 | 43.8320 | 18.0354 | 2.4303 | 0.0264 |
| Hillslope | 1.2208 | 0.5221 | 2.3383 | 0.0319 |

**Analysis of Variance:**

Analysis of Variance:

| | DF | SS | MS |
|---|---|---|---|
| Regression | 4 | 69360.9470 | 17340.2368 |
| Residual | 17 | 2612.3359 | 153.6668 |
| Total | 21 | 71973.2829 | 3427.2992 |

Corrected for the mean of the observations:

| | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Regression | 3 | 33248.5589 | 11082.8530 | 72.1226 | <0.0001 |
| Residual | 17 | 2612.3359 | 153.6668 | | |
| Total | 20 | 35860.8948 | 1793.0447 | | |

**Statistical Tests:**

| | | |
|---|---|---|
| **Durbin-Watson Statistic** | 1.2098 | Failed |
| **Normality Test (Shapiro-Wilk)** | | Passed (P = 0.2801) |

W Statistic= 0.9456 Significance Level = 0.0500

**Constant Variance Test** Passed (P = 0.6007)

**Regression Diagnostics:**

| Row | Predicted | Residual | Stud. Res. |
|---|---|---|---|

**95% Confidence:**

| Row | Predicted | 95% Conf-L | 95% Conf-U | 95% Pred-L | 95% Pred-U |
|---|---|---|---|---|---|

## *Lemna minor*: recovery plant count ANOVA

**One Way Analysis of Variance** Friday, December 24, 2010, 11:21:11 AM

**Data source:** Data 1 in LEMNA MINOR_RECOVERY ANALYSIS

Dependent Variable: # Plants

**Normality Test:** Passed (P = 0.340)

**Equal Variance Test:** Passed (P = 0.199)

| Group Name | N | Missing | Mean | Std Dev | SEM |
|---|---|---|---|---|---|
| 0.000 | 3 | 0 | 23.000 | 5.568 | 3.215 |
| 0.300 | 3 | 0 | 23.333 | 6.506 | 3.756 |
| 3.000 | 3 | 0 | 21.000 | 2.646 | 1.528 |
| 25.000 | 3 | 0 | 16.000 | 1.000 | 0.577 |
| 50.000 | 3 | 0 | 18.333 | 5.859 | 3.383 |
| 100.000 | 3 | 0 | 15.000 | 2.000 | 1.155 |
| 200.000 | 3 | 0 | 8.333 | 0.577 | 0.333 |

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Between Groups | 6 | 506.571 | 84.429 | 4.925 | 0.007 |
| Residual | 14 | 240.000 | 17.143 | | |
| Total | 20 | 746.571 | | | |

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.007).

Power of performed test with alpha = 0.050: 0.856

Multiple Comparisons versus Control Group (Dunnett's Method) :

Comparisons for factor: **Concentration**

| Comparison | Diff of Means | q' | P | P<0.050 |
|---|---|---|---|---|
| 0.000 vs. 200.000 | 14.667 | 4.338 | -- | Yes |
| 0.000 vs. 100.000 | 8.000 | 2.366 | -- | No |
| 0.000 vs. 25.000 | 7.000 | 2.071 | -- | Do Not Test |
| 0.000 vs. 50.000 | 4.667 | 1.380 | -- | Do Not Test |
| 0.000 vs. 3.000 | 2.000 | 0.592 | -- | Do Not Test |
| 0.000 vs. 0.300 | 0.333 | 0.0986 | -- | Do Not Test |

Note: The P values for Dunnett's and Duncan's tests are currently unavailable except for reporting that the P's are greater or less than the critical values of .05 and .01.

## *Lemna minor*: recovery frond count ANOVA

**One Way Analysis of Variance** Friday, December 24, 2010, 11:24:00 AM

**Data source:** Data 1 in LEMNA MINOR_RECOVERY ANALYSIS

Dependent Variable: # Fronds

**Normality Test:** Passed (P = 0.122)

**Equal Variance Test:** Passed (P = 0.448)

| Group Name | N | Missing | Mean | Std Dev | SEM |
|---|---|---|---|---|---|
| 0.000 | 3 | 0 | 132.333 | 17.898 | 10.333 |
| 0.300 | 3 | 0 | 147.667 | 9.452 | 5.457 |
| 3.000 | 3 | 0 | 137.333 | 13.204 | 7.623 |
| 25.000 | 3 | 0 | 112.333 | 10.599 | 6.119 |
| 50.000 | 3 | 0 | 118.000 | 11.269 | 6.506 |
| 100.000 | 3 | 0 | 93.000 | 12.166 | 7.024 |
| 200.000 | 3 | 0 | 55.000 | 8.888 | 5.132 |

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Between Groups | 6 | 17862.000 | 2977.000 | 19.840 | <0.001 |
| Residual | 14 | 2100.667 | 150.048 | | |
| Total | 20 | 19962.667 | | | |

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

Multiple Comparisons versus Control Group (Dunnett's Method) :

Comparisons for factor: **Concentration**

| Comparison | Diff of Means | q' | P | P<0.050 |
|---|---|---|---|---|
| 0.000 vs. 200.000 | 77.333 | 7.732 | -- | Yes |
| 0.000 vs. 100.000 | 39.333 | 3.933 | -- | Yes |
| 0.000 vs. 25.000 | 20.000 | 2.000 | -- | No |
| 0.000 vs. 0.300 | 15.333 | 1.533 | -- | Do Not Test |
| 0.000 vs. 50.000 | 14.333 | 1.433 | -- | Do Not Test |
| 0.000 vs. 3.000 | 5.000 | 0.500 | -- | Do Not Test |

Note: The P values for Dunnett's and Duncan's tests are currently unavailable except for reporting that the P's are greater or less than the critical values of .05 and .01.

## *Lemna minor*: recovery wet weight ANOVA

**One Way Analysis of Variance** Friday, December 24, 2010, 11:26:25 AM

**Data source:** Data 1 in LEMNA MINOR_RECOVERY ANALYSIS

Dependent Variable: Freshweight mg

**Normality Test:** Passed (P = 0.358)

**Equal Variance Test:** Passed (P = 0.848)

| Group Name | N | Missing | Mean | Std Dev | SEM |
|---|---|---|---|---|---|
| 0.000 | 3 | 0 | 135.500 | 20.129 | 11.622 |
| 0.300 | 3 | 0 | 163.400 | 12.275 | 7.087 |
| 3.000 | 3 | 0 | 154.367 | 14.624 | 8.443 |
| 25.000 | 3 | 0 | 127.333 | 17.457 | 10.079 |
| 50.000 | 3 | 0 | 127.333 | 8.844 | 5.106 |
| 100.000 | 3 | 0 | 109.867 | 21.826 | 12.601 |
| 200.000 | 3 | 0 | 75.433 | 19.156 | 11.060 |

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Between Groups | 6 | 15289.436 | 2548.239 | 8.937 | <0.001 |
| Residual | 14 | 3992.013 | 285.144 | | |
| Total | 20 | 19281.450 | | | |

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 0.995

Multiple Comparisons versus Control Group (Dunnett's Method) :

Comparisons for factor: **Concentration**

| Comparison | Diff of Means | q' | P | P<0.050 |
|---|---|---|---|---|
| 0.000 vs. 200.000 | 60.067 | 4.357 | -- | Yes |
| 0.000 vs. 0.300 | 27.900 | 2.024 | -- | No |
| 0.000 vs. 100.000 | 25.633 | 1.859 | -- | Do Not Test |
| 0.000 vs. 3.000 | 18.867 | 1.368 | -- | Do Not Test |
| 0.000 vs. 25.000 | 8.167 | 0.592 | -- | Do Not Test |
| 0.000 vs. 50.000 | 8.167 | 0.592 | -- | Do Not Test |

Note: The P values for Dunnett's and Duncan's tests are currently unavailable except for reporting that the P's are greater or less than the critical values of .05 and .01.

## *Lemna minor*: Recovery dry weight ANOVA

**One Way Analysis of Variance** Friday, December 24, 2010, 11:38:09 AM

**Data source:** Data 1 in LEMNA MINOR_RECOVERY ANALYSIS

Dependent Variable: Dryweight mg

**Normality Test:** Passed (P = 0.784)

**Equal Variance Test:** Failed (P < 0.050)

Test execution ended by user request, ANOVA on Ranks begun

**Kruskal-Wallis One Way Analysis of Variance on Ranks** Friday, December 24, 2010, 11:38:09 AM

**Data source:** Data 1 in LEMNA MINOR_RECOVERY ANALYSIS

Dependent Variable: Dryweight mg

| Group | N | Missing | Median | 25% | 75% |
|---|---|---|---|---|---|
| 0.000 | 3 | 0 | 14.700 | 12.375 | 18.000 |
| 0.300 | 3 | 0 | 14.300 | 14.075 | 15.425 |
| 3.000 | 3 | 0 | 13.100 | 11.600 | 14.525 |
| 25.000 | 3 | 0 | 10.300 | 9.475 | 11.650 |
| 50.000 | 3 | 0 | 11.800 | 11.275 | 12.850 |
| 100.000 | 3 | 0 | 9.800 | 7.250 | 9.800 |
| 200.000 | 3 | 0 | 4.700 | 4.025 | 5.750 |

H = 15.826 with 6 degrees of freedom. (P = 0.015)

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.015)

To isolate the group or groups that differ from the others use a multiple comparison procedure.

