Hexazinone dissipation in forest ecosystems and impacts on aquatic communities

Jerry L. Michael, Cliff Webber, Jr., David R. Bayne, Joseph B. Fischer, Hilliard L. Gibbs, and Wendy C. Seesock

Abstract: Hexazinone (active ingredient) was aerially applied as a pellet (Velpar ULW) and as a liquid (Velpar L) to watersheds in the Piedmont of Alabama, U.S.A., at the rate of 6.72 kg ha\(^{-1}\) (three times the prescribed rate for this site). An untreated watershed served as a control. We determined hexazinone half-life in days for Velpar ULW (plants, 26-59; litter, 55; bare soil, 68; soil under litter, 74) and for Velpar L (plants, 19-36; litter, 56; bare soil, 77; soil under litter, 275). Maximum stream concentrations of hexazinone (422 \(\mu\)g L\(^{-1}\) for Velpar ULW; 473 \(\mu\)g L\(^{-1}\) for Velpar L) were observed during application and resulted from direct overspray. Hexazinone stream concentrations peaked several times during stormflow in the first 30 days (56-70 \(\mu\)g L\(^{-1}\) for Velpar ULW, 145-230 \(\mu\)g L\(^{-1}\) for Velpar L) and were diluted three to five times 1.6 km downstream. Hexazinone metabolites were also monitored. Exposure of macroinvertebrates to hexazinone did not alter benthic community structure. Taxa richness, including pollution-sensitive insects, did not differ significantly between either hexazinone treatment and the control. Benthic macroinvertebrates in Piedmont streams of the southeastern United States appear insensitive to hexazinone at the exposures observed in this study.

Résumé : De l’hexazinone (ingrédient actif) a été appliqué par voie aérienne sous formes granulaire (Velpar ULW) et liquide (Velpar L) dans des bassins versants du piémont de l’Alabama, aux États-Unis, au taux de 6.72 kg ha\(^{-1}\), c’est-à-dire trois fois le taux recommandé pour ce site. Un bassin versant non traité a servi de témoin. Nous avons déterminé la demi-vie en jours de l’hexazinone pour le Velpar ULW (plantes, 26-59 ; litière, 55 ; sol nu, 68 ; sol recouvert de litière, 74) et le Velpar L (plantes, 19-36 ; litière, 56 ; sol nu, 77 ; sol recouvert de litière, 275). Les concentrations maximales d’hexazinone dans les ruisseaux (422 \(\mu\)g L\(^{-1}\) pour le Velpar ULW ; 473 \(\mu\)g L\(^{-1}\) pour le Velpar L) ont été observées lors de l’application et résultent directement de l’application aérienne. Les concentrations d’hexazinone dans les ruisseaux ont connu des pointes à plusieurs reprises lors des crues pendant les premiers 30 jours (56-70 \(\mu\)g L\(^{-1}\) pour le Velpar ULW ; 145-230 \(\mu\)g L\(^{-1}\) pour le Velpar L) et étaient diluées de trois à cinq fois à 1,6 km en aval. Les métabolites de l’hexazinone ont aussi été suivis. L’exposition des macro-invertébrés à l’hexazinone n’a pas altéré la structure de la communauté benthique. La richesse en taxons, incluant les insectes sensibles à la pollution, n’était pas significativement différente avec ou sans traitement à l’hexazinone. Les macro-invertébrés benthiques dans les ruisseaux du piémont du sud-est des États-Unis ne semblent pas affectés par l’hexazinone au niveau d’exposition observé dans cette étude.

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Introduction

Most environmental fate and impact concerns associated with the use of forest herbicides are related to offsite movement into aquatic ecosystems, during and after application. The fate and potential impacts of forest herbicides are governed by movement and transformation in the atmosphere, aboveground vegetation, soil surface, soil rooting zone, unsaturated zone below the rooting depth, and groundwater. Herbicides and their breakdown products are transported within ecosystems mainly through the water cycle. Drift, volatilization, photodecomposition, and other forms of degradation also affect movement, directly or indirectly. Precipitation and evaporation are the principal driving forces in the processes of runoff, leaching, and plant uptake. Hewlett (1982), Anderson et al. (1976), and Crossley and Swank (1988) have discussed these processes in great detail for forest watersheds. Many herbicide fate studies have been conducted in the southern pine forests of the United States. Michael and Neary (1993) have reviewed those studies, but worldwide, few studies have looked holistically at dissipation of forest herbicides.

The U.S. Environmental Protection Agency has registered hexazinone (3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1H,3H)-dione), the active ingredient (a.i.) in Velpar (RTM) Herbicide, for forestry use in the United States. Hexazinone is a white crystalline solid with a relatively low vapor pressure making it essentially nonvolatile. It is a potent inhibitor of photosynthesis in susceptible species. Hexazinone is very water soluble and readily leaches through soil. The principal routes of loss are from photodegradation and plant and microbial metabolism (USDA...
1984). Photodegradation occurs in aqueous solution with a half-life of approximately 4-5 weeks in river water (Rhodes 1980a). Metabolism of hexazinone into eight compounds has been described (Rhodes 1980a, 1980b; Rhodes et al. 1980).

Hexazinone is used for forest vegetation control once or twice per rotation (about 30 years). It is used around the world for silvicultural purposes (Reynolds and Roden 1995; Steele et al. 1995; Link and Allison 1995; Gous 1995; Adams and Dutkowski 1995; Obiaga 1985; Garcia-Holguin et al. 1991). Hexazinone is also used for weed control in hay and fodder (McCarty et al. 1996), lowbush blueberry (Vaccinium angustifolium Ait.; Jensen and Kimball 1987), and sugarcane (Saccharum officinarum L.; Fadayomi 1988) production. The near-global availability and use of hexazinone has spurred considerable research on various aspects of the environment potentially affected by its use. For example, soil leaching, soil persistence, microbial impacts, contamination of streams, and impacts on aquatic organisms have been reported.

