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Toxicity of the herbicide Tebuthiuron to Australian tropical freshwater organisms

Towards an ecological risk assessment

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Executive summary

One of the major recognised causes of wetland degradation and loss in Australia is the invasion of exotic species. Nowhere is the threat of weeds currently greater than in the wetland habitats of the wet/dry tropics of northern Australia. One of the major weed threats, *Mimosa pigra* (mimosa), grows as a leguminous shrub up to 6 m tall, forming dense, impenetrable monospecific stands in floodplain environments. It is known as the 'giant sensitive plant' due to its bipinnate leaves that close when touched, and has invaded vast areas of northern Australian floodplains.

Strategic control of mimosa has concentrated on integration of mechanical/physical, biological and chemical control methods. Chemical control has been the most widely used approach to date and involves the application of herbicides to mimosa stands, although in some situations all three methods of control are employed. Several herbicides have been used to control mimosa but probably the most widely used has been Tebuthiuron—the active ingredient of the commercial formulation Graslan®. Tebuthiuron is a thiadiazole urea herbicide that acts to kill plants by uncoupling electron transport and thereby inhibiting photosynthesis. Graslan® contains Tebuthiuron at concentrations of either 10, 20, or 30%, and is applied to soils in clay pellet form, with primary uptake by plants being through root absorption.

In 1991 approximately 62 000 kg of Graslan® (~12 000 kg Tebuthiuron) were applied to a mimosa infestation of approximately 5800 ha at Oenpelli in western Arnhem Land, highlighting the extensive use of the herbicide in one area of northern Australia. Such large-scale application of herbicides in northern Australian wetlands is of particular environmental concern, particularly considering that there were no toxicological data available on the effects of Tebuthiuron to non-target tropical freshwater species. Adding to concerns about northern Australian wetland environments, regulatory authorities and the product's manufacturer recommend that Tebuthiuron not be applied near established watercourses or where surface water is present.

While no data exist on the aquatic toxicity of Tebuthiuron to Australian tropical freshwater species, its effects on northern hemisphere temperate species have been extensively studied. The studies indicated that Tebuthiuron toxicity to aquatic animals was very low compared to aquatic plants. Nevertheless, considering the large amounts of Tebuthiuron used in northern Australia for mimosa control, it was imperative that an assessment of the sensitivity of local aquatic organisms to the herbicide be performed.

The aims of the present study were to:

1 Assess the toxicity of Tebuthiuron to the following freshwater animals:

- the purple spotted gudgeon, Mogurnda mogurnda
- the green hydra, *Hydra viridissima*
- the cladoceran, Moinodaphnia macleayi

2 Use the above toxicity results, and all other relevant information, to undertake a preliminary ecological risk assessment on the use of Tebuthiuron for the chemical control of mimosa in wetland habitats of northern Australia.

Toxicity test results showed that the toxicity of Tebuthiuron to the three organisms tested decreased in the following order:

cladoceran (*M. macleayi*) > hydra (*H. viridissima*) > gudgeon (*M. mogurnda*).

The 10% bounded effect concentration (BEC_{10}) and EC_{50} for *M. macleayi*, *H. viridissima* and *M. mogurnda* were 17.4 and 134, 40.6 and 153, and 108 and 214 (LC_{50}) mg L^{-1}, respectively. Overall there was little difference in the toxicity of Tebuthiuron to Australian tropical species compared to northern hemisphere temperate species, although *M. mogurnda* was approximately 1.3−1.9 times more sensitive than northern hemisphere fish species. However, it was recommended that further data on local species, including plants, be obtained in order to perform a more substantial comparison.

As a means of evaluating statistical endpoints for use in deriving water quality guidelines, a comparison of various statistical endpoint values for the three test species was undertaken. In all cases, the BEC_{10} was lower than the corresponding no-observed-effect concentration (NOEC). However, more confident estimates of the NOEC (ie when a large number of concentrations were tested and sample size (*n*) was high, eg in the hydra and cladoceran experiments) closely approximated the BEC_{10}, indicating that the BEC_{10} was an appropriate estimate of a no adverse biological effect concentration. The EC_{10} was not considered an appropriate indicator of such a 'no-effect' concentration for Tebuthiuron.

A literature review on the fate and behaviour of Tebuthiuron in the aquatic environment, particularly northern Australian floodplain environments, was carried out in order to estimate a likely level of exposure to local aquatic organisms. As this study represented only a preliminary phase of an overall ecological risk assessment of Tebuthiuron, and some data were still lacking, a precautionary principle approach was adopted for determining the likely exposure level. Thus the likely maximum level of Tebuthiuron aquatic organisms would be exposed to was estimated to be 4.9 mg L^{-1}, being the highest concentration of Tebuthiuron measured in the water column following a major application to a mimosa infestation in northern Australia.

While this level significantly exceeded the recommended Australian water quality guideline value for Tebuthiuron of 1 µg L^{-1} (ANZECC 1992), a comparison with the toxicity data from the three species assessed in the present study indicated that the risk of adverse effects to the non-target aquatic organisms was minimal. However, to date, insufficient local data are available to quantitatively assess the risks of Tebuthiuron to the tropical wetland environments of northern Australia. In particular, the response of local aquatic plants needs to be assessed, as does the ability of organisms and plants to recover from short-term exposure to potentially toxic Tebuthiuron concentrations. Experiments assessing the toxicity of Tebuthiuron to an aquatic macrophyte and green alga were already underway at the time of completion of the present study. It is anticipated that the results will be combined with those of the present study, in order to undertake a quantitative ecological risk assessment of the use of Tebuthiuron to control *Mimosa pigra*.

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1 Introduction

1.1 Weeds

There is no universally accepted definition of a weed. In simplified terms, weeds are plants that interfere with human activity in crop and non-crop areas (Labrada & Parker 1994). Weeds can cause problems in a large variety of situations ranging from terrestrial, man-made agricultural systems to aquatic weeds constricting waterways, or the invasion of natural ecosystems by alien plant species (Humphries et al 1991). The main research focus in weed science has traditionally been on agricultural weeds which may cause significant economic losses (Combellack 1989). In recent years, it has been realised that weeds invading natural ecosystems are also a serious threat and it is now acknowledged that weed invasion constitutes one of the most serious threats to the survival of natural ecosystems (Storrs & Lonsdale 1995).

One of the major recognised causes of wetland degradation and loss in Australia is the invasion of exotic species (Bunn et al 1997). Nowhere is the threat of weeds currently greater than in the wetland habitats of the wet-dry tropics of northern Australia (Miller & Wilson 1995). Exotic species such as mimosa (*Mimosa pigra*), salvinia (*Salvinia molesta*) and the pasture species, paragrass (*Brachiaria mutica*) have been rapid in their spread, and have infested many wetland regions throughout northern Australia (Miller & Wilson 1995).

M. pigra is a highly undesirable weed. It grows as a prickly, leguminous shrub up to 6 m tall forming dense, impenetrable monospecific stands under moist conditions in the tropics (Lonsdale et al 1995). It is an aggressive invader of wetlands and is now considered one of Australia's worst tropical weeds (Humphries et al 1991). Chemical control on Mimosa commenced in 1965 (Miller et al 1981), and since this time considerable funds have been allocated and used to purchase and apply herbicides to infestations, and to promote further investigation of mimosa control by government agencies. However, despite the long-term use of herbicides in Australian wetlands, there was an absence of toxicity data for Australian non-target freshwater species. A combination of these facts led to the selection of mimosa control by herbicides as a focus for the present study.

1.2 *Mimosa pigra*

Mimosa, also known as the 'giant sensitive plant' (due to its bipinnate leaves that react to touch) is a native of tropical Central and South America. Outside its native range mimosa has caused problems in tropical areas across northern Australia and other parts of the world including the wetlands of south-east Asia (Lonsdale & Forno 1994). For example, it is responsible for sediment accumulation in irrigation systems, reservoirs and fallow rice paddies in Thailand (Lonsdale 1992), while it also has the potential to cause serious threats to wetland ecosystems in southern Florida (Sutton 1994).

Mimosa is believed to have first been introduced into Australia at the Botanical Gardens in Darwin in the late 19th Century (Harley 1992). The plant caused occasional nuisance in the Darwin region (Lonsdale et al 1995), but since the late 1970s has undergone a dramatic population increase throughout the wet-dry tropics of northern Australia (Lane et al 1995). It now covers an estimated area of 80 000 ha, stretching from the Moyle River in the west to eastern Arnhem Land in the east (Finlayson et al 1996) (fig 1). Under favourable environmental conditions, infestations have been found to double in area each year (Lonsdale 1992).

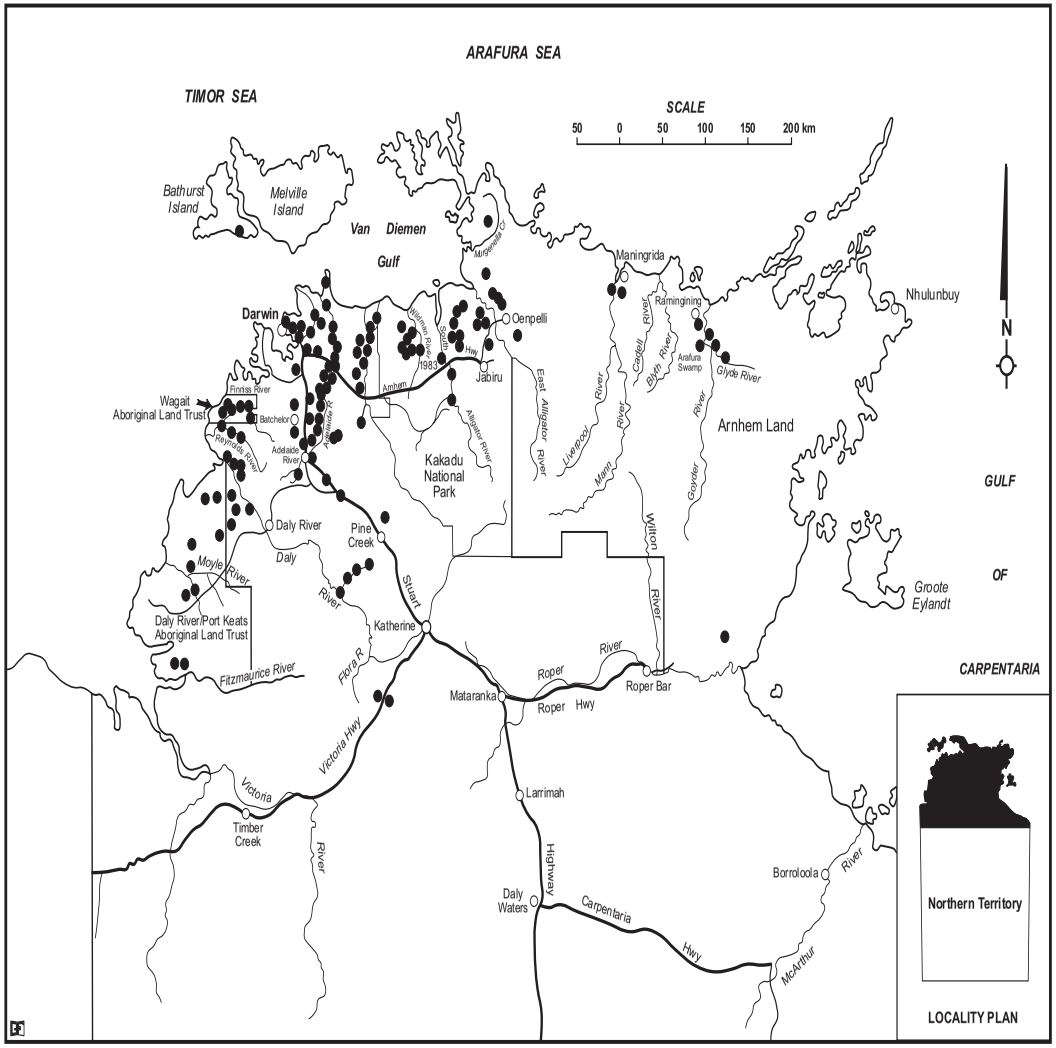

Figure 1 Documented locations of *Mimosa pigra* infestations in the Northern Territory as of February 1998

Mimosa is an opportunistic plant with great reproductive and dispersal capacities, mainly invading disturbed areas (for example, areas disturbed by water buffalo in tropical northern Australia). Within the floodplains of the Northern Territory, mimosa has had severe ecological and socio-economic impacts. Without doubt, large scale change has occurred as dense Mimosa thickets have advanced across sedgeland, grassland and swamp forest communities and replaced the native flora and fauna (DASETT 1991, Lonsdale et al 1995, Finlayson et al 1996). The dense thickets prevent access to water, thereby posing a threat to pastoral industries (Miller et al 1981). They also interfere with the traditional Aboriginal use of wetlands (ie fishing and food gathering). In addition, mimosa threatens to invade Kakadu National Park (Storrs & Lonsdale 1995)—a World Heritage site of great ecological and economic importance. Since its discovery in the Park in 1981, 160 mimosa infestations (ranging from individual plants to 4 ha stands) have been located and controlled (M Storrs pers comm).

