

Ecological risk assessment of tebuthiuron following application on northern Australian wetlands

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Table of Contents

Acknowledgments	iii
Summary	iv
1. Introduction	1
1.1 Background	1
1.2 Approach of the ecological risk assessment	1
2. Problem formulation	1
2.1 <i>Mimosa pigra</i>	1
2.2 Tebuthiuron	2
2.3 Wetland ecosystems potentially at risk	7
2.4 Endpoints	7
2.5 Working hypotheses	8
3. Analysis	8
3.1 Effects characterisation	8
3.2 Exposure characterisation	11
3.3 Risk characterisation	14
4. Management implications	20
4.1 Risk evaluation and research needs	20
4.2 Risk management and reduction	21
5. Conclusions	22
References	22

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Summary

The herbicide tebuthiuron is commonly used in Australia's Northern Territory to control the wetland weed, *Mimosa pigra*. The present study provided a probabilistic ecological risk assessment of tebuthiuron to freshwater fauna and flora of northern Australian wetlands. The following two working hypotheses were assessed: (i) that tebuthiuron may result in direct adverse effects to native freshwater biota at the site and downstream of treated *M. pigra* infestations, potentially resulting in adverse effects to community structure and function, and (ii) that long term and/or delayed effects to native freshwater biota may occur as a result of the residual properties of tebuthiuron. Indirect effects of tebuthiuron were recognised but could not be quantitatively assessed.

Effects characterisation involved assessment of the acute or chronic toxicity of tebuthiuron to five local freshwater species (three animals and two plants), and comparison of the results with toxicity data derived for northern hemisphere species. Chronic no-observed-effect concentrations (NOECs) for local plant and invertebrate species ranged from 0.05 to 50 mg L⁻¹, while the acute (96 h) LC50 of a local fish species was 214 mg L⁻¹. The data were similar to those derived for northern hemisphere species.

Exposure characterisation involved the use of historical field monitoring data of tebuthiuron concentrations following application of tebuthiuron to a large *Mimosa* infestation. Tebuthiuron concentrations in surface water ranged from 0.002 to 2.05 mg L⁻¹. The highest concentration of 2.05 mg L⁻¹, was measured three days following application. Tebuthiuron was still measurable in surface water three, four and five months following application, with the highest concentrations at these time points being 0.168, 0.037 and 0.034 mg L⁻¹, respectively.

Risk characterisation involved the comparison of cumulative probability distributions of environmental tebuthiuron concentrations and species sensitivity to tebuthiuron. Risks were estimated for freshwater plant chronic toxicity, invertebrate and vertebrate chronic toxicity, and vertebrate acute toxicity. The tebuthiuron concentrations for the 5th centiles of the species sensitivity distributions for the above scenarios were 0.012 (NOEC data), 9.1 (NOEC data) and 98 (LC50 data) mg L⁻¹, respectively. The probability of at least 5% of freshwater plant species experiencing chronic effects due to tebuthiuron was 73%. When only tebuthiuron concentrations measured more than three months following application were considered, this probability was only reduced to 63%. Overlap of the 95% confidence limits of the exposure and plant sensitivity distributions indicated substantial uncertainty in the risk estimates. The probabilities of direct chronic effects to freshwater animals and acute effects to freshwater vertebrates were < 1%.

The risk assessment concluded that tebuthiuron represents a significant and prolonged risk to native freshwater plant species, particularly phytoplankton and floating macrophytes, while the risks to freshwater invertebrates and vertebrates appear low. The risks of tebuthiuron (and other herbicides) must be weighed against the serious environmental impacts of the target weed, *M. pigra*.

1. Introduction

1.1 Background

The herbicide tebuthiuron has been used widely in the Northern Territory of Australia for control of the wetland weed, *Mimosa pigra* (Mimosa), since the late 1980s. Mimosa is an opportunistic and aggressive weed, forming dense mono-specific stands in tropical wetland habitats and replacing native vegetation (Lonsdale et al 1995). Thus, there is a need to effectively control and manage Mimosa in northern Australian wetlands. However, the control measures themselves may well impart some adverse impact on the local environment. Ideally, potential adverse impacts of control measures should be assessed prior to their implementation; where this has not occurred, appropriate assessments should be carried out as a priority. While the long term goal for the effective management of Mimosa in northern Australia is the establishment of a successful biological control program (Forno 1992), it is acknowledged that this will need to be used in conjunction with chemical and mechanical methods (Environment Australia 1997). Therefore, the current use of herbicides will continue in the long-term, and it is imperative that their risks to the local aquatic environment are assessed and understood.

Herbicides used for Mimosa control include tebuthiuron, fluoroxypr, metsulfuron methyl, hexazinone and dicamba (DASETT 1991; Miller & Siriworakul 1992; Ashley 1999). No site-specific assessments of the potential adverse effects of these herbicides were undertaken prior to their use as Mimosa control agents in the Northern Territory. Although an assessment of their potential effects was undertaken prior to a large scale control program in the region (Dames & Moore 1990), it was based solely on ecotoxicological and use data derived from northern hemisphere studies, with very little consideration of site-specific information. It was essentially a hazard assessment, providing no estimate of potential risks. Historically, tebuthiuron has been the most commonly used herbicide for Mimosa control in northern Australia (I Brown, pers. comm.), and for this reason was the focus of this risk assessment. Thus, the present study aimed to provide a quantitative estimate of the ecological risks of tebuthiuron to the aquatic fauna and flora of northern Australian wetlands.

1.2 Approach of the ecological risk assessment

The ecological risk assessment generally followed the probabilistic approach recommended by the U.S. Environmental Protection Agency (US EPA 1998). A detailed description of the approach is not provided here, but can be found elsewhere (Solomon et al 1996; US EPA 1998). The assessment involved the following three major steps: *problem formulation*, *analysis* and *risk characterisation*, each of which consisted of several components. A final section discusses *management implications*.

2. Problem formulation

2.1 *Mimosa pigra*

It is difficult to discuss and assess the use of tebuthiuron without placing it in the context of the broader issue of Mimosa invasion and infestation, and the threat the weed poses to the wetland flora and fauna communities of northern Australia. Mimosa forms dense, impenetrable stands up to several metres high, eliminating native plant species and displacing native wildlife (Braithwaite et al 1989), while economic (pastoralism and tourism) and

cultural resources are also largely impacted (Lonsdale 1992, NLC & ERISS 1997). The aggressive nature of the weed is further demonstrated by the fact that uncontrolled Mimosa infestations have been reported to double in size each year (Lonsdale 1992).

In 1989, it was estimated that Mimosa had already invaded over 80,000 ha of wetlands, primarily seasonally inundated floodplains and various swamplands, in the ‘Top End’ of the Northern Territory (Finlayson et al 1996, Environment Australia 1997). Documented infestations range over approximately a 1,000 km arc, from the Fitzmaurice River in the west, to the Phelps River (a tributary of the Roper River) in south-eastern Arnhem Land (Figure 1), although, the actual wetland area currently infested by Mimosa is generally unknown (Walden et al in press). Although Mimosa is yet to spread beyond the northern half of the Northern Territory, the estimated wetland area potentially susceptible to Mimosa invasion in northern Australia (based on its preferred wetland habitats and optimal average annual rainfall range of 750 – 2250 mm) has been estimated at over 4 million ha (van Dam et al 1999).

2.2 Tebuthiuron

2.2.1 Physicochemical properties

Tebuthiuron, the active ingredient of the formulation Graslan[®], belongs to the family of substituted urea herbicides. A summary of the physical and chemical properties of tebuthiuron 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).

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-yl]-N,N'-dimethylurea
Alternative names	Graslan [®] , Spike [®] , Perflan [®] , Herbec [®] , Herbic [®]
Chem Service Cat	F2243
Molecular weight	228.3 g mol ⁻¹
Molecular formula	C ₉ H ₁₆ N ₄ OS
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).