Leaching of hexazinone and movement through the soil profile has been determined for several soil types. Roy et al. (1989) found that hexazinone did not move laterally in the soil profile. Roy and Roy and Allender (1989) also found that 98% or more of the applied hexazinone remained in the upper 15 cm of soil for both clay (280% clay, 29% organic matter) and sand (>80% sand, 5% organic matter) soils in boreal forest sites. Stone et al. (1993) found hexazinone concentrations at 150 cm were about half that observed at 10 and 20 cm in coarse sandy soils in the Lake States region of the United States. In their study approximately 1% of the applied hexazinone leached to 150 cm. In Australia, Allender (1991) observed leaching of hexazinone to a depth of 45 cm in the soil profile. Allender found that at industrial application rates (12 kg ha⁻¹) hexazinone damaged vegetation up to 100 m offsite by lateral movement in soil and surface runoff. Jensen and Kimball (1987) investigated hexazinone movement in sandy loam and sandy soils in Nova Scotia and observed leaching below 45 cm in all soils. Feng and Navratil (1990) found at least 13-17% of the applied hexazinone leached to a depth in the soil of 15-30 cm. Clearly, soil factors, temperature, and precipitation duration and intensity play major roles in the leaching of hexazinone through soil profiles.

Hexazinone persistence has been determined both in field and laboratory studies. Rhodes (1980b) found the half-life of hexazinone in the soil to range from 1 month in Delaware (sandy loam) to 10-12 months in Mississippi (silty loam). But in the laboratory, hexazinone incubated in soil (silty loam) had a half-life of about 80 days. Rhodes (1980b) identified several metabolites in soil. Of these, compound C (3-(4-hydroxy-cyclohexyl)-6-(methyleneamino)-1-methyl-1,3,5-triazine-2,4(1H,3H)-dione) was the principal metabolite by-product in field studies while compounds A (3-(4-hydroxy-cyclohexyl)-6-(dimethy lamino)-1-methyl-1,3,5-triazine-2,4(1H,3H)-dione), B (3-cyclohexyl-6-(methyleneamino)-1-methyl-1,3,5-triazine-2,4(1H,3H)-dione), and D (3-cyclohexyl-1-methyl-1,3,5-triazine-2,4,6(1H,3H,5H)-trione) were the principal metabolites in greenhouse studies. Jensen and Kimball (1987) found half-lives for hexazinone in soils from Nova Scotia to be similar to those reported by Rhodes;

however, compound B was the major metabolite present except when soils were incubated or heat sterilized, then compound D was the predominant break-down product. Sung et al. (1981) determined the half-life of hexazinone to be 24 days in a sandy soil and up to 42 days in a clay soil in Alabama. Bouchard et al. (1985) measured the half-life to be less than 42 days in fine sandy loam soils in Arkansas and 77 days in the same soil incubated in the laboratory. In summary, the half-life of hexazinone in soils from field tests ranged from 24 to 365 days. In laboratory studies the half-life in soils ranged from 77 to 80 days. The high variability found in the field studies was probably a result of climatic differences among the sites, principally temperature and timing, intensity, and duration of rainfall coupled with antecedent soil moisture conditions.

Herbicides that leach through the soil profile and are very water soluble may reach aquatic environments. Michael and Neary (1993) summarized studies that monitored offsite movement of hexazinone from forested watersheds in the southern United States. When applied according to label directions, the maximum concentration observed in storm run-off was short lived and generally below 442 μg L⁻¹. In Australia, hexazinone contamination (up to 4 μg L⁻¹) of a stream draining a catchment treated with 2 kg a.i. ha⁻¹ was detected only on the day of application (Leitch and Flinn 1983).

Evaluation of the impacts of hexazinone on aquatic ecosystems has been conducted using static laboratory exposures (Abou-Waly et al. 1991; Peterson et al. 1994; Berrill et al. 1994; Wan et al. 1988), artificial enclosures in boreal forest lakes (Thompson et al. 1993a, 1993b; Solomon et al. 1988), and in artificial streams exposed to continuous addition of hexazinone (Schneider et al. 1995; Kreutzweiser et al. 1995). Abou-Waly et al. (1991) found considerable growth depression for two species of algae, Anabaena fos- aqueae (Lynx) and Selenastrum capricornutum (Printz), exposed to hexazinone concentrations from 0.035 to 2.0 mg L⁻¹. Within 5-7 days, however, growth recovery was complete for both species. Peterson et al. (1994) found nearly complete inhibition of photosynthesis with static exposures of 22 h to hexazinone at 2.867 mg L⁻¹. However, they did not evaluate post-exposure recovery. In a study of amphibians, Berrill et al. (1994) reported frog embryos and tadpoles were not affected by 8-day exposures to hexazinone at 100 mg L⁻¹. Wan et al. (1988) determined that hexazinone was toxic to juvenile Pacific salmonids exposed to concentrations exceeding 236 mg L⁻¹ for 96 h or more. Thompson et al. (1993a) found significant impacts on phytoplankton in boreal lake enclosures at chronic hexazinone exposures to concentrations exceeding 0.1 mg L⁻¹ and on zooplankton at chronic exposures to concentrations approximating 1 mg L⁻¹ (Thompson et al. 1993b). Solomon et al. (1988) found significant inhibition of photosynthesis in artificial enclosures at hexazinone concentrations of 0.02-0.2 mg L⁻¹. They observed recovery within 100 days after treatment (DAT) and concluded that hexazinone at these treatment levels would not be expected to cause long-term secondary effects on the biota.