Multiple Comparisons versus Control Group (Dunnett's Method) :

| Comparison | Diff of Ranks | q' | P<0.05 |
|---|---|---|---|
| 200 vs 0 | 44.000 | 2.895 | Yes |
| 100 vs 0 | 33.000 | 2.171 | No |
| 25 vs 0 | 24.000 | 1.579 | Do Not Test |
| 50 vs 0 | 13.500 | 0.888 | Do Not Test |
| 3 vs 0 | 7.500 | 0.493 | Do Not Test |
| 0.3 vs 0 | 3.000 | 0.197 | Do Not Test |

Note: The multiple comparisons on ranks do not include an adjustment for ties.

## *LEMNA GIBBA:* PULSE EXPOSURE/RECOVERY STATISTICS

### *Lemna gibba*: exposure plant count ANOVA

**One Way Analysis of Variance** Wednesday, January 12, 2011, 11:42:15 PM

**Data source:** Data 1 in Notebook1

Dependent Variable: # Plants

**Normality Test:** Passed (P = 0.139)

**Equal Variance Test:** Passed (P = 0.168)

| Group Name | N | Missing | Mean | Std Dev | SEM |
|---|---|---|---|---|---|
| 0.000 | 6 | 0 | 14.667 | 3.204 | 1.308 |
| 0.300 | 6 | 0 | 13.333 | 3.502 | 1.430 |
| 3.000 | 6 | 0 | 14.500 | 3.017 | 1.232 |
| 25.000 | 6 | 0 | 10.833 | 3.710 | 1.515 |
| 50.000 | 6 | 0 | 8.667 | 2.251 | 0.919 |
| 100.000 | 6 | 0 | 6.167 | 1.602 | 0.654 |
| 200.000 | 6 | 0 | 4.500 | 0.837 | 0.342 |

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Between Groups | 6 | 597.238 | 99.540 | 12.967 | <0.001 |
| Residual | 35 | 268.667 | 7.676 | | |
| Total | 41 | 865.905 | | | |

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

Multiple Comparisons versus Control Group (Dunnett's Method) :

Comparisons for factor: **Concentration**

| Comparison | Diff of Means | q' | P | P<0.050 |
|---|---|---|---|---|
| 0.000 vs. 200.000 | 10.167 | 6.356 | -- | Yes |
| 0.000 vs. 100.000 | 8.500 | 5.314 | -- | Yes |
| 0.000 vs. 50.000 | 6.000 | 3.751 | -- | Yes |
| 0.000 vs. 25.000 | 3.833 | 2.396 | -- | No |
| 0.000 vs. 0.300 | 1.333 | 0.834 | -- | Do Not Test |
| 0.000 vs. 3.000 | 0.167 | 0.104 | -- | Do Not Test |

Note: The P values for Dunnett's and Duncan's tests are currently unavailable except for reporting that the P's are greater or less than the critical values of .05 and .01.

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

## *Lemna gibba*: exposure frond count ANOVA

**One Way Analysis of Variance** Wednesday, January 12, 2011, 11:48:00 PM

**Data source:** Data 1 in Notebook1

Dependent Variable: # Fronds

**Normality Test:** Failed (P < 0.050)

Test execution ended by user request, ANOVA on Ranks begun

**Kruskal-Wallis One Way Analysis of Variance on Ranks** Wednesday, January 12, 2011, 11:48:00 PM

**Data source:** Data 1 in Notebook1

Dependent Variable: # Fronds

| Group | N | Missing | Median | 25% | 75% |
|---|---|---|---|---|---|
| 0.000 | 6 | 0 | 70.500 | 66.000 | 75.000 |
| 0.300 | 6 | 0 | 65.000 | 62.000 | 67.000 |
| 3.000 | 6 | 0 | 62.000 | 59.000 | 71.000 |
| 25.000 | 6 | 0 | 51.000 | 48.000 | 60.000 |
| 50.000 | 6 | 0 | 36.000 | 33.000 | 41.000 |
| 100.000 | 6 | 0 | 24.000 | 21.000 | 26.000 |
| 200.000 | 6 | 0 | 13.000 | 13.000 | 14.000 |

H = 35.123 with 6 degrees of freedom. (P = <0.001)

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001)

To isolate the group or groups that differ from the others use a multiple comparison procedure.

Multiple Comparisons versus Control Group (Dunnett's Method) :

| Comparison | Diff of Ranks | q' | P<0.05 |
|---|---|---|---|
| 200 vs 0 | 196.500 | 4.624 | Yes |
| 100 vs 0 | 160.500 | 3.777 | Yes |
| 50 vs 0 | 118.500 | 2.788 | Yes |
| 25 vs 0 | 76.000 | 1.788 | No |
| 3 vs 0 | 36.000 | 0.847 | Do Not Test |
| 0.3 vs 0 | 32.000 | 0.753 | Do Not Test |

Note: The multiple comparisons on ranks do not include an adjustment for ties.

## *Lemna gibba*: exposure fresh weight ANOVA

**One Way Analysis of Variance** Thursday, January 13, 2011, 12:10:02 AM

**Data source:** Data 1 in Notebook1

Dependent Variable: Freshweight (mg)

**Normality Test:** Passed (P = 0.256)

**Equal Variance Test:** Passed (P = 0.349)

| Group Name | N | Missing | Mean | Std Dev | SEM |
|---|---|---|---|---|---|
| 0.000 | 3 | 0 | 178.833 | 14.022 | 8.096 |
| 0.300 | 3 | 0 | 172.067 | 7.603 | 4.390 |
| 3.000 | 3 | 0 | 169.867 | 37.507 | 21.655 |
| 25.000 | 3 | 0 | 130.067 | 9.672 | 5.584 |
| 50.000 | 3 | 0 | 86.100 | 22.131 | 12.777 |
| 100.000 | 3 | 0 | 67.933 | 18.329 | 10.582 |
| 200.000 | 3 | 0 | 15.800 | 2.722 | 1.572 |

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Between Groups | 6 | 70274.136 | 11712.356 | 31.681 | <0.001 |
| Residual | 14 | 5175.753 | 369.697 | | |
| Total | 20 | 75449.890 | | | |

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

Multiple Comparisons versus Control Group (Dunnett's Method) :

Comparisons for factor: **Concentration**

| Comparison | Diff of Means | q' | P | P<0.050 |
|---|---|---|---|---|
| 0.000 vs. 200.000 | 163.033 | 10.385 | -- | Yes |
| 0.000 vs. 100.000 | 110.900 | 7.064 | -- | Yes |
| 0.000 vs. 50.000 | 92.733 | 5.907 | -- | Yes |
| 0.000 vs. 25.000 | 48.767 | 3.106 | -- | Yes |
| 0.000 vs. 3.000 | 8.967 | 0.571 | -- | No |
| 0.000 vs. 0.300 | 6.767 | 0.431 | -- | Do Not Test |

Note: The P values for Dunnett's and Duncan's tests are currently unavailable except for reporting that the P's are greater or less than the critical values of .05 and .01.

## *Lemna gibba*: exposure dry weight ANOVA

**One Way Analysis of Variance** Thursday, January 13, 2011, 12:13:26 AM

**Data source:** Data 1 in Notebook1

Dependent Variable: Dryweight

**Normality Test:** Passed (P = 0.074)

**Equal Variance Test:** Passed (P = 0.562)

| Group Name | N | Missing | Mean | Std Dev | SEM |
|---|---|---|---|---|---|
| 0.000 | 3 | 0 | 9.200 | 0.872 | 0.503 |
| 0.300 | 3 | 0 | 9.067 | 0.462 | 0.267 |
| 3.000 | 3 | 0 | 8.633 | 0.723 | 0.418 |
| 25.000 | 3 | 0 | 5.633 | 0.153 | 0.0882 |
| 50.000 | 3 | 0 | 3.567 | 0.681 | 0.393 |
| 100.000 | 3 | 0 | 1.833 | 0.0577 | 0.0333 |
| 200.000 | 3 | 0 | 0.867 | 0.0577 | 0.0333 |

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Between Groups | 6 | 224.651 | 37.442 | 131.705 | <0.001 |
| Residual | 14 | 3.980 | 0.284 | | |
| Total | 20 | 228.631 | | | |

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

Multiple Comparisons versus Control Group (Dunnett's Method) :

Comparisons for factor: **Concentration**

| Comparison | Diff of Means | q' | P | P<0.050 |
|---|---|---|---|---|
| 0.000 vs. 200.000 | 8.333 | 19.142 | -- | Yes |
| 0.000 vs. 100.000 | 7.367 | 16.922 | -- | Yes |
| 0.000 vs. 50.000 | 5.633 | 12.940 | -- | Yes |
| 0.000 vs. 25.000 | 3.567 | 8.193 | -- | Yes |
| 0.000 vs. 3.000 | 0.567 | 1.302 | -- | No |
| 0.000 vs. 0.300 | 0.133 | 0.306 | -- | Do Not Test |

Note: The P values for Dunnett's and Duncan's tests are currently unavailable except for reporting that the P's are greater or less than the critical values of .05 and .01.