2.2.2 Mode of Action

Tebuthiuron is absorbed by woody plants via the roots and translocated to its target sites in the stems and leaves (Steinert and 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.

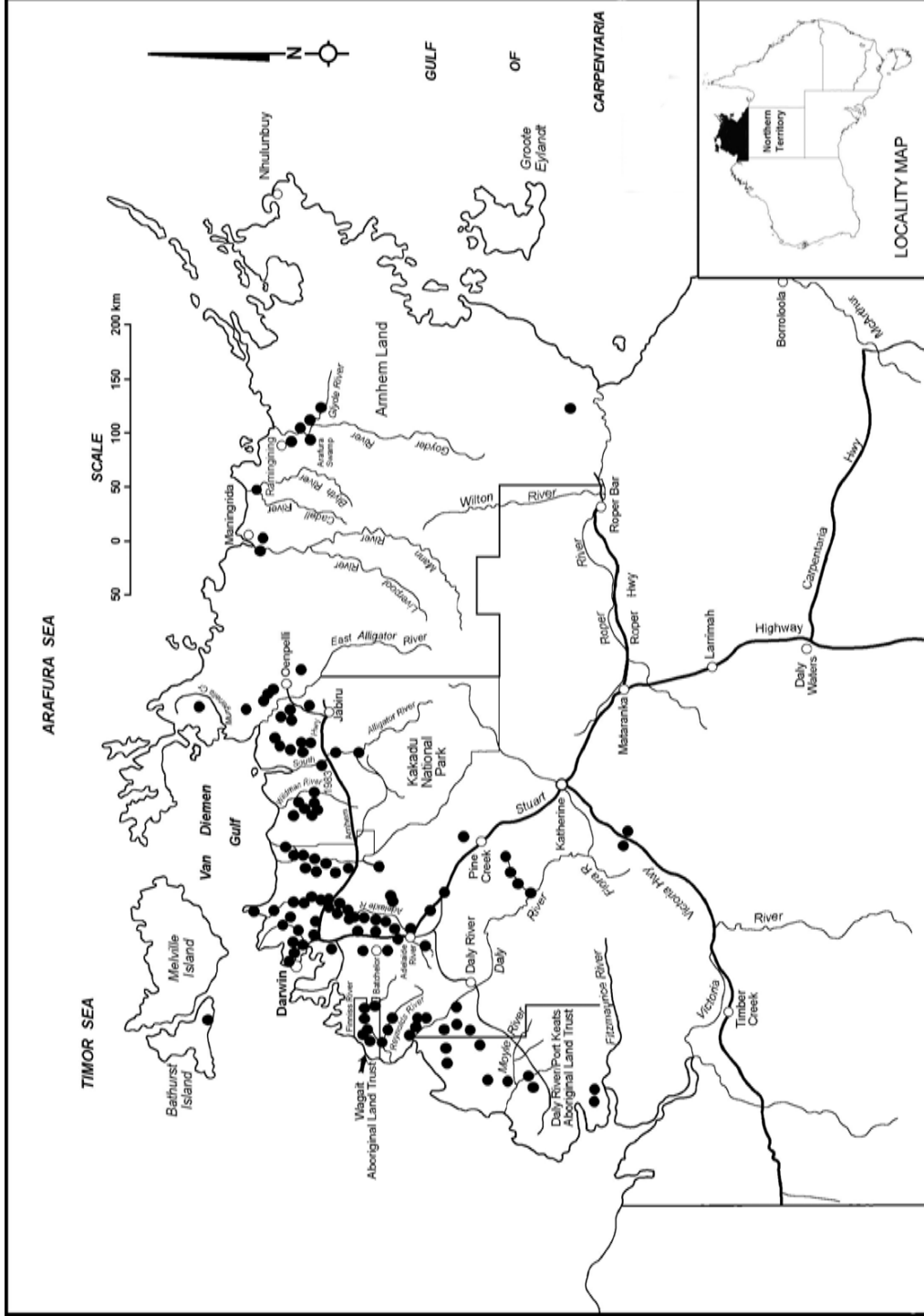


Figure 1 Documented locations of *Mimosa pigra* in the Northern Territory of Australia (adapted from map supplied by L.A. Hills, Northern Territory Department of Primary Industries and Fisheries, April 2001).

2.2.3 Use

The commercial product, Graslan[®], is applied to soils in pellet form, and typically contains tebuthiuron at concentrations of either 10, 20, or 30%. Between 1988 and 1996, approximately 30,000 kg of tebuthiuron (*ca* 150,000 kg Graslan[®]) was applied to a Mimosa infestation of almost 6,000 ha at Oenpelli in western Arnhem Land, in the Northern Territory of Australia (Cook 1996). Approximately 40% of this (12,000 kg tebuthiuron) was applied to the infestation in 1991 (Cook 1993), followed by a further 8,000 kg to 5,000 ha in 1993, and 4,000 kg to 2,500 ha in 1994 (NT DPIF 1993, 1994). Although the total amount of tebuthiuron applied to Mimosa infestations has decreased markedly since then, considerable quantities are still being used, with approximately 4000 kg applied in 1998 to an infestation at Koolpinyah Station, east of Darwin (G. Schultz, pers. comm.). On the Mimosa-infested wetlands of the Northern Territory, 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 US EPA (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 (Anon 1990).

2.2.4 Environmental behaviour

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 have considered the environmental fate of tebuthiuron under cracking clay floodplain environments characteristic of the wet/dry tropics of northern Australia (Parry & Duff 1990, Batterham 1992).

Degradation

The degradation 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. Reported degradation half lives in soil range from approximately 1 to 7 y (Anon, 1988; Johnsen and Morton 1989). 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 of continuous irradiation with a sunlamp (Rainey & Magnussen 1976, as cited by Caux et al 1997). 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 resultant metabolites are apparently either non-herbicidal or possess weak herbicidal activity (Anon 1988). 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). According to Emmerich et al (1984), microbial degradation of tebuthiuron in a loamy soil was slow, with 38% of the original tebuthiuron remaining after 21 months. The microbial degradation of tebuthiuron under simulated northern Australian floodplain conditions was also found to be slow, with over 95% remaining after 99 d (Batterham 1992).

Dissipation

Tebuthiuron is known to be a relatively persistent and mobile chemical, with the potential to leach to groundwater (Caux et al 1997) and be mobilised by surface water (Batterham 1992). Batterham (1992) investigated the dissipation of the herbicide under northern Australian floodplain conditions. A controlled field experiment 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 (Batterham 1992). However, tebuthiuron in the soil generally

accounted for less than 10% of the total applied tebuthiuron, with the large loss attributed to the presence of water, mostly in the form of runoff, but also by dilution through inundation. Due to their much lower infiltration rates, the characteristic heavy clay soils of northern Australian floodplains most likely serve to facilitate this (Batterham 1992).

The low $\log K_{ow}$ (1.8) of tebuthiuron indicates that adsorption by soils should be limited. According to Chang and Stritzke (1977) adsorption of tebuthiuron is greatest on soils with high organic matter content, followed by soils with high clay content. Dissipation of tebuthiuron in clay-dominated soils was concentrated within the top 50 mm, with a small amount of lateral dissipation to 150 mm (Batterham 1992). However, simulated flooding resulted in a greater lateral dissipation in soil, as well as substantial mobilisation in surface water (Batterham 1992). Taking into account the volume of water, this accounted for almost 60% of the total applied tebuthiuron, emphasising the ability of soil-bound tebuthiuron to mobilise into flood water (Batterham 1992). The limited persistence of tebuthiuron in soils under northern Australian floodplain conditions was attributed to poor infiltration and high intensity rainfall, resulting in the removal of tebuthiuron in surface runoff and through mobilisation into flood water (Parry and Duff 1990; Batterham 1992). The metabolism of the herbicide by the large vegetative biomass of the floodplain also contributes to its limited persistence in soils (Batterham 1992). Persistence of tebuthiuron in the water column or when bound to suspended sediment is greater to that in soils (Parry and Duff 1990; Batterham 1992; Caux et al 1997).