Environmental exposures of biota to herbicides in lotic systems are almost never static. Therefore, the results of static tests, either in the laboratory or in enclosures in lentic systems, are not easily extrapolated to lotic systems. Studies
Fig. 1. Study watersheds showing sampling stations for water and biota in Coosa County, Alabama, 1990-1991. Lower stations were located 75-100 m downstream of the H-flumes.

in lotic systems have attempted to overcome the disadvantages of static laboratory tests and field enclosures by establishing experimental stream channels through which flow could be carefully controlled (Schneider et al. 1995; Kreutzweiser et al. 1995). Schneider et al. (1995) added Velpar L to five stream channels for 24 h to effect mean treatment levels of 145132 μg a.i.:L−1. This range of exposures resulted in an 80% reduction in photosynthetic activity by the algal component of the periphyton, but photosynthetic activity returned to normal within 24 h after exposure was discontinued. Schneider et al. found that the concentration of hexazinone that reduced productivity by 50% (EC50) was 3.6 μg:L−1. Schneider et al. concluded that stream systems appear resilient to short-term hexazinone exposures, but chronic exposures or higher doses of Velpar L might have adverse effects on stream biota. During this study, periphyton biomass and macroinvertebrates were unaffected but the effects of repeated and chronic exposure on stream communities remained unexplored (Schneider et al. 1995). Similarly, Kreutzweiser et al. (1992) established treatment levels of 2700 μg a.i.:L−1 for 12 h in experimental channels and found that algal photosynthesis was reduced, but recovery of photosynthetic activity to levels in untreated control channels was complete within 3 h after treatment was discontinued. Kreutzweiser et al. (1995) also reported no significant drift for five of six insect species following exposure to hexazinone up to 80 mg:L−1. We found only one study that examined the responses of benthic macroinvertebrates to hexazinone in natural streams. Mayack et al. (1982) detected no adverse effects on benthic communities in streams treated at a rate of 1.68 kg a.i.:ha−1. The maximum concentration of hexazinone measured by Mayack et al. was 0.044 mg:L−1.

Forest ecosystems typically include both a terrestrial and aquatic component. The aquatic component is usually a stream that receives runoff of surface flow and groundwater. There is a need to better understand risks to aquatic communities associated with variable-term multiple exposures to low concentrations from the dissipation of pesticides applied to the terrestrial portion of the system. The purpose of this study was to describe the dissipation of hexazinone from the terrestrial component of the ecosystem and how that dissipation affected biota in the receiving stream. The study was also used in partial fulfillment of the U.S. Environmental Protection Agency reregistration requirements. The rate of dissipation of hexazinone in plant tissue, forest litter, and soil; offsite movement of hexazinone in water and sediment; and impacts of that movement on aquatic macroinvertebrate and fish populations were measured. The principal metabolites of hexazinone were also monitored during the study.

Materials and methods

Study area

This study was conducted in Coosa County, Alabama, in typical Piedmont pine forestland. The study area is a headwater drainage of the Coosa River and consists of numerous small watersheds with ephemeral and first-order perennial streams. The terrain is highly dissected with about 122 m of topographic relief. Mean watershed slope calculated using Van Haveren’s (1986) method is 4% for all three watersheds, but the hillside slope average is 15, 17, and 21% for the three watersheds. The three adjacent watersheds used in this experiment, each contained a perennial first-order stream (Fig. 1). Loblolly pines (Pinus taeda L.) were harvested from each watershed in 1988 and 1989. One watershed served as a control (96 ha, 17% hillside slope) and was not treated with the herbicide. Timber on the south side of the control watershed was not clearcut and consisted of mixed hardwoods and pines extending to the edge of the stream. On 23 and 24 April 1990 the other two watersheds received aerial applications of hexazinone at a rate of 6.72 kg ha−1, a rate three times that listed on the label for site preparation for this site. One treatment watershed (76 ha, 2% hillside slope) received a liquid formulation (Velpar L) and the other watershed (75 ha, 15% hillside slope) received pellets (Velpar ULW). All foliage was fully developed at the time of herbicide application. In each watershed a 5-6 ha streamside management zone (SMZ) was left unharvested. SMZs were 10 m wide on each side of the perennial stream and were not treated with herbicide. Ephemeral channels above the perennial streams were not protected by SMZs.

The dominant soils in these watersheds are the Tallapoosa series (loamy, micaceous, thermic, shallow Ochreptic Hapludults) on the moderately sloping terminal terraces and side slopes. Tallapoosa soils are shallow, well drained, moderately permeable, and low in natural fertility and organic matter. Much of the Tallapoosa series on this site is less than 90 cm in depth with rocky strata below 60 cm. Some of the broader ridges include Tatum soils (clayey, mixed, thermic Typic Hapludults). These well-drained soils are also moderately permeable and low in natural fertility and organic matter. The Chewacla soils (fine-loamy, mixed, thermic Dystrochrepts) occupy the floodplains and low stream terraces throughout the study area. Chewacla soils are deep, somewhat poorly drained with moderate organic matter and are subject to short-term flooding (L.E. McGhee, Soil Survey Project Leader, Coosa County, Alabama, personal communication).

Physicochemical variables

Rainfall was recorded in each watershed using standard recording rain gauges. Stream discharge was measured continuously in the lower portion of each tributary near stations 2, 4, and 6 through 30-cm H flumes built into the streams. Dissolved oxygen (DO) and temperature were measured in situ on six dates at about 1400 at stations 2, 4, and 6. In April and July 1990, duplicate 2-L grab samples were collected from each station for nutrient analyses.
Nutrient analyses were used to characterize water quality in these streams before and after treatment with hexazinone.

Herbicide application

Hexazinone was applied to two watersheds by helicopter at the rate of 6.72 kg ha\(^{-1}\); the first as a granular formulation, Velpar ULW (23 April 1990) and the second as the liquid formulation, Velpar L (24 April 1990), in a total of 168 L carrier (water) volume per hectare. Treated watersheds were flagged at 15-m intervals providing parallel flight lines. All flight lines marking the approximate center of each application swath were numbered for use in vegetation, litter, and soil sampling. Treatment rate was verified using herbicide traps strategically placed across randomly selected flight lines.

