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THE RESPONSE OF BIOTA IN EXPERIMENTAL STREAM CHANNELS TO A 24-HOUR EXPOSURE TO THE HERBICIDE, VELPAR L

by

Judith Schneider

Thesis submitted to the
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University of Ottawa
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en vue de l'obtention de la maîtrise ès sciences à

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LIST OF ABBREVIATIONS

EC_{50} - median effect concentration, the concentration of a chemical in the air or water that causes a 50 percent reduction in the measured variable, for example productivity or respiration.

EEC - expected environmental concentration, the concentration expected in a body of water (0.5 m depth) after a direct overspray of Velpar L at the full application rate.

HPLC - high performance liquid chromatography

LC_{50} - median lethal concentration, the concentration of a chemical in the air or water that causes death in 50 percent of the organisms exposed.

LD_{50} - median lethal dose, the dosage (mg kg^{-1} body weight) causing death in 50 percent of the exposed organisms.

NOEL - no effect level, a concentration or dosage at which no effect on the exposed organisms is detected.

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ABSTRACT

Velpar L (manufactured by Dupont Canada, Inc.) is one of a few forestry herbicides registered for aerial spraying in Canada. However, little is known about the impact of the active ingredient, hexazinone, on stream biota. Ten experimental stream channels, fed from a Laurentian stream were used to examine the short-term impact of a pulse of Velpar L on stream periphyton and invertebrates. Velpar L was added to five alternating channels for 24 hours with the other five channels serving as controls. The mean concentration of hexazinone in the treatment channels varied over time from 145 $\mu g\ L^{-1}$ to 432 $\mu g\ L^{-1}$.

The periphyton community was dominated by pennate diatoms (Bacillariophyceae). Periphyton chlorophyll *a*-specific productivity was significantly reduced during the Velpar L treatment. Within 24 hours after the termination of the Velpar L treatment, productivity returned to control levels. Mean periphyton biomass, measured as the quantity of chlorophyll *a* per unit area, was not affected by the Velpar L treatment. In addition, the concentration of hexazinone that reduced periphyton chlorphyll *a*-specific productivity by 50% after a four hour exposure was determined. This value was only 3.6 $\mu g\ L^{-1}$ of hexazinone. Thus, low concentrations of Velpar L affect stream periphyton but the impact appears to be temporary.

Velpar L did not cause an increase in the drift of insects or other macroinvertebrates. Mean density of benthic macroinvertebrates increased

slightly with time in the Velpar L-treated channels and nearly doubled in the control channels. The mean length of individual macroinvertebrates decreased slightly over time in the control channels but not in the treated channels. However, the changes in mean density, biomass and length were not statistically significant. The variance of the invertebrate lengths increased over time in both the control and Velpar L-treated channels, which suggests that the size distribution of the invertebrate community varied with time.

Although it appears that lotic systems are stable to short-term exposure to Velpar L, the effects of repeated and chronic exposures on stream biota have not yet been studied. Velpar L may have a greater effect on lakes and other receiving waters which may accumulate doses of Velpar L and which may retain it for longer periods of time.

RÉSUMÉ

Velpar L (produit par Dupont Canada, Inc.) est l'un des rares herbicides dont l'utilisation pour épandage aérien est permise au Canada. Cependant, peu d'information existe quant à l'impact de l'ingrédient actif, la triazine hexazinone, sur les communautés de ruisseau. Dix canaux expérimentaux, alimentés par l'eau d'un ruisseau des Laurentides, ont été utilisés pour étudier l'impact d'une courte exposition au Velpar L sur le périphyton et les invertébrés. Du Velpar L a été ajouté à 5 canaux pour une période de 24 heures. Les 5 autres canaux, intercalés avec ceux traités, ont servi de témoins. La concentration moyenne d'hexazinone dans les canaux traités a varié de 145 à 432 $\mu g\ L^{-1}$ durant la période de traitement.

La communauté périphytique était dominée par des Diatomées pennées (Bacillariophycées). La productivité par unité de chlorophylle *a* a été significativement réduite pendant l'exposition au Velpar L. Au cours des 24 heures suivant la fin de l'exposition au Velpar L, la productivité est remontée au niveau des témoins. La biomasse moyenne du périphyton, mesurée par la quantité de chlorophylle *a* par unité de surface, n'a pas été affectée par le Velpar L. La concentration d'hexazinone, provoquant une réduction de 50% de la productivité du périphyton par unité de chlorophylle *a* après une exposition de 4 heures, a été estimée à 3.6 $\mu g\ L^{-1}$. De faibles quantités de Velpar L affectent donc le périphyton, mais cet effet est de courte durée.

Le Velpar L n'a pas fait augmenter la dérive des insectes et autres

invertébrés. La densité moyenne des macroinvertébrés benthiques a augmenté légèrement durant la période suivant le traitement dans les canaux traités et a presque doublé dans les canaux témoins. La longueur individuelle moyenne a baissé dans les canaux témoins mais n'a pas changé dans les canaux traités. Ces variations de la biomasse, densité et longueur moyennne n'étaient cependant pas statistiquement significatives. La variance des longueurs des invertébrés a augmenté dans les canaux témoins et ceux traités. La structure en taille de la communauté a donc varié dans le temps et semble avoir été affectée par le Velpar L.

Quoiqu'il semble que les écosystèmes lotiques soient résilients à des expositions de courte durée à l'herbicide Velpar L, les effets de doses répétées et d'exposition chroniques restent à déterminer. Velpar L pourrait avoir un impact plus marqué sur les lacs et réservoirs accumulant les doses passant par les ruisseaux et pouvant retenir l'hexazinone pour de plus longues périodes.

GENERAL INTRODUCTION

Only a few herbicides are currently registered for forestry use in Canada. The most commonly used are 2,4-D, glyphosate, hexazinone and simazine (Campbell, 1990). Foresters are turning to these herbicides to prepare land for reforestation with conifers because these herbicides increase lumber yields by reducing competing vegetation. As the use of herbicides by foresters increases, there is a need to understand how stream ecosystems respond to these herbicides. Both direct impacts of the herbicides on macrophytes and algae and indirect impacts on primary and secondary consumers are of concern.

Velpar L (a registered trade mark of E. I. DuPont de Nemours & Co., Inc.) is a forestry herbicide that is one of the four most heavily used forestry herbicides in the United States (Neary *et al.*, 1993). The active ingredient of Velpar L is the triazine compound hexazinone. The other components of Velpar L were not divulged by E. I. Dupont de Nemours & Co. Hexazinone is effective against a wide range of annual herbaceous broadleaf weeds, perennial grasses and woody vines such as raspberry, goldenrod and brome grass. Spot applications control unwanted hardwoods such as aspen, birch and maple. Velpar L was first registered for use in Canada in 1984. In 1988, when Velpar L was registered only for ground application, it made up 2% of the total forestry herbicide use (Campbell, 1990). Four years after registration for aerial spraying glyphosate made up 81% of forestry herbicide use.

Velpar L has the potential to become a major forestry herbicide after aerial spraying registration because aerial spraying is the most practical and economical method of applying herbicide to large tracts of forest. Registration for aerial spraying of Velpar L in Canada was obtained in 1991. Aerial spraying and increased usage of Velpar L will increase the quantities of Velpar L entering stream ecosystems.

The active ingredient of Velpar L is hexazinone (3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1*H*,3*H*)-dione) (Fig. GI.1). Hexazinone is a triazine compound that inhibits photosynthesis by blocking the Hill reaction (Corbett *et al.*, 1984). Lipid and RNA synthesis have also been shown to be significantly inhibited by hexazinone in soybean leaf cells (Hatzios and Howe, 1982), although this may result from a reduction in metabolic energy subsequent to the inhibition of photosynthesis.

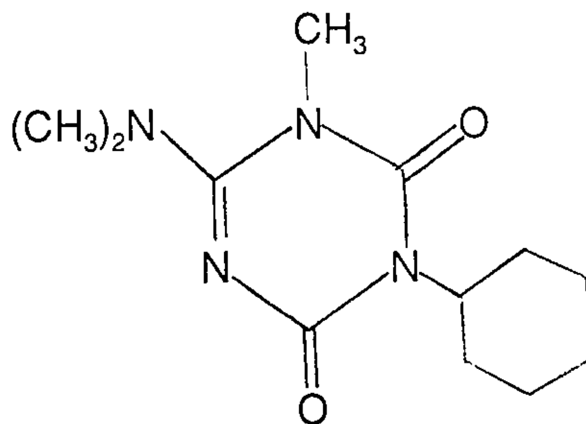

Figure GI.1. The molecular structure of hexazinone.

The physical and chemical characteristics of hexazinone (Table GI.1) make it susceptible to off-site movement. The solubility of hexazinone in water is 33 g L^{-1} at 25 °C (WSSA, 1983) which is high when compared to other common forestry herbicides such as glyphosate (12 g L^{-1}) (Neary *et al.*, 1993). The adsorption coefficient (K_d) of hexazinone is only 0.2, compared to 16.5 for glyphosate (Neary *et al.*, 1993). Rhodes (1980b) placed hexazinone in class 4 (very mobile) of the mobility classification scheme of Helling and Turner (1968). Finally, its octanol water partition coefficient (K_{ow}) is very low, ranging from 9.3-11.9 (Bouchard and Lavy, 1985), which suggests that hexazinone is unlikely to partition into organic matrices (Thompson *et al.*, 1992). The combination of high water solubility, low K_d, high mobility and low K_{ow} suggest that hexazinone has a very high potential for off-site movement to aquatic systems.

Hexazinone is broken down in the environment primarily by photodegradation but also by bacterial degradation (Neary *et al.*, 1993). The persistence of hexazinone in water and soil has been studied extensively. Estimates of its half-life in water range from a low of 3.8 days (Solomon *et al.*, 1988) to several weeks (Rhodes, 1980a) and up to 9 months (Thompson *et al.*, 1992). This extreme variability may be a result of different amounts of light as this would affect photodegradation. Thompson *et al.* (1992) reported low light levels and relatively short day lengths during their lake enclosure study. The half-life of hexazinone in soil is variable: 1 to 6 months (Rhodes, 1980b),

Table GI.1. The physical and chemical characteristics of hexazinone.

CHARACTERISTIC	VALUE	REFERENCE
Trade name	Velpar L	
Chemical family	triazine	
Chemical formula	$C_{12}H_{20}N_4O_2$	WSSA (1983)
Molecular weight	252.3	WSSA (1983)
Physical state	white, crystalline powder	WSSA (1983)
Specific gravity	1.25	WSSA (1983)
Melting point	115-117 °C	WSSA (1983)
Solubilty at 25 °C		
Water	33 g L^{-1}	WSSA (1983)
Acetone	792 g L^{-1}	WSSA (1983)
Methanol	2650 g L^{-1}	WSSA (1983)
pK_a	1.09-1.23	Bouchard and Lavy (1985)
Octanol water partition coefficient (K_{ow})	9.3-11.9	Bouchard and Lavy (1985)
Adsorption coefficient (K_d)	0.2	Neary *et al* (1993)
Mobility[a]	Class 4 (very mobile)	Rhodes (1980)
Maximum application rate (Canada)	4 kg ha^{-1}	Campbell (1990)
Maximum expected environmental concentration (EEC)[b]	800 µg L^{-1}	Thompson *et al* (1992)
Mode of action	inhibits photosynthesis	Corbett *et al* (1984)

[a] mobility classification scheme of Helling and Turner (1968).
[b] based on a direct overspray of a water body (0.5 m deep) at full application rate.

less than 3 months (Feng, 1987), 43 days (Roy *et al.*, 1989) and 1.5 to 18 months (Bouchard *et al.*, 1985). Neary *et al.* (1993) give the average as 30 days. The variation in results is attributed to soil type and climatic conditions.

Hexazinone has a low toxicity to mammals, birds and fish but is quite toxic to algae and aquatic macrophytes (Table GI.2). In laboratory experiments 0.5 mg L^{-1} of hexazinone was sufficient to kill the algae *Cladophora glomerata* and *Rhizoclonium hieroglyphium* and 1.0 mg L^{-1} was sufficient to kill the macrophytes *Myriophyllum verticillatum* and *Potamogeton pectinatus* (Fowler, 1977). Treatment of a small pond with 1 mg L^{-1} of hexazinone dramatically reduced the species diversity and density of periphyton on artificial substrates as well as causing a sharp decline in dissolved oxygen content of the pond (Anderson, 1981). An algal bioassay determined that 22.5 μg L^{-1} of hexazinone inhibited photosynthesis and reproduction by 50% in *Selenastrum capricornutum* (Williamson, 1988). Abou-Waly *et al.* (1991) reported 30 μg L^{-1} of hexazinone inhibited algal biomass growth and CO_2-fixation in *S. capricornutum* and *Anabaena flos-aquae*.