2.2.5 Aquatic toxicity

A summary of existing aquatic toxicity data for tebuthiuron is given in Table 2. These data indicate that tebuthiuron has low acute toxicity to temperate freshwater fish species, with 96 h LC_{50} values ranging from 112 to $> 160 \text{ mg L}^{-1}$ (Bionomics 1972; Todd et al 1972; Blaise & Harwood 1991). Acute toxicity to amphibians appears similarly low, with a 96 h LC_{50} for the bullfrog, *Rana catesbeiana* reported to be over 300 mg L^{-1} (Todd et al 1984). Chronic toxicity to fish and aquatic invertebrates is somewhat greater, with no-observed-effect concentrations (NOECs) ranging between about 10 and 30 mg L^{-1} (Todd et al 1972; Meyerhoff et al 1985). A preliminary toxicity assessment for two northern Australian freshwater species, green hydra (*Hydra viridissima*) and purple-spotted gudgeon (*Mogurnda mogurnda*), reported the lowest-observed-effect concentrations (LOECs) to be 75 and 270 mg L^{-1} , respectively (Pfeifle 1996).

The toxicity of tebuthiuron to freshwater algae and macrophytes is much greater than that reported for freshwater animals. For example, EC_{50} values (growth rate) for various algal and floating macrophyte species range from 0.08 to 0.235 mg L^{-1} (Negilsky & Cocke 1989a, 1989b; Blaise and Harwood 1991; Hickey et al 1991). The NOEC values for various algal and floating macrophyte species range from 0.01 to 0.31 mg L^{-1} (Adams et al 1985; Meyerhoff et al 1985; Negilsky & Cocke 1989a, 1989b; Negilsky et al 1989).

The toxicity of tebuthiuron in aquatic microcosms and mesocosms has also been studied. Seven days exposure of microcosm algal cultures to $\sim 6 \text{ mg L}^{-1}$ tebuthiuron resulted in $> 50\%$ inhibition of ^{14}C uptake in nine of the ten species assessed, and 100% growth inhibition in the floating macrophyte, *Lemna minor* (Peterson et al 1994). In another microcosm study, effects on eleven species of green algae exposed for 189 d to 0.18 mg L^{-1} tebuthiuron varied depending on their growth phase (Price et al 1989). Cell density of algal cultures in lag growth phase were significantly ($P \leq 0.05$) reduced, while cultures experiencing exponential growth were not affected (Price et al 1989). Temple et al (1991) investigated the effects of tebuthiuron ($0.01 - 1.9 \text{ mg L}^{-1}$) on aquatic productivity in large outdoor mesocosms. Phytoplankton primary production was negatively correlated with the

Table 2 Toxicity of tebuthiuron to northern hemisphere species (from Caux et al 1997).

Test organism	Duration	Life Stage	Endpoint	Effect (mg L ⁻¹)	Source reference
Fish					
Rainbow trout (<i>Oncorhynchus mykiss</i>)	96 h	Juvenile (1.5 g or 42 mm)	Survival	LC ₅₀ = 144	Bionomics (1972)
	96 h	Juvenile (10.3 ± 3.5 g)	Survival	LC ₅₀ = 115	Blaise & Harwood (1991)
	96 h	ND ¹	Survival	LC ₅₀ > 160	Tomlin (1994)
	24 h		Survival	LC ₅₀ = 193	Hamelink & Kehr (1976a)
	5-d		Survival	LC ₅₀ = 126	Hamelink & Kehr (1976a)
	~ 45-d	Embryo-larval	Growth and survival	NOEL ² = 26 LOEL ³ = 52	Todd et al (1981)
Bluegill sunfish (<i>Lepomis macrochirus</i>)	96 h	Juvenile (36 mm)	Survival	LC ₅₀ = 112	Bionomics (1972)
Goldfish (<i>Carassius auratus</i>)	96 h	Adult (50 mm)	Survival	LC ₅₀ > 160	Todd et al (1972)
Fathead minnow (<i>Pimephales promelas</i>)	96 h	Adult (50 mm)	Survival	LC ₅₀ > 160	Todd et al (1972)
	7 d		Survival	LOEL = 70 - 110	Hamelink & Kehr (1976b)
	33 d	Larvae	Growth	NOEC ⁴ = 9.3 LOEC ⁵ = 18	Meyerhoff et al (1985)
Amphibians					
Bullfrog (<i>Rana catesbeiana</i>)	72-h		Survival	LC ₅₀ = 316	Todd et al (1984)
Invertebrates					
Cladoceran (<i>Daphnia magna</i>)	21 d (~5 brood)	≤ 24 h	Body length; Fecundity	NOEL = 21.8 LOEL = 44.2	Meyerhoff et al (1985)
Chironomid (various species)	Meso-cosms 64 d	All	Density	Density reduced by 30% at 0.2	Temple et al (1991)
Plants					
Green alga (<i>Selenastrum capricornutum</i>)	96 h	Exponential growth phase	Growth rate	EC ₅₀ = 0.08	Blaise & Harwood (1991)
	96 h	Exponential growth phase	Growth rate	EC ₅₀ = 0.102	Hickey et al (1991)
	1 – 6 days	Exponential growth phase	Growth rate; cell number	NOEC = 0.01	Adams et al (1985)
	14 d	Exponential growth phase	Growth rate	NOEL = 0.033 LOEL = 0.079	Meyerhoff et al (1985)

Table 2 cont.

Diatom (<i>Navicula pelliculosa</i>)		Terminal biomass	NOEL = 0.056 LOEL = 0.11 EC ₅₀ = 0.213	Negilsky & Cocke (1989a)
Blue-green alga (<i>Anabaena flo-aquae</i>)		Exponential growth phase	Growth rate NOEL = 0.31 LOEL = 0.62	Negilsky et al (1989)
Duckweed (<i>Lemna gibba</i>)	14 d	Growth rate; terminal frond; plant counts; biomass	NOEL = 0.091 LOEL = 0.19 EC ₅₀ = 0.235	Negilsky and Cocke (1989b)

¹ ND: Not determined; ² NOEL: no-observed-effect level; ³ LOEL: lowest-observed-effect level; ⁴ NOEC: no-observed-effect-concentration; ⁵ LOEC: lowest-observed-effect concentration

concentration of tebuthiuron when sampled 42–64 d after exposure. Concentrations of < 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 *Selenastrum capricornutum*, an interim Canadian guideline value for tebuthiuron of 1.6 µg L⁻¹ was derived for the protection of freshwater life (Caux et al 1997). Similarly, a guideline of 2.2 µg L⁻¹ has recently been derived for the protection of freshwater ecosystems in Australia and New Zealand (ANZECC & ARMCANZ 2001). This value has been calculated using a statistical extrapolation method (Shao 2000) to protect 95% of species (ANZECC & ARMCANZ 2001).

2.3 Wetland ecosystems potentially at risk

The wetland ecosystems potentially at risk from tebuthiuron include those areas infested by *Mimosa*. These are predominantly the seasonally-inundated floodplains and paperbark swamps and forests in the wet-dry tropics of the Top End of the Northern Territory (see Figure 1). Within the floodplains and swamps exist a number of various permanent and intermittent aquatic habitats, including paleochannels and billabongs (backwaters and other natural inundated depressions or waterbodies) on the floodplains, and creeks and billabongs in the swamps. As tebuthiuron is often applied at the onset of the Wet season, ‘first-flush’ rains can result in transportation of the herbicide downstream of *Mimosa* infestations. Thus, both local and downstream aquatic ecosystems are potentially at risk from tebuthiuron.