## *Lemna gibba*: exposure reduction in plant yield regression

**Nonlinear Regression - Dynamic Fitting** **Thursday, January 13, 2011, 10:52:31 AM**

**Data Source: Data 2 in LEMNA GIBBA_FINAL_EXPOSURE**
**Equation: Standard Curves, Four Parameter Logistic Curve**
f1 = min + (max-min)/(1 + (x/EC50)^(-Hillslope))
f = if(x<=0, if(Hillslope>0,min,max), f1)

**Dynamic Fit Options:**

| | |
|---|---|
| Total Number of Fits | 200 |
| Maximum Number of Iterations | 1000 |

**Parameter Ranges for Initial Estimates:**

| | Minimum | Maximum |
|---|---|---|
| min | -233.3333 | 77.7778 |
| max | -88.8889 | 266.6667 |
| EC50 | 0.0000 | 0.6500 |
| Hillslope | -1.0000 | 3.0000 |

**Summary of Fit Results:**

| | |
|---|---|
| Converged | 100.0% |
| Singular Solutions | 41.0% |
| Ill-Conditioned Solutions | 1.0% |

**Results for the Overall Best-Fit Solution:**

| R | Rsqr | Adj Rsqr | Standard Error of Estimate |
|---|---|---|---|
| 0.8559 | 0.7325 | 0.7114 | 19.2606 |

| | Coefficient | Std. Error | t | P |
|---|---|---|---|---|
| min | 0.9678 | 5.1345 | 0.1885 | 0.8515 |
| max | 89.9072 | 25.3591 | 3.5454 | 0.0011 |
| EC50 | 44.2962 | 23.7957 | 1.8615 | 0.0704 |
| Hillslope | 1.2984 | 0.7858 | 1.6522 | 0.1067 |

**Analysis of Variance:**

Analysis of Variance:

| | DF | SS | MS |
|---|---|---|---|
| Regression | 4 | 83990.4104 | 20997.6026 |
| Residual | 38 | 14096.9260 | 370.9717 |
| Total | 42 | 98087.3364 | 2335.4128 |

Corrected for the mean of the observations:

| | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Regression | 3 | 38607.4257 | 12869.1419 | 34.6904 | <0.0001 |
| Residual | 38 | 14096.9260 | 370.9717 | | |
| Total | 41 | 52704.3518 | 1285.4720 | | |

**Statistical Tests:**

| | | |
|---|---|---|
| **Durbin-Watson Statistic** | 1.5089 | Passed |
| **Normality Test (Shapiro-Wilk)** | Failed | (P = <0.0001) |

W Statistic= 0.8347 Significance Level = 0.0500

**Constant Variance Test** Passed (P = 0.9372)

**Regression Diagnostics:**

| Row | Predicted | Residual | Stud. Res. |
|---|---|---|---|

**95% Confidence:**

| Row | Predicted | 95% Conf-L | 95% Conf-U | 95% Pred-L | 95% Pred-U |
|---|---|---|---|---|---|

## *Lemna gibba*: exposure reduction in frond yield regression

**Nonlinear Regression - Dynamic Fitting** **Thursday, January 13, 2011, 2:34:18 PM**

**Data Source: Data 3 in LEMNA GIBBA_FINAL_EXPOSURE**
**Equation: Standard Curves, Four Parameter Logistic Curve**
f1 = min + (max-min)/(1 + (x/EC50)^(-Hillslope))
f = if(x<=0, if(Hillslope>0,min,max), f1)

**Dynamic Fit Options:**

| | |
|---|---|
| Total Number of Fits | 200 |
| Maximum Number of Iterations | 1000 |

**Parameter Ranges for Initial Estimates:**

| | Minimum | Maximum |
|---|---|---|
| min | -9.8901 | 3.2967 |
| max | -93.4066 | 280.2198 |
| EC50 | 0.0000 | 143.5768 |
| Hillslope | -1.0000 | 3.0000 |

**Summary of Fit Results:**

| | |
|---|---|
| Converged | 100.0% |
| Singular Solutions | 26.5% |

**Results for the Overall Best-Fit Solution:**

| R | Rsqr | Adj Rsqr | Standard Error of Estimate |
|---|---|---|---|
| 0.9667 | 0.9345 | 0.9294 | 8.7151 |

| | Coefficient | Std. Error | t | P |
|---|---|---|---|---|
| min | 6.5721 | 2.2829 | 2.8789 | 0.0065 |
| max | 106.0360 | 15.4905 | 6.8452 | <0.0001 |
| EC50 | 58.1215 | 17.1287 | 3.3932 | 0.0016 |
| Hillslope | 1.2780 | 0.3319 | 3.8502 | 0.0004 |

**Analysis of Variance:**

Analysis of Variance:

| | DF | SS | MS |
|---|---|---|---|
| Regression | 4 | 102444.4158 | 25611.1039 |

| | | | |
|---|---|---|---|
| Residual | 38 | 2886.2444 | 75.9538 |
| Total | 42 | 105330.6602 | 2507.8729 |

Corrected for the mean of the observations:

| | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Regression | 3 | 41192.5101 | 13730.8367 | 180.7788 | <0.0001 |
| Residual | 38 | 2886.2444 | 75.9538 | | |
| Total | 41 | 44078.7545 | 1075.0916 | | |

**Statistical Tests:**

**Durbin-Watson Statistic** 1.8950 Passed

**Normality Test (Shapiro-Wilk)** Failed (P = 0.0002)

W Statistic= 0.8683 Significance Level = 0.0500

**Constant Variance Test** Failed (P = <0.0001)

**Regression Diagnostics:**

| Row | Predicted | Residual | Stud. Res. |
|---|---|---|---|

**95% Confidence:**

| Row | Predicted | 95% Conf-L | 95% Conf-U | 95% Pred-L | 95% Pred-U |
|---|---|---|---|---|---|

## *Lemna gibba*: exposure reduction in fresh weight yield regression

**Nonlinear Regression - Dynamic Fitting** **Thursday, January 13, 2011, 3:05:20 PM**

**Data Source: Data 4 in LEMNA GIBBA_FINAL_EXPOSURE**
**Equation: Standard Curves, Four Parameter Logistic Curve**
f1 = min + (max-min)/(1 + (x/EC50)^(-Hillslope))
f = if(x<=0, if(Hillslope>0,min,max), f1)

**Dynamic Fit Options:**

| | |
|---|---|
| Total Number of Fits | 200 |
| Maximum Number of Iterations | 1000 |

**Parameter Ranges for Initial Estimates:**

| | Minimum | Maximum |
|---|---|---|
| min | -17.3481 | 5.7827 |
| max | -101.0081 | 303.0242 |
| EC50 | 0.0000 | 119.0882 |
| Hillslope | -1.0000 | 3.0000 |

**Summary of Fit Results:**

| | |
|---|---|
| Converged | 100.0% |
| Singular Solutions | 28.0% |

**Results for the Overall Best-Fit Solution:**

| R | Rsqr | Adj Rsqr | Standard Error of Estimate |
|---|---|---|---|

0.9705 0.9419 0.9316 9.6637

| | Coefficient | Std. Error | t | P |
|---|---|---|---|---|
| min | 0.7455 | 4.3202 | 0.1725 | 0.8650 |
| max | 194.6251 | 156.7465 | 1.2417 | 0.2312 |
| EC50 | 197.4698 | 388.7139 | 0.5080 | 0.6180 |
| Hillslope | 0.7880 | 0.3574 | 2.2048 | 0.0415 |

**Analysis of Variance:**

Analysis of Variance:

| | DF | SS | MS |
|---|---|---|---|
| Regression | 4 | 55404.3209 | 13851.0802 |
| Residual | 17 | 1587.5905 | 93.3877 |
| Total | 21 | 56991.9113 | 2713.9005 |

Corrected for the mean of the observations:

| | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Regression | 3 | 25732.2257 | 8577.4086 | 91.8473 | <0.0001 |
| Residual | 17 | 1587.5905 | 93.3877 | | |
| Total | 20 | 27319.8161 | 1365.9908 | | |

**Statistical Tests:**

**Durbin-Watson Statistic** 3.0820 Failed

**Normality Test (Shapiro-Wilk)** Passed (P = 0.5394)

W Statistic= 0.9611 Significance Level = 0.0500

**Constant Variance Test** Passed (P = 0.3398)

**Regression Diagnostics:**

| Row | Predicted | Residual | Stud. Res. |
|---|---|---|---|

**95% Confidence:**

| Row | Predicted | 95% Conf-L | 95% Conf-U | 95% Pred-L | 95% Pred-U |
|---|---|---|---|---|---|

## *Lemna gibba*: exposure reduction in dry weight yield regression

**Nonlinear Regression - Dynamic Fitting** **Thursday, January 13, 2011, 3:06:24 PM**

**Data Source: Data 5 in LEMNA GIBBA_FINAL_EXPOSURE**
**Equation: Standard Curves, Four Parameter Logistic Curve**
f1 = min + (max-min)/(1 + (x/EC50)^(-Hillslope))
f = if(x<=0, if(Hillslope>0,min,max), f1)

**Dynamic Fit Options:**

| | |
|---|---|
| Total Number of Fits | 200 |
| Maximum Number of Iterations | 1000 |

**Parameter Ranges for Initial Estimates:**

| | Minimum | Maximum |
|---|---|---|
| min | -56.1762 | 18.7254 |
| max | -99.9247 | 299.7742 |
| EC50 | 0.0000 | 64.7553 |
| Hillslope | -1.0000 | 3.0000 |

**Summary of Fit Results:**

| | |
|---|---|
| Converged | 100.0% |
| Singular Solutions | 28.5% |
| Ill-Conditioned Solutions | 0.5% |

**Results for the Overall Best-Fit Solution:**

| R | Rsqr | Adj Rsqr | Standard Error of Estimate |
|---|---|---|---|
| 0.9882 | 0.9765 | 0.9724 | 6.7316 |

| | Coefficient | Std. Error | t | P |
|---|---|---|---|---|
| min | 0.7143 | 2.6206 | 0.2726 | 0.7885 |
| max | 110.7500 | 10.6684 | 10.3812 | <0.0001 |
| EC50 | 36.3599 | 6.7263 | 5.4056 | <0.0001 |
| Hillslope | 1.2740 | 0.3020 | 4.2189 | 0.0006 |

**Analysis of Variance:**

Analysis of Variance:

| | DF | SS | MS |
|---|---|---|---|
| Regression | 4 | 71347.8304 | 17836.9576 |
| Residual | 17 | 770.3490 | 45.3146 |
| Total | 21 | 72118.1794 | 3434.1990 |