1.3 Control of *Mimosa pigra*

Several methods have been used to control mimosa over the last two decades. The major control methods are described below.

1.3.1 Physical/mechanical control

The control of weeds by mechanical means involves the physical removal of plants or, in some aquatic situations, the drainage or diversion of a water body (AWRC 1982). One method used on the floodplains of Arnhem Land involved dragging heavy chains behind a tractor over infestations. The plants were then gathered and burned. Fire has also been used on intact mimosa infestations, however, application of a fuel such as gelled gasoline from aircraft must be used to facilitate the burn as there is little grassy understorey (Miller & Lonsdale 1992). This method is only effective if a follow-up control is carried out after the burn, as the seed bank remains viable within 5 cm of the soil surface, and germination is rapid and enhanced after clearing (Miller 1988, Miller & Lonsdale 1992).

1.3.2 Biological control

The biological control of weeds attempts to re-establish the balance between a weed and its natural enemies (AWRC 1982). This is achieved by introducing diseases, insects or other animals that specifically target the weed species. Australian and international agencies are involved in a collaborative biological control program of mimosa (Lonsdale & Forno 1994). A suite of six animal species has been identified for control, targeting particular life stages or aspects of plant physiology. However, there has been little discernible effect from these agents alone in controlling mimosa (Lonsdale & Forno 1994). Nevertheless, it is anticipated that an effective biological control can be found.

1.3.3 Chemical control

At present, the most effective and widely used method for the control of mimosa has been the use of herbicides. A wide range of herbicides, with different modes of action and application methods, have been shown to be effective in destroying mimosa. Following field trials, Miller and Siriworakul (1992) recommended a range of herbicides and application methods for the control of mimosa in different land-use situations in Australia and Thailand. The following five herbicides were selected by the Northern Land Council for use in large-scale mimosa control programs on Aboriginal land in the Northern Territory (DASETT 1991):

- Velpar® (active ingredient, hexazinone)
- Starane® (fluroxypyr)

- Brush-off® (metsulfuron methyl)
- Banvel® (dicamba)
- Graslan® (Tebuthiuron)

Hexazinone (Velpar®) is a relatively non-selective, post-emergent contact herbicide that is applied to cut stumps of mimosa or directly on the soil (DASETT 1991, Miller & Siriworakul 1992). It is relatively non-toxic to aquatic vertebrates, with an LC_{50} to the fish species, bluegill (*Lepomis macrochirus*) of 370–470 mg/L (Tomlin 1994). Fluroxypyr (Starane®) is a post-emergent chemical control agent applied to the foliage of actively growing mimosa plants (Miller & Siriworakul 1992). According to Tomlin (1994) it appears to have a low toxicity to birds, fish and aquatic invertebrates at the recommended rate of 600 g ha^{-1}. Metsulfuron methyl (Brush-off®) is a selective sulfonylurea herbicide which is applied to the foliage either from the ground or aerially (Miller & Siriworakul 1992). It has a toxicity to the non-target fish species, *L. macrochirus* of >150 mg L^{-1} (Tomlin 1994). Dicamba (Banvel®) is a selective post-emergent herbicide which is readily absorbed through both roots and leaves by most plants, thereby making it a very versatile herbicide (effective in soil, foliage, aerial application and stem injection). The LC_{50} of dicamba to *L. macrochirus* is 135 mg L^{-1} (Tomlin 1994). Within Kakadu National Park, Velpar®, Starane® and Banvel® have previously been used for the chemical control of mimosa (J Maddison pers comm). The quantities and timing of Tebuthiuron applications in northern Australia are of concern due to its application at the beginning of the wet season, onto dry floodplain that is subsequently covered by water, and at the end of the wet season when floodplains are still inundated with at least 30 cm of water (R Ansell pers comm). As a result it was considered that an assessment of the ecological risks associated with this application into the tropical freshwater aquatic environment should take priority over the other commonly used herbicides listed above.

1.4 Tebuthiuron

1.4.1 Physicochemical properties

Tebuthiuron, the active ingredient of the formulation Graslan®, belongs to the family of substituted urea herbicides. The structural formula of Tebuthiuron is shown in figure 2, while a summary of the physical and chemical properties is given in table 1. Technical grade Tebuthiuron (99% pure) is a colourless crystalline powder. It is stable when exposed to light, and has low vapour pressure and log K_{ow} indicating that it is non-volatile and relatively hydrophilic, respectively (Caux et al 1997).

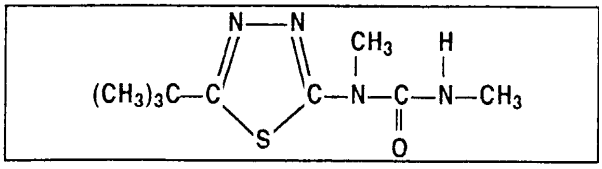

Figure 2 Structural formula of Tebuthiuron (from Tomlin 1994)

Table 1 Summary of physical and chemical characteristics of Tebuthiuron[a]

CAS number	34014-18-1
Chemical name	N-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-y1]-N,N'-dimethylurea
Alternative names	Graslan®, Spike®, Perflan®, Herbec®, Herbic®
Chem Service Cat	F2243
Molecular weight	228.3 g mol^{-1}
Molecular formula	$C_9H_{16}N_4OS$
Melting point	161.5–164°C
Water solubility	2.3 g L^{-1} at 25°C
Vapour pressure	2×10^{-6} mm Hg at 25°C
Log K_{ow}	1.8
Hydrolysis	Stable for up to 2 months at pH 3–9

a Data from Loh et al (1980) and Caux et al (1997)

1.4.2 Use

Tebuthiuron has been the most extensively used herbicide for mimosa control in northern Australia (I Brown pers comm). In 1991, approximately 12 000 kg of Tebuthiuron (*ca* 62 000 kg Graslan®) were applied to a mimosa infestation of approximately 5800 ha at Oenpelli in western Arnhem Land (Cook 1993). Treatment of the Oenpelli mimosa infestation has been described as the largest herbicide application to mimosa in the world, the largest Graslan® application in Australia, and probably the largest single application of Graslan® to a wetland environment in the world (Schultz & Barrow 1995). Although the total amount of Tebuthiuron applied to mimosa infestations has decreased markedly since 1991, considerable quantities are still being used, with approximately 4000 kg recently applied to an infestation at Koolpinyah Station, east of Darwin (G Schultz pers comm). The commercial product, Graslan®, is applied to soils in pellet form, and typically contains Tebuthiuron at concentrations of either 10, 20, or 30%. On the mimosa-infested wetlands of northern Australia, Graslan® has generally been applied at the onset of the first rains of the wet season, to facilitate dissolution of the pellet, thereby releasing the active ingredient. However, the USEPA (1994) reported that Graslan® should not be used in areas where surface water is present, while DowElanco recommended that there should be no application of product within 50 metres of established waterways (DowElanco 1990).

1.4.3 Mode of action

Tebuthiuron is absorbed by woody plants via the roots and translocated to its target sites in the stems and leaves (Steinert & Stritzke 1977). Here the herbicide inhibits photosynthesis by uncoupling electron transport (Caux et al 1997). Hatzios (1981) also suggested that Tebuthiuron may inhibit mixed function oxidase activity.

1.4.4 Environmental fate

Tebuthiuron, and its formulation, Graslan®, was originally designed for temperate rangeland areas and most of the environmental fate studies relate to such environmental conditions. Only a limited number of studies (eg Parry & Duff 1990, Batterham 1992) have considered the environmental fate of Tebuthiuron under cracking clay floodplain environments characteristic of the wet/dry tropics of northern Australia.

The persistence of Tebuthiuron varies with soil type, temperature and soil moisture. Chang and Stritzke (1977) found that greater degradation of Tebuthiuron in soil occurs at higher temperatures and at higher moisture levels. Under experimental conditions in the southern

United States, Tebuthiuron had a half-life in soil of 12.9 months (Elanco 1988). Under semi-arid conditions, Johnsen and Morton (1989) found that Tebuthiuron in soil had a half-life of 2–7 years. Batterham (1992) reported the photodegradation half-lives of Tebuthiuron under full sunlight in simulated northern Australian floodplain conditions to be 79 and 103 d in soil and water, respectively. The major pathway for microbial degradation of Tebuthiuron in soils is demethylation of the terminal nitrogen to form one major and at least three minor metabolites (Morton & Hoffman 1976). The resultant metabolites are apparently either non-herbicidal or possess weak herbicidal activity (Elanco 1988). However, the microbial degradation of Tebuthiuron under simulated northern Australian floodplain conditions was found to be negligible, a result confirmed by the analysis of field samples for metabolites (Batterham 1992).

The low log K_{ow} of Tebuthiuron indicates that adsorption by soils should be limited. Chang and Stritzke (1977) showed that adsorption of Tebuthiuron is greatest on soils with high organic matter content, followed by soils with high clay content. However, Tebuthiuron is known to be a relatively persistent and mobile chemical, with the potential to leach to groundwater (Caux et al 1997).

Parry and Duff (1990) highlighted the rather limited persistence of Tebuthiuron in soils under northern Australian floodplain conditions. Batterham (1992) attributed this to poor infiltration and high intensity rainfall, resulting in the removal of Tebuthiuron in surface runoff and through mobilisation into flood water. The metabolism of the herbicide by the large vegetative biomass of the floodplain also contributes to its limited persistence (Batterham 1992).

Apart from Pfeifle (1996), no data have been reported for the toxicity of Tebuthiuron to tropical or temperate Australian organisms. The majority of available data have been derived using temperate northern hemisphere rangeland organisms. Considering the extensive use of Tebuthiuron in tropical northern Australia, an assessment of the sensitivity of local species under relevant environmental conditions was necessary. As such, the major objectives of the present study was to provide aquatic toxicity data on the sensitivity of non-target tropical Australian organisms to Tebuthiuron, and to determine whether temperate North American toxicity data are applicable to the tropics of Australia. The available aquatic toxicity data are summarised below.