Sampling for hexazinone residues

Litter, sediment, vegetation, and soil matrices were sampled on 14 dates (-1, 0, 1, 3, 7, 14, 30, 45, 60, 90, 120, 180, 270, and 365 days relative to hexazinone treatment) for hexazinone and metabolite residue analysis. Chest-style freezers powered by portable generators were taken to the sites, and all samples were frozen immediately after collection and maintained frozen until analyzed. Samples for vegetation, litter, and soil were collected near the middle of established helicopter flight lines. Sampling locations at ridge, midslope, and toe-slope positions were marked with metal pins prior to application. At each position, a small area of bare ground was prepared. Five flight lines were randomly selected for sampling on any given date.

The plants sampled were bracken fern, Pteridium aquilinum (Kuhn); blueberry, Vaccinium spp.; dogwood, Cornus florida L.; and grasses. All four groups were analyzed separately. Whole tops were collected for grasses and bracken fern, but only the terminal, 15-30 cm of branches with foliage were collected for the remaining species.

Soil samples were taken from areas covered with litter and from bare soil. Litter samples were taken from the same place as soil under litter. The litter was first removed from a 30 x 30 cm square and labeled; the underlying soil was sampled to a maximum depth of 1 m. Bare soil was similarly sampled. Soils were sampled with 7.6 cm diameter PVC tubes. The tubes were ham-mered into the ground to a maximum depth of 90 cm, carefully extracted, and stored frozen until they were cut into appropriate lengths (15-cm increments of soil depth) in the laboratory prior to analysis.

Plant material, litter, and soil samples were composited as separate matrices by slope position for each watershed. In the laboratory, each matrix was composited according to slope position. For example, from five randomly selected flight lines, litter from ridge positions comprised one composite sample; for each matrix, a similar procedure was followed for midslope and toe-slope positions. Litter was dried for 24 h at 80°C and ground in a Waring blender. Each of the plant groups was composited by slope position as described for litter, yielding a total of 12 (3 slope positions x 4 plant groups) samples per date. Plant material was shredded into small pieces and ground in a Waring blender with dry ice. Plant residues were expressed on a fresh-mass basis. Soil samples were oven-dried at 80°C and ground to a fine powder. Subsamples from each soil depth were composited for each slope position producing a maximum of 36 composited soil samples at each sampling date (3 slope positions x 2 soil cover conditions x a maximum of 6 depth increments) for each watershed.

Water and sediment residues

Hexazinone residues were measured in water samples taken with automatic samplers attached to 30-cm H flumes and 1.6 km downstream. H flumes were located at the lower edge of the treated areas. Following removal from the automatic samplers, water samples were kept cool and in the dark until frozen. Sampling was conducted at 15-min intervals during herbicide application and most storm events. When swollen streams began to recede following storm events, sampling intensity was decreased to 6-h intervals. Sampling occurred at least daily through November 1990. Water samples collected at the H flumes on each site were normally analyzed without compositing through day 78 (10 July 1990); and then samples were composited to form daily or weekly samples. Sediment samples were taken from the approach to each H flume or from the stream bottom at the downstream station. Sediment samples were not composited.

Hexazinone analysis

Samples from each matrix were analyzed for hexazinone and metabolites by HPLC using reversed-phase gradient-elution and programmed wavelength ultraviolet detection; metabolite identity, and quantification were confirmed by thermospray ionization liquid chromatography - mass spectrometry (Fischer and Michael 1995). Hexazinone detection limits based on method blanks and detector response were: water, 2 \(\mu\)g L\(^{-1}\); plants, 17 \(\mu\)g kg\(^{-1}\); litter, 16 \(\mu\)g kg\(^{-1}\); soil, 4 \(\mu\)g kg\(^{-1}\); and sediment, 15 \(\mu\)g kg\(^{-1}\). Recovery of hexazinone and metabolites ranged from 86-97% in the various matrices analyzed.

Half-life, the time to dissipation of 50% of the parent material, was calculated from hexazinone residue data. Data for each matrix were subjected to simple linear regression of the log concentration versus time and half-life \(T_{0.5}\) was calculated from the slope of the regression line from the time of maximum concentration (Michael and Neary 1990, 1993). Hexazinone dissipation in the natural environment is a function of many different parameters that vary from site to site so dissipation normally does not follow first-order kinetics implied by the term half-life. Perhaps a better term is dissipation time or \(DT_{50}\) as used by Thompson et al. (1993b) but half-life is used throughout this paper to minimize confusion with previously published values.

Aquatic invertebrate communities

Benthic macroinvertebrates were sampled at all stations on six dates between 20 April 1990 and 26 February 1991 (~3, 7, 41, 69, 139, and 307 days relative to hexazinone treatment). Qualitative samples were collected in riffle and run habitats at each station using D-frame nets (mesh size about 1 mm). This method is recommended by other researchers (Lenat 1988; Plafkin et al. 1989; Barbour et al. 1996) for use in small lotic systems. It provides a reliable assessment of water quality that concentrates on estimating taxa richness of the macroinvertebrate communities.

At each station, two biologists collected macroinvertebrates from available habitat in a 10- to 20-m reach of stream. All organisms were combined for one composite sample for each biologist providing a total of two replicates per station. Netting effort was timed at 10 min for each biologist to insure similar collecting effort. From randomly selected subsamples of at least 100 macroinvertebrates, we determined taxa richness and the EPT index. The EPT index is the number of insect taxa in the orders Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies). This index measures the diversity of insect groups consisting mostly of pollution-sensitive species (Barbour et al. 1992). The percent composition of different functional-feeding groups was also analyzed from the subsamples as a measure of community structure. The other measure used to determine a difference among treatments, the Shannon-Weaver diversity index (Weber 1973), was based on macroinvertebrates from the whole sample collected at each station.
Fish communities
Fish communities were sampled at stations 2, 4, and 6 (Fig. 1) in April (prior to hexazinone application), June, and July 1990 and again in April 1991. A 100-m section of stream was blocked off with nets (6-mm bar mesh), and fish were sampled with two passes through the reach with a backpack electroshocker. Captured fish were identified, weighed, measured, and examined for physical abnormalities in the field. Most fish were released alive after sampling. Fish were further classified based on their tolerance to pollution and trophic level (Karr 1981).