Corrected for the mean of the observations:

| | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Regression | 3 | 32039.7705 | 10679.9235 | 235.6837 | <0.0001 |
| Residual | 17 | 770.3490 | 45.3146 | | |
| Total | 20 | 32810.1195 | 1640.5060 | | |

**Statistical Tests:**

| | | |
|---|---|---|
| **Durbin-Watson Statistic** | 2.0770 | Passed |

**Normality Test (Shapiro-Wilk)** Failed (P = 0.0033)

W Statistic= 0.8439 Significance Level = 0.0500

**Constant Variance Test** Passed (P = 0.3700)

**Regression Diagnostics:**

| Row | Predicted | Residual | Stud. Res. |
|---|---|---|---|

**95% Confidence:**

| Row | Predicted | 95% Conf-L | 95% Conf-U | 95% Pred-L | 95% Pred-U |
|---|---|---|---|---|---|

## *Lemna gibba*: recovery plant count ANOVA

**One Way Analysis of Variance** Thursday, January 13, 2011, 4:27:15 PM

**Data source:** Data 1 in Notebook1

Dependent Variable: # Plants

**Normality Test:** Passed (P = 0.275)

**Equal Variance Test:** Passed (P = 0.239)

| Group Name | N | Missing | Mean | Std Dev | SEM |
|---|---|---|---|---|---|
| 0.000 | 3 | 0 | 15.667 | 2.517 | 1.453 |
| 0.300 | 3 | 0 | 15.333 | 2.309 | 1.333 |
| 3.000 | 3 | 0 | 15.000 | 1.000 | 0.577 |
| 25.000 | 3 | 0 | 15.333 | 2.309 | 1.333 |
| 50.000 | 3 | 0 | 13.667 | 2.082 | 1.202 |
| 100.000 | 3 | 0 | 17.000 | 2.646 | 1.528 |
| 200.000 | 3 | 0 | 10.333 | 0.577 | 0.333 |

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Between Groups | 6 | 81.619 | 13.603 | 3.210 | 0.034 |
| Residual | 14 | 59.333 | 4.238 | | |
| Total | 20 | 140.952 | | | |

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.034).

Power of performed test with alpha = 0.050: 0.575

Multiple Comparisons versus Control Group (Dunnett's Method) :

Comparisons for factor: **Concentration**

| Comparison | Diff of Means | q' | P | P<0.050 |
|---|---|---|---|---|
| 0.000 vs. 200.000 | 5.333 | 3.173 | -- | Yes |
| 0.000 vs. 50.000 | 2.000 | 1.190 | -- | No |
| 0.000 vs. 100.000 | 1.333 | 0.793 | -- | Do Not Test |
| 0.000 vs. 3.000 | 0.667 | 0.397 | -- | Do Not Test |
| 0.000 vs. 0.300 | 0.333 | 0.198 | -- | Do Not Test |
| 0.000 vs. 25.000 | 0.333 | 0.198 | -- | Do Not Test |

Note: The P values for Dunnett's and Duncan's tests are currently unavailable except for reporting that the P's are greater or less than the critical values of .05 and .01.

## *Lemna gibba*: recovery frond count ANOVA

**One Way Analysis of Variance** Thursday, January 13, 2011, 4:33:08 PM

**Data source:** Data 1 in LEMNA GIBBA_FINAL_RECOVERY

Dependent Variable: # Fronds

**Normality Test:** Passed (P = 0.541)

**Equal Variance Test:** Passed (P = 0.242)

| Group Name | N | Missing | Mean | Std Dev | SEM |
|---|---|---|---|---|---|
| 0.000 | 3 | 0 | 90.667 | 14.012 | 8.090 |
| 0.300 | 3 | 0 | 95.000 | 20.664 | 11.930 |
| 3.000 | 3 | 0 | 94.667 | 10.408 | 6.009 |
| 25.000 | 3 | 0 | 92.333 | 4.509 | 2.603 |
| 50.000 | 3 | 0 | 86.667 | 5.132 | 2.963 |
| 100.000 | 3 | 0 | 93.000 | 2.000 | 1.155 |
| 200.000 | 3 | 0 | 77.333 | 4.933 | 2.848 |

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Between Groups | 6 | 699.619 | 116.603 | 1.012 | 0.456 |
| Residual | 14 | 1613.333 | 115.238 | | |
| Total | 20 | 2312.952 | | | |

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.456).

Power of performed test with alpha = 0.050: 0.052

The power of the performed test (0.052) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

## *Lemna gibba*: recovery fresh weight ANOVA

**One Way Analysis of Variance** Thursday, January 13, 2011, 4:19:12 PM

**Data source:** Data 1 in Notebook1

Dependent Variable: Fresh weight

**Normality Test:** Passed (P = 0.574)

**Equal Variance Test:** Passed (P = 0.533)

| Group Name | N | Missing | Mean | Std Dev | SEM |
|---|---|---|---|---|---|
| 0.000 | 3 | 0 | 0.279 | 0.0395 | 0.0228 |
| 0.300 | 3 | 0 | 0.268 | 0.0386 | 0.0223 |
| 3.000 | 3 | 0 | 0.283 | 0.0510 | 0.0294 |

| | | | | | |
|---|---|---|---|---|---|
| 25.000 | 3 | 0 | 0.288 | 0.0206 | 0.0119 |
| 50.000 | 3 | 0 | 0.228 | 0.0128 | 0.00739 |
| 100.000 | 3 | 0 | 0.181 | 0.0235 | 0.0136 |
| 200.000 | 3 | 0 | 0.163 | 0.0265 | 0.0153 |

| **Source of Variation** | **DF** | **SS** | **MS** | **F** | **P** |
|---|---|---|---|---|---|
| Between Groups | 6 | 0.0481 | 0.00802 | 7.487 | <0.001 |
| Residual | 14 | 0.0150 | 0.00107 | | |
| Total | 20 | 0.0631 | | | |

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 0.982

Multiple Comparisons versus Control Group (Dunnett's Method) :

Comparisons for factor: **Concentration**

| **Comparison** | **Diff of Means** | **q'** | **P** | **P<0.050** |
|---|---|---|---|---|
| 0.000 vs. 200.000 | 0.116 | 4.345 | -- | Yes |
| 0.000 vs. 100.000 | 0.0976 | 3.653 | -- | Yes |
| 0.000 vs. 50.000 | 0.0508 | 1.903 | -- | No |
| 0.000 vs. 0.300 | 0.0111 | 0.414 | -- | Do Not Test |
| 0.000 vs. 25.000 | 0.00957 | 0.358 | -- | Do Not Test |
| 0.000 vs. 3.000 | 0.00430 | 0.161 | -- | Do Not Test |

Note: The P values for Dunnett's and Duncan's tests are currently unavailable except for reporting that the P's are greater or less than the critical values of .05 and .01.

## *Lemna gibba*: recovery dry weight ANOVA

**One Way Analysis of Variance** Thursday, January 13, 2011, 4:24:04 PM

**Data source:** Data 1 in Notebook1

Dependent Variable: Lemna dry weight

**Normality Test:** Passed (P = 0.726)

**Equal Variance Test:** Passed (P = 0.243)

| **Group Name** | **N** | **Missing** | **Mean** | **Std Dev** | **SEM** |
|---|---|---|---|---|---|
| 0.000 | 3 | 0 | 0.0161 | 0.00318 | 0.00184 |
| 0.300 | 3 | 0 | 0.0162 | 0.00166 | 0.000956 |
| 3.000 | 3 | 0 | 0.0159 | 0.00160 | 0.000926 |
| 25.000 | 3 | 0 | 0.0180 | 0.000814 | 0.000470 |
| 50.000 | 3 | 0 | 0.0142 | 0.000503 | 0.000291 |
| 100.000 | 3 | 0 | 0.0122 | 0.000874 | 0.000504 |
| 200.000 | 3 | 0 | 0.0101 | 0.00125 | 0.000722 |

| **Source of Variation** | **DF** | **SS** | **MS** | **F** | **P** |
|---|---|---|---|---|---|
| Between Groups | 6 | 0.000135 | 0.0000225 | 8.430 | <0.001 |
| Residual | 14 | 0.0000373 | 0.00000267 | | |
| Total | 20 | 0.000172 | | | |

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 0.992

Multiple Comparisons versus Control Group (Dunnett's Method) :

Comparisons for factor: **Concentration**

| **Comparison** | **Diff of Means** | **q'** | **P** | **P<0.050** |
|---|---|---|---|---|
| 0.000 vs. 200.000 | 0.00600 | 4.500 | -- | Yes |
| 0.000 vs. 100.000 | 0.00390 | 2.925 | -- | Yes |
| 0.000 vs. 25.000 | 0.00197 | 1.475 | -- | No |
| 0.000 vs. 50.000 | 0.00183 | 1.375 | -- | Do Not Test |
| 0.000 vs. 0.300 | 0.000167 | 0.125 | -- | Do Not Test |
| 0.000 vs. 3.000 | 0.000133 | 0.100 | -- | Do Not Test |

Note: The P values for Dunnett's and Duncan's tests are currently unavailable except for reporting that the P's are greater or less than the critical values of .05 and .01.