Although single species laboratory tests may allow us to estimate chemical toxicity, they are generally not adequate predictors of the effects of chemicals on natural communities (Kimball and Levin, 1985). This inadequancy of single species tests arises because they do not take into account either the interactions between different species or the interactions

Table GI.2. Reported toxicity data for hexazinone and Velpar L
(1 Kennedy, 1984; 2 Kennedy and Kaplan; 3 Kreutzweiser et al., 1992; 4 St. Laurent et al., 1992; 5 Williamson, 1988; 6 Thompson et al., 1992; 7 Rhodes et al., 1980; 8 Thompson et al., 1993a; 9 Thompson et al., 1993b)

CLASSIFICATION	TAXON	TEST	SUBSTANCE	TEST PROCEDURE	RESULTS	REF.
MAMMALS	beagle dog	acute oral	hexazinone	single forced feeding	LD50 >3400 mg/kg	1
	dog	chronic oral	hexazinone	90-day feeding	NOEL 1000 ppm	2
	rat	acute oral	hexazinone	single forced feeding	LD50 1690 mg/kg	1
	rat	acute injection	hexazinone	intraperitoneal injection	LD50 530 mg/kg	1
	rat	chronic oral	hexazinone	90-day feeding	NOEL 1000 ppm	2
	rat	chronic oral	hexazinone	2-year feeding	NOEL 200 ppm	2
	rat	reproductive	hexazinone	3-generation study	NOEL 2500 ppm	2
	rat	teratogenic	hexazinone	in diet from day 6 to 15 of gestation period		
	maternal				NOEL 200 ppm	2
	fetal				NOEL 5000 ppm	2
	mouse	chronic oral	hexazinone	8-week feeding	NCEL 2500 ppm	2
	mouse	chronic oral	hexazinone	2-year feeding	NOEL 200 ppm	2
	guinea pig	acute oral	hexazinone	single forced feeding	LD50 800 mg/kg	1
	rabbit	teratogenic	hexazinone	force-fed from day 6 to 15 of gestation period		
	maternal				NOEL 50 mg/kg	2
	fetal				NOEL 125 mg/kg	2

Table GI.2. continued

CLASSIFICATION	TAXON	TEST	SUBSTANCE	TEST PROCEDURE	RESULTS	REF.
BIRDS	bobwhite quail	acute oral	hexazinone	single forced feeding	LD50 2258 mg/kg	1
	bobwhite quail	subacute oral	hexazinone	5000 ppm/day for 5 days	LC50 >5000 ppm	1
	mallard ducks	subacute oral	hexazinone	5000 ppm/day for 5 days	LC50 >5000 ppm	1
FISH	bluegill sunfish	acute	hexazinone	24 hour exposure	LC50 425 ppm	1
	bluegill sunfish	acute	hexazinone	96 hour exposure	LC50 370-420 ppm	1
	rainbow trout	acute	hexazinone	24 hour exposure	LC50 401 ppm	1
	rainbow trout	acute	hexazinone	96 hour exposure	LC50 320-420 ppm	1
	fathead minnow	acute	hexazinone	24 hour exposure	LC50 453 ppm	1
	fathead minnow	acute	hexazinone	96 hour exposure	LC50 274 ppm	1
INVERTEBRATES	fiddler crab	acute	hexazinone	96 hour exposure	LC50 >1000 ppm	1
	grass shrimp	acute	hexazinone	24 hour exposure	LC50 241 ppm	1
	grass shrimp	acute	hexazinone	48 hour exposure	LC50 94 ppm	1
	Daphnia magna	acute	hexazinone	48 hour exposure	LC50 152 ppm	1
	Ephemeroptera spp.	acute	hexazinone	1-hour exposure	LC50 >70 ppm	3
	Plecoptera spp.	acute	hexazinone	1-hour exposure	LC50 >80 ppm	3
	Trichoptera spp.	acute	hexazinone	1-hour exposure	LC50 >73 ppm	3
	Odonata sp.	acute	hexazinone	1-hour exposure	LC50 >80 ppm	3
	Diptera sp.	acute	hexazinone	1-hour exposure	LC50 >70 ppm	3
	zooplankton	abundance	hexazinone	lake enclosures	EC50 <0.6 ppm	9

Table GI.2. continued

CLASSIFICATION	TAXON	TEST	SUBSTANCE	TEST PROCEDURE	RESULTS	REF.
FUNGI	soil fungi	growth inhibition	hexazinone	10 ppm dose 8 week plate count	no reduction in population counts	7
ALGAE	*Selenastrum capricornutum*	growth inhibition	hexazinone	96-hour flask assay	EC50 27.7 ppb	4
	Selenastrum capricornutum	growth inhibition	hexazinone	96-hour microplate assay	EC50 24.5 ppb	4
	Selenastrum capricornutum	reproduction inhibition	Velpar L	18-day bioassay	EC50 22.5 ppb	5
	Selenastrum capricornutm	reproduction inhibition	Velpar L	4-day microtest	EC50 24 ppb	5
	Selenastrum capricornutum	acute	hexazinone	96-hour	LC50 22.5 ppb	6
	phytoplankton	biomass reduction	hexazinone	lake enclosures	EC50 0.01-0.07 ppm	8
BACTERIA	bacteria	growth inhibition	hexazinone	10 ppm 8 week plate count	no reduction in population counts	7

between organisms and the abiotic environment. Moreover, the results of laboratory toxicity tests may not be applicable in the field because the composition of the medium can affect toxicity, and because laboratory conditions can create artifacts that lead to carbon dioxide-limited growth (Nyholm and Kallqvist, 1989). Therefore toxicity tests which use natural communitites under natural conditions are needed to accurately predict environmental impacts of chemicals (Larsen *et al.*, 1986).

Periphyton productivity responds rapidly to chemical contaminations and changes in productivity are early indicators of stress (Blanck and Wängberg, 1988). In addition, subsequent changes in periphyton biomass are indicative of potential indirect effects on primary and secondary consumers. Therefore, research into the impact of Velpar L on periphyton productivity and biomass is required if the implications of increased Velpar L use are to be understood. In particular, dose-response relationships for the productivity and biomass of natural periphyton communitites are needed to be able to predict the impacts of Velpar L applications.

Pond macroinvertebrates have been shown to be adversely affected by hexazinone, although the effects appear to be indirect. An experimental pond treated with 1 mg L^{-1} of hexazinone had one half the macroinvertebrate species diversity of the control pond twenty-three days after the treatment (Anderson, 1981); indeed, macroinvertebrate species diversity did not increase until 80 days post-treatment, and 98 days post-treatment it was still less than

in the control pond. The initial decrease in macroinvertebrate diversity was probably the result of a steep decline in the dissolved oxygen content of the pond following the hexazinone application. Additional decreases in macroinvertebrate diversity were attributed to deterioration of habitat and food sources. In addition, Thompson *et al* (1993b) reported reductions in the abundance of zooplankton in lake enclosures treated with as little as 0.01 mg L^{-1} of hexazinone and in lake enclosures treated with 10 mg L^{-1} there was no significant recovery of zooplankton abundance at 77 days post-treatment. The impact of hexazinone of the zooplankton community was attributed to reductions in phytoplankton biomass and dissolved oxygen.

In contrast to lentic invertebrates, stream invertebrates do not appear to be adversely affected by hexazinone. Application of 16.8 kg ha^{-1} of hexazinone to a watershed exposed the stream to intermittent doses of hexazinone, with a peak concentration of 44 µg L^{-1}. This exposure had no adverse effects on stream invertebrates. This is not surprising, given the low concentrations of hexazinone reported. Kreutzweiser *et al.* (1992) reported 1-hour LC_{50}'s greater that 70 mg L^{-1} for 13 test species (5 orders) of aquatic insects and no increase in the drift of aquatic insects at 80 mg L^{-1} of hexazinone. They concluded that hexazinone contamination of streams at expected environmental concentrations (800 µg L^{-1}) would not have direct, toxic effects on aquatic insects. It is unclear, however, whether the reduction of primary productivity following treatment would have an indirect effect on

biomass and growth of stream invertebrates.

Recently there has been a trend towards the use of artificial streams to assess the impact of pesticides on stream biota and their functions (eg. Austin *et al.*, 1991; Krieger *et al.*, 1988; Moorhead and Kosinski, 1986; Hamala and Kollig, 1985; and Kosinski and Merkle, 1984). This is due in part to concerns about the ability of single species laboratory tests to accurately predict effects on complex, natural communities (Hansen and Garten, 1982). Experiments in artificial streams manipulate natural communitites under conditions that are controlled and replicated, and yet are realistic enough to allow the evaluation of the impacts of pesticides in the environment (Odum, 1985). The use of natural communitites in stream mesocosms allows the study of indirect and system-level effects that can not be predicted using single species tests. In some cases stream mesocosms are being used to obtain the information necessary for the registration of pesticides (Crossland *et al.*, 1991).

This research assesses the potential short-term impacts of Velpar L use on natural stream communitites. In the first chapter, the responses of periphyton and invertebrate communitites to a 24-hour exposure to Velpar L are examined. The dose and duration of the exposure to Velpar L mimics the pulse of herbicide entering a stream after the first major rain event following forest spraying. In the second chapter, the dose-response relationship for primary productivity of natural periphyton assemblages is examined. The concentration of formulated hexazinone (Velpar L) that reduces productivity by

50 percent (EC_{50}) during a four-hour exposure is determined. In addition, the ability of the periphyton community to return to control levels of productivity following exposure to Velpar L is assessed.

CHAPTER ONE

INTRODUCTION

Velpar L is a forestry herbicide recently registered for aerial spraying in Canada. The active ingredient of Velpar L, hexazinone, is present at a concentration of 240 g L^{-1} in the formulated product. Hexazinone is very soluble in water (33 g L^{-1} at 25 °C) (WSSA, 1983) and has a low ability to adsorb onto soil (Bouchard and Lavy, 1985). These characteristics make hexazinone very susceptible to off-site movement via runoff and subsurface flow. Mayack *et al.* (1982) reported a peak of 44 µg L^{-1} of hexazinone in a stream following ground application of Velpar L and Feng and Feng (1988) reported a similar peak (30.8 µg L^{-1}) during the first storm event following aerial application of Velpar L. Two additional papers report lower peak concentrations of hexazinone, 14 µg L^{-1} (Bouchard *et al.*, 1985) and 16 µg L^{-1} (Lavy *et al.*, 1989). But both values were detected during the high stream discharge following a storm event (upto a ten-fold increase over the normal discharge). Neary *et al.* (1983) reported a peak concentration of 442 µg L^{-1} in runoff and a hypothetical potential peak of 926 µg L^{-1}. Thus hexazinone is entering streams and its impact on stream biota must be assessed.

The impact of hexazinone on natural periphyton communities is not well known. In one study, the application of hexazinone to an experimental pond to give a final concentration of 1 mg L^{-1}, dramatically reduced the species diversity and density of the periphyton community (Anderson, 1981). As little

as 10 μg L^{-1} of hexazinone has been shown to suppress photosynthesis in phytoplankton (Thompson *et al*, 1993a) and the 96-hour LC_{50} for *Selenastrum* is 22.5 μg L^{-1} (Thompson *et al.*, 1992).

Current information suggests that stream invertebrates are unlikely to be directly affected by Velpar L, but indirect effects, such as food shortage and habitat loss have not been investigated. The 1-hour EC_{50} for 13 genera (5 orders) of aquatic insects is greater than 70 mg L^{-1} (Kreutzweiser *et al.*, 1992) and the 96-hour EC_{50} for *Daphnia* is 151.6 mg L^{-1} (Thompson *et al.*, 1992), several times the maximum expected environmental concentration. Other zooplankton taxa may be adversely affected by hexazinone. Thompson *et al.* (1993b) reported EC_{50} values for zooplankton as less than 0.6 mg L^{-1} and suggested that substantial reductions in zooplankton could result from chronic exposure to environmentally realistic concentrations.

This study examines the effects of a 24-hour exposure to Velpar L on primary and secondary producers in experimental stream channels. Rainfall subsequent to the application of Velpar L to a watershed carries a pulse of the herbicide to local streams. The concentration and duration of the Velpar L treatment was choosen to mimic this pulse. Primary productivity and biomass of the periphyton community, and drift, individual length, density and size distribution of macroinvertebrates were monitored to examine direct and indirect effects of herbicide exposure.

METHODS

Study Site and Experimental Channels

Experimental channels were constructed at the Station de Biologie des Laurentides near St-Hippolyte, 75 km north of Montreal, Québec on the southern edge of the Laurentian Shield (47°N, 71°W). The experimental channels were located about 50 m downstream of Lac Quatre on a second order, oligotrophic stream. The stream flows through a second-growth deciduous forest that partially shades the stream, although sunlight reaches the stream surface during mid-day. The water is acid (pH 5.8-6.2), and soft (<50 mg $CaCO_3$ L^{-1}) with low alkalinity and low conductivity (Lauzon and Harper, 1988).

The experimental system consisted of two units, each with a water supply pipe, water receiving box and five channels. The design, modified from Sérodes *et al.* (1984), is shown in Figure 1.1. The supply pipes were hard plastic sewage pipes with an internal diameter of 10 cm. The receiving boxes and channels were constructed from plywood and then painted with marine paint. The two units were installed side by side on wooden supports so that the channels were a few centimeters above the stream surface. Water was supplied to the channels by gravity through the water supply pipes that stretched 30 m upstream. The water passed from the pipes into the receiving boxes where it was partitioned among the channels and subsequently returned to the stream. The flow rate was controlled in each channel with a piece of

plexiglass that was adjusted to increase or decrease the size of the slit in the water receiving box which opened into the channel. The discharge in each channel was approximately 1 L s^{-1}.

The channels were filled with pint-size plastic mesh baskets containing dried gravel collected from the stream. The gravel was composed of stones with diameters ranging from approximately 2 to 32 mm, and a median diameter of 10 mm. The gravel substrate was left undisturbed in the channels for three weeks before the treatment to allow the establishment of the periphyton and macroinvertebrate communitites. The baskets provided uniform sampling units that could be removed without disturbing the remaining gravel.

Velpar L Treatment and Analysis of Hexazinone

Velpar L (supplied by Dupont Canada) was added to five channels, via peristaltic pumps, for a period of 24 hours starting at noon on June 8, 1992. The concentration of hexazinone was approximately 200 µg L^{-1}. The 5 treatment and 5 control channels were arranged such that every other channel was a control channel.

Water samples were collected in duplicate from the ends of the five treated channels and from one of the control channels at hours 2, 12, 22 of the treatment and at 22 hours after the termination of the treatment.