1.4.5 Toxicity of Tebuthiuron to non-target aquatic species

Acute toxicity data suggests that Tebuthiuron has low toxicity to temperate freshwater fish species (see review by Caux et al 1997). The 96 h LC_{50} values for rainbow trout (*Oncorhynchus mykiss*) range from 115–144 mg L^{-1} (Bionomics 1972, Blaise & Harwood 1991). Similarly, a 96h LC_{50} value of 112 mg L^{-1} has been reported (Bionomics 1972) for bluegill sunfish fry (*L. macrochirus*). Tebuthiuron appeared to be slightly less toxic to the fathead minnow (*Pimephales promelas*) and the goldfish (*Carassius auratus*), with 96 h LC_{50} values >160 mg L^{-1} (Todd et al 1972). Meyerhoff et al (1985) assessed the chronic toxicity of Tebuthiuron to the cladoceran *Daphnia magna* (21 d reproduction test) and larval fathead minnows (*P. promelas*; 33 d larval growth test). The no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) for *D. magna* and *P. promelas* were 21.8 and 44.2 mg L^{-1}, and 9.3 and 18.0 mg L^{-1}, respectively.

The toxicity of Tebuthiuron to freshwater algae and macrophytes is much higher than that reported for freshwater animals. For example, Adams et al (1985) reported a NOEC range of 0.01–0.05 mg L^{-1} for the green alga *Pseudokirchneriellia subcapitata*, using various biological endpoints (ie growth rate, cell number, area under growth curve) over a period of

1–6 d. Using the same species, Blaise and Harwood (1991) and Hickey et al (1991) reported 96 h EC_{50} values of 0.08 and 0.102 mg L^{-1}, respectively, using growth rate as an endpoint. A study of the toxicity of Tebuthiuron applied at expected environmental concentrations to ten algal species (assessing 24 h inhibition of ^{14}C uptake), and the floating macrophyte, *Lemna minor* (assessing 7 d growth inhibition), showed that 5.87 mg L^{-1} of Tebuthiuron resulted in >50% inhibition in 90% of the algae tested and 100% inhibition of growth in *L. minor* (Peterson et al 1994). In another study, eleven species of green algae typically found in North American playa lakes (found in arid regions which may intermittently fill with water), were exposed for 189 d to 0.18 mg L^{-1} of Tebuthiuron in microcosm cultures (Price et al 1989). It was found that if the algal cultures were exposed before active growth (ie in the lag phase of the culture) cell numbers were significantly ($P \leq 0.05$) reduced. However, similar levels of Tebuthiuron exposure during maximum growth rates (ie exponential growth) demonstrated no significant ($P \leq 0.05$) effect on the cultures (Price et al 1989).

Temple et al (1991) investigated the effects of Tebuthiuron on aquatic productivity in large outdoor multi-species test systems (mesocosms) simulating pools in Texas streams. Mesocosms were exposed to Tebuthiuron at concentrations ranging from 0.01–1.0 mg L^{-1}. Phytoplankton primary production was negatively correlated to the concentration of Tebuthiuron when sampled 42–64 d after exposure. Concentrations <0.2 mg L^{-1} had no effect on primary production or fish (fathead minnow) biomass. However, at 0.2 mg L^{-1} chironomid density was reduced by approximately 30% (Temple et al 1991).

Based on studies using *P. subcapitata*, an interim Canadian guideline value for Tebuthiuron of 1.6 µg L^{-1} was recently derived for the protection of freshwater life (Caux et al 1997). Similarly, a guideline of 1.0 µg L^{-1} has recently been derived for the protection of freshwater ecosystems in Australia and New Zealand (M Warne pers comm). However, a modified guideline value of 8.0 µg L^{-1} has been derived for freshwaters where algae are not considered important as primary producers (eg allocthonous streams) (M Warne pers comm).

As noted above, the majority of ecotoxicological data for Tebuthiuron have been derived from northern hemisphere species. Considering the large amounts of Tebuthiuron used in northern Australia for mimosa control, it is imperative that an assessment of the sensitivity of local aquatic organisms to the herbicide is performed. While the need to effectively control the spread of this highly invasive weed is unquestioned, equally important is a greater understanding of the risks of Tebuthiuron application to local aquatic species.

A preliminary investigation of the effects of Tebuthiuron on two local freshwater organisms, the green hydra, *Hydra viridissima*, and the purple-spotted gudgeon, *Mogurnda mogurnda*, has previously been undertaken (Pfeifle 1996). The results indicated that both organisms were not particularly sensitive to Tebuthiuron, with significant ($P \leq 0.05$) adverse effects (LOECs) occurring at 75 and 270 mg L^{-1} for *H. viridissima* and *M. mogurnda*, respectively (Pfeifle 1996). While the data represented the first assessment of the toxicity of Tebuthiuron to aquatic species native to northern Australia, they were insufficient to confidently predict the likelihood of Tebuthiuron having adverse effects on aquatic biota of the region. Subsequently, more data were required for the above two species, and at the very least, one other species, representing a different taxonomic group and trophic level.

1.5 Aims

The aims of the present study were to:

1 Assess the toxicity of Tebuthiuron to the following freshwater animals:

- the purple spotted gudgeon, *M. mogurnda*

- the green hydra, *H. viridissima*
- the cladoceran, *Moinodaphnia macleayi*

2 Use the above toxicity results, and all other relevant information, to undertake a preliminary ecological risk assessment on the use of Tebuthiuron for the chemical control of mimosa in wetland habitats of tropical northern Australia.

2 Materials and methods

2.1 Collection of control/diluent water

Natural (control/diluent) surface water was collected from two sites along Magela Creek, depending on seasonal availability. During the Wet season (January–June), water was collected from Georgetown Billabong (ie the creek-side monitoring field station site–G8210201), either from pump-operated header tanks or by hand when flow permitted. During the Dry season (July–November), when creek flow had ceased and water was unable to be collected from Georgetown Billabong, water was collected from Bowerbird Billabong (R8210008), a permanent water body in the headwaters of Magela Creek, approximately 20 km upstream of Georgetown Billabong. Water was collected in 20 L polyethylene containers. Each container was pre-cleaned using detergent (2% Neutracon), nitric acid (5% BDH AnalaR) and deionised water (Milli Q, <1 μS cm^{-1}), before being used to collect water. In the field, containers were thoroughly rinsed (three times) with surface water before being filled. The water was then transported to the laboratory (***eriss***) where pH, electrical conductivity (EC) and dissolved oxygen (DO) were measured in unfiltered and filtered sub-samples. All control and diluent water were filtered through a 10 μm paper filter (Postlip) to remove any wild zooplankton and reduce particulate matter.

The fresh surface waters of Magela Creek during the main Wet season are slightly acidic (mean pH (m), 6.0; range ®: 4.2–7.0) with a low buffering capacity, and generally characterised by low levels of hardness (m, 3.6; r, 1.3–16 mg L^{-1} as $CaCO_3$), alkalinity (m, 4.4; r, 0.83–19 mg L^{-1} as $CaCO_3$), conductivity (m, 17; r, 6.0–75 μS cm^{-1}), suspended solids (m, 7.1; r, 1.2–146 mg L^{-1}) and turbidity (m, 3.2; r, 0.6–61 NTU) (see review by Markich (1998)). A continuous flow of water during the main Wet season effectively flushes the creek channel and imparts a uniform and common water chemistry to all billabongs (Walker & Tyler 1982, Hart et al 1987). Consequently, chemical differences between surface and deeper waters of billabongs in Magela Creek are slight. Surface water from Bowerbird Billabong during the Dry season is typical of Wet season Magela Creek water (C leGras pers comm). Therefore, a relatively consistent water chemistry was used for all experiments.

2.2 Preparation of test solutions

Stock solutions of Tebuthiuron were prepared by reconstituting the chemical with high purity deionised water (Milli Q, 18 MΩ cm^{-1} resistivity), to achieve a final concentration of 2000 mg L^{-1}. They were prepared in pre-cleaned 2 L glass containers and refrigerated (4°C). Prior to use, the stock solution was allowed to equilibrate to room temperature. Test solutions were prepared by serially diluting the stock solution with filtered (<10 μm) Magela Creek water (ie from Georgetown or Bowerbird billabongs), which was collected as close as practicable to the commencement of each toxicity test. Test concentrations were determined from the preliminary results obtained by Pfeifle (1996) and range-finding experiments (for *M. macleayi*). Test solutions were prepared in pre-cleaned 5 L polyethylene screw-topped

containers immediately prior to test commencement. Throughout the test, the test solutions were kept at 4°C until required for daily solution renewals, when they were allowed to equilibrate to 27 ± 1°C inside a constant temperature incubator (Labec) for several hours.

2.3 Toxicity testing procedures

The toxicity of Tebuthiuron to the three test species was assessed using standard protocols. The protocols are summarised in table 2, and described in detail elsewhere (Hyne et al 1996, Markich & Camilleri 1997). Sections 2.1 and 2.2 described modifications to the standard protocols, which were initially designed to assess the toxicity of pre-release waste waters from the Ranger uranium mine (Hyne et al 1996). A total of six experiments were carried out for *M. mogurnda,* seven experiments for *H. viridissima*, and five experiments for *M. macleayi*.

Table 2 Summary of standard protocols used to assess the toxicity of Tebuthiuron

Test species	Test endpoint	Test duration (acute/chronic)	Protocol
Purple-spotted gudgeon (*M. mogurnda*)	Survival	96 h (acute)	BTT-E[a]
Green hydra (*H. viridissima*)	Population growth	96 h (chronic)	BTT-B[b]
Cladoceran (*M. macleayi*)	Reproduction	3 brood/5–6 d (chronic)	BTT-D[c]

a Protocol BTT-E is described by Markich & Camilleri (1997).
b Protocol BTT-B was shortened from 6 to 4 d (96 h), as described by Markich & Camilleri (1997).
c Protocol BTT-D is described by Hyne et al (1996).

2.3.1 Purple-spotted gudgeon (*M. mogurnda*) 96 h sac-fry survival

M. mogurnda sac-fry (<10 h-old) were exposed to Tebuthiuron concentrations ranging from 0 (control) to 300 mg L^{-1} for 96 h. Observations of sac-fry survival were recorded at 24 h intervals. Sac-fry were exposed to 30 mL of each test concentration in 40 mL glass Petri dishes. Each Petri dish contained 10 sac-fry. Three replicates were used for each test concentration, resulting in a total of 18 test dishes and 180 sac-fry for a given test run. The test dishes were kept in a constant temperature incubator at 27 ± 1°C, with a photoperiod of 12 h light:12 h dark. Tests solutions were renewed every 24 h, following recording of sac-fry survival. The sac-fry were not fed prior to, or during, the 96 h test period. The test was considered valid if control mortality did not exceed 20% after 96 h. pH, EC and DO were measured daily for fresh (t_0) and 24 h-old (t_{24}) test water.

2.3.2 Green hydra (*H. viridissima*) 96 h population growth rate

Asexually reproducing hydra, each with one relatively well developed bud, were exposed to Tebuthiuron concentrations ranging from 0 (control) to 800 mg L^{-1} for 96 h. Observations of population changes (ie one animal equals one hydroid plus any attached buds) were recorded every 24 h. Hydra were exposed to 30 mL of each test concentration in 40 mL glass Petri dishes. Each Petri dish initially contained 10 hydra. Three replicates were used for each test concentration, resulting in a total of 18 test dishes and 180 hydra for a given test run. The test dishes were kept in a constant temperature incubator at 27 ± 1°C, with a photoperiod of 12 h light:12 h dark. Tests solutions were renewed every 24 h, following recording of the number of hydra in each dish. Each hydra was individually fed with 3–4 live brine shrimp nauplii (*Artemia franciscana*) per day over the 96 h test period. PH, EC and DO were measured daily for fresh (t_0) and 24 h-old (t_{24}) test water.