**APPENDIX 4**

**Chickpea PRZM inputs**

| Parameter | PRZM 3.12.3 Variable | Value used | Source/Rationale |
|---|---|---|---|
| Simulation start date | ISDAY, ISMON, ISTYR | 8 191 | NSW Government DWE water quality program |
| Simulation end date | IEDAY, IEMONTH, IEYR | 123107 | NSW Government DWE water quality program |
| **Hydrologic Data** | | | |
| Precipitation (cm) | PRECIP | Daily | BOM (2008) |
| Air Temperature (oC) | TEMP | Daily | BOM (2011) |
| Pan Factor | IPEIND | 1 | FAO |
| Snow Factor (cm/oC) | SFAC | 0 | Does not snow in Gwydir River catchment |
| Pan Evaporation Flag | IPEIND | 0 | Australian Government, Bureau of Meteorology (monthly long-term averages used as inputs) |
| Evaporation Extraction, Minimum Depth (cm) | ANETD | 17 | USEPA |
| Flag for initial crop | INICRP | 1 | |
| Crop condition (initial) | ISCOND | 3 | USEPA |
| Erosion | ERFLAG | 0 | Not used. Solution phase was of concern |
| Area (ha) | AFIELD | 1 | |
| NRCS 24-hour hyetograph | IREG | - | Not applicable |
| **Crop data** | | | |
| No. Crops simulated | NDC | 1 | |
| Crop number for the different crop | ICNCN | 1 | USEPA |
| Maximum interception storage (cm) | CINTCP | 0.2 | USEPA |
| Maximum active root depth (cm) | AMXDR | 30 | USEPA |
| Maximum areal coverage (%) | COVMAX | 100 | USEPA |
| Soil surface condition after harvest | ICNAH | 3 | |
| Runoff curve number | CN | Based on soil | Taken from PRZM manual |

| | | | |
|---|---|---|---|
| for AM-II (fallow, crop, residue) | | hydrological group | (Carousel *et al*., 2005) |
| Max. Dry weight of crop (kg/m2) | WFMAX | - | Not modelled |
| Max. Canopy height | HTMAX | 40 | USEPA |
| Crop number | CROPNO | 1 | |
| Number of cropping periods | NCPDS | 17 | |
| Day for crop emergence | EMD | 20 | Felton *et al*. (1995); and Hulugalle *et al.*(2001) |
| Month for crop emergence | EMM | 6 | |
| Year for crop emergence | IYREM | 1991-2007 | Based on monitoring period |
| Day for crop maturation | MAD | 5 | Felton *et al*. (1995); and Hulugalle *et al.*(2001) |
| Month for crop maturation | MAM | 11 | |
| Year for crop maturation | IYRMAT | 1991-2007 | Based on monitoring period |
| Day for harvest | HAD | 10 | Assumed 5 days after maturity, similar to soybean in the PRZM manual (Carousel *et al*. 2005) |
| Month for harvest | HAM | 11 | |
| Year for harvest | IYRHAR | 1991-2007 | Based on monitoring period |
| Crop number associated with NDC | INCROP | 1 | |
| **Irrigation data** | | | **fraction available water irrigation cotton** |
| Irrigation flag | IRFLAG | 0 | |
| Irrigation type | IRTYP | - | Not modelled |
| Salinity leach factor requirement | FLEACH | - | Not modelled |
| Percent depletion | PCDEPL | - | Not modelled |
| Application efficiency | RATEAP | - | Not modelled |
| **Pesticide application data** | | | |
| Number of applications | NAPS | 1 | |
| Application month, day and year | APM,APD, APY | 06,15,71-76 | At sowing (NuFarm, 2009a) |
| Chemical application method | CAM | 1 | |
| Incorporation depth (cm) | DEPI | 4 | |
| Target application | TAPP | 0.9 | (NuFarm, 2009a) |

| | | | |
|---|---|---|---|
| rate (kg/ha) | | | |
| Application efficiency (fraction) | APPEFF | 1 | |
| Spray drift | DRFT | 0 | Not modelled |
| Filtration parameter for exponential foliar application model | FILTRA | 0 | |
| Disposition of foliar pesticide after harvest | IPSCND | | |
| Plant uptake factor | UPTKF | 0 | EPA default |
| **Pesticide fate data** | | | |
| Soil type | STITLE | | Source: Australian Government, 2007 |
| Soil-water adsorption coefficient (mL/g) | KD | Koc*OC | Computed based on OC in profile and Koc of 366 mL/g (average from Chapter 1) |
| Decay rate, dissolved (/day) | DWRATE | 0.0077 | Half-life of 74 days (DuPont) |
| Decay rate, adsrobed (/day) | DSRATE | 0.0077 | Half-life of 74 days (DuPont) |
| **Soil data** | | | |
| Number of horizons | NHORIZ | 3 | ABARE (2000b) |
| Depth of soil core (cm) | CORED | 100 | Arbitrarily set and profile was extended to 100 cm, where needed (See soil input section) |
| Horizon thickness | THKNS | 10 | First layer set to 10 cm and if needed split to a 10 cm later, a second layer and third layer are used to account for remaining depth |
| Layer thickness | DPN | 2nd and 3rd yayer location dependent | ABARE (2000b) |
| Bulk density (g/cm3) | BD | location dependent | ABARE (2000b) |
| Field capacity (cm3/cm3) | THEFC | Computed | Soil class dependent, and calculated using method of Saxton and Rawls (2011) |
| Wilting point (cm3/cm3) | THEWP | Computed | Soil class dependent, and calculated using method of Saxton and Rawls (2011) |
| Initial soil moisture (cm3/cm3) | THETO | Computed | Soil class dependent, and calculated using method of Saxton and Rawls (2011) |
| Organic Carbon (%) | OC | location | ABARE (2000b) |

| | | | |
|---|---|---|---|
| | | dependent | |
| Hydrodynamic dispersion (cm2/day) | DISP | 0 | |
| Soil drainage parameter (L/day) | AD | 0 | |

**Canola PRZM inputs**

| Parameter | PRZM 3.12.3 Variable | Value used | Source/Rationale |
|---|---|---|---|
| Simulation start date | ISDAY, ISMON, ISTYR | 8 191 | NSW Government DWE water quality program |
| Simulation end date | IEDAY, IEMONTH, IEYR | 123107 | NSW Government DWE water quality program |
| **Hydrologic Data** | | | |
| Precipitation (cm) | PRECIP | Daily | BOM (2008) |
| Air Temperature (oC) | TEMP | Daily | BOM (2011) |
| Pan Factor | IPEIND | 0.76 | FAO |
| Snow Factor (cm/oC) | SFAC | 0 | Does not snow in Gwydir River catchment |
| Pan Evaporation Flag | IPEIND | 0 | Australian Government, Bureau of Meteor (monthly long-term averages used as inputs) |
| Evaporation Extraction, Minimum Depth (cm) | ANETD | 12.5 | USEPA |
| Flag for initial crop | INICRP | 1 | Canola does not emerge well through crop re Therefore, assumed that the soil would be prepar fallow condition. |
| Crop condition (initial) | ISCOND | 3 | USEPA |
| Erosion | ERFLAG | 0 | Not used. Solution phase was of concern |
| Area (ha) | AFIELD | 1 | |
| NRCS 24-hour hyetograph | IREG | - | Not applicable |
| **Crop data** | | | |
| No. Crops simulated | NDC | 1 | |
| Crop number for the different crop | ICNCN | 1 | USEPA |
| Maximum | CINTCP | 0.1 | USEPA |

| | | | |
|---|---|---|---|
| interception storage (cm) | | | |
| Maximum active root depth (cm) | AMXDR | 120 | USEPA |
| Maximum areal coverage (%) | COVMAX | 100 | USEPA |
| Soil surface condition after harvest | ICNAH | 3 | |
| Runoff curve number for AM-II (fallow, crop, residue) | CN | Based on soil hydrological group | Taken from PRZM manual (Carousel *et al*., 2005) |
| Max. Dry weight of crop (kg/m2) | WFMAX | - | Not modelled |
| Max. Canopy height | HTMAX | 125 | |
| Crop number | CROPNO | 1 | |
| Number of cropping periods | NCPDS | 17 | |
| Day for crop emergence | EMD | 20 | Canola emergence was estimated to occur approxir 5 days (assumed the same as winter wheat in Table the PRZM manual) after the sowing date recomm by NSWDPI (2010) |
| Month for crop emergence | EMM | 5 | |
| Year for crop emergence | IYREM | 1991-2007 | Based on monitoring period |
| Day for crop maturation | MAD | 14 | Maturity occurs on approximaly 14 Decemeber acc to newspaper report (ABC NEWS 24/11/ (http://www.abc.net.au/rural/content/2009/s275190 |
| Month for crop maturation | MAM | 12 | |
| Year for crop maturation | IYRMAT | 1991-2007 | Based on monitoring period |
| Day for harvest | HAD | 24 | Harvest occurs when canola is dry enough. This o approximaly 10 days after maturity according to a article (ABC NEWS 24/11/ (http://www.abc.net.au/rural/content/2009/s275190 |
| Month for harvest | HAM | 12 | |
| Year for harvest | IYRHAR | 1991-2007 | Based on monitoring period |
| Crop number associated with NDC | INCROP | 1 | |
| **Irrigation data** | | | **fraction available water irrigation cotton** |
| Irrigation flag | IRFLAG | 0 | |
| Irrigation type | IRTYP | - | Not modelled |
| Salinity leach factor requirement | FLEACH | - | Not modelled |