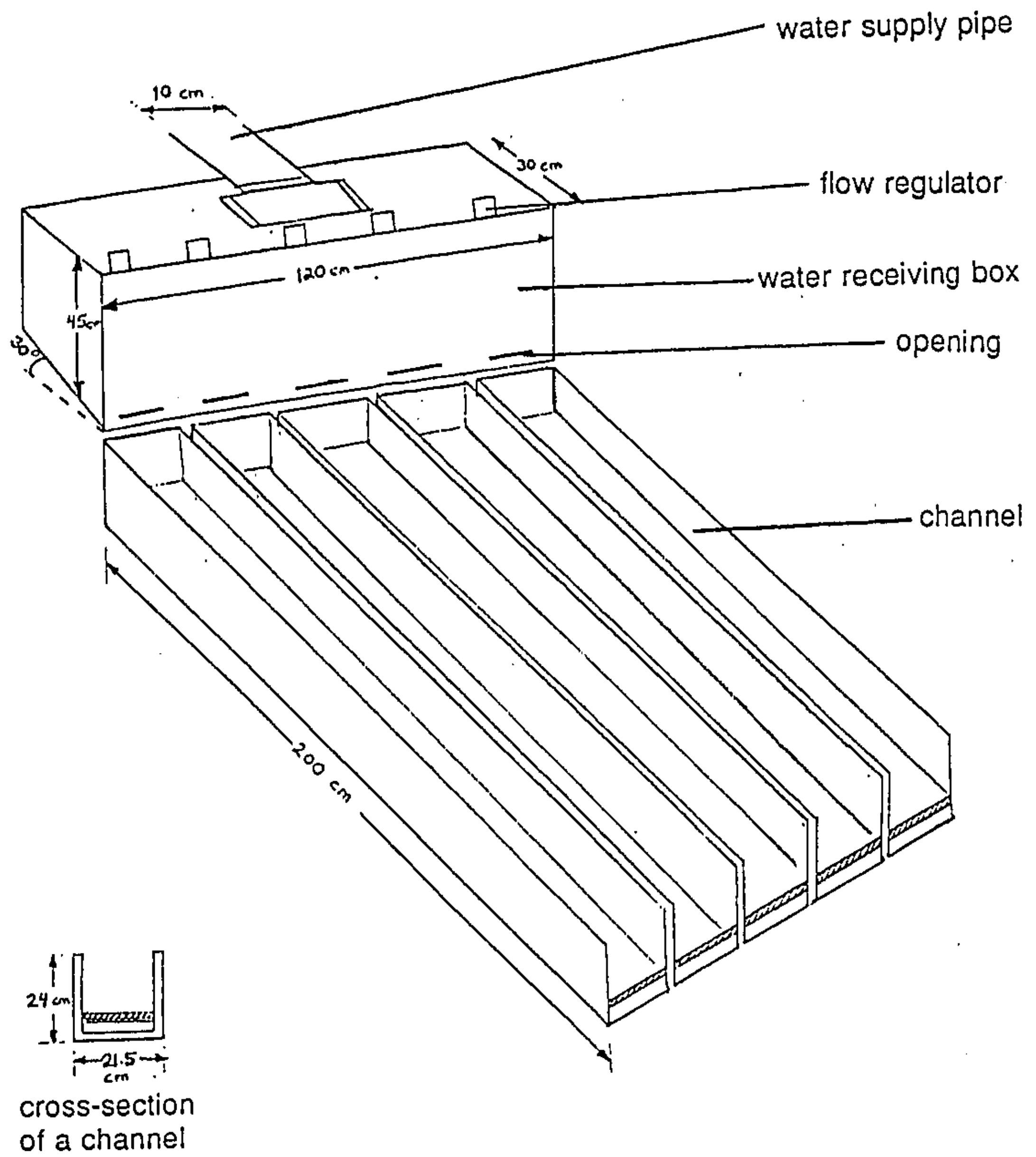

Figure 1.1 The experimental stream system (scale: 1 cm=20 cm).

These water samples were analyzed for hexazinone. Water samples were also collected in duplicate from the stream at two sites, 40 m and 200 m downstream, from the channels at hour 22 of the treatment.

Brown glass jars were used for all samples. Prior to use, the jars were washed and then rinsed with acetone to remove all organics. The jars were rinsed several times with the sample water before being filled and tightly capped. The samples were transported to the laboratory in a cooler and stored at 4 °C until extraction of the hexazinone. All extractions were completed within 10 hours of sampling.

Hexazinone was extracted from the water samples using solid phase extraction (SPE) cartridges containing C-18 bonded silica (Junk and Richard, 1988). The SPE cartridge was conditioned with 10 mL of methanol followed by 10 mL of distilled water immediately prior to the addition of 100mL of sample (Sherma, 1986). The cartridge was not allowed to dry before the addition of the sample. The sample passed through the cartridge under vacuum pressure, but at a slow rate (drop by drop). After all of the sample had passed through, the cartridge was centrifuged at 2500 rpm for ten minutes to remove any water and stored in a refrigerator.

In the laboratory hexazinone was eluted from the cartridges with 5 mLs of acetonitrile using vacuum pressure. The eluent was dried in a rotary evaporator (30 °C) and the residue was reconstituted in an acetonitrile:water (2:8 by volume) solution. The reconstituted sample was analyzed on a Varian

5000 liquid chromatograph/UV detector HPLC. The HPLC column was 25x4.5 mm and filled with Partisil 10 ODS-2. The mobile phase used was 60:40 (by volume) acetonitrile:1mM ammonium acetate and had a flow rate of 1 mL min^{-1}. Sample size was 100 µL. The UV detector was set at a wavelength of 245 nm because this wavelength is the most sensitive to hexazinone. The retention times for hexazinone varied from 5.34 to 5.44 minutes. Sample chromatographs and the calibration curve used to calculate hexazinone concentrations are given in Appendix A.

Recovery is the percentage of hexazinone actually recovered by the extraction and analysis processes. To determine recovery two additional samples of stream water from the water receiving box were collected at each sample time and spiked with 100 µg L^{-1} of formulated hexazinone. The processing of the spiked samples was identical to that of the other water samples. Recovery was calculated with the following equation:

$$R = \frac{H_{det} * 100\%,}{100\ \mu g\ L^{-1}}$$

where R is recovery and H_{det} is the concentration of hexazinone detected in the spiked sample. Low recovery values (<90%) indicate a loss of hexazinone during the storage period or during the extraction and analysis processes. High values (>105%) indicate that other chemicals are interfering with the analysis.

Periphyton Community Composition

Randomly selected stones were collected from several channels and preserved in Lugol's solution. Periphyton was removed from the stones by scrubbing with a brush. The sample was then blended at low speed for 5 seconds to break up any clumps of filamentous algae. Subsamples ranging from 0.5 to 4.0 mL, depending on periphyton density, were pipetted into sedimentation chambers and allowed to settle onto a counting chamber on a glass microscope slide for 24 hours prior to enumeration. Taxa were enumerated at 400 X using a Leitz Diaplan light microscope. A minimum of 300 cells per sample were counted using Utermöhl's transect technique (Lund *et al.*, 1958). Paul Hamilton (Botany Division, Canadian Museum of Nature, Ottawa, Canada) confirmed the taxonomic identifications.

Periphyton Productivity

The productivity of the natural periphyton community was measured four times: on the day before the start of the treatment, near the end of the treatment and on two consecutive days after the termination of the treatment.

Two or three stones (diameter 1-3 cm) were selected randomly from each channel and placed in labelled, clear plastic bags with 100 mL of stream water. These were then innoculated with 40 µL of $NaH^{14}CO_3$ for a final activity of approximately 10^5 DPMs mL^{-1} and incubated at the site for four hours during midday. The samples were secured in a large mesh basket that was placed in

shallow water at the edge of the stream. To correct for residual, unincorporated ^{14}C and non-photosynthetic uptake of ^{14}C, two dark samples were innoculated and incubated in a cooler at the ambient temperature. At the end of the incubation period all samples were placed into the cooler and transported to the laboratory.

The total amount of ^{14}C activity added to each sample bag was measured. For each sample 1 mL of the innoculated stream water was pipetted into a scintillation vial containing 12 mLs of scintillation cocktail (ecolume) to which 50 μL of ethanolamine was added to prevent loss of ^{14}C (Lean and Burnison, 1979). The radioactivity, measured in disintegrations per minute (DPM), was counted by a Beckman LS 6000IC liquid scintillation counter.

The stones were rinsed twice with clean stream water to remove any residual ^{14}C. Chlorophyll *a* and ^{14}C-labelled photosynthates were simultaneously extracted using 20 ml of 100% dimethyl sulfoxide (DMSO). DMSO extracts 76% of the total ^{14}C-labelled photosynthates from natural rock (Palumbo *et al.*, 1987). After a 24 hour extraction period in the dark, the ^{14}C activity of 2 mL of DMSO was measured by liquid scintillation.

The remaining 18 mL of DMSO were used to measure the chlorophyll *a* on each stone by fluorometry (Yentsch and Menzel, 1963). The fluorescence values were converted to mg of chlorophyll against a spectrophotometrically derived calibration curve (Appendix B). Surface areas of the stones were

determined using the calibration curve given in Appendix C.

The areal-specific productivity (P_{area}) of the periphyton was calculated from the formula:

$$P_{area}\ (\mu g\ C\ fixed\ cm^{-2}\ h^{-1}) = \frac{[DPM_s - DPM_d] * DIC * 1.05}{DPM_t * SA * T},$$

where DPM_s, DPM_d and DPM_t are the sample-incorporated, dark and total activitites (in DPM) respectively, DIC ($\mu g\ C\ L^{-1}$) is the dissolved inorganic carbon concentration of the stream water, derived from pH and alkalinity (APHA, 1980), 1.05 is the factor to correct for the differential uptakes of ^{13}C and ^{14}C, SA is the surface area of the stone (cm^2) and T is the incubation time (h).

Chlorophyll *a*-specific productivity (P_{chla}) was calculated from the formula:

$$P_{chla}\ (\mu g\ C\ (\mu g\ Chl\ a)^{-1}\ h^{-1} = \frac{P_{area}}{Chl\ a},$$

where Chl *a* is the chlorophyll *a* content ($\mu g\ cm^{-2}$).

Benthic Sampling

The gravel in the channels was sampled 24 hours before the treatment and at 1, 2, 4, 7 and 14 days after the treatment. For the first four sampling times fives baskets were collected from all channels. At the last sampling time five baskets were collected from three of the Velpar L-treated and three of the control channels because water had stopped flowing in the four other

channels. At each sample time, the five baskets were collected from pre-determined locations in each channel and the same sampling pattern was used in all channels. Baskets of gravel were lifted from the channels and the gravel was poured into sample bags with 200 mL of 95% ethanol to preserve macroinvertebrates and and extract chlorophyll *a*. To prevent the remaining baskets from shifting in the channels, sampled baskets were replaced by baskets containing dried gravel.

Twenty-four hours after collection, a 12 mL aliquot of ethanol was centrifuged and the chlorophyll *a* concentration estimated spectrophotometrically by the method of Ostrofsky and Rigler (1987).

The gravel was fractioned on three sieves (4 mm, 1 mm, 63 μm). All macroinvertebrates in the 4 mm sieve were washed into the 1mm sieve and the large gravel was discarded. The sample was repeatedly subsampled with a Folsom plankton splitter until 100 to 150 individuals remained. The macroinvertebrates were sorted from the debris under a stereo microscope. Individuals were counted and their body lengths (excluding appendages) were measured with an image analysis system connected to a stereo microscope. Dry mass (μg) was estimated as the cube of the body length (mm) (Morin and Nadon, 1991). The density and total biomass of the macroinvertebrates were calculated for each basket. In addition, the size distribution of the benthic macroinvertebrate community was assessed. Size distribution is the distribution of individuals among size classes where each size class

corresponds to a dry mass interval (μg).

Macroinvertebrate Drift

To monitor macroinvertebrate drift, 125 μm nets were placed at the end of each channel so that all water leaving the channel passed through the net. The nets were left in place for 10 minute intervals, several times before, during and after the treatment. After the nets were removed the discharge of each channel was measured by catching the discharge in a large bucket for a known time period and then measuring the volume of water. Captured individuals were preserved with formaldehyde, sorted and counted.

Statistical Analyses

A variety of statistical tests were used to determine the significance of differences between the treated and control channels, before and after the Velpar L treatment. Two-way analysis of variance (ANOVA) was used if the variable had been sampled twice, once before and once after the treatment because these sampling times were not correlated. Where a variable had been sampled three or more times, a repeated measures ANOVA was used to account for the correlation between sampling times. Results of a multivariate repeated measures ANOVA are reported except when there were insufficient degrees of freedom to perform the analysis. In this case univariate results are reported. Where multiple comparisons were necessary, Tukey's test was used

(Zar, 1984). Linear regression was used to fit lines through some data sets. To assess any changes in the size distribution of macroinvertebrates, the mean length of the macroinvertebrates and the variance of the mean length were analyzed because they provide information about the central tendency and the width of the size distribution curve. All statistical analyses used the software packages Quattro Pro and PC SAS 6.04 (SAS Institute, 1985). Results were deemed significantly different at the level $\alpha \leq 0.05$.

RESULTS

Hexazinone Analysis

The concentration of hexazinone in the experimental channels was determined four times, three times during the treatment and once after. Hexazinone was not detected in the control channels at any time. In the treatment channels, the hexazinone concentration could not be kept constant because of difficulties in controlling the flow of stream water in each channel throughout the experiment. In the treatment channels the mean hexazinone concentration was 206 $\mu g\ L^{-1}$ at 2 hours. It increased to 432 $\mu g\ L^{-1}$ at 10 hours and decreased to 145 $\mu g\ L^{-1}$ at 22 hours (Table 1.1). The mean at 10 hours was high because the flow in one of the channels was reduced by debris and the concentration of hexazinone reached 970 $\mu g\ L^{-1}$ in that channel. The low level at 22 hours is the result of an adjustment of the flow rate of Velpar L into the channels at 18 hours. Water collected in the stream at two sites downstream at 22 hours had low levels of hexazinone. At 40 m downstream 9.4 $\mu g\ L^{-1}$ and at 200 m downstream 6.5 $\mu g\ L^{-1}$ of hexazinone were detected at 22 hours. Hexazinone was not detected in any of the channels 22 hours after the end of the treatment. The mean recovery (± standard error) of hexazinone for the four sample times was 96.1% (± 1.9).

Table 1.1. Mean hexazinone concentrations in control and treated channels and at sites 40m and 200m downstream of the experimental channels at several sampling times during and after the treatment (hours 0-24). Standard errors are given in brackets (n=2 except n=10 for treated channels).

TIME (h)	HEXAZINONE CONCENTRATION ($\mu g\ L^{-1}$)				RECOVERY (%)
	Control	Treated	40 m	200 m	
2	nd	206 (41)	ns	ns	103 (3.7)
12	nd	432 (98)	ns	ns	94.5 (0.7)
22	nd	145 (17)	9.4 (0.2)	6.5 (0.1)	93.1 (4.9)
46	nd	nd	ns	ns	93.7 (1.3)

nd - not detected, detection limit <0.10 $\mu g\ L^{-1}$.
ns - not sampled

Periphyton

The periphyton community in the channels one day after the end of the treatment was dominated by pennate diatoms (Bacillariophyceae) especially *Tabellaria flocculosa* which represented 67.1% and 48.1% of the total abundance of the control and treatment communities respectively. Chlorophyta represented only 7.5% and 6.2% of the control and treatment communities respectively (Table 1.2).