2.3.3 Cladoceran (*M. macleayi*) 3 brood/5–6 day reproduction

Female *M. macleayi* neonates (<6 h-old) were exposed to Tebuthiuron concentrations ranging from 0 (control) to 250 mg L^{-1} until control cladocerans released their third brood offspring (ie usually 5–6 d). Observations were recorded every 24 h on the survival of each female, the number of neonates produced, and the number of surviving neonates. The numbers of neonates from all broods were summed for each adult cladoceran, resulting in a count for the total number of offspring per adult. Cladocerans were exposed to 30 mL of each test concentration in 50 mL glass beakers covered with clear Perspex trays. Each beaker initially contained one neonate. Ten replicates were used for each test concentration, resulting in a total of 60 test beakers and 60 neonates for a given test run. Test beakers were kept in a constant temperature incubator at 27 ± 1°C, with a photoperiod of 12 h light:12 h dark. Tests solutions were renewed every 24 h, following observation of the number of neonates in each beaker. Cladocerans were fed daily with the unicellular green alga, *Chlorella* sp. (at a cell density of 6 x 10^6 cells mL^{-1}), as well as 1 µL of fermented food and vitamins per mL of test solution. The test was considered valid if mortality in the controls did not exceed 20%, and reproduction in the controls averaged 30 or more neonates per surviving female over the test period. PH, EC and DO were measured daily for fresh (t_0) and 24 h-old (t_{24}) test water.

2.4 Chemical analysis

Tebuthiuron was analysed by high performance liquid chromatography. Samples were injected without pre-treatment onto a Vydac 201TP C_{18} column and eluted with 35% ammonium acetate (0.1 M) and 65% methanol. The peaks were identified by retention time and confirmed by matching the UV spectra with that of standard Tebuthiuron (Chem Service Cat F2243). Quantitation was achieved by calibrating peak areas of standard Tebuthiuron under identical chromatographic conditions. Each sample was analysed in duplicate or triplicate. Tebuthiuron analyses were performed for most control concentrations from each experiment, as well as a selection of other test concentrations which characterised the concentration-response curves of the test species. PH, EC and DO in the test waters were measured using the methods described by Markich and Camilleri (1997).

2.5 Statistical analysis

The BEC_{10} (10% bounded effect concentration), an alternative statistical measure to the NOEC, was estimated using the approach described by Hoekstra and van Ewijk (1993a). The MDEC (minimum detectable effect concentration), an alternative measure to the LOEC, was estimated using the approach described by Ahsanullah and Williams (1991). A four-parameter logistic regression model (Guardabasso et al 1987, Seefeldt et al 1995) consistently provided the best fit for the sigmoidal relationship between Tebuthiuron concentration and the selected responses of each organism. The concentration-response relationships are described by the following equation:

$$Y = \frac{a - d}{1 + (x / c)^b} + d$$

where Y is the response: x, the nominal (arithmetic) Tebuthiuron concentration: a, the minimum response: d, the maximum response: c, the EC_{50}, ie the concentration resulting in a response midway between a and d: and b is the 'slope factor' around the EC_{50}.

Using this logistic regression model, the EC_{50} (and its 95% confidence interval (CI)) was calculated for *H. viridissima* and *M. macleayi*. Model parameter estimates were derived by the method of maximum likelihood with a binomial probability distribution. Model adequacy was

evaluated using a (χ^2 goodness of fit test (Helsel & Hirsch 1992) and confirmed in all cases. By combining the definitive response data with the range-finding data, an LC_{50} was also calculated for *M. mogurnda* using the logistic regression.

Plots generated from the concentration-response data given in Appendix A showed that the regression relationships for each individual test-run of a given Tebuthiuron-organism exposure (eg six Tebuthiuron test-runs were performed with *M. macleayi*; appendix A) could be adequately described by linear models for the purpose of analysis of covariance (ANCOVA) (plots not provided here). ANCOVA was also used to compare the concentration-response curves of a given test organism exposed to Tebuthiuron using both test/diluent waters (ie Georgetown and Buffalo Billabong water). From ANCOVA, it was shown that both the regression slopes and intercepts did not significantly (P >0.05) differ for each test-run of a given Tebuthiuron-organism exposure. These results validated the use of pooled (mean) data for deriving BEC_{10}, MDEC and $E(L)C_{50}$ values, as well as parameter estimates for the logistic regression models.

Data from individual experiments were also analysed using one-way analysis of variance (ANOVA) to determine LOEC and NOEC values for each of the three test species. In addition, an EC_{10} value was calculated from the pooled concentration-response curve of each species. This was done to compare LOEC and NOEC estimates with BEC_{10} and EC_{10}, and MDEC estimates, respectively. Reporting of LOEC and NOEC values, together with alternative statistical endpoints has been recommended by Denton and Norberg-King (1996) as an interim step, until regression-based measures of toxic response are universally accepted. The comparison of several statistical approaches will assist in a better understanding of the benefits and limitations of each approach.

2.6 Preliminary assessment of Tebuthiuron exposure levels

To begin to understand the risks associated with the application of Tebuthiuron to aquatic systems, a preliminary assessment was undertaken to estimate the likely levels of exposure of local freshwater organisms to Tebuthiuron. This was achieved by way of a literature review, rather than using predictive modelling techniques, as a considerable amount of information on the environmental fate of Tebuthiuron, derived from both laboratory and field studies already existed. Since the effects of Tebuthiuron on non-target aquatic organisms from the floodplains and nearby waterways of northern Australia were of prime interest, the literature review concentrated on data relevant to this region. However, in some cases, data and information derived from other locations were also used.

3 Results and discussion

3.1 Physicochemical parameters

Physicochemical parameters remained within the acceptable limits prescribed in the standard protocols (see Hyne et al (1996) and Markich & Camilleri (1997)). Mean pH and conductivity of the control waters for each experiment are shown in appendix B. The major purpose for summarising these parameters was to assess potential differences in water chemistry of the two water collection sites (ie Georgetown and Bowerbird Billabongs) over the course of the experiments. Overall the maximum seasonal differences between the two sites varied by less than one pH unit (ie 6.2–7.1) and 15 $\mu S\ cm^{-1}$ (ie 12–27 $\mu S\ cm^{-1}$). At the end of the wet season (April–June 1996), pH values approached 7 for t_0 samples (where t_0 is the fresh diluent water measurement). PH values in t_0 samples (where t_0 is the fresh diluent water measurement) were at their lowest (6.2–6.5) at the beginning of the wet season (January–February 1997),

reflecting a high and slightly acidic rainfall, and highest (about 7) at the end of the wet season (April–June 1996). The mean t_0 values for pH and EC were only marginally lower in Bowerbird Billabong (pH 6.69, EC 16.0) compared with Georgetown Billabong (pH 6.81, EC 16.6) (see appendix B), indicating a similar water chemistry. The mean t_{24} values (where t_{24} is the test water 24 h after exposure to test organisms and conditions) for pH and EC increased slightly from the t_0 values in waters from both billabongs (ie pH 6.77–6.94, EC 16.4–18.5). Dissolved oxygen concentrations in the test containers were maintained at >90% saturation for all tests.

Nominal and measured concentrations of Tebuthiuron are presented in appendix C. Measured concentrations were typically within 6–7% of their nominal values, and always within 20%. Due to the overall accuracy of the nominal values, coupled with the fact that only selected Tebuthiuron samples were measured (due to financial constraints), nominal values were used in the concentration-response relationships (figs 3–5). This is consistent with the OECD (1984) recommendation that nominal concentrations may be used if measured concentrations are within 20%.

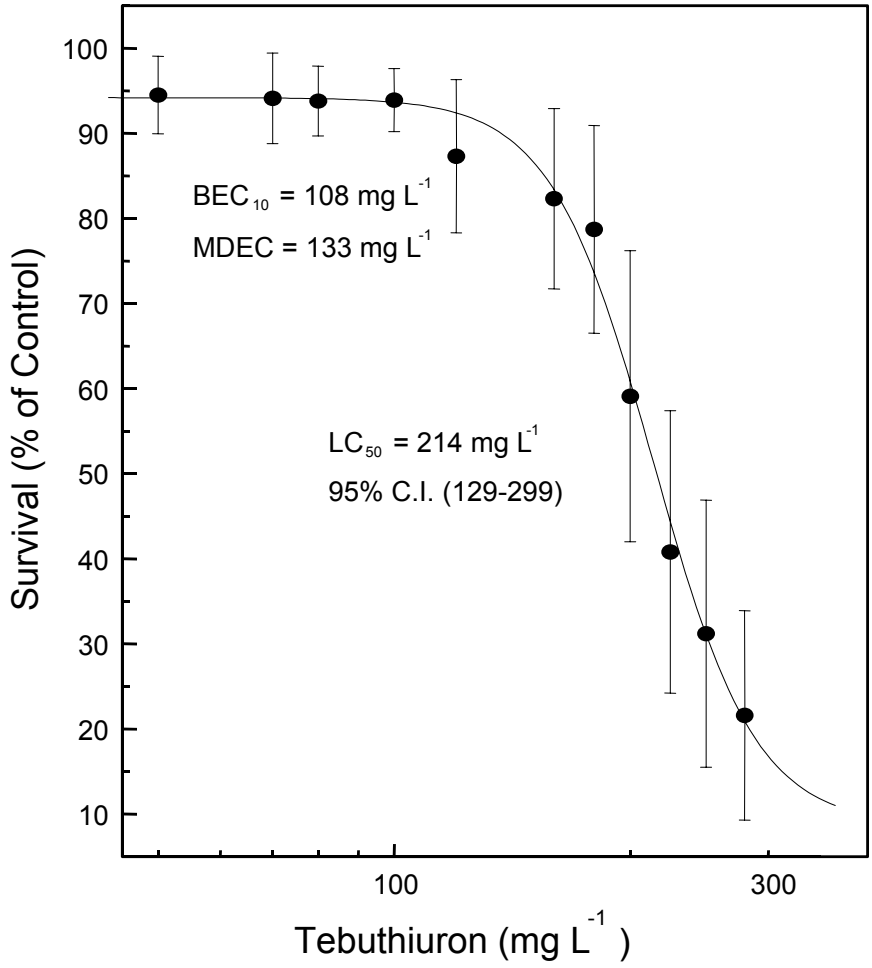

Figure 3 Survival of *M. mogurnda* as a percentage of mean Control survival, plotted against nominal Tebuthiuron concentration. Each plotted point represents the mean and 95% confidence interval. The toxicological endpoints (ie BEC_{10}, MDEC and LC_{50} at 96 h) are described in section 2.5.