Results and discussion
Precipitation
A light rainfall (4 mm) began at about 16:00 following application of the Velpar ULW treatment. No additional measurable precipitation occurred until 28 April (3 mm), 4 DAT for the Velpar L application. A total of 17 storms exceeding 12 mm occurred on the Velpar ULW watershed and 12 on the Velpar L site between application and 31 December (251 days). Within the first 28 DAT, four storms exceeded 12 mm on both sites. These storms were intensively monitored for herbicide runoff. Total precipitation measured on the two watersheds varied by only a few centimetres (Velpar ULW site, 45.4 cm; Velpar L site, 42.4 cm).

Plant residues
Hexazinone residues were much higher in vegetation from the Velpar L watershed than in plant tissues from the Velpar ULW site. Maximum hexazinone concentrations from the Velpar L site ranged from 384 mg kg$^{-1}$ in fems to 702 mg kg$^{-1}$ in dogwoods. On the Velpar ULW watershed, maximum concentrations ranged from 2 mg kg$^{-1}$ in blueberry plants to 32 mg kg$^{-1}$ in grass tissues. Also, peak hexazinone residues in vegetation occurred within a day of application on the Velpar L watershed but not until approximately 14 DAT on the Velpar ULW site (Table 1, Fig. 2). The reason for this difference in plant uptake of hexazinone between treatments relates to the formulations used on each site. Application of the liquid formulation of hexazinone resulted in direct contact with plant foliage and rapid absorption. However, the granular formulation (Velpar ULW) had to be dissolved by rain, washed or leached to soil, then absorbed by plants via root uptake. This process took about 14 days during which 10 mm of rain fell on the site. Dogwood on the Velpar ULW site was the exception. Highest concentrations occurred 2 DAT and was probably due to a Velpar ULW pellet stuck to some part of the foliage during sampling. While hexazinone residues were increasing in vegetation from the Velpar ULW watershed, residues declined by up to 93% in vegetation from the Velpar L site (Fig. 2).

Foliar hexazinone residues decreased by 51–79% for the four groups of vegetation during the first 7 days after application of the Velpar ULW treatment. No additional hexazinone residues were observed.

Table 1. Maximum observed hexazinone and principal metabolite concentrations in plant tissues from the Velpar ULW and Velpar L treated watersheds.

<table>
<thead>
<tr>
<th>Hexazinone</th>
<th>Metabolite</th>
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<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Velpar ULW</td>
<td></td>
</tr>
<tr>
<td>Blueberry</td>
<td>2.16 (14)</td>
</tr>
<tr>
<td>Dogwood</td>
<td>5.53 (2)</td>
</tr>
<tr>
<td>Fem</td>
<td>7.82 (14)</td>
</tr>
<tr>
<td>Grass</td>
<td>32.38 (14)</td>
</tr>
<tr>
<td>Velpar L</td>
<td></td>
</tr>
<tr>
<td>Blueberry</td>
<td>525.63 (1)</td>
</tr>
<tr>
<td>Dogwood</td>
<td>702.41 (0)</td>
</tr>
<tr>
<td>Fem</td>
<td>383.98 (0)</td>
</tr>
<tr>
<td>Grass</td>
<td>626.23 (1)</td>
</tr>
</tbody>
</table>

Note: Concentrations are expressed in mg kg$^{-1}$ fresh mass, and values in parentheses are the days after treatment when these values were observed.
application of Velpar L. Precipitation during this period to-taled only 3 mm and occurred 4 DAT. Michael et al. (1992) found that a 6-mm rain occurring 1 h after treating dogwoods with Velpar L washed 91% of the hexazinone from the foliage. Hexazinone photodegrades slowly in aqueous solution with a half-life of 4-5 weeks (Rhodes 1980a). Therefore, the rapid decline in hexazinone from vegetation on the Velpar L watershed the first 2 weeks after application was mostly due to mechanical loss and rain washing hexa-zinone from the foliage.

Hexazinone in plant tissues decreased rapidly on both wa-tersheds, especially for the Velpar L vegetation. Hexazinone was 99% dissipated within 180 DAT, except for dogwood on the Velpar ULW site. In dogwoods, hexazinone residues were 88% dissipated. The half-life for hexazinone residues in vegetation varied by species and ranged from 19 to 36 days on the Velpar L watershed and from 26 to 59 days on the Velpar ULW watershed.

Hexazinone was metabolized by plants on the two wa-tersheds and metabolites also dissipated during the study. Although peak concentrations were much lower, metabolites A, B, D, and E (3-(4-hydroxycyclohexyl)-1-methyl-1,3,5-triazine-2,4,6(1H,3H,5H)-trione) followed the same general pattern of dissipation observed for hexazinone. Maximum concentrations of metabolites usually peaked 1-7 days after that for hexazinone on the Velpar L site, but the pattern was more complicated and maximum concentrations took longer to develop in Velpar ULW treated plants (Table 1). Concentrations of metabolites were higher in plant tissues from the Velpar L sites than in plants from the Velpar ULW wa-tershed. We found interfering compounds in plant extracts from both sites that occasionally made identification and quantification difficult. However, use of mass spectrometry confirmed the presence of metabolites A, B, and D in vegetation at concentrations similar to those observed under HPLC conditions. Detection limits for metabolite E in this analyti-cal system were too high to permit confirmation. We were also unable to confirm the presence of metabolite C in vegetation samples.