Productivity, expressed per unit chlorophyll *a* or per unit area varied with time and with the Velpar L treatment (Appendix E). During the Velpar L treatment the rate of chlorophyll *a*-specific productivity was significantly less in the treated channels than in the control channels (Fig. 1.2). The presence of Velpar L reduced the ability of the periphyton chlorophyll *a* to fix carbon (Fig. 1.3). After the Velpar L addition was stopped the productivity of the periphyton recovered quickly. Twenty-four hours after the stop of the hexazinone treatment the level of productivity was the same in the treatment and control channels and 46 hours after the stop of the treatment the level of productivity was higher in the treated than the control channels. Areal-specific productivity is shown in Appendix D.

In Figure 1.3 chlorophyll *a* was measured on individual stones picked out of the baskets of gravel whereas in Figure 1.4 chlorophyll *a* was measured per basket of gravel. There was no easy method of measuring the surface area of the stones covered in periphyton in each basket. Therefore, for

simplicity and uniformity, the surface area of the basket (100 cm^2) was used as the surface area of the gravel. This is an underestimate of the actual surface area and accounts for the discrepancy between the chlorophyll *a* values in Figures 1.3 and 1.4.

Periphyton biomass, measured as the quantity of chlorophyll *a* per unit area, varied over time (F=4.14, 5df, 20df, p=0.01) but was not affected by the Velpar L treatment (F=0.11, 1df, 4df, p=0.8) (Appendix F). During the days immediately following the Velpar L treatment, periphyton biomass decreased but this decrease was evident in both the control and treatment channels (Fig. 1.4). From day 7 to day 14 the periphyton biomass started to increase and approached the levels present on the day before the treatment.

Table 1.2. Percent abundances of periphyton species in the control and treatment channels one day after the termination of the Velpar L treatment.

TAXON	CONTROL CHANNELS (%)	TREATED CHANNELS (%)
Chrysophyta		
Chrysophyceae		
Dinobyron sp.	3.0	28.7
Bacillariophyceae		
Tabellaria flocculosa	67.1	48.1
Nitzschia spp.	14.0	7.8
Eunotia spp.	3.0	0.9
Frustulia rhomboides	1.9	2.1
Achnanthes spp.	1.4	1.6
Synedra sp.	1.1	2.8
Diploneis sp.	0.8	0.2
Pinnularia viridis	0.2	1.4
TOTAL	92.5	93.6
Chlorophyta		
Pediastrum obtusum	4.5	5.3
Microspora sp.	3.0	0.9
TOTAL	7.5	6.2
Pyrrhophyta *Peridinium*	0.0	0.2
GRAND TOTAL	100.0	100.0

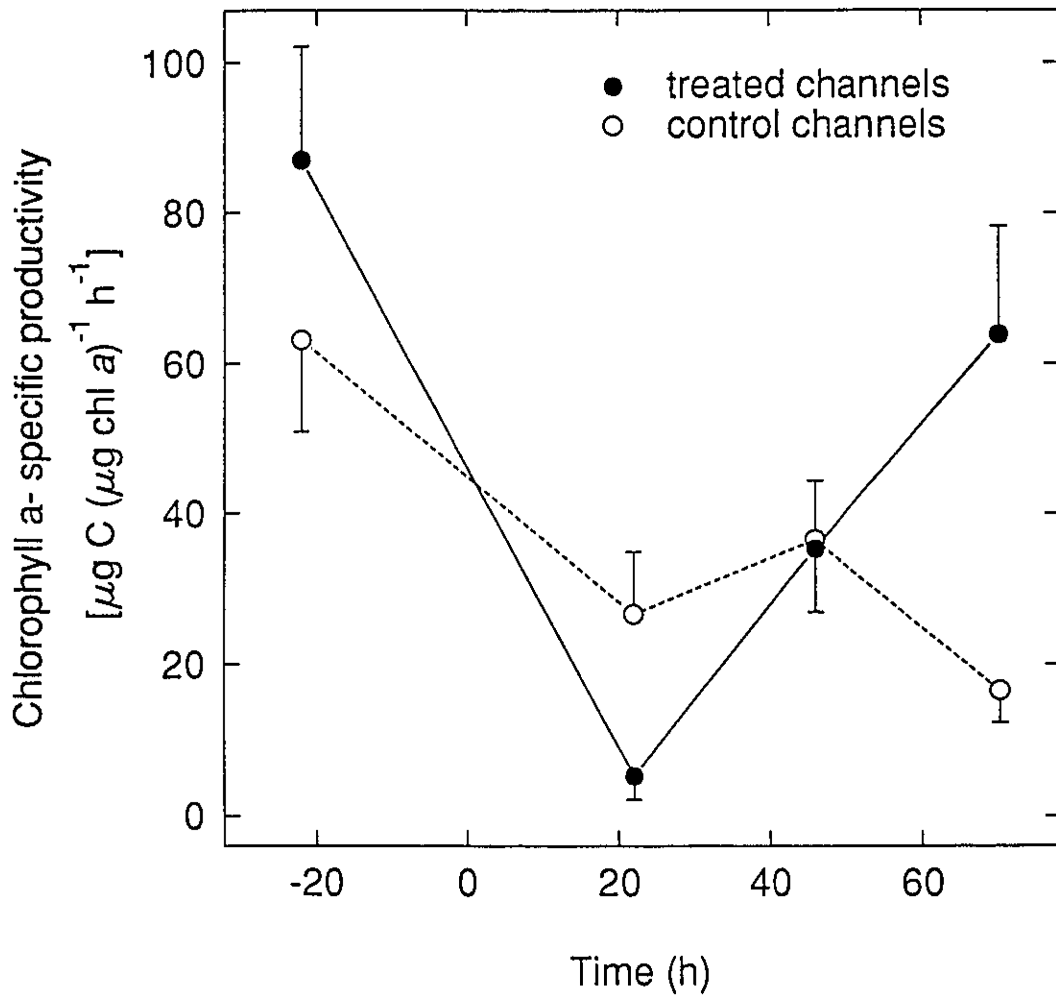

Figure 1.2. Changes in mean chlorophyll *a*-specific periphyton productivity in the control and Velpar L-treated channels before, during and after the treatment (hours 0-24). Vertical bars show standard errors (n=15 for times 1 and 2, n=10 for times 3 and 4).

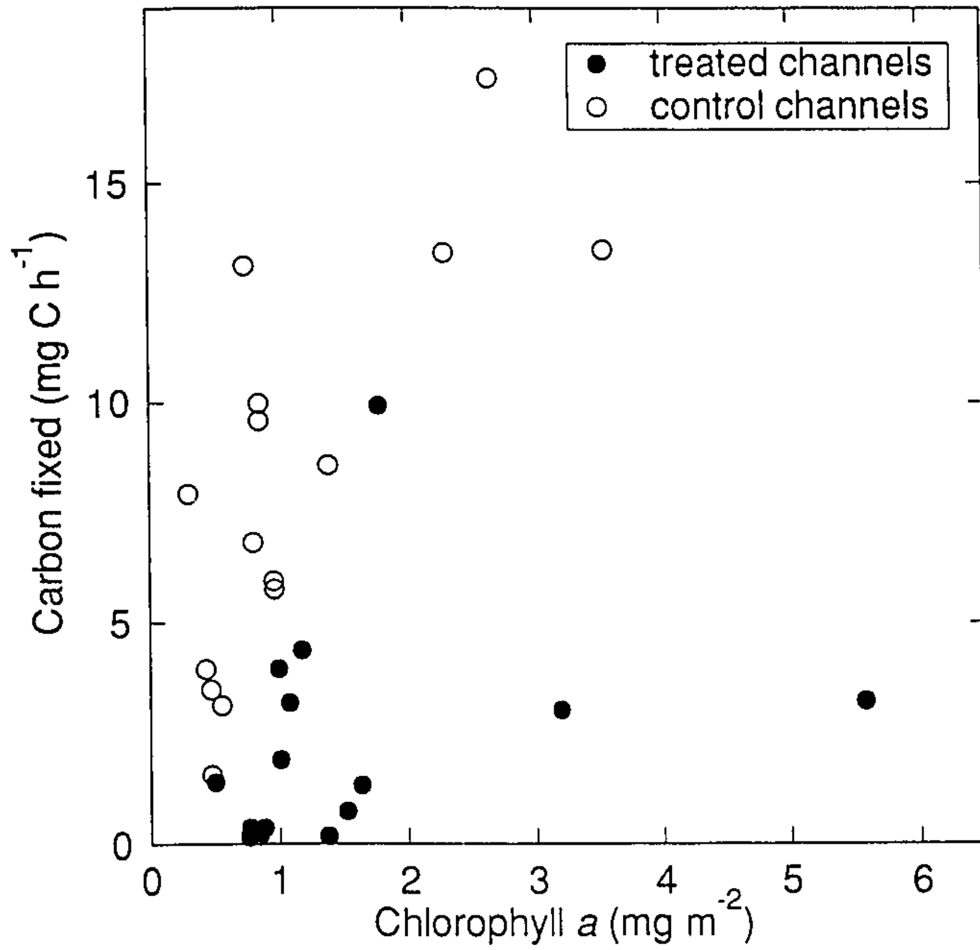

Figure 1.3. Carbon fixation by periphyton chlorophyll *a* in control and Velpar L-treated channels. Carbon fixation was measured at hour 22 of the Velpar L treatment. Each circle represents the periphyton on one stone.

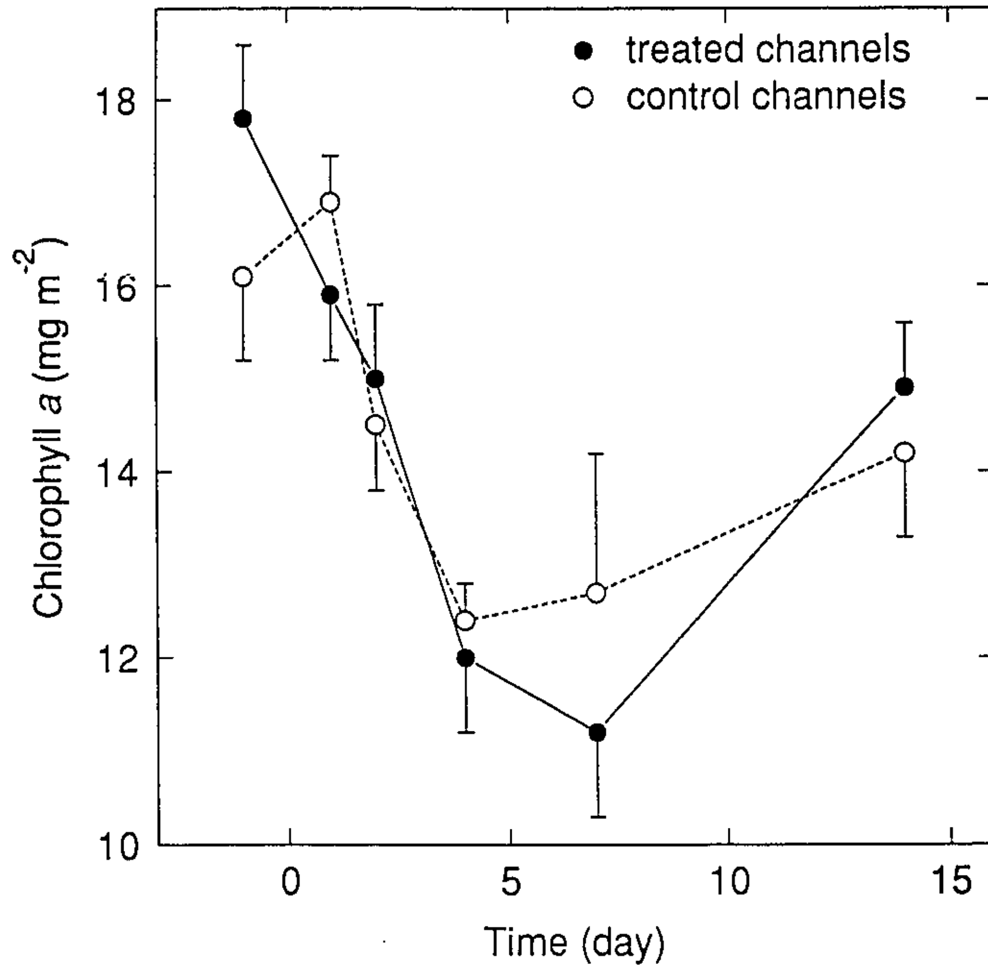

Figure 1.4. Periphyton biomass measured as chlorophyll *a* concentration in the control and the Velpar L-treated channels before, during and after the treatment (day 0). Vertical bars are standard errors (n=25 for times 1 to 4, n=15 for times 5 and 6).

Macroinvertebrates

The macroinvertebrate drift consisted mainly of Copepoda, *Hydra*, and Diptera. The total number of macroinvertebrates in the drift varied with time of sampling (F=7.69, 9df, 72df, p=0.0001) (Fig. 1.5A), although a repeated measures ANOVA showed that Velpar L had no effect on the number of drifting individuals (F=0.05, 1df, 8df, p=0.8) (Appendix F). When considered separately insect drift also varied with the time of sampling (F=2.29, 9df, 72df, p=0.03) (Fig. 1.5B) but was not affected by the addition of Velpar L (F=1.5, 1df, 8df, p=0.3) (Appendix G).

The benthic invertebrate community in the experimental stream channels was dominated by chironomids, oligochaetes and molluscs. Although mean biomass and mean density of the benthic invertebrates (Table 1.3) did not vary with time or with the Velpar L treatment (Appendix H), the mean density was higher in both the control and treated channels after the hexazinone addition. This increase in mean density, although not statistically significant was much larger in the control channels than the treated channels.