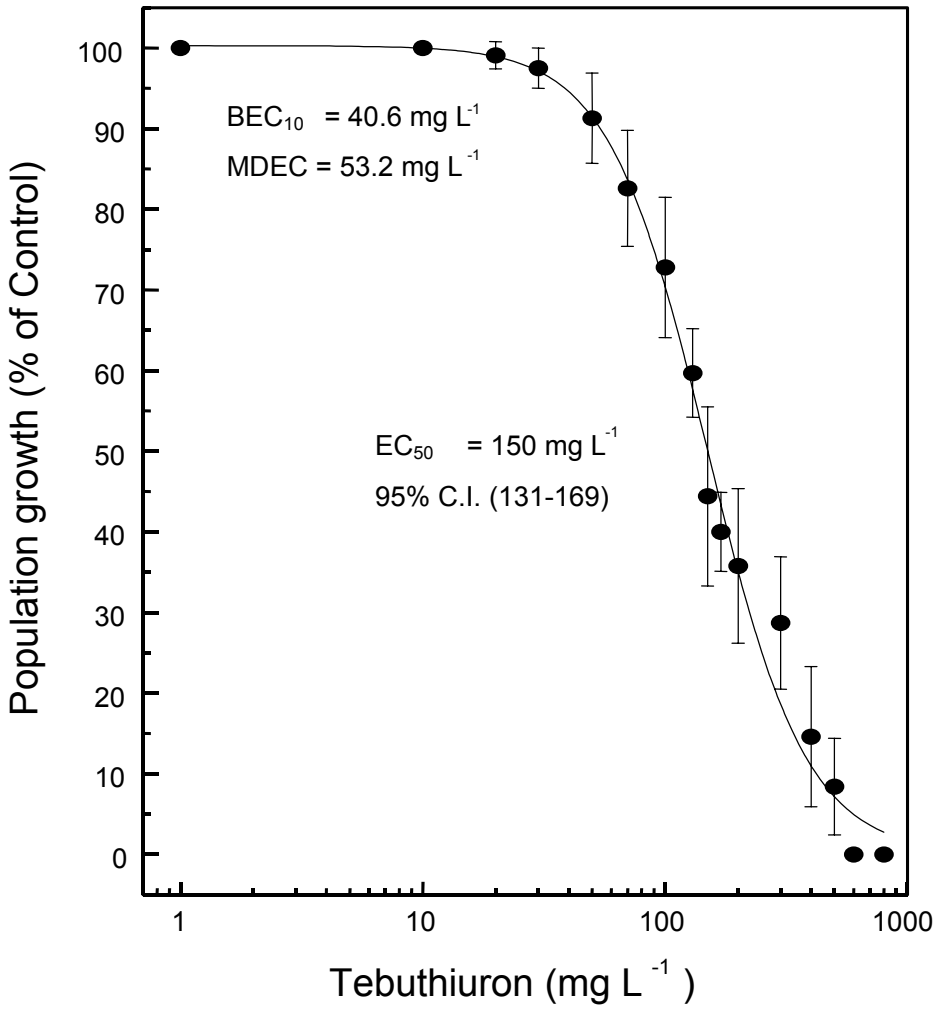

Figure 4 Population growth of *H. viridissima* as a percentage of mean Control growth, plotted against nominal Tebuthiuron concentration. Each plotted point represents the mean and 95% confidence interval. The toxicological endpoints (ie BEC_{10}, MDEC and EC_{50} at 96 h) are described in section 2.5.

3.2 Comparative toxicity of Tebuthiuron to Australian and northern hemisphere species

Raw toxicity data for each organism at the end of the test period are provided in appendix A. Figures 3–5 show the concentration-response relationships for the three test species, *M. mogurnda*, *H. viridissima* and *M. macleayi*, respectively. A comparison of the various statistical endpoints (ie BEC_{10} with NOEC and EC_{10}, and MDEC with LOEC, as well as the EC_{50}) are shown in table 3. The toxicity of Tebuthiuron to the three test organisms decreased in the following order:

cladoceran (*M. macleayi*) > hydra (*H. viridissima*) > gudgeon (*M. mogurnda*)

For *M. mogurnda*, the NOEC and LOEC were approximately twice the BEC_{10} and MDEC, respectively, while the EC_{10} better approximated the MDEC than the BEC_{10}. In contrast, the NOEC and LOEC values for *H. viridissima* and *M. macleayi* were similar to the BEC_{10} and MDEC values. Similarly, for both *H. viridissima* and *M. macleayi*, the EC_{10} was a better approximation of the MDEC than the BEC_{10}.

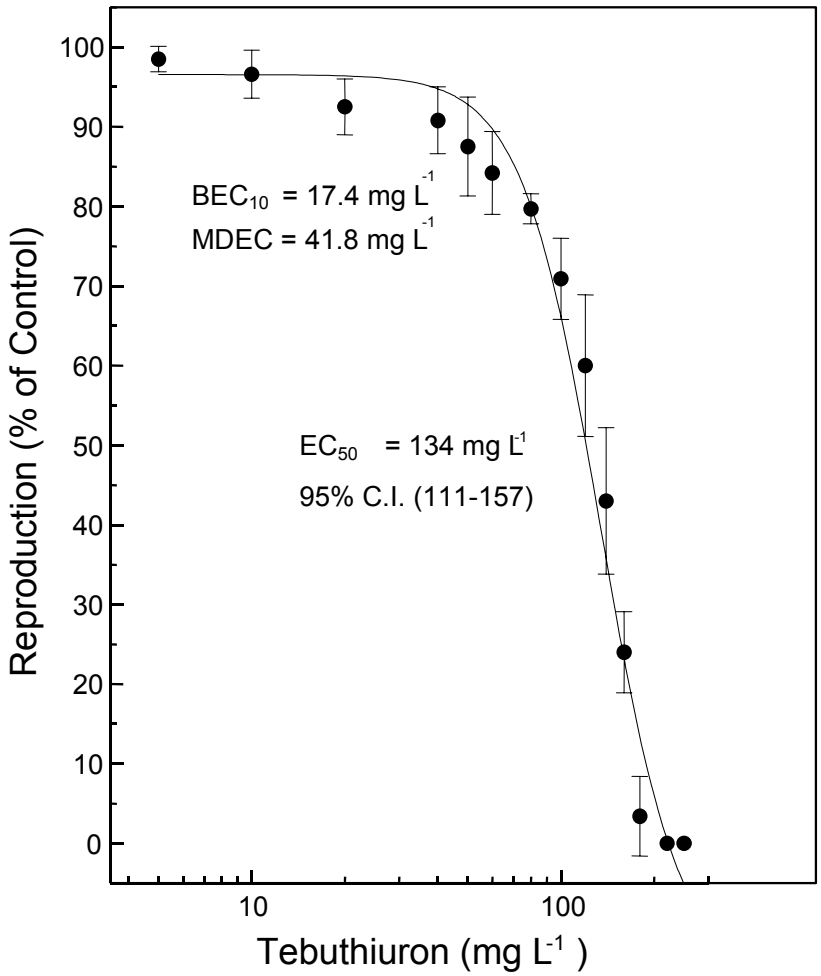

Figure 5 Reproduction (3 brood) of *M. macleayi* as a percentage of mean Control reproduction, plotted against nominal Tebuthiuron concentration. Each plotted point represents the mean and 95% confidence interval. The toxicological endpoints (ie BEC_{10}, MDEC and EC_{50} at production of three broods) are described in section 2.5.

Table 3 Comparison of statistical endpoint values for the three test species exposed to Tebuthiuron[a]

Test species	Test duration/endpoint	BEC_{10}[b]	NOEC[c]	EC_{10}[b]	MDEC[b]	LOEC[c]	EC_{50}[b]
M. mogurnda	96 h/sac-fry survival	108	200	137	133	225	214[d]
H. viridissima	96 h/population growth	40.6	50	53.2	53.2	75	150
M. macleayi	3 brood/reproduction	17.4	20	43.0	41.8	40	134

[a] All values are expressed in mg L^{-1}; [b] Value based on results of pooled data (see section 2.5);
[c] Value based on results of individual experiments (see section 2.5); [d] LC_{50} value

To compare the gudgeon endpoints (acute, lethal) with the cladoceran and hydra endpoints (chronic, sublethal), the gudgeon toxicity values (ie EC_{50}, BEC_{10}, NOEC) were divided by an extrapolation factor of two, as recommended by Hendriks (1995). Following this correction, the relative sensitivity of Tebuthiuron, based on NOEC/BEC_{10} values, remained constant; moreover, if EC_{50} are used, *M. mogurnda* becomes more sensitive than the cladoceran and hydra (ie 107, 134, and 150 mg L^{-1}, respectively). However, it is important to note that even with the change in relative sensitivity using the corrected EC_{50} value for *M. mogurnda*, the

EC_{50} values of all three species were not significantly (P >0.05) different based on overlapping 95% confidence limits (see figs 3–5). Additionally, it is the 'no-effect' endpoints, such as the NOEC and BEC_{10}, that are ideally used to derive water quality guidelines and/or criteria for protecting aquatic life, and hence, used as input into risk assessments.

A comparison of Tebuthiuron toxicity data for northern hemisphere temperate and Australian tropical test species is shown table 4. To facilitate an accurate comparison, the selected temperate data were based on a standard 96 h exposure period for fish species, and chronic, multiple brood (≥3 broods), reproduction tests for cladocerans. Where possible, data also included the life stage of the test species (eg sac-fry stage for fish). While fish life stage was not always consistent, it was considered that a reasonable indication of comparative toxicity could still be made.

In general, acute LC_{50} values of Tebuthiuron for northern hemisphere temperate freshwater fish (112–160 mg L^{-1}) were <1.3–1.9 times lower than the Australian tropical freshwater fish, *M. mogurnda* (table 4). The differences in Tebuthiuron toxicity may be due to inter-species variation, however, other factors, including the type of test water used (ie natural versus synthetic/reconstituted water) may have also played a role. Differences in water chemistry, including the concentration of dissolved organic matter, are known to influence the bioavailibility of Tebuthiuron (Caux et al 1997). In contrast to the fish species, there was very little difference in the toxicity of Tebuthiuron to the northern hemisphere temperate cladoceran, *D. magna*, and the smaller Australian tropical species, *M. macleayi*, with NOEC values of 21.8 and 20 mg L^{-1}, and LOEC values of 44.2 and 40 mg L^{-1}, respectively (table 4). A comparison could not be made for hydra as no comparable temperate data were available.

Table 4 Comparative toxicity of Tebuthiuron to northern hemisphere temperate and Australian tropical freshwater animals

Test organism	Duration	Life Stage	Effect (mg L^{-1})	Reference
Fish				
Rainbow trout (*Oncorhynchus mykiss*)	96 h	Juvenile (1.5 g or 42 mm)	LC_{50} = 144	Bionomics (1972)
	96 h	Juvenile (10.3 ± 3.5 g)	LC_{50} = 115	Blaise & Harwood (1991)
	96 h	ND	LC_{50} >160	Tomlin (1994)
Bluegill sunfish (*Lepomis machrochirus*)	96 h	Juvenile (36 mm)	LC_{50} = 112	Bionomics (1972)
Goldfish (*Carassius auratus*)	96 h	Adult (50 mm)	LC_{50} = 160	Todd et al (1972)
Fathead minnow (*Pimephales promelas*)	96 h	Adult (50 mm)	LC_{50} >160	Todd et al (1972)
Purple-spotted gudgeon (*Mogurnda mogurnda*)	96 h	Sac-fry	LC_{50} = 214	This study
Invertebrates				
Cladoceran (*Daphnia magna*)	21 d (~5 brood)	≤24 h	NOEL = 21.8 LOEL = 44.2	Meyerhoff et al (1985)
Cladoceran (*Moinodaphnia macleayi)*	5–6 d (3 brood)	≤6 h	NOEC = 20 LOEC = 40	This study
Hydra (*Hydra viridissima*)	96 h	Adult	NOEC = 50 LOEC = 75	This study

ND, Not determined.

Northern hemisphere temperate organisms Australian tropical organisms

Based on the available literature, it appears that the toxicity of Tebuthiuron to a limited number of Australian tropical freshwater organisms is similar to that of northern hemisphere temperate species. While it could be argued that northern hemisphere data could be used for deriving site-specific water quality guidelines, or for use in ecological risk assessment for Australian tropical environments, too few species have been tested and compared for this to be recommended at present. Thus, it is preferable that further data based on Australian species be obtained.

3.3 Comparison of statistical endpoints for assessing Tebuthiuron toxicity

A major issue currently being debated in the field of ecotoxicology is the choice of statistical endpoints to use in deriving water quality guidelines and/or criteria, and for ecological risk assessment (Hoekstra & van Ewijk 1993a,b, Denton & Norberg-King 1996, Dhaliwal et al 1997, Moore & Caux 1997). The rationale behind the comparison of the various statistical endpoints is outlined below.