The metabolism of hexazinone by plants appears to be a function of the species and the formulation used. In plants from the Velpar ULW watershed the principal metabolite (metabolite found in the highest concentration in each species) was present at concentrations up to 51% of the highest hexazinone concentration except metabolite D. When the principal metabolite was D, it was present at a concentration up to five times that of the parent hexazinone. Plants in this study (blueberry) that are most resistant to hexazinone, pro-duced metabolite D as the principal metabolite, while plants that are susceptible to hexazinone produced larger quantities of metabolite B, the one metabolite known to be phototoxic. Metabolite concentrations did not exceed 4% of the parent hexazinone in plants from the Velpar L watershed. The prin-cipal metabolite also varied by species.

On the Velpar ULW treated watershed, metabolite B was the principal metabolite in all but blueberry plants and was the only metabolite confirmed in dogwood and fern. On this same site, metabolite D was the principal metabolite in blueberry and was found in grasses at about the same concentration as metabolite B. Metabolite A was also identified in grasses. On the Velpar L watershed, metabolites B and D were found in all four species, but metabolite D was the principal metabolite only in blueberry. Metabolite B was the principal metabolite in both dogwood and grasses. Metabo-lite A was the principal metabolite in fern and was also found in grasses.

Differences in susceptibility to hexazinone may be related to different routes of metabolism. Sidhu and Feng (1993) found only metabolites A and B in six species of boreal vegetation, but they also found differences in metabolite distri-bution with metabolite B the principal metabolite in only two of the six species monitored. Thus, the species they treated with a pelleted formulation were similar to our Velpar ULW treated plants in the paucity of metabolites present in vegetation. Sidhu and Feng did not detect metabo-lite D in any of their vegetation. While Rhodes (1980b) sug-gested that metabolite D was a very minor metabolite, Jensen and Kimball (1987) found that metabolite D was formed by a chemical process in the absence of biological activity and suggested it can be a major degradation product chemically formed under warm, moist incubation conditions in soil. In our study, metabolite D was found in significant concentrations in blueberry and grass and was present as a minor metabolite in the remaining species.

The toxicity of hexazinone to wildlife species is very low and the potential for its consumption by wildlife have been discussed (USDA 1984; Sidhu and Feng 1993). In our study, defoliation made plant tissue essentially unavailable to wild-life on both sites within 2 weeks of application. Some re-sprouting of defoliated plants occurred during the following months. The presence of hexazinone was not confirmed in resprouting vegetation 365 DAT.

Litter

Absorption of hexazinone residues in litter followed a different pattern than that described for vegetation but was also related to the type of formulation applied to the site. The highest hexazinone residues in litter on the Velpar ULW site occurred on the day of application (Fig. 2). Hexazinone concentrations in litter on the Velpar L watershed increased to a maximum 7 DAT, as a result of transfer from foliage to litter by rain (Fig. 2). Hexazinone residues in litter from both sites peaked at similar levels, but residues declined at a slower rate in litter from the Velpar L site. Hexazinone in the liquid application had the greater potential for absorption by litter because much of the hexazinone fell directly onto the litter in solution and could be immediately absorbed. Hexazinone in the granular formulation moved (by mechanical action) down through the litter to soil and could not be absorbed until sufficient rain fell to release hexazinone from the granules. The different mechanisms of absorption and release that govern the dissipation of hexazinone in the two formulations used in this study led to different residue patterns in litter. The half-life for hexazinone in litter was 55 days on the Velpar ULW watershed and 56 days on the Velpar L watershed.

Metabolites B and D were the most frequently observed metabolites in litter and both occurred in highest concentrations on the Velpar L treated site. Metabolites A, C, and E were observed infrequently and at lower concentrations. There are no reports in the literature considering the rate of hexazinone in litter, but it is reasonable to assume that
metabolites measured within the first few days after application resulted from microbial action in the litter. Metabolites that appeared long after application likely resulted from metabolism in foliage of treated plants and appeared in litter as a result of defoliation.

**Table 2.** Mean hexazinone residues (mg·kg⁻¹; n = 3) in bare soil samples at five depths from the Weogufka, Ala., Velpar ULW site.

<table>
<thead>
<tr>
<th>Date</th>
<th>DAT</th>
<th>O-15</th>
<th>16-30</th>
<th>31-45</th>
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</tr>
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<td>NST</td>
<td>NST</td>
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<tr>
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<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
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<td>0.02</td>
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<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

*NST, no sample taken at this depth and date.

**Table 3.** Mean hexazinone residues (mg·kg⁻¹; n = 3) in litter-covered soil samples at five depths from the Weogufka, Ala., Velpar ULW treated site.

<table>
<thead>
<tr>
<th>Date</th>
<th>DAT</th>
<th>O-15</th>
<th>16-30</th>
<th>31-45</th>
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<th>61-75</th>
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<tbody>
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<td>0.02</td>
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<td>0.01</td>
</tr>
</tbody>
</table>

*NST, no sample taken at this depth and date.

Soil residues

Herbicide application was monitored with verification traps located on the site. Analysis of hexazinone in these traps indicated that the actual application rate was 83-109% of the intended rate of 6.72 kg a.i·ha⁻¹. The theoretical concentration of hexazinone in soil was calculated from the application rate and bulk density in the upper 15 cm of bare soil for both watersheds. Theoretical values on both watersheds were 3 ± 0.54 mg·kg⁻¹ (mean ± SD; n = 15 for each watershed).

The theoretical value on the Velpar ULW site was observed in bare soil and litter covered soil 1-7 DAT (Table 2). However, on the Velpar L watershed the theoretical value was not observed either in bare soil (maximum concentration was 1.95 mg·kg⁻¹ 7 DAT) or in soil under litter (Fig. 2, Tables 4 and 5). During application of herbicides, vegetation and litter intercept a portion of the applied material that is subsequently dislodged by wind action and precipitation. This dislodged material may then find its way to soil so that soil concentration actually decreases during the first week following treatment. This was the case with the Velpar L application in which the maximum

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Fig. 3. Mean daily hexazinone concentrations in streamflow from watersheds treated 23 and 24 April 1990 with 6.72 kg a.i.·ha⁻¹.