Aquatic species within these ecosystems include, phytoplankton, periphyton and macrophytes, which may be affected directly by tebuthiuron. In addition, aquatic invertebrates and vertebrates (eg fish, turtles) may be affected directly, but also indirectly, via decreased food supply or altered habitats. Indirect effects are not specifically assessed in the present study, but are acknowledged and discussed where possible. Other plant species, such as terrestrial vegetation that is subjected to seasonal inundation (eg *Melaleuca* spp.) are also susceptible (Cook 1993; 1996), but were not considered in this assessment.

2.4 Endpoints

Most ecological risk assessment frameworks recommend assessments be structured around two types of endpoints: assessment and measurement endpoints (Suter 1993, US EPA 1998). Assessment endpoints, are the actual environmental values to be protected, whilst

measurement endpoints are the measured responses to a stressor that can be linked with or used to predict risks to assessment endpoints (US EPA 1998).

The assessment endpoints for this risk assessment were considered to be the structure and function of native aquatic plant communities and native aquatic invertebrate and fish communities. Measurement endpoints used to predict the risks to the above assessment endpoints included chronic growth, reproduction, biomass and productivity response data for aquatic plants, invertebrates and vertebrates, and also acute toxicity data for fish.

2.5 Working hypotheses

From the above information, the following working hypotheses were developed:

- i. Tebuthiuron may result in direct adverse effects to native aquatic biota, particularly plants, both at the site and downstream of treated *M. pigra* infestations, potentially resulting in adverse effects to community structure and function;
- ii. Direct effects of tebuthiuron on aquatic biota, particularly plants, may result in adverse indirect effects to aquatic animals due to resource (ie food, habitat) depletion and/or changes;
- iii. Long term and/or delayed effects to native aquatic biota may occur as a result of the residual properties of tebuthiuron.

Due to the nature of the existing toxicity data for tebuthiuron, and the scope of this assessment, the risks of indirect effects of tebuthiuron (hypothesis ii.) could not be quantitatively assessed. For the remaining two hypotheses (hypotheses i. and iii.), a probabilistic approach for estimating risks was employed.

3. Analysis

3.1 Effects characterisation

The majority of available aquatic toxicity data for tebuthiuron has been derived for temperate, northern hemisphere species (Table 2). 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, a major objective 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 northern hemisphere toxicity data are comparable to data for the Australian tropics.

3.1.1 Tebuthiuron toxicity to Australian tropical species

Five freshwater species, covering a range of trophic groups, from primary producers to vertebrate predators, were selected for ecotoxicological assessment (Table 3). All are native to the region and are known to inhabit at least one of the habitats potentially at risk (see Table 3). Details of the toxicity test protocols are provided elsewhere (Hyne et al 1996; Markich & Camilleri 1997; Franklin et al 1998), although brief descriptions are provided below. All tests were conducted at a constant temperature of $27 \pm 1^\circ\text{C}$ with a 12 h light:12 h dark photoperiod, with the exception of the *Lemna aequinoctialis* growth test which was conducted at $30 \pm 1^\circ\text{C}$. Physico-chemical properties (pH, conductivity and dissolved oxygen) of the test waters were measured either daily or at the commencement and end of the experiments.

Table 3 Summary of test species, endpoints and the wetland habitats represented by each species

Test organism	Duration (acute/chronic)	Test endpoint	Wetland habitats represented
<i>Chlorella</i> sp. (green alga)	72 h (chronic)	Cell division rate	upland and lowland streams, billabongs, floodplains
<i>Lemna aequinoctialis</i> (duckweed)	96 h (chronic)	Growth (frond number)	billabongs, floodplains
<i>Moinodaphnia macleayi</i> (water flea)	3 brood (chronic)	Reproduction	billabongs and other lentic waters
<i>Hydra viridissima</i> (green hydra)	96 h (chronic)	Population growth	billabongs, floodplains
<i>Mogurnda mogurnda</i> (purple-spotted gudgeon)	96 h (acute)	Survival	upland streams, lowland streams, billabongs, floodplains

Green alga (Chlorella sp.) 72 h cell division rate

Due to difficulties with the laboratory cultures of *Chlorella* sp., assessment of the toxicity of uranium to the alga was undertaken by Stauber & Binet (1999). Exponentially growing *Chlorella* cells ($2-4 \times 10^4$ cells mL⁻¹) were exposed to tebuthiuron concentrations ranging from 0.0001 to 1 mg L⁻¹ for 72 h. Algal cell density was measured every 24 h using an electronic particle counter (Coulter® Multisizer II). *Chlorella* sp. cells were exposed to tebuthiuron in 250 mL borosilicate glass Erlenmeyer flasks containing 50 mL of test solution. The test was considered valid if the cell division rate in controls exceeded 1.4 ± 0.4 divisions per day, with the coefficient of variation in controls < 20%. A total of three experiments was carried out.

Duckweed (L. aequinoctialis) 96 h plant growth

Vegetatively reproducing *L. aequinoctialis* (Lemna) plants (each with three fronds), were exposed to tebuthiuron concentrations ranging from 0 (control) to 5 mg L⁻¹ for 96 h. Lemna were exposed to 100 mL of each test solution in sterilised 250 mL Erlenmeyer flasks, with each flask initially containing four plants (~ 12 fronds). Three replicates were used for the first test, and two replicates for subsequent tests, resulting in 42 or 28 flasks for a given test run. Test solutions were not renewed during the test. Daily observations for bacterial or fungal growth were made on the sealed, sterilised flasks. At the completion of the test (96 h) the number of fronds in each test container was counted, and the percent inhibition of plant growth compared with controls calculated. The test was considered valid if frond numbers in the control flasks were at least five times greater after 96 h (ie 60 fronds per control flask).

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 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 2×10^5 cells mL⁻¹), as well as 1 µL of fermented food and vitamins (FFV) 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. A total of five experiments was carried out.

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. 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. The test was considered valid if control population growth rate (K) exceeded 0.3 day⁻¹ (i.e. ≥ 34 individual hydra after 96 h). A total of seven experiments was carried out.

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. 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. A total of six experiments was carried out.

Chemistry

Tebuthiuron was analysed by high performance liquid chromatography. Samples were injected without pre-treatment onto a Vydac 201TP C₁₈ 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 treatments from each experiment, as well as a selection of other test concentrations which characterised the concentration-response curves of the test species. As measured concentrations were typically within 6–7% of their nominal values, nominal values were used in the analyses and are reported here.

Statistical analysis

EC/LC₅₀ values for all species were calculated using a logistic regression model (Guardabasso et al 1987, Seefeldt et al 1995). This model consistently provided the best fit for the sigmoidal relationship between tebuthiuron concentration and the selected responses of each organism.

Data from individual experiments were also analysed using one-way analysis of variance and Dunnett's multiple comparison test ($P \leq 0.05$) to determine LOEC and NOEC values for each of the five test species. Prior to this, all data were found to meet the assumptions of normality

(Shapiro-Wilk's test) and homogeneity of variance (Bartlett's test). For each species, the lowest NOEC from the experiments was used for risk characterisation.

3.1.2 Toxicity results

Table 4 summarises the results of the toxicity tests. Freshwater plant species were about 2 to 3 orders of magnitude more sensitive to tebuthiuron than the animal species. *L. aequinoctialis* was the most sensitive species tested, while *M. mogurnda* was the least sensitive, although the latter estimate was based on an acute response.