In all channels the density (ind. m^{-2}) of invertebrates increases up to the size classes around 0.1 µg and then plateaus before starting to decrease around the size class of 22 µg (Fig. 1.6). Visual examination of the size distribution after the Velpar L treatments suggests that the size distribution in the control channels may have shifted slightly to the left.

The mean individual length of benthic macroinvertebrates (Table 1.3) did

not vary with time or with the Velpar L treatment (F=1.8, 3df, 16df, p=0.2) (Appendix H). Although the decrease in mean length in the control channels was not statistically significant, the decrease supports the visual observation of a shift in the size distribution. Thus, the large, but not significant, increase in density in the control channels, while maintaining the same biomass, corresponds to a decrease in the mean length of the macroinvertebrates in the control channels.

The variance of individual lengths was analyzed because a change in the variance would be indicative of a change in the size structure of the macroinvertebrate community. Variance increased through time (F=5.9, 1df, 16df, p=0.03) in both the control and Velar L-treated channels (Appendix I).

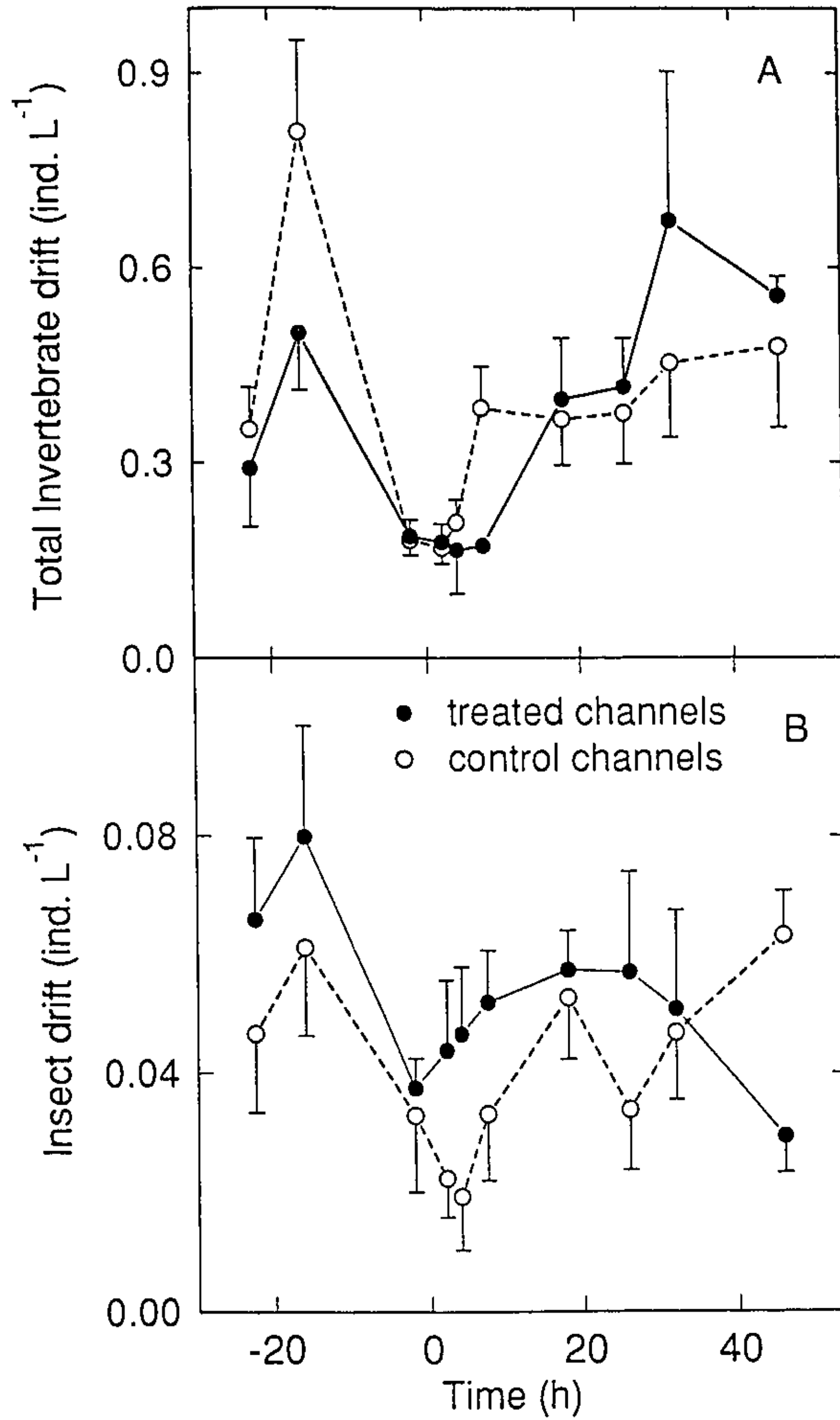

Figure 1.5. Total macroinvertebrate (A) and insect (B) drift in the control and Velpar L-treated channels before, during and after the treatment (hours 0-24). Vertical bars show standard errors (n=5).

Table 1.3. Mean biomass, mean density and mean individual length of benthic macroinvertebrates in the control and treated channels one day before treatment and one day after treatment. Standard errors are given in brackets (n=25).

	BEFORE		AFTER	
	Control	Treated	Control	Treated
Biomass (mg m^{-2})	4114 (578)	4355 (439)	4933 (340)	5664 (458)
Density (ind. m^{-2})	390916 (43367)	457828 (34738)	616864 (47262)	514888 (36585)
Length (mm)	1.80 (0.05)	1.73 (0.05)	1.63 (0.05)	1.75 (0.08)

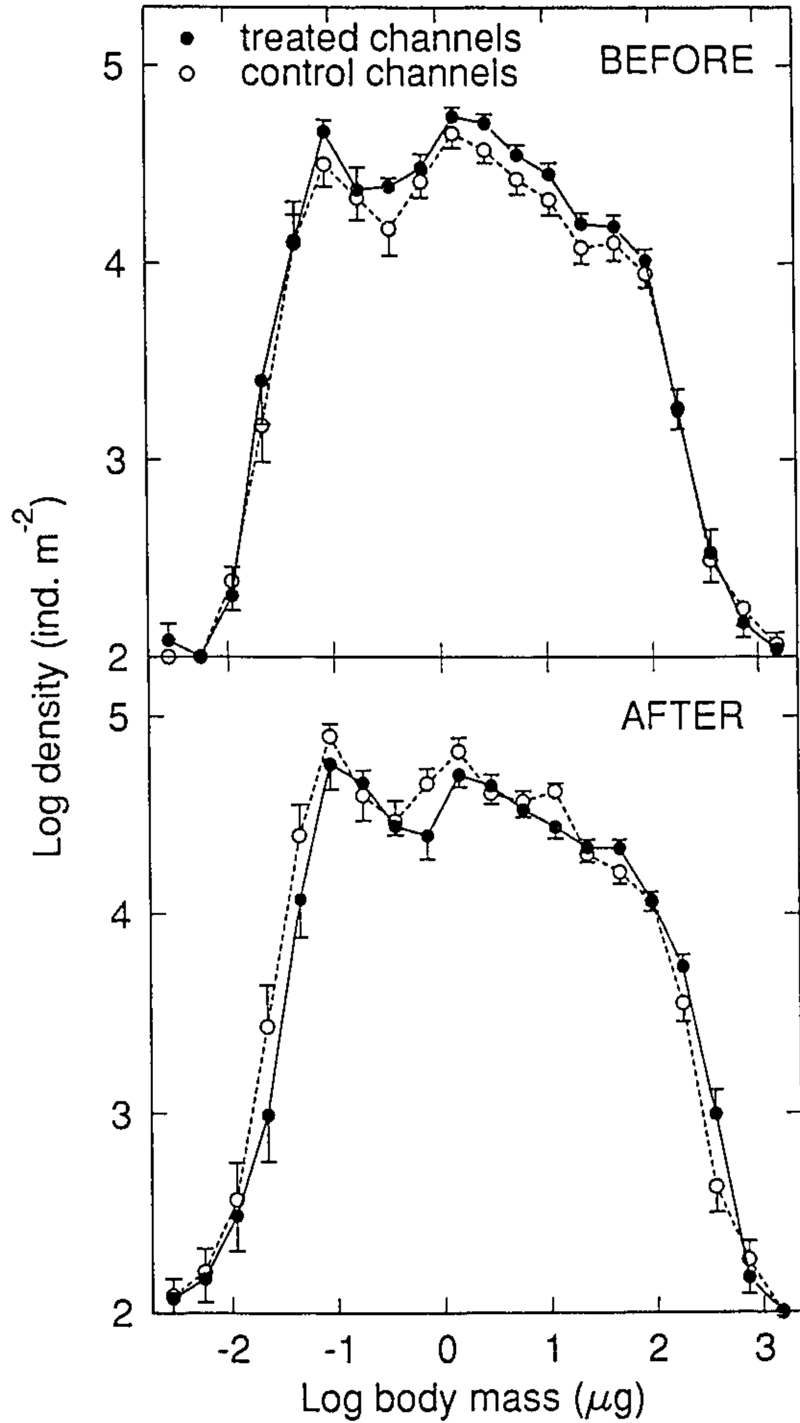

Figure 1.6. The size distribution of the macroinvertebrate community in the control and Velpar L-treated channels, before and after the addition of Velpar L. Vertical bars show standard errors (n=25).

DISCUSSION

The addition of hexazinone to the experimental channels had only short-term impacts on the stream biota. Hexazinone has a very low capacity to adsorb to sediments (Neary *et al.*, 1993) and therefore would be quickly flushed out of the system after the treatment. Twenty-two hours after the termination of the treatment no hexazinone was detected in any of the stream channel water samples.

The decrease in the rate of chlorophyll *a*-specific productivity during the hexazinone treatment resulted from a decrease in chlorophyll *a* efficiency (Fig. 1.3). Hexazinone, a photosynthetic inhibitor (Corbett *et al.*, 1984), prevented the fixation of carbon by the periphyton community in the treatment channels. This lead to the significant reduction in chlorophyll *a*-specific productivity in the treatment channels when compared to the control channels. However, this decrease was temporary and productivity recovered to control levels within 24 hours after the termination of the treatment.

Differences in lighting may account for the increase in the productivity rates (Fig. 1.2) over the first three days and the slight drop on the fourth. The amount of light varied between the ^{14}C incubations of the periphyton because the incubations were done at the site. The first measurement day was darkly overcast, the second was overcast but starting to clear, the third day was sunny and the final day was mostly sunny.

The drop in the chlorophyll *a*-specific productivity of the control channels

on the fourth sample day may result from self-shading. The mean chlorophyll *a* on the stones from the control channels was 1.5 ± 0.2 mg m^{-2}, three times the mean on the stones from the hexazinone treated channels (0.5 ± 0.1 mg m^{-2}). Thus self-shading by the periphyton in the control samples may have reduced the efficiency of the chlorophyll *a* leading to a reduction in the chlorophyll *a*-specific productivity in the control channels.

The decrease in periphyton biomass through time was consistent in both the control and hexazinone treated channels. The decrease may have resulted from changes in the discharge of the channels or from the sloughing off of the top layers of the periphyton mat. The fact that periphyton biomass in the treated channels did not differ from that in the control channels may explain how the rate of productivity recovered to control levels so quickly.

Other studies have shown that the impact of another triazine compound, atrazine, on periphyton communitites depends on the composition of the periphyton community at the time of exposure. Hamilton *et al.* (1987) reported that atrazine caused a shift from a Chlorophyte-dominated community to a Bacillariophyceae-dominated community. Herman *et al.* (1986) reported that while the impact of atrazine on Cyanophyta was drastic, there was no impact on Chlorophyta or Bacillariophyceae. If Bacillariophyceae are resistant to atrazine, then they are probably also resistant to hexazinone because both are triazines with similar modes of action. Therefore the impact of hexazinone on the periphyton of the stream channels may have been mitigated by the

presence of more-resistant Bacillariophyceae.

It is not surprising that the total macroinvertebrate and insect drift did not increase in the hexazinone treated channels. Kreutzweiser *et al.* (1992) reported no increase in the drift of five of six insect species exposed to concentrations of hexazinone up to 80 mg L^{-1}. The six species drifted only at the maximum hexazinone concentration and the survival of these individuals was not affected. The maximum concentration used by Kreutzweiser *et al.* (1992) was approximately 200 times the maximum mean hexazinone concentration in this study (Table 1.1). The separate analysis of insect drift was done because it gives a better idea of the response of benthic invertebrates to the Velpar L treatment because the majority of captured invertebrates were not benthic species. The majority of macroinvertebrates captured in the drift samples probably came from upstream through the water supply pipe rather than from the channels.

Observations of standing stock and size distribution of benthic invertebrates suggest that Velpar L may have had an impact on this community. The impact on the benthic invertebrates is inconclusive because there were no statistically significant differences between the control and treatment channels after the treatment. However, an increase in density in both control and Velpar L-treated channels may have resulted from colonization of the artificial stream channels by drifting and newly hatched invertebrates, that add little to the total biomass. Moreover, the insignificant

decrease in average length in the control channels and the lack of change in the treatment channels suggests that perhaps the number of small invertebrates did not increase as much in the treated channels as in the control channels. A loss of small invertebrates in the treatment channels could be possible, due to mortality of any newly hatched larvae or the smallest invertebrates in the treated channels.

Alhthough the productivity of the periphyton community was adversely affected by the exposure to an environmentally realistic concentration ($<800\ \mu g\ L^{-1}$) of Velpar L, periphyton biomass and community composition were not affected. The drift and mean biomass of invertebrates also were not affected. The changes in mean density, mean length and the size distribution, although slight, suggest that Velpar L has the potential to adversely affect the invertebrate community. However, given the quick recovery of the periphyton community, any impact on the invertebrate community will probably also be short-term. Thus it appears that stream communities are resilient to short-term exposures to Velpar L, although the impact of Velpar L may be greater on the periphyton communities dominated by Chlorophyta or Cyanophyta.

CHAPTER TWO

INTRODUCTION

Triazines are the most widely used class of pesticides in Canada (Trotter *et al.*, 1990). In the United States the triazine, atrazine, is the most heavily used pesticide (DeNoyelles *et al.*, 1982). Velpar L, whose active ingredient is the triazine compound hexazinone, is one of the most heavily used forestry herbicides in the United States (Neary *et al.*, 1993). Velpar L was recently registered for aerial spraying of forests in Canada and has the potential to become as widely used in Canada as it is in the United States.