| | | | |
|---|---|---|---|
| Percent depletion | PCDEPL | - | Not modelled |
| Application efficiency | RATEAP | - | Not modelled |
| **Pesticide application data** | | | |
| Number of applications | NAPS | 1 | |
| Application month, day and year | APM,APD, APY | 07,06,71-76 | |
| Chemical application method | CAM | | |
| Incorporation depth (cm) | DEPI | | |
| Target application rate (kg/ha) | TAPP | 0 | Not registered for use in canola (NuFarm, 2009a) |
| Application efficiency (fraction) | APPEFF | 1 | |
| Spray drift | DRFT | 0 | Not modelled |
| Filtration parameter for exponential foliar application model | FILTRA | 0 | |
| Disposition of foliar pesticide after harvest | IPSCND | | |
| Plant uptake factor | UPTKF | 0 | EPA default |
| **Pesticide fate data** | | | |
| Soil type | STITLE | | Source: Australian Government, 2007 |
| Soil-water adsorption coefficient (mL/g) | KD | Koc*OC | Computed based on OC in profile and Koc of 366 (average from Chapter 1) |
| Decay rate, dissolved (/day) | DWRATE | 0.0077 | Half-life of 74 days (DuPont) |
| Decay rate, adsrobed (/day) | DSRATE | 0.0077 | Half-life of 74 days (DuPont) |
| **Soil data** | | | |
| Number of horizons | NHORIZ | 3 | ABARE (2000b) |
| Depth of soil core (cm) | CORED | 100 | Arbitrarily set and profile was extended to 100 cm, needed (See soil input section) |
| Horizon thickness | THKNS | 10 | First layer set to 10 cm and if needed split to a later, a second layer and third layer are used to ac for remaining depth |
| Layer thickness | DPN | 2nd and 3rd yayer location dependent | ABARE (2000b) |
| Bulk density (g/cm3) | BD | location dependent | ABARE (2000b) |

| | | | |
|---|---|---|---|
| Field capacity (cm3/cm3) | THEFC | Computed | Soil class dependent, and calculated using meth Saxton and Rawls (2011) |
| Wilting point (cm3/cm3) | THEWP | Computed | Soil class dependent, and calculated using meth Saxton and Rawls (2011) |
| Initial soil moisture (cm3/cm3) | THETO | Computed | Soil class dependent, and calculated using meth Saxton and Rawls (2011) |
| Organic Carbon (%) | OC | location dependent | ABARE (2000b) |
| Hydrodynamic dispersion (cm2/day) | DISP | 0 | |
| Soil drainage parameter (L/day) | AD | 0 | |

**Dryland wheat PRZM inputs**

| **Parameter** | **PRZM 3.12.3 Variable** | **Value used** | **Source/Rationale** |
|---|---|---|---|
| Simulation start date | ISDAY, ISMON, ISTYR | 8 191 | NSW Government DWE water quality program |
| Simulation end date | IEDAY, IEMONTH, IEYR | 123107 | NSW Government DWE water quality program |
| **Hydrologic Data** | | | |
| Precipitation (cm) | PRECIP | Daily | BOM (2008) |
| Air Temperature (oC) | TEMP | Daily | BOM (2011) |
| Pan Factor | IPEIND | 0.7 | FAO |
| Snow Factor (cm/oC) | SFAC | 0 | Does not snow in Gwydir River catchment |
| Pan Evaporation Flag | IPEIND | 0 | Australian Government, Bureau of Meteorology (monthly long-term averages used as inputs) |
| Evaporation Extraction, Minimum Depth (cm) | ANETD | 10 | USEPA |
| Flag for initial crop | INICRP | 1 | |
| Crop condition (initial) | ISCOND | 3 | |
| Erosion | ERFLAG | 0 | Not used. Solution |

| | | | |
|---|---|---|---|
| | | | phase was of concern |
| Area (ha) | AFIELD | 1 | |
| NRCS 24-hour hyetograph | IREG | - | Not applicable |
| **Crop data** | | | |
| No. Crops simulated | NDC | 1 | |
| Crop number for the different crop | ICNCN | 1 | |
| Maximum interception storage (cm) | CINTCP | 0.2 | |
| Maximum active root depth (cm) | AMXDR | 110 | |
| Maximum areal coverage (%) | COVMAX | 99 | |
| Soil surface condition after harvest | ICNAH | 3 | |
| Runoff curve number for AM-II (fallow, crop, residue) | CN | Based on soil hydrological group | Taken from PRZM manual (Carousel *et al*., 2005) |
| Max. Dry weight of crop (kg/m2) | WFMAX | - | Not modelled |
| Max. Canopy height | HTMAX | 100 | |
| Crop number | CROPNO | 1 | |
| Number of cropping periods | NCPDS | 17 | |
| Day for crop emergence | EMD | 25 | Felton *et al*. (1995); Carousel *et al*. (2005) |
| Month for crop emergence | EMM | 5 | Felton *et al*. (1995); Carousel *et al*. (2005) |
| Year for crop emergence | IYREM | 1991-2007 | Based on monitoring period |
| Day for crop maturation | MAD | 25 | Felton *et al*. (1995); Carousel *et al*. (2005) |
| Month for crop maturation | MAM | 11 | Felton *et al*. (1995); Carousel *et al*. (2005) |
| Year for crop maturation | IYRMAT | 1991-2007 | Based on monitoring period |
| Day for harvest | HAD | 1 | Felton *et al*. (1995); Carousel *et al*. (2005) |
| Month for harvest | HAM | 5 | Felton *et al*. (1995); Carousel *et al*. (2005) |
| Year for harvest | IYRHAR | 1991-2007 | Based on monitoring period |
| Crop number associated with NDC | INCROP | 1 | |
| **Irrigation data** | | | **fraction available water irrigation cotton** |
| Irrigation flag | IRFLAG | 0 | |
| Irrigation type | IRTYP | - | Not modelled |
| Salinity leach factor | FLEACH | - | Not modelled |

| | | | |
|---|---|---|---|
| requirement | | | |
| Percent depletion | PCDEPL | - | Not modelled |
| Application efficiency | RATEAP | - | Not modelled |
| **Pesticide application data** | | | |
| Number of applications | NAPS | 1 | NuFarm (2009a) |
| Application month, day and year | APM,APD, APY | 07,06,71-76 | NuFarm (2009a) |
| Chemical application method | CAM | 6 | Soil applied, user defined incorporation depth (DEPI), linearly decreasing with depth |
| Incorporation depth (cm) | DEPI | 4 | |
| Target application rate (kg/ha) | TAPP | 0.45 | Maximum label rate (NuFarm, 2009a) |
| Application efficiency (fraction) | APPEFF | 1 | Assume all chemical applied |
| Spray drift | DRFT | | Not modelled |
| Filtration parameter for exponential foliar application model | FILTRA | 0 | |
| Disposition of foliar pesticide after harvest | IPSCND | 3 | Pesticide applied prior to emergence |
| Plant uptake factor | UPTKF | 0 | EPA default |
| **Pesticide fate data** | | | |
| Soil type | STITLE | | Source: Australian Government, 2007 |
| Soil-water adsorption coefficient (mL/g) | KD | Koc*OC | Computed based on OC in profile and Koc of 366 mL/g (average from Chapter 1) |
| Decay rate, dissolved (/day) | DWRATE | 0.0077 | Half-life of 74 days (DuPont) |
| Decay rate, adsrobed (/day) | DSRATE | 0.0077 | Half-life of 74 days (DuPont) |
| **Soil data** | | | |
| Number of horizons | NHORIZ | 3 | ABARE (2000b) |
| Depth of soil core (cm) | CORED | 100 | Arbitrarily set and profile was extended to 100 cm, where needed (See soil input section) |
| Horizon thickness | THKNS | 10 | First layer set to 10 cm and if needed split to a 10 cm later, a second layer and third layer are used to account for remaining depth |

| | | | |
|---|---|---|---|
| Layer thickness | DPN | 2nd and 3rd yayer location dependent | ABARE (2000b) |
| Bulk density (g/cm3) | BD | location dependent | ABARE (2000b) |
| Field capacity (cm3/cm3) | THEFC | Computed | Soil class dependent, and calculated using method of Saxton and Rawls (2011) |
| Wilting point (cm3/cm3) | THEWP | Computed | Soil class dependent, and calculated using method of Saxton and Rawls (2011) |
| Initial soil moisture (cm3/cm3) | THETO | Computed | Soil class dependent, and calculated using method of Saxton and Rawls (2011) |
| Organic Carbon (%) | OC | location dependent | ABARE (2000b) |
| Hydrodynamic dispersion (cm2/day) | DISP | 0 | |
| Soil drainage parameter (L/day) | AD | 0 | |

**Irrigated cotton summary of PRZM inputs**

| **Parameter** | **PRZM 3.12.3 Variable** | **Value used** | **Source/Rationale** |
|---|---|---|---|
| Simulation start date | ISDAY, ISMON, ISTYR | 8 191 | NSW Government DWE water quality program |
| Simulation end date | IEDAY, IEMONTH, IEYR | 123107 | NSW Government DWE water quality program |
| **Hydrologic Data** | | | |
| Precipitation (cm) | PRECIP | Daily | BOM (2008) |
| Air Temperature (oC) | TEMP | Daily | BOM (2011) |
| Pan Factor | IPEIND | 0.75 | USEPA North Carolina Cotton scenario |
| Snow Factor (cm/oC) | SFAC | 0 | Does not snow in Gwydir River catchment |
| Pan Evaporation Flag | IPEIND | 0 | Australian Government, Bureau of Meteorology (monthly long-term averages used as inputs) |
| Evaporation Extraction, Minimum Depth (cm) | ANETD | 17 | USEPA North Carolina Cotton scenario |
| Flag for initial crop | INICRP | 1 | USEPA North Carolina Cotton scenario |
| Crop condition (initial) | ISCOND | 1 | USEPA North Carolina Cotton |