Table 4 Summary of tebuthiuron toxicity to five tropical freshwater species

Test organism	EC ₅₀ (mg L ⁻¹)	NOEC (mg L ⁻¹)	LOEC (mg L ⁻¹)
<i>Chlorella</i> sp.	0.25	.092	0.19
<i>Lemna aequinoctialis</i>	0.14	0.05	0.1
<i>Moinodaphnia macleayi</i>	134	20	40
<i>Hydra viridissima</i>	150	50	75
<i>Mogurnda mogurnda</i>	214*	200	225

* LC₅₀

3.1.3 Comparative toxicity of tebuthiuron to Australian and northern hemisphere species

In general, there were no major differences in the acute and chronic toxicity of tebuthiuron between northern hemisphere and Australian tropical aquatic species. The acute LC₅₀ values of tebuthiuron for northern hemisphere temperate freshwater fish (112 – >160 mg L⁻¹; table 2) tended to be slightly lower than the Australian tropical freshwater fish, *M. mogurnda* (Table 3), although the maximum difference was less than two-fold. Similarly, chronic toxicity values for algae varied a little between the data sets, but were less than an order of magnitude different. A number of factors, including inter-species variation and the type of test water used (ie natural versus synthetic/reconstituted water), may have played a role in the slight differences in toxicity. Indeed, differences in water chemistry, including the concentration of dissolved organic matter, are known to influence the bioavailability of tebuthiuron (Caux et al 1997). A comparison could not be made for hydra, as no comparable temperate data were available.

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. Given this, it was considered appropriate to incorporate the existing, northern hemisphere toxicity data for the risk characterisation component of the risk assessment.

3.2 Exposure characterisation

The environmental fate of tebuthiuron on the 'cracking clay' floodplains in the Top End of northern Australia is reasonably well understood (Parry & Duff 1990; Batterham 1992; Cook 1992). Tebuthiuron is relatively persistent and is easily mobilised from soils into run-off and flood water by the high intensity rainfall that characterises the wet season. To characterise exposure, use was made of existing data from a chemical monitoring program previously

undertaken during large scale application of tebuthiuron to the Mimosa infestation on the Oenpelli floodplain, western Arnhem Land (Parry & Duff 1990; Cook 1992). These data provided a reasonable indication of the potential concentrations of tebuthiuron to which aquatic organisms could be exposed. Furthermore, they provided some, although not extensive, information on the likely duration of exposure of an aquatic organism to the herbicide.

In 1989, an area of approximately 1,000 ha of Mimosa was treated at a rate of 1.5 kg tebuthiuron (7.5 kg Graslan) per ha, representing a total of approximately 1,500 kg tebuthiuron (Parry & Duff 1990). Following this treatment, the concentrations of tebuthiuron were measured in 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 were negligible 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 concentrations of tebuthiuron in surface water (0.55 mg L^{-1}) and suspended sediment (4.39 mg kg^{-1}) were measured after 10 d in a small waterhole approximately 500 m outside the area treated with Graslan (Parry & Duff 1990). Tebuthiuron was also recorded in suspended sediment (0.072 mg L^{-1}) about 1 km downstream from the treatment area 22 weeks following application (Parry & Duff 1990). The highest recorded concentration of tebuthiuron in soil (2.91 mg kg^{-1}) was measured after 10 d within the treated area (Parry & Duff 1990).

In 1991, an area of approximately 5,800 ha of Mimosa was treated at a rate of approximately 2 kg tebuthiuron per 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. 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 kg^{-1} (Cook 1992). It was presumed that this represented residual tebuthiuron remaining from the 1989 application (Cook 1992), highlighting the persistence of the herbicide.

Overall, tebuthiuron was shown 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 substantial amounts being washed downstream with runoff water (Batterham 1992), either dissolved or bound to suspended particulate matter (Parry & Duff 1990). For the purposes of this assessment, it was considered that only the dissolved tebuthiuron in the water column would potentially be available for uptake by aquatic biota. However, it should be noted that some organisms, particularly filter feeders such as the cladoceran, *M. macleayi*, may be susceptible to particulate-bound tebuthiuron. The distribution of dissolved tebuthiuron concentrations measured on the Oenpelli floodplain between 1989 and 1992 is shown in Figure 2. Soil-bound tebuthiuron was not directly assessed.

Table 5 Highest recorded tebuthiuron concentrations 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 (days)											
		3	10	70	98	123	154	on-site	off-site	on-site	off-site	on-site	off-site
1989 ^b	Surface water (mg L ⁻¹)	-	- ^c	0.550	0.039	0.168	0.030	-	-	0.034	0.002	-	-
	Suspended sediment/ Microalgae (mg kg ⁻¹)	-	- ^c	4.39	BADL ^d	BADL ^d	BADL ^d	-	-	BADL ^d	0.072	-	-
	Soil (0-100 mm; mg kg ⁻¹)	-	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 and 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).

3.3 Risk characterisation

3.3.1 Risk estimation

The approach taken for this assessment involved comparison of a cumulative probability distribution of environmental concentrations with cumulative probability distributions of species sensitivity based on the toxicity assessment results, an approach recommended by SETAC (1994) and US EPA (1998), and described in detail by Solomon et al (1996) and US EPA (1998). The major assumptions of the approach are that the distributions of measured sensitivity (acute and chronic) are representative of all species in the ecosystems of interest, and that they are log-normal with respect to concentration (Solomon et al 1996). The degree of overlap between distributions of species sensitivity and environmental concentrations is used to estimate the risks to aquatic biota. Log-normal probability plots were constructed using Minitab 12.1[®], which uses the following formula to plot the data positions:

$$\text{Cumulative frequency} = \frac{\text{rank} - \frac{3}{8}}{n + \frac{1}{4}}$$

where *rank* is the rank of the datum in the set, and *n* is the total number of data points in the set. The reliance on the log-normal distribution has been recently criticised (Newman et al 2000), although there exists substantial support for its continued use (Burmester & Hull 1997; Solomon et al 2001; US EPA 1998).

Incorporation of the toxicity data for northern hemisphere species provided sufficient data points to construct distributions of species sensitivity based on either NOEC or LC₅₀/EC₅₀ data. Risks were estimated for three different freshwater scenarios: *plant chronic toxicity*; *animal chronic toxicity*; and *vertebrate acute toxicity* (Table 6). Where multiple values existed for the same endpoint for a single species, the geometric mean of the values was used for the risk estimation process (ANZECC & ARMCANZ 2001). Where multiple values existed for various endpoints for a single species, the lowest value was used (ANZECC & ARMCANZ 2001).

The probability of the environmental concentration of tebuthiuron exceeding the 10th, 5th, and 1st percentile of the species sensitivity distributions was calculated for each of the three scenarios (ie the probability that 10, 5 and 1% of species may be affected). The selection of a level of protection can vary depending on the characteristics of the stressor and the ecosystem at risk (Solomon et al 1996). For example, the Australian and New Zealand Water Quality Guidelines (ANZECC & ARMCANZ 2001) recommend deriving site-specific trigger (or guideline) values (based on chronic NOEC data) that protect at least 95% of species, but suggest a higher level of protection (eg 99%) for ecosystems of high conservation/ecological value. Alternatively, Solomon et al (1996) estimated the probability of atrazine in North American surface waters exceeding the 10th percentile of the species sensitivity distribution. Although the three percentiles are presented here for comparative purposes, the 5th percentile (ie protection of 95% of the species) was chosen as the level of protection for the assessment endpoints in this risk assessment, which is consistent with the recommendations of ANZECC & ARMCANZ (2001).