The 'traditional' measure of the no adverse biological effect concentration of a toxicant, the NOEC, has come under increasing criticism in recent years. This is mainly due to the NOEC being restricted to one of the test concentrations, and as such, does not necessarily represent a good measure of the actual toxicant concentration that causes no adverse biological effect (Hoekstra & van Ewijk 1993a,b, Chapman et al 1996, Moore & Caux 1997). In addition, the determination of the NOEC is heavily reliant on the power of a test (ie the probability (P) to conclude correctly that the control and treatment chemical concentrations are significantly different). As ecotoxicological tests often possess low power, in some cases below 30%, it is almost impossible to regard the NOEC as a true no adverse biological effect concentration (Hoekstra & van Ewijk 1993a,b, Chapman et al 1996). As a result of this criticism, numerous investigators have proposed the use of alternative statistical measures.

Hoekstra and van Ewijk (1993a,b) recommended the 10% bounded effect concentration (BEC_{10}) as an alternative statistical endpoint to the NOEC. The BEC_{10} is the highest concentration for which one may claim with 95% confidence that its biological effect does not exceed 10% of the observed effect (Hoekstra & van Ewijk 1993a). While the process of deriving the BEC_{10} does not utilise all the data (as point estimation does), it usually involves extrapolation to a concentration whose upper or lower 95% confidence interval would not have exceeded 10% of the observed effect. This is achieved by determining the BEC_{25}, being the concentration whose upper/lower 95% confidence limit does not exceed 25% of the observed effect, and subsequent linear extrapolation to the 10% effect level (ie the BEC_{10}). A more detailed explanation of the BEC_{10} is given by Hoekstra and van Ewijk (1993a).

In this study, all estimates of the BEC_{10} were lower than the corresponding NOEC and EC_{10} values (table 4). However, depending on the test species, differences between BEC_{10} and NOEC values ranged from almost 100% for *M. mogurnda* to just over 10% for *M. macleayi*. The large difference between the BEC_{10} and NOEC values for *M. mogurnda* is most likely due to the large inherent variability of response (ie survival) at each test concentration (see error bars in fig 3). The lowest NOEC for all the *M. mogurnda* experiments was 200 mg L^{-1}, just below the calculated EC_{50} (214 mg L^{-1}). It is difficult to confidently state that 200 mg L^{-1} is representative of the true no biological effect concentration. This could easily be attributed to the large variability surrounding the mean value of survival for *M. mogurnda*. However, evaluation of the BEC_{10} does not require the determination of a statistically significant (eg $P \leq 0.05$) difference from the control response, and thus, is not restricted by the variability in response. Consequently, the BEC_{10} (108 mg L^{-1}) was markedly lower than the NOEC.

In contrast, the BEC_{10} and NOEC for both *M. macleayi* and *H. viridissima* were relatively similar (table 4). This was reflected in the much lower variability around the mean responses of each species to Tebuthiuron (see figs 4 and 5, respectively), thus increasing the tests' power and ability to detect a statistically significant ($P \leq 0.05$) difference from the control responses (ie increased sensitivity in the estimation of the LOEC and NOEC). In addition, for the *M. macleayi* tests, the sample size (n) equalled 10, compared to 3 for the *M. mogurnda* and *H. viridissima* tests (see section 2.3), again increasing the power of the test, and increasing confidence in the NOEC estimate. It should be noted that up to six tests were carried out for each species, with each test including several Tebuthiuron concentrations. Consequently, the ability to obtain a more accurate estimate of the NOEC was increased. However, when time and financial constraints limit the number of experiments that can be carried out, and hence, the number of concentrations tested, it is unlikely that the NOEC estimate will be as reliable. Given that the NOEC better approximated the BEC_{10} when more concentrations were tested and n was increased (ie when greater time and effort were allocated to determining the NOEC), suggests that the BEC_{10} should be considered an appropriate statistical endpoint to evaluate a no adverse biological effect concentration.

However, some care should be taken that the BEC_{10} value does not result in an overly conservative estimate of the no adverse biological effect concentration. This may have important practical implications if the BEC_{10} becomes an acceptable estimate for the derivation of water quality guidelines and/or criteria for toxicants in the future. The major world-wide approach for deriving water quality guidelines and/or criteria for toxicants in aquatic ecosystems uses statistical extrapolation methods (eg Aldenberg and Slob method) to estimate 'safe' toxicant levels from available NOEC data, rather than utilising data only from the most sensitive species tested (Warne 1997). In the proposed revised Australian and New Zealand water quality guidelines for fresh and marine waters (ANZECC/ARMCANZ in prep), guideline trigger values for a particular toxicant derived using the Aldenberg and Slob method are often lower than those values based on the previous safety factor method (ANZECC 1992). In some cases the values are below natural background levels or analytical detection limits. However, for a number of toxicants, the use of the safety factor approach also provided the same problems. Consequently, concerns have been raised regarding the validity of both approaches (ANZECC/ARMCANZ in prep). While guideline values derived from the Aldenberg and Slob method are generally thought to be driven/determined by the magnitude of the standard deviation of the toxicity data (ANZECC/ARMCANZ in prep), the size of the values themselves will also have some influence. Therefore, some caution should be exercised if BEC_{10} values are to be considered as alternative statistical measures to the NOEC for deriving water quality guidelines for the protection of aquatic ecosystems, as they are generally lower than the NOEC.

Several investigators (Hoekstra & van Ewijk 1993a,b, Denton & Norberg-King 1996, Koepp 1997, Moore & Caux 1997) have recommended the use of regression-based techniques for estimating no adverse biological effect concentrations of toxicants. In most cases, the use of point estimation has been recommended (Denton & Norberg-King 1996, Koepp 1997, Moore & Caux 1997). That is, the calculation of the concentration associated with a specified biological effect or percentage change (p) from that observed under control conditions (referred to as the EC_p). As described in section 2.5, the EC_{10} was selected in this study for comparison with the NOEC, because estimates below the 10% biological effect level are often model-dependent and have large confidence intervals associated with them (Moore & Caux 1997), while the 10% effect level also corresponded to the BEC_{10} estimate. Furthermore, Hoekstra and van Ewijk (1993a) concluded that in most cases, the BEC_{10} would be lower (ie more conservative) than the EC_{10}, hence, a further interest in the comparison.

In this study, the EC_{10} values were all markedly higher than the respective BEC_{10} values (table 3). In fact, the EC_{10} values appeared to be much better estimates of the MDEC than the BEC_{10}, suggesting that the EC_{10} may not represent an appropriate choice of endpoint if an estimate of a no adverse biological effect concentration of a toxicant is desired. EC_5 estimates may represent a better option for this purpose, however, this may have other limitations associated with it, as outlined by Moore and Caux (1997). Denton and Norberg-King (1996) recommended that the biological effect level (p), should not be less than 5%. In contrast, a recent OECD workshop clearly stated that a zero percent (0%) biological effect level should be included in an evaluation of appropriate effect levels (Koepp 1997). While point estimation techniques are known to have great potential for use in deriving water quality guidelines and/or criteria, and in ecological risk assessment, it is generally accepted that a greater understanding of the methods and their application is required.

3.4 Assessment of Tebuthiuron exposure levels in northern Australia

3.4.1 Field monitoring

In an attempt to understand the maximum likely levels of Tebuthiuron exposure to the non-target test species, a 'worst-case scenario' approach was adopted. Chemical treatment of the mimosa infestation at Oenpelli in 1989 and 1991 represented two such worst-case scenarios, with the latter application of Tebuthiuron being substantially greater in terms of the size of infestation treated, and therefore, the quantity of herbicide applied, but not in terms of the density, or rate, of herbicide applied.

In 1989, an area of approximately 1000 ha of mimosa was treated at a rate of 1.5 kg Tebuthiuron (7.5 kg Graslan)/ha, representing a total of approximately 1500 kg Tebuthiuron (Parry & Duff 1990). Following this treatment, the concentrations of Tebuthiuron were measured in the various environmental compartments (ie surface water, suspended sediment/microalgae and soil/sediment) (Parry & Duff 1990). The highest concentrations of Tebuthiuron in the three compartments, both within (on-site) and outside (off-site) the treated area, at various times over a 22 week (154 d) period after application, are given in table 5. The data show that the majority of Tebuthiuron was found in suspended sediment/microalgae, although concentrations had dropped to zero by 70 d after application. A substantial amount of Tebuthiuron was also detected in soil samples (0–100 mm depth), which remained to some extent in the compartment over the 154 d monitoring period. Considerably less Tebuthiuron was found dissolved in surface water, regardless of its solubility, with the majority having disappeared by 70 d after application. The highest surface water and suspended sediment Tebuthiuron concentrations were measured after 10 d in a small waterhole approximately 500 m outside the area treated with Graslan (Parry & Duff 1990). The highest recorded concentration of Tebuthiuron in soil was measured after 10 d within the treated area (Parry & Duff 1990).

In 1991, an area of approximately 5800 ha of mimosa was treated at a rate of approximately 2 kg Tebuthiuron/ha (Cook 1992), representing a total of approximately 12 000 kg of Tebuthiuron. Concentrations of Tebuthiuron in the surface water only were measured at various sites at 3 and 123 d after application. The maximum Tebuthiuron concentrations in surface water at these times are given in table 5.

Table 5 Highest recorded Tebuthiuron concentrations (mg L^{-1}) in surface water, suspended sediment/microalgae and soil on the Oenpelli floodplain, western Arnhem Land, following treatment with Graslan® in November 1989 and November 1991[a]

Year	Compartment	Time after application (d)											
		3		10		70		98		123		154	
		on-site	off-site	on-site	off-site	on-site	off-site	on-site	off-site	on-site	off-site	on-site	off-site
1989[b]	Surface water	–	–	–[c]	0.550	0.059	0.039	0.168	0.030	–	–	0.034	0.002
	Suspended sediment/ Microalgae	–	–	–[c]	4.39	BADL[d]	BADL[d]	BADL[d]	BADL[d]	–	–	BADL[d]	0.072
	Soil (0-100 mm)	–	–	2.91	–	0.807	–	1.82	–	–	–	0.350	–
1991[e]	Surface water	2.05	0.016	–	–	–	–	–	–	0.015	0.037	–	–

a Measured values (mg L-1) are given for both within (on-site) and outside (off-site) the treatment areas. A dash indicates that measurements were not taken.
b Data from Parry & Duff (1990).
c Surface water and suspended sediment concentrations could not be determined due to the absence of surface water.
d BADL, Below analytical detection limit (ie 0.01 μg L-1).
e Data from Cook (1992).

Similar results for surface water were obtained in a North American study, where Tebuthiuron concentrations were recorded at 2.2 mg L^{-1} and 0.05 mg L^{-1} at two and approximately 100 d following treatment to a rangeland watershed, respectively (Bovey et al 1984). Cook (1992) also measured Tebuthiuron in soil samples prior to (but not following) the 1991 application, with the highest concentration being 1.38 mg L^{-1}. It was presumed that this represented residual Tebuthiuron remaining from the 1989 application (Cook 1992).

3.4.2 Dissipation

As a result of the extensive use of Tebuthiuron to control mimosa in northern Australia, Batterham (1992) investigated the dissipation of the herbicide under both field and laboratory conditions. A controlled experiment carried out in the field indicated that after 22 d and 169 mm of simulated rainfall, the highest concentrations of Tebuthiuron in soil were directly below the point of pellet application, with dissipation being mainly vertical, with only very limited lateral movement (Batterham 1992). However, Tebuthiuron in the soil generally accounted for less than 10% of the applied Tebuthiuron. This large loss in Tebuthiuron was attributed to the presence of water, mostly in the form of runoff, but also through dilution through inundation. In addition, the characteristic heavy clay soils of northern Australian floodplains most likely serve to facilitate this, due to the much lower infiltration rates (Batterham 1992).