| | | | |
|---|---|---|---|
| | | | scenario |
| Erosion | ERFLAG | 0 | Not used. Runoff dominated |
| Area (ha) | AFIELD | 1 | USEPA North Carolina Cotton scenario |
| NRCS 24-hour hyetograph | IREG | - | Not applicable |
| **Crop data** | | | |
| No. Crops simulated | NDC | 1 | |
| Crop number for the different crop | ICNCN | 1 | |
| Maximum interception storage (cm) | CINTCP | 0.2 | USEPA North Carolina Cotton scenario |
| Maximum active root depth (cm) | AMXDR | 150 | Cotton Australia |
| Maximum areal coverage (%) | COVMAX | 98 | USEPA North Carolina Cotton scenario |
| Soil surface condition after harvest | ICNAH | 3 | USEPA North Carolina Cotton scenario |
| Runoff curve number for AM-II (fallow, crop, residue) | CN | Based on soil hydrological group | Taken from PRZM manual (Carousel *et al*., 2005) |
| Max. Dry weight of crop (kg/m2) | WFMAX | - | Not modelled |
| Max. Canopy height | HTMAX | 120 | Cotton australia 2010 |
| Crop number | CROPNO | 1 | Hulugalle *et al*. (2001) |
| Number of cropping periods | NCPDS | 17 | |
| Day for crop emergence | EMD | 1 | Hulugalle *et al*. (2001) |
| Month for crop emergence | EMM | 10 | Hulugalle *et al*. (2001) |
| Year for crop emergence | IYREM | 1991-2007 | Based on monitoring period |
| Day for crop maturation | MAD | 1 | Hulugalle *et al*. (2001) |
| Month for crop maturation | MAM | 4 | Hulugalle *et al*. (2001) |
| Year for crop maturation | IYRMAT | 1991-2007 | Based on monitoring period |
| Day for harvest | HAD | 1 | Hulugalle *et al*. (2001) |
| Month for harvest | HAM | 5 | Hulugalle *et al*. (2001) |
| Year for harvest | IYRHAR | 1991-2007 | Based on monitoring period |
| Crop number associated with NDC | INCROP | 1 | |
| **Irrigation data** | | | **fraction available water irrigation cotton** |
| Irrigation flag | IRFLAG | 2 (0 for dryland) | |
| Irrigation type | IRTYP | 8 | Furrow irrigation (NA for |

| | | | |
|---|---|---|---|
| | | | dryland) |
| Salinity leach factor requirement | FLEACH | 0 | Not modelled |
| Percent depletion | PCDEPL | 0.55 | PRZM manual (Carousel et al., 2005); Not Applicable for Dryland |
| Application efficiency | RATEAP | 0.2 | Koech *et al*. (2010) |
| **Pesticide application data** | | | |
| Number of applications | NAPS | 1 | Farrell (2008); and NuFarm (2009a) |
| Application month, day and year | APM,APD, APY | 11,15,71-76 | Hulugalle *et al*. (2001) |
| | APD | 10 | Agronomist and Cotton Pest management guide |
| Chemical application method | CAM | 6 | Soil applied, user defined incorporation depth (DEPI), linearly decreasing with depth |
| Incorporation depth (cm) | DEPI | 4cm and 0.5cm | Pre-emergent chemical incorporated to depth of 4cm; Post-emergent, chemical applied to the top 0.5cm |
| Target application rate (kg/ha) | TAPP | 1.8 | Maximum label rate |
| Application efficiency (fraction) | APPEFF | 1 | Assume all chemical applied |
| Spray drift | DRFT | | Not modelled |
| Filtration parameter for exponential foliar application model | FILTRA | 0 | |
| Disposition of foliar pesticide after harvest | IPSCND | 3 | Pesticide applied prior to emergence |
| Plant uptake factor | UPTKF | 0 | EPA default |
| **Pesticide fate data** | | | |
| Soil type | STITLE | | Source: Australian Government, 2007 |
| Soil-water adsorption coefficient (mL/g) | KD | Koc*OC | Computed based on OC in profile and Koc of 366 mL/g (average from Chapter 1) |
| Decay rate, dissolved (/day) | DWRATE | 0.0077 | Half-life of 74 days (DuPont) |
| Decay rate, adsrobed (/day) | DSRATE | 0.0077 | Half-life of 74 days (DuPont) |
| **Soil data** | | | |
| Number of horizons | NHORIZ | 3 | ABARE (2000b) |
| Depth of soil core (cm) | CORED | 100 | Arbitrarily set and profile was extended to 100 cm, where needed (See soil input section) |

| | | | |
|---|---|---|---|
| Horizon thickness | THKNS | 10 | First layer set to 10 cm and if needed split to a 10 cm later, a second layer and third layer are used to account for remaining depth |
| Layer thickness | DPN | 2nd and 3rd yayer location dependent | ABARE (2000b) |
| Bulk density (g/cm3) | BD | location dependent | ABARE (2000b) |
| Field capacity (cm3/cm3) | THEFC | Computed | Soil class dependent, and calculated using method of Saxton and Rawls (2011) |
| Wilting point (cm3/cm3) | THEWP | Computed | Soil class dependent, and calculated using method of Saxton and Rawls (2011) |
| Initial soil moisture (cm3/cm3) | THETO | Computed | Soil class dependent, and calculated using method of Saxton and Rawls (2011) |
| Organic Carbon (%) | OC | location dependent | ABARE (2000b) |
| Hydrodynamic dispersion (cm2/day) | DISP | 0 | |
| Soil drainage parameter (L/day) | AD | 0 | |

**Dryland Cotton summary of PRZM inputs**

| **Parameter** | **PRZM 3.12.3 Variable** | **Value used** | **Source/Rationale** |
|---|---|---|---|
| Simulation start date | ISDAY, ISMON, ISTYR | 8 191 | NSW Government DWE water quality program |
| Simulation end date | IEDAY, IEMONTH, IEYR | 123107 | NSW Government DWE water quality program |
| **Hydrologic Data** | | | |
| Precipitation (cm) | PRECIP | Daily | Australian Government, Bureau of Meteorology |
| Air Temperature (oC) | TEMP | Daily | Australian Government, Bureau of Meteorology |
| Pan Factor | IPEIND | 0.75 | USEPA North Carolina Cotton scenario |
| Snow Factor (cm/oC) | SFAC | 0 | Does not snow in Gwydir River catchment |

| | | | |
|---|---|---|---|
| Pan Evaporation Flag | IPEIND | 0 | Australian Government, Bureau of Meteorology (monthly long-term averages used as inputs) |
| Evaporation Extraction, Minimum Depth (cm) | ANETD | 17 | USEPA North Carolina Cotton scenario |
| Flag for initial crop | INICRP | 1 | USEPA North Carolina Cotton scenario |
| Crop condition (initial) | ISCOND | 1 | USEPA North Carolina Cotton scenario |
| Erosion | ERFLAG | 0 | Not used. Runoff dominated |
| Area (ha) | AFIELD | 1 | USEPA North Carolina Cotton scenario |
| NRCS 24-hour hyetograph | IREG | - | |
| **Crop data** | | | |
| No. Crops simulated | NDC | 8 | |
| Crop number for the different crop | ICNCN | 1 | |
| Maximum interception storage (cm) | CINTCP | 0.2 | USEPA North Carolina Cotton scenario |
| Maximum active root depth (cm) | AMXDR | 150 | Cotton Australia |
| Maximum areal coverage (%) | COVMAX | 98 | USEPA North Carolina Cotton scenario |
| Soil surface condition after harvest | ICNAH | 3 | USEPA North Carolina Cotton scenario |
| Runoff curve number for AM-II (fallow, crop, residue) | CN | Based on soil | |
| Max. Dry weight of crop (kg/m2) | WFMAX | | |
| Max. Canopy height | HTMAX | 120 | Cotton australia 2010 |
| Crop number | CROPNO | 1 | |
| Number of cropping periods | NCPDS | 17 | |
| Day for crop emergence | EMD | | |
| Month for crop emergence | EMM | | |
| Year for crop emergence | IYREM | 1991-2007 | |
| Day for crop maturation | MAD | 1 | |
| Month for crop maturation | MAM | 4 | |
| Year for crop maturation | IYRMAT | 1991-2007 | |
| Day for harvest | HAD | 1 | |
| Month for harvest | HAM | 5 | |
| Year for harvest | IYRHAR | 1991-2007 | |
| Crop number associated with NDC | INCROP | 1 | |

| **Irrigation data** | | **Google search** | **fraction available water irrigation cotton** |
|---|---|---|---|
| Irrigation flag | IRFLAG | 2 | |
| Irrigation type | IRTYP | 2 | Furrow irrigation |
| | FLEACH | 0 | |
| Percent depletion | PCDEPL | 0.65 | |
| Application rate | RATEAP | | |
| **Pesticide application data** | | | |
| Number of applications | NAPS | 1 | Apply once per year. Cotton pest management guide |
| Application day and month | APD | 1_31 | Agronomist and Cotton Pest management guide |
| | APM | 10 | Agronomist and Cotton Pest management guide |
| Chemical application method | CAM | 6 | Soil applied, user defined incorporation depth (DEPI), linearly decreasing with depth |
| Incorporation depth | DEPI | 1 | |
| Target application rate (kg/ha) | TAPP | 1.0-1.5 | Agronomist and Cotton Pest management guide |
| Application efficiency (fraction) | APPEFF | 1 | |
| Spray drift | DRFT | | Not used. Runoff dominated |
| Filtration parameter for exponential foliar application model | FILTRA | 0 | |
| Disposition of foliar pesticide after harvest | IPSCND | 3 | Pesticide applied prior to emergence |
| Plant uptake factor | UPTKF | 0 | EPA default |
| **Pesticide fate data** | | | |
| Soil type | STITLE | | Source: Australian Government, 2007 |
| Soil-water adsorption coefficient (mL/g) | KD | Koc*OC | Computed based on OC in profile and Koc of 366 mL/g |
| Decay rate, dissolved (/day) | DWRATE | 0.0077 | Half-life of 74 days (DuPont) |
| Decay rate, adsrobed (/day) | DSRATE | 0.0077 | Half-life of 74 days (DuPont) |
| **Soil data** | | | |
| Number of horizons | NHORIZ | 3 | |
| Depth of soil core (cm) | CORED | 100 | Arbitrarily set and profile is extended to 100 cm if needed |
| Horizon thickness | THKNS | 10 | First layer set to 10 cm and |

| | | | |
|---|---|---|---|
| | | | if needed split to a 10 cm later, a second layer and third layer are used to account for remaining depth |
| Layer thickness | DPN | 2nd and 3rd yayer location dependent | Australian government 2007 |
| Bulk density (g/cm3) | BD | location dependent | Australian government 2007 |
| Field capacity (cm3/cm3) | THEFC | Computed | |
| Wilting point (cm3/cm3) | THEWP | Computed | |
| Initial soil moisture (cm3/cm3) | THETO | Computed | |
| Organic Carbon (%) | OC | location dependent | |
| Hydrodynamic dispersion (cm2/day) | DISP | 0 | |
| Soil drainage parameter (L/day) | AD | 0 | |