Freshwater plant chronic effects

As expected, risks of tebuthiuron to aquatic plants were far greater than to animal species. Based on the tebuthiuron levels measured in water on the Oenpelli floodplain following application in 1989 and 1991, the risks of chronic effects to native aquatic plant species can be considered high (Table 7; Figure 3). The probability of at least 5% of species experiencing

Table 6 Selected freshwater toxicity data used for risk characterisation

Species	Freshwater toxicity data (mg L ⁻¹)		
	Plant chronic toxicity		Animal chronic toxicity (NOECs)
	(NOECs)	(72-96 h EC ₅₀ s)	
<i>Oncorhynchus mykiss</i>			26
<i>Pimephales promelas</i>			9.3
<i>Lepomis machrochirus</i>			
<i>Carassius auratus</i>			
<i>Mogurnda mogurnda</i>			
<i>Rana catesbeiana</i>			
<i>Daphnia magna</i>			21.8
<i>Moinodaphnia macleayi</i>			20
<i>Hydra viridissima</i>			50
<i>Selanastrum capricornutum</i>	0.01	0.09	
<i>Chlorella</i> sp.	0.092	0.25	
<i>Anabaena flo-aquae</i>	0.31		
<i>Navicula pelliculosa</i>	0.056	0.213	
<i>Lemna gibba</i>	0.091	0.235	
<i>Lemna aequinoctialis</i>	0.05	0.14	

* geometric mean of three LC₅₀ values (115, 144, 126 mg L⁻¹; see Table 2)

** LC₅₀ value actually reported as >160 mg L⁻¹ (see Table 2)

some chronic effects due to tebuthiuron was 73%. Furthermore, the probability of at least 5% of species experiencing chronic effects equivalent to 50% reductions in population size (ie based on the species EC₅₀ distribution) was 27% (Table 7; Figure 3a).

To highlight the persistence of tebuthiuron in surface water, the comparison of effects and exposure distributions was repeated for aquatic plants using only tebuthiuron concentrations measured three months or more following application (Figure 3b). As can be seen, the risks of tebuthiuron to freshwater plant species remain high for some time following application, with the probability of at least 5% of species experiencing chronic effects still approximately 63%, while the probability of at least 5% of species experiencing chronic effects equivalent to 50% reductions in population size was still 12%.

Freshwater animal chronic effects

Data used to calculate risks of chronic effects to freshwater animal species included invertebrate, amphibian and fish chronic direct toxicity data. The risk of chronic direct effects to freshwater animal species can be considered low, with the concentrations estimated to affect even 1% of species being over 6 mg L⁻¹ (Table 7, Figure 4), well above the maximum recorded concentration on the Oenpelli floodplain of 2.05 mg L⁻¹. The concentration at which chronic, indirect effects were observed for chironomids in a mesocosm experiment (0.2 mg L⁻¹; Temple et al 1991) is displayed on the x axis of Figure 4. As the broken arrow indicates, the environmental concentrations of tebuthiuron exceed this concentration approximately 15% of the time, suggesting a likelihood for indirect effects to aquatic invertebrates.

Table 7: Risks of tebuthiuron to freshwater species in northern Australian wetlands

Scenario	Probability of x% of species being affected		
	10%	5%	1%
Plant chronic effects			
<i>NOEC data</i>	65% (0.018; 0.006-0.05)*	73% (0.012; 0.003-0.04)	85% (0.006; 0.001-0.03)
<i>EC₅₀ data</i>	24% (0.106; 0.067-0.167)	27% (0.092; 0.055-0.155)	32% (0.071; 0.037-0.136)
Animal chronic effects	<1% (11.1; 5.9-20.6)	<1% (9.1; 4.4-18.8)	<1% (6.3; 2.5-15.7)
Vertebrate acute effects	<1% (111; 78-159)	<1% (98; 65-148)	<1% (79; 47-131)

* Values in parentheses represent the corresponding tebuthiuron concentration (mg L⁻¹) and its associated 95% confidence limits.

Freshwater vertebrate acute effects

The risk of acute effects to freshwater vertebrate species is extremely low and of little concern (Table 7; Figure 4). From the available data, acute effects to fish are unlikely to occur below 100 mg L⁻¹ tebuthiuron, levels that would not occur in the aquatic environment as a result of Mimosa treatment.

3.3.2 Consideration of uncertainty

A range of factors associated with the effects and exposure characterisation contributed to the overall uncertainty in the risk assessment.

Effects

The use of single species laboratory toxicity tests to predict population-level impacts (at least) has been a long-running debate. The majority of toxicity data used for this risk assessment were derived from single-species laboratory tests of only a limited number of species. However, toxicity data were obtained for five local aquatic species, making the assessment relevant to the region of concern. In addition, data were included in the assessment only if they were derived from endpoints considered to possess some ecological relevance (eg growth, reproduction, mortality). Nevertheless, the subsequent prediction of effects on natural populations still carries some uncertainty, while there are also many other local species that have not been or cannot easily be tested. Related to this, the small sample sizes used to characterise the species sensitivity distributions added substantial uncertainty to the assessment, although they did exceed the minimum requirements for deriving site-specific water quality guideline values in Australia (ANZECC & ARMCANZ 2001). Newman et al (2000) estimated the optimal sample size for calculation of the 5th centile to range from 15 to 55, but acknowledged that it could be lower if estimates were made for specific, sensitive taxonomic groups, as conducted in the present study.

This study did not assess the indirect effects of tebuthiuron on freshwater ecosystems. However, such effects could include resource depletion in terms of food for aquatic herbivores (eg microcrustaceans, larval and some adult fish, tadpoles) or shelter and habitat due to the toxic effects of tebuthiuron to primary producers (eg phytoplankton, macrophytes, epiphytes). These types of ‘cascade’ effects could result in freshwater animals being more susceptible to the effects of tebuthiuron than this risk assessment was able to conclude from direct toxicity data alone. Highlighting this, Temple et al (1991) reported a 30% reduction in chironomid density in mesocosms exposed to 0.2 mg L⁻¹ tebuthiuron, possibly due to a shift

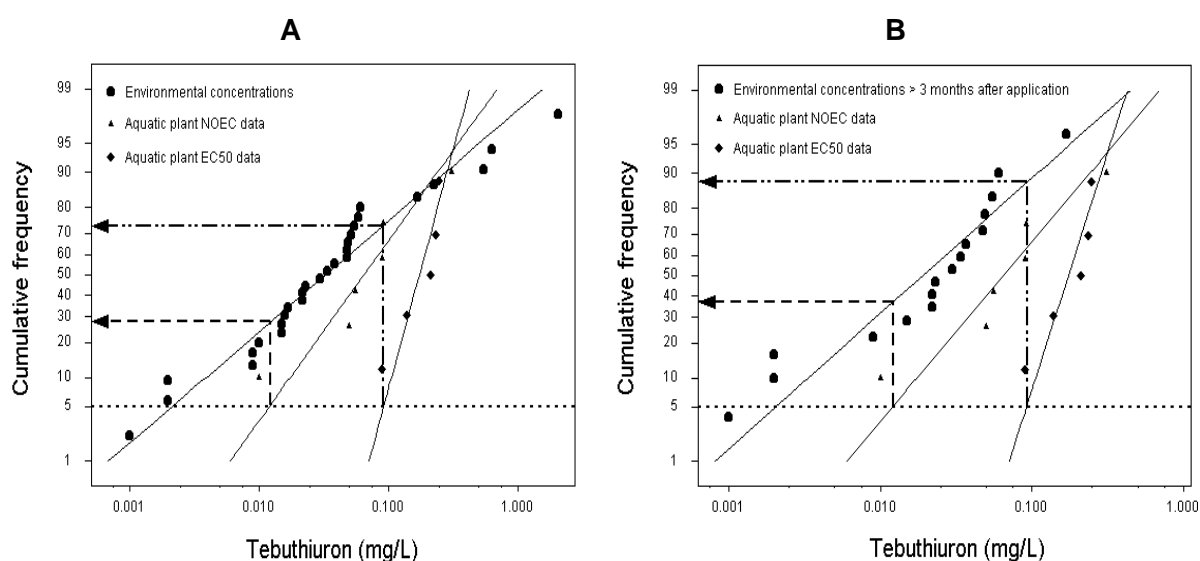


Figure 3 Comparison between chronic plant sensitivity distributions for tebuthiuron based on NOEC data and EC₅₀ data, and **(A)** the distribution of all environmental tebuthiuron concentrations, and **(B)** the distribution of environmental tebuthiuron concentrations measured three months or more following application. Broken line arrows indicate the point of overlap at the 5th percentile of the species sensitivity distributions with the distribution of environmental tebuthiuron concentrations.

in the algal assemblage to more unpalatable species, or decreased algal productivity. As was demonstrated by the risk characterisation in this study, 14% of the measured tebuthiuron concentrations on the Oenpelli floodplain exceeded the value of 0.2 mg L⁻¹ (Figure 4), suggesting that the potential for indirect effects does exist and should be investigated further.