Laboratory trials on the dissipation of Tebuthiuron in soils indicated that the majority of the herbicide was concentrated within the top 50 mm, with a small amount of lateral dissipation to 100 or 150 mm (Batterham 1992). Simulated flooding resulted in a greater lateral dissipation in soil, while the highest Tebuthiuron concentration in surface water was found to be 1.7 mg L^{-1}, 7 d after application of a 95 mg Graslan pellet (ie equivalent to approximately 19 mg Tebuthiuron). Taking into account the volume of water, this accounted for almost 60% of the applied Tebuthiuron, emphasising the ability of soil-bound Tebuthiuron to mobilise into flood water (Batterham 1992).

3.4.3 Degradation

Batterham (1992) demonstrated that under simulated floodplain conditions there was negligible microbial and photo-degradation of Tebuthiuron. Microbial degradation was estimated to be <5% over a 99 d test period, while the photodegradation half lives for Tebuthiuron in soil and water under continuous full sunlight were 79 and 103 d, respectively (Batterham 1992). According to Emmerich et al (1984), microbial degradation played a major role in the breakdown of Tebuthiuron in a loamy soil, although this was still a particularly slow process, with 38% of the original Tebuthiuron remaining after 21 months. Photolysis has also been reported as a means of Tebuthiuron degradation. Approximately 43% of a 2.5 mg L^{-1} solution of Tebuthiuron in natural water was degraded following 15 d continuous irradiation with a sunlamp (Rainey & Magnussen 1976, as cited by Caux et al 1997). As the experimental conditions used by Batterham (1992) simulated those experienced in northern Australian floodplain environments, the results of that study were considered to be the more reliable.

3.4.4 Estimated level of exposure

As stated above, a 'worst case scenario' approach was adopted for estimating the likely level of Tebuthiuron exposure to aquatic organisms assessed in this study. This was justified as this study represents only a preliminary phase towards a comprehensive ecological risk assessment of Tebuthiuron for northern Australian wetlands. Under northern Australian floodplain conditions, Tebuthiuron was found to be very stable and highly mobile in surface waters. Therefore, in considering the risks to the test organisms assessed in this study, the sum of the maximum concentrations of Tebuthiuron found in the surface water and suspended

sediment/microalgae at the same site 10 d following herbicide application in November 1989 (4.9 mg L^{-1}), was taken to be the maximum likely exposure level (table 5). The two sources of Tebuthiuron were combined (ie 4.39 and 0.55 mg/L), as both were considered to be potentially available for uptake by the aquatic organisms assessed.

3.5 Towards an ecological risk assessment of Tebuthiuron in northern Australia

It is not possible to quantitatively assess the ecological risks associated with the application of Tebuthiuron to northern Australian floodplains based on the toxicity data of three non-target freshwater animals. As stated previously, an assessment of the relative toxicity of Tebuthiuron to local aquatic plants (eg algae and macrophytes) is required to form a more comprehensive understanding of the range of toxicity exhibited by local aquatic species, particularly since plants are the primary target of Tebuthiuron. Nevertheless, this study does allow for a preliminary, qualitative assessment of the potential risks of Tebuthiuron to non-target aquatic animals.

Field monitoring of Tebuthiuron in various environmental compartments following two major Tebuthiuron applications to a northern Australian floodplain (Parry & Duff 1990, Batterham 1992, Cook 1992) provided a reasonable indication of the potential concentrations of Tebuthiuron that aquatic organisms could be exposed to. Furthermore, it provided some, although not extensive, information on the likely duration of exposure of an aquatic organism to higher concentrations of the herbicide. Overall, Tebuthiuron was found to be very stable and highly mobile in surface waters. The latter feature was exacerbated by the fact that Tebuthiuron was often applied just prior to storm events, resulting in large amounts being washed downstream with runoff water. As a result, the majority of Tebuthiuron could be measured in the water, either in its dissolved form, or bound to suspended sediment and/or microalgae. Regardless of the form, it was considered that the total Tebuthiuron in the water column would potentially be available for uptake by aquatic organisms, particularly filter feeders such as the cladoceran, *M. macleayi*. The highest measured concentration of Tebuthiuron in the water column was approximately 4.9 mg L^{-1}, 10 d after Graslan application, at a site approximately 500 m from the treatment area (Parry & Duff 1990). This concentration was selected as the likely exposure level because:

- This study assessed Tebuthiuron toxicity to three local freshwater animals only, and it was decided that a worst-case-scenario approach should be adopted;
- Tebuthiuron concentrations may have been higher (than 4.9 mg L^{-1}) prior to the 10 d post-application sampling period.

Thus, in the absence of more Tebuthiuron toxicity and field concentration data, a precautionary principle approach was adopted. Comparison between the estimated likely exposure level of 4.9 mg L^{-1} Tebuthiuron, and the BEC_{10} values of 17.4, 40.6, and 108 mg L^{-1} for *M. macleayi*, *H. viridissima*, and *M. mogurnda*, respectively, revealed no overlap, indicating a minimal risk of adverse effects of Tebuthiuron to these organisms. The BEC_{10} was selected for the comparison since it has been considered by some to be a better measure than the NOEC of the no adverse biological effect concentration (Hoekstra & van Ewijk 1993a,b, Markich & Camilleri 1997, see section 3.3). Moreover, because the BEC_{10} gave the lowest values of the three 'no-effect' statistical endpoints (ie BEC_{10}, NOEC, and EC_{10}), it best conformed to the precautionary principle approach (MacGarvin 1995). However, it is common to apply an application or safety factor to the lowest 'no-effect' value when attempting to determine 'safe' levels, to account for potentially more sensitive species that

may exist in the environment that were not assessed. When chronic 'no-effect' data are available, the general approach is to divide the lowest no-effect value, in this case the BEC_{10} for *M. macleayi* (17.4 mg L^{-1}) by a factor of 10, resulting in a 'safe' concentration of 1.74 mg L^{-1}. Based on this calculation, there appears to be potential risk to aquatic organisms associated with the application of Tebuthiuron in Australian tropical floodplain environments. However, all that can be concluded at this stage of the study is the likelihood of minimal risk (ie adverse effects) to non-target aquatic organisms such as *M. mogurnda*, *H. viridissima* and *M. macleayi*.

Once the toxicity of Tebuthiuron to local aquatic plant species is assessed, it is anticipated that the ecological risk will increase, given the much higher toxicity of Tebuthiuron reported for northern hemisphere aquatic plants (see review by Caux et al 1997). The next phase of this research will assess the effects of Tebuthiuron to the local aquatic floating macrophyte, *Lemna aequinoctialis*, and a local unicellular green alga, *Chlorella* sp. Tests using the former species were already underway at the time of completion of the present study. In addition, since elevated environmental concentrations of Tebuthiuron (eg 1−5 mg L^{-1}) in floodplain environments appear to be relatively short-lived (ie <70 d) (Parry & Duff 1990, Batterham 1992), the ability of organisms, and thus, populations and communities, to recover after such time needs to be considered, as do potential interactions between species. An experimental microcosm incorporating *Chlorella* sp., *M. macleayi* and *H. viridissima* will be established to determine the potential for interactions between species and trophic levels (ie indirect effects). Further, all concentrations of Tebuthiuron measured on the Oenpelli floodplain will be taken into account to better characterise likely exposure levels, while the implications of the coincidence of herbicide application with the onset of the Wet season will also be considered.

4 Conclusions

The herbicide Tebuthiuron was not particularly toxic to the three non-target Australian tropical freshwater organisms assessed in the present study. Overall, toxicity to the Australian species was relatively similar to that reported for northern hemisphere temperate aquatic organisms, although the Australian fish, *M. mogurnda*, appeared to be slightly less sensitive than northern hemisphere fish species. A literature review of the fate and concentrations of Tebuthiuron in the aquatic environment revealed that concentrations were not likely to exceed or even equal those reported to be toxic to aquatic fauna. Thus, the results indicated that there was little risk to the three non-target species tested in this study being directly affected by exposure to Tebuthiuron at environmentally relevant concentrations.

However, Tebuthiuron toxicity to local aquatic plant species is expected to be significantly greater, potentially resulting in a considerable degree of risk of adverse effects in the aquatic environment. Toxicity testing using a local aquatic macrophyte (*Lemna aequinoctialis*) and green alga (*Chlorella* sp.) was underway at the time of completion of the present study. The results are to be combined with those of the present study, along with a more comprehensive assessment of potential exposure levels, in order to quantitatively assess the ecological risks associated with the use of Tebuthiuron as a means of controlling *Mimosa pigra*.

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Appendices

Appendix A Raw data for all toxicity tests

Table A1 Raw data for *M. mogurnda*

CONC (mg/L)	0	50	70	80	100	120	150	160	175	180	200	210	225	240	250	280	300	310	500
Test No.	Control																		
330																			
rep1	10	7			10						8						0		0
rep2	10	10			10						10						0		0
rep3	2	10			10						10						0		0
mean(SE)	73.33 (0.27)	90.0 (0.08)			100 (0.07)						93.33 (0.07)						0(0)		0(0)
332																			
rep1	10									10		9			6	6		2	
rep2	9									9		9			10	5		2	
rep3	10									10		10			7	3		0	
mean(SE)	96.67 (0.03)									96.67 (0.03)		93.33 (0.03)			76.67 (0.12)	46.67 (0.09)		13.33 (0.07)	
337																			
rep1	10		10			8		7		5				6					
rep2	7		10			6		7		10				2					
rep3	9		6			6		9		5				4					
mean(SE)	86.67 (0.09)		86.67 (0.13)			66.67 (0.07)		76.67 (0.07)		66.67 (0.17)				40.67 (0.11)					
344																			
rep1	9	9		10		10		10		9	10			8					
rep2	9	10		9		10		10		10	10			6					
rep3	10	10		10		10		9		10	10			7					
mean(SE)	93.33 (0.03)	96.67 (0.03)		96.67 (0.03)		100 (0.0)		100 (0.0)		100 (0.0)	100 (0.0)			70.00 (0.06)					

Table A1 continued

CONC (mg/L)	0	50	70	80	100	120	150	160	175	180	200	210	225	240	250	280	300	310	500
Test No.	Control																		
350																			
rep1	7	0			2		0		2		6		2		0				
rep2	10	10			0		10		9		6		1		0				
rep3	10	5			9		10		7		5		2		0				
mean(SE)	95.00 (3.02)	74.17 (10.18)			58.33 (11.49)		79.17 (10.24)		76.67 (7.89)		72.50 (4.94)		41.67 (11.57)		0 (0)				
351																			
rep1	9	10			10		10		10		10		8		6				
rep2	10	10			10		10		10		10		9		2				
rep3	9	10			10		10		10		10		8		4				
mean(SE)	99.17 (0.75)	100 (0.0)			100 (0.0)		100 (0.0)		100 (0.0)		100 (0.0)		91.67 (2.42)		74.17 (7.09)				

NB: Individual replicate values represent the number of surviving sac-fry, while the mean (SE) values represent the percentage survival.