Streamflow residues

Hexazinone was detected in streams from both watersheds, but 1.6 km downstream these levels were diluted three to five times (Fig. 3). The highest concentration in streams was observed on the day of application at the flume on each treated watershed, and lasted 2-6 h. The maximum concentration observed on the Velpar ULW watershed (422 µg·L⁻¹) was similar to the maximum on the Velpar L watershed (473 µg·L⁻¹). However, during the day of application on the Velpar ULW watershed, hexazinone stream concentrations remained at 200-422 µg·L⁻¹ for 6 h before decreasing to a range of 79-130 µg·L⁻¹ for the remainder of the day. On the Velpar L watershed, hexazinone stream concentrations remained in the 200-473 µg·L⁻¹ range for 1.75 h and then decreased to a range of 11-23 µg·L⁻¹ for the remainder of the day. Maximum values were a result of direct application to ephemeral portions of the streams that were flowing on the day of application. In general, highest stormflow concentrations were observed on the Velpar L watershed, but they were shorter lived than those on the Velpar ULW watershed. Daily average concentrations of hexazinone in the two streams were two to three times higher in the Velpar ULW stream than in the Velpar L stream (Fig. 3).

Peak stormflow concentrations were measured for several precipitation events on each watershed (Table 6). The maximum observed stormflow concentrations were less than those observed on the day of application and generally decreased with successive storms, but they were higher for the Velpar L site than for the Velpar ULW site. In general, hexazinone concentrations were highest in stormflow at or near peak stream discharge for the Velpar L watershed and lagged behind peak discharge (descending limb of the
hydrograph) by 2.5-3.5 h on the Velpar ULW watershed. Peak runoff concentrations in the stream draining the Velpar L watershed were primarily from surface runoff, while subsurface flow was the primary route for peak hexazinone contributions reaching the stream from the Velpar ULW watershed.

Metabolites A and B were monitored throughout the study in both streams but were generally not detected or were at the detection limits 189 DAT. In general, metabolite occurrence downstream, when observed, was highly diluted. Metabolite B was the most frequently identified metabolite in water samples. It occurred at concentrations up to 23 µg L⁻¹ in the Velpar L treated watershed, but concentrations were usually much lower. Metabolite B was found more frequently in the Velpar ULW site, but concentrations were always much lower than observed in the Velpar L site. Metabolite B occurrence was correlated with stormflow and most concentrations above 2 µg L⁻¹ were also correlated with the presence of hexazinone. Metabolite A was infrequently detected in water from both watersheds. The maximum observed concentration for metabolite A was around 13 µg L⁻¹ in the Velpar ULW water but most values were near the detection limit. Its occurrence also coincided with storm runoff. Occurrence of both metabolites A and B in water may be a result of movement from the terrestrial component through surface and subsurface flow rather than a product of degradation in water.

Sediment
There was a bimodal distribution of hexazinone over a 365-day period in sediment samples, but it was much more obvious from the Velpar ULW site (Fig. 4). Sediment collected shortly after application was composed of fine, mostly organic or loam soil that contained hexazinone up to 1.7 mg kg⁻¹. Hexazinone dissipated more quickly in sediment from the Velpar L stream than that in the Velpar ULW stream, but by 180 DAT, hexazinone reached its lowest point in both streams. Then concentrations began to increase and by 360 DAT peaked at 0.5 mg kg⁻¹ in the Velpar L stream and 1.3 mg kg⁻¹ in the Velpar ULW stream. At 365 DAT, hexazinone concentrations in sediment began to decline again in both streams. Hexazinone concentration in streamflow did not increase in sediment concentration in either stream. The composition of the sediment changed during the study, and by 300 DAT it was composed mainly of very friable rock resembling coarse sand. This coarse friable rock was probably contaminated with hexazinone during the process of soil infiltration in the upper reaches of the watershed. Subsequently, erosion and overland flow during intense storms resulted in its deposition in streams as sediment. This sediment was then transported 1.5-1.7 km downstream to the flumes located at the bottom of the treated watershed during the following year. Hexazinone may have been stored in the fractures of this material and extracted during analysis accounting for the second node of the bimodal distribution.

Physicochemical variables in streams
Cobble, gravel, and sand comprised 70% or more of all riffle and run habitats sampled at each station. The first-order tributary width ranged from about 0.6 m at station 3 to 2 m at station 5. Riparian vegetation in the SMZ was similar at each station providing a canopy cover, prior to leaf fall, that ranged from partly shaded to shaded. Of the three streams, the control received more shading primarily because of the forested hillside bordering the southern edge of the stream. Consequently, on a given date water temperatures were usually 3°C colder in the control stream than in the other two treatments. Dissolved oxygen varied little among stations and values were always greater than 7 mg L⁻¹.

Water in each stream was slightly acidic (pH 6.2-6.9) and soft with low alkalinity and conductivity (Table 7). Seasonal changes in alkalinity, nitrate nitrogen (NO₃-N), soluble orthophosphate (PO₄-P) and conductivity were measured at all stations between pre- and post-treatment dates. No other pattern of change was discernible for alkalinity, PO₄-P, or conductivity. However, the magnitude of the NO₃-N increase in the Velpar L stream was greater than that in the control. In the Velpar ULW stream the magnitude of the increase in
Nitrate-N
ULW
4.30 4.10
Velpar L
ULW
Control of Georgia. Macroinvertebrates
16 4.13 3.88 3.85
6.8
Control 0.003 0.037 0.005 0.040
ULW per date.
to analyze the
effect,
were the
4.0
was
4.9
of the pollution-sensitive mayfly, stonefly, and
4.00
6.5 4.3 0.016 0.115
@nho.cm-'
37 33 33 19 16 16
(n
treatment of hexazinone ranging from 6 to 44
the Velpar L stream and 422
concentrations ranged from 1 to 60
benthic communities. In our study, mean daily hexazinone
no major
changes among streams within sample dates. Prior to and after
application of hexazinone, taxa richness in each stream was
different when compared with the control (Table 8). Based on the EPT index, hexazinone appeared to have no significant effect, in either treatment, on the community structure of the pollution-sensitive mayfly, stonefly, and caddisfly fauna. On each date these three groups of insects comprised from 41 to 58% of the overall taxa richness.