Another factor contributing to uncertainty is the unknown ability of aquatic biota to recover following exposure to tebuthiuron. The residual nature of tebuthiuron in soil and its mobility in water suggests that individuals of susceptible freshwater biota in the vicinity of treated Mimosa infestations will possibly die. Thus, recovery of individuals may not occur, and local population crashes may result. However, the annual drying and flooding cycle of the wet-dry tropical climate facilitates the active and passive dispersal of species throughout the wetlands. Thus, once tebuthiuron levels fall below those known to cause adverse effects, recolonisation and recovery of local populations may occur over several years. Local factors will determine the nature and extent of recovery and the subsequent species structure, and it cannot be assumed that a system will return to its previous state.

The influence of confounding stressors on biota exposed to tebuthiuron is also unknown. The wetlands of the Australian wet-dry tropics experience highly variable conditions annually and inter-annually (Butterworth 1995), with their species compositions changing in response to these conditions. It is difficult to predict the extent of impact tebuthiuron may have on such a naturally variable system. In addition, local physico-chemical factors (eg suspended sediment, nutrient levels) can also influence local populations and possibly confound the effects of tebuthiuron. While there is no evidence to support this for tebuthiuron, Solomon et al (1996)

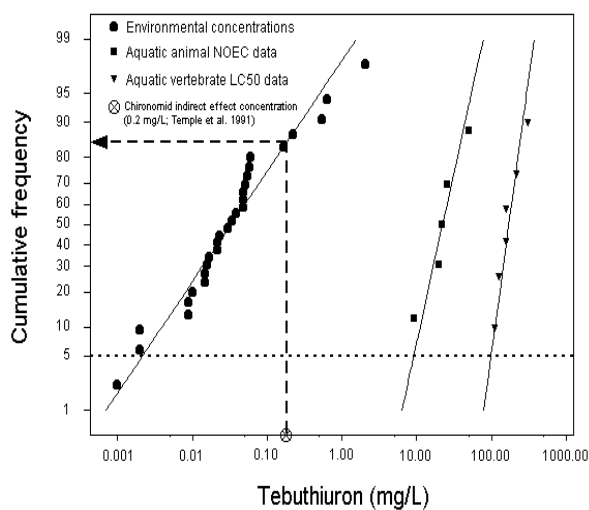


Figure 4 Comparison between the distribution of environmental tebuthiuron concentrations and the chronic animal sensitivity and acute vertebrate sensitivity distributions for tebuthiuron based on NOEC and LC₅₀ data, respectively. The broken line arrow indicates the point of overlap of a reported indirect effect on chironomids (Temple et al 1991) with the distribution of environmental tebuthiuron concentrations.

reported such confounding effects for the herbicide atrazine. Interactions between tebuthiuron and other chemicals are probably unlikely as there are usually few other chemical inputs to the wetland areas infested by *Mimosa*.

Exposure

Tebuthiuron exposure was estimated using data from only two previous, albeit the only, monitoring programs, both of which occurred at the same site (Oenpelli floodplain). *Mimosa* currently occurs on at least 15 different catchments in the Top End of northern Australia, constituting a range of soil types and water regimes that may affect the environmental fate and toxicity of tebuthiuron. Thus, as the persistence of tebuthiuron is known to vary with soil type, temperature and soil moisture (Batterham 1992, Chang & Stritzke 1977, Lane et al 1995, Miller 1988), the results from the Oenpelli floodplain monitoring programs may not necessarily reflect environmental fate in other catchments. For example, the effectiveness of tebuthiuron to *Mimosa* depends greatly on the soil type, with less efficacy reported in soils with high clay content (Lane et al 1995, Miller 1988). It is also recognised that the monitoring programs were undertaken following relatively large tebuthiuron applications, particularly in 1991, when 12,000 kg was applied (Cook 1993). Given that the 1991 tebuthiuron application was considered the world's largest to a wetland environment (Schultz & Barrow 1995), the monitoring data may provide an over-estimate of tebuthiuron concentrations or extent of contamination in other treated areas. In addition, timing of tebuthiuron application can also be a critical determinant of its fate, with up to 50% loss in surface water run-off being reported when applied to wet soils (Morton et al 1989).

Another uncertainty surrounding the exposure characterisation was the assumption that dissolved tebuthiuron represented the only bioavailable fraction. Tebuthiuron bound to soil and suspended material (including phytoplankton) may be available to mobilise into the water column and contribute to the overall bioavailable fraction, or may itself be bioavailable, particularly to benthic macroinvertebrates and filter feeding organisms such as cladocerans.

Previous studies have demonstrated that inorganic toxicants bound to algae and suspended particulate matter can in fact be bioavailable to filter feeding organisms (Taylor et al 1998, Weltens et al 2000). This is of particular importance because high tebuthiuron concentrations were recorded in suspended sediment and soil at various times following application (Parry & Duff 1990, Cook 1993). Therefore, further research on the bioavailability of tebuthiuron and its movement between the various environmental compartments would reduce this area of uncertainty.

Risk characterisation

The probabilistic estimates of risk calculated in this assessment incorporate many of the uncertainties associated with the exposure and effects characterisation described above. Those that are not incorporated relate to the ecological uncertainty surrounding indirect effects, recovery, ecological redundancy and confounding factors.

The 95% confidence limits (CLs) surrounding the predicted tebuthiuron concentrations for the 10th, 5th and 1st percentiles of the species sensitivity distributions provide an indication of the variability associated with the risk estimates. Although variability was evident, in all cases the upper and lower CLs were well within an order of magnitude of the predicted concentrations (Table 7). The uncertainty of the 5th percentile risk estimates for freshwater plant chronic toxicity (for both the NOEC and EC₅₀ data) were assessed by comparing the lower 95% CL of the relevant species sensitivity distribution with the upper 95% CL of the exposure distribution (ie a worst case scenario), and the upper 95% CL of the relevant species sensitivity distribution with the lower 95% CL of the exposure distribution (ie a best case scenario). The comparisons are presented in figure 5 (A and B), and indicate large uncertainty around the risk estimates.

3.3.3 Ecological significance

Acknowledging the uncertainties described in the preceding section, a number of conclusions can be made about the ecological implications of tebuthiuron in northern Australian wetland environments.

The predicted high (73%) probability of at least 5% of aquatic plant species being affected suggests a strong likelihood for changes to the structure, and possibly function, of the native freshwater plant communities following tebuthiuron application. It is probable that some phytoplankton and floating aquatic macrophyte species will be directly adversely affected both on- and off-site. By corollary, epiphytes may also be at risk. As the assessment did not investigate soil-bound tebuthiuron, little can be concluded about rooted aquatic macrophytes. However, experiments conducted by Lane et al (1997) found that while tebuthiuron was more toxic to Mimosa seedlings than to native seedlings, mortality was still reported for native herbaceous (12% mortality) and grass (5% mortality) seedlings at high concentrations (ie ~1 mg cm⁻³ soil). The results of the risk assessment provide supporting evidence for existing recommendations that tebuthiuron should not be used in the vicinity of surface water or established waterways (Anon 1990, US EPA 1994).

The degree of on-site impact will depend on the amount of water present at the time of application and, given the persistence of tebuthiuron, upon any subsequent inundation,. It is likely that any freshwater habitats existing under the closed Mimosa canopy will be biologically depauperate due to the shortage of light at ground level. Thus, there would be little native plant life to be affected. Larger, exposed water bodies within Mimosa infestations would certainly harbour susceptible species, and thus, effects would be expected. The degree of off-site impact will depend on a range of local conditions including weather (ie wind), and the amount and flow rate of water flowing downstream through the treated area.