Table A2 Raw data for *M. macleayi*

CONC (mg/L)	0	5	10	15	20	25	40	50	60	80	100	120	140	160	180	220
Test No.	Control															
345																
rep 1	48	46	44	49	9	46		45								
rep 2	0	47	44	48	10	44		0								
rep 3	47	49	46	49	47	43		44								
rep 4	50	47	46	48	45	45		0								
rep 5	48	9	46	46	45	43		40								
rep 6	48	45	0	50	46	23		42								
rep 7	49	45	47	46	45	47		43								
rep 8	45	47	48	47	48	10		10								
rep 9	45	49	49	50	48	46		0								
rep 10	48	43	47	12	46	46		10								
mean(SE)	42.8 (4.8)	42.7 (3.8)	41.7 (4.7)	44.5 (3.6)	38.9 (4.9)	39.3 (3.9)		23.4 (6.6)								
346																
rep 1	50		44		40		44		0	40	8					
rep 2	48		42		45		41		37	34	19					
rep 3	24		40		41		45		24	39	28					
rep 4	48		42		41		44		40	36	29					
rep 5	47		43		40		43		42	37	21					
rep 6	48		44		43		43		10	38	22					
rep 7	47		0		41		43		40	35	0					
rep 8	47		44		40		40		9	36	34					
rep 9	47		43		41		26		41	38	34					
rep 10	45		10		40		39		43	18	26					
mean(SE)	45.1 (2.4)		35.2 (5.1)		41.2 (0.5)		40.8 (1.8)		28.6 (5.2)	35.1 (2.0)	22.1 (3.5)					

Table A2 continued

CONC (mg/L)	0	5	10	15	20	25	40	50	60	80	100	120	140	160	180	220
Test No.	Control															
352																
rep 1	44				43			0		38	33		6		1	0
rep 2	44				44			37		25	33		15		0	0
rep 3	48				37			43		38	37		14		0	0
rep 4	50				40			37		37	7		19		3	0
rep 5	42				43			37		40	0		23		4	0
rep 6	42				41			41		36	19		20		0	0
rep 7	49				41			41		34	32		17		0	0
rep 8	47				40			43		40	19		11		0	0
rep 9	46				42			41		35	34		18		5	0
rep 10	50				44			37		36	39		18		0	0
mean(SE)	46.2 (1.0)				41.5 (0.7)			35.7 (4.0)		35.9 (1.4)	25.3 (4.2)		16.1 (1.5)		1.3 (0.6)	0 (0)
353																
rep 1	10		42		46		44			23	40	6	11		0	
rep 2	50		44		45		41			39	34	28	21		0	
rep 3	27		49		46		44			40	38	6	0		0	
rep 4	44		49		41		43			39	41	31	8		6	
rep 5	49		46		41		39			41	33	19	1		4	
rep 6	46		47		35		39			39	39	5	1		0	
rep 7	42		45		46		42			35	21	31	15		5	
rep 8	46		45		44		40			33	39	9	0		6	
rep 9	47		42		32		38			38	39	8	0		0	
rep 10	46		43		48		25			36	40	24	10		0	
mean(SE)	40.7 (4.0)		45.2 (0.8)		42.4 (1.7)		39.5 (1.8)			36.3 (1. 7)	36.4 (1.9)	16.7 (3.5)	6.7 (2.4)		2.1 (0.9)	

Table A2 continued

CONC (mg/L)	0	5	10	15	20	25	40	50	60	80	100	120	140	160	180	220
Test No.	Control															
354																
rep 1	45		45		42		9			38	7	14	18	5	0	
rep 2	48		40		43		10			33	30	22	6	11	0	
rep 3	48		43		41		48			38	34	31	4	2	8	
rep 4	45		44		42		45			39	31	29	12	12	0	
rep 5	44		43		43		24			37	21	27	19	13	3	
rep 6	10		44		43		46			34	32	26	10	7	4	
rep 7	45		43		23		42			21	28	19	22	1	0	
rep 8	46		44		43		41			36	29	16	12	0	0	
rep 9	46		44		40		41			37	33	27	3	7	1	
rep 10	45		42		40		40			34	35	0	13	16	8	
mean(SE)	42.2 (3.6)		43.2 (0.4)		40 (1.9)		34.6 (4.7)			34.7 (1.7)	28 (2.7)	21.1 (3.0)	11.9 (2.0)	7.4 (1.7)	2.4 (1.0)	

NB: All values represent the total number of offspring per adult.

Table A3 Raw data for *H. viridissima*

CONC (mg/L)	0	5	10	20	25	50	70	75	90	100	120	130	150	170	180	200	210	250	290	300	330	400	500	600	800
Test No.	Control																								
328																									
rep1	59	46	39	42		39		30																	
rep2	43	44	40	42		35		34																	
rep3	37	43	36	38		37		35																	
mean(SE)	0.38 (0.03)	0.37 (0.005)	0.34 (0.008)	0.35 (0.008)		0.33 (0.008)		0.30 (0.01)																	
330																									
rep1	31					27				28						0				10			0		
rep2	33					34				28						19				11			0		
rep3	23					37				31						17				11			0		
mean(SE)	0.26 (0.03)					0.29 (0.02)				0.27 (0.01)						0 (0)				0.02 (0.01)			0 (0)		
331																									
rep1	62									38			24			17				14		9			
rep2	63									39			23			19				15		12			
rep3	50									33			23			19				16		9			
mean(SE)	0.44 (0.02)									0.32 (0.01)			0.21 (0.01)			0.15 (0.01)				0.1 (0.01)		0 (0)			
334																									
rep1	43									25					18					16		12	11		
rep2	42									23					16					15		11	10		
rep3	43									31					17					16		12	11		
mean(SE)	0.36 (0.002)									0.24 (0.02)					0.13 (0.01)					0.11 (0.01)		0.04 (0.01)	0.02 (0.01)		

Table A3 continued

CONC (mg/L)	0	5	10	20	25	50	70	75	90	100	120	130	150	170	180	200	210	250	290	300	330	400	500	600	800
Test No.	Cont																								
341																									
rep1	45					42	27		31		23											0		0	0
rep2	37					44	33		40		17											0		0	0
rep3	42					34	31		27		22											0		0	0
mean(SE)	0.35 (0.01)					0.35 (0.02)	0.28 (0.02)		0.29 (0.03)		0.18 (0.02)											0 (0)		0 (0)	0 (0)
342																									
rep1	36					43			32			26		18			18	15	16		16	10			
rep2	51					43			39			24		19			18	15	13		13	9			
rep3	51					44			33			25		20			21	15	15		15	11			
mean(SE)	0.37 (0.03)					0.37 (0.01)			0.31 (0.02)			0.23 (0.01)		0.16 (0.01)			0.16 (0.01)	0.10 (0.0)	0.10 (0.02)		0.07 (0.03)	0 (0)			
349																									
rep1	40				43	33		33		33			21			11				3		0			
rep2	35				33	30		37		26			17			9				4		0			
rep3	36				34	34		33		26			16			6				1		0			
mean(SE)	0.32 (0.01)				0.32 (0.02)	0.29 (0.01)		0.31 (0.01)		0.26 (0.02)			0.15 (0.02)			0 (0)				0 (0)		0 (0)			

NB: Individual replicate values represent number of hydra, while the mean (SE) values represent the population growth rate.

Appendix B Summary of mean pH and conductivity values of control waters for each experiment

Billabong	Date	Test No and organism	pH		Conductivity ($\mu S\ cm^{-1}$)	
			t_0	t_{24}	t_0	t_{24}
Georgetown	24/4/96	328, hydra	6.95	7.04	14.4	17.2
Georgetown	6/5/96	330, gudgeon	7.10	7.19	16.2	18.0
Georgetown	6/5/96	330, hydra	7.10	7.09	16.2	18.5
Georgetown	14/5/96	331, hydra	7.02	7.07	16.9	19.8
Georgetown	14/5/96	332, gudgeon	7.02	7.17	16.9	20.9
Georgetown	11/6/96	334, hydra	6.91	7.05	15.7	17.1
Georgetown	1/7/96	337, gudgeon	6.87	6.66	15.0	17.6
Bowerbird	29/7/96	341, hydra	6.92	6.96	13.8	15.6
Bowerbird	6/8/96	342, hydra	6.76	6.85	13.1	15.2
Bowerbird	3/9/96	344, gudgeon	6.68	6.62	14.4	18.2
Bowerbird	21/10/96	345, cladoceran	6.93	7.47	21.0	21.5
Bowerbird	29/10/96	346, cladoceran	6.82	7.17	20.2	20.6
Bowerbird	3/11/96	349, hydra	6.38	6.66	19.2	20.7
Georgetown	18/1/97	350, gudgeon	6.48	6.83	13.1	20.2
Georgetown	1/2/97	351, gudgeon	6.18	6.58	11.6	12.7
Georgetown	10/2/97	352, cladoceran	6.74	6.82	15.8	17.0
Georgetown	24/2/97	353, cladoceran	6.33	6.97	26.6	26.8
Georgetown	15/3/97	354, cladoceran	6.67	6.83	15.0	15.0
Georgetown		**Mean (SE)**[a]	6.81 (0.08)	6.96 (0.08)	16.6 (0.8)	18.6 (0.7)
Bowerbird		**Mean (SE)**[a]	6.69 (0.10)	6.91 (0.04)	16.0 (2.1)	18.3 (1.9)
		Overall mean	6.77	6.94	16.4	18.5

a SE, standard error around the mean.

Appendix C Nominal and measured concentrations of Tebuthiuron for selected water samples from each experiment[a]

Sample ID	Nominal concentration(mg L^{-1})	Measured concentration (mg L^{-1})	% of Nominal concentration
330-1-BE	0	ND	–
330-2-BE	50	48 (1.0)	96
330-3-BE	100	104 (2.0)	104
330-4-BE	200	216 (7.0)	108
330-5-BE	300	326 (7.0)	109
330-6-B	500	541 (17.0)	108
331-1-B	0	ND	–
331-2-B	100	103 (3.0)	103
331-3-B	150	160 (4.0)	107
331-4-B	200	211 (15.0)	106
331-5-B	300	318 (19.0)	106
331-6-B	400	434 (16.0)	109
332-2-E	180	191 (2.0)	106
332-3-E	210	228 (3.0)	109
332-4-E	250	273 (6.0)	109
332-5-E	280	306 (6.0)	109
332-6-E	310	329 (16.0)	106
334B	0	ND	–
334B	100	106 (0.7)	106
334B	500	515 (4.3)	103
337E	0	ND	–
337E	70	74 (2.3)	106
337E	120	124 (1.0)	103
339D	50	54 (3.1)	108
339D	80	83 (1.0)	104
341B	0	ND	–
341B	50	57 (0.1)	114
341B	70	76 (0.2)	109
341B	400	420 (1.1)	105
342B	0	ND	–
342B	50	55 (0.9)	110
342B	90	97 (0.9)	108

Appendix C continued

Sample ID	Nominal concentration(mg L^{-1})	Measured concentration (mg L^{-1})	% of Nominal concentration
342B	130	139 (0.2)	107
342B	400	418 (6.6)	107
343D	0	ND	–
343D	10	12 (0.4)	120
343D	20	23 (0.5)	115
343D	40	33 (0.3)	83
343D	60	62 (3.8)	103
343D	100	110 (1.4)	110
344E	120	115 (3.7)	96
344E	240	233 (3.0)	97
345D	20	19 (0.1)	95
345D	50	49 (0.6)	98
349B	50	48 (0.8)	96
349B	75	72 (1.7)	96
349B	100	95 (0.6)	95
349B	150	143 (1.7)	95
350E	0	ND	–
350E	175	171 (1.0)	98
351E	50	50 (0.1)	100
351E	200	187 (1.0)	94
352D	100	96 (0.5)	96
352D	220	207 (2.0)	94
353D	40	40 (0.5)	100
353D	120	112 (4.3)	93
354D	80	78 (1.3)	98
354D	160	155 (2.0)	97

a Measured concentrations are given as the mean (and standard deviation) of duplicate or triplicate runs. ND: not detected. The typical analytical detection limit for Tebuthiuron during this study was 0.01 μg L^{-1}.