The few studies on the toxicity of hexazinone to algae suggest that it is at least as toxic as atrazine. Denoyelles *et al* (1982) reported that 20 $\mu g\ L^{-1}$ of atrazine caused a temporary decline in the rate of productivity in experimental ponds whereas 500 $\mu g\ L^{-1}$ of atrazine caused a significant reduction in productivity and biomass with little recovery after 136 days. Thompson *et al* (1993) reported that 10 $\mu g\ L^{-1}$ of hexazinone caused a temporary reduction in the dissolved oxygen concentrations in lake enclosures, and at 100, 500 and 1000 $\mu g\ L^{-1}$ hexazinone reduced biomass significantly from which there was no recovery even after 77 days. Although hexazinone has a large impact on phytoplankton, the response of stream periphyton communities to hexazinone has not been assessed.

The results of bioassays that quantify the short-term impact of hexazinone on a natural periphyton community that was developed in outdoor

experimental channels are reported. The use of a natural community ensures a complex periphyton assemblage and enables more realistic simulation of the impact of the herbicide on streams. Under these conditions the concentration of hexazinone that inhibits productivity by 50% after a 4 hour exposure (4-h EC_{50}) was determined and the ability of the natural periphyton community to return to control levels of productivity after exposure to different concentrations of formulated hexazinone is also measured.

METHODS

The periphyton community was characterized by counting and identifying the species on stones preserved in Lugol's solution, as described in Chapter One.

To determine the EC_{50}, randomly selected stones from the experimental channels were incubated with $NaH^{14}CO_3$ and Velpar L. Three stones were randomly assigned to each of seven hexazinone concentrations (0, 1, 10, 50, 100, 500 and 1000 µg L^{-1}. Three stones were used as dark samples. The stones were incubated for four hours, during mid-day, at the site. The incorporated ^{14}C and chlorophyll *a* of the periphyton was extracted overnight with DMSO and then measured by liquid scintillation and fluorometry, repectively, as described in Chapter One. The productivity of the periphyton at each concentration was calculated as a percentage relative to the control. An inverse logistic model of the productivities and hexazinone concentrations was used to interpolate the EC_{50} value. Linear regression models are recommended over probit and logit models for the statistical characterization of herbicidal effects on algal productitivity (Millie and Hersh, 1987). However when the data was graphed, its shape suggested that an inverse logistic model was appropriate. Therefore both models were considered. But the inverse logistic model seemed to fit the data better and is the one reported.

Four hexazinone concentrations 0, 100, 500 and 1000 µg/L were used to examine the recovery of periphyton productivity. Three stones were

incubated at each concentration for 20 hours at the stream site. Three stones were incubated for use as dark samples during the ^{14}C incubation. After 20 hours the stones were rinsed in stream water and placed in fresh, uncontaminated stream water and incubated with $NaH^{14}CO_3$ for four hours, during mid-day. The productivity of the periphyton was then determined and analyzed as described above.

RESULTS

The periphyton community that developed on the gravel in the experimental channels was dominated by pennate diatoms (Bacillariophyceae). The dominant taxa were *Eunotia* spp.(45.1%), *Achnanthes* spp (18.9%)., *Frustulia rhomboides* (14.5%) and *Tabellaria flocculosa* (10.6%).

The dose-response relationship of productivity of the natural periphyton community to Velpar L is shown in Figure 2.1. The inverse logistic model of the chlorophyll *a*-specific productivity gave a value of 3.6 µg L^{-1} for the 4-hour EC_{50} with 95% confidence intervals of 0 to 15.1 µg L^{-1}. Areal-specific productivity results were similar (Appendix J)

Whereas the periphyton community recovered to 70.9% of the control level of chlorophyll *a*-specific productivity during the 4 hour recovery period after the 100 µg L^{-1} exposure it recovered only 3% and 23% after the 500 and 1000 µg L^{-1} exposures, respectively (Fig. 2.2). Recovery of areal-specific productivity showed the same pattern (Appendix I). The hexazinone concentration at which chlorophyll *a*-specific productivity could be expected to recover to 50% of the pre-treatment level within 4 hours was 180 µg L^{-1}.

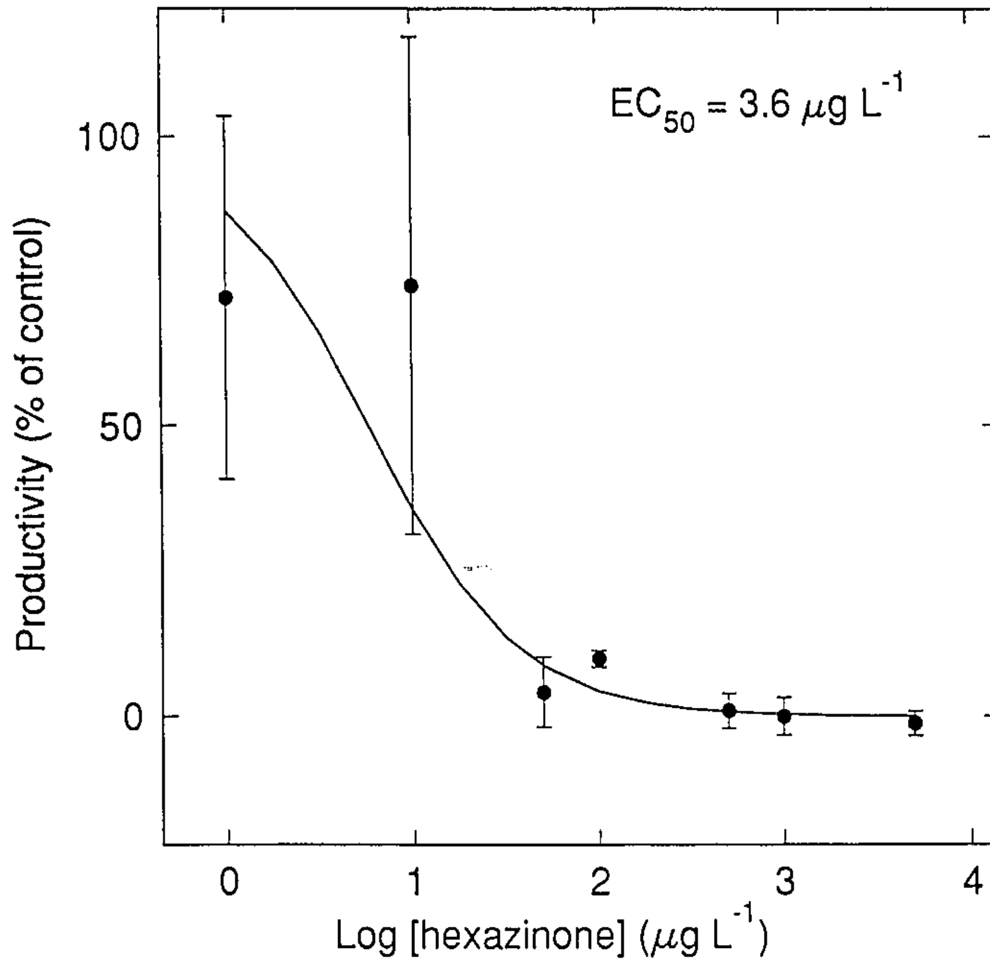

Figure 2.1. Inhibition of chlorophyll *a*-specific productivity of a natural periphyton community by formulated hexazinone in experimental stream channels. The line represents the best fit to an inverse logistic equation. Vertical bars are standard errors (n=3).

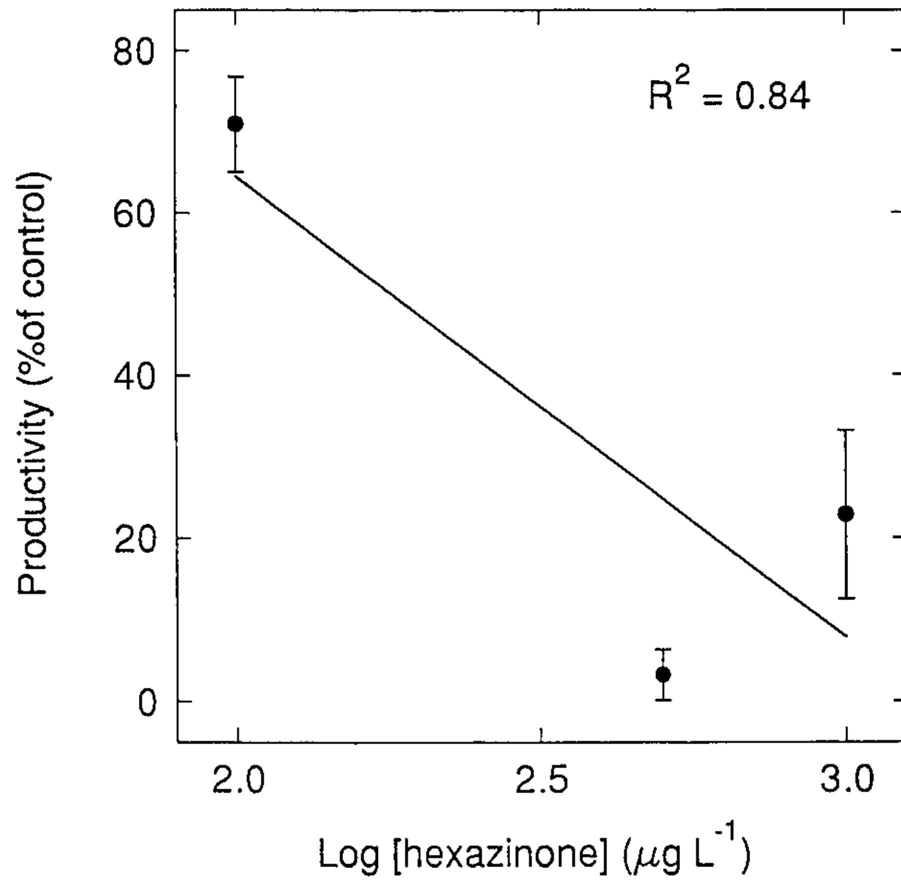

Figure 2.2. Inhibition of chlorophyll *a*-specific photosynthesis of a natual periphyton community, following a 20 hour exposure to formulated hexazinone. Vertical bars are standard errors (n=3).

DISCUSSION

The maximum expected environmental concentration (EEC) of hexazinone after a direct overspray of a 0.5 m deep body of water at the maximum application rate (4 kg ha^{-1}) is 800 $\mu g\ L^{-1}$. Given that the 4-hr EC_{50} for chlorophyll a-specific productivity is 100 times less (7.3 $\mu g\ L^{-1}$) than the EEC, environmentally realistic of hexazinone could have an impact on stream ecosystems.

Maule and Wright (1984) reported 96-hour EC_{50} for the growth inhibition of the green alga *Chlorella pyrenoidosa* at 60 $\mu g\ L^{-1}$ for atrazine. This is more than double the 96-hour EC_{50} (24.5 and 27.7 $\mu g\ L^{-1}$) for the growth inhibition of another green alga *Selenastrum capricornutum* reported for hexazinone (St. Laurent *et al.*, 1992).

Larsen *et al.* (1986) reported 24-hour EC_{50}'s for the productivity of a pond phytoplankton community that ranged from 24 to 131 $\mu g\ L^{-1}$ depending on the sample day. The 96-hour LC_{50} for *S. capricornutum* is 22.5 $\mu g\ L^{-1}$ for hexazinone (Thompson *et al.*, 1992). Comparison of the atrazine figures with the periphyton productivity 4-hr EC_{50} (7.3 $\mu g\ L^{-1}$, 3.6 $\mu g\ L^{-1}$) and 96-hour LC_{50} for *S. capricornutum* suggests that hexazinone is more toxic than atrazine to algae.

The ability of the periphyton to recover from short-term exposures to hexazinone is an important consideration when assessing the environmental impact of Velpar L. The productivity of this periphyton community had an

immediate 50% recovery from a 20 hour exposure to the environmentally realistic concentration of 180 μg L^{-1} of hexazinone. A 50% reduction in periphyton productivity could have possible indirect impacts on primary and secondary consumers.

After the 500 and 1000 μg L^{-1} exposures the productivity of the periphyton had only a slight recovery after 4 hours exposure. This lack of recovery may be the result of mortality of algal cells at these high concentrations. Alternatively, the periphyton community may have required a longer recovery period before being able to return to control levels of productivity.

The very low EC_{50} value for the productivity of a natural periphyton community and the potential for long-term inhibition of productivity, indicated by the lack of recovery from short exposures to environmentally realistic concentrations of formulated hexazinone, suggest that the use of hexazinone is cause for concern.

GENERAL CONCLUSIONS

This study looked at the potential impact of the forestry use of the herbicide, Velpar L on stream biota. The addition of Velpar L to the experimental stream channels had a temporary effect on periphyton productivity but periphyton biomass and community composition was not affected. Macroinvertebrate drift was not affected and the size structure of the macroinvertebrate community was slightly affected by the Velpar L addition. Thus, stream systems appear to be stable during short-term exposures to Velpar L.

However, this study also shows that very low concentrations (7.3 $\mu g\ L^{-1}$) of Velpar L will have a toxic impact on the productivity of natural periphyton communitites. In addition the ability of the periphyton to recover to pre-treatment levels of productivity after exposure to Velpar L is reduced as the concentration of Velpar L increases. Therefore longer exposures or higher doses of Velpar L has the potential to adversely affect stream biota.

Potential problems with Velpar L usage stem from the mobility and persistence of hexazinone in soil and water. Because hexazinone is mobile in soil there is the possibility that stream biota could receive longer term exposures to hexazinone, as the hexazinone moves towards the stream via subsurface and base flow. In addition rainfall events subsequent to Velpar L application will carry repeated doses of hexazinone to streams. The potential for adverse effects on the biota of lakes, which are exposed longer to

hexazinone and which receive accumulating doses, is high.

Further research could examine the impact of long-term (greater than 96 hours) low level (less than 100 μg L^{-1}) exposure to hexazinone on stream biota or the impact of several exposures to hexazinone with recovery periods in between exposures on stream biota.