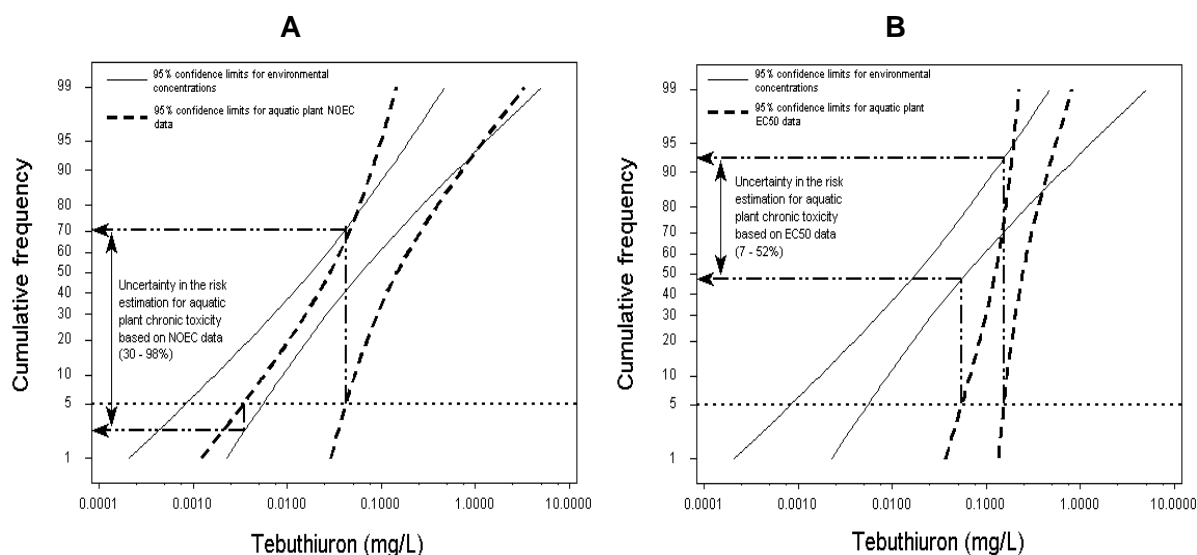


Figure 5 Estimate of uncertainty in the risk characterisation for plant chronic toxicity by comparison of the upper and lower 95% confidence limits (CLs) of the species sensitivity distribution based on **(A)** NOEC data and **(B)** EC₅₀ data, and the lower and upper 95% CLs of the distribution of environmental tebuthiuron concentrations.

The persistence of tebuthiuron suggests prolonged stress to aquatic plant life. This is supported by the estimate of risks to plant species three months or more following tebuthiuron application (Figure 5). However, the duration of effects will also depend on (i) the flow rate of water across the floodplains and other affected habitats, and (ii) the amount of residual tebuthiuron in soil available for mobilisation into water upon future flooding. Given that high tebuthiuron levels have been reported in soil approximately two years following its application (Cook 1992), reoccurrence of impacts could occur in following wet seasons.

Freshwater invertebrates and vertebrates appear not to be at direct risk to tebuthiuron, although evidence does exist elsewhere for indirect effects to invertebrates at environmental concentrations (Temple et al 1991), and these should not be discounted.

The ecological significance of the predicted risks of tebuthiuron must be considered within the broader issue of *Mimosa* invasion and infestation. Put in such a context, the risks of tebuthiuron may be considered of less concern than the known impacts of *Mimosa*. However, there are a number of other herbicides used to control *Mimosa*, whose risks are currently unknown, and could potentially be greater than those of tebuthiuron. This is an area that requires further investigation.

4. Management implications

4.1 Risk evaluation and research needs

The quality and quantity of monitoring and effects data available for tebuthiuron were limited in some aspects as described above. Although the effects assessment incorporated information on tebuthiuron toxicity to five local aquatic species, the data, along with those from northern hemisphere studies were largely derived from single species laboratory toxicity tests. Thus,

multispecies toxicity data (ie mesocosm or field) on the effects of tebuthiuron, particularly to native primary producers, but also aquatic invertebrates are desirable. Field data on tebuthiuron concentrations were available, but were somewhat limited in their spatial and temporal representativeness. Tebuthiuron monitoring coupled with field effects assessment would be ideal, while monitoring following tebuthiuron application on other catchments would provide further information on the herbicide's environmental fate. In addition, research is required to determine the bioavailable fractions of tebuthiuron. While the collection of further data would increase confidence in the estimates of risk, the results from this assessment are considered sufficient to make some recommendations for initial risk management actions as outlined below, pending further data acquisition.

4.2 Risk management and reduction

Ultimately, the need to reduce the ecological risks of tebuthiuron will be determined by the wider community. Stakeholders (eg pastoralists, tourist organisations, recreational fishermen, national park managers, traditional landowners) may be willing to accept some detriment to wetland biota as a result of tebuthiuron application if the outcome is containment and/or eradication of *Mimosa* from the area. While this is probably the most ecologically and economically sensible position to adopt, it should be noted that effective and ongoing management plans must initially be in place for *Mimosa* control in order for the benefits of its eradication to be realised and out-weigh the potential ecological costs of herbicide application. This point has often been emphasised in relation to *Mimosa* management in northern Australia (NLC & ERISS 1997, Rea & Storrs 1999, Storrs et al 1999, Walden et al in press).

The efficacy of tebuthiuron must also be considered when determining management options. Lane et al (1997) found that at twice the recommended application rate (15 kg ha⁻¹ Graslan), and under laboratory conditions ideal for the spread of tebuthiuron into the soil, almost 50% of exposed *Mimosa* seedlings survived. While tebuthiuron was more toxic to *Mimosa* seedlings than to native seedlings, some mortality was still reported for native herbaceous (12% mortality) and grass (5% mortality) seedlings (Lane et al 1997). While it was concluded that tebuthiuron can not be considered an effective herbicide against *Mimosa* seedlings (Lane et al 1997), it remains as a commonly used herbicide.

There is a need to assess the ecological risks of the other herbicides used to control *Mimosa*, particularly fluroxypyr and metsulfuron methyl, both of which are often applied aerially. By considering both the efficacy and risks of the major herbicides, it is possible that their usage can be managed to reduce the overall risks to the wetland habitats whilst retaining maximum efficacy for *Mimosa* control. In terms of reducing overall herbicide usage, the Northern Territory Department of Primary Industry and Fisheries (DPIF) and several land holders have made progress in developing integrated control programs that combine the use of chemical, biological and physical control measures (Rea & Storrs 1999, Walden et al in press). However, to date the relative risks of the major herbicides remain unknown. Following the identification of incidences of over-spraying and other application inefficiencies (Lonsdale 1992; Cook 1993; 1996), reductions in the risks of tebuthiuron have been made. This has been through major improvements to aerial application techniques, to minimise off-site contamination of natural wetland habitats, and a better understanding of the importance of timing of application (Cook 1996).

5. Conclusions

Tebuthiuron appears to represent a significant and prolonged risk to native freshwater phytoplankton and floating macrophyte communities following application to *Mimosa* infestations on northern Australian wetlands. The risks to native freshwater invertebrates and vertebrates appear low. Although of concern, the overall ecological risks of tebuthiuron are probably out-weighed by the known ecological and economic impacts caused by its target weed, *M. pigra*. Nevertheless, there is still a need to understand the relative risks of control measures, particularly the various herbicides used (eg fluroxypyr and metsulfuron methyl). Thus, site-specific ecological risk assessments for these herbicides would provide a sound basis for developing the most efficacious and 'environmentally friendly' chemical control regimes for *Mimosa*.

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