Several genera comprising the EPT index in this study were the same as those found by Mayack et al. (1982) in the Piedmont region of Georgia. Macrinovertebrates in the Mayack et al. study were exposed to intermittent concentrations of hexazinone ranging from 6 to 44 µg L⁻¹. They found no major changes in species composition or diversity in the benthic communities. In our study, mean daily hexazinone concentrations ranged from 1 to 60 µg L⁻¹ in the Velpar L treatment and from 4 to 126 µg L⁻¹ in the Velpar ULW treatment (Fig. 3). Direct application along sections of the stream resulted in maximum concentrations of 473 µg L⁻¹ in the Velpar L stream and 422 µg L⁻¹ in the Velpar ULW stream on the day of application. In both treatments, hexazinone concentrations peaked during storm runoff several times over the first 30 days. These short-duration peaks ranged between 145 and 230 µg L⁻¹ in the Velpar L stream and between 56 and 70 µg L⁻¹ in the Velpar ULW stream. After a month of this exposure to hexazinone, benthic community structure was not significantly altered. Even with rates applied in this study four times higher than those used by Mayack et al. (1982), hexazinone residues did not alter benthic community structure.

Concentrations measured in this experiment did not approach those used in artificial stream studies by Schneider et al. (1995) or Kreutzweiser et al. (1992, 1995). Schneider et al. (1995) found no significant effects on macroinvertebrate biomass, density, or drift from hexazinone at mean concentrations ranging from 145 to 432 mg L⁻¹ during a 24-h period (highest concentrations we observed lasted 15-30 min). However, their study did not involve chronic exposure of macroinvertebrates, and chironomids, oligochaetes, and mollusks dominated the fauna in their channels. In general, these macroinvertebrates are not as pollution sensitive as many of the representatives comprising the EPT fauna. The studies by Kreutzweiser et al. (1992, 1995) found no significant mortality for several macroinvertebrate taxa with 1 h (80 mg L⁻¹) and 12 h (2.7 mg L⁻¹) hexazinone exposures in flow-through systems. Both experiments by Kreutzweiser et al. included several EPT taxa that were common in our study.

No differences in Shannon-Weaver diversity were found among the streams indicating that hexazinone residues had little effect on the composition of the benthic communities (Table 8). Diversity values of 3 or higher in lotic systems are indicative of unstressed macroinvertebrate communities (Weber 1973). These results were similar to those found by Mayack et al. (1982).

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Table 7. Mean water quality measurements for stations in the control (1 and 2), ULW (3 and 4), and the Velpar L (5 and 6) treatments during 1990.

<table>
<thead>
<tr>
<th>Station</th>
<th>Alkalinity as CaCO₃ (mg L⁻¹)</th>
<th>Nitrate-N (mg L⁻¹)</th>
<th>Phosphate-P (mg L⁻¹)</th>
<th>Conductivity (µmho cm⁻¹)</th>
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<td>0.052</td>
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Note: Hexazinone was applied after the April sample. 1 mho = 1 S.


<table>
<thead>
<tr>
<th>Sample date</th>
<th>Taxa richness</th>
<th>EPT index</th>
<th>Shannon-Weaver diversity</th>
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Note: Hexazinone was applied on 23 and 24 April 1990. No difference (P > 0.05) was found among treatments on any date based on ANOVA (n = 4).
Benthic communities consisted predominantly of five functional feeding groups. Scrapers (i.e., macroinvertebrates that feed primarily on algae) and collector-gatherers (mostly Ephemeroptera) dominated the fauna in all streams, followed by predators (mostly Plecoptera), filtering collectors (mostly Trichoptera and species of the chironomid Tanytarsus spp.), and then shredders. Most scrapers were species of Stenonema, while species of Pseudocloeon and Baetis usually dominated the collector-gatherers. Scrapers are usually poorly represented in small headwater streams because of relatively low standing crops of periphytic algae, the result of heavy shading. Scraper numbers in our samples remained high in all streams suggesting that hexazinone had no effect on these organisms. Shredders and collectors often dominate in small headwater streams (Vannote et al. 1980). However, because watersheds in this study had been clearcut in 1988-1989, allochthonous inputs of leaf debris, or coarse particulate organic matter, to the streams was less than would have occurred if the systems were undisturbed. This may have contributed to the reduced number of shredders in these streams.

Biological impacts to benthic communities are often indicated by the absence of pollution-sensitive macroinvertebrate taxa, dominance by a particular taxon along with low taxon richness, and important shifts in community structure relative to the control condition (Barbour et al. 1996). Although the rates in our study provided exposures of benthic communities to variable concentrations of hexazinone over a period of 2 months, no changes in community structure were observed. Any changes in water quality resulting from the presence of hexazinone in the streams were short term and caused no decline in taxon richness. Benthic macroinvertebrates in Piedmont streams of the southeastern United States apparently are not sensitive to hexazinone at the application rates used in this study.

Fish communities

The first-order streams in this study did not contain a diverse fish community. This is typical of small streams in the Piedmont (Saylor and Scott 1987). The maximum number of species per stream at any one time was four. The dominant species comprising over 79% of the sample on all dates was Semotilus atromaculatus, the creek chub, a pollution-tolerant species common in Piedmont streams. Three pollution-intolerant species were also collected from one or more of the streams on at least one date following treatment. These included the Coosa shiner, Notropis xanoecephalus; the Alabama hogsucker, Hypentelium etowanum; and the Coosa darter, Etheostoma coosa, which was usually represented by only one or two individuals, if present at all. Based on the limited populations present in each stream, fish communities could not be used to evaluate hexazinone impacts.

Acknowledgments

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References