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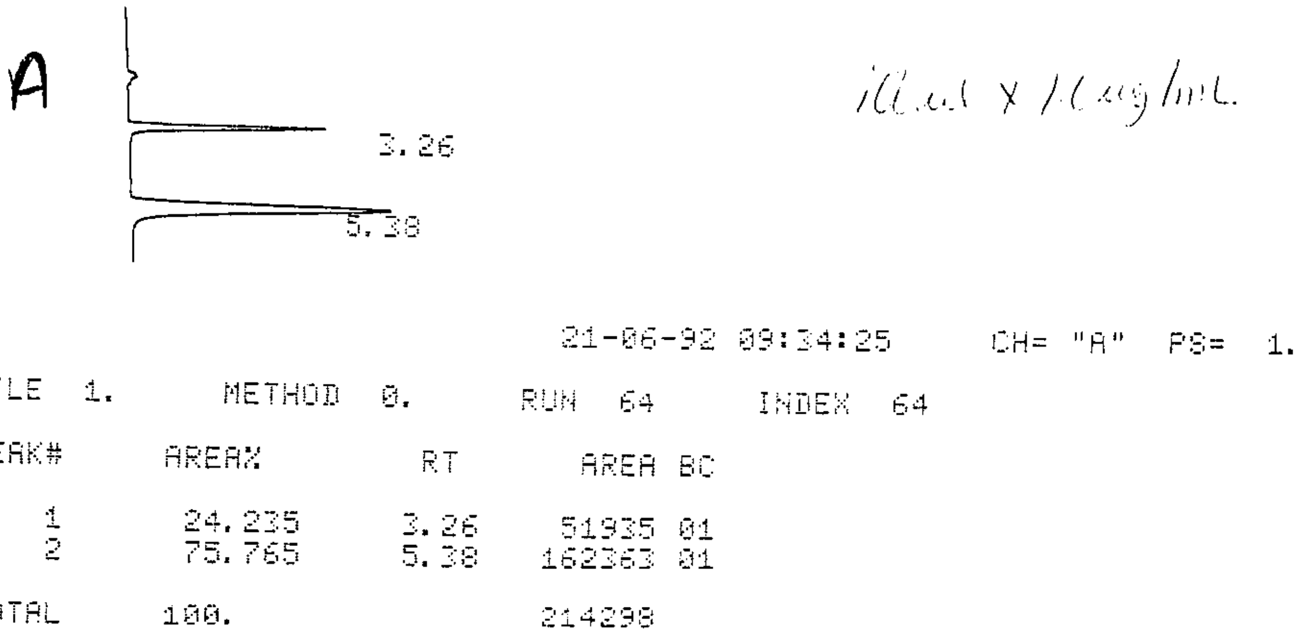
APPENDIX A. Sample HPLC chromatographs and the calibration curve used to calculate hexazinone concentrations.

The HPLC chromatographs of the standards, one spiked sample used to measure recovery and one water sample are shown in Figure A.1. These chromatographs show the consistency of the retention times and the increase in peak height as the hexazinone concentration increases.

Four different standard solutions of hexazinone were analyzed at regular intervals throughout the analysis to ensure the uniformity of the analysis. The means of the three replicates of each standard were used to develop the calibration curve used to convert the area under the peak to the corresponding hexazinone concentration.

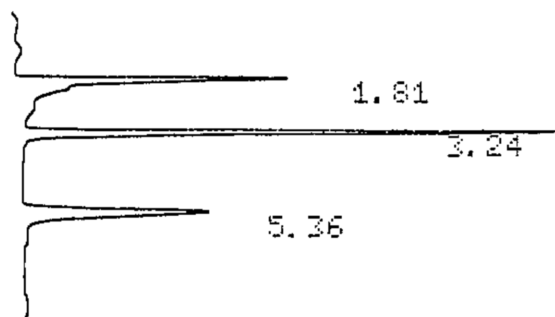


PEAK#	AREA%	RT	AREA BC
1	24.235	3.26	51935 01
2	75.765	5.38	162363 01
TOTAL	100.		214298

Figure A.1. Sample HPLC chromatographs produced during the hexazinone analysis: hexazinone standard solution of 1.0 µg mL^{-1} (A), a spiked sample used to calculate recovery (B) and a water sample collected from channel E during the Velpar L treatment (C).

B

1:4 dilution
Spike 2 618 1500

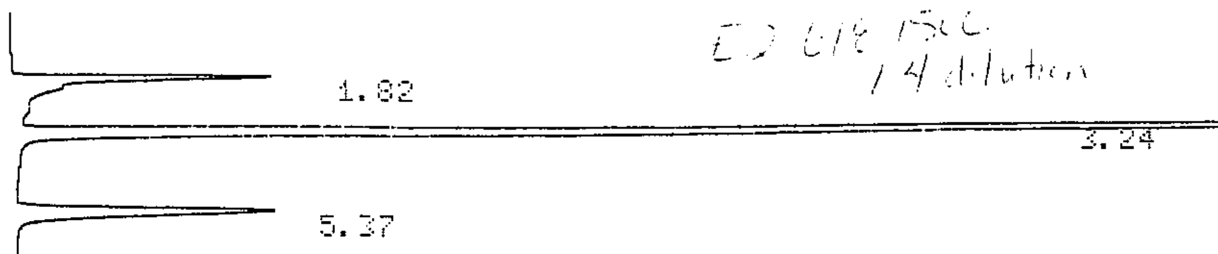


21-06-92 09:51:11 CH= "A" PS= 1.

FILE 1. METHOD 0. RUN 66 INDEX 66

PEAK#	AREA%	RT	AREA	BC
1	24.085	1.81	85432	01
2	43.795	3.24	155347	01
3	32.121	5.36	113937	01
TOTAL	100.		354716	

C



21-06-92 10:22:08 CH= "A" PS= 1.

FILE 1. METHOD 0. RUN 70 INDEX 70

PEAK#	AREA%	RT	AREA	BC
1	5.033	1.82	81885	01
2	85.254	3.24	1387128	01
3	9.713	5.37	158035	01
TOTAL	100.		1627048	

Figure A.1 (continued).

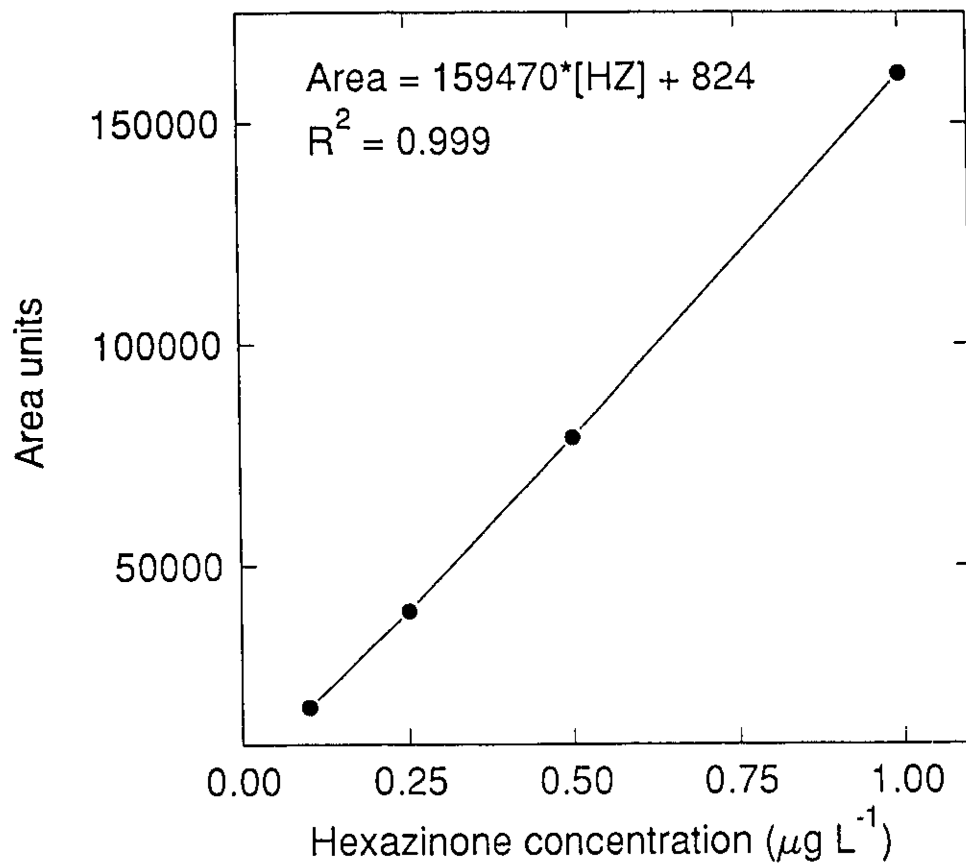


Figure A.2. The calibration curve developed to calculate the concentration of hexazinone.

APPENDIX B. The calibration curve developed to convert fluorescence to chlorophyll *a*.

A calibration curve was developed to convert fluroescence values to micrograms of chlorophyll *a* per stone. Stones with a range of periphyton biomass were selected. Chlorophyll *a* was extracted with DMSO for 24 hours and then measured both spectrophotometrically and fluorometrically. Linear regression analysis with fluroescence as the independent variable gave the calibration curve shown in Figure B.1

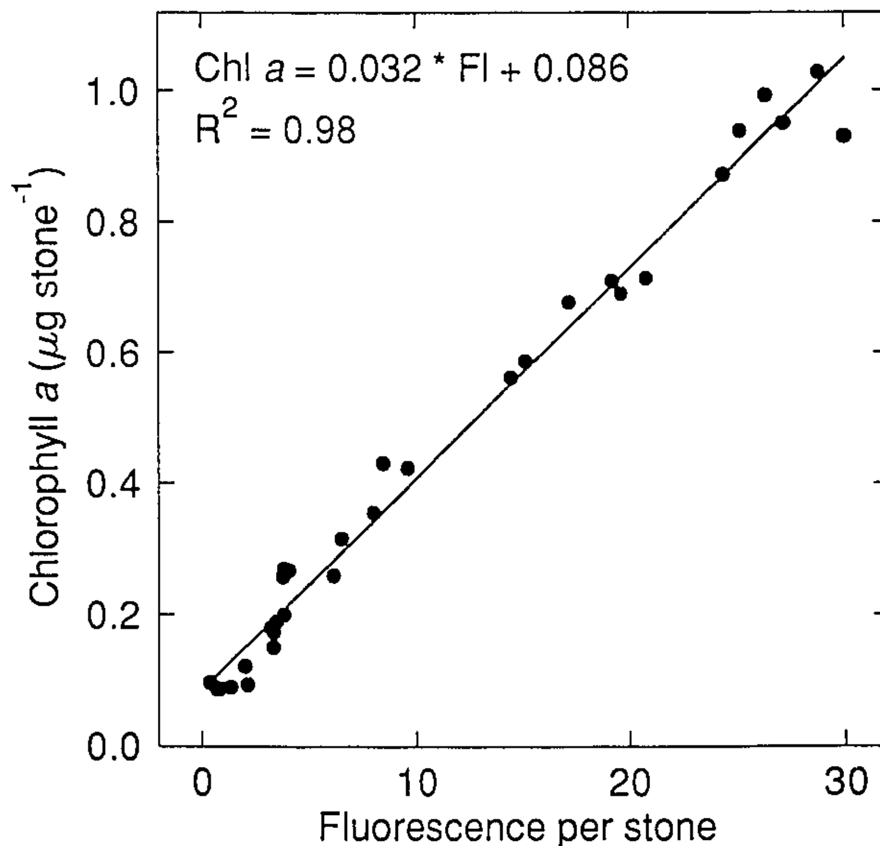


Figure B.1. The calibration curve used to convert fluorescence values to micrograms of chlorophyll *a* per stone.

APPENDIX C. The regression used to determine the surface area of the stones.

The surface area of the stones was measured by photocopying the stones, cutting out the shape and weighing it. The weight (mg) of the paper was then converted to surface area (cm^2) using a calibration curve. The curve was developed by weighing squares of paper with known surface areas. For all measurements the same type of paper was used. The calibration curve is shown in Figure C.1.

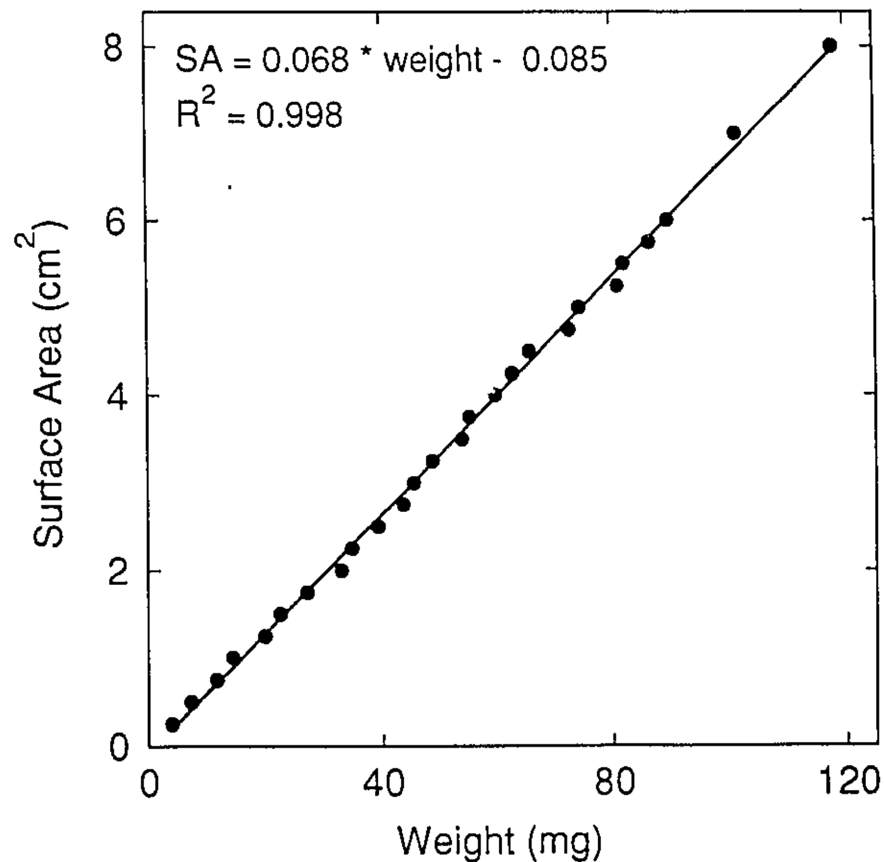


Figure C.1. The calibration curve used to convert the weight of the paper to surface area (cm_2).