**Pasture summary of PRZM inputs**

| **PRZM 3.12.3 Variable** | **Value used** | **Source/Rationale** |
|---|---|---|
| ISDAY, ISMON, ISTYR | 8 191 | NSW Government DWE water quality program |
| IEDAY, IEMONTH, IEYR | 123107 | NSW Government DWE water quality program |
| | | |
| PRECIP | Daily | BOM (2008) |
| TEMP | Daily | BOM (2011) |
| IPEIND | 0.74 | FAO |
| SFAC | 0 | Does not snow in Gwydir River catchment |
| IPEIND | 0 | Australian Government, Bureau of Meteorology (monthly long-term averages used as inputs) |
| ANETD | 10 | USEPA |
| INICRP | 1 | |
| ISCOND | 3 | USEPA |
| ERFLAG | 0 | Not used. Solution phase was of concern |
| AFIELD | 1 | |
| IREG | - | Not applicable |
| | | |

| | | |
|---|---|---|
| NDC | 1 | |
| ICNCN | 1 | USEPA |
| CINTCP | 0.1 | USEPA |
| AMXDR | 10 | USEPA |
| COVMAX | 100 | USEPA |
| ICNAH | 1 | |
| CN | Based on soil hydrological group | Taken from PRZM manual (Carousel *et al*., 2005) |
| WFMAX | - | Not modelled |
| HTMAX | 5 | USEPA |
| CROPNO | 1 | |
| NCPDS | 1 | |
| EMD | 1 | Assume planted from beginning |
| EMM | 8 | |
| IYREM | 1991 | Beginning modelling period |
| MAD | 2 | Assume immediate maturity |
| MAM | 8 | |
| IYRMAT | 1991 | Beginning modelling period |
| HAD | 31 | |
| HAM | 12 | |
| IYRHAR | 2007 | End of modelling period |
| INCROP | 1 | |
| | | **fraction available water irrigation cotton** |
| IRFLAG | 0 | |
| IRTYP | - | Not modelled |
| FLEACH | - | Not modelled |
| PCDEPL | - | Not modelled |
| RATEAP | - | Not modelled |
| | | |
| NAPS | 0 | |
| APM,APD, APY | - | Uncertain |
| CAM | - | |
| DEPI | - | |
| TAPP | - | Uncertain |
| APPEFF | - | |
| DRFT | 0 | Not modelled |
| FILTRA | 0 | |
| IPSCND | | |
| UPTKF | 0 | EPA default |
| | | |
| STITLE | | Source: Australian Government, 2007 |
| KD | Koc*OC | Computed based on OC in profile and Koc of 366 mL/g (average from Chapter 1) |

| | | |
|---|---|---|
| DWRATE | 0.0077 | Half-life of 74 days (DuPont) |
| DSRATE | 0.0077 | Half-life of 74 days (DuPont) |
| | | |
| NHORIZ | 3 | ABARE (2000b) |
| CORED | 100 | Arbitrarily set and profile was extended to 100 cm, where needed (See soil input section) |
| THKNS | 10 | First layer set to 10 cm and if needed split to a 10 cm later, a second layer and third layer are used to account for remaining depth |
| DPN | 2nd and 3rd yayer location dependent | ABARE (2000b) |
| BD | location dependent | ABARE (2000b) |
| THEFC | Computed | Soil class dependent, and calculated using method of Saxton and Rawls (2011) |
| THEWP | Computed | Soil class dependent, and calculated using method of Saxton and Rawls (2011) |
| THETO | Computed | Soil class dependent, and calculated using method of Saxton and Rawls (2011) |
| OC | location dependent | ABARE (2000b) |
| DISP | 0 | |
| AD | 0 | |

**Dryland cotton Input file example**

```
EXAMS - PRZM Exposure Simulation Shell v1.2.22, Apr 2003
*** Dryland Cotton; 15/07/2011 Title of input file  Existing
"Gwydir WL; GWL 413; Metfile: station3.MET
    0.75    0.00       0   17.00       1       3
       0
       1
       1    0.20  150.00   98.00       3  86  83  86    0.00  130.00
      17
   11070   1 471   1 571       1
   11071   1 472   1 572       1
   11072   1 473   1 573       1
   11073   1 474   1 574       1
   11074   1 475   1 575       1
   11075   1 476   1 576       1
   11076   1 477   1 577       1
   11077   1 478   1 578       1
   11078   1 479   1 579       1
   11079   1 480   1 580       1
   11080   1 481   1 581       1
```

```
   11081   1 482   1 582       1
   11082   1 483   1 583       1
   11083   1 484   1 584       1
   11084   1 471   1 571       1
   11085   1 486   1 586       1
   11086   1 487   1 587       1
RECORD 12: Chemical Input Data: Diuron
      17       1       0       0
Chemical_C
  151170  0 4 0.50  1.80 1.00 0.00
  151171  0 4 0.50  1.80 1.00 0.00
  151172  0 4 0.50  1.80 1.00 0.00
  151173  0 4 0.50  1.80 1.00 0.00
  151174  0 4 0.50  1.80 1.00 0.00
  151175  0 4 0.50  1.80 1.00 0.00
  151176  0 4 0.50  1.80 1.00 0.00
  151177  0 4 0.50  1.80 1.00 0.00
  151178  0 4 0.50  1.80 1.00 0.00
  151179  0 4 0.50  1.80 1.00 0.00
  151180  0 4 0.50  1.80 1.00 0.00
  151181  0 4 0.50  1.80 1.00 0.00
  151182  0 4 0.50  1.80 1.00 0.00
  151183  0 4 0.50  1.80 1.00 0.00
  151184  0 4 0.50  1.80 1.00 0.00
  151185  0 4 0.50  1.80 1.00 0.00
  151186  0 4 0.50  1.80 1.00 0.00
     0.0       1    0.00
Record 19: SoilID-1: Cracking clay; CSIRO2MG: 16455; HYDG: D
  130.00           0   0   0   0   0   0   0   0   0
 4300.00 5.4E-08   20.64
       3
       1  10.000   1.320   0.476   0.000   0.000   0.000
           0.009   0.009 0.00000
           0.100   0.476   0.358   0.310   1.240
       2  20.000   1.320   0.476   0.000   0.000   0.000
           0.009   0.009 0.00000
           0.100   0.476   0.358   0.310   1.240
       3  100.00   1.270   0.471   0.000   0.000   0.000
           0.009   0.009 0.00000
           1.000   0.471   0.352   0.342   1.368
       0
    WATR     YEAR       10     PEST     YEAR       10     CONC     YEAR       10
1
       3    DAY
    RFLX1   TSER 100 100   1.0E5
    RUNF1   TSER   1   1   1.0
```

```
    PRCP1   TSER   1   1   1.0
```

**Gwydir Wetlands RIVWQ input file**

```
***** FILENAME: GwydWL20110726.INP
***** Daily, rectangular
***** Dispersion added
*************************                SIMULATION                CONTROLS
*****************************
**  NJ  NH NPOI  NCHEM NPATHS  IDT  IDT3
    51   1    7      1      0    1     1
**  JM JD JY  KM KD KY    DT
     8  1 70  12 31 87    30.
**  Q1OPT   Q2OPT   Q3OPT COPT   POPT   SOPT   GOPT  MOPT  IDIFF
        1       0     2    0      1      0      1     0      2
** Q1FORM  Q2FORM  CFORM  PFORM  SFORM
        1       1      1      1      1
**  Q1UNIT  Q2UNIT  CUNIT  PUNIT  SUNIT  AGAGE IPDUR
       1.      1.     1.     1.    1.0     1.     0
************************              CHEMICAL                PROPERTIES
*******************************
**  CNAME     KW     KS    KD   VVOL  VBIND  VMIX  SOLUB
"Diuron"   .0700  .0231   1.3    0.0    0.0  .000  3.7E1
************************                SYSTEM                PROPERTIES
*********************************
** HW
  142
** POI
  142 136 104 163 153 178 166
** VSETL (SEDIMENT SETTLING RATE)
   1.0
** VEL1  VEL2   SS1    SS2 (SEDIMENT SCOUR RATING CURVE)
    0.8   1.8    0.  1.0e6
** ID   ND  IQ3   DX     DA DISP  TW    QC   QD  MUSK-K MUSK-X
** ID   DAS   POR    BD   QBASE    DBASE     DS   CSS0
** ID    CW1      CS1     PO1
*****  END OF DATA
```