APPENDIX D. The effect of the Velpar L treatment on the rate of areal-specific productivity of the natural periphyton community.

The rate of areal-specific productivity varied with time during the experiment (F=11.12, 3df, 6df, p=0.007) and there was a difference in the temporal pattern in the Velpar L-treated and control channels (F=5.4, 3df, 6df, p=0.04) (Appendix E). Twenty-four hours before the start of the treatment areal-specific productivity was the same in the treated and control channels (Fig. D.1). During the Velpar L treatment the rate of areal-specific productivity in the treated channels dropped to a level significantly less than that in the control channels which maintained their rate of productivity. At 22 and 46 hours after the stop of the treatment productivity was the same in both the control and treatment channels.

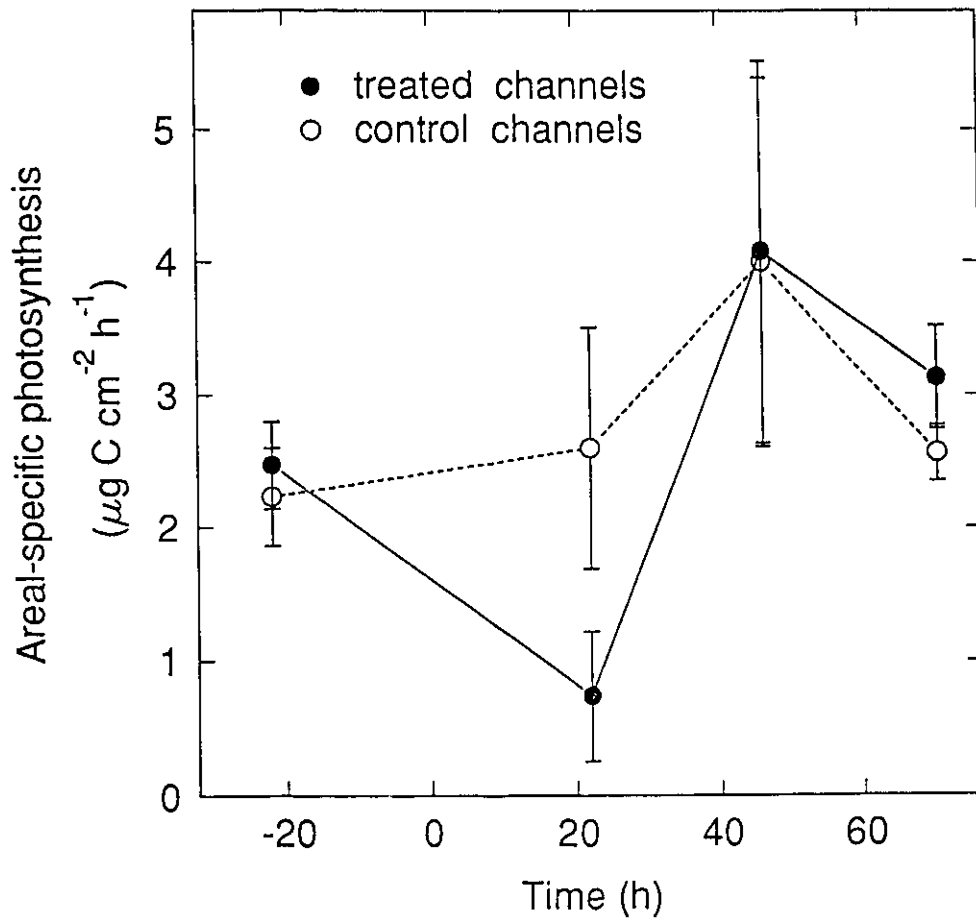


Figure D.1. Aeral-specific periphyton productivity in the control and Velpar L-treated channels before, during and after the treatment (hours 0-24). Vertical bars show standard errors (n=15 for times 1 to 4, n=10 for times 5 and 6).

APPENDIX E. Summary of the results of the multivariate repeated measures ANOVA models (n=40) for treatment (control and treated channels) with chlorophyll *a*-specific or areal-specific periphyton productivity as the independent variables.

Table E.1. Summary of the multivariate repeated measures ANOVA with chlorophyll *a*-specific productivity as the independent variable.

Within Subject Effects						
Source of variation	Statistic	Value	F	num df	den df	p
time	Wilks' Lambda	0.06	33.3	3	6	0.0004
treatment*time	Wilks' Lambda	0.12	15.1	3	6	0.003

Between Subject Effects				
Source of variation	df	MS	F	p
treatment	1	1640	9.9	0.01
error	8	1329		

Table E.2. Summary of the multivariate repeated measures ANOVA with area-specific productivity as the independent variable.

Within Subject Effects						
Source of variation	Statistic	Value	F	num df	den df	p
time	Wilks' Lambda	0.02	11.1	3	6	0.007
treatment*time	Wilks' Lambda	0.27	5.4	3	6	0.038

Between Subject Effects				
Source of variation	df	MS	F	p
treatment	1	0.59	0.3	0.60
error	8	2.01		

APPENDIX F. Summary of the results of the univariate repeated measures ANOVA model (n=52) for treatment (control and treated channels) with periphyton biomass (mg m^{-2}) as the independent variables. Multivariate analysis was not done due to insufficient degrees of freedom.

Table F.1. Summary of the univariate repeated measures ANOVA with the mean periphyton biomass for five baskets from each channels as the independent variable.

Source of Variation	df	MS	F	p
Within subject effects				
time	1	1.55	0.8	0.39
time*treatment	1	1.34	0.7	0.43
error	16	2.01		
Between subject effects				
treatment	1	1.24	0.1	0.76
error	4	11.50		

APPENDIX G. Summary of the results of the univariate repeated measures ANOVA model (n=100) for treatment (control and treated channels) with total macroinvertebrate drift (ind. L^{-1}) or insect drift (ind. L^{-1}) as the independent variables. Multivariate analysis was not done due to insufficient degrees of freedom.

Table G.1. Summary of the univariate repeated measures ANOVA using macroinvertebrate drift as the independent variable.

Source of Variation	df	MS	F	p
Within subject effects				
time	9	0.27	7.7	0.0001
time*treatment	9	0.06	1.6	0.13
error	72	0.04		
Between subject effects				
treatment	1	0.005	0.05	0.83
error	8	0.099		

Table G.2. Summary of the univariate repeated measures ANOVA using insect drift as the independent variable.

Source of Variation	df	MS	F	p
Within subject effects				
time	9	0.0014	2.3	0.03
time*treatment	9	0.0008	1.3	0.24
error	72	0.0006		
Between subject effects				
treatment	1	0.002	1.5	0.26
error	8	0.001		

APPENDIX H. Summaries of the results of the two-way ANOVA models (n=20) for time (one day before and one day after the treatment) and treatment (control and treated channels) with benthic macroinvertebrate biomass (mg m^{-2}) and density (ind m^{-2}) as the independent variables.

Table H.1. Summary of the two-way ANOVA using the mean biomass of five baskets for each channel as the independent variable.

Source of Variation	df	MS	F	p
time	1	$5.66*10^{6}$	2.9	0.11
treatment	1	$1.18*10^{6}$	0.6	0.45
time*treatment	1	$3.00*10^{5}$	0.2	0.70
error	16	$1.99*10^{6}$		

Table H.2. Summary of the two-way ANOVA using the mean density of five baskets for each channel as the independent variable.

Source of Variation	df	MS	F	p
time	1	$1.00*10^{11}$	12.5	0.003
treatment	1	$1.54*10^{9}$	0.2	0.67
time*treatment	1	$3.57*10^{10}$	4.5	0.05
error	16	$1.28*10^{11}$		

APPENDIX I. Summaries of the results of the two-way ANOVA models (n=20) for time (one day before and one day after the treatment) and treatment (control and treated channels) with benthic macroinvertebrate individual length (mm) and variance as the independent variables.

Table I.1. Summary of the two-way ANOVA using the mean individual length of five baskets for each channel as the independent variable.

Source of Variation	df	MS	F	p
time	1	0.003	2.2	0.16
treatment	1	0.033	0.2	0.65
time*treatment	1	0.045	3.0	0.11
error	16	0.015		

Table I.2. Summary of the two-way ANOVA using the variance of the mean individual lengths (mm) of five baskets for each channel as the independent variable.

Source of Variation	df	MS	F	p
time	1	82097	5.9	0.03
treatment	1	1812	0.1	0.72
time*treatment	1	0.4	0.0	1.00
error	16	13965		

APPENDIX J. The 4 hour EC_{50} and recovery experiment results expressed as areal-specific productivity.

The inverse logistic model gives a 4-hr EC_{50} for areal-specific productivity of 2.1 µg L^{-1} with a 95% confidence interval of 0 to 71 µg L^{-1} (Fig. J.1). This value is similar to the 4-hr EC_{50} for chlorophyll *a*-specific productivity (3.6 µg L^{-1}). Given that the maximum expected environmental concentration (EEC) of hexazinone is 800 µg L^{-1}, the EC_{50} value suggests that envrionmentally realistic concentrations of hexazinone will have an impact on stream periphyton.

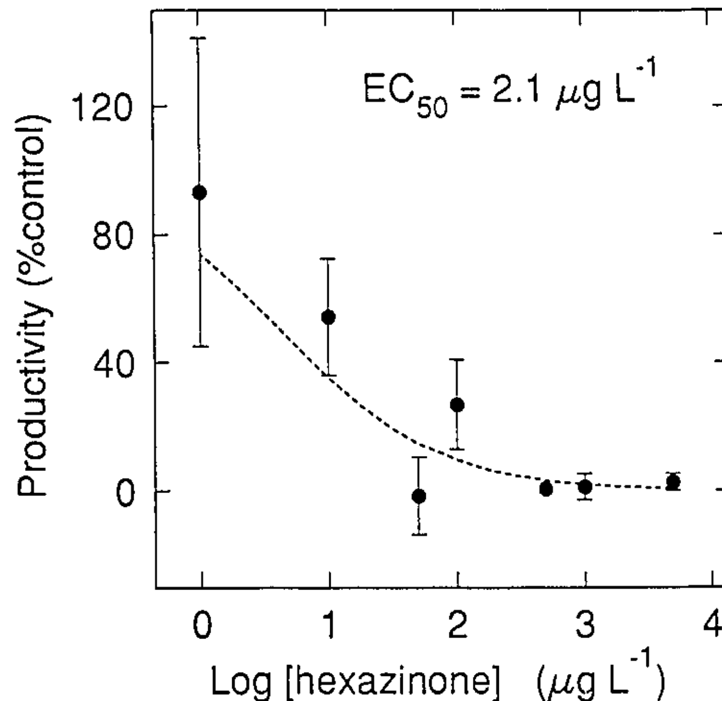


Figure J.1. Inhibition of areal-specific productivity by formulated hexazinone in a natural periphyton community developed in experimental stream channels. The line represents the best fit to an inverse logistic equation. Vertical bars are standard errors (n=3).

The extent of the impact will depend not only on the concentration but also the duration of the exposure and the ability of the periphyton to recover from the exposure. The concentration of formulated hexazinone at which the rate of areal-specific productivity would recover to 50% of the control level within 4 hours was 323 µg L^{-1} (Fig. J.2).

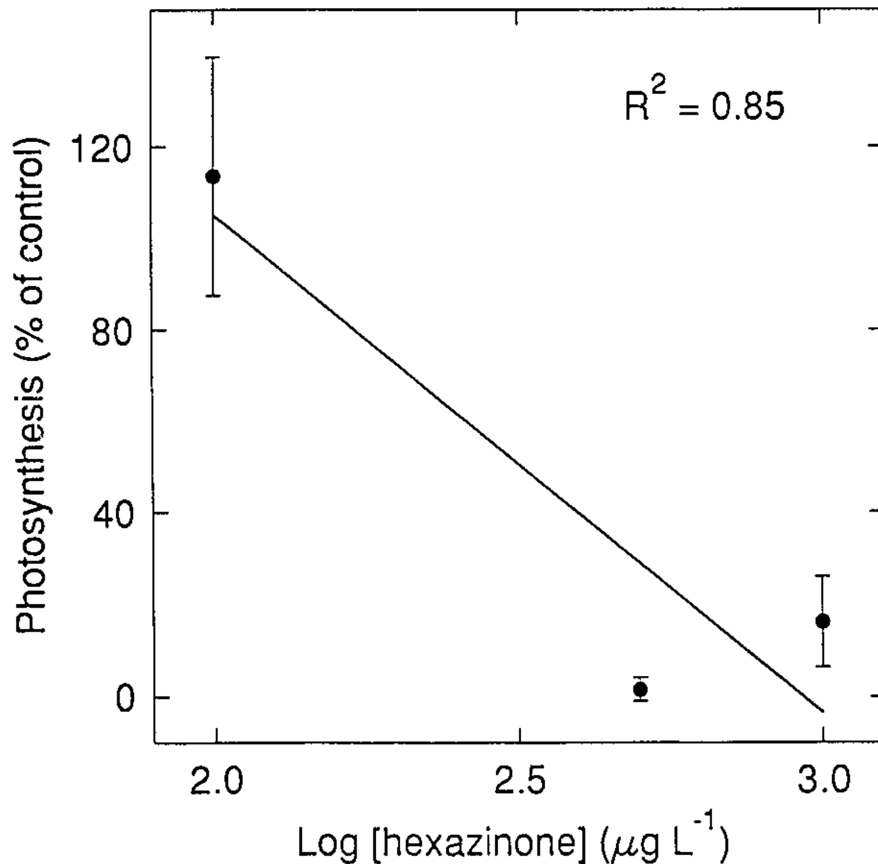


Figure J.2. Inhibition of areal-specific productivity of a natural periphyton community, developed in experimental stream channels, after a 20 hour exposure to formulated hexazinone followed by a 4 hour recovery period. Vertical bars show the standard errors (n=3).